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FREQUENCY OF HUMAN PAPILLOMA VIRUS IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA IN PATIENTS PRESENTING AT SKMCH PESHAWAR

Original Research

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ABSTRACT

Background: Oropharyngeal squamous cell carcinoma (OPSCC) is an increasingly recognized malignancy with a growing association to high-risk human papillomavirus (HPV) infections. While HPV-positive OPSCC cases are widely documented in Western populations, regional data, particularly from Pakistan, remain limited. Understanding local HPV prevalence is crucial for guiding preventive strategies, including vaccination and risk-based screening, especially in settings where sociocultural and environmental factors may influence disease presentation and progression.

Objective: To determine the frequency of human papillomavirus in patients diagnosed with oropharyngeal squamous cell carcinoma presenting at Shaukat Khanum Memorial Cancer Hospital, Peshawar.

Methods: This cross-sectional study was conducted from 03 November 2024 to 03 May 2025 and enrolled 89 patients aged between 40 and 80 years with histopathologically confirmed OPSCC. Patients with recurrent head and neck cancers, immunocompromised status, or coexisting malignancies were excluded. Clinical evaluation included detailed history-taking, ECOG performance status assessment, and radiological imaging (CT and/or MRI) to determine tumor staging. Histopathological confirmation was achieved through biopsy or FNAC, and DNA was extracted from formalin-fixed, paraffin-embedded tissue blocks. Polymerase chain reaction (PCR)-based assays were used for qualitative HPV-DNA detection.

Results: The mean age of participants was 53.38 ± 11.52 years, with 52 (58.4%) males and 37 (41.6%) females. HPV was detected in 14 patients, yielding a frequency of 15.7%. Advanced disease was prevalent, with 40 patients (44.9%) at stage III and 20 patients (22.5%) at stage IV. Tobacco use was observed in 12 (13.5%), alcohol in 5 (5.6%), and betel nut chewing in 10 (11.2%) cases.

Conclusion: The study demonstrated a moderate prevalence of HPV in OPSCC patients, with a majority presenting at advanced stages. The findings support the need for early detection efforts and highlight the importance of risk factor modification in reducing HPV-associated disease burden.

Keywords: Alcohol drinking, Betel nut, Human papillomavirus, Oropharyngeal neoplasms, Polymerase Chain Reaction, Risk factors, Tobacco use.

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INTRODUCTION

Oropharyngeal squamous cell carcinoma (OPSCC), often referred to as throat or tonsil cancer, is a malignancy arising in the oropharynx—the middle portion of the pharynx extending from the soft palate to the upper border of the hyoid bone. This anatomical region includes the base and posterior third of the tongue, soft palate, and the lateral and posterior pharyngeal walls. The vast majority of these tumors, over 90%, are histologically classified as squamous cell carcinomas, originating from the epithelial lining of the oropharynx (1,2). A substantial proportion of OPSCC cases are now known to be associated with persistent infection by high-risk subtypes of human papillomavirus (HPV), especially HPV16, which alone is responsible for approximately 90% of HPV-positive OPSCC cases (3,4). This viral link has dramatically altered the epidemiological and clinical landscape of oropharyngeal cancers in recent decades. Globally, OPSCC ranks as the sixth most common cancer, and its incidence is rising, particularly among younger, non-smoking populations, where HPV-related disease predominates (5). While HPV vaccination campaigns have successfully reduced the incidence of cervical cancer—by about 1.6% annually from 1999 to 2015 in countries such as the United States and across Europe—the incidence of HPV-positive OPSCC has increased during the same period, with a more pronounced rise in males (2.7% annually) than in females (0.8% annually) (6–8). This paradox highlights a shifting burden of HPV-associated disease from the cervix to the oropharynx, likely influenced by behavioral and immunological factors as well as suboptimal vaccine uptake in certain populations.

