



Published in final edited form as:

*Cancer Epidemiol Biomarkers Prev.* 2009 March ; 18(3): 863–865. doi:10.1158/1055-9965.EPI-08-0951.

## HPV types by age in cervical cancer precursors: predominance of HPV 16 in young women

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### Abstract

In a population-based study conducted in Guanacaste, Costa Rica, we investigated the HPV types detected in 233 cases of cervical intraepithelial neoplasia (CIN) 2/3 and cancer by age. CIN2+ and CIN3+ in young women were significantly more likely to be associated with HPV 16 than the same lesions in older women (80% of CIN3+ were associated with HPV 16 among women 18-26 years old compared to only 32% among women older than 55,  $p$  trend=0.018). There were no differences by age in HPV 18 positivity. Lesions in older women were mainly caused by other carcinogenic types.

This association was present for both prevalent and incident lesions and supports the notion that HPV 16 is a stronger carcinogen that causes disease faster than other types. It also has implications for prevention, including the need to vaccinate young women before exposure to HPV vaccine-containing types (HPV 16 and HPV 18) to prevent the majority of cervical cancer precursors.

### Keywords

Cervical neoplasia; HPV distribution; HPV16; HPV vaccines; population based

### Introduction

Persistent infection with carcinogenic HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, possibly others) is the necessary cause of cervical cancer (1,2). HPV 16 and HPV 18 infections have higher absolute risk of progression to precancer than other HPV types (3,4,5) and account for 70% of cervical cancers despite representing a modest fraction of infections in general population (6,7). Furthermore, up to 90% of many HPV-associated extra-cervical tumors are linked to HPV 16, and HPV 18 is overrepresented in cervical adenocarcinomas (8,9,10,11).

We hypothesized that, given a high frequency of HPV acquisition early after sexual debut, HPV 16 and HPV 18, being more carcinogenic than other types, should cause precancer and cancer relatively more frequently at younger than at older ages. Understanding these etiologic details has important public health implications and might affect the way health policy groups

consider the benefits of prophylactic HPV 16 and HPV 18 vaccination in different age groups and integration of screening using HPV tests.

We evaluated whether HPV 16 and HPV 18 cause CIN2+ in women at younger ages than other carcinogenic types in our 10049-women population-based natural history study in Guanacaste, Costa Rica (12,13,14).

## Materials and Methods

Briefly, a random sample of homes was ascertained by a census, and all resident women older than 18 years were invited, to ultimately include one sixth of the population. Participation rates were over 93%. All women signed informed consent at enrollment. The protocol was approved by IRBs at NCI and Costa Rica.

The cohort was intensively screened at enrollment (1993-94) and followed for seven years at varying intervals according to identified clinical risk. Participation rates during follow-up approached 90%. At each visit, cervical specimens for screening and HPV detection were collected. Women with abnormalities were referred to colposcopy. Biopsies and/or excisional treatments were performed as necessary and the histological final diagnosis was assigned after review by an expert panel including all available cytology and pathology data.

HPV detection and typing was performed using MY09/11 L1 degenerate primer PCR with AmpliTaq Gold polymerase followed by dot blot hybridization detecting more than 40 HPV types. There was extensive replicate testing in separate batches, and adjudication of discrepant data blinded to cytology and histology data.

We evaluated the HPV type distribution stratified in five age-groups (18-26, 27-30, 31-36, 37-45, 46-55,  $\geq 56$  years) among women with CIN2+, detected at enrollment or follow-up among non hysterectomized and sexually experienced women. For analyses focusing on HPV 16 versus other carcinogenic HPV types, if multiple types including HPV 16 were detected at the time of diagnosis, the “causative” type was assigned as HPV 16; otherwise the case was assigned to “other” carcinogenic HPV types. Similarly, for analysis that focused on HPV 18, if multiple HPV types were present, the “causative” type was assigned as HPV 18; otherwise to “other” carcinogenic HPV types. We also evaluated the HPV type distribution by age restricted to women with single infections (n=172) with similar findings (data not shown). Contingency tables were constructed for all cases and also restricted to incident cases; results are presented separately for CIN2+ and CIN3+. The proportion of lesions associated with HPV types of interest (HPV 16 and HPV 18, respectively) were each evaluated. The trend of HPV 16 positivity across age groups was estimated using Mantel-Haenszel Chi-Square (1 df).

## Results

At enrollment, we identified 140 women with prevalent CIN2+ among 8546 sexually experienced women. Among 8759 women in follow-up (including initially virginal women who initiated sexual activity during follow-up), 141 incident cases were identified. A total of 233 prevalent and incident CIN2+ cases were positive for any carcinogenic HPV type and included in the analyses. Analyses excluding invasive cancers yielded comparable results and are not presented.

Among the CIN2+ cases 38% were CIN2, 55% CIN3, and 7% screening-detected (not by symptoms) invasive cancer. The distribution of lesion severity did not differ by age ( $p=0.787$ ). Women diagnosed in Guanacaste due to symptoms tended to be substantially older on average than those found at screening.

