Oncoviral Signatures in Head and Neck Cancers: From Serological Analysis to Subtype-Specific Genomic Detection

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Posted Date: 19 June 2025

doi: 10.20944/preprints202506.1563.v1

Keywords: Head and neck cancer; viral STD agents; oncogenic viruses

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Article

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Abstract: Objective: This study aimed to investigate the cytomegalovirus (CMV), Epstein-Barr virus (EBV), human papillomavirus (HPV), and herpes simplex virus (HSV) in patients with head and neck cancer, in relation to age, sex, tumor type, and risk factors, at both molecular and serological levels. **Materials and Methods:** Data from 50 patients diagnosed histopathologically with head and neck cancer who were admitted to the Department of Otorhinolaryngology, Istanbul Faculty of Medicine, between April 2023 and April 2024 were reviewed. At the time of diagnosis, venous blood and tumor biopsy tissue were collected from all cases. CMV, EBV, HPV, and HSV DNA were investigated in both blood and biopsy samples using quantitative real-time PCR. Serologically, IgM and IgG levels were measured using the CMIA method. The data were analyzed along with demographic and clinical parameters. **Results:** In blood samples, CMV and HSV DNA were not detected, while EBV DNA was found to be positive in 2% and HPV DNA in 4% of the cases. In biopsy tissues, CMV DNA was detected in 8%, EBV DNA in 10%, and HPV DNA in 6% of the samples; HSV DNA was also not detected in tissue samples. HPV DNA positivity showed a statistically significant variation according to tumor type and was more frequently detected in the subtypes of tongue SCC and retromolar SCC ($p = 0.005$). In serological evaluation, IgG positivity for CMV, EBV, and HSV-1 was above 90%, whereas IgM positivity for all viruses was low and not statistically significant. **Conclusion:** Among patients with head and neck cancer, the overall positivity rates for HPV, EBV, and CMV DNA were low, and HSV DNA was not detected. The higher prevalence of HPV in specific tumor subtypes suggests a selective role of this virus in head and neck cancers. These findings highlight that the viral etiology of head and neck cancers may vary depending on tumor subtype and individual factors, emphasizing the importance of regional epidemiological data.

Keywords: head and neck cancer; viral STD agents; oncogenic viruses

1. Introductıon

Head and neck cancers constitute a significant public health concern worldwide in terms of both morbidity and mortality [1]. While environmental factors such as tobacco and alcohol use—

 doi:10.20944/preprints202506.1563.v1

particularly in squamous cell carcinomas (SCCs)—remain the predominant etiological contributors, recent studies have revealed that viral infections may also play a meaningful role in the pathogenesis of these tumors [2]. Human papillomavirus (HPV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and herpes simplex virus (HSV) are the main viral agents that have been investigated in association with various malignancies in the head and neck region [2–6].

Numerous studies and meta-analyses have demonstrated the oncogenic role of HPV, especially in specific subtypes of head and neck cancers such as oropharyngeal and oral cavity SCCs [3,4]. EBV is almost universally detected in nasopharyngeal carcinoma, while its presence in other head and neck tumors varies depending on geographic and ethnic factors [5,6]. CMV and HSV, on the other hand, are not primarily associated with direct oncogenic activity but are believed to contribute to tumor microenvironment modulation and the shaping of immune responses [7,8]. However, the prevalence of these viruses in head and neck cancers, their association with clinical and histopathological features, and their potential impact on tumor behavior remain limited and controversial in the literature.

Currently, both molecular methods (PCR-based viral DNA analysis) and serological tests (IgM/IgG antibody levels) are employed to detect viral agents in head and neck cancers. Nevertheless, the true clinical significance of viral DNA positivity and seroprevalence—particularly when considering regional epidemiological differences and tumor subtypes—has not yet been clearly established. Furthermore, most studies originate from Western countries or regions with high incidence rates, and comprehensive screenings including multiple microbial agents remain scarce in transitional regions such as Turkey.

The aim of this study is to evaluate the presence of HPV, EBV, CMV, and HSV at both molecular and serological levels, using blood and biopsy tissue samples from head and neck cancer patients admitted to the Department of Otorhinolaryngology at Istanbul Faculty of Medicine. Additionally, the study aims to correlate the findings with age, sex, tumor type, and risk factors, and to interpret the results in comparison with the existing literature. Ultimately, this study seeks to provide current, multifaceted, and original data regarding the role and clinical implications of viral etiology in head and neck cancers at a regional level.

