Human Papillomavirus Genotype Prevalence in Invasive Vaginal Cancer from a Registry-Based Population

Abstract

Objective—To describe the human papillomavirus (HPV) genotype distribution in invasive vaginal cancers diagnosed prior to the introduction of the HPV vaccine, and evaluate if survival differed by HPV status.
Methods—Four population-based registries and three residual tissue repositories provided formalin-fixed, paraffin-embedded tissue from microscopically confirmed primary vaginal cancer cases diagnosed between 1994 and 2005 that were tested by L1 consensus polymerase chain reaction with type-specific hybridization in a central laboratory. Clinical, demographic, and all-cause survival data were assessed by HPV status.

Results—Sixty cases of invasive vaginal cancer were included. HPV was detected in 75% (45) and 25% (15) were HPV negative. HPV 16 was most frequently detected (55%, 33/60) followed by HPV 33 (18.3%, 11/60). Only one case was positive for HPV 18 (1.7%) Multiple types were detected in 15% of the cases. Vaginal cancers in women < 60 were more likely to be HPV 16 or HPV 18 positive (HPV 16/18) than older women; 77.3% vs. 44.7% ($P= .038$). The median age at diagnosis was younger in the HPV16/18 (59 years) group vs. other HPV positive (68 years) and no HPV (77 years) ($P= .003$). The HPV distribution did not significantly vary by race or ethnicity or place of residence. The 5-year unadjusted all-cause survival was 57.4% for women with HPV-positive vaginal cancers vs. 35.7% among those with HPV-negative tumors ($p=0.243$).

Conclusion—Three quarters of all vaginal cancers in the United States had HPV detected, much higher than previously found, and 57% could be prevented by current HPV vaccines.

Introduction

Vaginal cancer accounts for less than two percent of all gynecologic malignancies, with an annual incidence rate of 0.7 per 100,000 and 1,178 new cases per year for 2005–2009.1,2 The 5-year relative survival rate for squamous cell carcinoma of the vagina is 54%3,4. The etiology of vaginal cancer varies by histology. Squamous cell carcinoma accounts for 80-90% of all cases and is associated with a prior history of cervical carcinoma, prior irradiation for anogenital cancer, and human papillomavirus 16 (HPV 16).5 Some of the risk factors for vaginal carcinoma are also indicators of either increasing acquisition or decreasing clearance of HPV (multiple lifetime sexual partners, age at first intercourse, immunosuppression, cigarette smoking). Others are non-HPV-related risk factors such as chronic pessary use, surgical menopause, or prior hysterectomy.67

HPV DNA has been detected in 55-64% of invasive vaginal cancers;8-10 however, these studies have been limited by small sample sizes, due to low numbers of vaginal cancers or HPV testing that is limited to HPV 16 or HPV 18 detection. Furthermore, these samples were convenience samples from single institutions and not population-based studies. HPV vaccines provide immunity to HPV 16 and HPV 18 and cross-immunity to other HPV types,11 and are expected to reduce the incidence of HPV-associated cancers, such as vaginal cancer. The HPV type distribution in these cancers should also shift following vaccine introduction. The aims of our study were to describe the HPV genotype distribution in invasive vaginal cancers diagnosed prior to the introduction of the vaccine and evaluate if survival differed by HPV status.

Materials and Methods

The Centers for Disease Control and Prevention Central Cancer Registries study was designed to provide a baseline prevalence of HPV types in HPV-associated cancer cases.
from participating population-based cancer registries. Four population-based registries and three residual tissue repositories provided formalin-fixed paraffin-embedded tissue from microscopically confirmed primary vaginal cancer cases diagnosed between 1994 and 2005. One formalin fixed, paraffin embedded tissue block from each case was provided.

Sample processing, extraction, and HPV testing have been previously described\textsuperscript{12,13}. Briefly, serial sections were cut using precautions to prevent contamination between cases. Hematoxylin and Eosin staining of first and last section was used to confirm presence of representative material. DNA extracts were tested with the Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, IN). Samples with negative or inadequate linear array results were re-tested with the INNO-LiPA HPV Genotyping Assay (LiPA, Innogenetics, Gent, Belgium). Samples failing both assays were considered inadequate and were not, therefore, included in the final analysis.

De-identified demographic (age, sex, population size), clinical (year of diagnosis, history of other cancers), pathologic (sub site, stage, grade), and survival data for cancer cases were available from each registry and tabulated. The Pearson chi-square test or Fisher’s exact test was used for discrete variables and the Kruskal-Wallis rank sum test for continuous variables. De-identified demographic (age, sex, population size), clinical (year of diagnosis, history of other cancers), pathologic (sub site, stage, grade), and survival data for cancer cases were available from each registry. Five-year all-cause survival estimates are presented as Kaplan-Meier estimates with statistical testing performed using the log-rank test. A Cox proportional hazards model was used to determine the effect of HPV positivity on five-year all-cause survival after adjusting for age. Due to the small number of events (26 deaths), additional covariates such as stage and treatment were not included to avoid over fitting the model. The statistical analyses were performed using SAS 9.2 (Statistical Analysis Software, Cary, NC) and R version 2.15.2. The Centers for Disease Control and Prevention and each participating state received approval for the institutional review board for the study.

