

Serologic Response to Oncogenic Human Papillomavirus Types in Male and Female University Students in Busan, South Korea

Gary M. Clifford,¹ Hai-Rim Shin,^{1,2} Jin-Kyoung Oh,² Tim Waterboer,³ Young-Hee Ju,² Salvatore Vaccarella,¹ Wim Quint,⁴ Michael Pawlita,³ and Silvia Franceschi¹

¹International Agency for Research on Cancer, Lyon, France; ²Research Institute for National Cancer Control and Evaluation, National Cancer Centre, Goyang, Republic of Korea; ³Research Program Infection and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany; and ⁴DDL Diagnostic Laboratory, Voorburg, the Netherlands

Abstract

In the absence of genital samples, human papillomavirus (HPV) serology may be useful to assess HPV infection in young men and women. HPV seroprevalence and determinants of seropositivity were assessed in 817 female and 518 male university students in Busan, South Korea, of whom 74% and 44%, respectively, reported never having had penetrative sexual intercourse. Type-specific HPV DNA status, assessed by a short PCR fragment primer set, was available from genital samples. Seropositivity to L1 proteins of HPV types 16, 18, 31, 33, 45, 52, and 58 were assessed using multiplex HPV serology. Among women, HPV seroprevalence was significantly higher among sexually active (26.1%) than nonsexually active students

[11.1%, odds ratio (OR) = 2.9; 95% confidence interval (95% CI), 1.8-4.7], although the association was weaker than that for HPV DNA prevalence (OR, 14; 95% CI, 4.7-42). Furthermore, HPV seroprevalence was higher among HPV DNA-positive (24%) than HPV DNA-negative women (13%), and there was a positive correlation of type-specific seroprevalence with the presence of HPV DNA of the same type. In contrast, HPV seropositivity among men was not associated with sexual behavior or the presence of HPV DNA. Seroprevalence correlates with genital HPV exposure in young women, but its meaning in young men is unclear. (Cancer Epidemiol Biomarkers Prev 2007; 16(9):1874-9)

Introduction

Human papillomavirus (HPV) DNA detection gives a measure of HPV infection at a given anatomic site, most commonly the female cervix. However, it underestimates cumulative exposure because most HPV infections are transient (1). The value of HPV DNA detection as an epidemiologic tool is also limited by sampling difficulties in men (2) and by the unwillingness of many unmarried women to undergo gynecologic examination necessary for the collection of exfoliated cells (3, 4).

HPV antibody response among women, on the other hand, has shown to be a useful epidemiologic marker of cumulative sexual exposure to HPV, being consistently linked to sexual debut, lifetime number of sexual partners, marital status, and herpes simplex virus type 2 seropositivity (5-16), as well as risk of cervical neoplasia/cancer (5, 7, 15, 17-21). Seroconversion is also linked to persistent HPV DNA infection (1, 14, 22), although no more than half of women infected with HPV seem to seroconvert (1, 7, 22, 23).

Seroprevalence data in men are far fewer and less consistent, but the limited data suggest that men are even less likely to mount a detectable antibody response than women (12, 24).

We report on HPV seroprevalence from a cross-sectional survey of female and male students aged 15 to 29 from Busan, South Korea (3), many of whom reported not, or only recently, to have initiated penetrative sexual intercourse. HPV DNA data was obtained from self-collected vaginal cells for women, and physician-collected genital cells for men (3). As major objectives in this report, we assess the gender-specific association of sexual behavior and genital HPV DNA status with seroprevalence of the seven most common HPV types in cervical cancer, detected simultaneously with a novel multiplex HPV antibody assay (25).

Subjects, Materials, and Methods

Study Procedures. The present survey was conducted during 2002 in three institutions of higher education in Busan, South Korea, the extensive details of which have been published previously (3). In summary, 1,500 students (900 women and 600 men), aged 15 to 29, were asked to complete a self-administered questionnaire including sociodemographic and lifestyle questions [e.g., smoking habits, alcohol consumption, and circumcision (male students only)]. Students were also asked to report whether they had engaged in penetrative sexual intercourse, their lifetime number of sexual partners, and

Received 4/17/07; revised 6/13/07; accepted 6/21/07.