The pathogenesis of HPV-associated OPSCC begins with the persistent presence of the virus in the oral mucosa, which in most individuals is cleared within 1–2 years. However, in a subset of patients, the virus evades immune surveillance and persists, potentially progressing over the course of a decade to invasive carcinoma through stages of dysplasia and pre-malignant transformation (9). This latency and the often-asymptomatic nature of early disease underscore the challenge of timely diagnosis and the critical importance of primary prevention strategies. Therapeutic approaches for OPSCC depend on disease stage and include surgical excision, radiotherapy, or a combination of both. For early-stage disease, single modality treatment—either surgery or radiotherapy—may suffice. In more advanced stages, combined modalities are often necessary to achieve optimal control. Recent advances in minimally invasive surgical techniques, such as transoral laser microsurgery, have gained prominence as effective first-line interventions with favorable outcomes and reduced morbidity (10,11). Despite robust international evidence linking HPV to oropharyngeal carcinogenesis, there remains a paucity of local data in Pakistan, particularly in the Khyber Pakhtunkhwa province. This gap limits the ability to develop region-specific public health strategies, including vaccination programs and early screening protocols. Therefore, this study was designed to determine the frequency of HPV infection among patients diagnosed with OPSCC at Shaukat Khanum Memorial Cancer Hospital, Peshawar. By generating regionally relevant epidemiological data, the findings aim to inform future policies for HPV vaccination and early detection efforts tailored to the local population.

METHODS

This cross-sectional study was conducted at Shaukat Khanum Memorial Cancer Hospital (SKMCH), Peshawar from November 3, 2024, to May 3, 2025, following formal approval from the Institutional Review Board (IRB) of the hospital. The primary objective was to assess the frequency of human papillomavirus (HPV) infection among patients diagnosed with oropharyngeal squamous cell carcinoma (OPSCC). A sample size of 89 patients was determined using the WHO sample size calculator, based on an expected HPV prevalence of 17.9% in OPSCC (11), a confidence level of 95%, and a margin of error of 8%. Non-probability consecutive sampling was used to recruit eligible participants. Inclusion criteria encompassed adult patients aged 40 to 80 years of either sex with histopathologically confirmed OPSCC. Patients with recurrent head and neck squamous cell carcinoma (HNSCC), known immunocompromised conditions such as HIV positivity or post-transplant immunosuppression, and those with coexisting secondary malignancies were excluded to reduce potential confounding factors. Written informed consent was obtained from all participants prior to enrollment, ensuring voluntary participation and confidentiality of data in compliance with ethical standards.

Each patient underwent a comprehensive clinical assessment, which included detailed history-taking, physical examination, and evaluation of performance status using the Eastern Cooperative Oncology Group (ECOG) scale. Radiological imaging—including computed tomography (CT) or magnetic resonance imaging (MRI) of the head and neck—was performed for tumor localization and staging. Where clinically indicated, additional imaging studies such as chest X-rays, thoracic CT scans, and abdominal ultrasonography

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were used to assess distant metastasis. Histological confirmation of OPSCC was achieved via fine-needle aspiration cytology (FNAC) of enlarged cervical lymph nodes or excisional biopsies of the primary lesion. Tissue specimens were reviewed by experienced pathologists to verify the presence of malignant squamous cells. For HPV detection, DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue blocks or fresh tissue scrapings. Qualitative analysis for HPV-DNA was conducted using polymerase chain reaction (PCR)-based assays. The results were interpreted as either HPV-positive or HPV-negative based on established criteria. Data were analyzed using SPSS version 23. Continuous variables such as age and duration of disease were presented as means and standard deviations, whereas categorical variables including gender, disease stage, HPV status, and risk factor profiles were summarized as frequencies and percentages. Chi-square tests were applied to examine associations between HPV status and demographic or clinical variables, with a p-value of ≤0.05 considered statistically significant.

