Cancer Causes Control (2009) 20:1663–1670
DOI 10.1007/s10552-009-9414-z

ORIGINAL PAPER

Human papillomavirus infection in honduran women with normal cytology

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Received: 10 February 2008 / Accepted: 29 July 2009 / Published online: 15 August 2009

Abstract
Objective This study was aimed at estimating type-specific HPV prevalence and its cofactors among Honduran women with normal cytology in order to provide valuable information to health policymakers about the epidemiology of this important sexually transmitted infection.
Methods A total of 591 women with normal cytology from Tegucigalpa, Honduras were interviewed and tested for HPV using the SPF10 LiPA25. A structured epidemiological questionnaire was administered to each woman.
Results The overall HPV prevalence was 51%. Twenty-three types of HPV were detected; HPV 16, 51, 31, 18, and 11 were the most common. The highest prevalence of cancer associated HPV types (15.0%) was found in the women less than 35 years. Besides the association with age, the main independent predictors of HPV infection were the lifetime number of sexual partners and having a low socioeconomic status and less than 5 previous Pap smears.
Conclusions In the population studied, there was a broad diversity of HPV infections, with high-risk types being the most common types detected. The establishment of a well-characterized population with regard to the community prevalence of type-specific HPV infection will provide a valuable baseline for monitoring population effectiveness of an HPV vaccine.

Keywords HPV · Honduras · Normal cytology · Risk factors

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Introduction

Carcinoma of the cervix is the most common type of cancer in the developing world and the leading cause of death from cancer among women. The estimated new cervical cancer cases per year is 500,000 [1], 80% of which is occurring in the developing countries [2]. In Central and South America, the incidence rate is about 5 times as high as in Western Europe [3]. In Honduras, cancer of the cervix is a major public health problem and also has the highest mortality rates due to cancer among women with a cervical cancer age standardized rate of 30.6/100,000 inhabitants [4].

There is overwhelming data from multiple epidemiologic and laboratory studies demonstrating the central etiologic role of human papillomavirus (HPV) in cervical cancer [5–10].

It is necessary to understand the global burden of HPV infections in order to establish effective prevention strategies such as improved screening programs, public health education, and vaccines [11]. In this context, surveys to

determine HPV prevalence in different age groups and circulating genotypes in the population are needed to understand the wide variations in the incidence of cervical cancer in populations worldwide. These will contribute to establish the proportion of women that could be effectively protected by vaccination.

Despite the high incidence of cervical cancer reported from Honduras, population-based studies on the HPV prevalence and genotype distribution are scarce [12, 13]. It is of fundamental importance to get insight into the development of this disease in order to design effective strategies to treat and prevent cervical carcinoma and its related mortality and morbidity.

This study was aimed at determining type-specific HPV prevalence and its cofactors among Honduran women with normal cytology in order to provide valuable information to health policymakers about the epidemiology of this sexually transmitted infection.

Methods

Study population and collection of specimens

The study population included a sample of 591 women, randomly selected, attending a communal cervical cancer screening centers and family planning clinics in low-medium socioeconomic settings in Tegucigalpa, the capital city of Honduras. In general, women may be referred for screening by their physicians or request screening on their own accord. Eligible women were those between 18 and 65 years of age, identified as being resident for at least 6 months in Tegucigalpa, without a history of preneoplasic or neoplasic lesions of the cervix, conization or hysterectomy, not currently pregnant, who were willing to participate and who signed an informed consent form.

The nature of the study was explained to the patients by trained medical staff

A thorough physical examination was performed, and a structured epidemiological questionnaire was administered to each woman. Questions pertained to sociodemographic variables like age, occupation, education level, smoking; contraceptive use, previous cervical smear history and exposure to wood smoke and also questions regarding sexual behavior to gain insight into risk factors for acquiring HPV were asked. The interviews lasted approximately 15 min. Assurance of confidentiality was obtained by gathering some of the information in two different questions and at the end of each interview the interviewer could address if the information given by the patient was reliable and if she appeared confident.

