Imaging, Diagnosis, Prognosis

Improved Survival with HPV among African Americans with Oropharyngeal Cancer

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Abstract

Purpose: A major limitation of studies reporting a lower prevalence rate of human papilloma virus (HPV) in African American patients with oropharyngeal squamous cell cancer (OPSCC) than Caucasian Americans, with corresponding worse outcomes, was adequate representation of HPV-positive African American patients. This study examined survival outcomes in HPV-positive and HPV-negative African Americans with OPSCC.

Experimental Design: The study cohort of 121 patients with primary OPSCC had 42% African Americans. Variables of interest included age, race, gender, HPV status, stage, marital status, smoking, treatment, and date of diagnosis.

Results: Caucasian Americans are more likely to be HPV positive (OR = 3.28; P = 0.035), as are younger age (age < 50 OR = 7.14; P = 0.023 compared with age > 65) or being married (OR = 3.44; P = 0.016). HPV positivity and being unmarried were associated with being late stage (OR = 3.10; P = 0.047 and OR = 3.23; P = 0.038, respectively). HPV-negative patients had 2.7 times the risk of death as HPV-positive patients (P = 0.004). Overall, the HPV-race groups differed (log-rank P < 0.001), with significantly worse survival for HPV-negative African Americans versus (i) HPV-positive African Americans (HR = 3.44; P = 0.0012); (ii) HPV-positive Caucasian Americans (HR = 3.11; P < 0.049); and (iii) HPV-negative Caucasian Americans (HR = 2.21; P = 0.049).

Conclusions: HPV has a substantial impact on overall survival in African American patients with OPSCC. Among African American patients with OPSCC, HPV-positive patients had better survival than HPV negative. HPV-negative African Americans also did worse than both HPV-positive Caucasian Americans and HPV-negative Caucasian Americans. This study adds to the mounting evidence of HPV as a racially linked sexual behavior life style risk factor impacting survival outcomes for both African American and Caucasian American patients with OPSCC. Clin Cancer Res; 19(9); 1–7. ©2013 AACR.

Introduction

There is abundant epidemiologic evidence that self-identified race/ethnicity is associated with differences in cancer incidence and mortality (1, 2). The high-mortality rate for head and neck squamous cell carcinoma (HNSCC) continues to be driven by the disparate unfavorable diagnosis and prognosis outcomes for African Americans (1–3). African Americans have been shown to have a worse overall survival compared with whites after controlling for age, disease stage, and treatment received (4). The 5-year relative survival is lower in African Americans than in Caucasian Americans for every stage of diagnosis for nearly every cancer site (5). There is no consensus on the causes of the differences in the higher incidence of and the mortality from HNSCC for African Americans when compared with Caucasian Americans, but they can include differences in access to care, stage at diagnosis, insurance status, attitudes of health providers, as well as human papilloma virus (HPV) infection status (2, 4, 6–9).

In African Americans with oropharyngeal squamous cell cancer (OPSCC), survival disparities were attributed to racial differences in the prevalence of HPV-positive tumors. Settle and colleagues (9) found that a worse survival outcome for African Americans versus Caucasian Americans in OPSCC was attributable to racial differences in the prevalence of HPV-positive tumors. This was also confirmed by Chernock and colleagues (10) with corresponding worse disease free survival in African Americans and a trend toward worse overall survival for African Americans. A major limitation of these studies was the lack of adequate...
representation of HPV-positive African American patients. For this study, we compared survival outcomes in HPV-positive and HPV-negative African Americans with OPSCC in a retrospective primary OPSCC cohort with 42% African Americans.

Materials and Methods

Patients

The study cohort of 121 patients with primary OPSCC was drawn from a large, clinically well-characterized multiethnic (42% African Americans), primary care patient population in the Detroit area (11). Patients were identified through tumor registry and ENT clinic records. Eligibility criteria included ages of 21 years or older, a primary HNSCC diagnosis (including OPSCC), and availability of tumor tissue blocks. For patients in this analysis, diagnosis dates ranged from 1990 to 2004, follow up dates from 1999 to 2008, and death dates from 1991 to 2007.

