

# Condom Use and the Risk of Genital Human Papillomavirus Infection in Young Women

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## ABSTRACT

*Background* To evaluate whether the use of male condoms reduces the risk of male-to-female transmission of human papillomavirus (HPV) infection, longitudinal studies explicitly designed to evaluate the temporal relationship between condom use and HPV infection are needed.

*Methods* We followed 82 female university students who reported their first intercourse with a male partner either during the study period or within two weeks before enrollment. Cervical and vulvovaginal samples for HPV DNA testing and Papanicolaou testing were collected at gynecologic examinations every four months. Every two weeks, women used electronic diaries to record information about their daily sexual behavior. Cox proportional-hazards models were used to evaluate risk factors for HPV infection.

*Results* The incidence of genital HPV infection was 37.8 per 100 patient-years at risk among women whose partners used condoms for all instances of intercourse during the eight months before testing, as compared with 89.3 per 100 patient-years at risk in women whose partners used condoms less than 5 percent of the time (adjusted hazard ratio, 0.3; 95 percent confidence interval, 0.1 to 0.6, adjusted for the number of new partners and the number of previous partners of the male partner). Similar associations were observed when the analysis was restricted to high-risk and low-risk types of HPV and HPV types 6, 11, 16, and 18. In women reporting 100 percent condom use by their partners, no cervical squamous intraepithelial lesions were detected in 32 patient-years at risk, whereas 14 incident lesions were detected during 97 patient-years at risk among women whose partners did not use condoms or used them less consistently.

*Conclusions* Among newly sexually active women, consistent condom use by their partners appears to reduce the risk of cervical and vulvovaginal HPV infection.

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Genital human papillomavirus (HPV) infections are common in sexually active young women,<sup>1,2,3,4</sup> and certain types of HPV are causally related to anogenital cancers<sup>5</sup> and warts.<sup>6</sup> Although evidence demonstrates that the use of condoms by men substantially reduces the risk of genital transmission of human immunodeficiency virus in women,<sup>7</sup> data on the effectiveness of condoms in reducing the incidence of other sexually transmitted infections are more limited.<sup>8,9</sup> In particular, several studies have found that condom use by men does not reduce the risk of HPV infection in women,<sup>1,4,10,11,12,13</sup> and recent congressional hearings have addressed changing Food and Drug Administration regulations on condom labeling.<sup>14</sup> Most data on condom use and HPV infection, however, are from cross-sectional studies,<sup>10,11,12,13,15</sup> and the prospective studies

reported to date were not explicitly designed to evaluate condom use.<sup>1,4</sup> Our longitudinal study was designed to evaluate more accurately the temporal relationship between condom use and HPV infection.

## Methods

### Study Design

We restricted eligibility to female University of Washington undergraduates who were 18 to 22 years old and who had never had vaginal intercourse or had first had intercourse with one male partner within the previous three months. In addition, the women had to have a cervix, could not be pregnant, had to be in good general health, and had to be able to provide written informed consent. Since the goal of the study was to enroll a population of healthy women (rather than women presenting to the student health clinic with gynecological problems), between December 2000 and June 2005, we mailed invitational letters to 24,201 women who met the age criterion and who released their names to the registrar. Given the restrictive eligibility criteria, we assumed that the number of participants would be low in relation to the number of letters mailed. We also provided informational flyers to contraceptive counselors at the student health clinic. Of the 243 eligible women who responded, 210 agreed to participate (86.4 percent) (Figure 1). The protocol was approved by the institutional review board at the University of Washington.

Participants completed a Web-based diary regarding their sexual behavior every two weeks and underwent gynecologic examination every four months. Diary features have been described previously.<sup>16</sup> The diary contained daily information regarding the number of instances of vaginal intercourse, the frequency of use of condoms by male partners, and the number of new partners. The first entry covered the two weeks before enrollment, and subsequent entries covered each successive two-week period. Missed diary entries could be made up within four months.

