

Multiple human papilloma virus infections predominant in squamous cell cervical carcinoma in Bandung

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ABSTRACT

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BACKGROUND

Persistent infection of high risk genotypes of human papilloma virus (hrHPV) has been established as the etiological cause for cervical cancer, and the most prevalent genotypes that infect the cervical tissue are HPV-16 and HPV-18. However, HPV genotype profile has been shown to differ according to geographical distribution across the globe. The present study aimed to determine the HPV genotype distribution in cervical cancer patients from Bandung, Indonesia.

METHODS

During the period of July – November 2010 viral DNA was extracted from randomly chosen cervical cancer biopsies and subjected to genotype determination using the diagnostic linear array genotyping test (Roche). The distribution of HPV genotypes was explored and the prevalence of HPV genotypes was mapped.

RESULTS

Of 96 cervical cancer tissue samples, 76 (79.2%) were histopathologically classified as squamous cell cervical carcinoma. Due to the high cost of HPV genotyping tests, only twenty-five samples were randomly genotyped. Almost 90% of the cervical cancer patients were multiply infected with HPV-16 in combination with HPV-18, HPV-45, or HPV-52. The HPV-16 genotype had the highest prevalence, all samples being infected with HPV-16.

CONCLUSION

The cervical cancer cases were predominantly infected by multiple hrHPVs with HPV-16 as the major genotype among other hrHPVs, supporting the carcinogenic role of this hrHPV. Therefore, screening for hrHPVs in the general population is urgently needed as a means of early detection of cervical cancer.

Keywords: Cervical cancer, HPV-16, multiple HPV infections, Bandung

Infeksi multipel human papilloma virus mendominasi kanker serviks tipe sel skuamosa di Bandung

ABSTRAK

LATAR BELAKANG

Infeksi virus human papilloma (HPV) tipe risiko tinggi yang kronik merupakan salah satu penyebab kanker serviks pada wanita. Genotipe yang tersering menginfeksi jaringan serviks adalah HPV genotipe 16 dan 18. Walaupun demikian, profil genotipe dapat berbeda-beda di seluruh dunia, tergantung dari geografinya. Penelitian ini bertujuan untuk menentukan distribusi genotipe HPV yang menginfeksi jaringan kanker serviks di Bandung, Indonesia.

METODE

Selama periode Juli – November 2010, dilakukan isolasi DNA virus HPV dari biopsi jaringan kanker serviks yang diambil secara acak, kemudian genotipe HPV ditentukan dengan menggunakan linear array HPV genotyping test (Roche). Selanjutnya dilakukan penelusuran distribusi genotipe HPV dan dibuat peta prevalensi genotipe HPV.

HASIL

Dari sejumlah 96 jaringan kanker serviks, 76 (79.2%) terdiagnosis secara histopatologis sebagai tipe squamous cell cervical carcinoma (SCC). Karena tingginya biaya uji genotipe HPV, penentuan genotipe HPV hanya dapat dilakukan pada 25 sampel SCC yang diambil secara acak Hampir 90% SCC terinfeksi secara multipel oleh HPV-16 dengan kombinasi HPV-18, HPV-45, atau HPV-52, sedangkan prevalensi HPV-16 pada jaringan kanker serviks mencapai 100%.

KESIMPULAN

Infeksi multipel HPV dengan genotipe risiko tinggi mendominasi jaringan kanker serviks, sesuai dengan peran genotipe HPV risiko tinggi sebagai agen karsinogenik, dengan prevalensi tertinggi untuk HPV-16. Untuk itu skrining genotipe risiko tinggi HPV menjadi hal yang sangat dibutuhkan sebagai upaya deteksi dini kanker serviks.

Kata kunci: *Kanker serviks, HPV-16, infeksi multipel HPV, Bandung*

INTRODUCTION

Cervical cancer is the second most prevalent female cancer after breast cancer, affecting more than 150,000 women worldwide annually, of which 80% occur in developing countries.⁽¹⁾ This cancer continues to impose a significant health burden in low- and medium-resourced countries of sub-Saharan Africa, Latin America, and South and South East Asia.⁽¹⁾ In Indonesia, cervical cancer ranks first among gynecological cancers and has become a major health problem in this country.⁽²⁾

Chronic human papilloma virus (HPV) infection is strongly associated with the development of cervical cancer. HPV infections in the cervix are frequently associated with intraepithelial neoplasia and invasive squamous cell carcinomas (SCC) with all their different histological variants i.e. large-cell keratinizing, large-cell nonkeratinizing and small-cell carcinoma.⁽³⁾ HPV types 16, 18, 31, 52, and 58 are considered carcinogenic or high-risk types, including other HPV types such as HPV type 33, 35, 39, 45, 51, 56, 59, 68, 73, and 82.⁽⁴⁾ Other HPV types 26, 53, and 66 are considered

as probably carcinogenic, and HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 are low-risk types.⁽⁴⁾ Persistent infection by high-risk HPV plays a pivotal role in the etiogenesis of cervical cancer, particularly HPV types 16 and 18. These two viruses were consistently identified in cervical cancer cases, regardless of geographical distribution.⁽⁵⁾ Interestingly, in a meta-analysis report, women with normal cytology in the general population have a different distribution of HPV infection compared to what is usually found in cervical cancer cases.⁽⁶⁾ There is also a shift in HPV distribution spanning from normal cervical cytology in the general population, to cervical intraepithelial neoplasia (CIN)1, CIN2, CIN3 and finally invasive cancer.^(7,8)