Results

One block from each of the 71 cases was cut and submitted. Of these, 60 cases had representative tissue and were successfully tested and 11 blocks did not contain representative tissue. The median age at diagnosis was 65, with 63% being 60 years of age or older (Table 1). At 76%, whites were the largest group in the sample. HPV DNA was found in 75% (45/60) of cases. HPV 16 was the most common type detected (55%, 33/60). The second most common was HPV 33, with 18% (11/60) of cases positive. Only one case was positive for HPV 18 (1.7%). In 15% of cases (9/60) multiple types were detected (Fig 1) but for 8 of 9 of those cases, HPV 16 was also detected and among 5/9 of those cases both HPV 16 and 33 were found .

Ninety one percent of women under the age of 60 were HPV positive, with most HPV types being HPV 16/18 (77.3%). Among the vaginal cancer cases in women 60 years of age and older, approximately two thirds were positive for HPV, and the proportion of HPV 16/18 was lower (44.7%); \( p=0.038 \) . The median age at diagnosis was younger in the HPV 16/18
group (59 years of age) vs other HPV positive (68 years) and no HPV (77 years) \( (P = .003) \). The HPV distribution did not significantly vary by race or ethnicity, or rural or urban residence (Table 1).

Among all cases, 86% (49/57) were found to be squamous cell carcinomas, whereas 14% (8/57) were adenocarcinomas. Of the squamous cell carcinomas, 31 cases (63.3%) were positive for HPV 16/18, as opposed to only 25% of adenocarcinomas \( (P = 0.08) \). Stage at diagnosis was not statistically different among the HPV subtypes.

The unadjusted all-cause survival for all patients with vaginal cancer who were positive for any HPV was 57.4% vs 35.7% for patients who were HPV negative \( (P = 0.243) \). The unadjusted hazard ratio comparing HPV positive to HPV negative was 0.62 (95% CI 0.28–1.39). However after adjusting for age, the hazard ratio was 1.57 (95% CI 0.63–3.91) (Fig 2)

**Discussion**

In this multicenter study which spanned 11 years, of the 60 samples examined, 75% were HPV positive, much higher than most previously published results \^8,10,14-16^9. Ostrow et al \^8^ found HPV in 21% of 14 patients by using in situ hybridization. Using southern blot hybridization, Ikenberg et al, found 55% of 18 patients positive for HPV\^10^9. Koyamatsu and colleagues\^15^10 and Ferreira et al \^16^11 both used polymerase chain reaction for detection and found HPV in 53% and 81% of 40 and 21 patients, respectively.

It is possible that our HPV detection rate, which is on the higher end of the reported spectrum, is secondary to careful tissue selection, optimized extraction and the inclusion of a second PCR assay if the first assay was unsatisfactory. \^13,17^15

HPV 16 was the most commonly detected type with 33/60 of the samples positive. HPV 33 was detected in 11/60 cases and HPV 18 was only detected in 1/60 cases. Of note, 45% of cases positive for HPV 33 were also co-infected with HPV 16. HPV 33 has not been previously reported to have such a high prevalence in vaginal cancer. The currently licensed HPV vaccines protect against HPV 16 and 18 and our study found that 57% of vaginal cancers could theoretically be prevented. These vaccines do not provide primary coverage for HPV 33, the second most common HPV genotype found in our study. However, Wheeler and co-workers showed vaccination with the quadrivalent vaccine, despite only including 4 HPV types, reduced the infection rate of related HPV types 31, 33, 45, 52, and 58 by 17.7% (95% confidence interval [CI], 5.1% to 28.7%) in pre-invasive cervical lesions.\^18^16 Cross-immunity secondary shared characteristics between the HPV subtypes have been proposed as the mechanism behind this reduction. These results could theoretically be extrapolated to vaginal cancer but further studies are needed.

Previous studies have attributed a longer overall survival for vaginal cancer cases that were HPV positive.\^9,19^9 Our study did not show a statistically significant longer unadjusted survival). The median age in our population for HPV 16 or 18 positive cancers was 18 years younger than those who were HPV-negative. After adjusting for age, the survival hazard ratio for HPV positivity changed from 0.62 to 1.57 but this remained statistically insignificant. This could be partially explained by the younger age at diagnosis of women
with HPV-positive tumors. Younger patients are likely to tolerate more aggressive treatment and have better functional status and less co-morbidity. These results, if validated by larger sample sizes, could show that age-adjusted HPV positivity in vaginal cancer is a poorer prognostic marker and a marker of more aggressive cancers. However, ideally, we would have a larger sample size to control for additional variables such as stage and treatment.

