Human papilloma virus in head and neck squamous cell carcinoma: A descriptive study of histologically confirmed cases at Kamuzu Central Hospital in Lilongwe, Malawi

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Abstract

Background
Head and neck squamous cell carcinoma (HNSCC) is common in sub-Saharan Africa, but the etiologic contribution of HPV is not well established.

Methods
We assessed HNSCC cases for HPV using p16 immunohistochemistry (IHC) in Malawi. Associations between p16 IHC and tumor site, behavioral risk factors, demographic characteristics, and HIV status were examined.

Results
From 2010 to 2014, 77 HNSCC cases were identified. Mean age was 52 years, 50 cases (65%) were male, and 48 (62%) were in the oropharynx (OP) or oral cavity (OC). HIV status was known for 35 patients (45%), with 5 (14%) HIV-infected. Substance use was known for 40 patients (52%), with 38% reporting any tobacco and 31% any alcohol. Forty-two cases (55%) had adequate tissue for p16 IHC, of which seven (17%) were positive, including 22% of OP/OC tumors.

Conclusions
Despite high cervical cancer burden, HPV-associated HNSCC is not very common in Malawi.

Introduction
Cancer burden is increasing in sub-Saharan Africa, where one-third of cancers are estimated to be caused by infectious agents.1-3 Head and neck squamous cell cancer (HNSCC) is the sixth most common malignancy in sub-Saharan Africa, including tumors in the oral cavity (OC), oropharynx (OP), nasopharynx, other pharynx, and larynx.1 Tobacco and alcohol exposure are established risk factors. However, human papilloma virus (HPV) is also a known cause particularly for OP cancer, especially in patients without tobacco or alcohol use. Incidence of HPV-associated OP SCC is increasing in high-income countries, and HPV now accounts for more than 70% of OP SCC compared to 20-25% of HNSCC at other sites.3-5

HPV is the causative agent of cervical cancer, for which burden is extremely high throughout sub-Saharan Africa.1 However, the contribution of HPV to HNSCC in the region is largely unknown. Prior studies have used varying methodologies to detect HPV [immunohistochemistry (IHC), polymerase chain reaction (PCR), or a combination], often without detailed characterization of anatomic site or simultaneous evaluation of other risk factors including HIV infection.6-9 We therefore sought to determine the presence of HPV using p16 IHC among histologically confirmed HNSCC cases at Kamuzu Central Hospital (KCH), a national teaching hospital in Malawi’s capital, Lilongwe.

Methods
Histologically confirmed HNSCC cases diagnosed at KCH between September 2010 and April 2014 were studied. HNSCC was diagnosed using hematoxylin and eosin staining of formalin-fixed paraffin-embedded (FFPE) tissue. All diagnoses were independently confirmed by senior pathologists in Malawi (NGL) and the US (WKF). Review by US pathologists was facilitated by the Aperio virtual microscopy system.10 Expression of p16 was visually assessed by IHC using five micron FFPE sections and the Roche CINtec Histology Kit per manufacturer specifications (Roche Products Ltd, Randburg, South Africa). All sectioning, staining, and IHC was performed manually in Malawi. Both study pathologists independently graded all specimens as negative (no p16 staining), intermediate (weak p16 staining in a minority of cells), or positive (strong p16 staining in a majority of cells). Only specimens considered positive by both pathologists were considered positive in these analyses.

Demographic and clinical data, including HIV status, anatomic site, and substance use, were obtained from medical records. Data were compiled using a standardized abstraction template. OP/OC sites were grouped together,
since most patients presented with bulky tumors making it
difficult to definitively assign tumor origin as OP or OC. In
addition, some patients were identified via the pathology
database alone without baseline assessment by dedicated
study clinicians. Anatomic site was therefore often not well
characterized for these patients.

Descriptive statistics were summarized and associations
between variables determined using a Fisher's exact test
and t-test. All data were analyzed using Stata 12 (StataCorp,
College Station, Texas, USA). The study was approved by
the Malawi National Health Science Research Committee
and Institutional Review Board of the University of North
Carolina.

Results
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Discussion
Despite a concentration of infection-associated cancers in
sub-Saharan Africa including an immense burden of HPV-
associated cervical cancer,11 and rising incidence of HPV-
associated OP SCC in high-income countries,3–5 we found few
HPV-associated HNSCC specimens at a national teaching
hospital in Malawi as assessed by p16 IHC. Our study was
limited by small sample size, as well as missing data, due
to reliance on routine medical records for abstracting HIV
status and clinical information. Nevertheless, these results
may be valuable in light of few studies specifically examining
HPV-associated HNSCC in sub-Saharan Africa, and no prior

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### Table 1: Characteristics of 77 confirmed head and neck squamous cell carcinoma cases in Lilongwe, Malawi

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>50 (65%)</td>
</tr>
<tr>
<td>Age, mean years (SD)</td>
<td>52.4 (16.8)</td>
</tr>
<tr>
<td>Tumor site, n (%)</td>
<td></td>
</tr>
<tr>
<td>Oropharynx/oral cavity</td>
<td>48 (62%)</td>
</tr>
<tr>
<td>Larynx</td>
<td>9 (12%)</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>HIV status, n (%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5 (6%)</td>
</tr>
<tr>
<td>Negative</td>
<td>30 (39%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>42 (55%)</td>
</tr>
<tr>
<td>Tobacco use, n (%)</td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>15 (20%)</td>
</tr>
<tr>
<td>None</td>
<td>25 (32%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>37 (48%)</td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>12 (16%)</td>
</tr>
<tr>
<td>None</td>
<td>27 (35%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>38 (49%)</td>
</tr>
</tbody>
</table>

