

# Human papillomavirus in malignant cervical lesions in Surinam, a high-risk country, compared to the Netherlands, a low-risk country

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**Abstract.** Krul EJT, van de Vijver MJ, Schuurings E, Van Kanten RW, Peters AAW, Fleuren GJ. Human papillomavirus in malignant cervical lesions in Surinam, a high-risk country, compared to the Netherlands, a low-risk country. *Int J Gynecol Cancer* 1999; 9: 206–211.

In various countries epidemiologic studies show an association between human papillomavirus (HPV) and cancer of the uterine cervix. We determined the presence of HPV and the distribution of the different HPV genotypes in cervical carcinomas from Surinam, a high-incidence country. The results were compared to the Netherlands where the incidence is five times lower. One hundred thirty cervical carcinomas from patients in Surinam were randomly selected and compared to an unselected group of 128 cervical carcinomas from caucasoid Dutch patients. Presence of HPV and distribution of HPV genotypes was determined in DNA extracted from paraffin-embedded specimens by polymerase chain reaction and sequence analysis. HPV DNA was detected in 82% of the Surinamese cervical cancer patients and in 87% of the Dutch patients. Thirteen different HPV genotypes were detected in the Surinamese group, and nine different HPV genotypes were detected in the Dutch group. Among the HPV-positive samples, HPV 16 was present in 68% in the Netherlands compared to only 49% in Surinam, where less common genotypes such as HPV 35, 45, and 58 were more prevalent. The results show a strong association between HPV and cervical cancer in both groups. However, the observed significant variation in distribution of the genotypes in the two populations with a large difference in cervical carcinoma incidence is important to the general understanding of the etiology of cervical cancer and to the development of HPV vaccination strategies.

**KEYWORDS:** cervical cancer, epidemiology, human papillomavirus, Surinam, the Netherlands.

Carcinoma of the uterine cervix is the second most common cancer in women worldwide<sup>(1)</sup>. Epidemiologic studies have shown differences in incidence rates

of cervical carcinoma throughout the world, with highest rates observed in developing countries<sup>(2,3)</sup>.

Geographic differences in the spread of cervical carcinoma can be explained not only by differences in socioeconomic factors and nonexistent or incomplete screening programs, but also by differences in the probability of being infected with specific types of hu-

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man papillomavirus (HPV). HPV, a sexually transmitted virus, has emerged as an important etiologic factor in the pathogenesis of cervical cancer<sup>(4,5)</sup>. Over 70 HPV genotypes have been identified and more than 20 of these HPV types have been associated with (pre) cancerous lesions<sup>(6)</sup>. Epidemiologic studies reveal that the vast majority of cervical carcinomas worldwide contain HPV DNA. In particular HPV 16 and HPV 18 are recognized as etiologic factors in the development of cervical cancer<sup>(7-11)</sup>.

In this report we present the results of a study on HPV DNA prevalence and HPV genotype distribution in cervical cancer specimens from patients from Surinam and the Netherlands. Surinam constitutes a high-risk area with an incidence rate of cervical cancer that is almost five times higher than in the Netherlands<sup>(12)</sup>. This study provides additional information on the prevalence of HPV and the occurrence of the various HPV genotypes in different geographic areas.

## Patients and methods

### Study populations

The Department of Pathology at University Hospital Surinam undertakes the histopathology for all hospitals in Surinam. Paraffin-embedded specimens of all newly diagnosed cases of invasive cervical lesions in Surinam between 1992 and 1994 were collected from the Department of Pathology, University Hospital Surinam, in Paramaribo for HPV typing ( $n = 130$ ). Material from newly diagnosed Caucasoid Dutch patients in the same period, including patients diagnosed in 1995 to make comparison more appropriate in terms of numbers of patients, was collected from the archives of the Department of Pathology in the Leiden University Medical Center, the Netherlands ( $n = 128$ ). Information on age, stage of disease at time of diagnosis according to the International Federation of Gynaecology and Obstetrics (FIGO)<sup>(13)</sup>, and ethnicity was extracted from patients' charts. The ethnic groups in Surinam were categorized as Creole, Hindustani, Javanese, Chinese, bush Negro, Amerindian, and "mixed". All histologic slides of the tumors were reviewed by an experienced pathologist (MJvdV). Tumor types were classified as squamous cell carcinoma, adenocarcinoma, or adenosquamous cell carcinoma<sup>(14)</sup>. Periodic acid-Schiff staining with diastase treatment and Alcian-blue staining were used to assign tumors with mucin production and squamous morphology to the adenosquamous category.

