Population-Based Study of Human Papillomavirus Infection and Cervical Neoplasia in Rural Costa Rica

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Background: **Human papillomavirus (HPV) is the main cause of cervical neoplasia. Because few population-based studies have investigated the prevalence of type-specific infection in relation to cervical disease, we studied a high-risk population, estimating the prevalence of HPV infection and the risk associated with various HPV types.** ***Methods:*** **We screened 9175 women in Guanacaste, Costa Rica, to obtain a referent standard final diagnosis, and tested 3024 women for more than 40 types of HPV with a polymerase chain reaction-based system.** ***Results:*** **Among women with normal cytology, HPV infections peaked first in women younger than 25 years, and they peaked again at age 55 years or older with predominantly non-cancer-associated types of HPV and uncharacterized HPV types. Low-grade squamous intraepithelial lesions (LSILs) (n = 189) decreased consistently with age. The prevalence of high-grade squamous intraepithelial lesions (HSILs) (n = 128) peaked first around age 30 years and again at age 65 years or older. Seventy-three percent of LSILs were HPV positive, with HPV16 being the predominant type (16% of positive subjects). HPV was found in 89% of HSILs and 88% of cancers, with HPV16 being strongly predominant (51% and 53% of positive subjects). Virtually all HSILs and cancers had cancer-associated HPV types, with high odds ratios (ORs) and attributable fractions around 80%. Risk for HPV16 was particularly high (OR for HSILs = 320, 95% confidence interval [CI] = 97–1000; OR for cancer = 710, 95% CI = 110–4500).** ***Conclusions:*** **We confirm the early decline of HPV infection with age but note increased prevalence after menopause, which could be related to a second peak of HSILs, an observation that warrants further investigation. At least 80% of HPVs involved in cervical carcinogenesis in this population have been characterized. Polyvalent vaccines including the main cancer-associated HPV types may be able to prevent most cases of cervical disease in this region. [J Natl Cancer Inst 2000;92: 464–74]**

Worldwide, about 200 000 deaths each year are caused by cervical cancer *(1),* and its prevention with cytologic screening programs requires enormous investments from health agencies. Certain types of human papillomavirus (HPV) are now recognized as the main cause of cervical cancer and its precursor lesions *(2).* The development of a prophylactic vaccine against these infectious agents now appears to be the most promising way of controlling cervical neoplasia.

HPV infection of the uterine cervix is one of the most common sexually transmitted diseases *(3),* which is usually acquired around the time sexual activity begins. Consequently, cervical infections are frequently detectable among young women *(4,5).* Although the majority of infections are detectable only with molecular techniques, the most common cytopathologic manifestations of cervical HPV infection are low-grade squamous intraepithelial lesions (LSILs), i.e., cervical intraepithelial neoplasia 1, including koilocytotic atypia and flat condyloma. These lesions occur in the transformation zone of the cervix. They are characterized typically by cytoplasmic cavitation and nuclear atypia, cytopathic effects of a productive HPV infection *(6).*

Generally, pathologic changes and the molecular evidence of infection (HPV DNA detection) regress spontaneously with time *(6,7),* as do cutaneous warts caused by HPV types that infect nongenital skin. For yet unknown reasons, when the infection does not resolve, high-grade squamous intraepithelial lesions (HSILs) can develop and progress to cancer over a period of several years. HSILs are characterized by more severe nuclear alterations, less evidence of productive HPV infection, a more restricted set of HPV types, and a higher tendency to progress to invasive carcinoma. It has been proposed that infections with certain HPV types (mainly, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) are most likely to progress to cancer. These types have thus been designated "cancer-associated," but other aspects of the virus and host are likely to be involved in progression.

A working model describing the natural history of HPV infection has been assembled from multiple sources. However, to our knowledge, no group has investigated the whole spectrum of disease (HPV infection, LSILs, HSILs, and cancer) in a truly unselected random sample of a large defined population.

Furthermore, the distribution of HPV types in defined populations and the association of each HPV type with the severity of cervical disease need to be described in detail.

We report the results of a population-based screening of 9175 randomly chosen women in a rural province of Costa Rica. The screening included an intensive diagnostic work-up and testing a large sample of subjects for more than 40 types of HPV. The population-based nature of this study provides previously un-

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See "Notes" following "References."

available unbiased estimates of the prevalence of the full spectrum of HPV infections. The cross-sectional information derived from this analysis, in conjunction with the expected prospective data from an ongoing follow-up of this cohort, should aid in the design of phase III trials of HPV vaccines. These trials will probably be conducted in high-risk populations, such as the one in Guanacaste.

SUBJECTS AND METHODS

Study Population

This study was conducted in Guanacaste, a rural province of Costa Rica with a population of about 240 000 inhabitants, who have a high incidence of invasive cervical cancer (average annual incidence rate in past 10 years = 33 cases per 100 000 women, adjusted for the age distribution of the world population).

Detailed methodologic aspects of this investigation have been reported *(8)*. A random sample of 16.4% (178 of 1083) of the smallest geographic divisions established in Guanacaste by the Costa Rican census bureau (i.e., censal segments) was selected to obtain approximately 10 000 women for a cohort study of the natural history of HPV infection and cervical neoplasia. Careful house-to-house enumeration of all adult women (≥18 years old) residing in those segments was conducted over a 6-week period by outreach workers of the Costa Rican Ministry of Health, under our supervision. The census data for the segments selected for the study were compared (in combination) with data from the national 1984 census (the last available census) with respect to age group, province of birth, nationality, social security affiliation, province of residence 5 years earlier, educational level, marital status, labor force participation, and children currently alive. Data from the combined segments and the whole province appeared to be similar for all variables examined. From June 22, 1993, through December 12, 1994, the 11 742 women identified in the 178 censal segments above were invited by mail or personal visits to participate in the study. They were given appointments at the nearest government clinic to participate in a research project that included cervical cancer screening. At the clinic, women with mental or language problems were identified and excluded, and eligible women were identified and given detailed explanations of the study. Women who agreed to participate then signed informed consent forms approved by Institutional Review Boards of Costa Rica and the U.S. National Cancer Institute.

Data and Specimen Collection

Female interviewers conducted private, standardized interviews in which data were collected on demographic factors, medical histories, and behaviors (sexual, reproductive, and smoking) related to the risk of cervical cancer. Women who reported previous sexual activity were given a pelvic examination by female nurses trained by expert clinical collaborators. Any woman with obvious lesions was referred to the study gynecologist (J. Morales) for immediate gynecologic evaluation and treatment. During the pelvic examination, the nurse collected exfoliated cervical cells with a Cervex brush (Unimar, Wilton, CT) by placing the tip of the brush in the endocervix and rotating it five times in one direction (1800 °). The cells were used for the preparation of a conventional Pap smear, which was fixed immediately (PapPerfect; Medscand, Hollywood, FL) and later stained and interpreted by our collaborating cytopathologists in Costa Rica (M. Alfaro and S. Mekbel, Caja Costarricense de Seguro Social, San Jose, Costa Rica). The brush was placed in a methanol-based medium (PreservCyt; CYTYC, Boxborough, MA) for the preparation and interpretation of thin-layer slides at Tufts University, Boston, MA (M. Hutchinson, formerly at Tufts University). After the Pap examination, additional cells were obtained for HPV testing with a Dacron swab that was rotated 180 ° inside the endocervical canal and then used to collect cells from the entire circumference of the ectocervix. The cells were preserved in specimen transport medium (STM; Digene, Silver Spring, MD) and frozen at −30 °C in Guanacaste and later at −70 °C, after transport, until testing for HPV. After cells were collected as described above, the cervix was rinsed twice with 5% acetic acid, and a cervigram examination was performed. A cervigram consists of two photographs of the cervix, which were later developed at National Testing Laboratories (Fenton, MO) and interpreted by expert colposcopists (M. D. Greenberg and M. Campion, formerly at the Graduate Hospital, Philadelphia, PA).

