

Prevalence of single and multiple HPV types in cervical carcinomas in Jakarta, Indonesia

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Abstract

Objectives. Cervical cancer is the second most frequently occurring type of cancer in women worldwide. A persistent infection with high-risk human papillomavirus (HPV) is a necessary causal factor in cervical carcinogenesis. The distribution of HPV types in populations has been studied worldwide.

In Indonesia, however, few data are available describing the prevalence of HPV. Cervical carcinoma is the most common female cancer in Indonesia and causes high morbidity and mortality figures. With HPV vaccination studies in progress, it is important to map the HPV status of a population that would benefit greatly from future prevention programs.

Methods. We tested 74 cervical cancer specimens from consecutive, newly diagnosed cervical cancer patients in the outpatient clinic of the Dr. Cipto Mangunkusumo Hospital, Jakarta.

After additional staining, the formalin-fixed, paraffin-embedded tissue samples were histologically classified. HPV presence and genotype distribution were determined by SPF10 polymerase chain reaction and line probe assay.

Results. HPV DNA of 12 different HPV types was detected in 96% of the specimens. The three most common types were 16 (44%), 18 (39%) and 52 (14%). In 14% of the specimens, multiple HPV types were present. The multiple HPV types were significantly more prevalent among adenosquamous carcinomas in comparison with squamous cell carcinoma or adenocarcinoma ($P = 0.014$).

Conclusion. Distribution of HPV types in Indonesia with a more prominent role for HPV 18 is slightly different from that in other parts of the world. The high amount of multiple HPV infections found in adenosquamous carcinomas may prompt further research on the pathogenesis of this type of cervical tumours.

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Introduction

Cervical cancer is the second most frequent cancer among women worldwide. In the year 2000, approximately 468,000 new cases were diagnosed, of which 80% was in developing countries. The average annual incidence of cervical cancer varies widely by geographic area, with highest incidences

reported in Latin America, sub-Saharan Africa and South Asia and Southeast Asia [1].

Indonesia, an archipelago in Southeast Asia, consists of more than 13,677 islands with a population of approximately 200 million people. Its capital Jakarta, situated on the most populous island Java, now harbours over 10 million people.

According to estimations, the incidence rate of cancer in Indonesia is about 100–190 per 100,000 people (Ministry of Health of the Republic of Indonesia. Guidelines for Cancer Control in Indonesia, 1989 and Ref. [2]). Cervical cancer is the most common malignancy among women, up to 22.5% of all cancer cases reported in governmental hospitals [3]. However, we expect this number to be an

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underestimation, because only 25–30% of all sick people enter these medical facilities [4].

Cervical cancer concerns a major health problem in Indonesia since most patients present in late stages of the disease in low resource settings where no screening program is available.

The association of high-risk HPV types (notably 16, 18, 31 and 45) and the aetiology of cervical cancer is now widely accepted, as HPV has been detected in almost all cervical cancers and much less in controls [5–8].

Indistinctness has abounded about the distribution of HPV in Indonesia. A predominant role for HPV 18 instead of 16 was reported [6], which is an exceptional ratio compared with data found in other geographical areas worldwide [9].

The viral origin for cervical cancer and its high morbidity and mortality figures give cause for the development of a vaccine against HPV. To design vaccination strategies suitable for the Indonesian population, an inventory of HPV prevalence is essential.

In this study, we report the distribution of HPV infections in a series of cervical cancer patients from Jakarta, the capital of Indonesia.

Methods

A group of 104 consecutive first attendants (see Table 1 for patient characteristics) with clinically a strong suspicion for 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.

Clinical and epidemiological data concerning risk factors for cervical cancer other than HPV were collected by interviewing the patient in a private interview setting after informed consent. An oncologic gynaecologist performed pelvic examination and staging of the women according to the FIGO classification [10], 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 after HE, PAS and Alcian Blue staining procedures by a classified pathologist. In five specimens, the representative tissue was not distinctive enough and in nine cases we found cervical intraepithelial neoplasia (CIN). These 14 cases were therefore excluded for further analysis.