### Extraction of DNA

Formalin-fixed, paraffin-embedded tissue sections were cut (4 sections of each 16  $\mu\text{m}$  length) and transferred to eppendorf tubes. Sections of a paraffin block without tissue, cut before each tissue sample, served as a negative control. DNA was extracted by overnight incubation at 56 °C of all sections in 250  $\mu\text{l}$  buffer containing proteinase K (600  $\mu\text{g}/\text{ml}$ ), boiled for 5 min to inactivate proteinase K and centrifuged for 5 min at 13,000 rpm. The aqueous solution was used directly for PCR analysis or stored at -20°C until use. Consecutive sections to the sections used for HPV DNA detection and typing were stained with hematoxylin and eosin and used for histologic confirmation.

### Consensus polymerase chain reaction (PCR)

Five  $\mu\text{l}$  DNA solution was added to 95  $\mu\text{l}$  reaction mixture containing 50 mM KCl, 5 mM MgCl<sub>2</sub>, 10 mM Tris HCl (pH 8.3), 0.06% bovine serum albumin (Organon Technica, Boxtel, the Netherlands), 25 mM of each dNTP (Pharmacia LKB Biotechnology, Uppsala, Sweden), 1 unit of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Emeryville, CA) and 10 pmol of each primer (Gibco BRL, Gaithersburg, MD).

Samples were amplified using the consensus primers CPI and CPIIG directed against the E1 open reading frame, which is highly conserved among the HPV genome and enables the detection of a broad spectrum of different HPV types<sup>(15)</sup>. Samples negative with the CPI/IIG primer set were additionally tested with the HPV-L1 consensus primer sets My09/11<sup>(16)</sup> and GP5 +/6 +<sup>(17)</sup>. As a control to confirm that the DNA was of suitable quality for PCR, all samples were screened using  $\beta$ -globin gene primers<sup>(18)</sup>. All standard precautions were taken to avoid contamination of amplification products.

Amplification with the CPI/IIG primer set was carried out in a touchdown PCR on a Perkin-Elmer Cetus thermal cycler with an initial denaturation for 7 min at 95 °C, followed by 40 cycles of denaturation for 30 s at 94 °C; annealing temperature starting at 60 °C and decreasing 1 °C every cycle to 56 °C for 3 cycles, 55 °C for 3 cycles followed by 30 cycles at 54 °C, and extension for 7 min at 72 °C. The GP5 +/6 + PCR was performed as described by de Roda Husman *et al*<sup>(17)</sup>. The My09/11 PCR was carried out according to the protocol described by Bauer *et al*<sup>(19)</sup>.

### Analysis of PCR products

PCR products were electrophoresed in a 2% agarose gel (Boehringer Mannheim, Mannheim, Germany)

and visualized with ethidium bromide under ultraviolet light. To determine the HPV genotype, the consensus-PCR products were subjected to sequence analysis. Amplification products of the consensus PCR were purified using the EasyPrep kit (Pharmacia). Three  $\mu$ l of the purified PCR product were used for sequence analysis with the same oligo nucleotides used for the PCR reaction, end-labeled with  $^{32}$ P dATP (Amersham, Buckinghamshire, UK) using a Perkin Elmer amplification cycle sequencing kit according to the manufacturers' protocol. For nucleotide sequence analysis and comparisons, the program Seqed and Fasta from the Genetics Computer Group sequence analysis software package (Gen Bank DNA database, version 8.1, Madison, WI) was used.

### Statistical analysis

Cross tables were analyzed with the chi-square test. A *p*-value < 0.05 was considered as statistically significant.

