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Randomized trial of HPV4 vaccine assessing the response to HPV4 vaccine in two schedules among Peruvian female sex workers

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ABSTRACT

Two hundred female sex workers (FSWs) in Lima, Peru were randomized to receive HPV4 vaccine in the standard (0, 2, 6 months) or a modified schedule (0, 3, 6 months). One hundred and eighty four (92%) participants completed 3 doses of vaccine. Baseline seropositive rates were 58% for HPV6, 22.5% for HPV11, 41.5% for HPV16, and 13% for HPV18. The final geometric mean antibody titer (GMT) following vaccination was significantly greater for women who were seropositive at baseline compared to seronegative women: HPV6 (GMT ratio = 2.3, $p < 0.01$), HPV11 (GMT ratio = 2.7, $p < 0.01$), HPV16 (GMT ratio = 1.3, $p = 0.04$), and HPV18 (GMT ratio = 2.4, $p < 0.01$). Antibody titers in the modified schedule were not inferior to those in the standard schedule, suggesting the modified schedule may be paired with required STD visits. Although all women benefit from vaccination, administration at a younger age and before sexual debut is needed to achieve maximum protection from vaccine.

1. Introduction

Approximately 500,000 women develop cervical cancer each year worldwide, and persistent human papillomavirus (HPV) infection is found in nearly all cases [1]. Studies of HPV vaccines were conducted in girls and young women 9–26 years of age with the primary objective to prevent cervical cancer [2]. HPV vaccines have been shown to be highly efficacious against cervical intraepithelial neoplasia associated with types 16 and 18 in women who were not infected at the time of immunization [3]. For each HPV4 associated genotype, antibody titer at 1 month following final vaccine dose was 27–145 times higher among placebo recipients who were seropositive at baseline [2].

Female sex workers (FSWs) are presumably at higher risk of HPV infection and cervical cancer than the general population due to their exposure to multiple sexual partners [4,5]. Studies of HPV among FSWs worldwide report cervical HPV DNA prevalence rates of 2.3–100% [6–10]. DNA prevalence of HPV4-associated genotypes among FSWs ranged from 3.4–45.8% in studies in Spain and Mexico [9,10]. We have identified one article which describes general HPV antibody prevalence among FSWs, but specific antibody values are not indicated [10].

HPV DNA prevalence among women in Peru is 17.7%, nearly twice the worldwide rate; cervical cancer is the leading cause of cancer death in Peruvian women, responsible for 20.6% of cancer deaths [11,12]. FSWs in Peru are required to receive STD and HIV testing every 3 months to obtain their health card and maintain their legal working status in brothels. Fewer than 10% of Peruvian FSWs were aware of HPV vaccine in previous studies [13].

Vaccination of new brothel-based FSWs at routine screening visits could increase completion rates, lower the risk of HPV related disease, and potentially decrease transmission to sex partners and clients [14]. We provided HPV vaccine to FSWs in Lima, Peru and collected serum before and after vaccination to evaluate the serologic response rates by baseline serologic status. We also investigated a modified immunization schedule and its effect on vaccine completion.

2. Materials and methods

FSWs 18–26 years of age were recruited between August 28, 2009 and March 3, 2010 from 49 different sex locales in Lima, Peru by trained medical staff and 8 health promoters. Inclusion criteria were: registered FSW aged 18–26 years, living in Lima, no

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reported immune deficiency (including HIV), not pregnant or planning a pregnancy in the next 7 months, having a uterus, and not having received HPV vaccine. Participants were randomized in a 1 to 1 ratio to receive HPV4 vaccine in the standard (0, 2, 6 months) or a modified schedule (0, 3, 6 months) which paired more closely with 3-month clinic visits to receive STI testing. Stata 9.0 (Statacorp, College Station, TX) was used by study investigators to generate a random allocation sequence for the two study arms in block sizes of 8 to maintain balance in treatment groups. Participants opened sequentially numbered sealed envelopes with a letter written on paper which corresponded to study arms (0, 2, 6) or (0, 3, 6). All women were asked to return for their next visit according to their schedule, and to return for a final study visit one month after the third vaccine dose.

Baseline surveys consisted of 52 questions including demographic data, sexual health, condom use, HPV knowledge, barriers to vaccination, and medical history. Surveys were administered in Spanish by a trained interviewer. All participants had a physical examination. A cervical swab was collected for HPV DNA testing using the Digene HPV sampling kit (Qiagen). Five milliliters of blood was collected at baseline and one month following final vaccination dose.

2.1. Data analyses

Survey data and laboratory results were analyzed in EpiInfo 3.5.1 and Stata 10.0. Pearson's chi-square tests were computed to test for differences in variables by baseline serostatus. The association between HPV DNA prevalence and serology was calculated using Fisher's exact tests. Comparison of antibody titers was done using *t*-tests on log transformed data. Associations of variables with antibody response were calculated using linear regression on log transformed antibody titer and *p*-values are from *F*-testing. Adherence was measured as receiving all 3 vaccine doses within a 30-day window of the scheduled vaccine dose.