2. Materials & Methods

2.1. Study Design and Participants

This study was conducted by including 50 patients who were admitted to the Otorhinolaryngology Outpatient Clinic of Istanbul Faculty of Medicine between April 2023 and April 2024 and were histopathologically diagnosed with head and neck cancer. The study was approved by the Clinical Research Ethics Committee of Istanbul University Faculty of Medicine on November 7, 2023 (Approval No: 2234909). The age, sex, tumor localization, clinical stage, and risk factors (smoking, alcohol consumption, comorbidities) of the included patients were recorded. Venous blood samples and tumor biopsy tissues were collected from all patients.

For molecular analyses, blood samples were collected in EDTA-containing anticoagulant tubes (Vacusera, Disera Ltd., Izmir, Turkey), while biopsy tissues were obtained in physiological saline under sterile conditions. For serological analyses, blood samples were drawn into gel-containing, anticoagulant-free tubes (Vacusera, Disera Ltd., Izmir, Turkey), and serum was obtained by centrifugation.

Patients who had previously received antiviral treatment for any viral infection, had immunodeficiency, systemic infections, or hematological malignancies were excluded from the study. Clinical and demographic data were obtained from the hospital information system and patient records.

 doi:10.20944/preprints202506.1563.v1

2.2. Viral DNA Analysis – Quantitative Real-Time PCR (qPCR)

The presence of viral DNA for CMV, EBV, HPV, and HSV was investigated in both venous blood samples and biopsy tissues using quantitative real-time polymerase chain reaction (qPCR), with a specific kit employed for each pathogen: HSV QLP 2.1 Real-Time PCR Kit, Fluorion HPV Screening QNS 1.1 Real-Time PCR Kit, Fluorion CMV QNP 3.0, and Epstein-Barr Virus QNP 1.0 Real-Time PCR Kit (IONTEK Pharmaceuticals, Diagnostics and Biotechnology R&D Corp., Istanbul, Turkey).

Nucleic acid extraction was performed using a commercial viral DNA isolation kit (MinElute® Virus Spin Kit, Qiagen, Germany) according to the manufacturer's protocol. The extracted DNA samples were subjected to qPCR analysis using primers and probes specific to each virus. Positive and negative control samples were included in each run. Results were interpreted based on Ct (Cycle threshold) values, and positivity was determined using the threshold values recommended by the manufacturer. Each sample was analyzed under double-blind conditions by at least two experienced researchers.

2.3. Serological Tests

IgM and IgG levels for CMV, EBV, HSV-1/2, and HPV were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The analyses were performed using kits and protocols recommended by the manufacturer, and the thresholds for positivity and negativity were determined based on the in-kit validation values. All samples were thawed only once prior to analysis and were not subjected to repeated freeze–thaw cycles. Results were reported both quantitatively and as positive/negative according to the manufacturer’s cut-off values.

2.3.1. CMV IgM and IgG Tests

A commercial kit based on the capture ELISA principle was used to measure CMV IgM and IgG levels (DIA PRO, Milan, Italy). For CMV IgM, according to the kit's evaluation criteria, samples with a signal-to-cutoff ratio (S/Co) <1.0 were considered negative, while those with an S/Co >1.2 were considered positive, based on optical density measurements at 450 nm wavelength. For CMV IgG, samples with levels <0.5 IU/mL were considered negative, and those >0.5 IU/mL were considered positive.

2.3.2. EBV IgM and IgG Tests

A commercial kit based on the capture ELISA principle was used to measure EBV IgM (targeting Epstein-Barr Virus Nuclear Antigen – EBNA) and IgG (targeting Viral Capsid Antigen – VCA) levels (DIA PRO, Milan, Italy). According to the kit's evaluation criteria, for EBV EBNA-IgM, samples with a signal-to-cutoff ratio (S/Co) <1.0 were considered negative, and those with S/Co >1.2 were considered positive, based on optical density measurements at 450 nm wavelength. For EBV VCA-IgG, samples with levels <0.5 arbU/mL were considered negative, and those >0.5 arbU/mL were considered positive.