The study was reviewed and approved by the correspondent ethical committee

Five women did no consent to participate in the study (<1%)

An Ayre spatula was used to obtain a cervical smear, which was immediately fixed for cytological examination. Following the Pap collection, additional material was obtained from the cervix for HPV analysis and placed in 5 mL sterile phosphate-buffered saline (PBS, 0.82% (w/v); NaCl, 0.19% (w/v); $Na_2HPO_42H_2O$, 0.03% (w/v); $NaH_2PO_42H_2O$, adjusted to pH 7.4 with HCL 1 M) 0.005% thimerosal. Upon arrival in the laboratory, cells were vortexed, centrifuged for 10 min at 4,500 rpm, then resuspended in 1 mL of PBS and centrifuged again for 10 min at 4,500 rpm, resuspended in 0.5 mL of PBS and stored at −20°C for further analysis. Extreme caution was taken to prevent cross-contamination of specimens. Pap smears were all reviewed by the same pathologist. Six women were excluded due to abnormal cytology results (1%).

HPV DNA detection

DNA extraction and HPV-PCR assay

DNA extraction from the cervical cells was performed according to the Boom method as previously described [14]. Two hundred microliters of material was isolated and resuspended in a final volume of 100 μL; an extraction control consisting of nuclease free water was placed after every 5th sample. About 10 μL of sample DNA or control were used for each of the various PCR analyses.

All samples were prescreened with the β-globin primers PCO3/PCO5 to assess sample integrity.

All work was performed in a laminar flow hood in a dedicated room free from plasmid DNA.

HPV SPF_{10} Line $Blot_{25}$ assay

(i) PCR amplification of HPV DNA. Broad-spectrum HPV DNA amplification was performed using a short PCR fragment assay (HPV SPF_{10} Line $Blot_{25}$ assay Labo Biomedical products B. V. Rijswijk, The Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame, and allows detection of at least 43 different HPV types. SPF_{10} PCR system was performed in a final reaction volume of 50 μL, containing 10 μL of the isolated DNA sample and 40 μL PCR mix, containing 10 mmol/L Tris–HCL (pH 9.0), 50 mmol/L KCL, 2.0 mmol/L $MgCl_2$, 0.1% Triton X-100, 0.01% gelatine, 200 μmol/L of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 15 pmol each of the forward and reverse primers

tagged with biotin at the 5′ end, and 1.5 units of AmpliTaq Gold® (Applied Biosystems, Foster City, CA, USA). Activation of AmpliTaq Gold for 9 min at 94°C was followed by 40 cycles of 30 s at 94°C, 45 s at 52°C and 45 s at 72°C, with a final extension of 5 min at 72°C. Appropriate negative and positive controls were used to monitor the performance of the PCR method in each experiment. To avoid contamination by PCR products, sample preparation and the amplification reaction were all performed in separate rooms.

(ii) HPV detection by DEIA. The presence of HPV DNA was determined by hybridization of SPF_{10} amplimers to a mixture of general HPV probes recognizing a broad range of high-risk, low-risk and possible high-risk HPV genotypes in a microtiter plate format, as described previously. All HPV DNA positive samples (by SPF_{10} DEIA) were genotyped using the HPV SPF_{10} Line $Blot_{25}$ genotyping assays.

(iii) HPV genotyping by reverse hybridization using the HPV SPF_{10} Line $Blot_{25}$ genotyping system. The 28 oligonucleotide probes which recognize 25 different types were tailed with poly(dT) and immobilized as parallel lines to membrane strips (Labo Bio-medical products B. V. Rijswijk, The Netherlands). The HPV genotyping assay was performed as described previously. The LiPA strips were manually interpreted using the provided reference guide.

The samples that tested positive using the DNA Enzyme Immuno Assay but showed no results on the LiPA strip were considered to be HPV X-type, i.e., genotypes not present on the LiPA strip.

To evaluate performance and reproducibility of the analysis, a quality control program from DDL was performed.

Data analysis

Statistical analyses were performed using the programs EpiInfo 6.02 (CDC) statistical program, Excell and LogXact version 4 (Cytel, Cambridge Ma).

Univariate statistics were calculated for all variables. Any p value less than 0.05 was considered significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained from multiple logistic regression models to evaluate the association between HPV infections and risk factors. Variables found to be significantly related to HPV infection by univariate analyses were entered into a multiple logistic regression model. We adjusted for age using five groups contrasted as dummy variables 18–24, 25–34, 35–44, 45–54, and 55–65) and for HPV infection status (only low risk types and any high risk types). We investigated the potential association with education, socioeconomic status, exposure to wood smoke, history of pregnancy, parity, age at first intercourse, lifetime number of sexual partners, number of previous screens, time since last Pap smear, history of sexually transmitted diseases, and occurrence of other genital infections that could influence the development of SIL. The variables that remained in the final multivariate models were: age, women's education, socioeconomic status, time since last Pap smear, and number of sexual partners.

