Head and Neck Squamous Cell Carcinomas in HIV-Positive Patients: A Preliminary Investigation of Viral Associations

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Received: 1 December 2009 / Accepted: 4 March 2010 / Published online: 24 March 2010
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Abstract Oncogenic human papillomaviruses (HPVs) are associated with oropharyngeal squamous cell carcinoma (SCC). Infection with human immunodeficiency virus (HIV) increases susceptibility to opportunistic infections and viral-promoted cancers. The prevalences of HPV, herpes simplex virus (HSV), Epstein-Barr virus (EBV), and human herpesvirus-8 (HHV-8) have not been established for head and neck squamous cell carcinoma in HIV-positive patients (HIV+ HNSCC). We have observed that HIV+ HNSCC tend to contain numerous multinucleated tumor giant cells, this finding has not been described previously. The goal of this study is to test for these oncogenic viruses in a small cohort of retrospectively identified patients with HIV infection, and to compare histologically these cancers

to a control group of HNSCC patients. Tumors were reviewed histologically and compared to a control group of 102 patients with HNSCC (serologically untyped or HIV negative). Polymerase chain reaction (PCR) was performed on formalin-fixed, paraffin-embedded HIV+ HNSCC samples from combined 25 patients in two institutions. In situ hybridization was performed to identify EBV (EBER) and immunohistochemistry was performed to detect HSV-1, HSV-2, HHV-8, and HIV-related proteins (Nef, p24). The study sample consisted of 34 HIV+ patients with HNSCC from Montefiore Medical Center, and six HIV+ HNSCC patients from Hospital Clinic, University of Barcelona; 24 (60%) men and 16 (40%) women. The larynx was most commonly involved (65%, $n = 26$); followed by the oropharynx (22.5%, $n = 9$). Four carcinomas arose from the oral cavity (10%) and one from the nasal cavity (2.5%). Histologically, multinucleated tumor giant cells were more common in the HIV+ group (39/40, 97.5%) than the control group (27/102, 26%, $p = 0.001$, chi-square). HPV was detected in 6 of 25 (24%) HNSCC tumors by PCR, five were typed as HPV 16 and one as HPV 26/69; five of these tumors (83%) were located in the oropharynx. EBV, HSV-1, HSV-2, and HHV-8 were detected only infrequently in tumor cells. Nef protein was detected in tumor cells in 7 of 21 (33.3%) cases; p24 was not detectable in 6 tumors studied. There were no significant associations between HPV positive tumors and co-infections with other viruses. This study is consistent with other reports that suggest an increased incidence of laryngeal carcinoma for HIV+ patients. HPV was detected in 24% of HIV+ HNSCC, however, the number of tumors with amplifiable DNA ($n = 25$) is too small to allow for conclusions. EBV, HSV-1, HSV-2, and HHV-8 are uncommon in HIV+ HNSCC; it is unlikely that these viruses have a promoting effect. MNTCG are significantly common in HIV+ HNSCC, but there is

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overlap in MNTCG counts with the control group and therefore this finding cannot be used as a biomarker of HIV infection.

Keywords AIDS · HIV · HNSCC · Squamous carcinoma · HPV

Introduction

Head and neck squamous cell cancer (HNSCC) is the sixth most common malignancy and the eighth leading cause of cancer death worldwide, HNSCC typically affects men in the sixth decade and risk factors include tobacco use, alcohol consumption, and oncogenic human papillomaviruses (HPV) [1–8]. HNSCC is the third most common head and neck malignancy for patients with human immunodeficiency virus (HIV) infection, after Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma [9, 10]. Although not an AIDS-defining illness, HNSCC is seen in excess among HIV-infected individuals [10]. Studying HNSCC in HIV-positive patients is important for optimizing treatment protocols and early detection strategies for this at-risk group.

Growing evidence confirms the causative relationship between oncogenic HPV and oropharyngeal squamous carcinoma in the general population [6–8, 11–14]. The prevalence of HPV in oropharyngeal cancer, especially tonsillar cancer, is as high as 90% [15–19], HPV 16 is most commonly involved [6, 12, 14, 19]. Some studies have found that HPV-positive HNSCC is associated with high-stage tumors with basaloid morphology, but paradoxically, better survival [6, 17, 18]. Oncogenic HPV, specifically types 16, 18, 31 and 45, have been established as the etiologic agents responsible for the majority of uterine cervical squamous carcinomas [20–23]. Molecular mechanisms for HPV-driven cervical carcinogenesis have been described, including those mediated by HPV E6 and E7 oncoproteins [24, 25].

