RESEARCH ARTICLE

Human Papillomavirus Genotypes Profile in Cervical Cancer Patients at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

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Abstract

Background: As in other developing countries, cervical cancer is the most frequent gynecologic malignancy in Indonesia. Persistent high risk genotypes of human papillomavirus (HPV) that infect the cervical tissue have been established as the etiology of cervical cancer. This study aimed to explore the profile of cervical cancer patients and the infected HPV genotypes at Dr. Hasan Sadikin General Hospital-Bandung. Materials and Methods: During the year 2010, 554 cervical cancer patients were registered. In a subset of the patients during July – November 2010, 40 randomized fresh biopsies were tested for HPV genotype after obtained informed consent. The distribution of HPV genotypes and the association to risk factors were analysed. Results: The result showed that 62.5% of the tested biopsies were infected by multiple HPV infections, with HPV-16 found in most of the cervical cancer patients (90%). Marriage at age younger than 16 years old was statistically significant in relation to multiple HPV infection (p=0.003), but not parity more than three times (p=0.59). Conclusions: Although high paritiy in our study was not associated with multiple HPV infection, good family planning programs and reproductive health education need to be emphasized in Indonesia as high parity and marriage at young age might increase the chance of cervical cancer development.

Keywords: Bandung - cervical cancer - HPV - multiple HPV infections - high parity - young marriage

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Introduction

Persistent human papillomavirus (HPV) infection has been established as a key determinant of cervical carcinogenesis (Castellsague, 2008). The new cases of cervical cancer remain high despite the HPV vaccine availability, and the cases are increasing being the second highest incidence rate in the world among women after breast cancer (Ferlay et al., 2012). Studies conducted in several countries have shown that highrisk (hr) HPV genotypes infect the cervical cancer tissue (de Sanjose et al., 2010), with genotype HPV-16 is the most commonly hrHPV genotype found in squamous cell carcinoma and HPV-18 in adenocarcinoma (Altekruse et al., 2003). The distribution in HPV genotype that infect the women differs in various regions, and the hrHPV infections can infect the cervical cancer tissue either in a single or multiple HPV infections (Vaccarella et al., 2010; Resende et al., 2014). Impication of knowing the status of hrHPV infection whether it is single or multiple HPV infection may give a clear prediction for successful therapy. Therefore, individual screening is needed, since multiple HPV infection is known to be associated with poor response of radiotherapy and bad prognosis in locally invasive cervical cancer (Munagala et al., 2009). Data on HPV distribution is necessary and should be available in every area for a better treatment and prevention of cervical cancer.

Next to persistent HPV infections, there are well known risk factors for having cervical cancer, known as reproductive risk factors such as early sexual intercourse, multiple partners, the number of parity, the number of abortions, the use of oral contraceptive (Kumar and Bhasker, 2013). Other riskfactors that may attribute to cancer developing are smoking behavior (Vaccarella et al., 2008), several host genetics determinants (Nunobiki et al., 2011; Mei et al., 2012) and other factors that may associate with socio-cultural factors of the population (Nessa et al., 2013).

Based on the histopathological data in Indonesia, cervical cancer is the most common gynecologic cancer occurring among women (Azis et al., 2009). Meta analysis in HPV prevalence across Asia region showed that only one study reported from Indonesia (Bruni et al., 2010). Therefore, we aimed to further explore the profile of

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cervical cancer patients and the infected HPV genotypes at Dr. Hasan Sadikin General Hospital, Bandung to add more data from Indonesia.