2.3.3. HSV 1/2 IgM and IgG Tests

A commercial kit based on the capture ELISA principle was used to measure HSV-1/2 IgM and IgG levels (DIA PRO, Milan, Italy). According to the kit's evaluation criteria, samples with a signal-to-cutoff ratio (S/Co) <1.0 were considered negative, and those with S/Co >1.2 were considered positive for HSV-1/2 IgM, based on optical density measurements at 450 nm wavelength. For HSV-1/2 IgG, samples with levels <0.5 arbU/mL were considered negative, and those >0.5 arbU/mL were considered positive.

 doi:10.20944/preprints202506.1563.v1

2.3.4. HPV IgM and IgG Tests

A commercial kit based on the capture ELISA principle was used to measure HPV IgM and IgG levels (Human Papillomavirus Antibody, HPV-IgG ELISA Kit, BT Lab, Shanghai Korain Biotech Co., China). According to the kit's evaluation criteria, samples with a signal-to-cutoff ratio (S/Co) <1.0 were considered negative, and those with S/Co >1.2 were considered positive for HPV IgM, based on optical density measurements at 450 nm wavelength. For HPV IgG, samples with levels <0.5 IU/mL were considered negative, and those >0.5 IU/mL were considered positive.

2.4. Statistical Analysis

All statistical analyses were performed using SPSS version 26.0 for Windows (IBM Corporation, Armonk, New York, USA). Categorical variables were presented as counts and percentages, while continuous variables were expressed as mean ± standard deviation and median values. Fisher's exact test was used for comparisons between two or more independent categorical groups, and for continuous non-parametric data, the Mann-Whitney U and Kruskal-Wallis tests were applied. Viral DNA and serological positivity rates were evaluated separately in relation to age group, sex, tumor type, and risk factors. A p-value of <0.05 was considered statistically significant.

3. Results

In this study, a comprehensive evaluation was conducted on demographic characteristics, tumor distribution, lifestyle factors, serological markers, and molecular-level viral DNA positivity based on data obtained from 50 patients diagnosed with head and neck cancer. The findings were assessed in relation to both blood and tissue viral loads as well as clinical variables such as age, sex, and tumor subtypes.

The mean age of participants was 57.9 ± 13.0 years, ranging from 24 to 86, indicating a patient population that may be influenced by age-related immunological changes. Regarding gender distribution, 73.1% of the patients were male (n=38) and 23.1% were female (n=12), consistent with existing literature indicating a higher prevalence of head and neck cancers in men. The most common histopathological diagnoses were laryngeal SCC (34.6%), tongue SCC (21.2%), and retromolar SCC (7.7%). Less frequent tumor types included hypopharyngeal SCC, tonsillar SCC, and nasopharyngeal carcinoma (Table 1).

Table 1. Demographic characteristics of all cases.

Demographics	Value
Total Cases (n)	50
Mean Age (±SD)	57.9 (±13.0)
Age Scale (min.-max.)	24.0 – 86.0
Male (%)	38 (%73.1)
Female (%)	12 (%23.1)
Common Tumor Subtypes	Laryngeal SCC (%34.6), Tongue SCC (%21.2), Retromolar SCC (%3.8)
Smoking Status (%)	%88
Alcohol Use (%)	%18

Among lifestyle factors, smoking was identified in 88% of patients (n=44), with a high mean pack-year index, supporting its significant role in the development of non-HPV-related head and neck cancers. Alcohol consumption was reported in only 18% (n=9) of patients; although lower than in Western populations, it remains notable given its synergistic carcinogenic effects.

In molecular testing, CMV and HSV DNA were not detected in any blood samples. EBV DNA was detected in one patient (2%), and HPV DNA in two patients (4%), all of whom were male and between 50–69 years old. However, subgroup analysis revealed no statistically significant association

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with age or sex ($p > 0.05$). These low positivity rates may be attributed to the short viremic phase or the challenge of detecting latent infections in peripheral blood using PCR. Latent viruses like EBV and HPV may be more accurately identified through tissue-based assessments (Table 2).

Table 2. CMV, EBV, HSV, and HPV DNA positivity in blood and tissue samples of patients with head and neck cancer.

