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Anal human papillomavirus infection and abnormal anal cytology in women with genital neoplasia ☆

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ABSTRACT

Objectives. Describe the type-specific prevalence of anal HPV infection in women with lower genital tract intraepithelial neoplasia and cancer. Describe the prevalence of abnormal anal cytology and identify risk factors for anal HPV infection and abnormal anal cytology in this population.

Methods. We performed a cross-sectional study of women attending 2 university-based colposcopy clinics with high-grade lower genital tract intraepithelial neoplasia or cancer. Participants received anal HPV testing/typing, anal cytology and completed a questionnaire detailing medical history and sexual behavior.

Results. Of the 102 women enrolled, 92 (90%) had adequate β-globin for analysis of HPV DNA status, and 47/92 women (51%) had detectable anal HPV. Of the 15 HPV types identified, 9 (60%) were oncogenic types and 6 (40%) were non-oncogenic or undetermined risk types. Overall, 9 women (9%) had abnormal anal cytology, and 7 of those had corresponding anal intraepithelial neoplasia grade I (AIN I). Women with vulvar disease had the highest proportion of abnormal anal cytology (21%) compared to women with cervical disease alone (7%), but this difference was not statistically significant ($p = 0.10$). Neither anal HPV infection nor abnormal cytology was significantly associated with anal sex practices, smoking or number of sexual partners.

Conclusions. Anal infection with high-risk HPV types is common in women with high-grade genital neoplasia, but was not associated with known risk factors for genital HPV infection. Other unidentified risk factors may play a role in the anal HPV infection in this population. Abnormal anal cytology was rare and larger studies are needed to identify risk factors associated with abnormal cytology and anal intraepithelial neoplasia in this population.

Introduction

Oncogenic types of the human papillomavirus (HPV) are the etiologic agents in cervical cancer [1] and have been associated with neoplasia of the vulva and vagina [2]. Anal cancer is also closely linked to HPV infection [3,4] with studies demonstrating presence of HPV in approximately 90% of squamous cell anal cancers and anal intraepithelial neoplasia (AIN), the putative anal cancer precursor [5,6].

Though there are few studies investigating the relationship between genital and anal HPV-related neoplasia, those that exist support the notion of HPV-related neoplasia as a multifocal disease process. Women with cervical intraepithelial neoplasia (CIN) and/or vulvar intraepithelial neoplasia (VIN) demonstrate a high prevalence of concurrent AIN when compared with disease-free controls [7,8]. Women with a history of high-grade CIN demonstrate nearly a five-fold greater incidence of anal cancer compared to the general population [9], which have lead some to recommend anal cancer screening in this population [10,11].

Though the licensure of the quadrivalent prophylactic HPV vaccine has great potential for the prevention of lower genital tract cancer and/or its precursors, its affect on anal HPV infection and prevention of anal cancer is unknown. Identification of the specific HPV types involved in anal infection and the risk factors for anal HPV infection and dysplasia may have implications for the potential efficacy of the HPV vaccine in preventing anal cancer and AIN in high-risk women. Therefore, our objective was to describe the type-specific prevalence

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of anal HPV infection in women with lower genital tract intraepithelial neoplasia and cancer, including vaccine and non-vaccine types. Our other objectives were to describe the prevalence of abnormal anal cytology and identify risk factors for anal HPV infection and abnormal anal cytology in this population.

Materials and methods

Participants were recruited from April 2006 through March 2007 in accordance with guidelines set forth by the University of Minnesota Institutional Review Board (IRB)-Research Subjects' Protection Program. All participants provided informed consent. Eligible subjects included females aged 18–85 years attending one of two university-based colposcopy clinics (University of Minnesota Medical Center-Fairview Women's Health Center or Boynton Health Service-Women's Health Clinic) and meeting any of the following inclusion criteria: 1) Biopsy confirming high-grade cervical intraepithelial neoplasia vaginal or vulvar intraepithelial neoplasia (CIN, VaIN, or VIN II–III) at the time of presentation 2) Biopsy-confirmed diagnosis of CIN, VaIN, or VIN II–III performed within 6 months prior to presentation, 3) Biopsy-confirmed diagnosis of cervical cancer, vulvar or vaginal cancer with biopsy performed within 6 months prior to presentation. Participants were excluded if they had any of the following: 1) History of prior pelvic irradiation for cancer (genital or rectal), 2) Previous diagnosis of anal intraepithelial neoplasia (AIN I–III) or anal cancer, or 3) Surgical absence of the anus. 4) Received one or more doses of HPV vaccine. All participants underwent anal cytology/HPV testing, and completed an anonymous, self-administered questionnaire on medical history (gravity/parity, history of gynecologic/colorectal cancer, HIV status, immunosuppressant medication use), tobacco use (lifetime and recent use), and sexual behavior (age of sexual debut, lifetime number of partners, recent and lifetime anal intercourse), and demographic factors (age, race/ethnicity, annual household income, education level). Sexual risk behavior items were taken from a previously validated questionnaire and the entire questionnaire was pilot tested from with members of the target population prior to its use in the study [12]. Questionnaires identified subjects by study number only.