RESULTS

The mean age of the patients diagnosed with oropharyngeal squamous cell carcinoma was 53.38 ± 11.52 years. The average duration of symptoms prior to diagnosis was 4.82 ± 2.32 months. Among the 89 participants, 52 (58.4%) were male and 37 (41.6%) were female. Regarding clinical staging, the majority of patients presented with advanced disease. Stage III was the most common with 40 cases (44.9%), followed by stage IV with 20 cases (22.5%). Earlier stages were less frequent, with 17 patients (19.1%) in stage II and 12 patients (13.5%) in stage I. HPV DNA was detected in 14 patients, yielding an overall frequency of 15.7% among OPSCC cases, while 75 patients (84.3%) tested negative for HPV. Assessment of potential risk factors revealed that tobacco use was reported in 12 patients (13.5%), alcohol consumption in 5 patients (5.6%), betel nut chewing in 10 patients (11.2%), poor oral hygiene in 7 patients (7.9%), occupational exposure in 3 patients (3.4%), radiation exposure in 2 patients (2.2%), and oral intercourse history in 2 patients (2.2%). Family history of malignancy was reported in 4 patients (4.5%). Stratified analysis of HPV-positive cases showed that 71.4% were aged 40–60 years and 28.6% were 61–80 years, with no significant association ($p = 0.96$). Among HPV-positive cases, 64.3% were male and 35.7% were female ($p = 0.62$). HPV was more frequently detected in stage III (50%) and stage IV (42.9%) patients, with only one case (7.1%) in stage II and none in stage I ($p = 0.08$). A significant association was observed between HPV positivity and tobacco use ($p < 0.0001$), alcohol use ($p = 0.005$), family history ($p = 0.001$), betel nut chewing ($p < 0.0001$), poor oral hygiene ($p < 0.0001$), occupational exposure ($p = 0.01$), radiation exposure ($p = 0.001$), and oral intercourse ($p = 0.001$). No significant relationship was found between HPV status and disease duration ($p = 0.50$).

Table 1: Stages of disease

Stages of disease	N	%
Stage I	12	13.5%
Stage II	17	19.1%
Stage III	40	44.9%
Stage IV	20	22.5%

Table 2: Risk factors of OPSCC

Risk factors of OPSCC		N	%
Tobacco	Yes	12	13.5%
	No	77	86.5%
Alcohol	Yes	5	5.6%
	No	84	94.4%
Family history	Yes	4	4.5%
	No	85	95.5%
Betel nut	Yes	10	11.2%
	No	79	88.8%
Poor oral hygiene	Yes	7	7.9%
	No	82	92.1%
Occupation exposure	Yes	3	3.4%
	No	86	96.6%
Radiation exposure	Yes	2	2.2%
	No	87	97.8%

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Risk factors of OPSCC		**N**	**%**
Oral intercourse	Yes	2	2.2%
	No	87	97.8%

Table 3: Frequency of Human papilloma virus

Human papilloma virus	**N**	**%**
Yes	14	15.7%
No	75	84.3%

Table 4: Stratification of HPV with various parameters

Parameters		**Human papilloma virus**				**P value**
		Yes		No		
		N	%	N	%	
Age distribution (Years)	40 to 60	10	71.4%	54	72.0%	0.96
	61 to 80	4	28.6%	21	28.0%	
Gender	Male	9	64.3%	43	57.3%	0.62
	Female	5	35.7%	32	42.7%	
Stages of disease	Stage I	0	0.0%	12	16.0%	0.08
	Stage II	1	7.1%	16	21.3%	
	Stage III	7	50.0%	33	44.0%	
	Stage IV	6	42.9%	14	18.7%	
Tobacco	Yes	9	64.3%	3	4.0%	0.0001
	No	5	35.7%	72	96.0%	
Alcohol	Yes	3	21.4%	2	2.7%	0.005
	No	11	78.6%	73	97.3%	
Family history	Yes	3	21.4%	1	1.3%	0.001
	No	11	78.6%	74	98.7%	
Betel nut	Yes	7	50.0%	3	4.0%	0.0001
	No	7	50.0%	72	96.0%	
Poor oral hygiene	Yes	5	35.7%	2	2.7%	0.0001
	No	9	64.3%	73	97.3%	
Occupation exposure	Yes	2	14.3%	1	1.3%	0.01
	No	12	85.7%	74	98.7%	
Radiation exposure	Yes	2	14.3%	0	0.0%	0.001
	No	12	85.7%	75	100.0%	
Oral intercourse	Yes	2	14.3%	0	0.0%	0.001
	No	12	85.7%	75	100.0%	
Duration of disease (Months)	1 to 5	9	64.3%	41	54.7%	0.50
	> 5	5	35.7%	34	45.3%	