### Clinical Measurements

At each visit to the clinic, a nurse practitioner conducted a face-to-face interview. Women were then instructed to obtain a vaginal sample with a Dacron-tipped swab and place it in a tube containing a specimen-transport medium for HPV testing. The nurse practitioner then performed a standardized pelvic examination. Separate cervical and vulvovaginal samples were obtained with Dacron-tipped swabs and placed in tubes containing transport medium for HPV testing. For Papanicolaou (Pap) testing, a cytobrush was used to collect cellular material from the endocervix and a plastic spatula was used to collect cells from the squamocolumnar junction and ectocervix.

Specimens were tested for HPV DNA with the use of a polymerase-chain-reaction (PCR) assay.<sup>17</sup> DNA was isolated with the use of the QIAamp DNA blood kit (Qiagen) according to the manufacturer's protocol. Of each sample, 1/250 was amplified and 10  $\mu$ l of PCR products was dotted onto nylon filters and probed with a biotin-labeled generic probe. Specimens that were found to be positive with the use of a generic probe were then typed with the use of a reverse line-blot assay (Roche Molecular Systems). Positive samples were first amplified simultaneously for HPV and  $\beta$ globin with the use of biotinylated PGMY09/PGMY11 and PC04/GH20 primers. A total of 70  $\mu$ l of denatured PCR product was added to each well of an Amplicor typing tray

containing 3 ml of preheated hybridization solution and a strip containing  $\beta$ globin and HPV oligonucleotide probes for 37 HPV types. Samples negative for  $\beta$ globin were considered insufficient for testing. Unless otherwise noted, PCR results for self-collected swabs and clinician-collected cervical and vulvovaginal swabs were combined to analyze acquisition and risk factors for genital infection. A cytotechnologist examined all Pap smears, and a pathologist reviewed all abnormal smears. Findings were classified according to the Bethesda system<sup>18</sup> as normal, atypical, low-grade squamous intraepithelial lesion, or high-grade squamous intraepithelial lesion.

## Statistical Analysis

Analyses were restricted to women with at least one follow-up visit after they first had intercourse. Excluded from the study were 65 women who reported having had no vaginal intercourse, 3 women who did not record any information in their diaries regarding sexual behavior, and 60 women who reported having first had intercourse more than two weeks before enrollment ([Figure 1](#)).

Incident HPV infection was defined as the first positive result for a specific type. The cumulative probability of HPV infection was estimated with the use of Kaplan–Meier methods. The time at risk was calculated from the date of first intercourse to the first detection of HPV. Kaplan–Meier methods were also used to estimate the cumulative probability of having cervical squamous intraepithelial lesions from the date of first intercourse.

Marginal Cox proportional-hazards models<sup>19</sup> were used to determine risk factors for HPV infection. Data from diaries were summarized into risk-factor variables during the eight months before HPV testing, since most infections associated with a first partner (before the report of a second partner) occurred within eight months after a woman first had intercourse. Data recorded less than 20 days before a given visit were excluded, because 20 days was the shortest observed interval between the time a woman first had intercourse and the detection of an incident HPV infection in this study ([Figure 2](#)). The time to an event was measured from the time a woman first had intercourse to the report of infection with each type of HPV or the last clinic visit, with each woman contributing at-risk time for each of 37 HPV types. Analyses were stratified according to the type of HPV, assuming common relative hazards across HPV types while allowing the baseline hazards to vary. Robust variance estimates were used to account for correlation within subjects. Analyses were restricted to intervals in which intercourse was reported.