Sample size was calculated using PASS 2008. With 80% power, type 1 error of 0.05, standard deviations of 0.6, and an equivalence margin of 0.3, 64 women were needed per study arm to detect non-inferiority. The primary outcome was antibody response following vaccination in the two study arms. Secondary outcomes included seroprevalence prior to vaccination and a comparison of seropositivity to cervical HPV DNA prevalence.

2.2. Cervical samples

Cervical samples were aliquoted, refrigerated at −20 °C, and sent to Johns Hopkins Bloomberg School of Public Health for testing. Aliquots of water without sampling were shipped from Peru and tested as negative controls. DNA was extracted using the QIAamp DNA Blood Kit (Qiagen). SiHa and K562 cells spiked into STM collection medium were used as positive and negative extraction controls, respectively in each extraction batch. Samples were analyzed for the presence or absence of HPV DNA and genotyped using the Roche HPV Linear Array test.

2.3. HPV antibody titer

Within 30 min of collection, blood was centrifuged at 20 °C for 5 min at 3000 rpm. Serum was removed, stored in 2 aliquots, and shipped to Pharmaceutical Product Development (Wayne, PA) for testing using the multiplexed competitive Luminex immunoassay [15,16]. All values were reported in milli Merck units (mMu). The established antibody cutoffs for seropositivity of the HPV competitive Luminex immunoassay are: HPV6 = 20 mMu, HPV11 = 16 mMu, HPV16 = 20 mMu, HPV18 = 24 mMu as per the analysis by Dias et al. [16].

2.4. Institutional review board (IRB) approval

This study was approved by IRBs at the Johns Hopkins Bloomberg School of Public Health in Baltimore, MD, and the Universidad Peruana Cayetano Heredia and Via Libre in Lima, Peru. All participants provided written informed consent. This clinical study was registered with clinicaltrials.gov identifier NCT00925288 under trial registry name "Acceptability of Human Papillomavirus (HPV) Vaccine in Female Sex Workers (Girasol)".

3. Results

Five hundred and ninety nine women were screened for eligibility between August 28, 2009 and March 3, 2010; 399 women were excluded ($n = 126$ non-eligible, $n = 273$ refused to participate). Two hundred participants were randomized to receive HPV vaccine in the standard or a modified schedule (Fig. 1). One hundred and eighty four (92%) participants completed 3 doses of vaccine, and 95% of those adhered to the schedule, with the final vaccine visit blood draw on December 3, 2010. There were no differences in vaccine completion by study arm, with 91 participants completing the 0, 2, 6 schedule and 93 completing the 0, 3, 6 schedule. Eleven participants were lost to follow-up (LFU) before the final blood draw in the 0, 2, 6 schedule, compared to 7 participants LFU in the 0, 3, 6 schedule, and 182 samples were analyzed for HPV antibody titer. No adverse events were experienced or reported by participants.

The average age of participants was 22.9 years. Mean age at first sex was 15.9 years. Seventy percent of women had previously heard of HPV. One hundred and fifty eight participants (79%) were seropositive for HPV6, 11, 16, or 18 at baseline. Presence of a HPV4 associated genotype (OR = 4.90, 95% CI 1.43–16.71), never having ever heard of HPV (OR = 0.42, 95% CI 0.21–0.86) and having a low or normal BMI (OR = 0.87, 95% CI 0.76–0.98) were significantly associated with HPV4-associated seropositivity (Table 1). Having an STD in the past year (OR = 2.18, 95% CI 0.91–5.26) and prevalence of any HPV DNA (OR = 1.94, 95% CI 0.96–3.89) was marginally associated with baseline serostatus. Age was not associated with HPV seroprevalence (OR = 1.26, 95% CI 0.64–2.51).

Baseline seropositive rates were 58% for HPV6, 22.5% for HPV11, 41.5% for HPV16 and 13% for HPV18. Four women (2%) were seropositive for all 4 genotypes, 31 (15.5%) had both types 6 and 11, and 12 (6%) women were seropositive for types 16 and 18. One hundred and fifty eight women were seropositive for any HPV4 type. In total, 11 women had genital warts at baseline, of which 7 were seropositive for HPV6. Baseline geometric mean antibody titers did not vary by study schedule ($p > 0.2$ for all HPV4 genotypes).

3.1. Comparison of HPV DNA and baseline seropositivity

We have cervical HPV DNA results from 199 participants and serology from 200 participants. Twenty three percent of participants were DNA positive for any of the HPV4-associated genotypes at baseline, compared to 79% who were seropositive for the same types (Table 2). No participants had more than one HPV4 genotype in DNA testing, while 42% of participants were seropositive for at least two HPV4 types, and 12% were seropositive for three HPV4 types.

Significantly more women were seropositive for HPV16 (OR = 3.6, 95% CI 1.54–8.48) and for any HPV4-associated genotype (OR = 4.9, 95% CI 1.43–16.7) than DNA positive. There were no significant differences in HPV6, 11, or 18 alone in DNA and sera