HHS Public Access

Author manuscript

J Med Virol. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

J Med Virol. 2016 November ; 88(11): 1944–1952. doi:10.1002/jmv.24555.

Prevalence and risk factors for oral DNA tumor viruses in HIV-infected youth

Jessica A. Kahn, MD, MPH[a], **Bret J. Rudy, MD**[b], **Jiahong Xu, MA**[c], **Bill Kapogiannis, MD**[d], **Elizabeth Secord, MD**[e], and **Maura Gillison, MD, PhD**[f]

Jessica A. Kahn: jessica.kahn@cchmc.org; Bret J. Rudy: Bret.Rudy@nyumc.org; Jiahong Xu: jiahongxu@westat.com; Bill Kapogiannis: kapogiannisb@mail.nih.gov; Elizabeth Secord: esecord@med.wayne.edu; Maura Gillison: maura.gillison@osumc.edu

[a]Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, OH, U.S., 45215

[b]New York University School of Medicine, New York, NY, U.S., 10016

[c]Westat, Rockville, MD, U.S., 20850

[d]Maternal and Pediatric Infectious Disease Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Rockville, MD, U.S., 20892

[e]Wayne State University, Detroit, MI, U.S., 48202

[f]Ohio State University, Columbus, OH, U.S., 43210

Abstract

Human papillomavirus (HPV), Epstein-Barr virus (EBV), and Kaposi sarcoma-associated herpes virus (KSHV) may promote oral cancers, especially among immunosuppressed individuals. The aims of this study were to examine whether demographic characteristics, medical history, sexual behaviors, substance use, CD4+ T-cell count, HIV viral load, and HPV vaccination were associated with HPV, EBV and KSHV infection and viral load. Multivariable modeling using logistic or linear regression examined associations between independent variables and infection or viral load, respectively. Among 272 HIV-infected 12–24 year-old youth, 19.5% were positive for oral HPV, 88.2% for EBV, and 11.8% for KSHV. In multivariable models, recent marijuana use (OR 1.97, 95% CI 1.02–3.82) and lower CD4+ T-cell count (< 350 vs. ≥ 350 cells/mm^3: OR 1.92, 95% CI 1.003–3.69) were associated with HPV infection; lifetime tobacco use (estimated coefficient [EC] 1.55, standard error [SE] 0.53, p=.0052) with HPV viral load; recent tobacco use (OR 2.90, 95% CI 1.06–7.97) and higher HIV viral load (≥ 400 vs. < 400 copies/mL: OR 3.98, 95% CI 1.84–8.74) with EBV infection; Black vs. White race (EC 1.18, SE 0.37, p=.0023) and lower CD4+ T-cell count (EC 0.70, SE 0.28, p=.017) with EBV viral load, male vs. female gender

Corresponding author: Jessica Kahn, MD, MPH, Division of Adolescent and Transition Medicine, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, MLC 4000, Cincinnati, OH 45229, Phone (513) 636-7764, Fax (513) 636-1129, jessica.kahn@cchmc.org.

These data were presented in part at the April 2014 network meeting for the Adolescent Medicine Trials Network for HIV/AIDS Interventions, Bethesda, MD

Competing interests: Drs. Kahn and Rudy co-chair an HPV vaccine clinical trial in HIV-infected young men, for which Merck & Co., Inc., provided vaccine and immunogenicity titers. Dr. Kahn chaired a grant review committee for the Society for Adolescent Health and Medicine evaluating public health demonstration project proposals to improve adolescent vaccination; grant funding for this program was from Merck, Inc. For the remaining authors none were declared.

(OR 10, 95% CI 1.32–100) with KSHV infection, and younger age at HIV diagnosis (1–14 vs. 18–20 years: EC 0.33, SE 0.16, p=.049; 15–17 vs. 18–20 years: EC 0.35, SE 0.13, p=.0099) with KSHV viral load. In conclusion, substance use and immunosuppression are associated with oral DNA tumor viruses in HIV-infected youth.

Introduction

Human papillomavirus (HPV), Epstein-Barr virus (EBV), and Kaposi sarcoma-associated herpesvirus (KSHV) contribute to the pathogenesis of diseases of the upper airway, including oral cancers. Compared to HIV-uninfected individuals, HIV-infected individuals have higher prevalence of oral HPV, EBV and KSHV infection as well as cancers linked to these viruses.[Gillison et al., 2012; Hille et al., 2002; McLemore et al., 2010; Webster-Cyriaque et al., 2006] Oral infection with cancer-associated or high-risk HPV types causes a subset of head and neck squamous cell carcinomas (e.g. oropharyngeal cancers) with increasing incidence, particularly among young men.[Chaturvedi et al., 2011] Hypothesized mechanisms for the increased risk of oropharyngeal cancers in HIV-infected individuals include immunosuppression and higher rates of oral HPV, tobacco use, and marijuana use. HIV-infected individuals are also at elevated risk for oral Epstein-Barr virus (EBV, associated with oral hairy leukoplakia and nasopharyngeal carcinoma),[Raab-Traub, 2002; Sand et al., 2002a] and for Kaposi's sarcoma-associated herpesvirus (KSHV, also known as human herpesvirus-8 [HHV-8], associated with Kaposi's sarcoma which may occur in the oral cavity).[Proceedings of the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1997] Furthermore, it has been hypothesized that EBV and HPV may interact to promote the development of nasopharyngeal carcinomas.[Al Moustafa et al., 2009]

Despite the role these DNA tumor viruses may play in oral carcinogenesis, little is known about their epidemiology among at-risk populations of HIV-infected youth, risk factors for oral infection and viral load in this population, and rates of co-infection. Also unknown are the effects of viral co-infection on the natural history of virus-associated oral cancers. Elucidation of the epidemiology of these viruses may lead to interventions that target the disease process. Our study aimed to: 1) examine the prevalence and viral loads for oral HPV, EBV and KSHV infection in HIV-infected youth; 2) define the behavioral, immunologic, and virologic factors associated with HPV, EBV and KSHV infection and viral loads; and 3) examine associations between HPV and EBV and between HPV and KSHV infection and viral loads.