Grant support: Bill & Melinda Gates Foundation (Grant no. 35537) and the Korean National Cancer Centre (Grant no. 0110250).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Hai-Rim Shin, IARC, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France. Phone: 33-4-72-73-84-18; Fax: 33-4-72-73-80-22. E-mail: shinhr@iarc.fr

Copyright © 2007 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-07-0349

use of contraceptive methods. Of those contacted, 860 female (95.6%) and 541 male (90.2%) students agreed to participate. Each returned, in a sealed envelope, the anonymous questionnaire. All participants signed an informed consent form, which, together with the study protocol, was approved by the ethical review committees of the National Cancer Center, Korea, and of the IARC.

Collection of Biological Samples. Ten ml of blood was taken from all consenting students (817 female and 518 male). Blood samples were centrifuged at $1,500 \times g$ for 10 min and aliquoted into plasma, buffy coats, and RBC. A majority of students consenting to blood collection also consented to provide samples of exfoliated genital cells (648 female and 366 male; ref. 3). Female students underwent self-collection of cervicovaginal cells, which has shown to be an appropriate alternative to physician-collected samples (26). Male students underwent collection of exfoliated genital cells from the scrotum, shaft, coronal sulcus, glans, urethral opening, and tip of the penis, done by an urologist (all placed into one tube and described in detail previously; ref. 3). Exfoliated genital cell samples from both female and male students were placed in PBS. Aliquots of blood and exfoliated genital cells were stored at -70°C .

HPV Serology Testing. Antibodies to the major capsid protein L1 of HPV types 16, 18, 31, 33, 45, 52, and 58 were tested for at the German Cancer Research Center, Heidelberg, Germany, using a multiplex HPV serology method based on a glutathione *S*-transferase (GST) capture immunosorbent assay (25). Viral antigens were expressed in *Escherichia coli* as double-fusion proteins with a NH_2 -terminal GST domain and a COOH -terminal peptide derived from the large T antigen of SV40. Glutathione-casein was coupled to internally fluorescence-labeled polystyrene beads (Luminex), and antigen fusion proteins were affinity purified on the beads directly in a one-step procedure. Beads coupled with GST only were prepared for background determination. Binding of the antigens (i.e., GST fusion proteins) to various beads was verified with a monoclonal antibody against the common COOH -terminal peptide. The differently labeled beads carrying different antigens were then mixed and incubated in 96-well plates with human serum that had been diluted 1:100 in blocking buffer. Antibodies bound to the beads via the viral antigens were then stained with biotinylated anti-human immunoglobulin and the fluorescent reporter conjugate streptavidin-*R*-phycoerythrin (Molecular Probes). Antibodies bound to antigens on beads were quantified in the Luminex analyzer, which also identified the antigen by the internal bead color. Antibody quantity was determined as the median *R*-phycoerythrin fluorescence intensity (MFI) from at least 100 beads of the same internal color.

MFI values were dichotomized as antibody positive or negative. Seropositivity cutoffs were calculated independently for each HPV type by analyzing MFI values obtained for 371 female students from the present study that reported never to have engaged in penetrative sexual intercourse and had no evidence of genital HPV DNA for 25 HPV types (see below). The mean and SD of MFI values for these 371 subjects were calculated, and values greater than the mean + 5 SDs were excluded as

outliers. This process was repeated on the remaining samples until no additional values could be excluded by this criterion, which occurred after one to four rounds of exclusion, depending on the HPV type. The final numbers of excluded outliers were 7, 13, 11, 1, 12, 4, and 3 for HPV types 16, 18, 31, 33, 45, 52, and 58, respectively. The cutoff was defined as 5 SDs above the mean of the final distribution and ranged from 394 to 714 MFI, depending on the HPV type. Alternative classifications of seropositivity using 3 or 4 SDs, with or without exclusion of outliers, or defining a 95% to 99% specificity threshold among virgins, yielded similar conclusions. For the following analyses, seropositivity for any HPV refers to that for types 16, 18, 31, 33, 45, 52, or 58, and multiple seropositivity as being positive for two or more of these types.