Virus	Sample Type	Positive (n)	Positive (%)	Negative (n)	Negative (%)
CMV	Blood	0	%0.0	50	%100
CMV	Bïopsiy	4	%8	46	%92
HSV	Blood	0	%0.0	50	%100
HSV	Bïopsiy	3	%6	47	%94
EBV	Blood	1	%2	49	%98
EBV	Bïopsiy	5	%10	45	%90
HPV	Blood	2	%4	48	%96
HPV	Bïopsy	3	%6	47	%94

Tissue-based DNA analysis showed higher positivity rates: CMV DNA was detected in 4 patients (8%), EBV DNA in 5 patients (10%), and HPV DNA in 3 patients (6%) (Figure 1). HSV DNA was not detected in any tissue samples. HPV DNA positivity varied significantly by tumor subtype and was found at higher rates in tongue SCC (18.2%) and retromolar SCC (50%) cases. This distribution was statistically significant ($p = 0.005$), supporting the oncogenic role of HPV particularly in oropharyngeal tumors. By sex, CMV and EBV DNA positivity was observed only in males, while HPV DNA positivity was detected in 16.7% of females and only 2.6% of males, a difference that, although not statistically significant ($p = 0.14$), is noteworthy.

Serological testing revealed high rates of IgG antibodies, reflecting past infections within the general population. CMV IgG (94%), EBV IgG (94%), and HSV-1 IgG (98%) positivity rates suggest widespread prior exposure to these viruses. Conversely, IgM antibodies—indicative of acute infections—were detected at low rates: CMV IgM (6%), EBV IgM (2%), HSV-1 IgM (0%), HSV-2 IgM (2%), and HPV IgM (8%). This suggests a low prevalence of active primary infections in the study cohort (Table 3, Figure 1).

Table 3. IgM and IgG positivity rates of CMV, HSV-1/2, EBV, and HPV in patients with head and neck cancer.

Serological Parameter	Positive (n)	Positive (%)
CMV IgM	3	%6
CMV IgG	47	%94
HSV 1 IgM	0	%0.0
HSV 1 IgG	49	%98
HSV 2 IgM	1	%2
HSV 2 IgG	15	%30
EBV IgM	1	%2
EBV IgG	47	%94
HPV IgM	4	%8
HPV IgG	3	%6

Age-stratified serological analysis revealed that IgG positivity rates approached 100% in the 50–69 age group. By gender, CMV IgG positivity was 100% in females and 92.1% in males. IgG antibodies against HPV were rare, detected in 6% of males and 0% of females. Comparisons of these serological markers by age and sex revealed no statistically significant differences ($p > 0.05$).

In summary, viral DNA positivity was low in blood samples but notably higher in tissue biopsies. Viruses such as HPV, EBV, and CMV may play a more prominent role in specific tumor subtypes, and such associations are better evaluated through tissue-based analyses. Serologically,

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past viral infections were found to be highly prevalent, while markers of active infection were observed at very low rates.

4. Discussion

While the dominant role of tobacco and alcohol in the etiology of head and neck cancers has been well established for decades, an increasing number of studies over the past decade have highlighted the significant contribution of viral infections. Although the exact impact of viral agents on the development, progression, and treatment response of head and neck tumors remains incompletely understood, several viruses—especially HPV and EBV—have been identified as potential key factors in certain tumor subtypes [11,12]. In this study, a comprehensive multi-viral screening at both molecular (blood and tissue PCR) and serological levels revealed the associations between viral positivity and clinical/demographic variables in a regional cohort of patients with head and neck cancer.

The significant variation in HPV DNA positivity across tumor subtypes in tissue samples ($p = 0.005$), particularly its higher detection in tongue SCC (18.2%) and retromolar SCC (50%) cases, suggests a central role for HPV in the pathogenesis of cancers located in these anatomical regions. This finding aligns with international data reporting HPV as a major oncogenic factor in oropharyngeal and oral cavity SCCs [13,14]. In a recent systematic review and meta-analysis, the overall prevalence of HPV-positive oropharyngeal squamous cell carcinoma (excluding tonsils and base of tongue) was found to be 20% (95% CI: 13–30%) [4]. In a study conducted by Uhlrich et al. in 2024, HPV positivity was reported in 65.8% of oropharyngeal squamous cell carcinomas [15]. This finding further highlights the prominent role of HPV in the development of oropharyngeal cancers and underscores the importance of vaccination and early detection strategies in this field. In contrast, HPV DNA detection rates in laryngeal SCC and other head and neck tumors were low in our study. Supporting this, In a meta-analysis published in 2024 by Vani VN et al., the proportion of HPV-positive oropharyngeal cancers was reported to be 45.8%. [16].