HPV-16 detection by real-time quantitative PCR

Whole 5 micron tissue sections with 70% or more tumor or microdissected tumor lesions were processed for DNA extraction (12). Tumor HPV DNA was determined using quantitative real-time PCR (qRT-PCR) as previously described (13). Briefly, primers and probes to a housekeeping gene (β-globin) are run in parallel to standardize the input DNA. By using serial dilutions, standard curves, adjustment curves are developed for the HPV viral copy number using CaSki (American Type Culture Collection) cell line genomic DNA, known to have 600 copies/genome equivalent (6.6 pg of DNA/genome). The cut-off value for HPV16-positive status was ≥ 0.03 (≥3 HPV genome copy/100 cells; ref. 13).

Statistical analysis

All analyses were done using SAS 9.2. Categorical data are presented as count (percent) and continuous data as mean (SD). Univariate Wilcoxon rank sum, χ², and Fisher exact tests were used to examine individual associations with HPV status. Multivariable logistic regression was used to examine the effects of all other variables of interest on the outcomes of interest (HPV status and stage). Kaplan–Meier plots and log-rank tests were used to compare the survival times of HPV-positive and HPV-negative patients and of African Americans and Caucasians with HPV as compared with those without HPV. Cox regression was used to model the risk of death given age, race, gender, HPV status, stage, smoking status, marital status, treatment, and year of diagnosis. Adjustment for multiple comparisons was done using Hochberg’s method(14). Statistical significance was set at P<0.05.

Results

Patient cohort characteristics

Of the 121 patients identified, 118 have HPV data, of which 67 are HPV negative and 51 HPV positive (43%). Patient characteristics (study variables) for the 118 OPSCC cohort are described in Table 1 and included 68 Caucasian Americans, 49 African Americans, and 1 unknown race. The median length of follow-up was 46.8 months (range 0.1 to 194 months).

HPV prevalence and associated outcomes

In univariate tests (Table 1), being HPV positive was associated with Caucasian race (P = 0.024), not smoking (P = 0.059), and being married (P = 0.004). In multiple logistic regression modeling of 88 OPSCC with complete data (Table 2), Caucasian Americans are more likely to be HPV positive (OR = 3.28; P = 0.035), as are younger age (age < 50 OR = 7.14; P = 0.023 compared with age > 65) or being married (OR = 3.44; P = 0.016). In a model using stage (early or late) as the outcome (Supplementary Table S1) and controlling for age, race, gender, smoking status, and year of diagnosis, late stage (stage III & IV) was associated with HPV-positive status [OR = 3.10; 95% confidence interval (CI) 1.01–9.45; P = 0.047] and as was being unmarried (OR = 3.23, 95% CI 1.06–9.78; P = 0.038).

HPV status and survival outcomes

Of the 121 patients with survival data available, 56 lived and 65 died. Patients who are HPV positive have significantly higher survival probability than patients who are HPV negative (univariate log-rank P = 0.003, Fig. 1). The median survival time for HPV-negative patients was 2.2 years (95% CI 1.3–5.2), but for HPV-positive patients median survival was over 13 years.

Cox regression given age, race, gender, HPV status, stage, treatment, smoking status, marital status, and year of diagnosis indicated that HPV-negative patients had 2.7 times the risk of death compared with HPV-positive patients (95% CI 1.37–5.31; P = 0.004; Table 3), late-stage patients had worse survival than early-stage patients (OR = 2.44; 95% CI 1.13–5.16; P = 0.020), and current smokers had 3.4 times the risk of death versus
never-smokers (95% CI 1.13–10.38; \( P = 0.030 \)). Race as African American was not independently associated with worse outcome in the entire cohort in multivariate analysis. No interactions were significant.

Survival of the HPV and race groups (combined to form 4 groups), differed significantly overall (log-rank \( P < 0.001 \), Fig. 2, Table 4). HPV-negative African Americans had worse overall survival than HPV-positive African Americans (HR = 3.44, raw \( P = < 0.001 \), Hochberg adjusted \( P = 0.0012 \)), worse survival than HPV-positive Caucasian Americans (HR = 3.11; raw \( P = 0.012 \); adjusted \( P = 0.0496 \)), and worse survival than HPV-negative Caucasian Americans (HR = 2.21; raw \( P = 0.02 \); adjusted \( P = 0.0496 \)). HPV-positive African American patients had no differences in survival from that of HPV-positive and HPV-negative Caucasian Americans (\( P = 0.84 \) and \( P = 0.66 \), respectively).

Also, HPV-positive Caucasian Americans and HPV-negative Caucasian Americans with OPSCC showed no differences in survival outcomes (\( P = 0.84 \)).