Diagnostic Procedures

Cytologic specimens (conventional smears and thin-layer slides) were classified with the modified Bethesda System *(9,10)* into normal, ASCUS (atypical squamous cells of unknown significance), LSIL (cervical intraepithelial neoplasia 1, including koilocytotic atypia), HSIL (cervical intraepithelial neoplasia 2 and 3), and cancer. After Costa Rican cytopathologists had read the conventional Pap smears, the smears were analyzed with the PapNet method, which makes digital tapes containing 128 video images of the most important computer-selected areas of the smear.

In this study, these images were then reviewed on a computer screen by a senior cytotechnologist (D. Kelly) at The Johns Hopkins University, Baltimore, MD. Any smears with cells suspected of being neoplastic were referred to the expert study pathologist (M. E. Sherman) for final diagnosis.

All women with an abnormal cytologic test (ASCUS or more severe) were referred to the study colposcopist, who performed a biopsy of visible lesions. The median period between enrollment visit and colposcopy visit was 13 weeks (range = 4–65 weeks). Biopsy specimens were analyzed by local pathologists and reviewed by the study pathologist (M. E. Sherman). Cervigrams were classified as negative, atypical, or positive; women with positive results were referred for colposcopic evaluation. In addition, a random sample of one in 50 women in the study was referred for colposcopy as a control group, irrespective of their screening diagnosis. All confirmed or highly suspicious high-grade or invasive lesions were treated at the collaborating hospitals with loop excision, surgical conization, hysterectomy, or radiotherapy, according to local protocols.

The final diagnoses of most cases of cervical neoplasia were readily evident from algorithms combining the various cytologic and histologic diagnoses (*see below*). When a diagnosis was unclear, the study pathologist evaluated all available cytologic and histologic specimens to determine the final diagnosis. Diagnostic categories used were as follows: 1) normal = women with normal cytologic screening results, including those with abnormal cervigrams who did not have abnormalities in other tests (in the absence of cytologic abnormalities, a positive cervigram was not associated with HPV detection); 2) ASCUS = women with an ASCUS cytologic diagnosis with no substantial disease confirmed by colposcopy and/or biopsy (normal colposcopy not requiring biopsy or abnormal colposcopy but a non-SIL biopsy); 3) conventional LSIL = women with only conventional cytologic evidence of LSILs (the most severe of conventional or PapNet diagnoses) that was not histologically confirmed (normal colposcopy not requiring biopsy or abnormal colposcopy but a non-SIL biopsy); 4) thin-layer LSIL = women with evidence of LSILs only in the thin-layer smear; 5) "confirmed" LSIL = women with histologically confirmed LSILs or with at least two of the three criteria of conventional LSIL, thin-layer LSIL, or a positive cervigram; 6) HSIL = women with histologically or unequivocal cytologically confirmed HSILs after review; or 7) cancer = women with histologic or unequivocal clinical evidence of invasive cervical cancer. Histologic confirmation was obtained for all cancers detected in the population-based sample, 93.0% of HSILs, and 39.2% of confirmed LSILs.

To supplement the anticipated small number of women with invasive cancers, a rapid detection system was established to identify all residents of Guanacaste who were diagnosed with invasive cervical cancer during the enrollment period (supplemental cancers). A network was set up for the rapid notification of study staff when such a patient was diagnosed at one of the three main cancer referral hospitals in Costa Rica (San Juan de Dios, Calderon Guardia, and Mexico), diagnosed at the regional hospitals in Guanacaste, or reported to the National Tumor Registry. Patients considered eligible for the study completed the study questionnaire, and specimens were collected as described above. Twenty-eight women were eligible as supplemental patients with cancer, and valid HPV results were available from 22 (79%) of them. Because these supplemental patients originated from the same study base, they were added to the 12 patients with cervical cancer identified among women in the study sample.

HPV Testing

HPV testing by polymerase chain reaction (PCR) was performed on exfoliated cervical cells from 3024 women, and valid results were available from 2974 after excluding those with inadequate specimens (*see below*). Subjects selected for HPV testing included all women with abnormal cervical diagnoses (1364 women). The following women were also selected for HPV testing: all women with positive cervigrams in the absence of cytologic abnormalities (n = 311), all

women who tested positive for HPV DNA with a less sensitive screening test [n = 301; a hybrid capture tube test *(11)*], all women with a higher than average number of sexual partners (n = 333), and women in a random sample selected as a control group from the entire cohort (n = 340; *see below*) regardless of diagnosis and who may belong to overlapping groups mentioned above. Finally, an additional random sample of the women not included in the above groups was also selected for HPV testing, for a total of 1610 normal women.

We tested these groups for HPV to obtain baseline HPV data on subjects with prevalent disease at enrollment and on their corresponding control subjects and to obtain data on women with the highest potential of developing cervical neoplasia during the follow-up period of the study.

The prevalence of HPV infection in the general population was estimated from the results of the various population samples by weighting according to sampling fractions to avoid bias (*see below*).

Cervical cells were processed in a BioSafety Cabinet (SterilGARD Hood, Baker Inc., Sanford, ME) in a laboratory physically separated from where the PCR amplification was performed as described *(5)*.

Aliquots of 400 μL were taken from the residual specimens previously tested by the hybrid capture tube test. Cells were removed with a disposable, sterile transfer pipette, placed in 100 μL of K buffer *(12)* containing proteinase K at 400 mg/mL, and incubated at 55 °C for 2 hours and at 95 °C for 10 minutes *(7,12)*. Ten microliters of this material was then amplified by PCR with the MY09/MY11 L1 consensus primers including HMB01 *(7)*, which amplifies a 450-base-pair HPV DNA fragment, and a control primer set, PC04/GH20 *(12)*, which simultaneously amplifies a 268-base-pair cellular β-globin DNA fragment and serves as an internal control. Ten microliters of PCR products or the entire reaction mixture was analyzed by gel electrophoresis in 3% NuSieve–0.5% SeaKem agarose (FMC BioProducts, Rockland, ME) and transferred to nylon filters. The filters were hybridized overnight with radiolabeled generic probes for HPV and an oligonucleotide for β-globin as described *(12,13)*. The filters were washed in 2× standard saline citrate (SSC; 1× SSC = 0.15 *M* sodium chloride and 0.015 *M* sodium citrate [pH 7])/0.1% sodium dodecyl sulfate at 55 °C and exposed to x-ray film.

