Prevalence and Type Distribution of Human Papilloma Virus (HPV) in Oral, Oropharyngeal, Cervical, and Vulvovaginal Cancers

Rahul G. Dholariya*, Ankur Tripathi, Amritha Prabha Shankar, M.S. Ganesh and Ravoori Harish Babu

Department of Surgical Oncology, Vydehi Institute of Medical Sciences and Research Centre, Bangalore, India

Abstract: *Background:* Human papillomavirus (HPV) is a major etiological agent in cervical cancer and is increasingly recognized as a driver of other anogenital and head and neck squamous cell carcinomas (HNSCCs). The oncogenic potential of HPV is primarily mediated by high-risk genotypes such as HPV16, HPV18, HPV31, and HPV45, whose E6 and E7 oncoproteins disrupt p53 and retinoblastoma pathways. While the burden of HPV-associated cervical cancer is well documented, data on the prevalence and genotype distribution of HPV in oral, oropharyngeal, vulvar, and vaginal cancers in India remain limited.

Objective: To identify the disease burden and determine the prevalence and genotype distribution of high-risk HPV (16, 18, 31, 45) in biopsy-proven squamous cell carcinomas of the cervix, oral cavity, oropharynx, vulva, and vagina.

Methods: We conducted a retrospective analysis of 131 patients with squamous cell carcinomas diagnosed between September 2022 and August 2024 at Vydehi Institute of Medical Sciences and Research Centre, Bangalore, India. PCR-based HPV detection was employed to determine the presence of high-risk HPV types 16, 18, 31, and 45 targeting the E6/E7 regions of high-risk HPV. Clinical and pathological data, including tumor stage and demographic variables, were analyzed.

Results: Overall HPV prevalence was 90.1%. Site-specific positivity was highest in cervical cancers (95.8%) and vulvovaginal cancers (100%), followed by oral (84.2%) and oropharyngeal (66.7%) cancers. HPV16 and HPV31 were the dominant genotypes across all tumor sites, whereas HPV18 and HPV45 were detected at lower frequencies. Cervical cancer cases predominantly presented in advanced stages (FIGO IIB–IIIB), while vulvovaginal cancers were diagnosed at earlier stages.

Conclusion: HPV infection, particularly with HPV16/31, is highly prevalent in multiple anogenital and head and neck squamous cell carcinomas in this Indian cohort. These findings reinforce the importance of HPV vaccination programs, highlight the need for comprehensive HPV screening strategies, and suggest that P16 immunohistochemistry (IHC) should be integrated with PCR-based detection to establish oncogenic causality.

Keywords: HPV, cervical cancer, vulvovaginal cancer, HPV16, HPV18, HPV31, HPV45.

INTRODUCTION

Global cancer rates have risen substantially in recent years, reflecting a complex interplay of multiple contributing factors. This upward trend stems from various influences including dietary patterns, tobacco and alcohol use, physical inactivity, workplace exposures, and infections. Viral infections, in particular, have been recognized as a significant causative element in many cancer types.

Viruses account for approximately 12% of cancers worldwide, including human papillomavirus (HPV), hepatitis B and C viruses, human immunodeficiency virus (HIV), human herpes simplex virus 8 (HHV-8), Epstein-Barr virus (EBV), and human T-cell lymphotropic virus 1 (HTLV-1) [1]. HPV infection is particularly notable, as it has been linked to various cutaneous and mucocutaneous squamous cell carcinomas affecting the cervix, vagina, vulva, penis, and oral, oropharyngeal, and anal regions.

HPV is a small, non-enveloped virus with circular DNA belonging to the papillomavirus family, with over 200 identified types [2]. Transmission typically occurs through sexual contact or skin-to-skin interaction via minor cuts or abrasions on skin or mucous membranes. Globally, about half to two-thirds of sexually active people get infected with HPV at some point [3]. Most infections are asymptomatic and resolve naturally through immune response. However, persistent infections can lead to benign warts, intraepithelial neoplasia, and in some cases, cancer development.