HPV DNA Testing. HPV DNA testing was done at DDL Diagnostic Laboratory (Voorburg, the Netherlands) as previously described (3). In brief, DNA was isolated from exfoliated genital cell samples, and HPV DNA was amplified by use of the short PCR fragment (SPF)₁₀ primer set (27). Amplification products were first tested by probe hybridization in a microtiter-plate assay to detect the presence of HPV DNA. SPF₁₀ amplimers from HPV DNA-positive samples were subsequently analyzed by reverse hybridization in an HPV line-probe assay (LiPA; ref. 28), permitting detection of 25 HPV types: HPV6, 11, 16, 18, 31, 33 to 35, 39, 40, 42 to 45, 51 to 54, 56, 58, 59, 66, 68/73, 70, and 74. Samples negative for a positive DNA control, the β -globin gene, were considered invalid (6 women and 72 men). DNA positivity is reported in the manuscript as that for (a) all 25 types or (b) only the seven types for which serology data were also available.

Statistical Analysis. Gender-specific odds ratios (OR) for HPV seropositivity and corresponding 95% confidence intervals (95% CI) were calculated by means of logistic regression, with adjustment for lifetime number of sexual partners where appropriate (Stata version 9). The statistical significance of trends was assessed by considering categorical variables as a continuous variable in the logistic model.

Results

A total of 817 female and 518 male students with valid HPV serology results were included in the following analyses. Of these, a subset of 648 women and 366 men also had valid results for genital HPV DNA. Self-reported information on sexual activity was available for a subset of 680 women and 466 men, of which 502 (74%) and 206 (44%), respectively, reported never having engaged in penetrative sexual intercourse.

Overall and type-specific HPV seroprevalence is shown in Fig. 1, stratified by gender and sexual activity status. Overall HPV seroprevalence did not differ significantly between female and male students (15% and 12%, respectively), which was also the case for type-specific HPV seroprevalence.

Among women, HPV seroprevalence was significantly higher among sexually active (25% for any HPV type) than nonsexually active students (13%), and this excess was significant for individual types HPV16, 18, and 33