Samples that hybridized the β-globin probe but not the generic probe were considered HPV negative. Subjects whose samples were negative for the β-globin probe and negative for the generic probe (n = 50; 1.7% of those tested) were excluded from the analysis. Samples that were β-globin negative but PCR positive were considered HPV positive. PCR products that hybridized to the HPV generic probe were tested with more than 40 specific types of HPV DNA. Seven-microliter aliquots of PCR products were denatured in 0.4 *M* NaOH–25 m*M* EDTA and applied to 10 replicate filters with a 96-well dot-blot apparatus (Bio-Rad Laboratories, Hercules, CA). Filters were individually hybridized, as described *(7,12,14)*, to biotinylated, type-specific oligonucleotide probes for the following types of HPV: 2, 6, 11, 13, 16, 18, 26, 31, 32, 33, 34, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61 (AE4), 62, 64, 66, 67, 68, 69, 70, 72, 73 (PAP238A), AE2, W13B, 83 (PAP291), and PAP155 [probes referenced or described in *(15)*], AE5 (CTGCAACTACTAATCCAGTTCC), AE6 (CCACAGAATACAGTTCTACACGCT), AE7 (AGCTACATCTGCTGCTGCA), and 71 (AE8) (CTGTGCTACCAAAACTGTTGAG). Samples that gave a positive result with the generic probe mixtures but a negative result with all type-specific probes were considered to have "uncharacterized" HPV types. In this analysis, the group of *a priori* cancer-associated HPV types includes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. These HPVs are also the 13 most common types in the International Biological Study on Cervical Cancer *(16)*. The group of "non-cancer-associated" HPV types includes all other HPV types tested. Some of these are recognized non-cancer-associated types (e.g., HPV2, HPV6, HPV11, HPV32, HPV40, HPV42, and HPV57), and others are HPV types with undetermined oncogenic potential (e.g., HPV53, HPV54, HPV61, HPV62, HPV64, HPV67, HPV69, HPV70, HPV72, HPV73, etc.). The strength of the hybridization signal was determined from the ethidium gel and autoradiogram, by taking into account the strength of the hybridization signal and the thickness of the band in the gel. Signal strength data were independently reviewed by two investigators, and discordant results were resolved by consensus.

Statistical Analysis

We used odds ratios (ORs) with 95% confidence intervals (CIs) to estimate relative risk and multiple logistic regression to adjust for potential confounding variables. In the logistic regression, we adjusted for age by using six age groups (<25 years old, 25–34 years old, 35–44 years old, 45–54 years old, 55–64 years old, and ⩾65 years old).

A random sample of 389 women was selected as a subcohort, without considering their screening results, to check the sensitivity of our screening protocol and to provide a random control group. Of these 389 women, 31 were excluded because of reported hysterectomies and 18 were excluded because their cervical specimens were β-globin negative, leaving a control group of 340 women.

In the calculation of ORs, subjects in the control group with the diagnosis under study were included as case subjects, but subjects with more severe diagnoses were excluded from the analysis. For example, when ORs were calculated for confirmed LSILs, control subjects with confirmed LSILs (n = 8) were considered case subjects, control subjects with HSILs (n = 2) were excluded, and control subjects with normal (n = 305), ASCUS (n = 18), thin-layer LSIL (n = 4), or conventional LSIL (n = 3) diagnoses were retained as control subjects. Risk associated with a single infection by individual HPV type was estimated by excluding women with multiple infections from the analysis (11 patients with cancer, 38 patients with HSILs, 59 patients with confirmed LSILs, and 20 control subjects). For the calculation of attributable fraction or proportion of disease attributable to individual or grouped HPV types, the following formula was used: attributable fraction = percent case subjects who are HPV positive × [1 − (1/OR)] *(17,18)*. ORs for the estimate of attributable fractions (*see* Table 4) were calculated by including each subject hierarchically in only one category of HPV type (HPV16; HPV18, HPV31, or HPV45; other cancer-associated, non-cancer-associated, and uncharacterized HPV types) and comparing them with HPV-negative subjects. Adjustment for other cervical cancer risk factors (education, age at first intercourse, number of sexual partners, number of pregnancies, duration of oral contraceptive use, and ever smoking) did not meaningfully modify the risk estimates. Therefore, for simplicity, only age-adjusted results are presented. Because HPV testing results were available from all subjects with abnormal diagnoses but were available from only a fraction of those with normal results, bias was prevented by weighting with a Horvitz–Thompson-type estimating function *(19)*. This function inversely weights the contribution of each subject by her probability of selection. This analysis was carried out by multiplying the percentage of women positive for HPV DNA in each subgroup of normal women tested (e.g., those selected on the basis of a known above-average number of sexual partners) by the prevalence of HPV in each individual subgroup, to arrive at the estimate for the normal category. This estimate was then added to the other categories to obtain the total. CIs for the prevalence estimates were calculated by the information sandwich technique *(20,21)* under the assumption that subjects were sampled from an infinite population. All *P* values are from two-sided tests.

Participation Rates

The original sample from the census of selected segments included 11 742 women, of whom 10 738 were eligible for the study and 10 049 were interviewed (94%). The majority of nonparticipants refused or did not show up for their appointments after multiple invitations.

Noneligible women included pregnant women who could not schedule a repeat visit by 3 months postpartum (2.6%), women who had moved out of Guanacaste (4.4%), and women who were physically ill (0.5%), mentally ill (0.7%), or dead (0.4%).

After exclusion of women without previous sexual experience, 9466 women of those interviewed were considered eligible for a pelvic examination, which was performed on 9175 women (97%), for an overall participation rate of 91%. The main reason for not performing a pelvic examination was refusal or physical problems associated with old age (41% of subjects not examined were older than 65 years), although more than 80% of older women received a pelvic examination. Satisfactory results of a conventional Pap test were available from 9093 women (99% of those with pelvic examinations), thin-layer diagnoses were available from 8694 (95%), PapNet results were available from 7375 (80%), and cervigram results were available from 9062 (99%). The reduced number of PapNet results occurred because of difficulties in shipping and processing specimens *(22)*. Detailed analyses of the performance of all methods used are reported elsewhere *(22–24)*.

For this analysis, women who reported hysterectomy (n = 621) were excluded, leaving 8582 women in the analytic dataset (8554 women from the population sample and 28 supplemental patients with invasive cancers). HPV results were available from more than 91% of subjects in each category of abnormal diagnoses (Table 1), except for women with invasive cancers, where

HPV results were available from 85% of the patients. HPV results also were obtained from 23% of subjects with normal cytologic diagnoses (n = 1610) selected as described above.

RESULTS

Characteristics of the Population

The median age of women included in this analysis was 37 years (range = 18–94 years). The median age at first sexual intercourse was 18 years, and more than half of the women reported only one lifetime sexual partner. A substantial proportion of the women reported multiple pregnancies (median = 4). Only 11% of the women in the sample reported ever having smoked. Most women reported having used oral contraceptives (63%) and having had a Pap test (87%). Characteristics of the control group of 340 randomly selected women tested by PCR were compared with characteristics of all 8554 women from the population sample, and no statistically significant difference was noted for any variable discussed above (data not shown).