HIV infection increases susceptibility to opportunistic infections and virally promoted cancers. The associations between HIV and HPV infections in general, and in the context of cervical neoplasia, are well established [9, 26–28]. Strickler demonstrated that low CD4 counts and high HIV viral loads are significantly associated with HPV DNA detection and development of cervical squamous intra-epithelial neoplasia [29]. HIV-infected patients are at increased risk for developing Burkitt's lymphoma and Hodgkin's disease (Epstein-Barr viral (EBV)-mediated malignancies), Kaposi's sarcoma (KS; associated with human herpesvirus-8 (HHV-8) infection), and anogenital carcinoma (associated with oncogenic HPV) [26, 27, 30]. EBV is known to produce persistent, reactivated infection of the lateral tongue (hairy leukoplakia) in the immunocompromised populations, particularly in HIV-positive

individuals [31, 32], and promotes the majority of non-keratinizing nasopharyngeal carcinomas (NPC), as well as some oral squamous carcinomas [33–35].

Based on the above associations, the purpose of this study was to test the hypothesis that viruses normally affecting the upper airway may be commonly present in HIV+ HNSCC, due to lack of immune surveillance and possibly increased exposure, thereby potentiating carcinogenesis. Our goal is to test for the presence of these oncogenic viruses in a small cohort of retrospectively identified patients with HIV infection. This represents the first systematic analysis of viral infections in HIV+ HNSCC. Additionally, we have observed that HIV+ HNSCC tend to contain many multinucleated tumor giant cells, this finding has not been described previously. Our second objective is to compare histologically these cancers to a control group of HNSCC patients.

Methods

Case Identification

This is a retrospective research investigation from two institutions, Montefiore Medical Center (MMC), Bronx, NY, and Hospital Clinic University of Barcelona (HCUB) Barcelona, Spain. The MMC patients were identified through the multidisciplinary Head and Neck Cancer Program, 1997–2007, and the HCUB patients were identified through the Department of Infectious Diseases, Hospital Clinic, University of Barcelona, 1998–2007. This study was not intended as a comprehensive review of *all* HIV positive patients with HNSCC for this period.

This study was approved by the Institutional Review Boards for each institution. Inclusion criteria were as follows: patients are at least 18 years old and have evidence of HIV infection (elevated HIV viral load) and histologically confirmed HNSCC. At MMC, HIV viral loads were analyzed by branch DNA testing (Siemens Healthcare Diagnostics, Inc, Deerfield, IL). At HCUB, HIV viral loads were analyzed by quantitative PCR (HIV Monitor test procedure, Amplicor PCR Diagnostic, Hoffman-LaRoche, Basel Switzerland) with a limit of quantification of log 2.3 copies/ml.

Histological Review

Hematoxylin and eosin slides from 40 HNSCC were reviewed and subtyped (keratinizing SCC, basaloid SCC, verrucous SCC, etc.). Multinucleated tumor giant cells (MNTGC) were commonly identified; here we consider any tumor cell with three or more nuclei to constitute a MNTGC. To test the hypothesis that MNTGC are significantly associated with HIV+ HNSCC, we counted 10

unique high-power fields (HPF) with the greatest MNTGC densities, and compared this to control cohort, consisting of 102 patients with HNSCC (serologically untyped or HIV-negative) followed clinically at MMC as part of our Head and Neck Research Program.

DNA Extraction, Polymerase Chain Reaction, and Dot Blot Hybridization

Montefiore/Albert Einstein College of Medicine Studies

Archival FFPE samples from 25 HNSCC were obtained from the Department of Pathology at MMC. Hematoxylin and eosin-stained slides were reviewed to confirm location of HNSCC; up to five 10- μ m sections of HNSCC were procured from the mapped areas in the corresponding FFPE tissue blocks using sterile technique. Each case was deparaffinized with 1 ml of octane and shaken gently at room temperature for 15–30 min. Tissue was pelleted by centrifugation for 5 min at 12,000 revolutions per minute. Each pellet was re-extracted with 1 ml of octane and washed with 500 μ l of 100% ethanol. Ten microliters of acetone were then added prior to drying each tube at 55°C. The dried pellet was dissolved in a digestion buffer (2 mM EDTA, 2% Laureth-12, 100 mM Tris–Cl, pH 8.0) that included 400 μ g/ml of proteinase K. Each tube was incubated overnight at 37°C and then for 10 min at 95°C to inactivate proteinase K.

