The Prevalence of Human Papillomavirus–Positive Oropharyngeal Squamous Cell Carcinoma at One of the Largest Tertiary Care Centers in Sub-Saharan Africa

Gloria Dapaah, BSc, BDS, MDS; Jos Hille, BDS, MDent; William C. Faquin, MD, PhD; Judith Whittaker, MBChB, MMed; Corneli M. Dittrich, MBChB, MSc; Abdul-Kader Ebrahim, MBChB, MMed; Johann W. Schneider, MBChB, MMed; Abraham C. van Wyk, MBChB, MMed; Johan Opperman, BSc, BChD, MDS; Marc Merven, MBChB, MMed; Komeela Naidoo, MBChB, MSc, MMed; James W. Loock, MBChB, MMed; Amir H. Afrogheh, BChD, MSc, MChD, PhD

• ***Context.***—**Limited data exist on the prevalence of human papillomavirus (HPV)–positive oropharyngeal squamous cell carcinoma in sub-Saharan Africa.**

Objective.—**To determine the prevalence of HPV-positive oropharyngeal squamous cell carcinoma at a large tertiary care center in South Africa.**

Design.—**A total of 266 oropharyngeal squamous cell carcinomas diagnosed during an 11-year period (2007–2017) were selected for evaluation. Cases staining positive for p16 immunohistochemistry were evaluated for high-risk HPV using the BD Onclarity assay (BD Diagnostics, Sparks, Maryland).**

Results.—**Of 266 oropharyngeal squamous cell carcinomas, 14% (n = 36) were positive for p16. Polymerase chain reaction for high-risk HPV performed on the p16-positive cases was negative in 23 cases and positive in 13 cases (13 of 266; 5%). p16 showed a positive predictive value of 36.1%. The HPV subtypes were HPV-16 (n = 10), HPV-18 (n = 1), HPV-52 (n = 1), and HPV-31 (n = 1). Human papillomavirus–positive cases occurred in 10 men and 3 women (mean age, 51 years) and arose from the tonsil (n = 10) or base of the tongue (n = 3). The HPV-positive cases were non-keratinizing (n = 10) or partially keratinizing (n = 1). Partially/nonkeratinizing cases revealed a modest improvement in p16 positive predictive value (11 of 21; 52.4%).**

Conclusions.—**The presence of high-risk HPV in 5% of cases suggests that high-risk HPV is a minor etiologic agent in oropharyngeal squamous cell carcinoma in this region. Given its suboptimal positive predictive value, p16 is not a reliable marker for high-risk HPV infection in this region. When p16 is positive, HPV-specific testing is necessary. The identification of less common high-risk HPV types, HPV-52 and HPV-31, may influence current local vaccination strategies.**

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The discovery of human papillomavirus (HPV) DNA in oropharyngeal squamous cell carcinoma (OPSCC) can be traced back to the mid-1980s, but its role in the induction of oropharyngeal malignancy was not conclusively established until 2000.[1] In 2007, the International Agency for Research on Cancer (IARC) concluded that there was ample evidence to support a causal role for HPV-16 in oropharyngeal cancers.[2] Since then the global burden of the disease has gradually increased, and it has surpassed cervical cancer in some developed countries.[3,4] The Centers for Disease Control and Prevention (CDC) reported that OPSCC was 1 of the 5 cancer types to have shown an increased incidence since 1975.[5] The expanding important role of high-risk HPV (HR-HPV) in the development of OPSCC has led to a noticeable shift in the profile of OPSCC patients.