Materials and Methods

In this cross sectional study, data on cervical cancer patients at Dr. Hasan Sadikin General Hospital, Bandung, during the year 2010 were collected. In a subset of the patients during the period of July-November 2010, patients were consented for HPV genotyping test from their biopsy material. Clinical data was analysed in association with the HPV genotype and the risk factors for having HPV infection was explored. HPV genotype detected from DNA HPV were performed using linear array genotyping tests as described previously (Panigoro et al., 2013). In brief, part of fresh cervical cancer biopsy from patients visited the Department Obstetrics and Gynecology Dr. Hasan Sadikin General Hospital, Bandung, was sent to Department of Pathology for histopathological examination. The other part of the biopsy was sent to Health Research Unit Faculty of Medicine Universitas Padjadjaran for HPV genotyping test using linear array according to the manufacturer's protocol (Roche Molecular Systems, Inc., Branchburg, NJ USA). The linear array genotyping test can detect 37 high- and low-risk HPV genotypes, i.e. 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. Distribution and prevalence of HPV genotypes were explored and riskfactors in association with HPV genotypes were analysed. Statistical analyses were performed using chi-square and p-value<0.05 was considered to be statistically significant.

Study permission for this study was given from Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital, Bandung.

Results

In the year of 2010, 554 cervical cancer patients were registered, with mean age of 48.4 years old (s.d. 10.03, range 23-82 years old, data of age only n=475 available). Due to incomplete data in the medical record, data were presented in percentage. Of those data collected, the majority was histopsthologically classified as squamous cell carcinoma (79.3%) and the rest was classified as adenocarcinoma (20.7%). The risk factors attributed to the chance of cervical cancer development were explored; i.e. the patients acknowledged to have multiple partners (35.7%), married at age younger than 16 years old (37.1%), have children more than two (74.2%), and ever had abortion (34.7%). Stratifying the risk factors into two histopathologically groups of cervical cancer in squamous cell carcinoma group and adenocarcinoma group showed no significant different (data not shown).

During the period of July to November 2010, 40 cervical cancer patients were randomly recruited and agreed to participate in the study after informed consent. The mean age of these women was 49.4 years old (s.d. 9.3, range 33-70 years old, data of age only n=36 available). Of these patients, 94.4% had squamous cell carcinoma. These patients had multiple partners (30.6%), married at age younger than 16 years old (33.3%), had multiple parity of three or more (72.2%), and had abortions (52.7%). Infection with multiple HPV genotypes was predominantly occurred in 62.5% of cervical cancer patients (Table 1). All patients were infected with high risk HPV genotype, with HPV-16 dominated in 90.0% of the infected cases (Table 2). One patient was infected with low risk HPV genotype, however, this was in combination with other high risk genotype HPV-16. The prevalence of the HPV genotype that infected the cancer tissue was as followed: HPV-16, HPV-18, HPV-45 and HPV-52 in the percentage of 90%, 50%, 32.5%, and 30%, respectively (Table 2).

Further analyses have been performed in the groups of patients that were infected with a single or multiple HPV infection. There was no difference in the mean age of patients with single HPV infection (48.4 years old, s.d. 10.0, range 33-64) compared to patients with multiple HPV infection (49.9 years old, s.d. 9.1 range 34-70). Both groups (~70%) acknowledged of having only one partner. Interestingly, marriage at young age had shown a strong association with multiple infections (p 0.003), however, the OR can not be further calculated since no one who married younger than 16 years old had single

Table 1. Human papillomavirus Genotypes Profile in Cervical Cancer Patients at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

	N	%
Negative	1	2.5
Single HPV infection	14	35.0
HPV 16	11	
HPV 18	2	
HPV 35	1	
Multiple HPV infection	25	62.5
HPV 16,18	7	
HPV 16,51	1	
HPV 16,61*	1	
HPV 16,18,45	3	
HPV 16,18,51	1	
HPV 16,18,52	2	
HPV 16,45,52	4	
HPV 16,33,45,52	1	
HPV 16,18,45,52	5	
TOTAL Cervical Cancer Patients	40	100

*HPV 61 belongs to the low risk HPV genotype; **HPV 16 was the most dominant in this study

Table 2. The Total Cervical Cancer Patients were 40

HPV genotype	N*	%
HPV 16	36	90
HPV 18	20	50
HPV 33	1	
HPV 35	1	
HPV 45	13	32.5
HPV 51	2	
HPV 52	12	30
HPV 61**	1	

*The total cervical cancer patients were 40; **HPV 61 belongs to the low risk HPV genotype