Results

Among the 591 women with normal cytology enrolled in this study, 29 were negative for the β-globin PCR and were eliminated from the study; from the remaining 562 samples, 289 (51%) were positive for HPV DNA. Overall, single genotypes were found in 67% of the 289 HPV positive women (36% of the entire sample) and multiple genotypes were detected in 33% of the HPV positives women (15.8% of total).

Twenty-three HPV types were ascertained; HPV 16 (25%), HPV 18 (12%), HPV 51 (10%), HPV 31 (8%), and HPV 11 (8%) were the most common types identified (Fig. 1).

Figure 2 shows the age-specific prevalence of HPV detection. HPV DNA was detected in 58% of women aged less than 25 years, with prevalence decreasing in older women to a minimum of 30% among women older than 55.

Overall of the 51% HPV positive women, 35.7% were infected with high risk (HR) types and 15.3% with low risk (LR) types only. Women who were infected with both HR/LR types were grouped as HR types. HR HPV age—specific prevalence was highest among women below age 25 (46.5%), and again lowest among women aged 55 or more years (22.7%; Fig. 3).

When considering all HPV types and all age groups combined, besides the association with age, we observed a trend for HPV infection and the lifetime number of sexual partners (Table 1). The only other factors that remained significant were having a low socioeconomic status (SES) and less than five previous Pap smears, and women who had six or more previous screens had a reduced risk of HPV infection. In HPV positive women, risk factors for detection of single and multiple HPV infections for all types were analyzed (Table 2). A total of 200 (69%) of 289 HPV-positive women had a single infection, whereas 89 women (31%) had multiple HPV type detected. For single HPV infection, the highest prevalence was found among women less than 25 years (48.8%), whereas for multiple infections the highest percentage was observed in women between 25 and 34 years (19.3%). Both single and multiple infections were associated with low socioeconomic status (SES) and with reporting more than one lifetime sexual partner. When analyzing the number of previous screens, an OR of 1.9, for women who had undergone cytological screening 6–10 month prior to the interview, was observed.

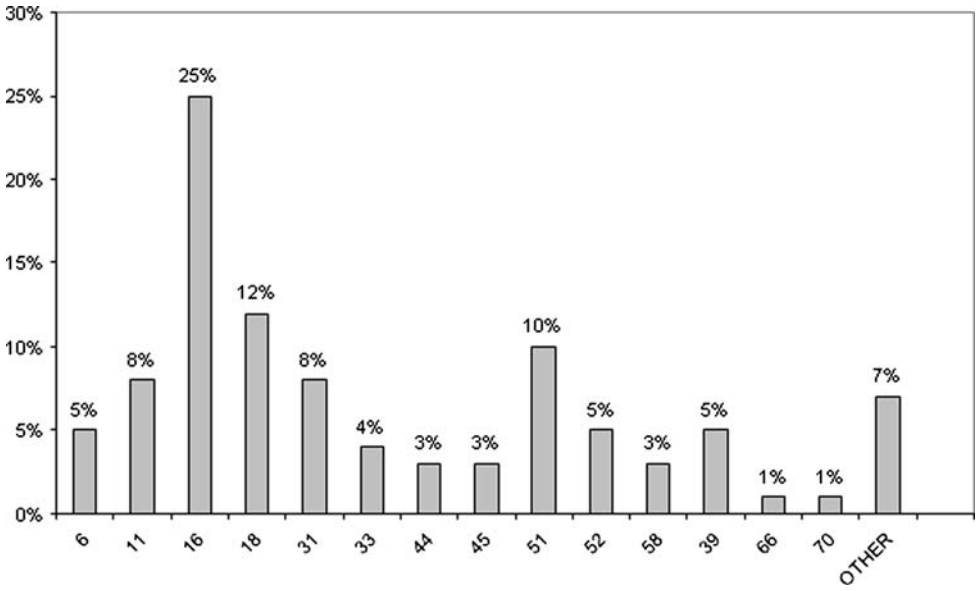

Fig. 1 Prevalence of individual HPV types among Honduran women with normal cytology