### Discussion

HPV is now regarded, in addition to tobacco and alcohol (15), as a causative agent for OPSCC (16) and an independent risk factor for OPSCC (17, 18). The association between HPV and HNSCC (for both incidence and prognosis) is strongest for OPSCC. HPV-positive OPSCC has been noted as a distinct variant of HNSCC characterized by

<table>
<thead>
<tr>
<th>Variable</th>
<th>ORs (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (51–65 vs. &gt;65)</td>
<td>1.25 (0.41–3.80)</td>
<td>0.690</td>
</tr>
<tr>
<td>Age (≤50 vs. &gt;65)</td>
<td>7.14 (1.31–39.00)</td>
<td>0.023</td>
</tr>
<tr>
<td>Race (White vs. Black)</td>
<td>3.28 (1.09–9.87)</td>
<td>0.035</td>
</tr>
<tr>
<td>Gender (Male vs. Female)</td>
<td>1.63 (0.42–6.32)</td>
<td>0.476</td>
</tr>
<tr>
<td>Stage (Early vs. Late)</td>
<td>1.74 (0.12–24.29)</td>
<td>0.681</td>
</tr>
<tr>
<td>Smoke (Never vs. Current)</td>
<td>1.03 (0.22–4.83)</td>
<td>0.970</td>
</tr>
<tr>
<td>Smoke (Past vs. Current)</td>
<td>1.48 (0.48–4.57)</td>
<td>0.501</td>
</tr>
<tr>
<td>Married (Yes vs. No)</td>
<td>3.44 (1.26–9.33)</td>
<td>0.016</td>
</tr>
<tr>
<td>Year of diagnosis</td>
<td>1.10 (0.96–1.26)</td>
<td>0.189</td>
</tr>
</tbody>
</table>

NOTE: Bold, \( P < 0.05 \).
high prevalence of HPV infection, better patient outcome, nonkeratinizing histology, and overexpression of p16 (19), with worse outcomes in OPSCC for African Americans compared with Caucasian Americans partially explained by less HPV-positive cases in African Americans (9, 10).

Overall HPV prevalence in this cohort was 43%, with diagnosis years ranging from 1990 through 2004 and younger patients <50 years of age were significantly more likely to be HPV positive. Chaturved and colleagues (18) reported a higher HPV prevalence in OPSCC of approximately 70% during 2000 to 2004. They found that HPV-positive patients diagnosed from 1984 to 2004 were significantly younger and more likely to be white. The study attributed increases in the population-level incidence and survival of OPSCC in the United States since 1984 to HPV infection, with an estimation that, by 2020, HPV will cause more oropharyngeal cancers than cervical cancers in the United States (18).

In this study, with 42% African Americans, prevalence of HPV was lower in African American than Caucasian American patients with OPSCC. Caucasian Americans patients were significantly more likely to be HPV positive than African American patients supporting HPV tumor status as a disparate determinant in African American patients with OPSCC (9, 10, 20, 21). There is conclusive evidence that HPV has a strong association between sexual behavior and OPSCC, which has been linked to an increase in the number of oral sex partners (22–25). Studies of racial differences in sexual behaviors (26, 27) suggest that a higher proportion of whites engage in oral sex. These sexual behavior differences are thought to be one piece of the puzzle (28) for the reported racial disparity of HPV prevalence in OPSCC and might explain the higher rate of HPV-positive oropharyngeal cancers in whites (vs. blacks; refs. 9, 10, 20, 21).

In this study, married patients were more likely to be HPV positive than unmarried OPSCC. The latter is the opposite of what was found in a report that never-married status may be a surrogate for sexual practices associated with HPV transmission based on the dramatically elevated and reciprocal risk of second primary anogenital and oral cavity/pharyngeal cancers risk among never-married men compared with ever-married men (29). Significant changes in marriages and living together dynamics have occurred over the years, resulting in changes in sexual behavior and cohabitation choices emphasizing the need for larger studies that address sexual practices to further define the interaction between marital status and HPV infection in OPSCC.

Our study supports improved survival for HPV positive as compared with HPV-negative OPSCC. Tumor HPV status has been shown to be the single strongest predictor, followed by measures of tobacco exposure and tumor stage (30). Overall, in multivariate associations, HPV16-negative patients had a significantly poorer survival probability than HPV positive, a finding that concurs with several reported studies (HR = 2.9; P = 0.003). Also, late-stage patients had better survival than early-stage OPSCC (OR = 2.21; P = 0.034) and support studies of late stage as an important predictor of poor survival with patients dying of uncontrolled loco-regional disease (31).