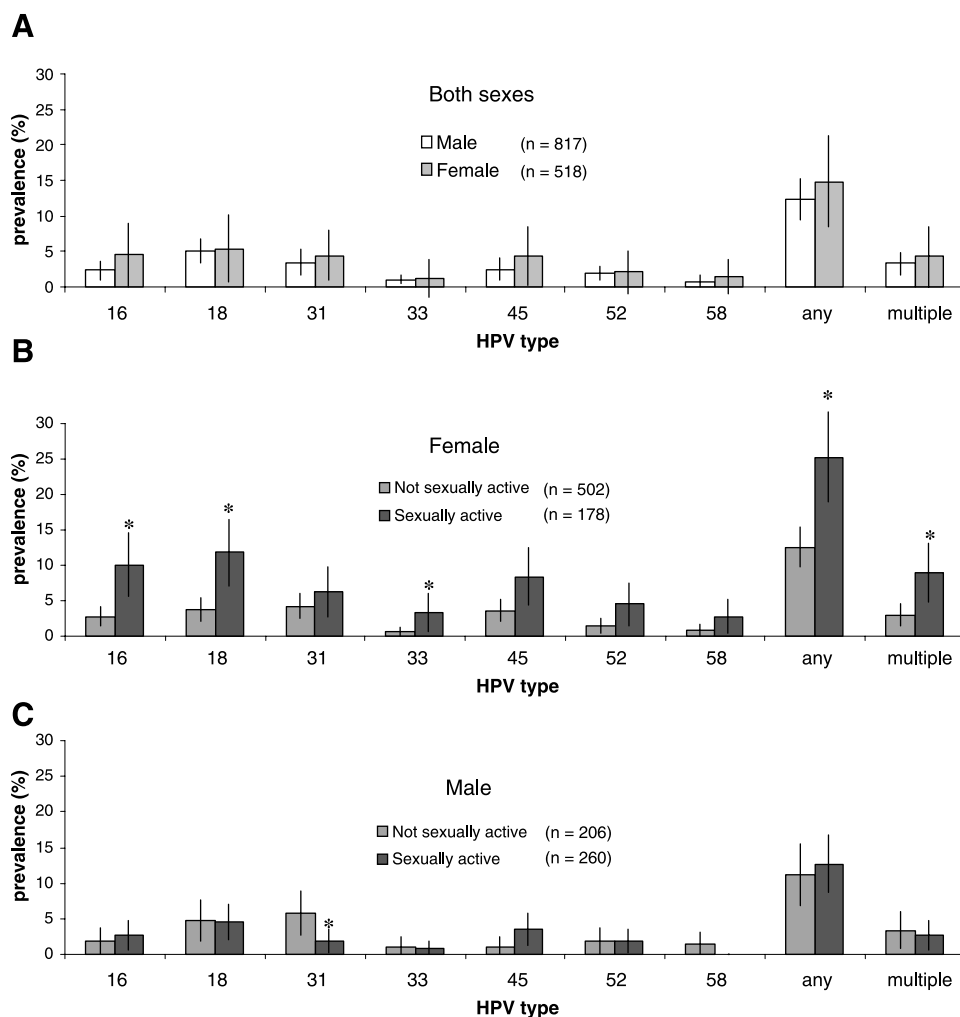


Figure 1. HPV type-specific seroprevalence among 680 female and 466 male university students by gender (A) and sexual activity status (B and C). (Data not shown for 137 female and 52 male students of unknown sexual activity status in parts B and C. *, $P < 0.05$ for comparison of not sexually active and sexually active.)

and for multiple-type HPV seropositivity. Among men, in contrast to their female peers, no significant increase in HPV seroprevalence was observed among those reporting to be sexually active (13%) compared with non-sexually active (11%). This meant that among sexually active students, HPV seroprevalence was significantly higher among women (25% for any HPV type) than men (13%), whereas it was similar among nonsexually active men and women.

Type-specific HPV seroprevalence is shown in Table 1 stratified by genital HPV DNA status (25 HPV types) and gender. Among women, there was a significant trend of increasing HPV seroprevalence from HPV DNA-negative women (12.9% for any HPV type), through women DNA positive for HPV types other than 16, 18, 31, 33, 45, 52, and 58 (17.4%), to that among women DNA positive for 16, 18, 31, 33, 45, 52, or 58 (40.7%). Furthermore, among HPV DNA-positive women, type-specific seroprevalence was always higher among women positive for the same HPV type (with the exception of HPV33 and 45 where the comparison was limited by only one and two HPV

DNA-positive cases, respectively). For men, type-specific seroprevalence is only shown for HPV16 and 18 and the combination of 16, 18, 31, 33, 45, 52, and 58, on account of the low level of HPV DNA and seropositivity (Table 1). No positive correlation between HPV seropositivity and genital HPV DNA status was observed for any HPV type nor for HPV16 or 18 alone among men.

Table 2 shows the relationship of HPV seroprevalence, genital HPV DNA prevalence (to those types for which serology was also available: 16, 18, 31, 33, 45, 52, or 58), and combinations thereof, with lifetime number of sexual partners stratified by gender. Among women, there was a significant association between engaging in penetrative sexual intercourse and HPV seroprevalence (OR, 2.9; 95% CI, 1.8-4.7), although the strength of this association was weaker than that for HPV DNA prevalence (OR, 14; 95% CI, 4.7-42). However, among sexually active women, HPV seroprevalence varied little by number of lifetime sexual partners (28.6% for ≥ 4 partners versus 26.8% for 1 partner), in contrast to HPV DNA prevalence (22.9% versus 5.6%). The strongest association with lifetime

Table 1. HPV type-specific seroprevalence among 648 female and 366 male university students, stratified by genital HPV DNA status

HPV type	<i>n</i> seropositive/ <i>n</i> total (% seropositive)			<i>P</i> for trend
	HPV DNA negative*	HPV DNA positive*		
		Nonconcordant type	Concordant type	
Females				
16	15/552 (2.7)	9/88 (10.2)	2/8 (25.0)	<0.01
18	26/552 (4.7)	10/89 (11.2)	2/7 (28.6)	<0.01
31	16/552 (2.9)	7/92 (7.6)	1/4 (25.0)	<0.01
33	6/552 (1.1)	2/94 (2.1)	0/2 (0.0)	0.45
45	20/552 (3.6)	9/95 (9.5)	0/1 (0.0)	0.02
52	9/552 (1.6)	2/88 (2.3)	1/8 (12.5)	0.14
58	7/552 (1.3)	1/91 (1.1)	1/5 (20.0)	0.18
Any [†]	71/552 (12.9)	12/69 (17.4)	11/27 (40.7)	<0.01
Males				
16	8/334 (2.4)	0/30 (0.0)	0/2 (0.0)	—
18	18/334 (5.1)	1/30 (3.3)	0/2 (0.0)	0.57
Any	42/334 (12.6)	1/21 (4.8)	1/11 (9.1)	0.40

NOTE: Data not shown for 169 female and 152 male students of unknown HPV DNA status.