Among women with incident or prevalent CIN2+ (table 1), significant differences in the distribution of HPV types were observed by age, with a decline in HPV 16 positivity from 67.6% among women 18-26 years to 27.6% among women  $\geq 56$  years and a corresponding increase in positivity for other carcinogenic types (p trend= 0.002). Similar results were observed in analyses restricted to incident CIN2+ (p trend= 0.001) (Table 1) and when restricting to all CIN3+ cases (p trend= 0.018) or only incident CIN3+ cases (p trend= 0.009) (Table 2). The pattern of declining HPV 16 with age was clear, with the exception of an apparent increase at ages 46-55; however, despite this increase in HPV 16 positivity observed at ages 46-55, positivity rates were still lower in this age group than those observed among women 30 and younger, particularly in analyses restricted to incident disease. There were no consistent or significant patterns by age for HPV 18 or any other individual type. Of note, over 35% of CIN2+ cases were detected by age 30, while less than 24% of our cases were diagnosed at ages above 45 years.

## Discussion

HPV 16 is known to cause about 50% of cervical cancers and its precursors, but until now it was not fully recognized that the fraction of lesions associated with this HPV type is higher among young women. In a large pooled analysis of invasive cervical cancers (1), HPV 16 related cancers were significantly but not markedly more common in younger women, and a recent report documented younger ages for invasive cancers associated with HPV 16, 18 and 45 (15).

Vaccines against HPV 16 and 18 are now being incorporated in prevention programs, and there is discussion about the appropriate ages to vaccinate. It is widely accepted that vaccinating adolescents, is the most cost effective approach for prevention, considering that most HPV infections occur early after initiation of sexual activity. However, some groups advocate vaccinating older women, claiming that exposure and new disease continue to occur into mid-adulthood. In this analysis we demonstrated that cervical cancer precursors occurring in younger women are significantly more likely to be associated with HPV 16 than lesions occurring in older women. These findings, together with the fact that the peak of cervical cancer precursors is also relatively early in life clearly indicate that the vaccine benefit will be maximal when given to young women, and that programs aimed at older women will have a reduced impact on the burden of cervical disease. HPV 16 and 18 are considered stronger carcinogens, as demonstrated in laboratory and epidemiologic studies (15,16,17,18) and it would therefore not be surprising if they cause disease earlier. Interestingly, in our study we did not observe a similar age association for HPV 18, in concordance with previous findings indicating an "occult" progression of HPV 18 in the initial years after infection.

A cohort effect could explain our findings if HPV had only recently been introduced in this population, but this is not supported by comparing these data with a more recent cohort recruited 10 years later in the same population (19). HPV 16 is known to produce identifiable high-grade cytologic (20) and colposcopic abnormalities (21) more readily, and increased detectability could partly explain our results. When interpreting these findings we need to keep in mind that HPV 16 related CIN2 and CIN3 in younger women may be more likely to regress than similar lesions in older women. However, in our study the association with age remained when restricting to CIN3+, which are less likely to regress.

These analyses need to be replicated for each individual type of HPV in other studies of precursors and invasive disease. A better understanding of age patterns in cervical lesions by HPV types will help inform decisions regarding the ideal target groups for vaccination and screening programs.

## Acknowledgments

We thank the women who participated in this study and the staff for their dedicated work.

**Funding:** The Guanacaste cohort was supported by National Institutes of Health (contracts numbers NO1-CP-21081, NO1-CP-33061, NO1-CP-40542, NO1-CP-50535, NO1-CP-81023 and CA78527 to RB). This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Division of Cancer Epidemiology and Genetics.

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Table 1

HPV types detected among HPV positive women with CIN2+ by age.

Age (years)	Incident and prevalent cases (n=233)					
	No. Women	HPV category <sup>‡</sup> (%)		No. Women	HPV category <sup>‡</sup> (%)	
		HPV 16	Other carcinogenic		HPV 16	Other carcinogenic
18-26	37	67.6	32.4	19	68.4	31.6
27-30	45	57.8	42.2	24	62.5	37.5
31-36	48	47.9	52.1	19	47.4	52.6
37-45	49	38.8	61.2	27	25.9	74.1
46-55	25	56.0	44.0	14	35.7	64.3
≥56	29	27.6	72.4	14	28.6	71.4
p trend			0.002			0.001

<sup>‡</sup> Each woman was counted only once; if multiple HPV types were detected including HPV 16, she was counted as HPV 16 positive; otherwise the woman was assigned to other carcinogenic HPV type.

Table 2

HPV types detected among HPV positive women with CIN3+ by age.

Age (years)	Incident and prevalent cases (n=148)					
	No. Women	HPV category <sup>‡</sup> (%)		No. Women	HPV category <sup>‡</sup> (%)	
		HPV 16	Other carcinogenic		HPV 16	Other carcinogenic
18-26	24	79.2	20.8	12	83.3	16.7
27-30	25	52.0	48.0	13	53.8	46.2
31-36	29	41.4	58.6	10	40.0	60.0
37-45	31	45.2	54.8	16	31.2	68.8
46-55	20	60.0	40.0	11	45.5	54.5
≥56	19	31.6	68.4	11	27.3	72.7
p trend			0.018			0.009

<sup>‡</sup> Each woman was counted only once; if multiple HPV types were detected including HPV 16, she was counted as HPV 16 positive; otherwise the woman was assigned to other carcinogenic HPV type.