HPV was detected and typed by polymerase chain reaction (PCR) and dot blot hybridization using the MY09/MY11 and GP5+/GP6+ consensus primers as previously described [36–39]. MY09/MY11 and GP5+/GP6+ primers detect HPV L1 regions, amplifying 450 and 150 bp fragments, respectively. The MY09/MY11 primer system includes a primer set (PC04/GH20) to amplify a ~150 base-pair fragment of the human beta-globin gene to confirm the presence of amplifiable DNA.

Gel electrophoresis was performed on PCR products transferred to nylon filters and hybridized overnight with radiolabeled generic probes for HPV DNA and β -globin, as described [38]. All samples were tested by oligonucleotide hybridization for over 40 different HPV types as described.

Hospital Clinic, University of Barcelona Studies

Three 10 μ m thick sections of formalin-fixed, paraffin embedded tissue were prepared after cutting deep into the block and confirming the presence of HNSCC using sterile technique. Each case was deparaffinized in xylene and alcohol and digested with proteinase K in a volume of 0.20 ml at 56°C overnight and then for 10 min at 70°C to inactivate proteinase K.

The samples were processed using the QIAGEN QIAamp DNA mini kit (QIAGEN, Germany) protocol and stored in

distilled water. To confirm integrity of the DNA, 5 μ l of DNA aliquot were amplified in PCR reaction volume of 25 μ l with primers specific for β -globin using taq polymerase (Promega Biotech Iberica, Alcobendas, Spain). Broad-spectrum HPV DNA amplification was performed using the short PCR fragment (SPF10) primer set (Innogenetics Diagnostica, Spain). The SPF10 primers amplify a 65-bp fragment from the L1 region of the HPV genome. PCR was performed in 50 μ l reaction following standard conditions. For HPV amplification, a 9 min denaturation step at 94°C was followed by 40 cycles of amplification using 1.5 U.I. DNA polymerase (Amplitaq Gold DNA polymerase, Applied Biosystems) with a thermocycler (PTC-200, Peltier Thermal Cycler, MJ Research, Waltham, MA). Each cycle included denaturation at 94°C for 30 s, primer annealing at 52°C for 45 s, and chain elongation at 72°C for 45 s. The final elongation step was prolonged by 5 min.

PCR products were subjected to electrophoresis in a 3% agarose gel and the 65-bp product was visualized with ethidium bromide staining. Confirmation of HPV type-specific sequences for the HCUB cases was performed using the INNO-LIPA HPV assay which is a microliter plate-based hybridization enzyme immunoassay. This assay which can identify 25 HPV genotypes (high-risk HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70 and low-risk: 6, 11, 34, 40, 42, 43, 44, 53, 54, 74) in a single test. Ten μ l of denatured HPV PCR product was hybridized to the genotype-specific probes immobilized as parallel lines on a nitrocellulose strip. After washing, the products of hybridization were detected by a color reaction with alkaline-phosphatase-streptavidin conjugate and substrate (5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium), which results in a purple precipitate, assessed visually by comparing to the standard grid.

All tumors with PCR samples that hybridized with the β -globin probe but not the generic HPV probes were considered negative for HPV infection. Samples that were negative for both HPV and β -globin were deemed technically insufficient and excluded from further analysis.

In situ Hybridization (ISH) and Immunohistochemistry (IHC) Studies

Tissue microarrays (TMA) were constructed from 25 HIV+ HNSCC tumors from the MMC patients using triplicate 1 mm cores.

ISH assays were performed on samples from both MMC and HCUB to detect latent EBV infection using the Ventana probe for EBV early RNA (EBER; fluorescein-labeled oligonucleotide INFORM^R probe, Ventana Medical Systems, Inc., Tucson, AZ). The ISH iVIEWTM BLUE detection kit with alkaline phosphatase was utilized for EBV localization, the slides were processed with the

Ventana BenchMark™ Automated Slide Stainer; NBT/BCIP was used as a chromogen. A fluorescein-labeled positive RNA control probe, recognizing the polyA mRNA tail, was used to confirm mRNA in the archival tissue. A negative RNA probe recognizing a tail sequence specific for Ventana oligonucleotide probes was used to assess endogenous background staining. Slides were counterstained with nuclear Fast Red. Human tissue with known EBV infection was included as a positive probe control. The ISH procedures for EBV detection are also available from Genzyme and the probe manufacturer.

