Papillomavirus Research 3 (2017) 50–56

Contents lists available at ScienceDirect

Papillomavirus Research

journal homepage: www.elsevier.com/locate/pvr

HPV serostatus pre- and post-vaccination in a randomized phase II preparedness trial among young Western Cape, South African women: The evri trial

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ARTICLE INFO

Keywords:
HPV vaccine
EVRI
Seroprevalence
cLIA

ABSTRACT

Background: HPV antibodies are a marker of past exposure to the virus. Our objective was to assess HPV serostatus pre- and post-vaccination among HIV-negative women.
Methods: Women aged 16–24 years old were randomized in a placebo controlled trial utilizing the 4-valent HPV (4vHPV) vaccine (NCT01489527, clinicaltrials.gov). Participants (n=389) received the 4vHPV vaccine or placebo following a three dose schedule. Sera were collected at Day 1 and Month 7 for assessment of HPV 6, 11, 16, and 18 neutralizing antibody levels using a multiplex competitive Luminex immunoassay (Merck) based on detecting the L1 capsid antigen for each HPV type.
Results: Seroprevalence was 73% for HPV6, 47% for HPV11, 33% for HPV16, and 44% for HPV18. Seroprevalence for any HPV type did not significantly differ by age or lifetime number of partners. The majority of participants (64%) had two or more 4vHPV antibodies present at enrollment and 12% had antibodies to all four. Among women in the vaccine arm, those that were seropositive for HPV16 at enrollment had higher titers at month 7 compared to women that were seronegative for HPV16 at enrollment; this trend holds for the other HPV types as well. Seroconversion among baseline seronegative participants in the placebo group ranged from 5% for HPV16 to 23% for HPV6.
Conclusion: HPV seroprevalence was high in this population, emphasizing the need to vaccinate prior to sexual debut.

1. Introduction

Women and men residing in southern African countries have a high burden of human papillomavirus (HPV) infection and related cancers [1–4]. Age-standardized incidence rate for cervical cancer in Southern Africa is 31.5 per 100,000 women and the age-standardized mortality rate is 17.9 per 100,000 women [3]. Cervical cytology screening can dramatically decrease the incidence of cervical cancer. South Africa has a national policy for cervical cancer screening which has been implemented primarily in the private sector but is lacking in the state sector [5]. A small proportion of South African women are screened for cervical cancer and an even smaller portion return for treatment of lesions [5]. These low trends in cervical screening coupled with the high HIV prevalence likely explain why cervical cancer incidence is high in South Africa.

HPV is a common infection among women worldwide and is the necessary cause of cervical cancer. Once infected with HPV, the majority of individuals will naturally clear the infection through an immune response. A proportion of those individuals that clear the infection will develop a detectable L1 capsid antibody to the specific HPV genotype 9–24 months after infection [6–8]. Antibody titers detectable after a natural infection are significantly lower than the

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http://dx.doi.org/10.1016/j.pvr.2017.02.001
Received 16 September 2016; Received in revised form 9 January 2017; Accepted 2 February 2017
Available online 16 February 2017

Table 1
Baseline characteristics of women seropositive for at least one of the 4vHPV (HPV6/11/16/18) types compared to women that were seronegative for all four HPV types.

	Seronegative (n=47), n (%)	Seropositive (n=342), n (%)	Pvalue*
Age in years			0.68
16 – 18	12 (25.5)	67 (19.6)	
19 – 21	22 (46.8)	172 (50.3)	
22 – 24	13 (27.7)	103 (30.1)	
Survey language			0.08
English	43 (91.5)	331 (96.8)	
Xhosa	2 (4.3)	2 (0.6)	
Afrikaans	2 (4.3)	9 (2.6)	
Marital status			**0.007**
Single/Widowed	41 (87.2)	332 (97.1)	
Married/Living Together	6 (12.8)	10 (2.9)	
Education			0.51
≤ Grade 7	1 (2.1)	19 (5.6)	
Grade 8–12	29 (61.7)	181 (52.9)	
Passed Grade 12	10 (21.3)	70 (20.5)	
me college/tech	7 (14.9)	72 (21.1)	
Ever been pregnant	21 (44.7)	172 (50.3)	0.54
Current birth control use			
Oral Contraceptives	1 (2.1)	13 (3.8)	0.71
IUD/Loop/Coil	1 (2.1)	1 (0.3)	0.24
Depo Provera	11 (23.4)	89 (26.0)	0.73
Condoms	6 (12.8)	88 (25.7)	0.07
Age at first vaginal sex; median (range)[a]	16.5 (14–21)	17.0 (12–21)	0.93
Lifetime no. of male sexual partners[b]			0.87
1	8 (22.2)	48 (18.3)	
2	10 (27.8)	79 (30.3)	
3+	18 (50.0)	134 (51.3)	
Ever received presents/money/drugs for sex	1 (2.1)	11 (3.2)	1.00
Diagnosis of STI through clinical test			
Gonorrhea	5 (10.6)	38 (11.1)	1.00
Chlamydia	10 (21.3)	117 (34.2)	0.10
Herpes Simplex	11 (23.4)	168 (49.1)	**0.001**
Syphilis	2 (4.3)	23 (6.7)	0.57
Cervical Cytology			0.50
Normal	43 (91.5)	300 (87.7)	
Abnormal	4 (8.5)	42 (12.3)	