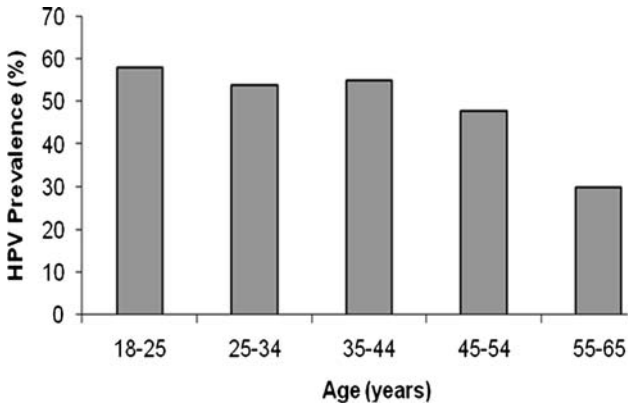

Fig. 2 HPV prevalence in Honduran women with normal cytology according to age

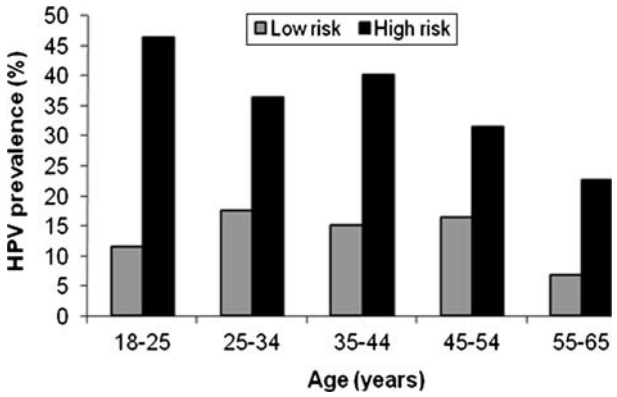

Fig. 3 Prevalence of high- and low-risk HPV detection among Honduran women

A stronger association was found in the women with multiple HPV infection that had the last Pap smear taken 0–5 months before (OR = 2.6).

When doing multivariate logistic regression analysis, a statistically significant association for HPV infection was ascertained for the women who had a low SES (OR = 1.8, 95%CI = 1.2–2.5).

Risk of HPV infection was reduced for women who were 45 years or older and had only one lifetime sexual partner (OR = 0.7, 95%CI = 0.5–1.0).

Discussion

In our survey, the prevalence of genital HPV infections in the studied population was very high (51%) when compared to what has been reported internationally. It is higher than the prevalence found among women with normal cytology in several other Latin American countries: Costa Rica (16%), Mexico (14.5%), Colombia (14.9%), and Chile (14.0%) but similar to the one observed previously in Honduras (39%) considering that in the present study a more sensitive PCR detection system was used [13, 15–18].

As it happens in most developing countries, in Honduras some cultural factors can be related to this high HPV prevalence, especially lack of education; early start of sexual activity; multiparity; and the prevalence of attitudes, misconceptions and beliefs that constrain people from discussing diseases of the genital tract. In most developing countries, access to health services is limited and screening for cervical cancer either is nonexistent or reaches few of the women who need it. A well-functioning health system, with the necessary equipment and trained providers, is essential for prevention activities, screening and diagnosis, linkages for follow-up and treatment, and palliative care. [7, 9, 10, 12, 13, 15].

Table 1 Risk factors for HPV DNA detection among Honduran asymptomatic women

	HPV positive (289)		Low-risk HPV types		High-risk HPV types	
	%	OR	%	OR	%	OR
Age (years)						
<25	4.4	3.31	0.2	0.86	3.6	3.44
25–34	16.9	2.80	1.8	1.91	11.4	2.45
35–44	16.9	2.94	1.9	2.21	12.3	2.78
45–54	10.9	2.20	1.8	2.35	7.1	1.88
>55	2.3	1.00	0.4	1.00	1.8	1.00
Women′s education (years)						
≥7	19	1.00	4.7	1.00	15.8	1.00
<7	32	1.05 (0.78–1.41)	7.3	0.99 (0.53–1.85)	27.0	1.08 (0.78–1.49)
Socioeconomic status						
Medium	15.1	1.00	4.6	1.00	10.8	1.00
Low	35.6	1.28 (0.88–1.85)	6.6	0.78 (0.35–1.71)	30.7	1.54 (1.01–2.37)
Exposure to wood smoke						
No	18.0	1.00	5.7	1.00	13.5	1.00
Yes	35.2	0.79 (0.53–1.16)	6.4	0.45 (0.21–0.99)	30.6	0.91 (0.59–1.40)
Parity						
0–3	33.7	1.00	7.8	1.00	26.7	1.00
≥4	19.1	0.72 (0.50–1.05)	4.8	0.79 (0.35–1.74)	17.8	0.84 (0.56–1.27)
Age at first intercourse						
>18	22	1.00	6.0	1.00	16.0	1.00
≤18	31	0.95 (0.66–1.36)	6.0	0.67 (0.31–1.45)	27.3	1.14 (0.76–1.71)
Lifetime number of sexual partners						
1	23.7	1.00	6.7	1.00	20	1.00
>1	29.4	1.33 (0.93–1.90)	5.3	0.85 (0.39–1.84)	24.5	1.35 (0.91–2.00)
History of oral contraceptive use						
No	17.6	1.00	5.2	1.00	28.3	1.00
Yes	32.7	1.05 (0.73–1.52)	9.2	2.20 (1.01–4.79)	13.5	0.93 (0.62–1.41)
No. previous screens						
>10	6.2	1.00	2.5	1.00	3.5	1.00
6–10	17.8	1.04	4.4	0.62	14.2	1.47
0–5	29.2	1.42	5.5	0.64	26.2	2.27
Time since last Pap smear (months)						
0–11	40	1.00	7.9	1.00	10.4	1.00
≥12	13.2	0.94 (0.61–1.43)	4.5	1.62 (0.70–3.70)	33.0	0.88 (0.55–1.42)

