Burden of Cervical, Anal, and Oral HPV in an Inner-City Pre-vaccine Adolescent Population

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INTRODUCTION

HPV is the central etiologic agent in cervical and anal cancers and a percentage of head and neck tumors. The FDA-approved prophylactic quadrivalent vaccine (GARDASIL®, Merck & Co., PA) targets the most common types of HPV found in cervical cancers (HPV16/18) and genital warts (HPV6/11).^{1,2} Current recommendations are to vaccinate young adolescents prior to exposure, after which the vaccine may be much less efficacious.³ However, catch-up vaccination is also underway for women up to the age of 26, and HPV coverage among adolescent females in the U.S.A. is lagging, with only one third having received the full three-dose series.⁴ Furthermore, while the prevalence of cervical HPV is at its highest in adolescent women, how this relates to other orifices involved in sex (i.e., anal canal and oral cavity) and to vaccine effectiveness in high-risk young women, is the focus of ongoing studies. We set out to assess the prevalence of HPV within the cervix, anal canal, and oral cavity in adolescent women prior to institution of the HPV vaccine from a high-risk inner-city minority population.

METHODS

Prior to release of the HPV vaccines, we conducted a survey of 97 sexually active female patients between the ages of 15 to 22 attending the Mount Sinai Adolescent Health Center, the largest adolescent-specific primary care facility in the U.S.A., between July 2005 and March 2006. Adolescent women undergoing a routine gynecological exam including sexually transmitted infections screening, follow-up for genital warts, and/or atypical cervical Pap smears were included. No participants had received the HPV vaccine at the time of enrollment. Subjects underwent a detailed clinical interview and cervical and anal specimens were collected in ThinPrep® medium using a Cytobrush®, and oral specimens were obtained from an oral rinse with Scope® mouthwash. Written informed consent was collected from all participants prior to enrollment, and the study was approved by the Institutional Review Boards of the participating institutions.

Samples were tested for the presence of HPV DNA following isolation of DNA, using a polymerase chain reaction (PCR) protocol that amplifies a highly conserved

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450-basepair segment in the L1-HPV gene using the MY09/MY11 primer system following a standardized protocol.⁵

Prevalence of HPV was assessed for approximately 40 different specific types of HPV separately and grouped according to oncogenic potential and phylogenetic relatedness. High-risk HPV types include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 reported in recent evidence-based reviews.^{2,6} Individual types were also grouped into related *Alpha papillomavirus* (α) species groups established from phylogenetic relatedness to the vaccine types (i.e., 6, 11, 16, and 18).⁷

We assessed the concordance in HPV detection between cervical and extra-cervical sites within the same person using kappa statistics.⁸

RESULTS

The cohort consisted predominantly of African-American or Hispanic young women, with a median age of 19 years (Table 1). Only 7 % of subjects identified themselves as non-Hispanic White. The majority of subjects (82 %) had had at least three sexual partners at the time of enrollment, and 53 % had four or more partners. The overall median age at first vaginal intercourse was 14 years (range 12–19), and history of sexually transmitted infections (STIs; 47 %) was high, including for Chlamydia (33 %). Over a third of cervical Paps collected at enrollment showed atypical cells of undetermined significance (ASCUS; 23 %) or presence of squamous intraepithelial lesions (LSIL 15 %/HSIL 1 %). Anal Pap results were available for 62 subjects, of which 11 had ASCUS.

HPV DNA was detected in 59 % of cervical, 57 % of anal, and 12 % of oral specimens (Table 2). We identified 30 different HPV types in the cervix, 24 in the anal canal, and 6 in the oral cavity; for a total of 32 different types across all three sites. Types most frequently identified in the cervix included HPV6, 16, 31, 51, 52, 61, and 81; HPV6, 51, and 52 in the anal canal; and HPV16 in the oral cavity. HPV types detected at all three anatomic sites included 16, 32, 33, and 51. Twenty percent of cervical, 17 % of anal, and 2 % of oral specimens harbored HPV types included in the quadrivalent vaccine (i.e., HPV6, 11, 16, and 18). In addition, 23 % of cervical, 18 % of anal, and 3 % of oral samples tested positive for HPV16-related types from the *alpha*-9 species (e.g., HPV31, 33, 35, 52, 58, and 67), and 10 % of cervical and 1 % of anal samples tested positive for HPV18-related types from the *alpha*-7 species (e.g., HPV39, 45, 68, and 59). HPV6/11-related types that were detected included HPV55 and 93 from the *alpha*-10 species in the cervix and oral cavity, respectively.

