Screening in low resource settings, towards a world without cervical cancer

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Chapter 2

Human papillomavirus type 18 and other risk factorsfor cervical cancer in Jakarta, Indonesia

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Abstract

Infection with human papillomavirus (HPV) has now been established as a necessary cause of cervical cancer. Indonesia is a country with a high cervical cancer incidence and with the world's highest prevalence of HPV 18 in cervical cancer. No information exists about the prevalence of HPV 18 or other HPV types in the Indonesian population. We conducted a hospital-based case-control study in Jakarta, Indonesia. A total of 74 cervical carcinoma cases and 209 control women, recruited from the gynecological outpatient clinic of the same hospital, were included. All women were HPV typed by the line probe assay, and interviews were obtained regarding possible risk factors for cervical cancer. HPV was detected in 95.9% of the cases and in 25.4% of the controls. In the control group, 13.4% was infected with a high-risk HPV type. HPV 16 was detected in 35% of the case group and in 1.9% of the control group and HPV 18 was identified in 28% of the case group and in 2.4% of the control group, suggesting that the oncogenic potentials of HPV 16 and HPV 18 in Indonesia are similar. In addition to HPV infection, young age at first intercourse, having a history of more than one sexual partner, and high parity were significant risk factors for cervical cancer. Within the control group, we did not identify determinants of HPV infection. We hypothesize that the high prevalence of HPV 18 in cervical cancer in Indonesia is caused by the high prevalence of HPV 18 in the Indonesian population.

Introduction

Cervical cancer is the second most common cancer in females worldwide. The incidence is highest in developing countries, largely as a result of lack of screening programs and poor access to medical care. The prevalence of human papillomavirus (HPV) and the distribution of its types probably plays an important role as well (1–3). Little is known about viral and epidemiologic factors in Indonesia, a country with a high cervical cancer incidence (4).

Infection with HPV has now been established as a necessary but not sufficient cause of cervical cancer (5). More than 80 HPV types have been identified and about 40 types can infect the genital tract. HPV types can be divided into low-risk and high-risk types according to their ability to induce cancer. Worldwide, HPV 16 is the most common high-risk type, present in 50%, followed by HPV 18, present in 14% of cervical cancers (6). Indonesia was described to have the highest prevalence of HPV 18 in cervical cancer in the world (6). The prevalence of HPV 18 in the Indonesian population is not known yet.

Apart from infection with HPV, several additional risk factors for cervical cancer have been studied. High parity, high number of sexual partners, smoking, young age at first intercourse, limited education, history of sexual transmitted infections, and low socioeconomic status have been proposed as additional risk factors (7–11). Yet, the importance of these factors seems to differ between populations and has never been studied in Indonesia. In this hospital-based case–control study, we assessed the risk associated with individual HPV types and possible additional risk factors in the etiology of cervical cancer in Indonesia.

Material and methods

Study population and specimen collection

The present study was a hospital-based case–control study. The case group consisted of patients diagnosed with invasive cervical cancer and was previously described by Schellekens *et al.* (4). A group of 104 first attendants with clinically a strong suspicion of cervical cancer was formed in the period October 2001 to March 2002 in the outpatient clinic of the National General Hospital "Dr Cipto Manungkusumo" in Jakarta, Indonesia. An oncologic gynecologist performed pelvic examination, staged according to FIGO classification, and biopsy specimens of the cervical lesion were taken and subsequently formalin-fixed and paraffin-embedded. Sixteen samples were not available for further analysis. The remaining 88 biopsy samples were histopathologically classified. In five specimens, the tissue was not distinctive enough, and in nine cases, we found cervical intraepithelial neoplasia. These 14 cases were therefore excluded from further analysis.

The control group consisted of 209 women without cervical cancer who were identified among patients attending the gynecological outpatient clinic of the same hospital for nonmalignant disease in the period December 2003 to January 2004.

Exclusion criteria included: diagnosis or suspicion of anogenital tract cancers, a history of hysterectomy or conization, current pregnancy, and mental incompetence. Accordingly, after informed consent was given, in a group of 213 women cervical scrapes of the endo and ectocervix were collected by sampling the cervix with an endocervical brush and a wooden Ayre spatula for the preparation of two Pap smears. The first smears were shipped to Leiden for DNA isolation and HPV typing. To prevent contamination, this smear was packed in a separate box for each patient and did not go through any staining procedures. The second smear was stained for cytologic classification. Trained technicians performed cytologic diagnosis according to the Bethesda classification. Their diagnoses, which were not used as a criterion for exclusion, were normal (*n*= 202), atypical cells of undetermined significance (ASCUS) (*n*= 1), atypical glandular cells of undetermined significance (AGUS) (*n*= 3), atypical glandular cells (*n*= 1), low-grade intraepithelial lesion (LSIL) (*n*= 1), high-grade intraepithelial lesion (HSIL) (*n*= 2) and one suspicious of adenocarcinoma. Two noninformative smears were excluded from further analysis.