Prevalence of HPV Infection and Cervical Neoplasia

The estimated overall prevalence of HPV in this population was 16% (95% CI = 15–18). Table 1 presents the distribution of subjects, the overall prevalence of each diagnostic category, the number of subjects tested for HPV, the overall prevalence of HPV, and the prevalence of cancer-associated (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), non-cancer-associated (all other HPV types investigated), and uncharacterized HPV types. Age-adjusted ORs and 95% CIs associated with having any type of HPV are presented also. Prevalence of detection of any type of HPV was 73% in LSILs, 89% in HSILs, and 88% in cancers. More severe disease was associated with higher prevalence of HPV DNA, higher prevalence of cancer-associated HPV types, and higher ORs in association with HPV detection. Women with final diagnoses of normal, ASCUS, conventional LSILs, and thin-layer LSILs were more likely to have non-cancer-associated or uncharacterized HPV types than cancer-associated HPV types; the opposite was true for confirmed LSILs, HSILs, and cancer.

Fig. 1, A, shows the estimated age-specific prevalence (and 95% CIs) of any type of HPV infection among women with normal or ASCUS diagnoses. Prevalence was highest (around 20%) for women under age 25 years, decreased to about 5% for women 35–54 years old, and then increased to almost 20% for women 65 years old or older. Fig. 1, B, presents the estimated prevalence for cancer-associated, non-cancer-associated, and uncharacterized types of HPV. The last types were included as a separate group because of their strong association with risk of HSILs and cancer (*see below* and *see* Table 4). Among women younger than 25 years, cancer-associated types predominated slightly, followed by non-cancer-associated types. Among women 55 years old or older, the pattern seemed to be reversed, with non-cancer-associated and uncharacterized types predominating. This pattern was driven mainly by the HPV type distribution among women with normal diagnoses because women with ASCUS diagnoses had a predominance of cancer-associated types in both age groups. Among women with ASCUS, the prevalence of cancer-associated and non-cancer-associated types increased after age 55 years, with cancer-associated types predominating. Estimates for that age group, however, were based on a smaller sample (n = 72 older women with ASCUS; data not shown).

The median age of patients with confirmed LSILs was 29 years. The prevalence of this diagnosis was highest in women younger than 25 years (Fig. 2), where it reached 5.2% and then decreased rapidly and consistently with age to a low of 0.4% among women 65 years old or older. When women with conventional LSILs and thin-layer LSILs were included in the group with confirmed LSILs, prevalence increased to 10% in women younger than 25 years and to 2.4% in women older than 65 years (data not shown).

The median age of patients with HSILs was 34 years. An

Table 1. Diagnostic categories, human papillomaviruses detected, and age-adjusted odds ratios*

	No.	Prevalence of diagnosis, %	No. with HPV results†	Detection of any HPV type,‡ %	Cancer-associated HPV type,§ %	Non-cancer-associated HPV type,‖ %	Uncharacterized HPV types, %	Age-adjusted ORs for any HPV	95% CI
All diagnoses	8582	100	2974	16¶	7.6¶	6.7¶	3.7¶	N/A	N/A
Normal	7131	83	1610#	11¶	3.9¶	4.7¶	3.3¶	1.0	
ASCUS	764	8.9	698	20	10	8.0	4.9	1.6	1.1–2.3
Conventional LSIL	186	2.2	182	31	15	16	3.3	2.6	1.6–4.0
Thinprep LSIL	144	1.7	144	65	35	31	9.7	11	6.7–18
Confirmed LSIL	189	2.2	181	73	54	34	5.0	15	9.0–24
HSIL	128	1.5	125	89	80	20	4.0	42	22–82
Cancer**	40	0.14	34	88	79	24	5.9	46	14–150

*HPV = human papillomavirus; OR = odds ratio; CI = confidence interval; N/A = not applicable; ASCUS = atypical squamous cells of unknown significance; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion.

†Excludes women who tested negative for β-globin.

‡The sum of the prevalence of cancer-associated, non-cancer-associated, and uncharacterized HPV types is higher than the sum for any HPV because of multiple infections.

§Includes HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

‖Includes all other HPV types investigated, except uncharacterized types.

¶The prevalence in the entire population and the prevalence among normal subjects were estimated from extrapolation of results from a sample of 1610 women with normal cytologic diagnoses selected for HPV testing (*see* text).

#The sample of 1610 normal subjects includes the 305 randomly selected control subjects with normal diagnoses and the additional 1305 subjects selected by criteria described in the text.

**The number of 40 cases includes screen-detected and supplemental cases of cancer, which were combined to calculate prevalence of HPV and the ORs; 12 prevalent cases of cancer were used to estimate the prevalence of 0.14 (*see* text).

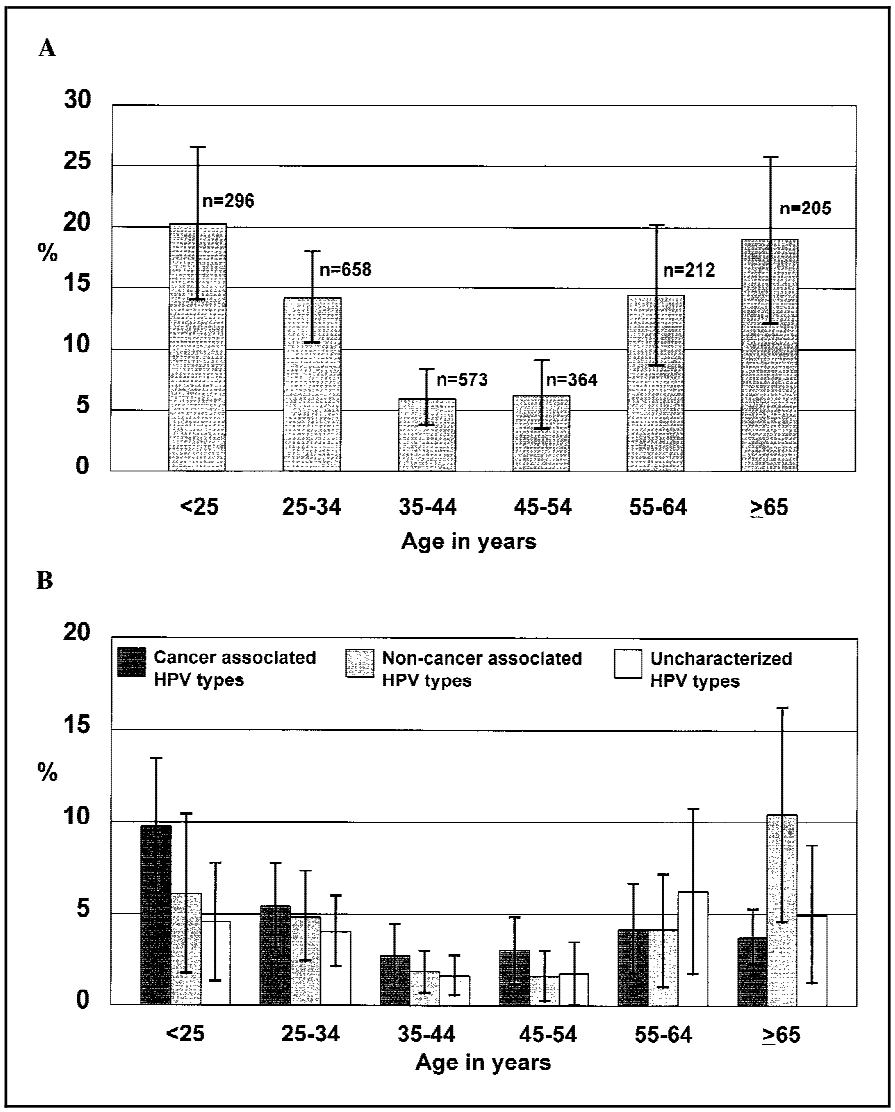


Fig. 1. A) Estimated prevalence and 95% confidence intervals of human papillomavirus (HPV) DNA detection among women with normal or ASCUS (i.e., atypical squamous cells of unknown significance) diagnoses. The number of women in each age group (n) are shown. **B**) Estimated prevalence and 95% confidence intervals of specific types of HPV among women with normal or ASCUS diagnoses. Cancer-associated HPV types include HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Non-cancer-associated HPV types include other HPV types identified.