Of note and in contrast to conventional smoking- and alcohol-related head and neck squamous cell carcinoma (HNSCC), HPV-positive OPSCC is frequently observed in younger (age <60 years), White, college-educated, and married men of higher socioeconomic status who tend to drink or smoke less and report more oral sex partners.[6–9]

Compared with HPV-negative HNSCCs, HPV-positive OPSCCs are smaller tumors that present with early nodal dissemination.[10,11] Secondary primary tumors or locoregional recurrences are infrequently seen with HPV-positive OPSCC, possibly because of the lack of field cancerization effect, because transcriptionally active HPV is not detected

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From the Department of Oral and Maxillofacial Pathology, National Health Laboratory Service, Tygerberg Academic Hospital, University of the Western Cape, Cape Town, South Africa (Dapaah, Hille, Opperman, Afrogheh); the Pathology Service, Massachusetts General Hospital, Harvard Medical School, Boston (Faquin); Lancet Laboratories, Cape Town, South Africa (Whittaker, Dittrich); the Department of Ear, Nose and Throat (Ebrahim, Merven, Loock), Division of Anatomical Pathology, National Health Laboratory Service (Schneider, van Wyk), and the Division of Radiation Oncology, Department of Medical Imaging and Clinical Oncology (Naidoo), Tygerberg Academic Hospital, University of Stellenbosch, Faculty of Medicine and Health Sciences, Cape Town, South Africa.

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Corresponding author: Amir H. Afrogheh, BChD, MSc, MChD, PhD, Division of Anatomical Pathology, Room 581, Tygerberg Hospital, Francie van Zijl Drive Tygerberg, Cape Town 7505, South Africa (email: amir.afrogheh@nhls.ac.za).

Reported Prevalence of High-Risk Human Papillomavirus (HPV) in Oropharyngeal Squamous Cell Carcinoma (OPSCC) in Sub-Saharan Africa								
Author	Year	OPSCC	HPV-Positive OPSCC	Methods	HPV-16	HPV-18	Other HPV	Overall, %
Current study (South Africa)	2021	266	13	p16, PCR	10	1	HPV-52 (n = 1) and HPV-31 (n = 1)	5
Aswani et al[19] (Kenya)	2019	8	0	PCR	0	0	0	0
Rettig et al[20] (Cameroon)	2019	7	2	p16, mRNA ISH	N/A	N/A	N/A	29
Kofi et al[21] (Central African Republic)	2019	NK	1	PCR	1	0	0	NK
Aboagye et al[22] (Ghana)	2018	12	6	PCR	12	0	0	50
Faggons et al[23] (Malawi)	2017	23[a]	5	p16	N/A	N/A	N/A	22
Blumberg et al[24] (Mozambique)	2015	22	0	p16, PCR	0	0	0	0
Ndiaye et al[25] (Senegal)	2013	5	0	p16, PCR	0	0	0	0

Abbreviations: ISH, in situ hybridization; N/A, not applicable; NK, not known; PCR, polymerase chain reaction.

[a] The sample contained a mix of OPSCC and oral squamous cell carcinoma.

in the peritumoral mucosa.[12] Overall, patients with HPV-positive OPSCC have better clinical outcomes compared with patients with conventional HPV-negative HNSCC when treated by similar modalities.[6–9] Human papillomavirus status has been shown to be an independent predictor of overall survival in OPSCC patients.[7]

There is significant geographic heterogeneity in the prevalence of HPV-positive OPSCC.

The IARC estimates that there are 29 000 new cases of HPV-positive OPSCC in the world each year.[13] According to the CDC, there are more than 16 000 cases of HPV-positive OPSCC per year in the United States, representing slightly more than 50% of all OPSCCs worldwide.[14]

The estimated proportion of oropharyngeal cancers attributable to HPV varies between 17% in Southern Europe and 38% to 39% in Northern, Western, and Eastern Europe.[15] In England, it has been estimated that the incidence of OPSCC will increase by 239% from 2011 to 2025, at which point OPSCC would comprise 35% of all HNSCCs.[13] Rates are more variable in other parts of the world but are significantly lower than the rates reported in the United States and Europe. For example, the rates are 36% and 17% for South America and Asia, respectively.[16–18]

Data regarding the prevalence of HPV-positive OPSCC in sub-Saharan Africa in particular are scarce. A few studies have reported variable detection rates for HR-HPV in OPSCC that ranged from 0% to 50% (Table).[19–25] The marked variation in HPV prevalence rates among studies of OPSCC may be explained in part by variations in study design and techniques used to identify HPV in biologic specimens.