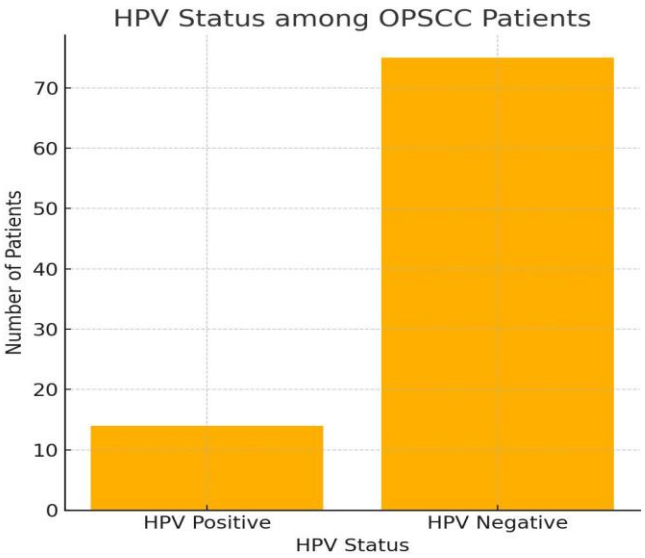

Figure 1 HPV Status among OPSCC Patients

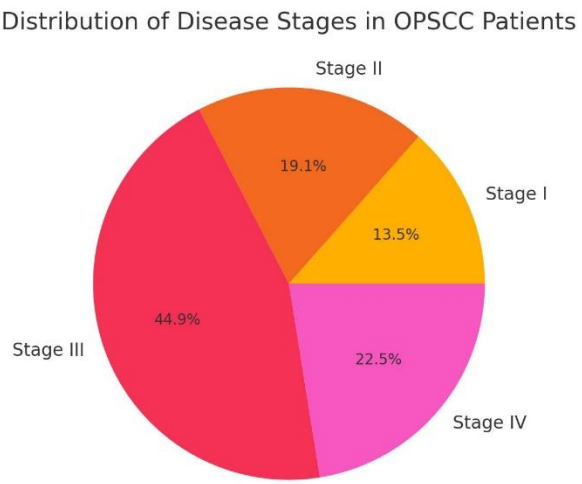

Figure 2 Distribution of Disease Stages in OPSCC Patients

DISCUSSION

In the current study, HPV DNA was detected in 15.7% of patients with oropharyngeal squamous cell carcinoma (OPSCC), reflecting a relatively low prevalence when compared to high-incidence regions such as North America, where HPV-positive OPSCC rates have escalated dramatically from 20.9% before 1990 to over 65% after 2000 (12,13). Similar findings of low HPV frequency have been observed in oral cavity squamous cell carcinomas in some low- to middle-income countries, where all detected cases were linked to high-risk subtypes such as HPV16. The variation in HPV detection rates across regions likely stems from differences in sexual behavior, cultural practices, or even diagnostic techniques. PCR-based assays used in the present study, known for their high sensitivity, might have enhanced detection compared to less sensitive methods like in situ hybridization, yet the prevalence remained low, suggesting a genuine regional difference in HPV burden (14,15). A striking pattern observed in the current cohort was the predominance of advanced-stage presentation, with 44.9% of patients presenting at stage III and 22.5% at stage IV. This finding aligns with other regional observations where HPV-positive patients were frequently diagnosed at later stages, often exhibiting significant nodal involvement and larger tumor sizes (16). However, contrasting data from other populations indicated no significant correlation between HPV status and disease stage, pointing toward the influence of non-viral factors such as tobacco exposure, limited healthcare access, and delays in diagnosis. In regions like Pakistan, barriers such as lack of awareness, cultural hesitancy, and economic constraints may contribute to late-stage diagnosis, underscoring the critical need for community-based screening and awareness programs (17,18).