Potential risk factors included the total number of instances of vaginal intercourse (continuous variable), the number of new partners (0, 1, or >1), the frequency of condom use by partners (<5 percent, 5 to 49 percent, 50 to 99 percent, or 100 percent), the partner's circumcision status (circumcised, uncircumcised, or unknown), and the partner's number of previous partners (0,  $\geq 1$ , or unknown). The frequency of condom use was calculated by dividing the number of condoms used for vaginal intercourse by the number of instances of vaginal intercourse during the eight-month study period. If multiple new partners were reported during an eight-month period, the circumcision status and previous number of partners were summarized ([Table 1](#)). The percentage of days that lacked diary entries was calculated to assess potential confounding created by missing data. The effect of the frequency of condom use was evaluated by multivariate analysis

with the inclusion of other variables that were found to be significant ( $P < 0.10$ ) in univariate analyses. Similar analyses were performed to evaluate risk factors for high-risk types of HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 73, and 82),<sup>20,21</sup> low-risk types (6, 11, 40, 42, 54, 55, 57, 61, 62, 64, 69, 70, 71, 72, 81, 83, 84, and CP6108),<sup>20,21</sup> and types 6, 11, 16, and 18 (which are included in quadrivalent vaccine trials<sup>22</sup>). To evaluate whether the effect of condom use on the risk of HPV infection differed according to the location in the genital tract, similar analyses were performed to examine risk factors for incident cervical and vulvovaginal infections.

In subanalyses, subjects whose partners used condoms 100 percent of the time were divided into two categories on the basis of their responses to the question "Did your partner's penis ever touch the opening of your vagina without a condom?" If subjects always answered "no" for every act of vaginal intercourse during the eight-month period, they were coded as having had "no genital contact without a condom." Analyses were performed as described above, with five categories of the frequency of condom use (100 percent without any genital contact without a condom, 100 percent with some or an unknown frequency of genital contact without a condom, and three groups below 100 percent, as described above).

Similarly, Cox proportional-hazards models were used to determine risk factors for the detection of incident cervical squamous intraepithelial lesions during the eight months before a given Pap test (on the basis of a previous observation that the majority of lesions occur soon after incident infection<sup>23</sup>). A 51-day lag before each visit was incorporated because 51 days was the shortest observed interval between the time a woman first had intercourse and the date of detection of an incident lesion in this study. Data for such subjects were censored after the first detection of an incident lesion.

## Results

The mean ( $\pm$ SD) age of the 82 subjects at enrollment was  $19.3 \pm 0.7$  years. The subjects completed 709 visits. The mean follow-up time was  $33.9 \pm 11.8$  months, the mean number of visits per woman was  $8.6 \pm 2.9$ , and the median time between visits was 4.1 months. Diary data were 90.7 percent complete, and condom data were recorded on 99.3 percent of days that intercourse was reported.

The 12-month cumulative incidence of a first HPV infection after first intercourse was 37.2 percent (95 percent confidence interval, 27.2 to 49.4 percent). A total of 126 incident type-specific infections were identified in 40 women after they first had intercourse ([Table 2](#)). Incident infections were identified in three women before they reported having intercourse for the first time, including one positive for HPV type 51, one for HPV type 6, and one for HPV types 40, 61, and 66. The 24-month cumulative incidence of cervical squamous intraepithelial lesions was 15.0 percent (95 percent confidence interval, 8.3 to 26.2 percent). Incident lesions were identified in 15 sexually active women, including 1 high-grade lesion and 14 low-grade lesions.

The report of new partners in the previous eight months was associated with an increased risk of HPV infection. More frequent condom use by partners and a report of partners with no previous partners were both associated with a decreased risk ([Table 3](#)). Not significant were the number of

instances of vaginal intercourse (hazard ratio for each additional instance, 1.0; 95 percent confidence interval, 0.99 to 1.01), having a partner who was circumcised (hazard ratio for the comparison with having an uncircumcised partner, 0.7; 95 percent confidence interval, 0.3 to 1.5), and the percentage of missing diary days. In the multivariate model, two categories associated with a partner's number of previous partners ("1 or more" and "unknown") were collapsed into one category because incident infections were identified among women whose partners were estimated to have had one or more or an unknown number of previous partners but not among women whose partners reportedly had had no previous partners.