Regarding EBV, the absence of a significant difference in tissue DNA positivity among tumor subtypes indicates that EBV does not play a selective or dominant role in head and neck SCC. While EBV is almost universally associated with nasopharyngeal carcinoma—with positivity rates exceeding 90% in studies from East Asia and Africa [17,18] —its presence in oral and laryngeal SCCs is more limited in Western populations. Our findings are consistent with this literature.

As for CMV, existing studies tend to focus not on its direct oncogenic potential in head and neck cancers but rather on its secondary roles in immunosuppression, tumor microenvironment modulation, and chemotherapy resistance [19]. Although some reports have linked CMV detection in tumor tissue to disease progression or poor prognosis, in our case series, CMV DNA positivity was low and similarly distributed across subtypes.

In the case of HSV, no positivity was observed in either blood or tissue samples. Recent meta-analyses suggest that HSV is not significantly associated with head and neck SCC and that its detection is more likely due to coinfection or viral reactivation [20].

The detection of viral DNA predominantly in patients aged 50 years and older was noteworthy; however, age-based subgroup analysis did not reveal a statistically significant difference. Previous literature suggests that HPV-related head and neck cancers are more frequent among younger adults, whereas classical risk factor-associated cases tend to occur at older ages [21]. Chaturvedi et al. reported an increasing trend of HPV-positive oropharyngeal cancers in individuals under 50, suggesting that viral factors may be more prominent in this subgroup. In our study, however, HPV, EBV, and CMV DNA positivity in tissue samples was also observed in older individuals, supporting the hypothesis that latent viral infections may reactivate with age or that age-related immune changes could facilitate viral oncogenesis [22].

Serological IgG positivity was also higher in older age groups, indicating widespread past or persistent infections. These results are likely associated with immunological changes due to aging and cumulative lifetime exposure to these viruses [23].

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The noticeably higher HPV DNA positivity in biopsy samples among female patients (16.7%) compared to males (2.6%), although not statistically significant ($p = 0.14$), is an important observation. Some studies have suggested that HPV prevalence may differ by sex. In a multicenter study conducted by D'Souza et al., the prevalence of HPV positivity in oropharyngeal squamous cell carcinoma (OPSCC) cases was found to be similar between women and men, with a significant increase observed over time in both sexes; however, no statistically significant difference was detected between the sexes ($p = 0.34$) [24]. However, most studies from Western populations report a higher prevalence of HPV-associated head and neck cancers in men [25]. These differences may stem from geographic and cultural factors, as well as variations in risk behaviors, sexual practices, and biological immune responses. Our findings suggest that the impact of gender on HPV positivity may be context-dependent, and the potential risk of HPV-related head and neck cancer in women should not be overlooked. EBV and CMV DNA positivity were observed only in male patients, but the limited number of cases prevented statistical significance. The literature contains limited data on this matter, and gender differences are generally attributed to risk exposures and behavioral patterns.

This study has several limitations, including a relatively small sample size and being a single-center study. In particular, the small number of cases in certain subgroups (e.g., rare tumors like retromolar SCC) limited statistical power and generalizability. Additionally, potential confounding variables such as immune status, treatment history, and tumor stage could not be comprehensively analyzed in this cohort. Nevertheless, the findings provide valuable and original insights into the presence and distribution of viral agents in head and neck cancers.

5. Conclusıons

Our study demonstrates that viral agents in head and neck cancer cases may exhibit significant variations according to clinical variables such as tumor subtype, age, and sex. Notably, HPV was detected at higher rates in oral cavity and tongue SCCs, whereas EBV and CMV did not show significant distribution patterns across these tumor types. HSV was not detected in any patient. Serologically, high IgG positivity rates reflect widespread past infections in the population, while IgM positivity rates—and thus evidence of acute or persistent infections—were quite low. These findings suggest that the viral etiology of head and neck cancers may vary depending on tumor subtype and individual factors, emphasizing the importance of considering tumor subtypes in clinical management.