initial peak of 2.0% at around age 30 years was observed, and a second peak of a similar magnitude was evident among women 65 years old or older. Although the number of older subjects with HSILs was small (17 women aged 65 years or older), the difference between the prevalence of HSILs in women 55–64 years old and women 65 years old or older was marginally statistically significant (two-sided Fisher's exact test, $P = .05$). In this context, women in the study population 55 years old or older were less likely to have been screened previously than younger women (75% versus 90%, $P<.001$).

The median age of the 12 women with screen-detected cancers was 39.5 years, but their age distribution was difficult to interpret because of their small numbers in our study. The 28 women with supplemental cases of cancer had a median age of 58 years (data not shown). As expected, 75% of the screen-detected cancers were diagnosed at early stages (International Federation of Gynecology and Obstetrics stages I and II) compared with only 19% of supplemental cancers.

Prevalence of HPV Types in Each Diagnostic Category

The prevalence of specific HPV types was calculated for the 305 control women with normal cytologic findings selected randomly from the cohort (excluding final diagnoses of ASCUS or more severe).

Twenty-six types of HPV were detected among women with normal diagnoses (Table 2), and HPV16 and HPV18 were uncommon (each at 1.0%; 9.1% of positive subjects), as were the condyloma-associated types HPV6 and HPV11. Multiple infections were present in 4.3% of normal women, corresponding to 39% of the infections.

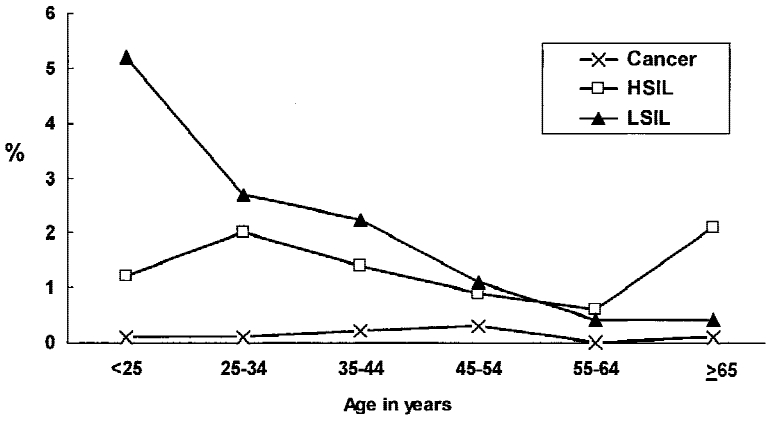
In confirmed LSILs, 32 HPV types were detected. The most common was HPV16 (prevalence = 12%; 16% of positive subjects), followed by HPV types 51, 56, 58, 52, 31, 70, 39, 53, and 6. Uncharacterized types were common (prevalence = 5.0%; 6.8% of positive subjects). Almost all other types of HPV investigated were found in at least one LSIL. Multiple infections were detected in 33% of LSILs, corresponding to 45% of positive subjects. The proportion of HPV-positive subjects with cancer-associated HPV types was similar among women with LSIL in different age groups (data not shown).

Twenty-eight HPV types were detected in HSILs. The most common type by far was HPV16, found in 45% of HSILs (51% of positive subjects), followed by HPV58, detected in 10% of HSILs. Most HSILs had at least one previously identified cancer-associated type. However, in one HSIL, HPV72 was detected alone; in another HSIL, HPV83 (pap291) was detected alone. In addition, HPV70, HPV53, HPV67, and AE5 (the last two in the same subject) were detected in some HSILs without cancer-associated types. Uncharacterized types were present in 4.0%, with multiple infections in 30% (34% of positive subjects).

In the 34 cancers, 18 HPV types were detected. All cancers had previously identified cancer-associated types, except for one in which HPV66 was detected.

As observed for HSILs, HPV16 was the most common type (47%; 53% of positive subjects), followed by HPV18 (15%; 17% of positive subjects) and HPV58 (12%; 14% of positive subjects). Multiple infections were detected in 32% of the cancers (36% of positive subjects). Among women with HPV-positive HSILs and cancers, the proportions with cancer-associated types were similar in different age groups, except for women 65 years old or older, who had a somewhat higher prevalence of non-cancer-associated HPV types detected as the only infection (22% versus <8% in all other age groups combined).

Table 3 shows the HPV types detected in HSILs and cancers among women with multiple infections and indicates which type(s) had the strongest signal. Each cancer tested had at least one high-risk HPV type; of the 11 cancers with multiple HPV



LSIL	5.2	2.7	2.2	1.1	0.4	0.4
HSIL	1.2	2.0	1.4	0.9	0.6	2.1
Cancer	0.1	0.1	0.2	0.3	0.0	0.1

Fig. 2. Prevalence of cervical neoplasia by age group in Guanacaste. LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion.

types, seven had at least two high-risk types. Similarly, of 38 HSILs with multiple types, 34 (89%) had recognized cancer-associated HPV types. For cancers and HSILs, cancer-associated HPV types almost invariably had the strongest PCR signal.

ORs and Attributable Fractions

Table 4 presents the prevalences and ORs for HPV types associated with various diagnoses. Subjects were considered hierarchically positive in only one of the groups, and HPV-negative subjects were the referent category.

Cancer-associated HPV types were present in more than 50% of confirmed LSILs and were associated with double-digit ORs. Non-cancer-associated HPV types were detected in about 15% of confirmed LSILs and were associated with lower ORs. The attributable fraction associated with HPV of any type was 68%. HPV16 was detected in almost 50% of the HSILs and was associated with a 320-fold increase in risk (95% CI = 97–1000). The attributable fraction for cancer-associated types of HPV was almost 80%; for any HPV, the attributable fraction reached 87%. A similar pattern was observed for cancers, with an attributable fraction for cancer-associated HPVs close to 80%, the presence of HPV16 in about 50% of the cancers, and an even higher OR for HPV16 of 710 (95% CI = 110–4500).

ORs associated with single HPV infections were calculated after excluding multiple infections. With the exception of a few HPV types, the number of lesions with single HPV types was relatively small and produced increased, but statistically nonsignificant, risk estimates, particularly for cancer-associated types (data not shown). For LSILs, statistically significant ORs were observed for HPV types 16, 39, 51, 52, and 58, with magnitudes between OR = 8.9 (95% CI = 1.6–49) for HPV58 and OR = 41 (95% CI = 4.9–340) for HPV51.