Table 3. Clinical Characteristic In Cervical Cancer Patients Stratifed by Single or Multiple Infections at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia

	Single HPV Infection (n=14)		Multiple HPV Infection (n=25)		p value
Number of husband/ sexual partner					
	12	**	24	*	
1	8	66.7%	17	70.8%	0.79
≥ 2	4	33.3%	7	29.2%	
Age at first marriage	12	**	24	*	0.003
≤ 16 years old	0		12	50%	
> 16 years old	12	100%	12	50%	
Number of parity	12	**	24	*	
1-2	4	33.3%	6	25%	0.59
≥ 3	8	66.7%	18	75%	
Number of abortion	12	**	24	*	
Never	6	50%	11	45.8%	0.81
Ever (Once or more)	6	50%	13	54.2%	
Histopathological findings	12	**	24	**	
Squamous cell carcinoma	12	100%	22	91.7%	0.30
Adenocarcinoma	0	%	2	8.3%	

*Data of one participant missing; **Data of two participants missing

HPV infection. Further analysis in women with two parities or more showed a tendency to have multiple hr HPV infections, but no statistically significant difference was noted (p 0.59) as depicted in Table 3.

Discussion

Persistent infection of highrisk human papillomavirus (hrHPV) genotypes is acknowledged as an aetiology of cervical cancer (Bruni et al., 2010). The distribution of HPV infections is known to vary in different area in the world. Although our participants in our study were small, our result showed that hrHPV infected the cervical cancer tissue in almost all cervical tissue, with HPV-16 is the most dominant (90%) virus. HPV-16 and HPV-18 are the most prevalent HPV genotypes in our study and this result is slightly higher compared to the meta-analysis study by Bao et al. (2008). Interestingly, our study result is contradictory to the previous study conducted in other place in Indonesia i.e. in Jakarta where HPV-18 was the most predominant. The discrepancy in this result might be due to the different tests used in various study sites which may have different specificity for HPV detection (Panigoro et al., 2013). For example, in the neighboring countries of Indonesia several tests were used i.e LightPower iVAHPV Genotype RDB Kit in Vietnam (Vu et al., 2012), INNO-LiPA HPV genotyping Extra Amp in Thailand (Swangvaree, et al., 2013), the biotin labeled PCR genotyped using reverse line blot hybridization (RLBH) in North Thailand (Natphopsuk et al., 2013), and many other tests available in the market. The biospecimens used in HPV genotypes detection were also various ranging from cervical swab, parafine block, fresh or frozen biopsy like in our study. However, women from Bandung have similar ethnic background as ethnic Malays in Malaysia and the HPV prevalence in Bandung is similar to the study done recently in Malaysia (Raub et al., 2014).

The infection with genotypes that are not covered by vaccines might affect the HPV distribution in several area, thus, it is important to consider second-generation HPV prophylactive vaccines by including the most prevalent genotypes in the area for optimal population coverage. Data in Asia shows that after HPV-16 and HPV-18, there are other most common HPV genotypes infecting the women i.e. HPV-58, HP-33, HPV 45, HPV-31 and HPV-35 (Bruni et al., 2010). Research about prevalence of HPV in other place in Indonesia showed that HPV-16 has the highest percentage, followed by HPV-18 and HPV-52, and this study recommended HPV-52 to be included for the next generation of HPV vaccine (Vet et al., 2008). Interestingly, the percentage of HPV-45 in our study is more than the HPV-52, though we had much smaller number of genotyping tests available. Thus, next to HPV-52 we propose to include HPV-45 in the new generation of vaccine as well.