Given the paucity of data and widely variable detection rates, the prime objective of this study was to determine the prevalence of HPV-positive OPSCC in one of the largest tertiary care centers in sub-Saharan Africa (Tygerberg Hospital [TBH], Cape Town, South Africa) using p16 immunohistochemistry (IHC), which is well documented to be a highly sensitive marker,[26] as a screening test, followed by polymerase chain reaction (PCR)–based HPV typing as an HPV-specific confirmatory test. To the best of our knowledge, this is the largest study to date that examines the presence of HR-HPV in OPSCC in sub-Saharan Africa.

MATERIALS AND METHODS

This study was approved by the Biomedical Research Ethics Committee of the University of the Western Cape (BM/16/5/3). Following approval and standard procedures, relevant data were retrieved from the National Health Laboratory Service at TBH, one of the largest hospitals in sub-Saharan Africa that primarily serves low-income (Black and mixed-race) South African patients. Sequential surgical samples of 266 cases of OPSCC diagnosed during an 11-year period (2007–2017) were selected for evaluation, and relevant patient characteristics were documented. Patients of all ages and both sexes with the following criteria were included: (1) patients with primary lesions of the oropharynx and its subsites, specifically the palatine tonsils, base of tongue, soft palate, uvula and posterior pharyngeal wall; and (2) patients with primary lesions of the oropharynx with extension into neighboring sites, confirmed by thorough radiologic examination.

Patients with nonoropharyngeal head and neck cancers; patients with non–squamous cell tumors (eg, lymphomas); patients with metastatic disease to the oropharynx; and patients who did not have any records or pathologic specimens at TBH were excluded. The formalin-fixed, paraffin-embedded tissue blocks and hematoxylin-eosin–stained sections of these 266 cases were collected from the pathology archives of TBH. The hematoxylin-eosin–stained sections were reviewed to confirm the histologic diagnosis and assess the histomorphologic features of each tumor. Unstained 5-μm formalin-fixed, paraffin-embedded sections from the surgical samples were evaluated for HR-HPV by immunohistochemical staining for p16, and positive cases were then assessed by PCR. Relevant patient clinical information was obtained from detailed review of the TBH medical records.

p16 Immunohistochemistry

Immunohistochemical expression of the cyclin-dependent kinase inhibitor p16 was evaluated in all cases. In brief, deparaffinized formalin-fixed, paraffin-embedded sections of all cases were subjected to antigen retrieval using the Leica Bond protocol (Leica Biosystems) with proprietary Retrieval ER2 (ethylene diamine tetraacetic acid solution, pH 9.0) for 20 minutes. A mouse monoclonal antibody against p16 (E6H4 clone, CINtec, Ventana Medical Systems, Tucson, Arizona) was used with a 1:4 dilution, detected by the Polymer Refine Kit (Leica Biosystems) on a Leica Bond autostainer. For positive immunohistochemical controls, a tonsil squamous cell carcinoma with positive p16 expression was used. The threshold for p16 positivity was met in cases where ≥70% of tumor cells demonstrated strong diffuse nuclear and cytoplasmic staining; other staining patterns were considered negative.

HR-HPV Status by PCR (BD Onclarity HPV Assay)

All cases with a p16-positive result (n = 36) were evaluated for HR-HPV using PCR.