*A total of 25 HPV types.

[†]HPV16, 18, 31, 33, 45, 52, or 58.

number of sexual partners was observed for positivity to both genital HPV DNA and HPV antibodies (11.4% versus 0.3%; OR, 50; 95% CI, 5.4-461). Among males, in contrast, there was no relationship of engaging in penetrative sexual intercourse (OR, 1.2; 95% CI, 0.6-2.5) or lifetime number of sexual partners with HPV seroprevalence, genital HPV DNA prevalence, or combinations thereof.

After adjustment for lifetime number of sexual partners, there was no significant association in women between HPV seropositivity and age (OR, 1.1; 95% CI, 0.7-1.6, for 20-30 versus 16-19 years), age at first penetrative intercourse (OR, 2.1; 95% CI, 0.8-5.8, for ≥ 20 versus ≤ 17 years), time since first penetrative intercourse (OR, 0.8; 95% CI, 0.2-2.6, for ≥ 3 versus ≤ 1 years), smoking habits (OR, 1.1; 95% CI, 0.6-1.9, for ever versus never) nor alcohol consumption (OR, 1.0; 95% CI, 0.6-1.7, for ever versus never). Similarly for men, no

significant associations were observed with the above variables (data not shown) nor for circumcised ($n = 321$, 88%) versus uncircumcised ($n = 42$, 12%) men (OR, 0.8; 95% CI, 0.3-2.1).

Discussion

Type-specific HPV seroprevalence was estimated in a young student population in South Korea, including many unmarried and/or virgin women that are normally unwilling to undergo gynecological examination and a comparative sample of men, with extensive validation against HPV DNA in genital samples.

Of ~500 female Korean students reporting no history of penetrative sexual intercourse, seroprevalence to HPV16, 18, 31, 33, 45, 52, or 58 was 13% (Figure 1), whereas prevalence of genital HPV DNA for these seven types was only 1%. Other studies have reported HPV

Table 2. ORs for seropositivity, DNA positivity, and combinations thereof, to HPV types 16, 18, 31, 33, 45, 52, and 58 among 648 female and 366 male university students by lifetime number of sexual partners

Lifetime number of sexual partners	<i>n</i>	HPV antibodies*		HPV DNA [†]		HPV antibodies or DNA [†]		HPV antibodies and DNA [†]	
		% + ve	OR (95% CI)	% + ve	OR (95% CI)	% + ve	OR (95% CI)	% + ve	OR (95% CI)
Females									
0	388	11.1	1	1.0	1	11.9	1	0.3	1
≥ 1	153	26.1	2.9 (1.8-4.7)	12.7	14 (4.7-42)	32.5	3.7 (2.3-5.8)	6.4	26 (3.3-207)
1	71	26.8	2.9 (1.6-5.4)	5.6	5.7 (1.4-24)	31.0	3.3 (1.8-6.0)	1.4	5.5 (0.3-89)
2-3	47	23.4	2.4 (1.2-5.2)	17.0	20 (5.7-68)	29.8	3.2 (1.6-6.3)	10.6	46 (5.3-404)
≥ 4	35	28.6	3.2 (1.4-7.1)	22.9	28 (8.0-100)	40.0	5.0 (2.4-10)	11.4	50 (5.4-461)
<i>P</i> for trend			0.84		0.02		0.35		0.06
Not reported	107	10.3	0.9 (0.5-1.8)	2.8	2.8 (0.6-13)	13.1	1.1 (0.6-2.1)	0.0	—
Males									
0	124	10.5	1	2.4	1	12.9	1	0.0	—
≥ 1	201	12.7	1.2 (0.6-2.5)	3.9	1.6 (0.4-6.3)	16.1	1.3 (0.7-2.5)	0.5	—
1	44	15.9	1.6 (0.6-4.4)	4.6	1.9 (0.3-12)	18.2	1.5 (0.6-3.8)	2.3	—
2-3	63	15.9	1.6 (0.7-3.9)	3.2	1.3 (0.2-8.1)	19.1	1.6 (0.7-3.6)	0.0	—
≥ 4	94	9.6	0.9 (0.4-2.2)	4.3	1.8 (0.4-8.2)	13.8	1.1 (0.5-2.4)	0.0	—
Not reported	41	12.2	1.2 (0.4-3.6)	0.0	—	12.2	0.9 (0.3-2.7)	0.0	—

*Restricted to women with valid HPV DNA results.