Materials and Methods

Adolescents and young adults 12–24 years of age with primarily behaviorally acquired HIV infection were enrolled in a cross-sectional study of the Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) after approval of the protocol by local Institutional Review Boards. Participants were recruited from clinics affiliated with the ATN, which provide medical care for HIV-infected youth. Participants completed a survey through audio computer-assisted self-interview (ACASI) assessing demographic information,

substance use, gynecologic history, sexual behaviors, and antiretroviral adherence. HIV-1 viral load, CD4+ T-cell count and HPV vaccination status were obtained by medical chart review. Participants provided an oral rinse sample by swishing and gargling with 10 mL of Scope mouthwash (or sterile saline if preferred) for 30 seconds.

DNA purification from oral rinse samples was accomplished in a central laboratory using the Qiagen Virus/Bacteria Midi Kit (Qiagen Inc.; Hilden, Germany) on the Qiasymphony SP instrument.[Broutian et al., 2011] The presence of any of 37 HPV DNA types and beta-globin was detected by PGMY primer polymerase chain reaction (PCR), followed by reverse line blot hybridization (Roche Linear Array HPV Genotyping Test, Roche Molecular System, Inc.).[Gravitt et al., 1998] Beta-globin positive samples were considered evaluable and classified as HPV-positive if any of the 37 HPV DNA types were detected. Positivity and viral load for EBV was determined using TaqMan quantitative (q)PCR.[Jebbink et al., 2003] Positivity (i.e. detection of viral shedding) and viral load for KSHV was determined using TaqMan quantitative (q)PCR.[Casper et al., 2004] Viral load in samples positive for HPV was determined by HPV type-specific qPCR, and viral loads were normalized to the number of diploid human cells in the specimen (i.e. copies/cell) as estimated by TaqMan PCR to ERV3 (ERV = endogenous retrovirus), and analyzed as a transformed continuous variable.[Koshiol et al., 2011]

We examined whether the following independent variables were associated with HPV, EBV and KSHV infection and viral load: demographic characteristics, gynecologic history, sexual behaviors (same-sex and opposite-sex), substance use, CD4+ T-cell count, length of HIV infection, HIV viral load, adherence to antiretroviral medications, and HPV vaccination status. Univariable logistic regression was used to examine associations between independent variables and HPV, EBV, or KSHV infection, and univariable linear regression was used to examine associations between independent variables and HPV, EBV, or KSHV viral load. All independent variables associated with outcomes at $p < .15$ were entered into multivariable logistic or linear regression models, and variables associated with the outcomes at $p < .05$ were retained in the final models. We examined associations between HPV and EBV or KSHV infection using chi-square and between HPV and EBV or KSHV viral load using correlation analysis.

Results

The mean age of the 272 participants (213 male and 59 female) was 21.5 years (standard deviation 2.0 years, median 22 years, range 14–24), 15.8% were non-Hispanic White, 64% non-Hispanic Black, and 20.2% Hispanic. Self-reported route of HIV infection was as follows: perinatal (0.4%), sex with a man (88.6%), sex with a woman (4%), and other/don't know (7%). Mean length of time since HIV diagnosis was 1.9 years, 74.2% had a CD4+ T cell count ≥ 350 cells/mm^3 (mean 515.5 cells/mm^3, median 479 cells/mm^3), 23.2% had a non-detectable HIV viral load, and 132 (48.9%) were taking antiretroviral medications. Approximately half (50.8%) of women and 33.3% of men had received at least one HPV vaccine dose. Oral HPV prevalence results, but not viral load results, have been described previously.[Kahn et al., 2015] Overall, 53 (19.5%) of participants were positive for at least one HPV type, 8 (2.9%) for HPV16 and 1 (0.4%) for HPV18. EBV was detected in 240

(88.2%) and KSHV in 32 (11.8%). HPV and EBV prevalence did not differ significantly by gender, but KSHV prevalence differed by gender: 31 (14.6%) of men vs. 1 (1.7%) of women (p=.005).

HPV, EBV and KSHV viral load results are shown in Table 1. A total of 84 HPV infections were detected, and the mean HPV viral load (log-transformed) was calculated for single as well as multiple HPV types. The mean log_{10}HPV viral load for multiple types ranged from 0.90–4.77, the mean EBV viral load was 854,694 (SD 4,139,721) copies/mL and the mean KSHV viral load was 1,173 (SD 8,253) copies/mL.

In univariable analyses, substance use and HIV-related immunosuppression were the variables most frequently associated with oral tumor virus infection and viral load. Independent variables associated at $p < .15$ with HPV, EBV and KSHV infection and viral load are summarized in Table 2. Variables not associated with the outcomes at $p < .15$, including same-sex sexual behaviors, are not included in the Table. The results of multivariable logistic and linear regression analyses are shown in Table 3. Recent marijuana use (OR 1.97, 95% CI 1.02–3.82) and lower CD4+ T-cell count (< 350 vs. ≥ 350 cells/mm^3: OR 1.92, 95% CI 1.003–3.69) were associated with HPV infection and lifetime tobacco use (estimated coefficient [EC] 1.55, standard error [SE] 0.53, p=.0052) was associated with HPV viral load. Recent tobacco use (OR 2.90, 95% CI 1.06–7.97) and higher HIV viral load (≥ 400 vs. < 400 copies/mL: OR 3.98, 95% CI 1.84–8.74) were associated with EBV infection, and Black vs. White race (EC 1.18, SE 0.37, p=.0023) and lower CD4+ T-cell count (EC 0.70, SE 0.28, p=.017) were associated with EBV viral load. Male vs. female gender (OR 10, 95% CI 1.32–100) was associated with KSHV infection, and younger age at HIV diagnosis (1–14 vs. 18–20 years: EC 0.33, SE 0.16, p=.049; 15–17 vs. 18–20 years: EC 0.35, SE 0.13, p=.0099) was associated with KSHV viral load.

Correlations were not significant between HPV and EBV infection (p=.14) and between HPV and KSHV infection (p=.56). Similarly, correlations were not significant between HPV and EBV viral load (p=0.63) and between HPV and KSHV viral load (p=0.35).