HPV typing

After isolation of the DNA of 74 cervical tissue samples, we tested if the samples were suitable for amplification with a β -globin PCR using primers RS40 and RS42 [11]. HPV DNA was amplified with the short-fragment PCR for which the SPF10 primer set was used. These primers are in the L1 open reading frame and amplify a 65-bp fragment [12]. The presence of HPV amplimers was determined in a microtiter plate test format [13].

HPV genotyping was done with type-specific probes of the HPV amplimers positive samples by the INNO-LiPA HPV prototype research genotyping assay, a reverse hybridisation assay, as described previously [14]. This assay can simultaneously identify the following mucosal high, intermediate or low-risk HPV genotypes: HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70 and 74.

The samples, in which multiple HPV types were detected, underwent additional HPV detection tests by a type-specific PCR followed by type-specific hybridisation. The primers for HPV 16 (TGCTAGTGCTTATGCAGCAA and ATT-TACTGCAACATTGGTAC) and 18 (AAGGATGCTG-CACCGGCTGA and CACGCACACGCTTGGCAGGT) were in the L1 region as well as the probes for HPV 16 (GCAAACCACCTATAGGGGAACACTGGGGCA) and HPV 18 (TGGTTCAGGCTGGATTGCGTCCG-CAAGCCCA). The used primers and probes for HPV types other than 16 and 18 were located at the E7 region as previously described [15].

Statistical analysis

For statistical analysis, the Chi-square test and the Fisher’s Exact Test were used. *P* values < 0.05 were considered to be statistically significant.

Results

HPV and histopathology

Of the specimens, 95.6% was positive for HPV DNA, and 11 different HPV types were detected (Table 2). HPV type 16, 18 and 52 were found as most prevalent types in this population. The percentage of two or three HPV types

Table 1
Patient characteristics

Average age (range)	47.2 years (29–70)
Ethnicity	
Javanese	73.6%
Chinese	2.8%
Sumatran	13.9%
Sulawesian	5.6%
Mean years of education (range)	6.4 years (0–16)
Illiterate	9.5%
Ever smoked	23.8%
Mean parity (range)	4.7 (1–13)
Mean number of sexual partners (range)	1.85 (1–10)
Mean sexarche	20 years
FIGO stage	
I	23.9%
II	46.2%
III	28.4%
IV	1.5%

Table 2
Distribution of HPV types

HPV type	Number in single HPV infections	Number in multiple HPV infections	Total number	Percentage of total HPV+ (%)
16	25	6	31	43.7
18	21	7	28	39.4
52	6	4	10	14.1
45	4	1	5	7.0
31	1	1	2	2.8
59	2	0	2	2.8
33	1	0	1	1.4
39	0	1	1	1.4
56	1	0	1	1.4
58	0	1	1	1.4
11	0	1	1	1.4
Total	61	22	83	
Multiple infections			10	14.1
Negative for HPV			3	
(% from N = 74 samples)				4.1

Distribution of HPV types among 74 Indonesian cervical cancer samples of which 71 were HPV positive. Multiple infections were counted double or triple, which explains that the sum is higher than 71 or 100%. Total number and percentage of multiple infections (of all HPV-positive samples) and the percentage HPV-negative samples are stated in the last two rows.

in one specimen among HPV-positive samples was 14.1%. The type-specific PCR confirmed the HPV types as found by the LiPA in 6 out of the 10 multiple HPV-infected samples. Histopathologically, the tumours were divided in

Table 3
HPV genotypes by histopathological diagnosis

HPV type	Histopathological diagnosis			Total
	Squamous cell carcinoma	Adenocarcinoma	Adenosquamous carcinoma	
Neg HPV	2	1		3
16	23	1	1	25
18	6	9	6	21
31	1			1
33	1			1
45	2	2		4
52	5	1		6
56	1			1
59		1	1	2
11 + 16	1			1
16 + 18	1	1	1	3
16 + 52			1	1
18 + 45			1	1
18 + 52			1	1
31 + 52	1			1
16 + 18 + 39	1			1
18 + 52 + 58			1	1
Total	45	16	13	74
	60.8%	21.6%	17.6%	100%

HPV genotypes as detected by reverse hybridization Line Probe Assay distributed by histopathological diagnoses. All HPV types combinations in multiple infections are shown.