Clinical examination

All participants were screened for AIN with anal cytology. A moistened polyester swab was inserted into the anal canal up to 3 cm. The swab was rotated in a circular motion and gentle pressure was applied to the walls of the anal canal as the swab was withdrawn in order to sample the entire anal transformation zone. The swab was then placed in liquid cytology media (SurePath, Burlington, NC). Anal cytology results were classified according to the Bethesda classification system as normal, ASCUS (atypical squamous cells of undetermined significance), LSIL (low grade squamous intraepithelial lesion), or HSIL (high-grade squamous intraepithelial lesion [13]. Anal cytology was read by a single cytopathologist; in rare cases where the diagnosis was in question, a second cytopathologist was consulted and a consensus diagnosis was obtained.

All women with cytologic abnormalities of any type (ASCUS or worse) were asked to return for high-resolution anoscopy (HRA). All HRA was performed by a trained anoscopist with assistance from co-investigators who received formal training in HRA. With the aid of 3% acetic acid, areas suspicious for AIN were biopsied and then graded by the pathologist as normal or AIN I–III. Women with AIN were referred to a colon and rectal surgeon for treatment or follow-up examination as appropriate.

Anal HPV DNA testing

After completion of cytology testing, residual liquid media was tested for HPV DNA utilizing polymerase chain reaction (PCR). PCR was performed with MY09/MY11 consensus HPV L1 primers as well as primers for human β-globin which were utilized as an internal control to assure for specimen adequacy. After 35 amplification cycles, samples with presence of both the 450 bp HPV band and 268 bp β-globin control band were considered HPV positive. Samples with absence of the 450 bp HPV band and presence of the 268 bp β-globin control band were considered HPV negative. Samples without the presence of the 268 bp β-globin internal control were considered inadequate for analysis, regardless of HPV result.

Procedures were also in place to avoid lab contamination and possible false positive results. In addition to standard procedures for PCR, specimens were run against two positive controls as well as a negative control (water) to detect reagent contamination. Of note, none of the negative control samples were positive for HPV DNA.

Samples that were both HPV positive and had adequate DNA for analysis were genotyped using restriction fragment analysis, including oncogenic/probable oncogenic types (HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51–53, 56, 58, 59, 66, 68, 73, 82, and IS39), non-oncogenic types (HPV types 6, 11, 40, 42, 54, 61, 70, 72, 81, and CP6108), and types with undetermined risk status (HPV types 55, 62, 64, 67, 69, 71, 83, and 84 [14,15]. All HPV typing was performed by technicians who were blinded to the subject's clinical history or questionnaire responses.

Table 1
Characteristics of subjects in study population ($N = 102$).

	N (% of total)
Age at enrollment (years)	
18–24	30 (29)
25–38	37 (36)
>38	35 (34)
Mean age (±SD)	35.2 (±15.0)
Education	
<High school	6 (6)
High school/GED	16 (16)
Some college	42 (41)
Bachelor's degree/grad school	38 (37)
Income (missing = 2)	
<$20,000	33 (33)
$20,001–40,000	27 (27)
$40,001–60,000	19 (19)
>$60,000	21 (21)
Race/ethnicity	
Black/African-American	1 (1)
White non-Hispanic	88 (87)
Hispanic	5 (5)
Asian	6 (6)
Multiethnic/other	2 (2)
Gender of sexual partners (missing = 3)	
Men only	92 (93)
Men and women	7 (7)
Age of sexual debut (missing = 3)	
≤16 years	33 (33)
≥17 years	66 (67)
Lifetime # sexual partners (missing = 4)	
1–2	19 (20)
3–9	42 (42)
≥10	37 (38)
Smoking status	
Current or former smoker	57 (56)
Never smoked	45 (44)
Location/type of neoplasia	
Cervix (CIN II, CIN III, AIS)	58 (57)
Cervix (cancer)	10 (10)
Vulva (VIN II, VIN III)	9 (9)
Vulva (cancer)	5 (5)
Vagina (VaIN II, VaIN III)	5 (5)
Multifocal[a] (cervix + vagina or vulva)	15 (15)

Percentages may not total 100 due to rounding.
[a] Multifocal dysplasia = at least 1 foci of high-grade dysplasia.