The BD Onclarity HPV Assay (BD Diagnostics, Sparks, Maryland) is a real-time PCR assay that detects type-specific E6 and E7

genomic DNA. It simultaneously detects all 14 HR-HPV types and can provide genotyping information on 6 individual genotypes (HPVs 16, 18, 31, 45, 51, and 52), reporting the remaining HPV types in 3 distinct groups (33 and 58; 56, 59, and 66; and 35, 39, and 68). DNA extraction was done on each of the formalin-fixed, paraffin-embedded biopsy samples using the automated workflow on the Viper LT system (BD Diagnostics). The section was combined with 0.5 mL of distilled water and added directly to a tube with a pierceable cap containing a proprietary diluent. The sample was then lysed directly using the Viper LT Pre-warm station (BD Diagnostics) before being transferred onto the deck of the instrument where it underwent automated sample processing and PCR detection. Briefly, the DNA was extracted using BD FOX (BD Diagnostics) magnetic particles, and the eluate-containing DNA was used to set up 3 PCR genotyping reactions: G1 detects HPV-16, HPV-18, and HPV-45 plus the internal β-globin control; G2 detects HPV-31, HPV-33, HPV-58, HPV-56, HPV-59, and HPV-66 plus the internal β-globin control, and G3 detects HPV-51, HPV-52, HPV-35, HPV-39, and HPV-68 plus the internal β-globin control. After 40 PCR cycles, any Ct score for a specific genotype and/or the internal β-globin control was considered positive for that channel.

Data Analysis

Associations between HPV status and demographic, clinical, and histologic characteristics were assessed using the analysis of variance test for continuous variables and the Fisher exact test for categoric variables. A two-sided P value less than .05 was considered statistically significant. All statistical analysis was performed using Statistica 14.0 (Data Science Workbench, version 14, TIBCO Software Inc, http://tibco.com).

RESULTS

A total of 266 patients with OPSCC who met the above-cited criteria were included in the current study. Overall, mean age at diagnosis was 57.6 years. Most patients were male (80%; n = 213). A history of smoking and alcohol consumption was documented in 20% (n = 53) and 8% (n = 22) of the patients, respectively. The most common primary tumor site was in the tonsil (56%; n = 149), followed by the base of the tongue (22%; n = 59). A single subsite was not specified in 18% (n = 47) of the cases. Only 11 tumors originated from the soft palate (n = 9), pharyngeal wall (n = 1), and uvula (n = 1), respectively.

p16 immunohistochemistry was positive in 14% of the cases (n = 36). Of 36 p16-positive cases, 23 were negative and 13 (13 of 266; 5%) were positive for HR-HPV when evaluated by PCR. p16 was found to have a positive predictive value (PPV) of only 36.1% (13 of 36). Human papillomavirus subtypes included HPV-16 (n = 10), HPV-18 (n = 1), HPV-52 (n = 1), and HPV-31 (n = 1). One case was positive for both HPV-16 and HPV-31.

In addition, p16 status was further stratified by morphology (keratinizing and partially keratinizing/nonkeratinizing) and subsite (tonsil and base of tongue). Of 36 p16-positive cases, 15 were keratinizing and 21 were partially keratinizing or nonkeratinizing. A total of 2 keratinizing and 11 partially keratinizing or nonkeratinizing p16-positive cases were positive for HR-HPV. p16 was found to have a PPV of 13.3% (2 of 15) and 52.4% (11 of 21) for keratinizing and partially keratinizing or nonkeratinizing OPSCC, respectively. Of 208 OPSCC cases from the tonsil and base of tongue, 15% (n = 31) were p16 positive. Of 31 p16-positive cases, 13 were positive for HR-HPV. Based on these findings, p16 was found to have a PPV of 42% (13 of 31) for tonsillar and base of tongue OPSCC.

All HPV-positive OPSCCs arose from the tonsil (n = 10) and base of tongue (n = 3). Human papillomavirus–positive OPSCCs occurred in 10 men and 3 women (male to female ratio, 3.3:1). There were statistically significant differences with respect to demographic and clinical variables or histologic features when comparing HPV-positive and HPV-negative groups. Patients with HPV-positive OPSCC had a mean age of 51 years (range, 33–72 years). By comparison, the mean age of patients with HPV-negative OPSCC was 58 years ($P = .02$).