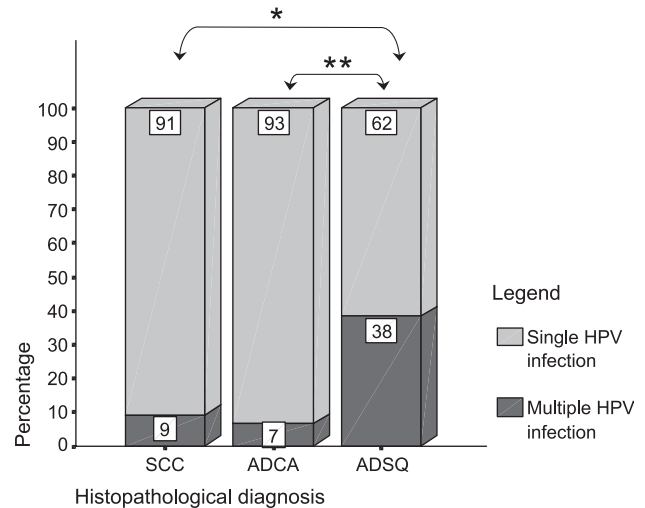


Fig. 1. Bar chart. Bars represent histopathological diagnosis of the cervical samples (ADCA = adenocarcinoma; SCC = squamous cell carcinoma; ADSQ = adenosquamous carcinoma). The stacks show the percentage of single vs. multiple HPV infections vs. HPV-negative samples in the histopathological diagnoses. The differences between percentages of multiple infections between the three histopathological diagnoses are statistically significant ($P = 0.023$). Separately, the SCC group differs significantly with the ADSQ group (*Chi-square test: $P = 0.012$; Fisher's Exact Test: $P = 0.024$) but the numbers in the ADCA group are too small to make a conclusion with a statistically significant difference (**Chi-square test: $P = 0.41$; Fisher's Exact Test: $P = 0.56$).

squamous cell carcinoma (60.8%), adenocarcinoma (21.6%) and adenosquamous carcinoma (17.6%). The distribution of the HPV types according to histopathological diagnosis is presented in Table 3.

Fig. 1 shows that the number of multiple HPV infections in adenosquamous carcinoma was significantly higher than in squamous cell carcinoma and adenocarcinoma ($P = 0.023$).

Discussion

Cervical cancer is the most frequent cancer among women in Indonesia. Extensive epidemiological evidence shows that HPV plays a necessary but not sufficient role in the origin of cervical cancer [8]. To plan future management of cervical cancer like vaccination against HPV, geographical differences in HPV distribution should be assessed, especially in high incidence areas that would benefit greatly from future prevention programs.

Bosch et al. [6] described in 1995 the HPV prevalence of an Indonesian series of 45 HPV-positive samples in which HPV 16 and 18 were found in 31.9% and 48.9% of the cases, respectively, using a consensus PCR HPV detection method. Since no other data about the Indonesian HPV status was reported, this country stayed recorded as the only geographical area with HPV18 as the most common HPV type. The present study was designed to observe the Indonesian HPV distribution in a larger sample. We used the SPF1/2 PCR

primer set with a confirmed high HPV DNA detection rate in formalin-fixed, paraffin-embedded cervical cancer specimens, because with this method a broad spectrum of HPV genotypes in single or multiple HPV infections can be detected with higher sensitivity than consensus primer sets [13,14].

