International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.11, pp 379-385, 2018

Human Papilloma Virus Type 16 & 18 Gene Identification In Plasma of Cervical Squamous Cell Carcinoma and Adenocarcinoma Patients Athaji Adam Malik General Hospital Medan

Tri Puji Asmiati[1]*, Delyuzar[1], Hidayat[2]

[1]Department of Anatomical Pathology, Faculty of Medicine, University of Sumatera Utara, Indonesia.
[2]Department of Biochemistry, Faculty of Medicine, University of Sumatera Utara, Indonesia.

Abstract : Cervical cancer, the forth most common cancer leading cause of death by HPV infection and the most common sexually transmitted infection. HPV type 16 & 18 are responsible for up to 70% cervical cancer compared to another high-risk group type. The aim of this study was to identify gene HPV type 16 & 18 in the plasma patients according to histopathology of squamous cell carcinoma and adenocarcinoma cervix. This descriptive study with cross sectional study from 46 cervical cancer blood patients. Gene HPV identification using polymerase chain reaction (PCR) methods. Majority of samples (80.4%) were infected with HPV-18, but none of them were infected with HPV-16. Most of them were commonly diagnosed squamous cell carcinoma (81.8%) and they generally aged >45 years old. From 13% of cervical cancer patients with metastases, all of them were infected with HPV 18. Detection of HPV type in cervical cancer is important, because HPV 16 and 18 is not always founds in squamous cell carcinoma and adenocarcinoma cervix.

Keywords : Human Papilloma virus, HPV tipe 16, HPV tipe 18, squamous cell carcinoma, adenocarcinoma.

Introduction

Cervical cancer was the fourth most frequent cancer in women with anestimated 528,000 new cases, and was also the fourth leading cause death, whichaccounted for more than 266,000 deaths in 2012[1].The International Agency forResearch on Cancer(IARC) showed that around 80% of cervical cancer cases occured in the less developed regions. In Indonesia, cervical cancer ranked thefirst (8/10.000 people) most common cancer. Meanwhile, the absolute casesestimation was 4,694 in North Sumatera[2,3].

DOI= http://dx.doi.org/10.20902/IJCTR.2018.111143

Human papillomavirus (HPV) is one of the most common sexually transmitted infections and can cause infection in the cervix. More than 100 typesof HPV have been identified, in which 40 of them causing various types ofcancers in the male and female reproductive system (vulva, vagina, cervix, andpenis). Sexually transmitted HPV is divided into 2 categories: low-risk group (6,11, 30, 34, 40, 42, 43, 44, 54, 55, 57, 61, 62, 64, 67, 70, 71, 72, 74, 79, 81, 83, and84) that usually found in benign tumor, and high-risk group (16, 18, 31, 33, 35,39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) that are associated with malignancies[4].There are two two strains in particular, HPV-16 (50 – 70%) and HPV-18 (7 –20%), account for most of the cervical and penile cancer cases[5].

Until these days, the exact origin of DNA found in the plasma remainsunclear. However, the presence of circulating tumor DNA is likely to be a reflection of tumor load or metastasis. Several hypotheses have been proposed, although they were still controversial. There are three possible sources that may be responsible for the occurrence of circulating DNA, namely: apoptosis, necrosis, and active cellular secretion. One of the hypotheses describes micrometastases as the source of circulating cancer cells. Nevertheless, several studies denied this statement, showing that the increasing number of circulating DNA was not associated with the number of cancer cells in the circulation[6].

This study aims to identify the HPV-16 and HPV-18 genes in the peripheral blood of cervical squamous cell carcinoma (SCC) and adenocarcinoma patients, and demonstrate the demographic distribution in those groups.

Experimental

This descriptive This was an analytic descriptive studystudy with cross sectional study from 46 peripheral blood specimens with the suspicion of cervical cancer ingynecologic oncology clinic at the Haji Adam Malik Medan General Hospital. The samples were collected between March and April 2018 after the approval from health research ethics committee of the Medical Faculty of North Sumatera University and Haji Adam Malik Medan General Hospital.

Theblood specimens were isolated using Promega Wizard Genomic DNA Purification Kit. The isolating procedures were done according to the kit protocol. The isolated samples were kept while waiting for the histopathology results.