IHC was performed at MMC to detect HSV-1 (polyclonal rabbit anti-HSV-1 antibody, 1:6 dilution, DakoCytomation, Inc., Carpinteria, CA), HSV-2 (polyclonal rabbit anti-HSV-2 antibody, 1:6 dilution, DakoCytomation, Inc.), and HHV8 (monoclonal rat antibody to latent nuclear antigen [LNA-1] ORF-73 of HHV-8, 1:500 dilution, Advanced Biotechnologies, Inc., Columbia, MD). The Dakocytomation EnVision+™ System was used according to manufacturer-recommended procedures.

IHC for the Nef HIV protein was conducted at Ohio State University Medical Center to evaluate the HIV-positive HNSCC tumors cells directly for HIV infection (monoclonal mouse anti-Nef antibody, clone AG11, 1:700 dilution, Millipore, Billerica, MA). The BenchMark™ Automated Staining System was utilized for this purpose (Ventana Medical Systems, Inc., Tucson, AZ). Manufacturer-recommended IHC procedures were followed.

IHC performed at HCUB utilized the TechMate 500® automated system (Dako Co, Carpinteria, CA), using the EnVision system (Dako). The following primary antibodies were studied: HIV-p24 (Dako Co, Glostrup, Denmark. Clone KAL-1; dilution 1:5), Latent Membrane Protein of Epstein Barr Virus (LMP-1; Dako Co. Clone CS 1--4, dilution 1:100), herpes virus-8 (HHV-8; Advanced Biotechnologies, Columbia, MD, USA, dilution 1:100).

Appropriate positive and negative controls were used for each antibody.

Results

Patient Demographics and Clinicopathologic Characteristics

There were 40 patients in total, 34 from MMC and 6 from HCUB. There were 24 males (60%) and 16 females (40%), ages 33–63 years (median 49 years). All MMC patients had detectable HIV viral loads (87–467,310 copies/ml), however, there was variation with respect to the dates of HIV serology and tumor diagnosis. Serum HIV loads were known at the time of tumor diagnosis for all 6 HCUB patients; HIV was detectable for 2 patients (log 5.08 and

5.41 copies/ml). The remaining 4 patients had viral counts below the limit of detection (log 2.3 copies/ml), however, detectable HIV viral loads were previously documented for these patients prior to initiating HAART therapy.

Tobacco exposure history was known for 29 patients, 27 (93%) had smoked.

The larynx was the most common site (26/40, 65%), followed by the oropharynx (9/40, 22.5%). Four tumors (10%) arose from the oral cavity, and one (2.5%) from the nasal cavity.

Histologically, 35 cases were classified as typical keratinizing SCC, four were basaloid SCC, and one was verrucous SCC. MNTGC were present in 39/40 tumors (97.5%) ranging from 4 to 67 cells/10 HPF (mean 18). MNTGC had three or more centralized nuclei with distinct nuclear membranes. Nuclei molded to each other or overlapped one another. Multiple nuclei occasionally formed chains or rings within tumor cells. Some MNTGC were keratinizing and located in the superficial maturing layers of tumor islands or ribbons. Alternatively, some MNTGC had clear cytoplasm imparting “popcorn”-like appearances. MNTGC in basaloid squamous carcinomas had limited cytoplasm and a basaloid appearance (Fig. 1).

No MNTGC were found in 74% of HNSCC in the control group; MNTGC were present in 27/102 cancers (26%), ranging from 3 to 55 cells in 10 HPF (mean 15). No obvious differences in size or number of nuclei were seen between the two groups. Figure 2 demonstrates the percent frequency distribution of tumors per MNTGC range, for the HIV+ HNSCC group and the control group. Chi-square analysis revealed that MNTGC were significantly associated with HIV+ HNSCC ($P = 0.0001$).

HPV Detection and Typing

HPV PCR analysis at MMC (MY09/MY11 primers and/or GP5+/GP6+ primers) detected HPV DNA in six of 19 tumors with amplifiable DNA; six positive cases were oropharyngeal, and one tumor was from the nasal cavity (Table 1). HPV typing by dot blot analysis demonstrated HPV16 in five tumors and HPV 26/69 in one tumor. Concordance between the two primer sets was high, the MY09/11 primer missed one HPV16 positive case detected by the GP5+/GP6+ primer set.

HPV analysis by PCR at HCUB, using the SPF10 primers, revealed no HPV in the six tumors with amplifiable DNA.