[†]DNA refers to HPV types 16, 18, 31, 33, 45, 52, and 58.

seroprevalence of 2% to 3% (HPV16 only) in young women who were virgins (6, 29), and others report 2% to 8% in children with a significant linear increase by age for girls (2, 30). It remains that sexual activity was a self-reported measure in the present study and may have been under-reported, particularly among women. Nevertheless, taken together, these data suggest that exposure and seroconversion to oncogenic HPV types may occur, to a certain extent, before penetrative sexual intercourse (10).

HPV seroprevalence doubled to 25% among sexually active women in this study, and this increase was seen independently for each of the seven HPV types tested. Although a strong increase in HPV seropositivity with increasing number of sexual partners has been widely reported (5-16), far fewer studies have allowed a comparison with women reporting to be nonsexually active (6, 29).

Nevertheless, the association of the lifetime number of sexual partners remained much weaker for HPV seroprevalence than for genital HPV DNA prevalence. This association was strongest for women that were both HPV seropositive and DNA positive, but these subjects were few (2%) among the pool of women positive for either marker (17%).

HPV DNA-positive women were more likely to be seropositive for antibodies to the same HPV type, confirming the type-specific nature of the immune response and the serologic assay. Nevertheless, the overall individual concordance between anti-HPV seropositivity and DNA positivity remained modest. At least three quarters of women infected with a given HPV type were not seropositive for the same HPV genotype. This proportion of non-seroconversion is higher than among older women from the same Korean population (31) and in some other studies (13, 14, 32), perhaps reflecting the effect of a time lag required for seroconversion in this young population. The 12-month cumulative incidence of seroconversion after cervical HPV infection has been estimated at ~50% (1, 7, 22), and the majority of the sexually active women in this study had initiated sexual activity only within the preceding 12 months.

In contrast to their female peers, becoming sexually active and having multiple lifetime sexual partners had no observable effect on HPV seroprevalence or, as already shown, on genital HPV DNA prevalence among young men (3), despite higher self-reported levels of sexual activity than women. Higher seroprevalence among sexually active women than sexually active men has previously been reported from various populations for HPV (6, 10-12) and also for herpes simplex virus type 2 (10, 33). This discrepancy may reflect greater transience of HPV infection in male external genitalia than in the female vagina and cervix, as persistent exposure to the virus over time seems to be required for seroconversion in women (1). On the other hand, some previous studies have observed an association of HPV seroprevalence with the number of sexual partners, albeit among men of a much broader age range (11, 12). This apparent discrepancy could reflect an importance of duration of exposure in determining seroconversion or differences in HPV infection status of sexual partners, for example, if young men have sex with young women of low HPV prevalence.

This seroepidemiologic study is the first to correlate seroprevalence in men with genital HPV DNA for a wide range of types and shows that the correlation between these two markers is very poor, as shown previously for HPV16 (12, 34). This may partly reflect the difficulty to accurately classify HPV DNA status in males in whom a broader area of external genitalia need to be sampled in comparison to women.

HPV16 accounts for more than half of cervical cancers in Korea and worldwide (35). Likewise, HPV16 DNA predominates over that of other oncogenic HPV types in cancer-free women in South Korea (31). However, this predominance does not seem to be reflected in seroprevalence, for which HPV16, 18, and 31 are equivalent, as reported previously in Korea (31) and elsewhere (14, 36). The reason for the difference in type distribution at the serologic and DNA levels is unclear, but may be related to type-specific differences in duration of infection or propensity to elicit a serologic response. Cross-reactivity of serotypes cannot be ruled out, but only one quarter of all HPV-seropositive cases had multiple seropositivity, and there were too few to allow a statistical comparison of concordance between different serotypes. Using a less or more stringent cutoff for seropositivity while having the effect to increase or decrease, respectively, overall HPV seropositivity did not alter this type-specific equivalence or any other key differences by gender.