Identificationwas done using HPV-16 (TCA AAA GCC ACT GTG TCC TGA-3' (*Forward*) and 5'-CGT GTT CTT GAT GAT CTG CAA-3' (*Reverse*)) and HPV-18 (HPV 18 5'-TCG TTT TCT TCC TCT GAG TCG CCT-3' (Forward) and 5'-CCG AGG ACG ACA GGA ACG ACT-3' (Reverse)) specific primers, to detect 450 and 174 bp PCR products from HPV 16 and 18 forms, respectively. PCR mix solution (by Go Taq® PCR Core System I) was prepared, containing 4 µl DNA Template, 12.375 µl ddH_2O, 0.5 µl Forward primer 20 pmol, 0.5 µl Reverse primer 20 pmol, 0,5 µl DNTPs 10 mM, 5 µl 5X GoTaq® Flexi Buffer, 2 µl $Mgcl_2$ (25 mM), 0.125 µlTaq polymerase, resulting in 25 µl solution in each tube. These mixture was spin downed and the PCR amplifications were performed as follows: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 59°C for 1 minute, extension at 72°C for 2 minutes and a final extension at 72°C for 5 minutes. The product was analysed using agarose gel electrophoresis, stained by 2 µl of Ethidium Bromide at 80 V for 70 minutes, and was visualised under UV transiluminator 2 (Uvitec Cambridge).Gene ruler 100-bp DNA Ladder (Bench Top 100bp DNA Ladder, Promega Corporation, Madison, USA) and Gene ruler 50-bp DNA Ladder (Bench Top 50bp DNA Ladder, Promega Corporation, Madison, USA) were used to assess the size of amplification product.

The data was analysed using statistical software and the results were presented in frequency tables.

Results

There was no (0%) HPV-16 positive specimen obtained from this study, whileHPV-18 positive was shown in 80.4% specimens (Table 1). The electrophoresisresults of HPV-16 and -18 genes PCR products were shown in Figure 1 and figure 2.

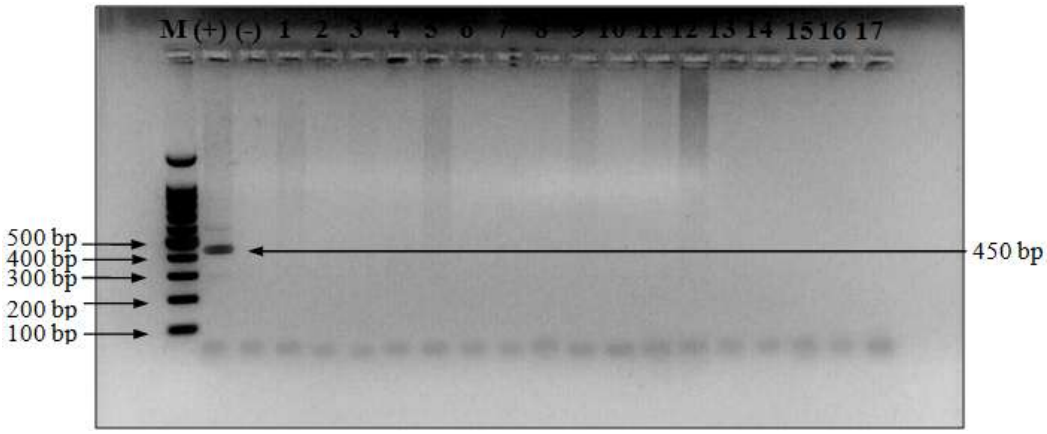

Figure 1. Electrophoresis results of PCR generating fragments of HPV-16 gene on a 2% agarose gel stained 20 well: Marker100 bp (line 1), positive control (line 2), negative control (line 3), samples (line 3 - 20).