In our study, HPV 16 (41.9%) and 18 (37.8%) were the most common types in cervical cancers in Indonesia, together accounting for almost 80% of the cases. This marginal difference between the HPV 16 and 18 deviates from data found by a worldwide meta-analysis by Clifford et al. [9], who describe the overall predominant role for HPV 16. However, we could not confirm the data as previously described by Bosch et al., in which HPV 18 was the preponderant type. HPV 52 (13.5%) was the third most frequent genotype, followed by HPV 45 (6.8%). As found in studies worldwide [9,16–19], HPV 52 and 58 seem to play a more prominent role in cervical cancer in Asia than HPV 31, 33 and 45, which are more common in the other continents. Our data agree with this, except that HPV 58 was only found once in a multiple infected tumour. Since few studies were conducted in Southeast Asia, this finding could refer to an intracontinental difference in HPV distribution. We found more than one HPV type in 10 (14.1%) samples. In six cases, we could confirm the presence of these types with type-specific PCR and hybridisation. In the remaining four cases, most likely the HPV DNA amount of one of the HPV types was too little to be picked up by the less-sensitive, type-specific PCR, in contrast with the very sensitive LiPA.

After mucin-staining procedures, we divided the 74 carcinomas in three histopathological groups: squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma, in contrast with most other studies that considered the latter two as one group. Five percent to twenty-five percent of all cervical carcinomas have an adenosquamous differentiation and are associated with a high prevalence of HPV DNA presence [20]. We found a significantly higher amount of multiple HPV infections in adenosquamous carcinoma in comparison with squamous cell carcinoma and adenocarcinoma. This finding gives rise to further investigation of the origin of this histopathological group. With the HPV detection method we used, the detection of multiple HPV types in one sample has been made more sensitive. This could explain why in other studies, with less-sensitive HPV detection methods, no clustering of multiple HPV infections with histological type was found.

As described in literature, we observed a significant difference in the HPV 16–18 ratio in squamous cell carcinoma and in adenocarcinoma. In squamous cell carcinoma, we found HPV 16 to be the most common type followed by HPV 18 (54.2% vs. 16.7%, $P = 0.000$); in adenocarcinoma, this ratio was reversed (12.5% vs. 62.5%) ($P = 0.018$).

Glancing at this HPV–tumour type association and the fact that adenosquamous tumours (with components of both squamous cell carcinoma and adenocarcinoma) are significantly more often infected with multiple HPV types, one

might argue that in these carcinomas, more than one HPV type reflects the bipartite character of adenosquamous tumours.

Determining which HPV type (or types) is responsible for the development of cervical cancer among patients found to have multiple HPV types remains difficult. It cannot be excluded that only one subtype caused the cancer and that the other types found merely represent HPV infection.

The FIGO stage of our patients was as expected in a developing country without an adequate disease education or screening program. The presented sexarche and number of sexual partners could be underestimated since raising items concerning sexual behaviour still is taboo in this mainly Muslim population. The cultural habits of the country comprehend young age of marriage, high parity and low contraceptive use. It is feasible that our population has been exposed to direct and indirect risk factors to incur HPV and subsequently cervical cancer.

Indonesia is a large country with a huge population with widespread ethnic diversity. Jakarta, as commercial capital of Indonesia, acts as a melting pot for migrants of all islands and its ethnicities. To gain more detailed insight in the HPV distribution representative for the whole of this country, future research should include more areas and a larger sample size, both of patients with invasive cervical cancer, as well as those with CIN 2/3 or persistent HPV infection, both precursor lesions of invasive cervical cancer.

In conclusion, the data presented in this paper give a clear indication of the HPV status in an area with a high incidence of cervical cancer. The high prevalence of HPV 18 in Indonesia deserves special attention in future vaccination programs to effectively lessen the burden of cervical cancer in Indonesia.

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