Data collection

Personal interviews were obtained using standardized questionnaires that included information on ethnic background, socioeconomic status, smoking habits, reproductive history, use of contraceptives, and sexual behavior. Two female doctors administered the questionnaires in the hospital.

DNA isolation

DNA was isolated from the cervical tumor tissue as previously described (4). The isolation of DNA from cervical smears was performed as previously described by Jacobs *et al.* (12) using the commercially available High Pure PCR Template Preparation Kit (Boehringer-Mannheim, Indianapolis, IN). This method was described to provide a reliable means to extract DNA from archival smears with a minimal susceptibility to contamination(12). To test the quality of the isolated DNA, we performed a polymerase chain reaction on the human genomic β-globin gene. Two samples in the control group tested negative and were therefore excluded. All samples in the case group tested positive for β-globin. The final groups consisted of 74 case subjects (45 squamous cell carcinomas and 29 adenocarcinomas/adenosquamous carcinomas) and 209 control subjects. Characteristics of the patient and control group are depicted in Table 1.

HPV typing

HPV DNA detection and genotyping were performed as previously described (4). Shortly, DNA was amplified with the SPF10 primer, and the presence of HPV amplimers was tested on an agarose gel. Samples that tested negative were retested in a 1:10 dilution. HPV genotyping of positive products was performed by the INNO-line probe assay prototype research genotyping assay (Innogenetics, Gent, Belgium),

a highly sensitive hybridization assay that can simultaneously detect 25 HPV types (13).

Statistical analysis

Statistical analysis was performed using the SPSS 10.0.7 software package. To estimate the risk of cervical cancer associated with individual HPV types or lifestyle factors, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) by using unconditional logistic regression. The Pearson Chi-square test for independence was calculated to detect statistically significant differences in the proportion of women who had a risk factor among the HPV positives versus HPV negatives. Differences were considered to be statistically significant when *P* values were below 0.05.

Table 1. Patient characteristics of case and control group

	Cervical cancer, $N = 74$	Normal controls, $N = 209$
Age, mean (years)	46.5	45.0
Ethnical group (%)		
Java	23	38
Sunda	31	23
Betawi	18	12
Sumatra	14	19
Sulawesi	5	2
Chinese	3	4
Other	4	2
Unknown	3	0

Results

HPV prevalence and distribution

The mean age for patients in the case group was 46.5 years and 45.0 years for women in the control group. Patients were distributed similarly by ethnicity. In the patient group, a total of 71/74 (95.9%) was proved to be HPV positive, and in the control group, 53/209 (25.4%) samples proved HPV positive. Of these control women, 13.4% were infected with a high-risk type, 7.7% with low-risk types, and 4.3% by an uncharacterized type (HPV X). We detected 11 different HPV types in the case group and 17 different types in the control group. The distribution of HPV types in the case group and control group is depicted in Table 2.

The most common HPV types in the case group were HPV 16 and HPV 18 in almost equal percentages, followed by HPV 52 and HPV 45. In the control group, the most common types were HPV 51, 74, 18, 16, 44, and 54, in descending order. The overall low prevalence of high-risk types in the control group resulted in an OR for cervical cancer for infections with a high-risk HPV type of 153.0. (95% CI, 45–519). The OR (with 95% CI) for HPV 16 was 26.1 (8.7–78.5) and 16.2 (5.8–44.9) for HPV 18. The distribution of HPV 16 and HPV 18 were not statistically different (Fisher's exact test $P = 0.72$).

Nine samples from the control group were positive by the SPF-10 primer set but could not be assigned to any of the known HPV types by line probe assay, and therefore referred to by HPV X. These samples were retested by the My09/11 primers. Three samples tested positive and through sequencing they were identified as HPV 62 in two cases and HPV 83 in one case. Multiple HPV types were present in 14% of the case group and in 2.4% of the control group. In the control group, the HPV 18-positive infections were all single infections, and one of the HPV 16-positive samples was a multiple infection with HPV 43.