Author Contributions: Conceptualization, M.O., H.K., M.C..; methodology, M.O., H.K., M.C., M.U., A.A., A.M.U..; software, K.S., Y.A.U., O.Y., F.G.K., A.M.U., A.A..; validation, M.O., A.A, H.K..; formal analysis, M.O., H.K.U..; investigation, M.O., H.K., A.A., M.C., U.A., A.M.U..; resources, M.O., H.K., K.S., Y.A.U., A.M.U..; data curation, K.S., Y.A.U., O.Y., F.G.K., U.A..; writing—original draft preparation, M.O., H.K., M.C., A.A..; writing—review and editing, M.O., H.K., M.C., Y.A.U., A.A..; visualization, M.O., H.K., O.Y., F.G.K..; supervision, M.O..; project administration, M.O.; All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by the Scientific Research Projects Unit of Istanbul University on 27.03.2024 (Project ID: 40491).

Institutional Review Board Statement: This study was approved by the Clinical Research Ethics Committee of Istanbul University Faculty of Medicine on 7 November 2023 (Approval No: 2234909).

Informed Consent Statement: Since test samples belonging to patients were used in the study, patient consent forms were used.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: This study was performed in the Istanbul Faculty of Medicine, Department of Medical Microbiology.

Conflicts of Interest: The authors declare no conflicts of interest.

 doi:10.20944/preprints202506.1563.v1

Abbreviations

The following abbreviations are used in this manuscript:

EBV	Ebstein Barr Virus
CMV	Cytomegalovirus
HSV	Herpes Simplex Virus
HPV	Human Papilloma Virus
SCC	Squamous Cell Carcinoma
ELISA	Enzyme Linked Immunosorbent Assay
qPCR	Quantitative Polymerase Chain Reaction

References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A.; Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 2018 Nov;68(6):394-424. doi:10.3322/caac.21492
2. Anderson, R.; Mkhize, N. M., Kgokolo, M. M., Steel, H. C., Rossouw, T. M., Anderson, L., & Rapoport, B. L. (2025). Current and Emerging Insights into the Causes, Immunopathogenesis, and Treatment of Cutaneous Squamous Cell Carcinoma. *Cancers*, *17*(10), 1702.
3. Gillison, M.L.; Koch, W.M.; Capone, R.B.; Spafford, M.; Westra, W.H.; Wu, L.; Zahurak, M.L.; Daniel, R.W.; Viglione, M.; Symer, D.E.; Shah, K.V.; Sidransky, D.; Alberg, A.J.; Sidransky, D.; Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst*, 2000 May 3;92(9):709-20. doi:10.1093/jnci/92.9.709
4. Al Fadel, A. M., Jakobsen, K. K., Jensen, L. H., Carlander, A. L. F., Grønhøj, C., & von Buchwald, C. (2025). The epidemiological trends and survival of HPV-related oropharyngeal cancer other than tonsils and base of tongue– a systematic review and meta-analysis. *Oral Oncology*, *165*, 107311.
5. Liu, W., Chen, G., Gong, X., Wang, Y., Zheng, Y., Liao, X., ... & Zhang, X. (2021). The diagnostic value of EBV-DNA and EBV-related antibodies detection for nasopharyngeal carcinoma: a meta-analysis. *Cancer cell international*, *21*, 1-13.
6. Khan, G., Hashim, M.J.; Global burden of deaths from Epstein–Barr virus attributable malignancies 1990–2010. *Infect Agent Cancer*, 2014 Mar 19;9:38. doi:10.1186/1750-9378-9-38
7. Yan, M., Xiao, L. Y., Gosau, M., Smeets, R., Feng, H. C., Burg, S., ... & Friedrich, R. E. (2024). The role of herpes simplex virus infection in the etiology of head and neck cancer–a Mendelian randomization study. *Frontiers in Immunology*, *15*, 1278327.
8. Verma, D.; Thompson, J.; Swaminathan, S.; CMV and cancer: more than meets the eye?. *Trends Microbiol*. 2021 Nov;29(11):980-999. doi:10.1016/j.tim.2021.03.010
9. Syrjänen, S.; Detection of human papillomavirus in head and neck carcinomas. *Methods Mol Biol*, 2015;1249:221-30. doi:10.1007/978-1-4939-2004-4_18
10. D'Souza, G.; Kreimer, A.R.; Viscidi, R.; et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med*, 2007 May 10;356(19):1944-56. doi:10.1056/NEJMoa065497
11. Park, J. C., Ye, G. H., Merkin, R., & Roberts, T. (2025). Outcomes for recurrent or metastatic head and neck cancer by HPV status: a systematic review and meta-analysis. *The Oncologist*, *30*(4), oyaf043.
12. Blanco, R., Carrillo-Beltran, D., Corvalán, A. H., & Aguayo, F. (2021). High-Risk Human Papillomavirus and Epstein–Barr Virus Coinfection: A Potential Role in Head and Neck Carcinogenesis. *Biology*, *10*(12), 1232.
13. Pirmoradi, Z., Nazari, K., Shafiee, N., Nikoukar, N., Minoo, S., Ghasemi, H., ... & Allahyartorkaman, M. (2024). Oral cancer and HPV. *Asian Pacific Journal of Cancer Biology*, *9*(1), 87-95.
14. Ghossein, R. A., Dogan, S., Cohen, M. A., Katabi, N., & Xu, B. (2024). Histologic spectrum and outcome of human papillomavirus (HPV)-associated oral cavity squamous cell carcinoma: a single center experience and a survey of The Cancer Genome Atlas (TGCA) cohort. *Virchows Archiv*, *485*(4), 665-674.