Furthermore, studies in the capital city of Indonesia, Jakarta, have shown different results from those in other parts of the world, showing that in Indonesia HPV-18 is more predominant in adenocarcinoma, followed by HPV-16.⁽⁹⁾ Studies in three other regions of Indonesia have reported that even though HPV-16 and HPV-18 were equally common in the general population, HPV-52 was the most prevalent type.⁽¹⁰⁾ Thus, it is recommended that HPV-52 should be included in prophylactic HPV vaccination.⁽¹⁰⁾

Studies on the distribution of HPV types in a particular area are useful to determine the type of HPV vaccine necessary for that area. However, the Indonesian archipelago covers a large area, making such a study unrealistic. A smaller study, covering a smaller area was undertaken to determine the distribution of HPV in cervical cancer in Bandung, Indonesia.

METHODS

Research design

This study was part of the HPV prevalence survey undertaken in Bandung and the surrounding areas which was conducted by the Department of Obstetrics and Gynecology, Hasan Sadikin Hospital, Bandung, Indonesia, from July to November 2010.

Study subjects and sample collection

All newly-diagnosed cervical cancer patients during this period, with International Federation of Gynecology and Obstetrics (FIGO) classification stage IIA/B, were counseled to take part in the study. Biopsies from the cervical tissues were diagnosed by the hospital pathologists and classified according to the World Health Organization (WHO) classification.⁽¹¹⁾

HPV genotyping test

After diagnosis, the tissues were sent to the Dharmas National Cancer Hospital for HPV genotyping using linear assay HPV genotyping according to the manufacturer's protocol (Roche Molecular Diagnostics, Branchburgh, New Jersey, USA). The assay detects 37 high- and low-risk HPV genotypes. The HPV genotypes detected include 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108. Briefly, DNA was extracted from the tissue specimen using the AmpliLute liquid medium extraction kit (Roche Molecular Diagnostics, Branchburgh, New Jersey, USA). The PCR amplicons were denatured using the denaturation solution and subjected to hybridization with a single HPV genotyping strip that was coated with HPV type-specific and human α -globin probes. The biotin-labeled amplicons were hybridized to the probes only if the type-specific sequence matched the amplicons. The biotin-labeled amplicons were detected by colorimetric development and the results were read visually by comparing the pattern of colored lines to the provided reference guide. The available number of assays for this study was very limited due to high cost of HPV genotyping tests. To make the sample homogenous, we decided to randomize only the non-keratinizing cases for this study.

Ethical clearance

Ethical clearance was granted by the Faculty of Medicine, Padjadjaran University, Bandung.

RESULTS

During the period of the study between July and November 2010, a total of 100 women who came to the outpatient clinic of the Department of Obstetrics and Gynecology, Hasan Sadikin General Hospital, Bandung, Indonesia were newly diagnosed with cancer of the cervix with FIGO classification IIA/IIB. They were all counseled to take part in the study and 96 finally agreed to be involved. Histological examination of the cervical tissues confirmed that 76 (79.2%) were squamous cell carcinoma (SCC), which were mostly of the non-keratinizing type (n=70, 92.1%). The distribution of biopsy classification of these patients is as shown in Table 1.

Biopsy samples classified as non-keratinizing and moderately differentiated samples (n=59) were then randomly genotyped (n=25). The tests revealed that all the samples were infected by HPV-16. However, only three samples showed a single infection by HPV-16. The rest of the samples (n=22) had multiple infections; HPV-16 infected the tissue in various combinations with other high-risk HPV types, with the most prevalent types being HPV-18, HPV-45 and HPV-52 as shown in Table 2. Low-risk HPV type HPV-61 was also detected in combination with high-risk type HPV-16 (n=1). Thus, our random genotyping of squamous cell carcinoma of the cervix during the period of study was dominated by high-risk type HPV-16, followed by high-risk HPV types 18, 45, and 52, occurring in equal percentages (Table 3).