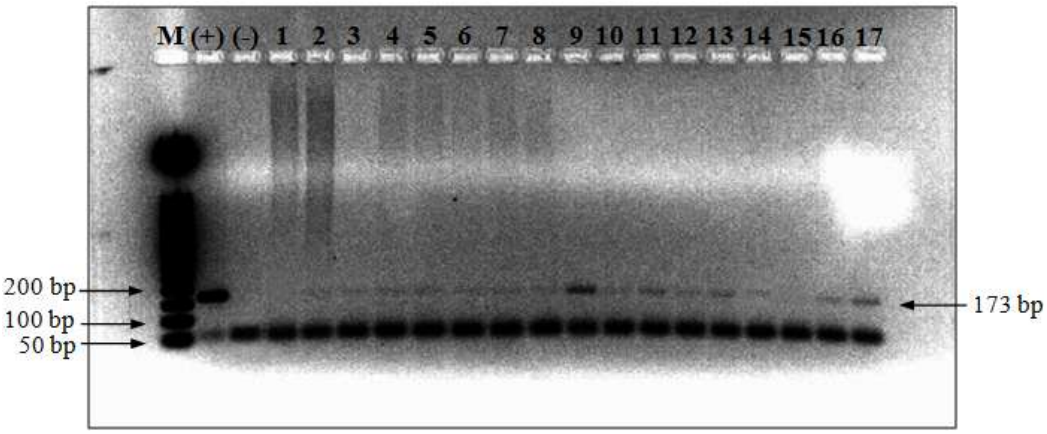

Figure 2. Electrophoresis results of PCR generating fragments of HPV-18 gene on a 2% agarose gel stained 20 well: Marker 50 bp(line 1), positive control (line 2), negative control (line 3), samples (line 3 – 20).

By all of 33 patients diagnosed with cervical SCC, most (84.6%) patientsage ≤ 45 year-old, while majority of cervical adenocarcinoma patients were > 45year-old. Percentage of cervical SCC patients who did not use any contraceptionwas high (91.7%), and percentage of cervical adenocarcinoma patient who tookcontraceptive pill was 42.3%. There was only one patient who had co-infection(HIV) diagnosed with cervical SCC (Table 2).

Table 1. Detection of HPV-16 and HPV-18

HPV strain	Total	Percentage (%)
HPV-16		
Positive	0	0
Negative	46	100
HPV-18		
Positive	37	80.4
Negative	9	19.6
Total	46	100

Table 2. Demographic Characteristics of Cervical Cancer Patients Related to Histopathologic Diagnosis

Variable	Diagnosis				Total
	SCC	%	Adenocarcinoma	%	
Age (years)					
• ≤ 45	11	84.6	2	15.4	13
• > 45	22	66.7	11	33.3	33
Contraceptive method					
• Pill	15	57.7	11	42.3	26
• Injection	7	87.5	1	12.5	8
• None	11	91.7	1	8.3	12
Co-infection					
• Positive	1	100	0	0	1
• Negative	32	71.1	13	28.9	45
Total	33	71.7	13	28.3	46

Among 37 cervical cancer patients who were infected by HPV-18, 84.8%aged > 45 and 84.6% taking contraceptive pill. Among those who were notinfected by HPV-18, there was one (100%) sample accompanied by co-infection.All patients who suffered from metastases were infected by HPV-18. Most(81.8%) patients were diagnosed with SCC (Table 3).

Table 3. Demographic Characteristics of Cervical Cancer Patients Related toHPV-18 Infection

Variable	HPV-18 Infection				Total
	Positive	%	Negative	%	
Age (years)					
• ≤ 45	9	69.2	4	30.8	13
• > 45	28	84.8	5	15.2	33
Contraceptive method					
• Pill	22	84.6	4	15.4	26
• Injection	6	75	2	25	8
• None	9	75	3	25	12
Co-infection					
• Positive	0	0	1	100	1
• Negative	37	82.2	8	17.8	45
Metastasis					
• Yes	6	100	0	0	6
• No	31	77.5	9	22.5	40
Diagnosis					
• SCC	27	81.8	6	18.2	33
• Adenocarcinoma	10	76.9	3	23.1	13
Total	37	80.4	9	19.6	46

Discussion

HPV-16 and -18 are the most frequent causes of cervical cancer around the world, which constitute of estimated 70% of the cases, compared to other types (31, 33, 35, 45, 52, and 58) which are only around 20%[7]. No samples in this study that was infected by HPV-16, while majority (80.4%) of the samples were infected by HPV-18. The authors have tried to replaced the HPV-16 specific primer thrice, yet no positive result was found. This could conclude that among 100% HPV-16 negative or 19.6% HPV-18 negative cases have the chance to be infected by other high risk HPV genotypes. Our results were quite different from the study conducted by Arias et al (2010), which reported: 54% cervical cancer were caused by HPV-16 and 8.8% were infected by

HPV-18[8]. Pornthanakasem et al (2001) reported that HPV-16 and -18 positive were found in 4% and 8% cases, respectively; Yang et al (2004) reported HPV-16 and -18 positive were found in 50% and 8%, respectively; and Simada et al (2010) reported that 30% cases were HPV-16 infected[9,10].