## Results

### Characteristics of the populations studied

No substantial difference in age distribution between the two populations was observed. In Surinam the age ranged from 23 to 98 (mean 50.3, median 47) and in the Netherlands from 24 to 80 (mean 49, median 45). Of the Surinamese and Dutch patients, respectively, 44 (48%) vs. 92 (80%) presented with Stage I disease, 29 (32%) vs. 13 (11%) with Stage II, 13 (14%) vs. 5 (4%) with Stage III, and 5 (6%) vs. 5 (4%) with Stage IV. Information on clinical stage was missing for 30% of the Surinamese patients and 10% of the Dutch patients due to inadequate description of tumor dissemination outside the cervix in the patients' charts. Of the 130 Surinamese cases, 94 (72%) were squamous cell carcinomas, 16 (12%) adenocarcinoma, and 20 (15%) adenosquamous cell carcinoma vs. 90 (70%), 31 (24%), and 7 (6%) of the 128 Dutch cases, respectively. Among the patients with known ethnicity, 30 were Creole, 28 Hindustani, 25 Javanese, 10 bush Negro, 7 Amerindians, 2 Chinese, and 7 'mixed.' This is representative of previously described distribution of cervical cancer over the different ethnicities<sup>(12)</sup>. Twenty patients were recorded with unknown ethnicity because no formal specific definition of ethnicity was used in the patients' charts.

### HPV prevalence and genotype distribution

Table 1 shows the results of HPV presence and prevalence of HPV genotypes in both populations. The

**Table 1.** Overall prevalence of HPV DNA positivity and distribution of genotypes in cervical cancer patients from Surinam and the Netherlands

	Surinamese		Dutch		p value
	No.	(%)	No.	(%)	
Total no. of patients	130		128		
HPV negative	24	(19)	17	(13)	0.26
HPV positive	106	(82)	111	(87)	
HPV 16	52	(49)	75 <sup>1</sup>	(68)	0.009 <sup>2</sup>
HPV other types	54	(51)	36	(32)	
HPV 18	20	(19)	20	(18)	
HPV 31	4	(4)	3	(3)	
HPV 33	4	(4)	5	(5)	
HPV 35	4	(4)	0	(0)	
HPV 45	7	(7)	3	(3)	
HPV 51	3	(3)	0	(0)	
HPV 52	3	(3)	1	(1)	
HPV 56	1	(1)	0	(0)	
HPV 58	5	(5)	1	(1)	
HPV 59	1	(1)	4	(4)	
HPV 68	1	(1)	1	(1)	
HPV 70	1	(1)	0	(0)	

<sup>1</sup>In two patients a double infection was identified; HPV 16 + 18 and HPV 16 + 45. For this comparison they are classified as HPV 16 only.

<sup>2</sup>Chi-square test shows a significant difference in the prevalence of HPV 16 versus HPV types other than HPV 16 between the two populations.

overall HPV prevalence was 82% in Surinam and 87% in the Netherlands. Thirteen different HPV genotypes were detected in the Surinamese population, and in the Dutch population nine different HPV genotypes were detected. Among the HPV positive samples, HPV 16 accounted for 49% (52/106) in Surinam vs. 68% (75/111) in the Netherlands. A chi-square test showed a statistically significant difference in distribution of HPV 16 vs. HPV types other than HPV 16 between the two populations (*P* = 0.009). HPV 18 was the second most frequent type in both groups: 15% in Surinam and 16% in the Netherlands. The four genotypes that were found in the Surinamese patients but not detected in specimens of the Dutch patients were HPV 35, HPV 51, HPV 56, and HPV 70.

In Table 2 the overall HPV prevalence and HPV genotype distribution among the HPV positive cases in the Surinamese population is given according to ethnicity. Three ethnic groups consisted of a sufficient number of cases for statistical analysis, *i.e.*, Creoles, Hindustani, and Javanese. The prevalence of HPV 16, HPV 18, and HPV types other than HPV 16 or 18 was not significantly different between the three groups.