The extended period between initial infection and the emergence of precancerous or cancerous lesions provides an opportunity for early screening and detection of persistent HPV infection. Regular screening and appropriate treatment of HPV infections can help reduce HPV-associated cancer incidence. The effectiveness of HPV vaccination in reducing

*Address correspondence to this author at the Department of Surgical Oncology, Vydehi Institute of Medical Sciences and Research Centre, Bangalore, India; E-mail: rahul.dholariya25@gmail.com

ISSN: 1927-7210 / E-ISSN: 1927-7229/25

cervical cancer is well-documented, with females immunized before age 17 experiencing approximately 90% lower cervical cancer risk [4]. While HPV has been associated with numerous squamous cell carcinomas beyond cervical cancer, definitive causal relationships and the potential impact of vaccination on reducing these cancers remain incompletely established in current literature. This research aims to determine the prevalence and type distribution of HPV-associated oral, oropharyngeal, cervical, and vulvovaginal cancers within the local population.

MATERIALS AND METHODS

Source of Data

Data were obtained from consecutive patients diagnosed with squamous cell carcinomas (SCC) of the cervix, vagina, vulva, oral cavity (including buccal mucosa, tongue, and palate), and oropharynx at Vydehi Institute of Medical Sciences and Research Centre, Bangalore. The hospital has maintained a prospective HPV database for these cancers since May 2022. Demographic and clinical data were extracted and analyzed using Chi-square or Fisher's exact test where appropriate.

Eligibility Criteria

Inclusion Criteria

Biopsy-proven squamous cell carcinomas of cervix, vulva, vagina, oral cavity, and oropharynx.

Exclusion Criteria

Patients with recurrent disease.

Patients who had received prior heavy treatment (chemotherapy/radiotherapy).

Patients with known immunodeficiency syndromes.

Study Design

Type: Retrospective analysis of a prospectively maintained database.

Study period: September 2022 to August 2024.

Data collection period: 2 years.

Data analysis and reporting period: 3 months.

Sample Size

All eligible patients diagnosed with squamous cell carcinoma at the above sites during the study period were included (n=131).

Sample Collection and Handling

Biopsies were collected from treatment-naïve patients during diagnostic procedures. Each specimen was immediately placed in sterile normal saline and transported under cold-chain conditions (4–8°C) to the molecular pathology laboratory within two hours of collection. Samples were rinsed with sterile saline to remove surface contaminants and then homogenized using a sterile mortar and pestle under aseptic conditions. Negative controls (saline blanks) were processed intermittently to monitor potential contamination during handling.

DNA Extraction and Quality Control

Approximately 500 µL of homogenized tissue suspension was transferred into lysis buffer tubes, vortexed for 10 minutes to ensure complete cellular disruption, and processed through automated extraction. The elution step (20 minutes) yielded purified DNA while minimizing PCR inhibitors. DNA concentration and purity were assessed using spectrophotometry (A260/280 ratio), and samples with ratios <1.7 were re-extracted. Randomly selected samples were further tested on agarose gel electrophoresis to confirm DNA integrity.

PCR Assay and Validation

High-risk HPV detection was performed using a chip-based real-time PCR assay targeting the E6 and E7 oncogenes of HPV types 16, 18, 31, and 45. Each run included:

Positive controls (plasmids with cloned HPV DNA fragments for each genotype).

Negative controls (no-template controls and HPV-negative human DNA).

Internal controls (β-globin amplification) to verify DNA adequacy and rule out PCR inhibition.

Each reaction used 6 µL of purified DNA in a total reaction volume of 20 µL. Amplification was carried out under standard cycling conditions recommended by the manufacturer. Viral load was semi-quantitatively estimated using cycle threshold (Ct) values, categorized into high, medium, and low based on pre-validated cut-offs (Figure **1**).

Assay validation was performed prior to the study by confirming sensitivity (limit of detection: 50 viral copies/reaction), specificity (no cross-reactivity with

low-risk HPV or other human DNA viruses), and reproducibility (intra- and inter-run variability <5%).