After accounting for confounding by number of sexual partners, we could not show associations of seropositivity in women with age or age at first penetrative sexual intercourse as in previous studies (14, 31), but the range in these variables was small in this student population. Oral contraceptive use was too rare to be evaluated as a determinant of seropositivity.

In summary, although seroprevalence may underestimate cumulative exposure to HPV due to low seroconversion rates, it seems to be a useful molecular epidemiologic tool to assess HPV infection in young women unwilling to undergo gynecological examination, with potential application in monitoring the effect of HPV vaccines. The utility of seroprevalence has been increased further with the development of cost-efficient high-throughput assays, of the type used in this study, that allow the simultaneous analysis of antibodies against a large number of HPV types (25, 37). As is the case for HPV DNA prevalence, the significance of HPV seroprevalence among young men remains unclear.

References

1. Ho GY, Studentsov YY, Bierman R, Burk RD. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. *Cancer Epidemiol Biomarkers Prev* 2004;13:110-6.
2. Dunne EF, Nielson CM, Stone KM, Markowitz LE, Giuliano AR. Prevalence of HPV infection among men: a systematic review of the literature. *J Infect Dis* 2006;194:1044-57.
3. Shin HR, Franceschi S, Vaccarella S, et al. Prevalence and determinants of genital infection with papillomavirus, in female and male university students in Busan, South Korea. *J Infect Dis* 2004;190:468-76.
4. Sukvirach S, Smith JS, Tunsakul S, et al. Population-based human papillomavirus prevalence in Lampang and Songkla, Thailand. *J Infect Dis* 2003;187:1246-56.
5. Castle PE, Wacholder S, Lorincz AT, et al. A prospective study of high-grade cervical neoplasia risk among human papillomavirus-infected women. *J Natl Cancer Inst* 2002;94:1406-14.