An important risk factor for developing cervical cancer is among others a coinfection with more than one HPV genotype, especially the high risk type (Wentzense et al., 2009). Type of HPV infection detected in a patient seems to be specific in certain types of cervical cancer. Multiple infection of HPV was commonly found in adenosquamous carcinoma of cervix than any other histopathological findings of cervical cancer (Chaturvedi, et al., 2011). Our study showed that multiple genotypes of HPV infection had a higher percentage (65% of the cases) in the cervical cancer patients that the majority consists of squamouscell carcinoma, while in study conducted in Jakarta multiple HPV infection were found to be more prevalent in patients whose cancers' type was adenosquamous cell carcinoma (Schellekens et al., 2004). In our study, we could not rule out the difference in these groups because we have limited source of HPV typing tests for adenosquamous cell carcinoma. Further study is needed to explore this difference.

Understanding the distribution of HPV genotypes is needed as the HPV virus infected in early phase may have attributed in the cervical cancer progression (Wentzensen et al., 2009). Multiple HPV infection is associated with several risk factors among others sexual behivour of the patients and their partner (Vaccarella, 2006). The risk factors that may play roles are multiple sexual partners, coital frequency, multiparity, contraceptive use, marital status, abnormal vaginal discharge, post-coital bleeding and menopausal bleeding (Ojiyi et al., 2013; Soto-de Leon et al., 2011). For example, young age of first sexual intercouse was known as a major risk factor (Louie et al., 2009). In our study we grouped the patients for first sexual intercourse into younger or older than 16 years since the legal marriage in Indonesia for women is 16 years old. The result of our study showed a strong association with the time of the first sexual intercourse younger than 16 years old. This result seems to be consistent with the study that HPV infection could be spread out through sexual contact (Plummer et al., 2012). However, result by other studies showed that no association between age of first marriage and the number of parity among the patients with different type of HPV infection (Munagala et al., 2009). Other study showed that high parity is associated

with the risk of developing cervical cancer, based on large number of births that may increase the number of lesions in the birth canal or causes immunosuppression in the area of cervix (Soto-de Leon et al., 2011). However, an interesting study with a large number of participants had shown that these factors enhance the probability of malignant transformation of HPV infections and not the acquisition of the HPV infection (Vaccarella, et al., 2006). Therefore, a good family planning program in Indonesia need to re-introduce continuously since in our study most of the women has high parity. The role of the stakeholders to prevent the cervical cancer is highly appreciated (Domingo et al., 2008).

Our other result in risk factors such as number of partner and abortions among patients showed no significant difference between a single or multiple HPV infections. But it is worth noting that individual with multipartner sexual or having sexually transmitted disease seems to have higher risk of HPV infection than individual with only one or none sexual partner. Thus, women infected with one HPV genotype are more likely to harbor additional genotypes when they have several partners as genital HPV are sexually transmitted (Chaturvedi et al., 2011), though our study with limited number of patients did not show this association. In any means, sexual education in Indonesia needs to be emphasized to minimize these risk factors.

Other riskfactors in cervical cancer is smoking (Vaccarella et al., 2008). All of our study participants acknowlegde as non smokers. Here, we cannot rule out the possibility of a moderate effect of passive smoking since Indonesia is a country with the third largest tobacco production. Further data on smoking status of the partners may give a complete information as passive smokers may increase chance for cancer.

The small number of cervical cancer patients who had HPV genotype data in our study was one of the study limitations due to the high cost of HPV genotyping linear array test. Moreover, discordance results from different type of linear array genotyping tests may occur (Rai et al., 2014). Our previous study showed that two linear array for HPV genotyping tests had discrepancy in results (Panigoro et al., 2013). Therefore, the same design and HPV genotyping tests are needed for baseline data to get clear epidemiology on HPV genotype. The regular screening on HPV genotype distributions around the globe for surveillance and vaccine effectiveness is needed using the same genotyping test platform. The poor and incomplete data in medical records are challenging factors in collecting data. Attempt has been made in mechanism of cancer registry in Indonesia (Wahidin et al., 2012), and this action need to be implemented well to get more baseline data to have accurate epidemiology data on HPV genotype national wide.

To conclude, this study showed a strong association of young age of first sexual intercourse and multiple HPV infection, suggesting that further approach must be undertaken to educate women for reproductive health. The prevention of cervical cancer is needed by exploring the risk factors to provide comprehensive base line data for future screening strategies.

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