ISH and IHC

Due to core drop out, ISH and IHC data was available on 21 patients from MMC.

Fig. 1 Various appearances of multinucleated tumor giant cells in HIV+ HNSCC. *Left top* low power view of multiple MNTGC. *Right top*: MNTGC with multiple dark overlapping nuclei. Careful examination reveals distinct contours of nuclear membranes. *Left middle* the overlapping nuclei here form elongated chains. *Right middle* basaloid MNTGC. *Bottom left* “Popcorn” MNTGC with clear cytoplasm

Fig. 2 Histogram showing the ranges of MNTGC counts/10 HPF with percent frequency distribution for 41 HIV+ HNSCC (blue) versus 102 HNSCC in the control group (purple). No MNTGC were seen in 74% of the control group

EBV

Latent EBV infection was detected in 2 tumors (Table 1). Both of these cases showed distinct nuclear staining in tumor cells from laryngeal squamous cell carcinoma (Fig. 3). IHC for LMP-1 on the HCUB patients was negative in all six cases.

HSV-1, HSV-2, HHV-8, and HIV

Two tumors were positive by IHC for HSV-1 (Table 1), with distinct focal nuclear staining in tumor cells (Fig. 3). One tumor demonstrated distinct focal cytoplasmic staining in tumor cells for HSV-2.

Table 1 Summary of results for viral studies

Site	Age	Sex	HPV	EBV	HSV1	HSV2	HHV8	HIV
Oropharynx	59	M	N	N	N	N/A ^a	N	N
	61	F	HPV16	N	N	N	N	Pos
	56	M	HPV16	N	N	N	N	N
	59	F	N	N	N	N	N	N
	56	M	N	N	N	N	N	N
	55	M	N	N	N	N	N	N
	48	M	HPV16	N	N	N	N	N
	59	M	HPV16	N/A ^a				N
	47	M	HPV26/69					N
Larynx	46	F	N	N	N	N	N	Pos
	61	M	N	Pos	Pos		N	N
	47	M	N	N	N	N	N	N
	52	M	N	N	Pos	N	N	Pos
	45	F		N	N	N	N	N
	33	F	N					Pos
	53	M		N	N	N	Pos	N
	46	F						Pos
	44	M	N	N	N	N	N	N
	49	F	N					N/A ^a
	49	F	N					N
	49	F	N					Pos
	54	M	N	N			N	N
	46	M	N	N			N	N
	42	M	N	N			N	N
	37	F	N	N			N	N
	45	M	N	N			N	N
	47	M	N	N			N	N
Oral Cavity								
Nasal	33	F	16	N	N	Pos	N	Pos

Key: Black data not available due to poor DNA quality for PCR or TMA core drop out. Cases in light turquoise were from the HCUB cohort; HPV PCR was performed using SPF10 primers. The remaining patients are from the MMC cohort; HPV PCR was performed in these cases using both GP5/GP6 and MY09/MY11 primer sets. The results in Table 1 are with the GP5/GP6 primers. HIV IHC on the HCUB cohort used the antibody to p24; the remainder cases of studied cases used the antibody to Nef protein

HHV-8 was investigated in both the MMC and HCUB patients; one of 21 tumors was positive for HHV-8, with distinct focal nuclear and cytoplasmic staining. Seven tumors demonstrated distinct cytoplasmic staining for the HIV Nef protein, usually >10% of tumor cells in each case (Fig. 4). IHC for HIVp24 on the HCUB patients was negative in all cases.

Discussion

To date, few studies have focused on HNSCC in HIV+ patients [40–44]. In this study, the larynx was the most common site for HIV+ HNSCC, which is consistent with other reports [9, 43, 44]. Age-matched analysis of the New York State Cancer Registry and the New York

Fig. 3 *Top left* low power H + E of laryngeal squamous carcinoma. *Top right* same region, ISH for EBER, note blue nuclear positivity. *Inset* higher power revealing tumor nuclei positive for EBER. *Bottom left* occasional tumor cells are positive for HHV-8 by IHC. *Bottom right* IHC HSV-1 expression in a tumor nucleus

City AIDS registries demonstrate that the adjusted relative risk (RR) for developing laryngeal cancer after the diagnosis of AIDS is 10.6 (95% CI 2.19, 31.04) for women, and 2.0 (95% CI 0.95, 3.65) for men, compared to the general population [45]. In addition, HIV+ laryngeal cancer is significantly associated with younger age at diagnosis [46].