6. Stone KM, Karem KL, Sternberg MR, et al. Seroprevalence of human papillomavirus type 16 infection in the United States. *J Infect Dis* 2002;186:1396–402.
7. Carter JJ, Koutsky LA, Wipf GC, et al. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. *J Infect Dis* 1996;174:927–36.
8. Dillner J, Kallings L, Brihmer C, et al. Seropositivities to human papillomavirus types 16, 18, or 33 capsids and to *Chlamydia trachomatis* are markers of sexual behavior. *J Infect Dis* 1996;173:1394–8.
9. Hagensee ME, Slavinsky J III, Gaffga CM, Suros J, Kissinger P, Martin DH. Seroprevalence of human papillomavirus type 16 in pregnant women. *Obstet Gynecol* 1999;94:653–8.
10. Kreimer AR, Alberg AJ, Viscidi R, Gillison ML. Gender differences in sexual biomarkers and behaviors associated with human papillomavirus-16, -18, and -33 seroprevalence. *Sex Transm Dis* 2004;31:247–56.
11. Strickler HD, Kirk GD, Figueroa JP, et al. HPV 16 antibody prevalence in Jamaica and the United States reflects differences in cervical cancer rates. *Int J Cancer* 1999;80:339–44.
12. Svare EI, Kjaer SK, Nonnenmacher B, et al. Seroreactivity to human papillomavirus type 16 virus-like particles is lower in high-risk men than in high-risk women. *J Infect Dis* 1997;176:876–83.
13. Viscidi RP, Kotloff KL, Clayman B, Russ K, Shapiro S, Shah KV. Prevalence of antibodies to human papillomavirus (HPV) type 16 virus-like particles in relation to cervical HPV infection among college women. *Clin Diagn Lab Immunol* 1997;4:122–6.
14. Wang SS, Schiffman M, Shields TS, et al. Seroprevalence of human papillomavirus-16, -18, -31, and -45 in a population-based cohort of 10,000 women in Costa Rica. *Br J Cancer* 2003;89:1248–54.
15. Wideroff L, Schiffman MH, Hoover R, et al. Epidemiologic determinants of seroreactivity to human papillomavirus (HPV) type 16 virus-like particles in cervical HPV-16 DNA-positive and -negative women. *J Infect Dis* 1996;174:937–43.
16. Wang ZH, Kjellberg L, Abdalla H, et al. Type specificity and significance of different isotypes of serum antibodies to human papillomavirus capsids. *J Infect Dis* 2000;181:456–62.
17. Nonnenmacher B, Hubbert NL, Kirnbauer R, et al. Serologic response to human papillomavirus type 16 (HPV-16) virus-like particles in HPV-16 DNA-positive invasive cervical cancer and cervical intraepithelial neoplasia grade III patients and controls from Colombia and Spain. *J Infect Dis* 1995;172:19–24.
18. Chua KL, Wiklund F, Lenner P, et al. A prospective study on the risk of cervical intra-epithelial neoplasia among healthy subjects with serum antibodies to HPV compared with HPV DNA in cervical smears. *Int J Cancer* 1996;68:54–9.
19. Strickler HD, Dillner J, Schiffman MH, et al. A seroepidemiologic study of HPV infection and incident cervical squamous intraepithelial lesions. *Viral Immunol* 1994;7:169–77.
20. Dillner J, Lehtinen M, Bjorge T, et al. Prospective seroepidemiologic study of human papillomavirus infection as a risk factor for invasive cervical cancer. *J Natl Cancer Inst* 1997;89:1293–9.
21. Sigstad E, Lie AK, Luostarinen T, et al. A prospective study of the relationship between prediagnostic human papillomavirus seropositivity and HPV DNA in subsequent cervical carcinomas. *Br J Cancer* 2002;87:175–80.
22. Carter JJ, Koutsky LA, Hughes JP, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis* 2000;181:1911–9.
23. Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. *J Natl Cancer Inst* 1994;86:494–9.
24. Thompson DL, Douglas JM, Jr., Foster M, et al. Seroepidemiology of infection with human papillomavirus 16, in men and women attending sexually transmitted disease clinics in the United States. *J Infect Dis* 2004;190:1563–74.
25. Waterboer T, Sehr P, Michael KM, et al. Multiplex human papillomavirus serology based on *in situ*-purified glutathione S-transferase fusion proteins. *Clin Chem* 2005;51:1845–53.
26. Ogilvie GS, Patrick DM, Schulzer M. Diagnostic accuracy of self collected vaginal specimens for human papillomavirus compared with clinician collected human papillomavirus specimens: a meta-analysis. *Sex Transm Infect* 2005;81:207–12.
27. Kleter B, van Doorn LJ, ter Schegget J, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. *Am J Pathol* 1998;153:1731–9.
28. Kleter B, van Doorn LJ, Schrauwen L, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *J Clin Microbiol* 1999;37:2508–17.
29. Studentsov YY, Ho GY, Marks MA, Bierman R, Burk RD. Polymer-based enzyme-linked immunosorbent assay using human papillomavirus type 16 (HPV16) virus-like particles detects HPV16 clade-specific serologic responses. *J Clin Microbiol* 2003;41:2827–34.
30. Dunne EF, Burstein GR, Stone KM. Anogenital human papillomavirus infection in males. *Adolesc Med* 2003;14:613–32.
31. Shin HR, Lee DH, Herrero R, et al. Prevalence of human papillomavirus infection in women in Busan, South Korea. *Int J Cancer* 2003;103:413–21.
32. Andersson-Ellstrom A, Dillner J, Hagmar B, et al. Comparison of development of serum antibodies to HPV16 and HPV33 and acquisition of cervical HPV DNA among sexually experienced and virginal young girls. A longitudinal cohort study. *Sex Transm Dis* 1996;23:234–8.
33. Fleming DT, McQuillan GM, Johnson RE, et al. Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med* 1997;337:1105–11.
34. Hagensee ME, Kiviat N, Critchlow CW, et al. Seroprevalence of human papillomavirus types 6 and 16 capsid antibodies in homosexual men. *J Infect Dis* 1997;176:625–31.
35. Smith JS, Lindsay L, Keys J, et al. HPV type distribution in invasive cervical cancer and high-grade cervical neoplasia: an update of meta-analyses and identification of global data gaps. *Int J Cancer* 2007;121:621–32.
36. Marais DJ, Rose RC, Lane C, et al. Seroreponses to human papillomavirus types 16, 18, 31, 33, and 45 virus-like particles in South African women with cervical cancer and cervical intraepithelial neoplasia. *J Med Virol* 2000;60:403–10.
37. Dias D, Van Doren J, Schlottmann S, et al. Optimization and validation of a multiplexed luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. *Clin Diagn Lab Immunol* 2005;12:959–69.