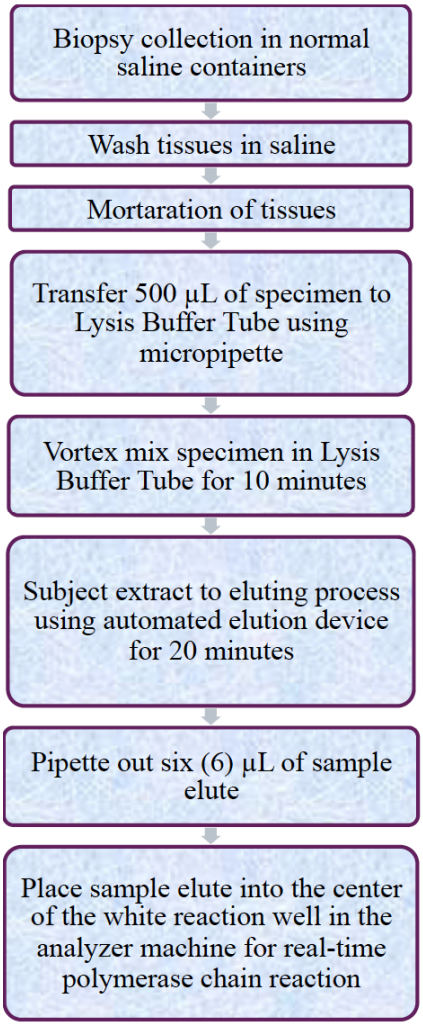

Figure 1: Standard operating protocol (SOP).

Data Interpretation

Samples were classified as HPV-positive if amplification curves crossed the detection threshold within 40 cycles. Viral load categories were interpreted cautiously as semi-quantitative indicators, not absolute quantification. Only results passing all internal and external control checks were included in the final analysis.

RESULTS

A total of 131 patients with squamous cell carcinomas were included, comprising 74.8% females and 25.2% males (Figure **2**).

Cervical cancer was the most common site (54.9%), followed by oral cavity (29%), oropharynx (9.2%), vagina (4.6%), and vulva (2.3%) (Table **1**).

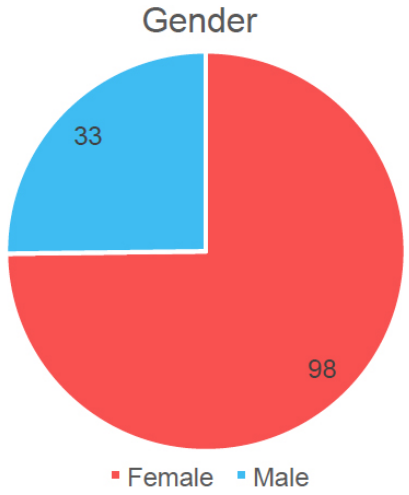

Figure 2: Gender distribution of various cancers.

Table 1: Cancer Distribution as Per the Sites

Site of cancer	Frequency	Percentage
Cervix	72	54.9
Oral	38	29
Oropharynx	12	9.2
Vagina	6	4.6
Vulva	3	2.3
Total	131	100

Overall HPV Prevalence

Overall HPV positivity was 90.1% (Figure **3**). Site-wise comparison revealed the highest prevalence in vulvovaginal cancers (100%) and cervical cancers (95.8%), followed by oral cavity (84.2%) and oropharyngeal cancers (66.7%). The difference in HPV positivity between sites was statistically significant (χ^2 test, $p = 0.021$).

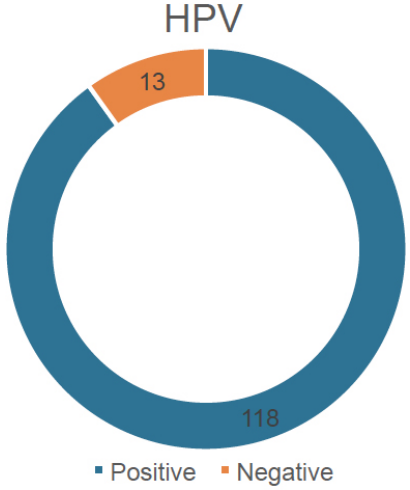

Figure 3: Total HPV positivity and negativity.

Genotype Distribution

HPV 16/31 was detected in 90.1% of cases, significantly higher than HPV 18/45 (45.0%; $p < 0.001$). Across sites, HPV 16/31 remained dominant. In cervical cancers, 95.8% were positive for HPV 16/31 compared to 47.3% for HPV 18/45 ($p < 0.001$). Vulvovaginal cancers demonstrated 100% HPV 16/31 positivity versus 44.4% HPV 18/45 ($p = 0.041$). Oral cancers also showed significantly higher detection of HPV 16/31 (84.2%) compared with HPV 18/45 (44.7%, $p = 0.003$). Oropharyngeal cancers followed a similar trend (66.7% vs 33.3%, $p = 0.046$).