Table 1. Histopathological distribution of cervical cancer patients with FIGO Stage IIA/B (n=96)

Histopathology classification*	n	%
Adenocarcinoma	11	11.5
Adenosquamous carcinoma	1	1.0
Squamous cell carcinoma:		
Keratinizing	6	6.3
Non keratinizing		
Moderate differentiated	59	61.5
Poor differentiated	11	11.5
Others	8	8.3

*Classification according to WHO classification

Table 2. Distribution of HPV genotypes of non-keratinizing moderately differentiated squamous cell carcinoma (n=25)

HPV Infections	NK SCC	
Single Infection		
HPV-16	3	12%
Multiple Infections	22	88%
HPV-16, -18	5	
HPV-16, -18, -45	1	
HPV-16, ---, -45, -52/33/38/58	6	
HPV-16, -18, -45, -52/33/38/58	5	
HPV-16, -18, ----, -52/33/38/58	2	
HPV-16, -----, -51	1	
HPV-16, -18, ----, -51	1	
HPV-16, --- ----, -----61*	1	

NK SCC= non-keratinizing moderately differentiated squamous cell carcinoma; *HPV-61 belongs to the low-risk HPV genotypes

Table 3. The prevalence of HPV genotypes in 25 patients with non-keratinizing moderately differentiated squamous cell carcinoma

HPV genotype	NKSCC	%
HPV-16	25	(100.0)
HPV-18	14	(56.0)
HPV-45	12	(48.0)
HPV-52	13	(52.0)
HPV-51	2	(8.0)
HPV-61*	1	(4.0)

NK SCC is non-keratinizing moderately differentiated squamous cell carcinoma; *HPV-61 belongs to the low-risk HPV genotypes

DISCUSSION

Studies have shown that even a cervix with normal cytology may harbor early infection of human papillomavirus (HPV).⁽¹²⁾ It is estimated that around 291 million women worldwide with normal cervical cytology are actually carriers of the HPV DNA, of whom 32% are infected with HPV types 16, 18 or even with both.⁽⁶⁾ However, HPV infection is usually a self-limiting infection as cell-mediated response immunity is mounted to clear the infection and induce lesion regression.⁽¹³⁾ There seems to be a shift in HPV distribution from normal cytology to CIN that develops into invasive cancer and it has been

found that the high-risk HPV type 16 remains the major HPV infection in cervical cancer.⁽⁸⁾

The current study showed that HPV type 16 was detected in all non-keratinizing squamous cell cervical carcinomas from Bandung, either as a single infection or in combination with other high-risk HPVs. The distribution in our biopsy samples from women with cervical cancer was similar to the meta-analysis conducted by Munoz et al.⁽¹⁴⁾ where the most common HPV types in the patients, in descending order of frequency, were types 16, 18, and 45. However, our results also showed the presence of HPV type 52 infection, occurring at an equal percentage as did types 18 and 45. These results are contrary to the result of a similar study from Jakarta, Indonesia.⁽¹⁰⁾ The difference in the results between these two studies may be due to the different types of carcinoma found in each study. The study from Jakarta has reported multiple infections that were mostly found in adenosquamous carcinomas,⁽¹⁵⁾ while in Bandung the cervical samples recruited were histopathologically classified as non-keratinizing squamous cell cancer, with only around 10% being adenocarcinoma. The high number of multiple HPV infections in this area need to be further explored to have a better insight on HPV genotype distribution in Indonesia.

More studies on HPV genotypes in cervical cancer might give accurate prevalence rates of HPV infection in cervical cancer in Bandung. Moreover, the distribution of single HPV infections in the Bandung samples also differs significantly when compared with the global data. This could be explained by the different techniques used in the HPV genotyping in each study. The study in Jakarta had used the INNO-line probe assay prototype from Innogenetics (Gent, Belgium) for the genotyping assay⁽¹⁰⁾ while the study in Bandung had used the linear assay for DNA genotyping from Roche Molecular Diagnostics (Branchburgh, New Jersey, USA). Different HPV type-specific profiles may be observed during genotyping using different methods on the same sample.^(16,17) Consistency in the DNA purification protocol is

required to guarantee intra-assay reproducibility.⁽¹⁸⁾ The limitation of the linear assay probe used in our study is in the detection of the HPV genotype 52. The assay cannot differentiate type 52 from types 33, 35 and 58. Since this test can separately detect HPV 33, 35 and 58, we have concluded that our samples were co-infected with HPV 52 since no other bands were shown at the HPV 33, 35 and 58 locations. Based on this assumption, 52% of our samples were co-infected by HPV-52. Another limitation of this study is the limited number of biopsies screened for HPV genotyping. Linear assay for HPV genotyping test is currently too costly, making it not readily available to the general population in Bandung.

It is clear that the most prevalent HPV infection in cervical cancer is the high-risk type HPV-16. Therefore, HPV genotype screening for the general population is necessary to detect those that are at high risk to develop cancer of the cervix,⁽¹⁹⁾ even though it is worth noting that a cervix with normal cytology may harbor early high-risk type HPV infection that can be cleared by natural immunity of the host. It is even more important that the current introduction of various products of HPV genotype detection into the health system will yield different distributions with each product specifically suitable for certain HPV types only.⁽¹⁶⁾ It should borne in mind that the types of HPV associated with the cervical cancer that is prevalent in the local scenario would determine which HPV vaccine product is suitable for that particular region. To give such a vaccine to a population without knowing the HPV types in the area would be a wasteful effort.

CONCLUSIONS

The cervical cancer cases were predominantly infected by multiple hrHPVs with HPV-16 as the major genotype, which is in accord with the role of hrHPVs as carcinogenic agents. Therefore, screening for hrHPVs in the general population is urgently needed as a means of early detection of cervical cancer.

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