The current study also provided insight into the risk factor landscape for OPSCC in the local population. Tobacco use was observed in 13.5% of patients, which is considerably lower than global averages reported in similar cohorts. Instead, habits such as betel nut chewing (11.2%) and poor oral hygiene (7.9%) appeared to be more prevalent and may play a more substantial etiological role in this setting. Alcohol use was reported by only 5.6% of patients, a figure that diverges sharply from international data where alcohol is a prominent co-factor in OPSCC pathogenesis (19). This deviation is likely due to religious restrictions and limited legal access to alcohol in Pakistan, reflecting how sociocultural norms can shape exposure profiles. Notably, HPV positivity showed a strong association with several modifiable risk factors including tobacco, alcohol, betel nut, and poor oral hygiene, supporting the multifactorial nature of HPV-related carcinogenesis and reinforcing the need for public health interventions aimed at reducing these behaviors. The mean age of patients was 53.38 ± 11.52 years, and no significant correlation between age and HPV status was identified. This diverges from patterns observed in high-income countries, where HPV-positive OPSCC typically affects younger adults with fewer traditional risk factors. These findings suggest that in this region, HPV-related oropharyngeal carcinomas may follow a different epidemiological trajectory, perhaps influenced by unique lifestyle and environmental exposures (19,20). Although younger patients with HPV-related disease have been associated

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with better treatment response and prognosis, such implications could not be explored here due to the absence of outcome and survival data, which represents a significant limitation of the study.

Among the strengths of this study was the use of sensitive PCR-based molecular techniques for HPV detection, along with a structured stratification of risk factors and staging. Moreover, the inclusion of a well-defined cohort with confirmed histopathological diagnoses and detailed demographic profiling enhanced the reliability of the data. However, several limitations must be acknowledged. The absence of HPV genotyping, particularly for high-risk subtypes like HPV16 and HPV18, limits the depth of analysis. Furthermore, the lack of follow-up data on treatment outcomes, recurrence, or survival prevents assessment of the prognostic significance of HPV status. The use of non-probability sampling and a single-center design also restrict generalizability. Future research should aim to incorporate multi-center data, detailed viral subtyping, and longitudinal follow-up to provide a more comprehensive understanding of the clinical behavior and public health implications of HPV-related OPSCC in this region.

CONCLUSION

In conclusion, this study highlighted the presence of human papillomavirus as a contributing factor in oropharyngeal squamous cell carcinoma within the local population, with notable associations observed between HPV positivity and modifiable risk factors such as tobacco use, betel nut chewing, and alcohol consumption. These findings underscore the multifactorial nature of OPSCC in this region and emphasize the importance of integrating HPV awareness, behavioral risk reduction, and targeted prevention strategies into future public health policies. By generating region-specific data, this study provides a foundation for informed screening, early detection efforts, and potential consideration of HPV vaccination as a preventive measure in high-risk groups.

AUTHOR CONTRIBUTION

Author	Contribution
Faraz Faisal Khan*	Data Entry, Data Acquisition, Data Analysis, Manuscript Writing, and Manuscript Revision
Fattaullah Khan Hassanzai	Critical Input, Study Design Conception, Final Approval of Draft

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