Viral Load Patterns

In cervical cancers, high viral load of HPV 16/31 was most frequent (48.6%), while HPV 18/45 was mostly low load (34.7%). In oral cancers, medium and low loads predominated for HPV 16/31 (63.1% combined), whereas HPV 18/45 was mostly negative or low. Similar low-load predominance was observed in oropharyngeal cancers for both genotypes.

Stage Distribution and HPV Association

Stage distribution varied significantly across sites (Fisher's exact, $p < 0.01$) (Tables **2** to **5**). Cervical cancers mostly presented in locally advanced stages (IIB–IIIC, ~60%), while vulvovaginal cancers were predominantly early-stage (T1–T2 without nodal disease, 77.7%). Oral and oropharyngeal cancers largely presented at intermediate to advanced stages with nodal involvement.

Table 2: Cervical Cancer Stage at Presentation

Stage	Frequency	Percentage
IA	1	1.4
IB	5	7
IIA	2	2.8
IIB	21	29.2
IIIa	2	2.8
IIIB	19	26.4
IIIC1	14	19.4
IIIC2	3	4.2
IIIC3	1	1.4
IV A	1	1.4
IVB	3	4.2
Total	72	100.0

Table 3: Vulvovaginal Cancer: Stage at Presentation

Stage	Frequency	Percentage
Vagina		
T1BN0M0	4	66.7
T2N0M0	2	33.3
Total	6	100.0
Vulva		
IB	1	33.3
IIB	1	33.3
IIIC1	1	33.3
Total	3	100.0

Table 4: Oral Cancer: Stage at Presentation

Stage	Frequency	Percentage
T1N0M0	2	5.3
T2N0M0	12	31.5
T2N1M0	6	15.8
T2N2M0	1	2.6
T2N3M0	1	2.6
T2N0M0	4	10.5
T3N1M0	1	2.6
T4aN0M0	1	2.6
T4aN1M0	3	7.9
T4aN2aM0	4	10.5
T4aN2bM0	1	2.6
T4aN2cM0	2	5.2
Total	38	100.0

Table 5: Oropharyngeal, Larynx and Hypopharyngeal : Stage at Presentation

Stage	Frequency	Percentage
T1N0M0	1	8.3
T2N0M0	6	50
T2N2aM0	2	16.7
T2N2bM0	1	8.3
T4aN2cM0	1	8.3
T4aN3bM0	1	8.3
Total	12	100

When stratified by HPV genotype, cervical cancers positive for HPV 16/31 were significantly more likely to present at advanced stages (IIB–IIIC) compared to HPV 18/45-positive cases ($p = 0.037$). In oral and oropharyngeal cancers, no statistically significant

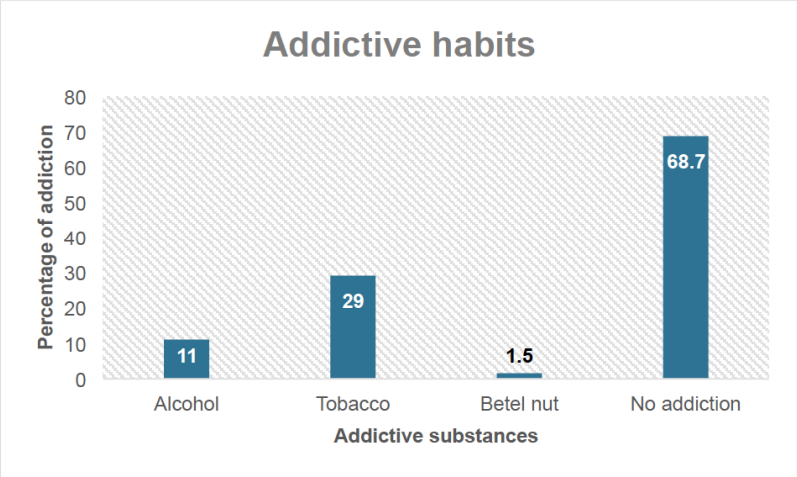

Figure 4: Addiction habits amongst the study population.

association between genotype and stage was observed, although HPV-negative cases showed a trend toward more advanced presentation.

Addictive Habits

Only 31.3% of patients reported addictive habits (tobacco 29%, alcohol 11%, betel nut 1.5%). HPV positivity was not significantly associated with addictive habit status ($p = 0.62$), suggesting an independent role of HPV infection in carcinogenesis across sites (Figure **4**).

