Original Research

Human papillomavirus 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 aetiologic contribution of human papillomavirus (HPV) is not well established.

Methods

We assessed HNSCC cases for HPV using p16 immunohistochemistry (IHC) in Malawi. Associations between p16 IHC and tumour site, behavioural 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 7 (17%) were positive, including 22% of OP/OC tumours.

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.¹² Head and neck squamous cell cancer (HNSCC) is the sixth most common malignancy in sub-Saharan Africa, including tumours in the oral cavity (OC), oropharynx (OP), nasopharynx, other pharynx, and larynx.¹ Tobacco and alcohol exposure are established risk factors. However, human papillomavirus (HPV) is also a known cause particularly for OP cancer, especially in patients without tobacco or alcohol use. Incidence of HPV-associated OSCC is increasing in high-income countries, and HPV now accounts for more than 70% of OP SCC compared to 20% to 25% of HNSCC at other sites.²⁻⁵

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 characterisation 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 haematoxylin 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 5-µm 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 standardised abstraction template. OP/OC sites were grouped together.
Table 1: Characteristics of 77 confirmed head and neck squamous cell carcinoma cases in Lilongwe, Malawi

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male, n (%)</th>
<th>Age, mean years (SD)</th>
<th>Tumour site, n (%)</th>
<th>Oropharynx/oral cavity</th>
<th>Unknown primary</th>
<th>Larynx</th>
<th>Nasopharynx</th>
<th>Hypopharynx</th>
<th>HIV status, n (%)</th>
<th>Smoking status, n (%)</th>
<th>Alcohol use, n (%)</th>
<th>Unknown primary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>50 (65%)</td>
<td>52.4 (16.8)</td>
<td>48 (62%)</td>
<td>13 (17%)</td>
<td>9 (12%)</td>
<td>6 (8%)</td>
<td>1 (1%)</td>
<td>37 (48%)</td>
<td>5 (6%)</td>
<td>25 (32%)</td>
<td>15 (20%)</td>
<td>42 (55%)</td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td>Any</td>
<td>15 (20%)</td>
<td>48 (62%)</td>
<td>13 (17%)</td>
<td>9 (12%)</td>
<td>6 (8%)</td>
<td>1 (1%)</td>
<td>37 (48%)</td>
<td>Positive</td>
<td>5 (6%)</td>
<td>0 (0%)</td>
<td>25 (32%)</td>
</tr>
<tr>
<td>Tobacco use, n (%)</td>
<td>Any</td>
<td>15 (20%)</td>
<td>48 (62%)</td>
<td>13 (17%)</td>
<td>9 (12%)</td>
<td>6 (8%)</td>
<td>1 (1%)</td>
<td>37 (48%)</td>
<td>Negative</td>
<td>30 (39%)</td>
<td>0 (0%)</td>
<td>25 (32%)</td>
</tr>
<tr>
<td>HIV status, n (%)</td>
<td>Positive</td>
<td>5 (6%)</td>
<td>48 (62%)</td>
<td>13 (17%)</td>
<td>9 (12%)</td>
<td>6 (8%)</td>
<td>1 (1%)</td>
<td>37 (48%)</td>
<td>Unknown</td>
<td>42 (55%)</td>
<td>12 (16%)</td>
<td>27 (35%)</td>
</tr>
<tr>
<td>Unknown primary</td>
<td>42 (55%)</td>
<td>12 (16%)</td>
<td>27 (35%)</td>
<td>6 (8%)</td>
<td>1 (1%)</td>
<td>30 (39%)</td>
<td>50 (65%)</td>
<td>8 (11%)</td>
<td>Tobacco use</td>
<td>25 (32%)</td>
<td>27 (35%)</td>
<td>42 (55%)</td>
</tr>
</tbody>
</table>

SD = standard deviation

since most patients presented with bulky tumours making it difficult to definitively assign tumour 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 summarised and associations between variables determined using a Fisher's exact test and t-test. All data were analysed 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

We identified 77 cases of histologically confirmed HNSCC at KCH between September 2010 and April 2014 (Table 1). Fifty (65%) cases were in men, with a mean age of 52 years. OP/OC was the most common anatomic site (n = 48; 62%), with hypopharynx least common (n = 1). HIV status was known for 35 patients, of whom 5 (14%) were HIV-infected. Tobacco history was known for 40 cases (52%), with 15 (38%) reporting any history of tobacco use. Alcohol history was known for 39 cases (51%), among whom 12 (31%) reported any history of alcohol use.

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, n (%)</td>
<td>5 (71%)</td>
<td>18 (51%)</td>
<td>0.43</td>
</tr>
<tr>
<td>HIV status, n (%)</td>
<td>Positive</td>
<td>1 (14%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>3 (43%)</td>
<td>12 (34%)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>3 (43%)</td>
<td>22 (63%)</td>
</tr>
<tr>
<td>Tobacco use, n (%)</td>
<td>Any</td>
<td>0 (0%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>1 (14%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>6 (86%)</td>
<td>29 (83%)</td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td>Any</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>1 (14%)</td>
<td>6 (17%)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>6 (86%)</td>
<td>29 (83%)</td>
</tr>
</tbody>
</table>

SD = standard deviation

Forty-two cases (55%) had suitable FFPE specimens for p16 IHC. Across all anatomic sites, 7 (17%) were positive. Although limited by small numbers, statistically significant differences by p16 IHC status were not observed for sex, age, primary tumour site, or HIV status (Table 2). Numerically higher proportions of OP/OC cancers (71% versus 51%) and HIV positivity were observed (14% versus 3%) among tumors that were positive by p16 IHC. No case with positive p16 IHC was documented to have prior tobacco or alcohol use. Of 7 positive tumours, 5 occurred in the OP/OC and 2 in the larynx (Table 3), but p16 IHC positivity was low across all anatomic sites including 5 of 23 (22%) tumors documented to have OP/OC origin.

Discussion

Despite a concentration of infection-associated cancers in sub-Saharan Africa including an immense burden of HPV-associated cervical cancer, and rising incidence of HPV-associated OP SCC in high-income countries, 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 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 tumours among 51 OP/OC cases using p16 IHC as well as PCR detection of E6 and E7 oncogene products. 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. Other studies from Sudan and South Africa have detected HPV DNA by PCR...
in 27% to 49% of OP/OC tumours. 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. 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.

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, including in Malawi specifically. 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. 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.

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% to 11% prevalence in the Malawi general population. Although HIV is associated with an approximately 2- to 3-fold increase in HNSCC risk in resource-rich settings, 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). Among HPV-infected persons in resource-rich settings, higher rates of tobacco and alcohol use and HPV use have contributed to increased HNSCC compared to HIV-negative people. 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 differences in sexual practices, which are major determinants of oral HPV infection and HPV-associated HNSCC risk.

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.

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

References