Women whose partners used condoms 100 percent of the time during the previous eight months were significantly less likely to acquire HPV than were those whose partners used condoms less than 5 percent of the time ([Table 3](#)). A linear categorical dose–response effect was observed, since the risk of incident HPV infection decreased with the increasing percentage of time a condom was used for intercourse ( $P=0.005$  by the test for trend). Similar trends were observed through an analysis of risk factors for high-risk types of HPV, low-risk types of HPV, and HPV types 6, 11, 16, and 18 ([Table 4](#)) and for incident cervical and vulvovaginal HPV infection. Among the subgroup of women reporting 100 percent condom use by their partners during the previous eight months, the association between condom use and HPV infection was similar, regardless of whether any unprotected genital skin-to-skin contact was reported.

Although incident cervical squamous intraepithelial lesions were positively associated with reports of new partners, associations with the prespecified categories of condom use were not significant ([Table 3](#)). However, as shown in [Table 1](#), no lesions were detected during 32 patient-years at risk among women reporting 100 percent condom use by their partners during the previous eight months, whereas 14 lesions were detected during 97 patient-years at risk among women reporting less consistent use or nonuse of condoms by their partners. The number of previous partners reported for the partner, the number of instances of vaginal intercourse, circumcision status, and the percentage of missing diary days were not significant.

## Discussion

Previous studies have suggested that the use of condoms by men offers women little if any protection against genital HPV infection.<sup>1,4,10,11,12,13</sup> The results of our study, which was specifically designed to collect detailed and current data on condom use, suggest that male condoms effectively reduce the risk of male-to-female genital HPV transmission. Women whose partners used condoms for all instances of vaginal intercourse during the previous eight months were 70 percent less likely to acquire a new infection than were women whose partners used condoms less than 5 percent of the time, after adjustment for the number of new partners and the estimated number of previous partners of the male partner. Even women whose partners used condoms more than half the time had a 50 percent risk reduction, as compared with those whose partners used condoms less than 5 percent of the time.

Design features unique to our study may explain why we detected a significant inverse association between the frequency of condom use and HPV infection, whereas previous studies did not. First, the longitudinal design allowed us to evaluate the temporal relationship between

condom use and HPV infection. Cross-sectional studies measuring prevalent infection may be misleading, since a temporal sequence cannot be established and most HPV infections are detected transiently.<sup>2,4</sup> Second, previous studies (including our previous longitudinal study of a different cohort of women<sup>1</sup>) tended to rely on broad measures of condom use such as "always" or "always or sometimes" as compared with "never" or "never or sometimes," recalled months or years later. By collecting information every two weeks, we minimized the potential for measurement error and more precisely measured the frequency of condom use.

Furthermore, evidence suggests that computer-assisted questionnaires yield more truthful reporting of sensitive behavior than do face-to-face interviews.<sup>24,25</sup> With electronic diaries, we aimed to reduce reporting biases related to social desirability (such as the overreporting of condom use) that could dilute any true analysis of the effect of condoms. By asking women to report information about their partners, we also minimized potential confounding by sexual behavior. For example, women may be more likely to use condoms with partners they perceive as "risky."<sup>26</sup> Indeed, women who reported that their partners had had one or more or an unknown number of previous partners were more likely to have their partners use condoms than were women who reported that their partners were virgins. Although ideally one would measure the HPV status of all male partners, the fact that no incident infections were detected among women reporting previously virginal partners suggests that this variable is a useful indicator of a partner's infection status in a university setting. Finally, given the ubiquity of HPV infection among sexually active young women, we restricted our analyses of risk factors to women who first had intercourse within two weeks before enrollment or during the study. The enrollment of women with minimal previous exposure helped ensure that the cohort was susceptible to new infections and that infections detected during follow-up were truly incident.