DISCUSSION

Human papillomavirus (HPV) is a well-established etiological factor in cervical cancer and is increasingly recognized in other anogenital and head and neck malignancies. The present study adds important epidemiological evidence from India, where HPV-associated cancers remain a major public health challenge and genotype distribution data outside cervical cancer are limited.

Our findings reaffirm the near-universal association of HPV with cervical carcinoma, with positivity rates exceeding 95%, in line with international data [5,6]. The predominance of HPV16 and HPV31 underscores the critical importance of vaccination strategies that provide coverage beyond the traditional HPV16/18 focus. Similarly, the universal detection of HPV DNA in vulvovaginal cancers supports the strong etiological role of HPV at these sites, although confirmatory biomarker analysis would further strengthen these observations [7].

A key finding of this study is the very high prevalence of HPV DNA in oral cavity cancers (84.2%), which is substantially higher than reported in most global meta-analyses, where true biologically active HPV positivity is typically 5–15% [8,9]. This discrepancy likely reflects methodological differences, as PCR can detect even small fragments of viral DNA that may represent transient infection or contamination rather than causal oncogenesis. Hence, while PCR provides valuable sensitivity, its results must be interpreted with caution, particularly for head and neck cancers. Establishing causality requires additional markers such as p16 immunohistochemistry or E6/E7 mRNA expression, which directly assess viral oncogene activity [10,11]. The absence of these confirmatory assays represents a limitation of the current work and will be critical for future studies in this population.

Despite this limitation, the present study makes several novel contributions. First, it provides comprehensive, site-specific HPV prevalence and genotype data across cervical, oral, oropharyngeal, and vulvovaginal cancers from an Indian cohort, an area where evidence remains sparse. Second, by reporting viral load distribution across cancer sites, it offers preliminary insights into heterogeneity of HPV involvement that may inform future biomarker research. Third, it highlights the predominance of advanced-stage cervical cancer presentation and the relatively earlier detection of vulvovaginal cancers, emphasizing the need for improved screening access and awareness in India.

From a public health perspective, these findings reinforce the urgency of strengthening HPV vaccination programs, particularly with the rollout of India's indigenous quadrivalent vaccine. Local genotype mapping, as demonstrated here, is essential for shaping vaccine policy, monitoring coverage adequacy,

and evaluating impact on multiple HPV-driven cancers [12-14].

In summary, while PCR-based detection revealed strikingly high HPV prevalence across cancer types, confirmatory testing with p16 IHC or E6/E7 mRNA is indispensable for distinguishing oncogenic infection from incidental viral presence. The study's novel contribution lies in providing much-needed Indian data on HPV genotype distribution beyond cervical cancer, with direct relevance for guiding vaccination, screening, and diagnostic strategies in the region.

Limitations and Future Directions

This study has several limitations that should be acknowledged. First, HPV DNA detection by PCR, while highly sensitive, does not establish the biological activity of the virus. Consequently, the high prevalence of HPV DNA observed in oral cavity tumors in our study may not truly reflect oncogenic HPV involvement. Incorporation of surrogate markers such as p16 immunohistochemistry (IHC) or direct E6/E7 mRNA detection is essential to confirm transcriptionally active infection, particularly in head and neck cancers. Second, the interpretation of viral load based on cycle threshold (Ct) values remains assay-dependent and lacks universal standardization, which limits the comparability of our findings with other studies. Third, the relatively small number of cases in the vulvar and vaginal cancer subgroups restricts the generalizability of those observations. Additionally, our cohort was derived from a single-center setting, which may not capture the broader regional or national epidemiological diversity.

Future research should aim to integrate multiple diagnostic modalities, including p16 IHC and E6/E7 mRNA assays, to reliably define HPV-driven tumors across different anatomical sites. Correlating Ct-derived viral load with histopathological features, treatment response, and long-term outcomes could provide valuable insights into its prognostic significance. Larger, multicentric studies would also enhance statistical power and improve the representativeness of findings across different populations. Finally, given the predominance of HPV-16 and HPV-31 in our cohort, further studies exploring genotype-specific outcomes and the potential impact of India's expanding HPV vaccination programs on cancer epidemiology are warranted.

CONCLUSION

Our results show different HPV prevalence patterns for different types of squamous cell carcinomas, which have important public health ramifications. The high proportion of HPV positive in cervical malignancies, especially for HPV 16, emphasizes the need for universal early screening procedures to detect the illness at precancerous stages and the vital significance of comprehensive vaccination programs. There is an urgent need for better healthcare accessibility, as evidenced by the prevalence of advanced-stage presentations.