For HSILs, statistically significant associations with risk were detected for HPV types 16, 58, 51, and 52, with magnitudes between OR = 20 (95% CI = 3.8–100) for HPV51 and OR = 1400 (95% CI = 120–16 000) for HPV16. For the 34 women with cancer, statistically significant risk estimates were observed for HPV16 (OR = 470; 95% CI = 68–3300) and HPV18 (OR = 120; 95% CI = 6.7–2200).

ORs were calculated by comparing women with multiple HPV type infections and women with single infections. No statistically significant increases in risk were observed for any of the diagnoses (data not shown).

To investigate the effect of HPV16 on risk of HSIL or cancer combined (HSIL/cancer) in the presence of other types of HPV, we estimated ORs for HSIL/cancer associated with HPV16 infections alone, associated with infections of multiple HPV types not including HPV16, or associated with infections of multiple HPV types including HPV16 (Table 5). A multiple infection not including HPV16 was associated with an OR of 29 (95% CI = 13–66), and an infection with HPV16 alone was associated with an OR of 450 (95% CI = 100–2000). However, a multiple infection including HPV16 was not associated with a higher risk than the other categories (OR = 190; 95% CI = 39–920).

Fig. 3 presents ORs associated with increasing PCR signals, with the highest signal detected used for each individual, independent of the HPV type. ORs increased with increasing signals in confirmed LSILs and HSIL/cancers. For confirmed LSILs, higher signal intensities were associated with higher ORs for cancer-associated and non-cancer-associated HPV types (data not shown).

Discussion

To our knowledge, this is the first population-based study to investigate the prevalence of HPV types in all grades of cervical neoplasia observed in a large sample of a high-risk population. We used multiple screening techniques and carried out extensive diagnostic work-ups to ensure completeness of case identification and classification. More than 90% participation in all components of the study was attained, assuring the representativeness of our study population and allowing population-wide estimates by sampling. The use of a randomly selected control group also permitted unbiased assessment of ORs and attributable fractions for specific HPV types. Women in Guanacaste were characterized by frequent monogamy (>50%), high parity, limited education, and a high frequency of previous screening. Notably, the persistently high incidence of cervical cancer in Guanacaste indicates the limited effectiveness of previous screening.

The estimated overall prevalence of HPV infection was 16% in the entire population. Among women with normal cervical diagnoses, the estimate was 11%, corresponding to the point prevalence of current HPV DNA detection. The cumulative incidence of infection is certainly higher and will be examined with HPV serologic markers and follow-up data from the same population.

We created diagnostic categories to indicate the severity of cervical disease, without regard to HPV infection. We observed, however, that increasingly severe diagnostic categories were strongly associated with increasing overall detection of HPV in the lesion, increasing prevalence of cancer-associated HPV types, and increasing ORs, when compared with the control group. Theoretically, all LSILs are the result of a productive

Table 2. Prevalence of type-specific human papillomavirus infection by final diagnosis*

Type	Normal, %* (n = 305)	Confirmed LSIL, % (n = 181)	HSIL, % (n = 125)	Cancer, % (n = 34)
Any type	11†	73	89	88
6	0.7‡	6.1	2.4‡	2.9‡
11	0	3.9	0	2.9‡
16	1.0	12	45	47
18	1.0	4.4	5.6	15
26	0.3‡	2.8‡	0	0
31	0.3‡	7.2	6.4	5.9
32	0.3‡	0.6‡	0.8‡	0
33	0.7	1.1	3.2	8.8‡
35	0.3‡	2.2‡	3.2	2.9‡
39	0.7	6.6	3.2‡	0
40	0	1.1‡	0	0
45	0	3.9‡	2.4	0
51	0.3	9.9	7.2	2.9‡
52	1.0	7.2	7.2	0
53	1.0	6.1	4.0‡	0
54	1.0	0	2.4‡	0
55	0.7	1.7	0	2.9‡
56	0	8.8	3.2	2.9‡
58	1.6	8.8	10	12
59	0	1.7‡	0.8‡	2.9‡
61	1.3	3.9	0.8‡	2.9‡
66	0	4.4	0	2.9
67	1.0‡	1.1	1.6‡	0
68	0.3	1.7	0.8	5.9‡
70	0.7‡	6.6	3.2‡	0
72	0	1.1‡	0.8	5.9‡
73	0.7‡	2.8	0.8‡	0
AE2 (IS39)	0.3	1.1	0.8‡	2.9‡
AE5	1.0‡	1.1‡	3.2‡	0
AE6 (CP6108)	1.3‡	1.1	0.8‡	0
AE7 (CP8304)	1.0	2.2	4.0‡	0
AE8 (HPV71)	2.0	2.8‡	1.6‡	2.9‡
pap155	0.3‡	2.8‡	0	0
pap291 (HPV83)	0	0	2.4	0
Uncharacterized	2.0	5.0	4.0	5.9
Multiple infections	4.3	33	30	32

*Includes subjects in the random control group of 340 subjects, after exclusion of diagnoses of ASCUS (i.e., atyical squamous cells of unknown significance) or more severe. HPV = human papillomavirus; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion.

†The prevalence of any HPV was estimated from the sample of 1610 cytologically normal women tested with a polymerase chain reaction-based assay, and the prevalence of individual types was estimated from the 305 normal subjects in the control group (*see* text).

‡Indicates HPV types detected only in multiple infections.

Table 3. Multiple-type human papillomavirus (HPV) infections in high-grade squamous intraepithelial lesions (HSILs) and cancers*

HSILs	Cancers
6, ***51***	***18, 51,*** *52,* 73, AE7, **291**	6, **11,** *33*
6, *56,* **70**	***18,*** AE5	*16,* ***18,*** **51**
6, 70	*31, 52,* **AE5**	***16,*** *33*
16, *18*	***31,*** 53	***16,*** *35*
16, ***31***	*31,* 67, **AE5, AE6, AE8**	*16,* AE8
16, 32	*33, 52*	*18, 31*
16, *33*	*33,* ***58***	***18,*** *59,* 72
16, *39*	*35, 39,* ***52,*** *56,* ***58***	**33,** *58,* AE2
16, *39, 51*	*39,* **53**	55, *68*
16, *45*	*45,* ***58***	56, ***58,*** **61,** *68*
16, 52	*51,* ***58***	***58,*** 72
16, **53**	***51,*** 291	
16, 54	*52,* 54, ***58***	
16, *59*	***52,*** *58*	
16, 70	*52,* AE8	
16, AE7	**53,** 61, AE2, AE7	
16, AE7	53, **70**	
16, AE7	54, ***56***	
18, ***35***	67, **AE5**	

***Boldface type** = highest signal intensity; **lightface type** = not predominant; **italic type** = cancer-associated HPV types (type 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, or 68). Each group represents a different lesion or cancer.

HPV infection; thus, HPV DNA should always be detectable. However, we observed that HPV DNA was not detected in almost 30% of LSILs, which indicates some misclassification of disease or HPV status. Because the main goal of our study was to detect HSILs with high sensitivity by the use of multiple screening techniques, we referred more than 20% of subjects to colposcopy and thus complicated the final case definition.