* Pvalue calculated from Pearson chi-square or Fisher Exact test and Mann-Whitney tests.
[a] Sample size reduced due to errors in reporting sexual behavior: seronegative (n=36) and seropositive (n=261).
[b] Sample size reduced due to errors in reporting sexual behavior: seronegative (n=36) and seropositive (n=272).

antibody titers achieved following HPV vaccination [9] and titers among the vaccinated remain detectable year post-vaccination [10]. The licensed HPV vaccines are efficacious in preventing HPV infection and pre-malignant anal, cervical, vulvar, and vaginal lesions [10–12]. In countries without adequate cervical cancer screening programs, uptake of the vaccine could have a major public health impact given the high incidence of cervical cancer in those countries.

HPV prevalence is highest in younger women but the age trends differ by country and world region with infection remaining high through older ages in some countries [13,14]. We have previously reported a high cervical HPV prevalence of 71% among HIV-negative 16–24 year olds in Western Cape, South Africa [15]. HPV prevalence was highest in the youngest aged women (83% among age 16–17 years) and decreased with age (60% among 24 year olds) [15]. HPV incidence was also higher among 16–18 year olds compared to 22–24 year olds [16]. HPV antibodies to conformational L1 epitopes are a marker of past HPV exposure to that specific HPV type. Nested within a Phase II vaccine trial, our objective was to assess HPV seroprevalence and seroconversion among HIV-negative women.

2. Methods

The Efficacy of HPV Vaccine to Reduce HIV Infection (EVRI) Trial (NCT01489527, clinicaltrials.gov) preparedness study enrolled women residing in the Western Cape, South Africa from November 2012 to July 2013. A full description of study procedures and conduct of the trial has been published elsewhere [15]. Briefly, women aged 16–24 years that were HIV-negative and non-pregnant were randomized 1:1 in a Phase II controlled trial of Gardasil (4-valent HPV (4vHPV) vaccine) vs. placebo (saline).

This study was conducted in accordance with ethics committee review and approved by the Institutional Review Boards of The University of South Florida and Stellenbosch University. South African policies and ethics approval regarding parental permission for children to take part in research studies were followed.

All staff and study investigators were blinded to participants' vaccine status except the pharmacist dispensing the vaccine (S.K.). Vaccine was administered at enrollment, month 2, and month 6. Study participants were followed for one month after the last vaccine dose (through month 7). At the 7-month visit, individual unblinding occurred, and women randomized to the placebo group were offered the Gardasil vaccine.

At each trial visit after randomization, urine pregnancy tests and rapid HIV tests were performed. Women with positive pregnancy tests were referred to care and excluded from the study. Women with a positive rapid HIV test were retested with two different confirmatory tests. Participants with a confirmed positive HIV test after the enrollment visit were referred to care and remained on trial [17]. At the enrollment and 7-month visits, sexual history, health, and socio-demographic characteristics were assessed by a tablet-based questionnaire using a computer-assisted self-interview.

2.1. Laboratory analyses

Sexually transmitted infection (STI) testing methods for chlamydia, gonorrhea, syphilis, and HSV-2 have previously been reported [15]. For HPV analyses, DNA was extracted from cervical cell specimens using the Qiagen Media Kit and amplified by polymerase chain reaction (PCR) with the PGMY09/11 L1 consensus primer system and AmpliTaq Gold polymerase (Perkin-Elmer) [15,16]. HPV genotyping was conducted on all specimens, regardless of PCR results, using the Linear Array HPV Genotyping Test (Roche Diagnostics), which detects 37 HPV genotypes [18,19].

Sera were collected at Day 1 and Month 7 for assessment of HPV neutralizing antibody levels. Immune response was measured using a multiplex competitive Luminex immunoassay (anti-HPV-6, −11, −16, −18, −31, −33, −45, −52, and −58 cLIA; Merck) at Pharmaceutical Product Development, Inc [20]. This assay simultaneously quantitates neutralizing antibodies to nine HPV types based on the L1 capsid antigen in 50 μL of serum. Antibody levels were reported as milli-Merck units (mMU) per milliliter (mL) serum. Cut points for determination of antibody positivity for HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 were 16, 6, 12, 8, 4, 4, 3, 3, and 4 mMU/mL, respectively.

2.2. Statistical analysis

Demographic and sexual behavior characteristics at enrollment were compared between women that were seropositive for at least one of the 4vHPV types to women that were seronegative to all four types (Table 1) using Pearson chi-square or Fisher Exact test for