Twenty-three different HPV types were detected in women in this study, which confirmed that there is great variability in the range of HPV types detected in exfoliated cervical cells at a population level in Latin America [15, 19, 20].

As was seen in previous studies examining prevalence of various HPV types among large numbers of women presenting for reasons other than cervical neoplasia or known gynecologic problems, we found HPV 16 to be the most frequently detected HPV type, followed by HPV 18, HPV 51, and HPV 31 [16, 21, 22]. In a Colombian-based study, HPV 16 was followed by HPV 58, 56, 18, and 51 [17]. In studies of Dutch, Mexican, and British women, the second most frequently detected HPV type was HPV 31, whereas among Brazilian women and women in the United States, HPV 53 was detected most frequently after HPV 16 [21]. In the Costa Rican population, oncogenic types 58, 51, and 52 were also relatively abundant, followed by types 31 and 18 [15]. It seems that the family of HPV 50s is common in Latin America and should be considered when developing HPV vaccines tailored to this population.

A slightly higher prevalence of infection was found in women less than 25 years, with a strong predominance of cancer-associated HPV types and single HPV infections.

Table 2 Risk factors for HPV DNA single and multiple detection among Honduran asymptomatic women

	HPV single detection ($n = 183$)		HPV multiple detection ($n = 93$)	
	%	OR	%	OR
Age (years)				
<25	7.4	5.74	1.5	0.98
25–34	19.5	3.38	12.5	1.86
35–44	23.2	4.23	9.6	1.50
45–54	15.1	3.21	6.6	1.21
>55	2.2	1.00	2.6	1.00
Women's education (years)				
≥7	15	1.00	9.0	1.00
<7	25.5	1.07 (0.77–1.49)	16.4	1.14 (0.75–1.75)
Socioeconomic status				
Medium	10.6	1.00	8.0	1.00
Low	28.6	1.47 (0.95–2.28)	17.2	1.16 (0.68–1.99)
Exposure to wood smoke				
No	14.6	1.00	8.2	1.00
Yes	27.1	0.74 (0.48–1.15)	18.8	0.92 (0.53–1.60)
Parity				
0–3	26.2	1.00	16.1	1.00
≥4	16.0	0.78 (0.51–1.19)	11.5	0.90 (0.54–1.53)
Age at first intercourse				
>18	18.0	1.00	9.8	1.00
≤18	23.6	0.88 (0.58–1.33)	16.9	1.16 (0.68–1.96)
Lifetime number of sexual partners				
1	19.0	1.00	11.4	1.00
>1	22.5	1.27 (0.85–1.91)	15.5	1.46 (0.88–2.44)
History of oral contraceptive use				
No	24.3	1.00	17.0	1.00
Yes	15.0	1.20 (0.79–1.82)	8.2	0.95 (0.55–1.61)
No. previous screens				
>10	9.2	1.00	2.3	1.00
6–10	21.8	0.86	11.9	1.87
0–5	34.9	1.14	19.9	2.61
Time since last Pap smear (months)				
0–11	31.0	1.00	20.8	1.00
≥12	10.5	0.96 (0.59–1.55)	6.5	0.89 (0.48–1.63)

HPV detection decreases among the women over 55 years (Fig. 2). This result has been interpreted as an indicator of the sexual transmission as it coincides with the initiation of sexual activity. It is a known fact that HPV prevalence decreases with age, as has been shown in several studies of younger women [23–25].