Current smokers were nearly 4 times more likely to die than never smokers (OR = 3.8; P = 0.019); no difference in survival for past smokers was noted. Tobacco exposure has been associated with clinical trial outcome (32) and nicotine has been reported to reduce the cytotoxic effects of cisplatin and radiation of HNSCC cell lines (33). A recent finding of an increase of risk of OPSCC progression and death as a direct function of tobacco exposure at diagnosis and during therapy, independent of tumor p16 status and treatment (34) concurs with tumor HPV status as a strong and independent prognostic factor for survival in OPSCC (17). Thus, the most important risk factors for development of head and neck cancer, HPV and smoking, have utility as predictors of response to therapy and patient survival and likely determine the molecular profile of this disease.

Late-stage OPSCC were more likely to be HPV-positive status (OR = 3.10; P = 0.47) and unmarried (OR = 3.23; P = 0.038) as compared with early-stage patients. HPV-positive HNSCC are more likely to be detected as late-stage cancers,
which traditionally indicate poor prognosis (24, 35, 36). Despite this, survival has been shown to be better for patients with HPV-positive when compared with HPV-negative HNSCC (37), underscoring HPV as a reliable biomarker that can be used to not only help diagnose HNSCC, but to also risk stratify patients and help direct treatment plans based on disease behavior and prognosis (37, 38).

A unique contribution of this study is the observation that 4 groups differed significantly overall (log-rank \( P < 0.001 \)). HPV-negative African Americans (AA) had worse overall survival than HPV-positive African Americans, worse survival than HPV-positive Caucasian Americans (CA), and worse survival than HPV-negative Caucasian Americans. HPV-positive African American patients had no differences in survival from that of HPV-positive and HPV-negative C. Also, HPV-positive Caucasian Americans and HPV-negative Caucasian Americans with OPSCC showed no differences in survival outcomes.

Table 4. Cox proportional survival hazard model for race and HPV with contrasts (\( N = 116 \))

<table>
<thead>
<tr>
<th>Model</th>
<th>HR (95% CI)</th>
<th>Raw P value</th>
<th>Adjusted P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV- CA (Ref)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV- AA vs. HPV- CA</td>
<td>2.21 (1.19–4.08)</td>
<td>0.012</td>
<td>0.0496</td>
</tr>
<tr>
<td>HPV+ C vs. HPV- CA</td>
<td>0.71 (0.28–1.80)</td>
<td>0.469</td>
<td>0.8403</td>
</tr>
<tr>
<td>HPV+ AA vs. HPV- CA</td>
<td>0.64 (0.32–1.30)</td>
<td>0.220</td>
<td>0.6600</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contrasts</th>
<th>HR (95% CI)</th>
<th>Raw P value</th>
<th>Adjusted P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV- AA vs. HPV- CA</td>
<td>3.11 (1.28–7.58)</td>
<td>0.012</td>
<td>0.0496</td>
</tr>
<tr>
<td>HPV- AA vs. HPV+ CA</td>
<td>3.44 (1.79–6.60)</td>
<td>&lt;0.001</td>
<td>0.0012</td>
</tr>
<tr>
<td>HPV+ CA vs. HPV+ AA</td>
<td>1.10 (0.42–2.88)</td>
<td>0.840</td>
<td>0.8403</td>
</tr>
</tbody>
</table>

Abbreviations: AA, African American; CA, Caucasian Americans

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: M.J. Worsham
Development of methodology: M.J. Worsham, J.K. Stephen, K.M. Chen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.J. Worsham, J.K. Stephen, K.M. Chen, V. Schweitzer
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.J. Worsham, J.K. Stephen, K.M. Chen, M. Mahan, V. Schweitzer, G. Divine
Writing, review, and/or revision of the manuscript: M.J. Worsham, J.K. Stephen, K.M. Chen, M. Mahan, V. Schweitzer, G. Divine

Figure 2. Survival of the HPV and race groups (combined to form 4 groups) differed significantly overall (log-rank \( P < 0.001 \)). HPV-negative African Americans (AA) had worse overall survival than HPV-positive African Americans, worse survival than HPV-positive Caucasian Americans (CA), and worse survival than HPV-negative Caucasian Americans. HPV-positive African American patients had no differences in survival from that of HPV-positive and HPV-negative C. Also, HPV-positive Caucasian Americans and HPV-negative Caucasian Americans with OPSCC showed no differences in survival outcomes.

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