Table 2. Distribution of HPV types in cancer patients versus controls. The classification in high- and low-risk types was performed according to Munoz *et al.*[19]

	Case subjects, *n* (%)	Control subjects, *n* (%)	OR (95% CI)
HPV negative	3 (4.1)	156 (68.4)	
Positive, any HPV type	71 (95.9)	53 (25.4)	69.5 (21–229.9)
Positive, any high-risk type[a]	71 (95.9)	28 (13.4)	153.0 (45.0–519.2)
HPV 16	25 (35.0)	4 (1.9)	26.1 (8.7–78.5)
HPV 18	21 (28.0)	5 (2.4)	16.2 (5.8–44.9)
HPV 52	6 (8.0)	1 (0.5)	18.4 (2.1–155.0)
HPV 45	4 (5.0)	2 (1.0)	5.9 (1.1–33.0)
HPV 51	0 (0.0)	6 (2.9)	0
HPV 74 (low risk)	0 (0.0)	5 (2.4)	0
Other high-risk types	5 (3.0)	3 (1.4)	5.0 (1.2–21.5)
Probable high-risk[b]	0 (0.0)	2 (0.5)	0
Multiple infections	10 (14.0)	5 (2.4)	6.4 (2.1–19.3)
HPV X	0 (0.0)	9 (4.3)	0
Other low-risk types	0	11 (5.3)	0

[a]Also including probable high-risk types and multiple infections that include high-risk types.
[b]Both HPV 53.

Other risk factors

In univariate regression analysis reporting young age at first intercourse, more than one sexual partner, and high parity were associated with risk of cervical cancer (Table 3). Smoking was more frequent in the case group, yet was not a significant risk factor.

Table 3. ORs for cervical cancer and corresponding 95% CI by sexual and reproductive risk factors and smoking

	Case subjects, $N = 66^a$, (%)	Control subjects, $N = 209$, (%)	OR (95% CI)	P value
Age at first intercourse (years)				
≤19	27 (56.1)	85 (40.7)	1.0 (referent)	0.012
≥20	29 (43.9)	124 (59.3)	0.48 (0.28–0.85)	
Number of sexual partners				
=1	40 (60.6)	188 (90.0)	1.0 (referent)	<0.0001
>1	26 (39.4)	21 (10.0)	5.83 (2.98–11.36)	
Parity				
0–3	27 (40.9)	136 (65.1)	1.0 (referent)	<0.001
>3	39 (59.1)	73 (34.9)	2.7 (1.55–4.72)	
Smoking status				
Never	47 (75.8)	175 (83.7)	1.0 (referent)	0.16
Ever	15 (24.2)	34 (16.3)	1.64 (0.81–3.31)	
Unknown	4			

[a]Eight cases from the case group were excluded because not all data were available.

Determinants of HPV infection

Among control subjects, HPV positivity was not associated with age, with 25% positive among women under 35 years, 25% in women aged 35–54 years, and 22% HPV positive in women of 55 years and older. Trends for HPV positivity were observed with decreasing parity (P= 0.08) and with older age at first intercourse (P= 0.08). We did not find an association of HPV prevalence with the number of sexual partners, the use of hormonal contraceptives, religion, or smoking. Determinants of HPV infection within the control group are presented in Table 4.

Table 4. Determinants of HPV infection within the control group

	HPV positive[a], $N = 53$	HPV negative, $N = 156$	P value[a]
Age (years)			0.79
<35	10 (18.9)	29 (18.6)	
35–44	19 (35.8)	45 (28.8)	
45–54	18 (34.0)	62 (39.7)	
≥55	6 (11.3)	20 (12.8)	
Parity			0.08
0–1	14 (26.4)	38 (24.4)	
2–3	27 (50.9)	57 (36.5)	
≥4	12 (22.6)	61 (39.1)	
Age at first intercourse (years)			0.08
<16	7 (13.2)	24 (15.4)	
17–19	8 (15.1)	46 (29.5)	
>20	38 (71.7)	86 (55.1)	
Sexual partners			0.48
=1	49 (92.5)	139 (89.1)	
>1	4 (7.5)	17 (10.9)	
Oral contraceptives			0.4
Never	29 (54.7)	75 (48.1)	
Ever	24 (45.3)	81 (51.9)	
Religion			0.82
Islam	41 (25.0)	123 (75.0)	
Other	12 (26.7)	33 (73.3)	
Smoking			0.79
Never	45 (84.9)	130 (83.3)	
Ever	8 (15.1)	26 (16.7)	

[a]HPV positive includes high-risk types, probable high-risk types, low-risk types, and HPV X.