In the general population, the expected ratio for new cancers favors the oral cavity over the larynx with a ratio of ~2:1 [3], combined for gender. However, the New York State AIDS registry database reveals that the ratio of oral cavity to laryngeal cancers is closer to 1, combined for gender [45]. Only modest increases in the incidence of oral and pharyngeal carcinoma (ICD-9 140–149) have been seen in AIDS patients [45, 47]. However, a dramatic increase in the RR of oral and pharyngeal carcinoma (ICD-9 140–149) is observed for women, especially around the time of AIDS diagnosis [45].

This study represents the first systematic analysis of viral infections in HIV+ HNSCC. The worldwide prevalence of HPV16 in HNSCC in the general population ranges from 16 to 30% [19]. HPV was detected in 6 of 25 tumors with amplifiable DNA, most commonly HPV16, and

most often in oropharyngeal tumors. HPV was not detected in any laryngeal cancers here. However, the number of samples studied here is too small to allow for any conclusions. Given the relatively rarity of HIV+ HNSCC, a multicenter case-controlled study is warranted to determine the incidence of HPV in HIV+ HNSCC.

Overall, infections with EBV, HSV-1, HSV-2, and HHV-8 are uncommon in this group. Only a few tumors were focally positive for HSV-1 or HSV-2, with a slight predilection for tumors along the larynx. MNTGC were not positive for HSV, or any other viruses studied. Interestingly, we detected Nef protein in tumor cytoplasm in seven carcinomas; this is the first report of Nef expression in solid tumors. The significance of finding Nef protein in HIV+ HNSCC is presently unknown. Only a few studies have reported HIV DNA in oral mucosa [48, 49]. The mechanisms by which HIV may co-promote tumor carcinogenesis remain to be elucidated and warrant further investigation. Some studies have suggested that oncogenic viruses may synergistically interact to promote some cancers, for instance EBV and HPV in nasopharyngeal carcinoma, and HSV and HPV in oral and laryngeal HNSCC [50–53]. These observations lead us to hypothesize that

Fig. 4 *Top* Nef expression by IHC (red) in stromal lymphocytes. *Bottom* cytoplasmic expression of Nef in tumor cytoplasm

HPV, EBV, HSV-1, HSV-2, and HHV-8 may synergistically promote the development of HNSCC in HIV-infected patients. Based on our findings, we conclude that this mechanism of viral carcinogenesis is not present in excess for this small series of HIV+ HNSCC patients. More likely, tobacco exposure (93%) promoted carcinogenesis in this group.

There are a number of limitations for this study. The relative rarity of HIV+ HNSCC necessitates a retrospective approach. Only 25 tumors had amplified DNA, which does not allow for conclusions regarding viral prevalence in HIV+ HNSCC; further study of larger patient groups is justified. The availability of HIV viral loads and CD4 counts was variable with respect to the date of tumor diagnosis. Therefore we cannot correlate immune status, HIV loads, HPV status, other viruses, or MNTGC counts. It is possible that the rates of viral infections differ for HIV+ HNSCC patients who are noncompliant with HAART, however, this issue cannot be addressed here. Different PCR primers were used between the two institutions, therefore direct comparison between the MMC and HCUB patients cannot be made.

Lastly, screening HIV serology is not routinely offered at MMC. The control group for MNTGC counts consisted of patients actively followed at MMC as part of our Head and Neck Research Program. It is very reasonable to assume that they are HIV negative. However, uniform data on their immune status and comorbidities are lacking. It is possible that some control patients are immunosuppressed for other reasons. The immune status for control HNSCC patients with MNTGC is largely unknown. Some HNSCC control patients with MNTGC had HBV and/or HCV infections, but liver studies are incomplete for many patients in this group. These issues limit our conclusions regarding the finding of MNTGC in HIV+ HNSCC. We do see that MNTGC are significantly common in HIV+ HNSCC (98%) compared to the untested control group (26%). However, the overlap in MNTGC counts between the two groups limits any practical predictive value for this finding.

Acknowledgment The authors thank Dr. Robert D. Burk, MD, Departments of Microbiology & Immunology, Obstetrics, Gynecology, & Women's Health at Albert Einstein College of Medicine, Bronx, NY for performing the HPV PCR studies, and Nicolas F. Schlecht, PhD, Department of Epidemiology & Population Health, and Michael B. Prystowsky, MD, PhD, Department of Pathology, Albert Einstein College of Medicine, Bronx, NY, for critical review of this manuscript.

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