Most HPV-positive OPSCCs were nonkeratinizing (n = 10) or partially keratinizing (n = 1). Two HPV-positive OPSCCs were keratinizing. In contrast, HPV-negative OPSCCs were predominantly keratinizing (n = 218; $P < .001$).

Treatment information was available in nearly half of the HPV-positive OPSCC patients (n = 6). Three patients were treated with radiation therapy, and 3 had a combination of radiation therapy and chemotherapy. Of the 6 HPV-positive patients, 1 elderly female patient (age 70 years with HPV-52 status) who discontinued radiation therapy early during the course of the treatment died within 1 year from the time of diagnosis. Another patient (a 49-year-old man with HPV-18 status) treated with chemoradiation developed local recurrence within a year from the time of diagnosis. The remaining 4 HPV-positive patients were documented to be disease free on the date of their last follow-up visit to the hospital.

DISCUSSION

The prevalence of HPV-positive OPSCC appears to vary widely in different geographic regions, potentially linked to varying sexual practices in different cultures around the globe. The largest number of studies on HPV-positive OPSCC have been conducted in North America and Europe, and data regarding the prevalence of HPV-positive OPSCC in sub-Saharan Africa in particular are scarce. A few studies have reported variable detection rates for HR-HPV in OPSCC that ranged from 0 to 50% (Table 1).[19–25] The marked variation in HPV prevalence rates among studies of OPSCC may be explained in part by variations in study design and techniques used to identify HPV in biologic specimens. Additionally, sample sizes were often small or limited.

Given the paucity of data and widely variable detection rates, the prime objective of this study was to determine the prevalence of HPV-positive OPSCC in one of the largest tertiary care centers in sub-Saharan Africa using p16 IHC as a screening test, followed by specific confirmatory PCR-based HPV typing. To the best of our knowledge this is the largest study to date that examines the presence of HR-HPV in OPSCC in sub-Saharan Africa.

Multiple methods for HPV detection in OPSCC samples have been described. In general, DNA PCR-based methods have yielded a significantly higher detection of HR-HPV, in comparison with other testing modalities.[27] Aboagye et al[22] from Ghana reported a remarkably high prevalence rate of 50% for HR-HPV in OPSCC using DNA PCR. However, PCR detection alone does not distinguish HPV infections that are truly causative (ie, transcriptionally active) from those that are not (ie, so-called passenger HPV).[28]

Evaluation for HPV E6/E7 mRNA transcripts by in situ hybridization (ISH) is considered the gold standard to confirm the presence of transcriptionally active HPV. In a

study from Cameroon, Rettig et al[20] showed a prevalence rate of 29% using mRNA ISH. This is the only study from Africa to have employed a highly sensitive and specific method of HPV testing (mRNA ISH). Nevertheless, their sample size of only 7 cases does not provide for a more accurate and reliable prevalence rate.

p16 IHC, a surrogate marker of HR-HPV infection, is the most cost-effective and widely employed HPV testing modality, demonstrating a high sensitivity (almost 100%) for the presence of transcriptionally active HPV, with a statistically similar performance to E6/E7 mRNA ISH in OPSCC.[26,29] p16 is overexpressed in tumor cells that harbor transcriptionally active HPV because viral E7 oncoprotein targets and degrades pRb tumor suppressor protein, with subsequent transcription of *E2F* genes and suppression of pRb-induced cell cycle arrest.[30] E7 also activates histone demethylases, KDM6A and KDM6B, which are involved in the induction of p16.[31,32] p16 IHC appears to fulfill all the criteria listed for an ideal test. Several prospective and randomized controlled studies, including studies with large numbers of patients, have demonstrated that patients with p16-positive OPSCC have considerably better outcomes than p16-negative patients.[7,8,18,33–37] Unlike in Western countries where the prevalence of HPV-positive OPSCC is much higher, data from our sub-Saharan African cohort suggest that p16 alone should not be used as the sole method to determine the HPV status of OPSCC.