Given the significant HPV presence for oral/oropharyngeal cancers, research into extending vaccination techniques beyond cervical cancer prevention is warranted. A complex carcinogenic process, in which HPV may be one of several contributing components, is suggested by the observed heterogeneity in viral loads. The significant HPV positive in vulvovaginal malignancies also suggests a fundamental etiological involvement, and the tumors' primarily early-stage appearance suggests benefits for focused screening methods.

Importantly, PCR-based detection alone cannot establish causality between HPV presence and oncogenesis. Confirmatory biomarkers such as P16 immunohistochemistry are essential to distinguish between truly HPV-driven carcinogenesis and incidental viral presence. This distinction is crucial for accurately informing public health initiatives, optimizing early detection approaches, and developing appropriate treatment strategies. Future prevention efforts should consider HPV's role across these anatomically diverse malignancies while avoiding overinterpretation of molecular findings without functional validation.

FUNDING STATEMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST STATEMENT

The Author(s) declare(s) that there are no relevant financial or non-financial competing interests to report.

REFERENCES

[1] Warowicka A, Nawrot R, Broniarczyk J, Węglewska M, Goździcka-Józefiak A. Oncogenic viruses and cancer. Postepy Biochem 2020; 66(4): 336-55.

[2] Soheili M, Keyvani H, Soheili M, Nasseri S. Human papilloma virus: A review study of epidemiology, carcinogenesis, diagnostic methods, and treatment of all HPV-related cancers. Med J Islam Repub Iran 2021; 35: 65. https://doi.org/10.47176/mjiri.35.65

[3] Hathaway JK. HPV: diagnosis, prevention, and treatment. Clin Obstet Gynecol 2012; 55(3): 671-80. https://doi.org/10.1097/GRF.0b013e31825caa36

[4] Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005; 14(2): 467-75. https://doi.org/10.1158/1055-9965.EPI-04-0551

[5] Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, *et al*. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health 2020; 8(2): e191-203. https://doi.org/10.1016/S2214-109X(19)30482-6

[6] de Sanjosé S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, *et al*. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010; 11(11): 1048-56. https://doi.org/10.1016/S1470-2045(10)70230-8

[7] de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer 2017; 141(4): 664-70. https://doi.org/10.1002/ijc.30716

[8] Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. J Clin Oncol 2015; 33(29): 3235-42. https://doi.org/10.1200/JCO.2015.61.6995

[9] Isayeva T, Li Y, Maswahu D, Brandwein-Gensler M. Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review. Head Neck Pathol 2012; 6(Suppl 1): S104-20. https://doi.org/10.1007/s12105-012-0368-1

[10] Lewis JS Jr, Beadle B, Bishop JA, Chernock RD, Colasacco C, Lacchetti C, *et al*. Human papillomavirus testing in head and neck carcinomas: guideline from the College of American Pathologists. Arch Pathol Lab Med 2018; 142(5): 559-97. https://doi.org/10.5858/arpa.2017-0286-CP

[11] Westra WH. The changing face of head and neck cancer in the 21st century: the impact of HPV on the epidemiology and pathology of oral cancer. Head Neck Pathol 2014; 8(1): 11-20.

[12] O'Rorke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. Oral Oncol 2012; 48(12): 1191-201. https://doi.org/10.1016/j.oraloncology.2012.06.019

[13] Chaturvedi AK, Graubard BI, Broutian T, Pickard RKL, Tong ZY, Xiao W, *et al*. Effect of prophylactic human papillomavirus (HPV) vaccination on oral HPV infections among young adults in the United States. J Clin Oncol 2018; 36(26): 2629-37.

[14] Basu P, Malvi SG, Joshi S, Bhatla N, Muwonge R, Lucas E, *et al*. Vaccine efficacy against persistent human papillomavirus (HPV) 16/18 infection at 10 years after vaccination in women in India: a multicentre, cluster-randomised trial. Lancet Infect Dis 2021; 21(11): 1448-56. https://doi.org/10.1200/JCO.2017.75.0141

Received on 06-08-2025 Accepted on 10-09-2025 Published on 07-10-2025

https://doi.org/10.30683/1927-7229.2025.14.07