Table 3 shows the relationship between HPV type and age, clinical stage, and histologic type for both ethnicities. HPV status was not associated with age in Surinam. In the Netherlands, HPV 16 prevalence was decreased in patients in the age-class > 45 years in

**Table 2.** HPV genotype prevalence by ethnicity in Surinam

Ethnicity	Total no. of HPV pos. (%)	HPV 16 No. (%)	HPV 18 No. (%)	other types No. (%)
Creoles	22/30 (73)	13/22 (59)	2/22 (9)	7/22 (32)
Hindustani	25/28 (89)	18/25 (72)	2/25 (8)	5/25 (20)
Javanese	20/25 (80)	12/20 (60)	2/20 (10)	6/20 (30)
bush Negro	9/10 (90)	3/9 (33)	3/3 (33)	3/9 (33)
Amerindians	7/8 (87)	2/7 (29)	4/7 (57)	1/7 (14)
Chinese	2/2 (100)	0/2 (0)	1/2 (50)	1/2 (50)
Mixed	6/7 (86)	1/6 (17)	2/6 (33)	3/6 (50)
Total	91/110 <sup>1</sup> (83)	49/91 (54)	16/91 (18)	26/91 (29)
p value <sup>2</sup>				
Creoles vs. Hindustani	0.62			
Creole vs. Javanese	0.90			
Hindustani vs. Javanese	0.69			

<sup>1</sup>The 20 patients with unknown ethnicity were not represented in this table.

<sup>2</sup>Chi-square tests were used to compare the distribution of HPV 16, HPV 18 and HPV types other than HPV 16 or 18 between Creoles, Hindustani and Javanese. HPV negative specimens were excluded from statistical analysis.

**Table 3.** HPV status according to age, clinical stage, and histologic type

	Surinamese				p <sup>1</sup>	Dutch				p <sup>1</sup>
	HPV 16 No. (%)	HPV 18 No. (%)	other types No. (%)	HPV negative No. (%)		HPV 16 No. (%)	HPV 18 No. (%)	other types No. (%)	HPV negative No. (%)	
No. of patients	52 (40)	20 (15)	34 (26)	24 (18)		75 (59)	19 (15)	17 (13)	17 (13)	
Age										
≤45 yr	27 (48)	9 (16)	8 (14)	12 (21)		45 (69)	12 (19)	4 (6)	4 (6)	
>45 yr	25 (36)	9 (13)	25 (36)	10 (15)	0.05	30 (48)	7 (11)	13 (21)	13 (21)	0.003
Clinical stage										
≤IIA	23 (42)	9 (16)	13 (24)	10 (18)		64 (64)	16 (16)	9 (9)	11 (11)	
>IIB	15 (42)	3 (8)	12 (33)	6 (17)	0.6	8 (53)	1 (7)	1 (7)	5 (33)	0.1
Histology										
squamous	41 (44)	9 (10)	26 (28)	18 (19)		58 (64)	11 (12)	12 (13)	9 (10)	
adeno	5 (31)	5 (31)	2 (13)	4 (25)		15 (48)	6 (19)	3 (10)	7 (23)	
adenosquamous	6 (30)	6 (30)	6 (30)	2 (10)	0.09	2 (29)	2 (29)	2 (29)	1 (14)	0.2

<sup>1</sup>Chi-square tests were used to assess statistical significance of HPV genotypes distribution by age, clinical stage, and histologic type in both populations respectively.

favor of HPV types other than HPV 16 and 18 and HPV negative tumors ( $P = 0.003$ ). No relationship was observed between viral type and clinical stage of disease in the two groups. In the adenocarcinomas and adenosquamous carcinomas, a higher proportion of the tumors were HPV 18 positive. However, the correlation was not statistically significant in either population.

## Discussion

Carcinoma of the uterine cervix is one of the most common malignancies seen in Surinam with an incidence rate almost five times the incidence in the Netherlands<sup>(12)</sup>. There is extensive epidemiological evidence, as reviewed by Muñoz and Bosch, that HPV plays a central role in the development of cervical can-

cer<sup>(20)</sup>. The present study was designed to compare the prevalence of HPV DNA in cervical carcinomas of patients from Surinam, a high-risk area, and the Netherlands, a low-risk area, and to investigate variations in the distribution of the different HPV genotypes.