p16 IHC as a surrogate marker for HR-HPV is not without some major limitations. Although p16 IHC is highly sensitive for the presence of transcriptionally active HPV (approaching 100%),[26] it is only 85% to 95% specific because other molecular events, such as pRb mutation in HPV-negative tumors, may result in p16 overexpression.[38,29,35] In fact, reports from low-prevalence regions with a high incidence of smoking- and alcohol-related disease suggest p16 to be a less reliable surrogate marker for HPV infection.[39]

Comparably, only 13 of the 36 p16-positive cases (PPV = 36.1%) in our study were HR-HPV positive when evaluated by PCR.

Several groups have recently published pooled analyses describing HPV in head and neck cancer.[40,41] Ndiaye et al[40] described the global burden of HPV in multiple head and neck subsites (oropharynx, hypopharynx/larynx, and oral cavity).[40] They compared various HPV detection techniques and demonstrated a high correlation between p16 IHC and HPV DNA PCR-based approaches for identifying HPV-positive oropharyngeal cancers. Using a combination of p16 IHC and HPV DNA PCR, we were able to demonstrate a prevalence rate of 5%, which is comparable to the lower rates reported in this region. Blumberg et al[24] failed to detect HR-HPV in 22 cases of OPSCC from Mozambique using a combination of p16 IHC and PCR. Using a similar methodology and study design, Ndiaye et al[25] could not demonstrate the presence of HR-HPV in 5 cases of OPSCC from Senegal. In a study from Malawi, Faggons et al[23] also reported a low prevalence rate of 22% using p16 IHC as a surrogate marker of HPV infection. Nevertheless, their sample in addition to OPSCC also included oral squamous cell carcinoma; thus, the true prevalence of HPV-positive OPSCC remains unknown.

The low prevalence of HPV-positive OPSCC in sub-Saharan Africa is most likely attributed to the nature of sexual practices in this region. Oral-genital contact has been implicated in the transmission of oral oncogenic HPV infection and subsequent development of OPSCC.[42] Although there have been no large population-based studies examining oral sexual practices in this region, observations are that they are less commonly practiced compared with the developed world. In a study from Kenya that examined the prevalence of HR-HPV in a group of 160 HNSCC patients, only 1 patient admitted to having engaged in oral-genital sex.[19] Davidson et al,[43] who examined the prevalence of oral HPV infection in a cohort of South African Black male mine workers aged 17 to 64 years, reported oral sex to be an uncommon practice among the study participants. A similar trend has been observed in African Americans.

An American study, including an equal number of Black and White sexually active adolescent males, showed that sexually active adolescent White males were 2.7 times more likely to engage in oral sex with a female than their sexually active adolescent Black male counterparts.[44] It has therefore been proposed that in the United States, HPV-positive OPSCC is predominantly a disease of young White males who often report multiple oral sex partners. In contrast, HPV-negative OPSCC is more common among non-White, urban, and poorly insured individuals because of higher smoking rates in these populations.[45,46] Comparatively, in the current study, most of the OPSCC patients were low-income Black/mixed-race South African males with a significant history of tobacco use and alcohol consumption.

Some authors have proposed that unknown genetic and environmental factors may provide immunologic protection against HPV infection in this region, akin to the immunologic protection conferred by hemoglobin S of sickle cell disease against *Plasmodium falciparum*, the causative agent of malaria.[47]

Most HPV-positive patients in our study were male (male to female ratio, 3.3:1). The most common sites affected in the HPV-positive group were the tonsil (n = 10) and base of tongue (n = 3). The tonsillar tissues (lingual and palatine tonsils) are hot spots for HPV-induced carcinogenesis.[48] At these sites, HR-HPV, and particularly HPV-16, is detected in more than 80% of squamous cell carcinomas.[49] The high affinity of HPV-16 for the specialized lymphoepithelium lining the tonsillar crypts might be explained by the activation of critical immune checkpoint pathways in the epithelium, creating a potential "immune-privileged" site for HR-HPV infection.[50]