Discussion

In this case–control study, we studied the prevalence of HPV 18 in a hospital-based population in Indonesia, the only country in the world described to have a similar or higher prevalence of HPV 18 compared to HPV 16 in cervical cancer patients. We found that the high prevalence of HPV 18 in cervical cancer patients is related to the high prevalence of HPV 18 in our control group.

The prevalence of HPV DNA in cervical smears is reported to be closely correlated with cervical cancer incidence rates (2). The prevalence of HPV among control women in our study 21.5% was comparable to the prevalence in high incidence countries like India 27.7% (8), Nigeria 26.3% (14), Chile 14.0% (15), and Colombia 14.8% (16). Consequently, the high HPV prevalence in Indonesia is in agreement with the high estimated incidence rate of cervical cancer in Indonesia of 25–40 per 100,000 women per year (4). Yet, our results may be biased because our control group was a selected group, consisting of women attending a gynecological outpatient clinic, both symptomatic and asymptomatic, and therefore may not be generalized to the general population.

The total number of multiple infections found was 2.4%, which is high compared with 1.2% as described in a worldwide study by Munoz (5). Yet, in areas with a high overall prevalence of HPV in the normal population, the percentage of multiple infections in the same group seems to be higher as demonstrated by Pham *et al.*(17).

Comparing the HPV prevalence in the case and the control group, we calculated an OR for HPV infection of 69.5 that is in agreement with risk estimates of 50–100 as reported in literature (18). The relative risk for infection with high-risk HPV types we found was 153.0. The distribution of HPV types in cervical cancer in this study was largely similar to that in normal smears. HPV 16 was observed in 35% of the cervical cancers, and HPV 18 was observed in 28%, whereas the prevalences in the control group were 1.9% and 2.4%, respectively. The ORs of HPV 16 and HPV 18 for progression to cervical cancer were within comparable ranges (HPV 16: OR, 26.1 (95% CI 8.7–78.5; HPV 18: OR, 16.2 (95% CI 5.8–44.9). Similar risks of progression to cervical cancer for HPV 16 and HPV 18 were observed in a worldwide study by Munoz *et al.*(19) as well. We conclude that, although the number of HPV 18–positive carcinomas is much larger in Indonesia compared to the rest of the world, the risk estimates for infection for progression to cervical cancer do not seem to be different and are similar to the risk of infection with HPV 16. Because the number of HPV 16– and HPV 18–positive samples in the control group is small, we cannot totally exclude other possible explanations for the high rate of HPV 18 in cervical cancer in Indonesia, like the prevalence of more oncogenic HPV variants and physiologic or immunologic causes. More research is needed, including also the distribution in precancerous disease, to draw definite conclusions.

HPV 51 had a high prevalence in our control group (2.9%) and was not identified in our case group, so behaves like a low-risk type in our group, yet was previously described as a high-risk type (19). In describing the prevalence of individual HPV

types, we recognize that the numbers in the control group are small, so risk calculations need to be addressed with caution. Also, the control group has not been collected in the same period as the case group.

In addition to HPV infection, first intercourse at the age of 19 or younger, having a history of more than one sexual partner, and high parity were significant risk factors for cervical cancer in Indonesia. Our numbers were too small to draw definite conclusions about additional risk factors; yet, results are concordant with world literature (7–10). By conducting a hospital-based case–control study, we tried to make case and control groups as comparable as possible. As shown in Table 1, the two groups are largely comparable as far as age and ethnic background are concerned. Still, patients who present at this government hospital with cervical cancer might be different compared to the women who visit the hospital with other health problems. Within the control group, we did not find an association between age and HPV positivity, probably because only a small number of women younger than 35 years were included. Muslim women have been described to have a low prevalence of cervical cancer and of HPV infection (20). Male circumcision is thought to provide a protective shelter to their female partners by getting fewer HPV infections (20). In our study, we did not identify differences between Muslim women and women with other religions, mostly Christians, neither was such a difference identified in a study conducted in India (20).

In conclusion, in Indonesia infection with a high-risk HPV type, young age at first intercourse, history of more than one sexual partner, and high parity were identified as risk factors for cervical cancer. The high prevalence of HPV in our control group is in agreement with the high estimated incidence rate of cervical cancer in Indonesia. The high prevalence of HPV 18 in cervical cancer patients is supposedly related to the high prevalence of HPV 18 in the general population and not due to a higher oncogenic potential of HPV 18 as compared to other HPV types.

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