With the use of consensus primer-mediated PCR, analysis of HPV DNA presence revealed no significant difference between Surinam and the Netherlands (82 vs. 87% HPV DNA positive). This is in agreement with previously reported findings that the association between HPV infection and cervical cancer is consistent worldwide<sup>(7,9-11,21)</sup>. The few HPV negative tumor samples in both populations may represent conditions of decreased detectability<sup>(22)</sup>. Interruptions or deletions at the primer binding site during the process of integration of HPV DNA may prevent HPV detection<sup>(23,24)</sup>. Moreover, the existence of hitherto uniden-

tified HPV genotypes which are not detected by the consensus primer PCRs used in this study is not excluded. Yet the prevalence of HPV DNA in our study is somewhat lower than that reported from other series of invasive cervical cancer. However, we detected HPV DNA in formaldehyde fixed, formalin-embedded cervical specimens whereas others who had described higher prevalences used frozen biopsy specimens or exfoliated cervical cells stored in transport medium at  $-70^{\circ}\text{C}$  until DNA testing<sup>(9-11,25)</sup>. Although the quality of the extracted DNA was determined by PCR with  $\beta$ -globin primers, it is likely that in our study fixatives had a significant effect on target DNA fragments in the amplification.

Substantial differences were found in the distribution of the different HPV genotypes between Surinamese and Dutch patients. In the Netherlands, HPV 16 accounted for 68% of the total of HPV positive samples whereas in Surinam only 49% of the HPV DNA positive samples contained HPV 16. The observed prevalence of HPV 16 in both populations is in concordance with other studies carried out in different countries. High HPV 16 rates are observed in western European countries, varying from 58% to 82%<sup>(8,9,26)</sup>. In contrast, substantially lower HPV 16 rates are described in Southeast Asia, Africa, and Central and South America<sup>(9,10,27)</sup>.

Less common types were more often represented in the Surinamese population. Differences in HPV type distribution by geographic region has been described in several previous studies. HPV 52 and 58 were detected in a large proportion of cervical neoplasia specimens from Taiwan<sup>(25)</sup> as well as in cervical cancers from Chinese women living in Shanghai<sup>(28)</sup>, while HPV 18 was the predominant type in Indonesia<sup>(9)</sup>. HPV 45, on the other hand, has shown to be more prevalent in precancerous and invasive cervical lesions in populations of African origin<sup>(9,29)</sup>. Thus, especially in non-European populations, the proportion of HPV 16 is lower in favor of other HPV types, compared to European countries. The meaning of these differences in type distribution remains to be clarified.

A population-based cross-sectional study is necessary to determine whether the HPV type distribution in cervical cancer in Surinam reflects the prevalence of these viruses in the general population of Surinam. The observed distribution, however, may be the result of distinct oncogenic potentials of diverse HPV types.

Of particular interest is whether the observed HPV genotype distribution varies by ethnic group within Surinam. No statistically significant variation, however, was observed between Creoles, Hindustani, and Javanese.

In the Netherlands, HPV 16 was observed significantly more often in younger patients while the prevalence of HPV types other than HPV 16 and HPV negatives were increased in the age groups of 45 years or older. Nakagawa *et al.* observed a higher prevalence of HPV types other than HPV 16 and 18 in older patients and suggested that the length of time from primary HPV infection to the development of cervical carcinoma may differ for different HPV types<sup>(30)</sup>.

In conclusion, as has been shown for other populations, this study confirmed the relation of HPV with cervical cancer in Surinam and the Netherlands, two countries at different risk for this disease. However, the distribution of the HPV genotypes differed significantly between both populations.

## Acknowledgments

We thank Professor J.P. Vandenbroucke of the Department of Clinical Epidemiology at Leiden University Medical Center, for critical reading the manuscript. We are grateful for excellent technical assistance of Y.A. Zomerdijk-Nooyen and S. Uljee, and we acknowledge the many contributions of J. Ter Schegget (Academic Medical Center, Amsterdam, the Netherlands).

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Accepted for publication March 23, 1999.