We did not find a second peak of HPV after 55 years of age as observed in a cohort study being conducted in Guanacaste, Costa Rica [15], in Morelos, Mexico [16], Our results are well in accordance with Cuschiere et al. [26], who did not find a second peak in peri-menopausal women in a survey conducted in Edinburgh and with Beby-Defaux et al. [27] in women who attended a Health Examination Center of the French social security; they observed that HPV prevalence gradually decreased with age. Differences in HPV DNA prevalence found in different studies, overall and by age, may be partly accounted for by differences in cohort effects and PCR methods used for HPV detection. In our study, it could also be that the group of women over 55 years was too small (13 women) to exhibit a second peak.

The presence of multiple infections (33%) was higher than previously observed among control subjects in the IARC studies done in the Philippines (14.3%), [28] Thailand (9.8%) [29], Morocco (5.3%) [30], Paraguay (16.7%) [22],

and The Netherlands (28%) [21], but lower than in a population-based study from Costa Rica (39%) [15] and similar to the results from a study done in Colombia (29.7%) [17]. The extent and importance of multiple HR-HPV infections in the progression of cervical neoplasia and its management remain unknown. Some studies have described multiple HR-HPV infections to be most prevalent in young women and to be more frequent in low grade than in high grade cervical neoplasia, which could reflect common sexual transmission of multiple HR-HPV [22, 26, 31, 32]. On the other hand, Bachtiary et al. [33] demonstrated that the presence of multiple HPV types is associated with poor response and with reduced survival in cervical cancer patients who receive radiotherapy as the primary treatment.

We explored risk factors for acquiring HPV infection and found that the number of sexual partners was weakly related to HPV infection, in contrast to what has been demonstrated in previous studies [34–36] and not well in accordance with data described before in Honduran women [37]. This could be the result of bias, since for cultural reasons women might not tell the real number of sexual partners or it could be explained by the predominance of "male role" in the transmission of HPV infections to women committed to one sexual partner. In this context, a woman's risk of cervical cancer may depend less on her own sexual behavior than on that of her husband or other male partners. Unfortunately, no information was available on the sexual behavior of the partners in our study population.

In the present study there was no association between HPV infection and the use of wood for cooking, considering that bias could have been introduced when they answered the question and the time of exposure to wood smoke was not measured. On the other hand, Ferrera et al. [37] found in a case–control study performed in Honduran women, a strong association between the use of wood as cooking fuel for many years and cervical cancer even after adjustment for education, strongly suggesting that exposure to wood smoke is not just an indicator of SES, but inclined them to believe that there could be a biologic effect.

Our results also showed that there was an association of HPV infection with number of previous screens, especially for multiple infections, in which women who had less than five previous screens had a two times higher risk of HPV infection than women who had more than 10 previous screens, supporting the preponderant role of cervical screening in the effective control of cervical cancer. This is in complete concordance with previous findings in Honduran women in a case–control study; cytological screening conferred a protective effect [13]. Prospective studies are required to assess the impact of multiple HR-HPV infections on neoplastic progression.

There are limitations in the design of the study; one of them is the small sample size, especially in women over 55 years of age. As a health care center-based study, there is a potential bias related to the selection of the study subjects, as they were primarily seeking cervical cancer screening services. It would be necessary to compare some of the variables, like the education level of women participating in the study with women in the general population to see how representative the sample of women participating in the study is of the general population.

The recent approval by the US FDA and the European EMEA of an HPV vaccine to prevent high-risk HPV infection and development of cervical cancer represents a major landmark to eliminate the suffering and death due to cancer, reduce the need for costly medical procedures and provide both women and communities throughout the world with substantial benefits. HPV 16–18 vaccination will prevent HPV 16–18 incident infection, and subsequently decrease in 90% the frequency of abnormal Pap attributable to these types and in about 50% overall abnormal Pap [38]. Besides, HPV vaccination will reduce the number of women who require colposcopy, biopsy, and cervical treatment for precancerous cervical lesions [39].

To maximize the cost-effectiveness of a HPV vaccination programmes, it is important to understand the distribution of the major HPV types in various geographic regions. The results emanating from this research may assist the public health authorities in planning prophylactic and therapeutic strategies to prevent cervical cancer, including HPV diagnosis and rational vaccine strategies. The establishment of a well-characterized population with regard to the community prevalence of type-specific HPV infection will provide a valuable baseline for monitoring population effectiveness of an HPV vaccine.

Acknowledgments This study was supported by The Netherlands Foundation for the Advancement of Tropical Research (WOTRO number WB 92-215).

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