 doi:10.20944/preprints202506.1563.v1

15. Uhlrich, E., Klijanienko, J., Martin, J., Jeannot, E., Vincent-Salomon, A., Freneaux, P., ... & Dubray-Vautrin, A. (2024). Prevalence of human papilloma virus in head and neck mucous squamous cell carcinoma and genotypes by location: an observational study. *European Journal of Cancer Prevention*, 10-1097.
16. Vani, N. V., Rama, R., Madhanagopal, R., Vijayalakshmi, R., & Swaminathan, R. (2024). Human Papillomavirus–Attributable Head and Neck Cancers in India—A Systematic Review and Meta-Analysis. JCO global oncology, 10, e2300464.
17. Young, L.S.; Rickinson, A.B., Epstein–Barr virus: 40 years on. *Nat Rev Cancer*, 2004;4(10):757-768.
18. Xu, M., Feng, R., Liu, Z., Zhou, X., Chen, Y., Cao, Y., ... & Lin, X. (2024). Host genetic variants, Epstein-Barr virus subtypes, and the risk of nasopharyngeal carcinoma: Assessment of interaction and mediation. *Cell genomics*, *4*(2).
19. Verma. D.; Thompson, J.; Swaminathan, S.; CMV and cancer: more than meets the eye?. *Trends Microbiol*, 2021;29(11):980-999.
20. Cox, M.A.; Cairns, T.M.; Willis, V.C.; et al. Herpesviruses in head and neck cancer: epidemiology, biology, and therapeutic implications. *J Dent Res*, 2022;101(8):853-862.
21. Chaturvedi, A.K.; Anderson, W.F.; Lortet-Tieulent, J.; et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol*, 2013;31(36):4550-4559.
22. Baba, S. K., Alblooshi, S. S. E., Yaqoob, R., Behl, S., Al Saleem, M., Rakha, E. A., ... & Mirza, S. (2025). Human papilloma virus (HPV) mediated cancers: an insightful update. *Journal of Translational Medicine*, *23*(1), 483.
23. Permar, S. R., & Gantt, S. (2025). Cytomegalovirus. In *Remington and Klein's Infectious Diseases of the Fetus and Newborn Infant* (pp. 640-687). Elsevier.
24. D'Souza, G., Westra, W. H., Wang, S. J., Van Zante, A., Wentz, A., Kluz, N., ... & Fakhry, C. (2017). Differences in the prevalence of human papillomavirus (HPV) in head and neck squamous cell cancers by sex, race, anatomic tumor site, and HPV detection method. *JAMA oncology*, *3*(2), 169-177.
25. Anantharaman, D.; Abedi-Ardekani, B.; Beachler, D.C.; et al. Geographical heterogeneity in the prevalence of human papillomavirus in head and neck cancer. *Int J Cancer*, 2017;140(9):1968-1975.