Discussion

In this study, we examined factors associated with oral DNA tumor virus infection and viral load in HIV infected youth. We found that although specific risk factors differed for HPV, EBV, and KSHV infection and viral load, substance use and HIV-related factors were the factors most commonly associated with all three oral tumor viruses. Neither infection with nor viral load for HPV, KSHV, and EBV infections were significantly correlated. To our knowledge, this is the first study to examine the epidemiology of all three of these oral tumor viruses in HIV-infected youth, including risk factors for oral infection and viral load and rates of co-infection.

Lifetime tobacco use was associated with HPV viral load and recent tobacco use with EBV infection. Previous studies, primarily conducted in adults, support these associations. Tobacco use has been shown to be a risk factor for anogenital HPV viral load, HPV persistence, and HPV-associated anogenital cancers.[Smith et al., 2010; Trottier and Franco,

2006; Wang et al., 2004] Furthermore, HPV viral load is a strong predictor of persistent HPV infection and cervical dysplasia,[Fakhry et al., 2010; Gravitt et al., 2007] and persistence of high-risk HPV infection is an established surrogate for risk of cervical disease progression.[Moscicki et al., 2001; Schiffman and Kjaer, 2003] It has been proposed that the immunologic changes caused by smoking may lead to increased HPV viral replication and higher viral load.[Chaturvedi et al., 2011; Moscicki et al., 2001; Xi et al., 2009] In addition, a number of studies have demonstrated that smoking is associated with oral HPV infection in HIV-uninfected[Beachler et al., 2012; D'Souza et al., 2007; Gillison et al., 2012; Kreimer et al., 2011; Ragin et al., 2011; Read et al., 2012; Smith et al., 2007] and HIV-infected[D'Souza et al., 2007; Fakhry et al., 2010][Beachler et al., 2012; Read et al., 2012] individuals, and tobacco use has been identified as a risk factor for development of HPV-positive oropharyngeal cancers.[Smith et al., 2010; Stoler et al., 2013] In contrast to this evidence for an association between tobacco use and HPV infection, there have been no consistent associations demonstrated between tobacco use and oral EBV infection.[Jalouli et al., 2012; Sand et al., 2002b]

Recent marijuana use was associated with HPV infection in this population. Some previous studies have demonstrated an association between marijuana use and oral HPV as well as oropharyngeal cancer,[Gillison et al., 2008; Marks et al., 2014] though in one study marijuana use was associated with a reduced risk of tongue cancers.[Marks et al., 2014] Marijuana contains carcinogens that may cause molecular changes in the airway epithelium and may also suppress humoral and cell-mediated immune responses, including anti-tumor immunity. Zhang et al. reported that in low doses, delta-9-tetrahydrocannabinol (D9-THC) facilitated KSHV infection in endothelial cells in vitro through enhancement of cell-cell interactions and endocytosis and upregulated the expression of the lytic switch gene ORF50 and the carcinogenic KSHV G protein-coupled receptor, increasing viral titers in culture and inducing endothelial cell transformation.[Zhang et al., 2007] The association between marijuana use and HPV infection is concerning given the high rates of use in youth, including HIV-infected youth: in a larger study of youth in the same research network, 28% used marijuana weekly or daily.[Fernandez et al., 2015] Further study is needed in order to examine whether – and by what mechanism – marijuana use may increase the risk of HPV infection or oral cancers in HIV-infected individuals.

HIV-related factors, including CD4+ T-cell count, HIV viral load, and age at HIV diagnosis, were also associated with oral tumor virus infection and viral load. A lower CD4+ T-cell count was associated with HPV infection and EBV viral load; higher HIV viral load was associated with EBV infection, and younger age at HIV diagnosis was associated with KSHV viral load. These associations likely reflect immunosuppression with increased susceptibility to oral virus infection or replication, and are consistent with the higher prevalence rates of both HPV and EBV in HIV-infected individuals.[Gillison et al., 2012; Hille et al., 2002; McLemore et al., 2010; Miller et al., 2006; Santos et al., 2014; Webster-Cyriaque et al., 2006] Overall CD4+ T-cell counts were relatively high, suggesting that even subtle perturbations in CD4+ T-cell count may affect HPV and EBV positivity. The findings imply that improving access and adherence to antiretroviral therapy in youth is essential. The lack of a consistent association between sexual behaviors and oral tumor viruses was

unexpected, given that both HPV and KSHV are usually sexually transmitted.[Martin et al., 1998]

Rates of HPV, EBV and KSHV found in this study are consistent with prior studies. Although the 19.5% prevalence for oral HPV infection was higher than previously reported for HIV-uninfected youth,[Beachler et al., 2012; D'Souza et al., 2007; Fakhry et al., 2006; Gillison et al., 2012; Kero et al., 2012; Kreimer et al., 2010; Kreimer et al., 2011; Paolini et al., 2013; Pickard et al., 2012; Smith et al., 2004; Smith et al., 2007; Summersgill et al., 2001] it was similar to that among youth at high-risk for sexually transmitted infections.[Du et al., 2012; Schlecht et al., 2012] The oral EBV prevalence (88.2%) in our study population was consistent with estimates previously reported in HIV-infected adults (42.1% to 90%), [Ammatuna et al., 2001; Miller et al., 2006] and healthy adults (90%),[Ikuta et al., 2000] and KSHV prevalence (11.8%) was also consistent with prior reports (11.6% to 57%).[de Franca et al., 2011; Del Mistro et al., 2012; Widmer et al., 2006]