A strong association between the presence of HR-HPV and a nonkeratinizing histomorphology is well known in OPSCC.[51,52]

Similarly, in our cohort of tumors, most HPV-positive OPSCCs were nonkeratinizing (n = 10) or only partially keratinizing (n = 1). A modest improvement in the p16 PPV was observed for OPSCCs with nonkeratinizing or partially keratinizing histomorphology (52.4%). HPV-16 was the most common HR-HPV in OPSCC (prevalence rate of >80%), followed by HPV-18 (prevalence rate of 3%) in a prospective US-based study.[49] Similarly, HPV-16 was the most common genotype identified in the current study (10 of 13; 85%), followed by HPV-18 (8%; n = 1), HPV-52 (8%; n = 1), and HPV-31 (8%; n = 1). The identification of the less common HR-HPV types, HPV-52 and HPV-31, in our cohort of HPV-positive OPSCC cases may have implications for HPV ISH cocktails used to identify HR-HPV in OPSCC specimens and the current HPV vaccination strategies in this region.

In April 2014, a national-based HPV vaccination program was rolled out in South Africa, targeting grade 4 schoolgirls

older than 9 years.[53] A bivalent vaccine with a 2-dose schedule (6 months apart) was used. The bivalent vaccine is targeted against HPV-16 and HPV-18 and may not provide effective broad-spectrum protection against other HR-HPV types, such as 52 and 31 detected in the present study. The new-generation vaccine (GARDASIL 9),[54] which aims to prevent HPVs 16, 18, 31, 33, 45, 52, and 58, may be employed as part of a comprehensive approach for the prevention of cervical-positive and subsequently HPV-positive oropharyngeal cancers in this region.

In OPSCC, HPV-16 integration status has been linked to increased survival and improved outcome.[2,6,7] Current clinical data suggest that patients with HPV-positive oropharyngeal SCC may benefit from new treatment regimens and modalities, such as de-escalation radiotherapy and immunotherapy.[8,9]

In our cohort of HR-HPV OPSCC, the extracted clinical outcome information for patients was insufficient to draw definitive conclusions regarding future management. Nevertheless, of the 6 patients with available clinical information, 4 were successfully treated and were disease free on their last follow-up visit. Local recurrence was identified in 1 patient. One elderly female patient (age 70 years with HPV-52 status) died of OPSCC within 1 year from the time of diagnosis, consistent with recent reports suggesting an attenuated survival benefit for older HPV-positive OPSCC patients in comparison with younger patients.[55]

The strength of our study is that it is the largest OPSCC study in sub-Saharan Africa using p16 IHC and PCR-based HPV typing. However, the limitation of the present study is the exclusion of OPSCC patients from private hospitals and clinics in Cape Town, where higher numbers of HPV-positive OPSCC patients are expected to be seen among insured White college-educated patients. Thus, future multi-institutional studies are needed to explore the exact prevalence of HPV-positive OPSCC in this region.

CONCLUSIONS

The presence of HR-HPV in 5% of OPSCC cases, in one of the largest tertiary care centers in sub-Saharan Africa (Tygerberg Hospital), suggests HR-HPV as a minor etiologic agent in OPSCC in this region. Because of its suboptimal PPV, p16 IHC is a less reliable marker for HR-HPV infection, because of the high incidence of tobacco- and alcohol-related disease in this region. The low PPV for p16 suggests that unlike in Western countries, it should not be used alone to determine the HPV status of OPSCC in sub-Saharan Africa. Therefore, when p16 is positive, HPV-specific testing should be performed using one of the available platforms. The identification of the less common HR-HPV types, HPV-52 and HPV-31, in our cohort of HPV-positive OPSCC cases may have implications for ISH HPV cocktails and the current vaccination strategies in this region.

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