HPV detection was strongly associated with age for women with normal or ASCUS diagnoses, being high in the youngest women and declining rapidly to a low in women around 35 years, as described by other investigators *(4,5)*. However, in this population, the prevalence of HPV types, particularly non-cancer-associated HPV types, increased again among women 55 years old or older. For women with ASCUS diagnosis, cancer-associated types predominated at all ages, but the prevalence of cancer-associated and non-cancer-associated HPV types also increased after age 55 years. We have described *(8)* a similar but less marked pattern in the same population by use of the hybrid capture method. The second peak of prevalence is intriguing and has not been consistently noted by others, in part because some studies have not included enough older women. It is interesting that Muñoz et al. *(25)* have reported similar data for age and the detection of HPV DNA among control women in their studies in Spain and Colombia, although the predominant HPV types in those women were cancer associated and uncharacterized *(26,27)*.

One possible explanation for this second peak would be a cohort effect, with older women having been exposed more intensely to HPV. Alternatively, the second peak could indicate reactivation of a latent HPV infection, a possibility that has been proposed for women also infected with human immunodeficiency virus *(28)*.

Another possibility could be that the detection of HPV increases as atrophic changes occur in the postmenopausal cervix. Currently, we cannot explain the marked increase in HPV detection among older women, and more investigation in different populations, including risk factors for infection by different types *(29,30,31)*, and in different age groups is needed. Such studies should incorporate markers of immune suppression and HPV type-selective tests for viral latency when available.

The 11% overall estimated prevalence of HPV infection in women with normal diagnoses in our study population was somewhat lower than values observed in other case–control studies in high-risk countries, particularly among middle-aged women *(25,32)*. This difference could be explained by our strict criteria for definition of normal diagnoses, differences in the population selected, or limited sensitivity of our PCR.

The prevalence of LSILs was highest among the youngest women, with a median age of 29 years, and coincided with the first peak of HPV infection among normal women. However, we did not observe a second peak of LSILs in the older women. This could indicate that older women are less prone to develop overt cervical intraepithelial neoplasia because of cervical atrophy or mature metaplastic epithelium in the transformation zone.

Table 4. Prevalence, age-adjusted odds ratios (ORs), and attributable fractions (AFs) of selected human papillomavirus (HPV) types or combinations

HPV type(s)	Prevalence of HPV, %	Age-adjusted OR*	95% confidence interval	AF, %	Cumulative AF, %
Low-grade lesions (n = 181)					
16	12	29	8.4–100	12	12
18, 31, or 45	13	45	12–160	12	24
Other cancer-associated†	29	17	9.0–34	28	52
Non-cancer-associated‡	14	7.7	3.7–16	12	64
Uncharacterized	5.0	6.2	2.1–18	4.2	68
High-grade lesions (n = 125)					
16	45	320	97–1000	45	45
18, 31, or 45	12	56	18–180	12	57
Other cancer-associated	23	31	14–70	22	79
Non-cancer-associated	4.8	5.4	1.8–16	4.0	83
Uncharacterized	4.0	12	3.4–44	3.6	87
Cancer (n = 34)					
16	47	710	110–4500	47	47
18, 31, or 45	15	150	22–1000	15	62
Other cancer-associated	18	20	4.5–90	17	78
Non-cancer-associated	2.9	2.2	0.2–23	1.6	80
Uncharacterized	5.9	27	3.5–210	5.7	86

*Corresponding to risk associated with single or multiple infections.
†Includes HPV types 33, 35, 51, 52, 56, 58, 59, and 68.
‡Includes all other types of HPV.

Table 5. Age-adjusted odds ratios (ORs) for high-grade squamous intraepithelial lesions and cancer combined associated with single human papillomavirus 16 (HPV16) infections, multiple HPV infections not including HPV16, and multiple infections including HPV16

	OR (95% confidence interval)	
Multiple HPV infection*	HPV16 negative	HPV16 positive
No	1.0† (referent)	450‡ (100–2000)
Yes	29§ (13–66)	190‖ (39–920)

*Includes infection with cancer-associated and non-cancer-associated HPV types.
†Based on 18 case subjects and 279 control subjects. Case and control subjects with single infections associated with types other than HPV were excluded from this analysis.
‡Based on 53 case subjects and two control subjects.
§Based on 30 case subjects and 18 control subjects.
‖Based on 19 case subjects and two control subjects.

The observation that the prevalence of HSILs peaked in women 25–34 years old is consistent with previous findings *(33)* and the hypothesis of a disease continuum with progression from HPV infection to HSIL to cancer. In fact, we could roughly divide the HSILs into the equivalent of cervical intraepithelial neoplasia 2 and 3, with corresponding median ages of 33 and 37 years, respectively, which would corroborate the hypothetical transition time of more than 5 years from HPV infection (including LSILs) to HSILs. In this population, a second peak of HSILs is observed in older women, which could be partially explained by a cohort effect in screening behavior. Fewer women older than 55 years had a history of being screened, but this explanation would imply the existence of long-term lesions that do not progress to cancer. Alternatively, this could reflect the second peak of HPV DNA, which might result from reactivation of latent HPVs, particularly some types of yet unknown carcinogenic potential.

The number of cancers detected in our study was too small to allow conclusions about prevalence in our sample. However, the median age of women with screen-detected cancers was 39 years, which is 5 years older than the median age of women with HSILs. It has been proposed that HSILs progress to subclinical cancer in 9–10 years and that subclinical invasive cancer progresses to symptomatic invasive cancer in 4–5 years *(33)*. Our findings are consistent with the progression time from HSIL to subclinical cancer if only the early peak at age 30 years is considered. However, the median age of women with supplemental cases of cancer was 58 years, which, if compared with the mostly early-stage cancers detected in the sample, would indicate a very slow progression from subclinical to symptomatic cancer, although this difference could be explained by chance. These findings also probably indicate inadequate follow-up and treatment of dysplasia, despite a frequent history of screening among women in Guanacaste.

In normal women, we detected almost all HPV types for which we tested, and no type was clearly predominant. HPV16 was detectable in only about 1% of normal subjects. Because HPV16 is rare among normal women but common in women with HSILs and cancers, its predictive value may be even higher than suspected, particularly in this population. It could also partially reflect the fact that our definition of "normal" is stricter than definitions in other studies, given our highly sensitive screening.

In LSILs, almost all HPV types were detected, and HPV16 was the most common. Cancer-associated HPV types were present in more than 50% of the lesions (corresponding to 75% of the HPV-positive lesions). The LSILs harboring cancer-associated HPV types are probably the most likely to persist and progress *(34)*, but the clinical value of cancer-associated HPV detection as a predictor of the behavior of LSILs has not been determined.