In this sample, we did not find significant associations between HPV and either EBV or KSHV infection or viral load. Investigators have suggested that oral tumor viruses may interact in the development of HPV-related oral cancers. For example, Al Moustafa et al. hypothesized that human oral epithelial cells, especially nasopharyngeal cells, are susceptible to high-risk HPV and EBV co-infections, and that E6/E7 oncoproteins of high risk HPVs and Epstein-Barr virus nuclear antigen 1 (EBNA1), Latent membrane protein 1 (LMP1), Latent membrane protein 2 (LMP2), and BARF1 oncoproteins cooperate to induce neoplastic transformation of human oral epithelial cells.[Al Moustafa et al., 2009] Lo et al. similarly demonstrated that high-risk HPV types were commonly detected in naspharyngeal carcinomas.[Lo et al., 2010] In a multinational study of oral squamous cell carcinomas, 55% were positive for EBV and 35% for HPV. In 34% of the samples, co-infection by two (30%) or three (4%) virus specimens was detected; the most frequent co-infection was HPV with EBV in 21% of all cancers.[Jalouli et al., 2012] Jiang et al. found that a high proportion of base-of-tonsil and tonsil cancers were co-infected with HPV and EBV, that co-infection was significantly associated with cancer status, and that co-infected cell lines demonstrated a higher degree of cell proliferation and invasiveness.[Jiang et al., 2015] In contrast, McLemore et al. demonstrated that while HPV was detected in 24% of head and neck squamous cell carcinomas in a sample of HIV-infected individuals, EBV and KSHV detection were uncommon, not supporting a promoting effect of co-infection.[McLemore et al., 2010] We examined associations between oral tumor viruses detected in saliva samples, not in carcinomas, so the lack of a significant correlation between tumor viruses does not preclude interaction between these viruses to promote carcinogenesis. Further research is needed to explore whether co-infection potentiates carcinogenesis.

Finally, only one-third of young men in this study had received at least one HPV vaccine dose. Evidence is emerging that HPV vaccination prevents oral HPV infection as well as anogenital infection.[Herrero et al., 2013] Vaccination was not associated in adjusted regression models with oral HPV infection, perhaps because most men were not vaccinated or because they were already infected with oral HPV before vaccination. These findings suggest that improving HPV vaccination rates in young men, particularly young men who have sex with men or who are HIV-infected, should be a public health priority.

Limitations of this exploratory study include the small sample size, limiting the power to detect associations between independent variables and oral tumor viruses as well as correlations between oral tumor viruses. Furthermore, the associations identified between independent variables and oral tumor viruses do not imply causation.

In conclusion, common risks for these three oral tumor viruses were identified, including substance use and HIV-related factors. These associations are concerning given the high prevalence of substance use as well as oral tumor viruses and the high rates of poor adherence to antiretroviral therapy in HIV-infected youth. HPV was not correlated with EBV or KSHV infection. Longitudinal studies are needed to provide more insight into causal associations between substance use and oral tumor viruses; mechanisms by which substance use may influence carcinogenesis; and the role of HIV-related cofactors. Understanding co-infections in this population and identifying specific contributions resulting from HIV infection and any interaction with other viruses in the development and pathogenesis of HPV-related cancers is important, as early interventions may be needed to prevent viral-associated cancers in HIV-infected individuals.

Acknowledgments

This work was supported by The Adolescent Trials Network for HIV/AIDS Interventions from the National Institutes of Health through the National Institutes of Health [U01 HD 040533 and U01 HD 040474] through the National Institute of Child Health and Human Development (B. Kapogiannis, C. Worrell), with supplemental funding from the National Institutes of Drug Abuse (S. Kahana) and Mental Health (P. Brouwers). The study was scientifically reviewed by the ATN's Therapeutic Leadership Group. Network, scientific, and logistical support was provided by the ATN Coordinating Center (C. Wilson, C. Partlow) at the University of Alabama at Birmingham. Network operations and data management support was provided by the ATN Data and Operations Center at Westat, Inc. (J. Korelitz, B. Driver). Laboratory testing was supported by a grant from the Viral Oncology Program, Ohio State University Comprehensive Cancer Center. We acknowledge the contribution of the investigators and staff at the following ATN 114 sites that participated in this study: Baylor Collect of Medicine (Mary E. Paul, M.D., Nancy Calles, MSN, RN, PNP, ACRN, MPH, Norma Cooper, RN); Wayne State University Children's Hospital of Michigan (Elizabeth Secord, M.D., Monique L Green-Jones, MPH, Charnell Cromer, MSN); Johns Hopkins University (Allison George Agwu, M.D., Thuy C Anderson, BSN, Kaye Park, MPH); The Fenway Institute (Kenneth Mayer, M.D., Marcy S Gelman, CRNP, Emily George, RN); University of Colorado Denver (Elizabeth McFarland, M.D., Kerry A Hahn, BS, CCRP, Emily A Barr, CPNP,CNM,MSN); We sincerely thank the other ATN 114 Protocol Team Members (Nancy Calles, MSN, RN, PNP, ACRN, MPH and Charnell Cromer, MSN), and the youth who participated in the study. Laboratory testing was supported by a grant from the Viral Oncology Program, Ohio State University Comprehensive Cancer Center.

Funding: This work was supported by The Adolescent Trials Network for HIV/AIDS Interventions from the National Institutes of Health through the National Institute of Child Health and Human Development, with supplemental funding from the National Institutes of Drug Abuse and Mental Health [grant numbers U01-HD040533, U01 HD 040474].

References

Al Moustafa AE, Chen D, Ghabreau L, Akil N. Association between human papillomavirus and Epstein-Barr virus infections in human oral carcinogenesis. Med Hypotheses. 2009; 73(2):184–186. [PubMed: 19361933]

Ammatuna P, Campisi G, Giovannelli L, Giambelluca D, Alaimo C, Mancuso S, Margiotta V. Presence of Epstein-Barr virus, cytomegalovirus and human papillomavirus in normal oral mucosa of HIV-infected and renal transplant patients. Oral diseases. 2001; 7(1):34–40. [PubMed: 11354920]

Beachler DC, Weber KM, Margolick JB, Strickler HD, Cranston RD, Burk RD, Wiley DJ, Minkoff H, Reddy S, Stammer EE, Gillison ML, D'Souza G. Risk factors for oral HPV infection among a high prevalence population of HIV-positive and at-risk HIV-negative adults. Cancer Epidemiol Biomarkers Prev. 2012; 21(1):122–133. [PubMed: 22045700]

Broutian TR, He X, Gillison ML. Automated high throughput DNA isolation for detection of human papillomavirus in oral rinse samples. J Clin Virol. 2011; 50(4):270–275. [PubMed: 21273118]

Casper C, Redman M, Huang ML, Pauk J, Lampinen TM, Hawes SE, Critchlow CW, Morrow RA, Corey L, Kiviat N, Wald A. HIV infection and human herpesvirus-8 oral shedding among men who have sex with men. J Acquir Immune Defic Syndr. 2004; 35(3):233–238. [PubMed: 15076237]

Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, Jiang B, Goodman MT, Sibug-Saber M, Cozen W, Liu L, Lynch CF, Wentzensen N, Jordan RC, Altekruse S, Anderson WF, Rosenberg PS, Gillison ML. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol. 2011; 29(32):4294–4301. [PubMed: 21969503]

D'Souza G, Fakhry C, Sugar EA, Seaberg EC, Weber K, Minkoff HL, Anastos K, Palefsky JM, Gillison ML. Six-month natural history of oral versus cervical human papillomavirus infection. Int J Cancer. 2007; 121(1):143–150. [PubMed: 17354235]

de Franca TR, de Araujo RA, Ribeiro CM, Leao JC. Salivary shedding of HHV-8 in people infected or not by human immunodeficiency virus 1. J Oral Pathol Med. 2011; 40(1):97–102. [PubMed: 20969629]

Del Mistro A, Baboci L, Frayle-Salamanca H, Trevisan R, Bergamo E, Lignitto L, Sasset L, Cecchetto MG, Cattelan AM, Calabro ML. Oral human papillomavirus and human herpesvirus-8 infections among human immunodeficiency virus type 1-infected men and women in Italy. Sex Transm Dis. 2012; 39(11):894–898. [PubMed: 23064540]

Du J, Nordfors C, Ahrlund-Richter A, Sobkowiak M, Romanitan M, Nasman A, Andersson S, Ramqvist T, Dalianis T. Prevalence of oral human papillomavirus infection among youth, Sweden. Emerging infectious diseases. 2012; 18(9):1468–1471. [PubMed: 22932445]

Fakhry C, D'Souza G, Sugar E, Weber K, Goshu E, Minkoff H, Wright R, Seaberg E, Gillison M. Relationship between prevalent oral and cervical human papillomavirus infections in human immunodeficiency virus-positive and -negative women. J Clin Microbiol. 2006; 44(12):4479–4485. [PubMed: 17021055]

Fakhry C, Sugar E, D'Souza G, Gillison M. Two-week versus six-month sampling interval in a short-term natural history study of oral HPV infection in an HIV-positive cohort. PloS one. 2010; 5(7):e11918. [PubMed: 20689835]

Fernandez MI, Huszti HC, Wilson PA, Kahana S, Nichols S, Gonin R, Xu J, Kapogiannis BG. Profiles of Risk Among HIV-Infected Youth in Clinic Settings. AIDS and behavior. 2015; 19(5):918–930. [PubMed: 25117556]

Gillison ML, Broutian T, Pickard RK, Tong ZY, Xiao W, Kahle L, Graubard BI, Chaturvedi AK. Prevalence of oral HPV infection in the United States, 2009–2010. JAMA. 2012; 307(7):693–703. [PubMed: 22282321]

Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, Viscidi R. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst. 2008; 100(6):407–420. [PubMed: 18334711]

Gravitt PE, Kovacic MB, Herrero R, Schiffman M, Bratti C, Hildesheim A, Morales J, Alfaro M, Sherman ME, Wacholder S, Rodriguez AC, Burk RD. High load for most high risk human papillomavirus genotypes is associated with prevalent cervical cancer precursors but only HPV16 load predicts the development of incident disease. Int J Cancer. 2007; 121(12):2787–2793. [PubMed: 17722112]

Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol. 1998; 36(10):3020–3027. [PubMed: 9738060]

Herrero R, Quint W, Hildesheim A, Gonzalez P, Struijk L, Katki HA, Porras C, Schiffman M, Rodriguez AC, Solomon D, Jimenez S, Schiller JT, Lowy DR, van Doorn LJ, Wacholder S, Kreimer AR, Group CVTV. Reduced prevalence of oral human papillomavirus (HPV) 4 years after bivalent HPV vaccination in a randomized clinical trial in Costa Rica. PloS one. 2013; 8(7):e68329. [PubMed: 23873171]

Hille JJ, Webster-Cyriaque J, Palefski JM, Raab-Traub N. Mechanisms of expression of HHV8, EBV and HPV in selected HIV-associated oral lesions. Oral diseases. 2002; 8(Suppl 2):161–168. [PubMed: 12164651]

Ikuta K, Satoh Y, Hoshikawa Y, Sairenji T. Detection of Epstein-Barr virus in salivas and throat washings in healthy adults and children. Microbes and infection / Institut Pasteur. 2000; 2(2):115–120. [PubMed: 10742683]

Jalouli J, Jalouli MM, Sapkota D, Ibrahim SO, Larsson PA, Sand L. Human papilloma virus, herpes simplex virus and epstein barr virus in oral squamous cell carcinoma from eight different countries. Anticancer Res. 2012; 32(2):571–580. [PubMed: 22287747]

Jebbink J, Bai X, Rogers BB, Dawson DB, Scheuermann RH, Domiati-Saad R. Development of real-time PCR assays for the quantitative detection of Epstein-Barr virus and cytomegalovirus, comparison of TaqMan probes, and molecular beacons. The Journal of molecular diagnostics : JMD. 2003; 5(1):15–20. [PubMed: 12552075]

Jiang R, Ekshyyan O, Moore-Medlin T, Rong X, Nathan S, Gu X, Abreo F, Rosenthal EL, Shi M, Guidry JT, Scott RS, Hutt-Fletcher LM, Nathan CA. Association between human papilloma virus/Epstein-Barr virus coinfection and oral carcinogenesis. J Oral Pathol Med. 2015; 44(1):28–36. [PubMed: 25040496]

Kahn JA, Rudy BJ, Xu J, Secord EA, Kapogiannis BG, Thornton S, Gillison ML. Behavioral, immunologic, and virologic correlates of oral human papillomavirus infection in HIV-infected youth. Sexually transmitted diseases. 2015 In press.