Younger age was found to be associated with the type of HPV, in which Sargent et al (2008) stated that the most common HPV type infected < 30 year old patients was HPV-16 (35.5%)[11]. Meanwhile according to Bahmanyar et al (2012), the most frequent type found in < 25 year old patients was HPV-18 (20%)[12]. Nonetheless, the DNA test used in those two studies were done using cervical cytology smear. No one in this study was aged < 30 year old and majority was > 45 year old, and 84.8% were HPV-18 positive. Lifestyle was suspected to play an important role. The habit of free sex might be the reason why cervical cancer often found in younger age in overseas studies. Technologic advancement might probably influence those who live in developed areas to be more concerned about their health issues and willing to undergo a screening test.

Oral contraceptives tend to be more associated with SCCs than adenocarcinomas[13]. The study conducted by Anderson et al (2008) revealed that 35% of pill-takers suffered from cervical cancers, and the histopathology results were highly associated with high grade squamous intraepithelial lesion (HGSIL) ($p < 0.032$)[14]. Their findings were not consistent with ours, in which 91.7% patients who did not use any contraception were diagnosed with cervical SCC. The oral contraceptives was also associated with HPV strain. Baudu et al (2014) reported that 65.1% pill-takers were HPV (-16/-18/-45) positive[15]. The study did not specifically divide the types of HPV, thus the distribution of pill-taker in each strain infection was not known. On the other hand, the oral contraceptives users in this study were more common (84.8%) in HPV-18 infection.

Several studies showed that there was a correlation between HIV co-infection and HPV in cervical SCC. Parham et al (2006) found that HSIL were detected in 79% HIV infected women[16]. HPV that was often found in HIV patients was HPV-58 strain (18.8%), as described by Hanisch et al (2013)[17]. There was only one HIV case found in this study, with a diagnosis of cervical SCC, and did not shown a significant association neither with HPV-16 nor -18.

According to histopathologic, cytologic, and CT scan assessments, thirteen percent of this study patients had metastasis, involving left and right parametrium, vaginal wall, groin, upper clavicle, and liver. All of these metastatic cases were in HPV-18 group. Nevertheless, not all patients in this study underwent further investigations. Only one patient had CT scan assessment and the rest had chest X-ray to exclude metastasis. Interestingly, each metastatic case in this study showed clearer band (sample -9, -12, -13, -26, -34, and -36) than those without metastasis. This could be possibly caused by higher DNA concentration in metastatic cases. Pornthanakasem et al (2007) study found 50% of metastatic cases were HPV positive[9].

The proportion of cervical SCC case was shown higher in a study conducted by Chan et al (2010), which is 82.1%, while 14% were cervical adenocarcinoma and cervical mixed adenosquamous cell carcinoma. The range of patients'age was 21 – 93 year-old, mean 45.5 year-old[18]. In addition, Pornthanakasem et al (2001) stated 84% of their study's samples were diagnosed with cervical SCC and 16% were cervical adenocarcinoma. Their findings were along with ours, in which 71.1% patients were diagnosed as cervical SCC and 28.3% as cervical adenocarcinoma[19]. However, cervical SCC was still the most common diagnosis in both age ≤ 45 group and age > 45 group.

An interesting finding in this study was 76.9% cervical adenocarcinoma patients was HPV-18 infected, lower than in cervical SCC (81.8%). Based on other studies, cervical adenocarcinoma and adenosquamous cell carcinoma were tightly associated with HPV-18, and SCC was highly associated with HPV-16, although these statements have not been explained specifically[19]. Meantime, other literature presented that HPV DNA was detected in almost 100% of SCC cases, and 62 - 100% of adenocarcinoma cases[20]. This observation had no particular explanation as well. Other study found that HPV-18 was shown to be higher in adenocarcinoma (~ 37%) than SCC (~ 12%). Apparently, HPV-18 has various variants, which are classified by the region (geographical and ethnic groups). However, the authors have not been able to explain whether HPV-18 tends to be positive in adenocarcinoma or SCC[21].

Conclusion

In this study, HPV-18 DNA was detected in most of the samples, and none was detected with HPV-16 DNA. Most of the HPV-18 positive patients aged > 45, taking oral contraceptives, and partly had metastasis. Moreover, we concluded that detection of HPV type in cervical cancer is important, because HPV-16 and -18 are not always found in both cervical SCC and adenocarcinoma

References:

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, RebeloM,et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359–E386.
2. Omran OM, Alsheeha M. Human papillomavirus early protein E6 (HPV 16/18-E6) and cell cycle marker P16 (INK4a) are useful prognostic marker in uterine cervical carcinomas in Qassim region-Saudi Arabia. Pathol. Onchol. Res. 2015;2:157-166.
3. KementrianKesehatan RI. InfodatinPusat data daninformasiKementerianKesehatan RI. 2015.1-6
4. Senba M, Mori N. Oncogenesis of human papillomavirus (HPV) associated with cancers and host immune responses. International journal of cancer research and prevention, International Journal of Cancer Research and Prevention, Volume 9. Number 1. 2016
5. Smith JS, Lindsay L, Hoots B, Keys J, FranceschiS,Winer R, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer* 2007;121:621–32.
6. Singh N, Saraya A. Circulating DNA in cancer: an overview. Chemo Open Acces. 2015;4:4.
7. Ghedira R, Mahfoudh W, Hadhri S, Gabbouj S, Bouanene I, Khairi H, et al. Human papillomavirus genotypes and HPV-16 variants distribution among Tunisian women with normal cytology and squamous intraepithelial lesions. Infectious Agents and Cancer. 2016;11:61.
8. Arias Y, Carillo E, Aristizabal F. Human papillomavirus (HPV) detected in restored plasma DNA from woman diagnosed with pre invasive lesions and invasive cervical cancer. Colombia Medica, North America. 2010. Hamasaki N. The role of band 3 protein in oxygen delivery by red blood cells. Indian Journal of Clinical Biochemistry. 1999;14(1): 49-58.
9. Pornthanakasem W, Shotelersuk K, Termrungruanglert W, Voravud S, Niruthisard S, Mutirangura A. Human papillomavirus DNA in plasma of patient with cervical cancer. BMC Cancer. 2001;1:2.
10. Shimada T, Yamaguchi N, Nishida N, Yamasaki K, Miura K, Katamine S et al. Human papillomavirus DNA in plasma of patiens with HPV16 DNA-positive uterine cervical cancer. Jpn J ClinOncol. 2010;40(5):420-424.
11. Sargent A, Bailey A, Almonte M, Turner A, Thomson C, Peto J, et al. Prevalence of type-specific HPV infection by age and grade of cervical cytology: data from the ARTISTIC trial. British Journal of Cancer.2008;98:1704-1709.
12. Bahmanyar ER, Paavonen J, Naud P, Salmeraon J, Chow SN, Apter D, et al. Prevalence and risk factors for cervical HPV infection and abnormalities in young women at enrolment in the multinational PATRICIA trial. Gynecologic Oncology. 2102;127:440-450.
13. Hellberg D, Stendahl U. The biological role of smoking, oral contraceptive use and endogenous sexual steroid hormones in invasive squamous epithelial cervical cancer. Anticancer Research. 2005;25:3041-3046.
14. Anderson NM, Bazuaye PE, Jackson MD, Smikle M, Fletcher HM. Cervical dysplasia and cancer and use of hormonal contraceptives in Jamaican women. BMC Women's Health. 2008;8:9. 1-6 doi: 10.1186/1472-6874-8-9.
15. Baudu A, Pretet JL, Riethmuller D, Chotard M, Mougin C, Mercier M. Prevalence and risk factors of human papillomavirus infection types 16/18/55 in a cohort of French females age 15-23 years. Journal of epidemiology and global health.2014;4:35-43.
16. Parham GP, Sahasrabuddhe VV, Vermud SH. Prevalence and predictors of squamous intraepithelial lesions of the cervix in HIV-infected women in Lusaka, Zambia. GynecolOncol. 2006;103(3):1017-1022.
17. Hanisch RA, Sow PS, Toure M, Dem A, Dembele B, ToureP,et al. Influence of HIV-1 and/or HIV-2 infection and CD4 count on cervical HPV DNA detectionin women from Senegal, West Africa. J ClinVirol. 2013;58(4):696-702.

18. Chan PKS, Chang AR, Yu MY, Li WH, Chan MY, Yeung AC et al. Age distribution of human papilloma virus infection and cervical neoplasia reflects caveats of cervical screening policies. Int J Cancer. 2010;126(1):297-301.
19. Bosch FX, Lorincz A, Munoz N, Meijer CJLM, Shah KV . The causal relation between human papillomavirus and cervical cancer. J ClinPathol. 2002;55(4):244-265.
20. Pirog EC. Cervical adenocarcinoma, diagnosis of Human Papillomavirus-positive and Human Papillomavirus-negative tumors. Arch Pathol Lab Med. 2017;141:1653-1667.
21. Chen AA, Gheit T, Franceschi S, Tommasino M, Clifford GM. Human papillomavirus 18 genetic variation and cervical cancer risk worldwide. Journal of virology. 2015;89:10680-10687.

*****