HPV prevalence at any anatomic site was 73 % with many adolescents having HPV infection at two sites (44 %, $n=41$), and 9 % at three sites ($n=8$). Assessment of type-specific co-infection across anatomic sites showed that 53 % of anal HPV-positive and 1 % of oral HPV-positive subjects had the same HPV type in the cervix. Type-specific concordance between cervical and anal HPV for the quadrivalent vaccine types HPV16 and 18 was lower than for HPV6 and 11; i.e., 30 % of HPV16-positive subjects ($n=3/10$) were positive for both the cervix and anal canal (kappa=0.43, 95 % CI 0.1–0.8), 20 % ($n=1/5$) were positive for HPV18 (kappa=0.31, 95 % CI –0.2–0.8), 42 % ($n=5/12$) for HPV6 (kappa=0.55, 95 % CI 0.2–0.8), and 50 % ($n=1/2$) for HPV11 (kappa=0.66, 95 % CI 0.04–1.0). No individuals had the same vaccine type at all three sites.

TABLE 1 Study sample demographic and sexual history characteristics

Characteristic	N	%
Race/ethnicity		
African American	52	54
Hispanic	38	39
White non-Hispanic/other	7	7
Sexual activity		
Number of vaginal sexual partners		
1	17	18
2–3	27	28
4–9	34	35
10–25	17	18
Age at first intercourse		
12–15	68	70
16–19	26	27
Anal intercourse ever		
Yes	24	24
Oral sex ever		
Yes	58	60
History of contraceptive use		
Condom use during vaginal sex		
Always	19	20
Sometimes	61	64
Never	12	13
Other contraceptives		
None	18	19
Any prescription contraceptive ^b	74	76
Pill	50	52
Patch	29	30
Depo-Provera shot	24	25
Number of pregnancies		
0	52	54
1	27	28
2–5	14	14
History of STI ^a		
None	49	51
Any STI ^b	46	47
Chlamydia	32	33
Bacterial vaginosis	11	11
Trichomoniasis	8	8
Genital warts	5	5
Cervical Pap ^c		
Negative/within normal limits	52	54
Atypical cells of undetermined significance	22	23
Low-grade squamous intraepithelial lesions	15	15
High-grade squamous intraepithelial lesions ^d	1	1
Anal Pap ^c		
Negative/within normal limits	51	53
Atypical cells of undetermined significance	11	11
Non-diagnostic/unsatisfactory sample	21	22

^aAny sexually transmitted infection (STI) excluding HPV

^bSubjects may have used more than one form of contraceptive and had more than one STI

^cCategory totals may not add up to 97 due to missing data

^dIncludes one case of low-grade squamous intraepithelial lesion (SIL) with foci of high-grade SIL

DISCUSSION

This study detected a high rate of cervical HPV infection among pre-vaccinated inner-city adolescent females seeking gynecological care, including HPV vaccine types and related types. The current quadrivalent HPV vaccine is recommended for females 9–26 years of age for prevention of cervical and anal diseases caused by

TABLE 2 HPV types detected across different anatomic sites in adolescent females

HPV type	Cervix (n=96)	Anal (n=92)	Oral (n=93) ^a	All sites (n=88) ^b	Kappa (95 % CI) ^c
HPV6	8 (8 %)	9 (10 %)	—	12 (14 %)	0.55 (0.2–0.8)
HPV11	2 (2 %)	1 (1 %)	—	2 (2 %)	0.66 (0.04–1.0)
HPV16	9 (9 %)	4 (4 %)	2 (2 %)	12 (14 %)	0.43 (0.1–0.8)
HPV18	3 (3 %)	3 (3 %)	—	4 (5 %)	0.31 (–0.2–0.8)
All vaccine types (6, 11, 16, 18)	19 (20 %)	16 (17 %)	2 (2 %)	24 (27 %)	0.54 (0.3–0.8)
HPV alpha species					
α10	10 (10 %)	10 (11 %)	1 (1 %)	15 (17 %)	0.55 (0.3–0.8)
α10 (not HPV6/11)	1 (1 %)	—	1 (1 %)	2 (2 %)	0.00 (—)
α9	28 (29 %)	19 (21 %)	3 (3 %)	34 (39 %)	0.54 (0.3–0.7)
α9 (not HPV16)	22 (23 %)	17 (18 %)	1 (1 %)	27 (31 %)	0.54 (0.3–0.7)
HPV31	7 (7 %)	4 (4 %)	—	8 (9 %)	0.52 (0.2–0.9)
HPV52	8 (8 %)	7 (8 %)	—	11 (13 %)	0.49 (0.2–0.8)
HPV58	6 (6 %)	3 (3 %)	—	7 (8 %)	0.42 (0.1–0.8)
α7	12 (13 %)	4 (4 %)	—	13 (15 %)	0.20 (–0.1–0.5)
α7 (not HPV18)	10 (10 %)	1 (1 %)	—	10 (11 %)	0.16 (–0.1–0.5)
HPV45	4 (4 %)	1 (1 %)	—	4 (5 %)	0.39 (–0.1–0.9)
α11	4 (4 %)	4 (4 %)	—	8 (9 %)	–0.05 (–0.1–0.01)
α1/α13	1 (1 %)	2 (2 %)	1 (1 %)	4 (5 %)	–0.02 (–0.05–0.02)
α5/α6	19 (20 %)	12 (13 %)	1 (1 %)	21 (24 %)	0.50 (0.3–0.7)
α3	17 (18 %)	10 (11 %)	—	19 (22 %)	0.38 (0.1–0.6)
HPV risk group					
All high-risk HPV	38 (40 %)	28 (30 %)	4 (4 %)	44 (50 %)	0.44 (0.3–0.6)
High-risk HPV (not HPV16/18)	33 (34 %)	25 (27 %)	2 (2 %)	39 (44 %)	0.45 (0.3–0.6)
All low-risk HPV ^d	41 (43 %)	30 (33 %)	7 (8 %)	52 (59 %)	0.15 (–0.1–0.4)
Low-risk HPV (known types)	37 (39 %)	24 (26 %)	2 (2 %)	45 (51 %)	0.24 (0.04–0.4)
Low-risk HPV (not HPV6/11)	32 (33 %)	21 (23 %)	2 (2 %)	41 (47 %)	0.17 (–0.04–0.4)
All HPV (total)	57 (59 %)	52 (57 %)	11 (12 %)	64 (73 %)	0.44 (0.3–0.6)