Kero K, Rautava J, Syrjanen K, Grenman S, Syrjanen S. Oral mucosa as a reservoir of human papillomavirus: point prevalence, genotype distribution, and incident infections among males in a 7-year prospective study. European urology. 2012; 62(6):1063–1070. [PubMed: 22771267]

Koshiol J, Rotunno M, Gillison ML, Van Doorn LJ, Chaturvedi AK, Tarantini L, Song H, Quint WG, Struijk L, Goldstein AM, Hildesheim A, Taylor PR, Wacholder S, Bertazzi PA, Landi MT, Caporaso NE. Assessment of human papillomavirus in lung tumor tissue. J Natl Cancer Inst. 2011; 103(6):501–507. [PubMed: 21293027]

Kreimer AR, Bhatia RK, Messeguer AL, Gonzalez P, Herrero R, Giuliano AR. Oral human papillomavirus in healthy individuals: a systematic review of the literature. Sex Transm Dis. 2010; 37(6):386–391. [PubMed: 20081557]

Kreimer AR, Villa A, Nyitray AG, Abrahamsen M, Papenfuss M, Smith D, Hildesheim A, Villa LL, Lazcano-Ponce E, Giuliano AR. The epidemiology of oral HPV infection among a multinational sample of healthy men. Cancer Epidemiol Biomarkers Prev. 2011; 20(1):172–182. [PubMed: 21148755]

Lo EJ, Bell D, Woo JS, Li G, Hanna EY, El-Naggar AK, Sturgis EM. Human papillomavirus and WHO type I nasopharyngeal carcinoma. The Laryngoscope. 2010; 120(10):1990–1997. [PubMed: 20824783]

Marks MA, Chaturvedi AK, Kelsey K, Straif K, Berthiller J, Schwartz SM, Smith E, Wyss A, Brennan P, Olshan AF. Association of Marijuana Smoking with Oropharyngeal and Oral Tongue Cancers: Pooled Analysis from the INHANCE Consortium. Cancer Epidemiology Biomarkers & Prevention. 2014; 23(1):160–171.

Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH. Sexual transmission and the natural history of human herpesvirus 8 infection. N Engl J Med. 1998; 338(14):948–954. [PubMed: 9521982]

McLemore MS, Haigentz M Jr, Smith RV, Nuovo GJ, Alos L, Cardesa A, Brandwein-Gensler M. Head and neck squamous cell carcinomas in HIV-positive patients: a preliminary investigation of viral associations. Head and neck pathology. 2010; 4(2):97–105. [PubMed: 20333562]

Miller CS, Berger JR, Mootoor Y, Avdiushko SA, Zhu H, Kryscio RJ. High prevalence of multiple human herpesviruses in saliva from human immunodeficiency virus-infected persons in the era of highly active antiretroviral therapy. J Clin Microbiol. 2006; 44(7):2409–2415. [PubMed: 16825357]

Moscicki AB, Hills N, Shiboski S, Powell K, Jay N, Hanson E, Miller S, Clayton L, Farhat S, Broering J, Darragh T, Palefsky J. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. Jama. 2001; 285(23):2995–3002. [PubMed: 11410098]

Paolini F, Rizzo C, Sperduti I, Pichi B, Mafera B, Rahimi SS, Vigili MG, Venuti A. Both mucosal and cutaneous papillomaviruses are in the oral cavity but only alpha genus seems to be associated with cancer. J Clin Virol. 2013; 56(1):72–76. [PubMed: 23092620]

Pickard RK, Xiao W, Broutian TR, He X, Gillison ML. The prevalence and incidence of oral human papillomavirus infection among young men and women, aged 18–30 years. Sex Transm Dis. 2012; 39(7):559–566. [PubMed: 22706220]

Raab-Traub N. Epstein-Barr virus in the pathogenesis of NPC. Seminars in cancer biology. 2002; 12(6):431–441. [PubMed: 12450729]

Ragin C, Edwards R, Larkins-Pettigrew M, Taioli E, Eckstein S, Thurman N, Bloome J, Markovic N. Oral HPV Infection and Sexuality: A Cross-Sectional Study in Women. International journal of molecular sciences. 2011; 12(6):3928–3940. [PubMed: 21747715]

Read TR, Hocking JS, Vodstrcil LA, Tabrizi SN, McCullough MJ, Grulich AE, Garland SM, Bradshaw CS, Chen MY, Fairley CK. Oral human papillomavirus in men having sex with men: risk-factors and sampling. PloS one. 2012; 7(11):e49324. [PubMed: 23173054]

Sand LP, Jalouli J, Larsson P-A, Hirsch J-M. Prevalence of Epstein-Barr virus in oral squamous cell carcinoma, oral lichen planus, and normal oral mucosa. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2002a; 93(5):586–592.

Sand LP, Jalouli J, Larsson PA, Hirsch JM. Prevalence of Epstein-Barr virus in oral squamous cell carcinoma, oral lichen planus, and normal oral mucosa. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2002b; 93(5):586–592.

Santos L, Azevedo K, Silva L, Oliveira L. Epstein-Barr virus in oral mucosa from human immunodeficiency virus positive patients. Revista da Associacao Medica Brasileira. 2014; 60(3): 262–269. [PubMed: 25004273]

Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr. 2003; (31):14–19. [PubMed: 12807940]

Schlecht NF, Burk RD, Nucci-Sack A, Shankar V, Peake K, Lorde-Rollins E, Porter R, Linares LO, Rojas M, Strickler HD, Diaz A. Cervical, anal and oral HPV in an adolescent inner-city health clinic providing free vaccinations. PloS one. 2012; 7(5):e37419. [PubMed: 22624027]

Smith EM, Ritchie JM, Summersgill KF, Klussmann JP, Lee JH, Wang D, Haugen TH, Turek LP. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. Int J Cancer. 2004; 108(5):766–772. [PubMed: 14696105]

Smith EM, Rubenstein LM, Haugen TH, Hamsikova E, Turek LP. Tobacco and alcohol use increases the risk of both HPV-associated and HPV-independent head and neck cancers. Cancer Causes & Control. 2010; 21(9):1369–1378. [PubMed: 20401530]

Smith EM, Swarnavel S, Ritchie JM, Wang D, Haugen TH, Turek LP. Prevalence of human papillomavirus in the oral cavity/oropharynx in a large population of children and adolescents. Pediatr Infect Dis J. 2007; 26(9):836–840. [PubMed: 17721381]

Stoler DL, Smaldino PJ, Darbary HK, Sullivan MA, Popat SR, Hicks WL Jr, Merzianu M, Gaile DP, Anderson GR, Loree TR. Human papillomavirus and tobacco use in tongue base cancers. Ear, nose, & throat journal. 2013; 92(8):372–380.