SD = standard deviation

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### Table 2: Characteristics by p16 immunohistochemistry status for 42 head and neck squamous cell carcinoma cases in Lilongwe, Malawi

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>p16 positive (n = 7)</th>
<th>p16 negative (n = 35)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>4</td>
<td>23</td>
<td>0.68</td>
</tr>
<tr>
<td>Age, mean years (SD)</td>
<td>49.1 (8.3)</td>
<td>54.3 (18.0)</td>
<td>0.47</td>
</tr>
<tr>
<td>Oropharynx/oral cavity</td>
<td>5 (71%)</td>
<td>18 (51%)</td>
<td>0.43</td>
</tr>
<tr>
<td>HIV status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (14%)</td>
<td>1 (3%)</td>
<td>0.43</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (43%)</td>
<td>12 (34%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (43%)</td>
<td>22 (63%)</td>
<td></td>
</tr>
<tr>
<td>Tobacco use, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>0 (0%)</td>
<td>3 (9%)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (14%)</td>
<td>3 (9%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (86%)</td>
<td>29 (83%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (14%)</td>
<td>6 (17%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (86%)</td>
<td>29 (83%)</td>
<td></td>
</tr>
</tbody>
</table>

SD = standard deviation
Table 3: p16 immunohistochemistry status by anatomic site for 42 head and neck squamous cell carcinoma cases in Lilongwe, Malawi

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Total</th>
<th>p16 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Oropharynx/oral cavity</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>Unknown primary</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Larynx</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>All sites</td>
<td>42</td>
<td>7</td>
</tr>
</tbody>
</table>

studies in Malawi to our knowledge.

Despite its limitations, our results are generally consistent with the few other contemporary studies existing from the region. A recent study from Mozambique found no HPV-associated tumors among 51 OP/OC cases using p16 IHC as well as PCR detection of E6 and E7 oncoproteins.5 Similarly, a study from Senegal found 3% HPV DNA detection by PCR among 117 HNSCC cases, with no PCR-positive cases being positive by p16 IHC.7 Other studies from Sudan and South Africa have detected HPV DNA by PCR in 27–49% of OP/OC tumors.6,8 However, PCR-based detection alone can often identify bystander oral HPV that may not be causally implicated in oncogenesis, a process for which p16 IHC is a more reliable surrogate.3 Our findings are also consistent with global epidemiologic data suggesting that increases in OP cancer, the HNSCC site which has the closest causal association with HPV, are largely confined to more economically developed countries rather than less developed countries.5,5

If these data are collectively correct in suggesting low frequency for HPV-associated HNSCC in sub-Saharan Africa, this is despite an extremely high burden of HPV-associated cervical cancer,11 including in Malawi specifically.12–14 While this is speculative, one reason for this discrepancy may be differences in sexual practices, which are major determinants of oral HPV infection and HPV-associated HNSCC risk.5,15 Such data are absent from Malawi and were not specifically assessed in our study, but other regional literature have suggested that oral intercourse and oral HPV infection are uncommon at least in South Africa.16

In our study, although HIV status was missing for many patients thereby introducing potential bias, we found an HIV prevalence of 14% among cases with known HIV status, roughly similar to 10-11% prevalence in the Malawi general population.17 Although HIV is associated with an approximately 2-3-fold increase in HNSCC risk in resource-rich settings,18,19 our data do not suggest a major association between HIV and HNSCC in Malawi, a country with high HIV prevalence. This may reflect generally low rates of tobacco and alcohol use in our setting (14% and 7% in the Malawi general population respectively).20 Among HIV-infected persons in resource-rich settings, higher rates of tobacco and alcohol use and HPV contribute to increased HNSCC compared to HIV-negative people.18,19

Also, despite remarkable progress in antiretroviral therapy (ART) scale-up, coverage is still lower in Malawi than high-income countries with typically more advanced HIV illness prior to ART initiation. Therefore, Malawi may have still to realize epidemiologic transitions in its HIV-infected population that have occurred in other countries, where opportunistic infections and classical AIDS-defining cancers have been supplanted by non-AIDS-defining cancers and cardiovascular disease as principal causes of morbidity and mortality, among aging HIV-infected populations effectively treated with ART.

In summary, HPV-associated HNSCC as assessed by p16 IHC was uncommon in our small series at a national teaching hospital in Malawi. Continued HNSCC surveillance in Malawi will be important to fully understand the importance of this disease moving forward, ideally through larger regional collaborations across sub-Saharan Africa which can provide more definitive results. This will be particularly important as ART coverage increases for HIV-infected individuals, behavioral practices change, and HPV vaccination becomes available. Finally, if established risk factors including tobacco, alcohol, and HPV do not collectively account for most HNSCC in sub-Saharan Africa, high-quality epidemiologic studies are urgently needed to identify etiologic agents in HNSCC development which may be unique to the region. These vital data can inform prevention efforts in settings where treating established HNSCC is difficult due to resource limitations, including exceedingly scarce radiotherapy.

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Competing interests

All authors declare that they have no competing interests related to this work.

References