We also observed an inverse association between the frequency of condom use and the incidence of cervical squamous intraepithelial lesions, although no significant associations with the prespecified categories of condom use were observed. However, no incident lesions were detected among women reporting 100 percent condom use by their partners during the previous eight months, whereas 14 incident lesions (14.5 per 100 patient-years at risk) were detected among women whose partners used condoms less consistently or never used condoms (although this analysis was conducted a posteriori). Although this trend is consistent with previous data indicating that condom use by their male partners protects some women against high-grade cervical neoplasia<sup>27,28</sup> and invasive cervical cancer,<sup>29,30</sup> larger data sets would be needed to determine the significance of the observed trend associated with 100 percent condom use.

Even though our primary aim was to evaluate the frequency of condom use in relation to HPV infection, we were also interested in evaluating whether unprotected nonpenetrative genital contact would diminish the effectiveness of condoms. The question "Did your partner's penis ever touch the opening of your vagina without a condom?" was intended to capture the incidence of unprotected genital contact while keeping the time required for making diary entries to a minimum. Women who reported 100 percent condom use by their partners and no genital skin-to-skin contact and those reporting some or an unknown amount of such contact had a similar incidence of HPV infection. Therefore, either our question was not a sensitive or specific marker of unprotected genital contact or brief episodes of skin-to-skin contact are not particularly efficient for male-to-female HPV transmission. Questions that specifically address condom

breakage or slippage<sup>31</sup> (problems particularly common among inexperienced users<sup>32</sup>) may be important, though such indicators may be more relevant for infections transmitted primarily through secretions.

Some limitations of the study should be noted. First, it is difficult to determine the optimal time frame for an assessment of risk factors for acquiring HPV infection. In retrospect, the 20-day lag period was relevant not only because it was the shortest observed interval between first intercourse and the detection of HPV infection in this study but also because it is within the range of time estimated for epithelial cells to mature and differentiate,<sup>33</sup> cellular events that are required for HPV replication.<sup>34</sup> Although an eight-month period was used because the majority of first infections associated with a first partner occurred within this time frame, not all partners are infectious, a partner's infectivity may change, and infection could have occurred at any time within the eight-month period. If infection occurred early, for example, condom use for intercourse reported later in the interval would be unrelated to HPV status, and the true effect of condoms might be diluted.

Furthermore, incomplete reporting could have created a misclassification of the frequency of condom use, although such an effect probably would have been minimal, given the small percentage of days with missing data regarding sexual behavior. Incomplete follow-up and delayed or missed clinical visits were other potential sources of bias. For example, outcomes of short duration could be missed with less frequent follow-up, potentially leading to underestimates of the cumulative incidences of infection and lesions. However, when we compared women with average visit intervals of less than four months with those with intervals of four months or more, we observed similar rates of HPV infection ( $P=0.43$ ). In a comparison of women who were eventually lost to follow-up with those who were not, similar rates of HPV infection were observed during the study ( $P=0.56$ ).

Finally, although the newly sexually active women in our cohort reported a yearly median number of instances of intercourse (48) and a yearly median number of new partners (1) that were similar to those reported in a large national survey of a random sample of women of a similar age,<sup>35</sup> our results may not be generalizable to populations of older women or women of lower socioeconomic status.

Our study demonstrates an inverse, temporal association between the frequency of condom use by male partners and the risk of HPV infection in women. The association was strong and increased with the increasing frequency of condom use, suggesting a causal, protective effect. Given that HPV is transmissible through nonpenetrative sexual contact with both male<sup>1</sup> and female<sup>36</sup> partners and that imperfect condom use does occur, it is not surprising that some infections were still detected among women reporting consistent use. It is encouraging, however, that the women in this cohort, who were new to sexual intercourse and condom use, were able to reduce their risk of HPV infection through the consistent use of condoms by male partners. Furthermore, our results suggest that consistent condom use offers similar protection against both high-risk and low-risk types of HPV. Even after the quadrivalent vaccine for HPV types 6, 11, 16, and 18 becomes available, consistent condom use by their partners may protect women against infection with other high-risk types of HPV that put them at risk for cervical cancer.