Summersgill KF, Smith EM, Levy BT, Allen JM, Haugen TH, Turek LP. Human papillomavirus in the oral cavities of children and adolescents. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2001; 91(1):62–69.

Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine. 2006; 24(Suppl 1):S1–15. [PubMed: 16406226]

Wang SS, Schiffman M, Herrero R, Carreon J, Hildesheim A, Rodriguez AC, Bratti MC, Sherman ME, Morales J, Guillen D, Alfaro M, Clayman B, Burk RD, Viscidi RP. Determinants of human papillomavirus 16 serological conversion and persistence in a population-based cohort of 10 000 women in Costa Rica. Br J Cancer. 2004; 91(7):1269–1274. [PubMed: 15292929]

Webster-Cyriaque J, Duus K, Cooper C, Duncan M. Oral EBV and KSHV infection in HIV. Advances in dental research. 2006; 19(1):91–95. [PubMed: 16672557]

Widmer IC, Erb P, Grob H, Itin P, Baumann M, Stalder A, Weber R, Cathomas G. Human herpesvirus 8 oral shedding in HIV-infected men with and without Kaposi sarcoma. J Acquir Immune Defic Syndr. 2006; 42(4):420–425. [PubMed: 16791117]

Xi LF, Koutsky LA, Castle PE, Edelstein ZR, Meyers C, Ho J, Schiffman M. Relationship between cigarette smoking and human papilloma virus types 16 and 18 DNA load. Cancer Epidemiol Biomarkers Prev. 2009; 18(12):3490–3496. [PubMed: 19959700]

Zhang X, Wang JF, Kunos G, Groopman JE. Cannabinoid Modulation of Kaposi's Sarcoma–Associated Herpesvirus Infection and Transformation. Cancer research. 2007; 67(15):7230–7237. [PubMed: 17671191]

Table 1

Log-transformed viral load for HPV among subjects infected with HPV, and viral load for EBV and KSHV among subjects infected with EBV and KSHV, respectively

			Log_{10} HPV viral load (single type)[a]		Log_{10} HPV viral load (multiple types)[a]	
HPV type	**# subjects infected with this type**	**# subjects with single[b] / multiple[c] infections**	**Mean (SD[c])**	**Median (IQR[e]: 25th – 75th)**	**Mean (SD)**	**Median (IQR: 25th – 75th)**
6	3	1/2	1.81 (--[f])	1.81 (1.81 – 1.81)	3.06 (1.73)	3.06 (1.83 – 4.28)
11	2	1/1	0.82 (--)	0.82 (0.82 – 0.82)	1.83 (--)	1.83 (1.83 – 1.83)
16	8	5/3	0.00 (0.00)	0.00 (0.00 – 0.00)	3.12 (1.81)	4.05 (1.03 – 4.28)
18	1	1/0	4.22 (--)	4.22 (4.22 – 4.22)	--	--
26	1	1/0	3.72 (--)	3.72 (3.72 – 3.72)	--	--
35	4	0/4	--	--	4.77 (1.06)	4.72 (4.03 – 5.51)
39	5	3/2	2.64 (2.58)	2.76 (0.00 – 5.16)	4.47 (0.27)	4.47 (4.28 – 4.66)
45	3	2/1	0.59 (0.83)	0.59 (0.00 – 1.18)	1.64 (--)	1.64 (1.64 – 1.64)
51	1	0/1	--	--	1.64 (--)	1.64 (1.64 – 1.64)
53	3	1/2	2.89 (--)	2.89 (2.89 – 2.89)	0.90 (1.28)	0.90 (0.00 – 1.80)
55	3	0/3	--	--	3.15 (1.79)	3.82 (1.13 – 4.51)
56	2	0/2	--	--	3.77 (1.25)	3.77 (2.89 – 4.66)
58	4	0/4	--	--	4.77 (1.06)	4.72 (4.03 – 5.51)
59	9	5/4	3.18 (1.70)	3.44 (1.86 – 3.50)	2.79 (1.62)	2.93 (1.42 – 4.17)
62	2	0/2	--	--	2.52 (0.97)	2.52 (1.84 – 3.20)
66	2	0/2	--	--	3.05 (0.22)	3.05 (2.89 – 3.20)
68	3	1/2	5.44 (--)	5.44 (5.44 – 5.44)	0.82 (1.16)	0.82 (0.00 – 1.64)
Overall	53	21/32			1.79 (1.96)	1.18 (0.00 – 3.50)

			Mean viral load (SD)[i]	**Median viral load (Range)[i]**
EBV	240[g]	N/A[h]	854,694 (4,139,721)	16,240 (0–53,381,900)
KSHV	32[j]	N/A	1,173 (8,253)	0 (0–109,507)

[a] Viral loads for HPV are expressed as log_{10} HPV viral load, and were normalized to the number of diploid human cells in the specimen; the normalized viral load unit is number of viral copies per cell.

[b] # subjects with single infection: number of subjects positive for specified HPV type but negative for all other HPV types.

[c] # subjects with multiple infections: number of subjects positive for specified HPV type and at least one additional HPV type

[d] SD = standard deviation

[e] IQR = interquartile range

[f] -- indicates that mean, SD, median, and IQR may not be able to be estimated if there are 0 or 1 observations

[g] Number positive for EBV

[h] N/A = not applicable

[i] Viral load units are copies/mL

[j] Number positive for KSHV

J Med Virol. Author manuscript; available in PMC 2016 November 01.