In addition to the cancer-associated HPV types, several other types of HPV were also frequently detected in LSILs, indicating that a subset of LSILs is caused by non-cancer-associated HPV types. Such HPV types theoretically would not have the poten-

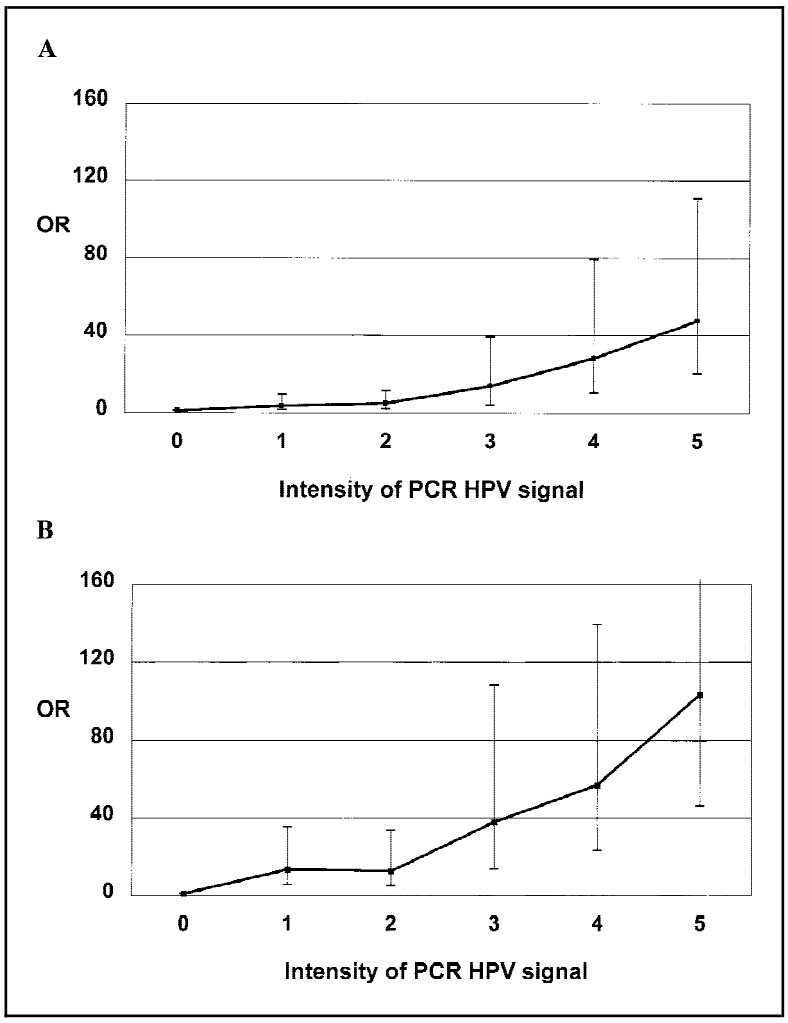


Fig. 3. A) Odds ratio (OR) of confirmed low-grade squamous intraepithelial lesions by polymerase chain reaction (PCR) signal intensity of human papillomavirus (HPV). Intensity 0 = HPV-negative specimen. **B**) OR of high-grade squamous intraepithelial lesions and cancer by intensity of PCR HPV signal.

tial to cause progressive disease despite being able to cause apparently similar cytologic abnormalities. Whether the LSILs produced by such HPVs have distinctive colposcopic or microscopic characteristics is unclear.

The majority of HSILs and cancers were associated with previously identified cancer-associated HPV types, particularly HPV16, which was detected in almost 50% of both types of lesions. This finding is consistent with previous reports *(35–38)*. HPV58 was the second most common HPV type in HSILs and the third most common type in cancers in our population. This finding is in contrast with the findings of Bosch et al. *(16)*, who found that HPV58 was not common among cancer patients from Central America or South America. However, two reports from China *(39,40)* indicate that HPV58 is common in patients with cancer in the Pacific region.

In our study, HPV18 was the second most common type in cancers but was not so in HSILs. These findings could indicate that an HPV18 infection could lead more rapidly to cancer than infection with other cancer-associated HPV types. In several studies *(41–43)*, the survival of patients with cervical cancer was worse for women harboring HPV18, independent of the stage at diagnosis.

In HSILs, besides known cancer-associated types, HPV72 and HPV83 (pap291) were identified as the only HPV types present (each in only one patient). HPV70, HPV53, HPV67, and AE5 were detected in HSILs containing multiple types of HPV but no cancer-associated HPV types. HPV66 was detected alone in one cancer. Thus, HPV66, HPV70, HPV72, HPV53, HPV67, HPV83, and AE5 should be regarded as potentially cancer associated. HPV53 and HPV66 have previously been classified as cancer associated based on phylogenetic analysis *(44)*, and HPV70 has been the only HPV type detected in some invasive carcinomas *(45)*. Some of these findings could be explained by multiple infections in which cancer-associated HPVs have integrated in the host genome and abolished L1 expression *(46)* or by the inability of our PCR to detect certain cancer-associated HPV types.

Multiple HPV-type infections were found in many cervical neoplasias, including invasive carcinomas, but they were not associated with increased risk of disease above that associated with a single HPV-type infection. The proportion of multiple HPV infections that we detected was somewhat higher than that detected by Kalantari et al. *(37)* but was similar for all grades of cervical intraepithelial neoplasia. It is unknown whether these multiple infections are associated with coexisting cervical lesions of different grades, an issue that could be important in formulating a vaccine.

Bosch et al. *(16)* detected multiple infections in only a few patients, but that study used biopsy material where only the clonally expanded HPV type would be expected. We would expect to detect multiple infections in samples of exfoliated cells because these cells come from a wider area of the cervix and vagina. Similar to our findings, Ho et al. *(38)* did not find a substantially increased risk associated with multiple infections, supporting the view that cervical neoplasia is the result of clonal expansion of a cell infected with a single type of HPV. Additional support for the absence of an interaction between types is provided by our findings that the risk associated with HPV16 alone is similar to or higher than the risk associated with HPV16 in the presence of other HPV types. However, small numbers of HPV16-positive control subjects limited our ability to investigate this issue further. The complex interrelationship of multiple HPV types requires further analysis because it can have a direct impact on the outcome of vaccination.

We found evidence that only one HPV type was clonally expanded because generally one HPV type, usually a cancer-associated HPV type, had a stronger PCR signal. For example, the HPV16 signal was the strongest signal in 13 (68%) of 19 HSILs and cancers with multiple HPV types including HPV16. Of 49 HSILs and cancers with multiple HPV infections, 24 had other high-risk HPV types, but again there was generally only one strong signal, indicating that one type was predominant. It is unknown if subjects with multiple infections including several cancer-associated HPV types would be protected by a vaccine not including all cancer-associated types.

This study provides further evidence for the role of HPV in cervical carcinogenesis, as demonstrated by high ORs and attributable fractions associated with various pathologic states, particularly the most advanced lesions. The highest ORs were

associated with HPV16 and other cancer-associated types. A higher risk of cervical disease was also associated with increasing signal strength in a PCR-based HPV assay, an indirect measure of viral load. This finding may have implications for screening programs, given the importance of properly defining the threshold of HPV detection to maximize sensitivity and specificity of the test *(47)*. The high attributable fractions observed argue that cancer-associated HPV types have a preponderant role in the development of HSILs and cancer, since the attributable fractions for cancer-associated HPV types and for any HPV were almost the same. Thus, we have identified at least 80% of the HPV types responsible for the cervical cancer in this population and, therefore, should be able to formulate a vaccine against the correct combination of HPV types to reach our ultimate goal of controlling this worldwide devastating disease.

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NOTES

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