We acknowledge several limitations to our study, one of which is the small sample size, which is attributable to the rarity of the disease. This small sample size yields low statistical power and limits the ability to interpret the data. Vaginal cancer is, however, an extremely rare disease and this is the cumulative data of 7 centers over 10 years. Another limitation in this data is that variables were missing from some cases such as stage and residence (12 and 6 samples respectively). This is an unfortunate problem with retrospectively collected data.

Similar to other reports, 76% of the samples were obtained from Caucasian patients. 76% of the samples were obtained from Caucasian patients, which potentially limits the generalizability of this data to other races. As with all studies based on extracted DNA, the detection of HPV DNA does not necessarily prove its involvement in malignant transformation versus its incidental presence. Another limitation is the use of formalin-fixed tissue blocks, which could potentially underestimate the HPV prevalence rate.

This study describes the prevalence of HPV in vaginal cancer. Given that the majority of vaginal cancers are positive for HPV 16, it is expected that if girls are vaccinated as recommended and coverage is high, we may anticipate a decrease in vaginal cancers by up to 57%.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.

References
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Figure 1. HPV Genotype Distribution in Vaginal Cancer Cases (n = 60) HPV Typing of Cancers Study

Fig 1*Multiple types includes human papillomavirus (HPV) 16/33 (n = 5 56%) HPV 16 / 35 (n = 1, 11%) HPV 16/66 (n = 1, 11%) HPV 16/81 (n = 1, 11%), HPV 51 / 82 (n = 1, 11%).
Figure 2.
5-year all-cause survival by HPV status among vaginal cancer patients. Note: Five-year unadjusted survival estimates are presented as Kaplan-Meier estimates with statistical testing performed using the log-rank test.
| Demographic and Clinical Characteristics of Vaginal Cancer Cases by Human Papillomavirus Status |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|-----------------|
|                                | Overall (N = 60) | HPV 16/18* Positive (n = 34) | Other HPV DNA† Positive (n = 11) | HPV DNA Negative (n = 15) | P               |
| Age at diagnosis               |                  |                                |                                |                               |                 |
| Median (25th, 75th percentiles) | 65 (56, 77)     | 69 (53, 72)                   | 68 (54, 77)                     | 77 (69, 85)                | 0.003           |
| <60                            | 22 (37%)         | 17 (77%)                      | 3 (14%)                         | 2 (9%)                      | 0.04            |
| ≥60                            | 38 (63%)         | 17 (45%)                      | 8 (21%)                         | 13 (34%)                   |                 |
| Race or ethnicity‡ (n=59)      |                  |                                |                                |                               | 0.17            |
| White                          | 45 (76%)         | 23 (51%)                      | 8 (18%)                         | 14 (31%)                   |                 |
| African American               | 4 (7%)           | 2 (50%)                       | 2 (50%)                         | 0 (0%)                     |                 |
| Hispanic                       | 8 (14%)          | 7 (88%)                       | 0 (0%)                          | 1 (13%)                    |                 |
| Asian                          | 2 (3%)           | 1 (50%)                       | 1 (50%)                         | 0 (0%)                     |                 |
| Residence*‡(n=54),            |                  |                                |                                |                               | 0.31            |
| Urban                          | 7 (13%)          | 2 (29%)                       | 2 (29%)                         | 3 (43%)                    |                 |
| Rural                          | 47 (87%)         | 27 (57%)                      | 9 (19%)                         | 11 (23%)                   |                 |
| SEER Summary Stage‡(n=48)     |                  |                                |                                |                               | 0.89            |
| Localized                      | 23 (48%)         | 16 (70%)                      | 3 (13%)                         | 4 (17%)                    |                 |
| Regional                       | 18 (38%)         | 10 (56%)                      | 3 (17%)                         | 5 (28%)                    |                 |
| Distant                        | 7 (15%)          | 4 (57%)                       | 1 (14%)                         | 2 (29%)                    |                 |
| Histology Type‡ (n=57)         |                  |                                |                                |                               | 0.08            |
| Squamous Cell Carcinoma        | 49 (86%)         | 31 (63%)                      | 8 (16%)                         | 10 (20%)                   |                 |
| Adenocarcinoma                 | 8 (14%)          | 2 (25%)                       | 2 (25%)                         | 4 (50%)                    |                 |

Statistical testing was performed using the Pearson chi-square test or Fisher's exact test for discrete variables and the Kruskal-Wallis rank sum test for continuous variables.

* Samples positive for HPV 16 or HPV 18

† Samples positive for any HPV except HPV 16 or HPV 18

‡ Covariate-specific frequencies may sum to less than 60 due to incomplete data, total for race or ethnicity (n=59), residence (n=54), surveillance, epidemiology, end results summary stage (n=48), and histology type, (n=57).