^aCells with zero observations are denoted by “—”

^bAdequate HPV and beta-globin (control DNA) signal was obtained for cervical, anal, and oral samples from 88 subjects

^cKappa statistic and 95 % confidence interval (CI) for concordance between cervical and anal HPV detection

^dIncludes uncharacterized types

HPV. While vaccine uptake is increasing among U.S. adolescents,⁴ vaccine efficacy is reportedly only observed among women without evidence of prior or current HPV infection with the vaccine types.^{3,9–11} Therefore, the full benefit of vaccination may not be seen for high-risk populations with prior cervical or extra-cervical exposure to HPV vaccine types. This highlights the importance of early vaccination, although vaccinating sexually active adolescents may still provide a benefit, either against other HPV types targeted by the vaccine (e.g., for HPV16 among those HPV-DNA positive for HPV18 only and vice versa, which still include the majority of adolescents tested), or for phylogenetically related *alpha*-9 and -7 HPV types (e.g., HPV31 and 45) that may benefit from cross-protection.^{12,13}

This study shows only moderate concordance in detection of HPV vaccine high-risk (HPV16 and 18) and related types (HPV31 and 45) between cervical and extra-cervical sites. Although the implications of prevalent anal or oral HPV infections on vaccine efficacy are unknown, this suggests post-vaccine surveillance studies of cervical, anal, and oral HPV in real-world settings and high-risk populations are warranted.

The reported study has strengths and limitations. First, subjects were not randomly selected and do not represent adolescents overall. All subjects were sexually active and were on average older than the targeted group for vaccination (i.e., 9–12 years). In addition to a history of STI and genital warts, 30 % ($n=29/97$) of subjects also had a history of atypical or abnormal cervical Paps within the 3 years before participation, reflecting the high-risk nature of the study group. Secondly, we did not assess what proportion of infections persisted, nor did we test for antibody levels of HPV vaccine types, which would indicate prior exposure. These data nevertheless underscore for both clinicians and patients that future cervical screening and gynecological exams will still be necessary in vaccinated adolescents, particularly sexually active women.

Among the strengths, the current study evaluated HPV at multiple anatomical sites known to harbor HPV. While it is generally understood that acquisition and prevalence of cervical HPV is at its highest in adolescent women, how this relates to the prevalence and acquisition of HPV in other tissues susceptible to high-risk HPV-associated tumorigenesis (i.e., the anal epithelium and oral cavity), is not currently known. Furthermore, the association of HPV with anal and oropharyngeal papillomas and neoplasia is also an important public health concern. Given recent reports on the increasing burden of anal and oral HPV and associated neoplasia,^{14–17} and lower efficacy of the vaccines against anal HPV,^{18,19} examination of samples from extra-cervical sites adds to our understanding of the HPV burden.

In conclusion, the detection of extra-cervical, as well as cervical HPV, in high-risk adolescent women highlights the public health importance of early vaccination. However, the degree to which the vaccine may be effective in preventing extra-cervical infection in high-risk adolescent populations and that infection at extra-cervical sites might impact effectiveness in the cervix, and vice versa, is currently under investigation.

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