Table 2

Risk factors associated with HPV, EBV, and KSHV infection and viral load at p < 0.15: results of unadjusted logistic and linear regression models

	HPV		EBV		KSHV	
	Infection	Log_{10} viral load[a]	Infection	Log_{10} viral load	Infection	Log_{10} viral load
	Unadjusted OR[b] (95% CI[c]) p value	Estimated coefficient (SE[d]) p value	Unadjusted OR (95% CI) p value	Estimated coefficient (SE) p value	Unadjusted OR (95% CI) p value	Estimated coefficient (SE) p value
Race						
Black vs. White (ref.)	1.84 (0.85–4.01) .122	_ [e]	-	1.14 (0.38) .0044	-	-
Ethnicity						
Hispanic vs. Non-Hispanic (ref.)	-	-	-	0.90 (0.38) .022	-	-
Gender						
Male vs. female (ref.)	-	-	-	-	10 (1.31–100) .026	−0.21 (0.13) .107
Marijuana use, past 3 months						
Almost daily/daily vs. weekly or less often (ref.)	1.83 (0.95–3.52) .069	-	-	-	-	−0.21 (0.11) .052
Tobacco use, past 3 months						
Almost daily/daily vs. weekly or less often (ref.)	-	-	2.70 (1.0–7.27) .0496	0.53 (0.35) .132	-	−0.25 (0.11) .029
Tobacco use, lifetime						
Yes vs. no (ref.)	-	1.55 (0.53) .0052	-	-	-	-
Alcohol use, past 3 months						
Weekly or more often vs. monthly or less often (ref.)	-	-	-	-	2.14 (0.99–4.59) .052	-
Alcohol use, lifetime						
Yes vs. no (ref.)	-	1.27 (0.74) .092	-	-	-	-
Number of heterosexual sex partners, lifetime						

	HPV		EBV		KSHV	
	Infection	**Log_{10} viral load[a]**	**Infection**	**Log_{10} viral load**	**Infection**	**Log_{10} viral load**
	Unadjusted OR[b] (95% CI[c]) p value	**Estimated coefficient (SE[d]) p value**	**Unadjusted OR (95% CI) p value**	**Estimated coefficient (SE) p value**	**Unadjusted OR (95% CI) p value**	**Estimated coefficient (SE) p value**
1+ vs. 0 (ref.)						0.19 (0.12) .116
Oral sex (performed/ received) with condom, past 3 months[f]	1.06 (1.00–1.13) .069					
Oral sex (performed/ received) without a condom, past 3 months						
1+ vs. 0 (ref.)						0.22 (0.13) .108
Unprotected oral/anal sex, past 3 months[g]						
1+ vs. 0 (ref.)	0.43 (0.17–1.12) .086	-	-	-	-	0.28 (0.14) .056
CD4+ T cell count						
<350 cells/mm^3 vs. ≥350 cells/mm^3 (ref.)	1.83 (0.96–3.48) .066	-	2.67 (0.90–7.90) .076	0.66 (0.31) .037	-	-
HIV viral load						
≥400 copies/mL vs. <400 copies/mL (ref.)	-	-	3.79 (1.74–8.26) .0008	-	-	-
Age at HIV diagnosis						
1–14 years vs. 18–20 years (ref.)	-	-	-	−1.20 (0.47) .014	-	0.33 (0.16) .049
15–17 years vs. 18–20 years (ref.)	-	-	-	−0.71 (0.38) .069	-	0.35 (0.13) .0099
HPV vaccination history						
< 2 doses vs. ≥ 2 doses (ref.)	2.54 (0.74–8.69) .137					

[a]Viral loads were normalized to the number of diploid human cells in the specimen as estimated by TaqMan PCR to ERV3 (ERV = endogenous retrovirus), and analyzed as a transformed continuous variable

[b] OR = odds ratio

[c] CI = confidence interval

[d] SE = standard error

[e] Dash indicates that the association was not significant at $p < .15$

[f] Frequency of oral sex with a condom during the past 3 months with heterosexual partners

[g] Frequency of oral vaginal sex (females only) or anal sex (females and males) without a condom or washing sex toys in between

J Med Virol. Author manuscript; available in PMC 2016 November 01.

Table 3

Risk factors associated with HPV, EBV, and KSHV infection and viral load: results of adjusted logistic and linear regression models

	HPV		EBV		KSHV	
	Infection	**Log_{10} viral load[a]**	**Infection**	**Log_{10} viral load**	**Infection**	**Log_{10} viral load**
	Adjusted OR[b] (95% CI[c]) p value	**Estimated coefficient (SE[d]) p value**	**Adjusted OR (95% CI) p value**	**Estimated coefficient (SE) p value**	**Adjusted OR (95% CI) p value**	**Estimated coefficient (SE) p value**
Race						
Black vs. White (ref.)				1.18 (0.37) .0023		
Gender						
Female vs. male (ref.)					10 (1.32–100) .026	
Marijuana use, past 3 months						
Almost daily/daily vs. weekly or less often (ref.)	1.97 (1.02–3.82) .045					
Tobacco use, past 3 months						
Almost daily/daily vs. weekly or less often (ref.)			2.90 (1.06–7.97) .039			
Tobacco use, lifetime						
Yes vs. no (ref.)		1.55 (0.53) .0052				
CD4+ T cell count						
<350 cells/mm^3 vs. ≥350 cells/mm^3 (ref.)	1.92 (1.003–3.69) .049			0.70 (0.28) .017		
HIV viral load						
≥400 copies/mL vs. <400 copies/mL (ref.)			3.98 (1.84–8.74) .0006			
Age at HIV diagnosis						
1–14 years vs. 18–20 years (ref.)						0.33 (0.16) .049

	HPV		EBV		KSHV	
	Infection	**Log_{10} viral load[a]**	**Infection**	**Log_{10} viral load**	**Infection**	**Log_{10} viral load**
	Adjusted OR[b] (95% CI[c]) p value	**Estimated coefficient (SE[d]) p value**	**Adjusted OR (95% CI) p value**	**Estimated coefficient (SE) p value**	**Adjusted OR (95% CI) p value**	**Estimated coefficient (SE) p value**
15–17 years vs. 18–20 years (ref.)						0.35 (0.13) .0099

[a] Covariates with a pluded in the initial full model for multivariable model selection, and covariates with Type III p-values < 0.05 were retained in the final model. Covariates deleted either during the model selection or from the final multivariable model are not shown.

[b] OR = odds ratio

[c] CI = confidence interval

[d] SE = standard error

J Med Virol. Author manuscript; available in PMC 2016 November 01.