The Associations between HPV 18 Gene in The Blood, Contraception, Age of Menarche, Parity with Cervical Carcinoma

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

[*] Department of Anatomical Pathology, Faculty of Medicine,Universitas Sumatera Utara, Medan, Indonesia
[**] Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

DOI: 10.29322/IJSRP.9.06.2019.p9087
http://dx.doi.org/10.29322/IJSRP.9.06.2019.p9087

***Abstract*- Background:** Cervical cancer is the second most frequent cancer in women wordwide and the leading cause of death in several developing countries. It is well established that the risk factors of cervical cancer are infection of Human Papillomavirus, contraception, age of menarche, and parity.
Objective: This study was aimed to analyse the association between HPV 18 gene in the blood, contraception, age of menarche, parity, and cervical cancer.
Methods: This is an observational analytic study with cross sectional approach, involving 46 blood samples from cervical cancer patients (case) and 46 pap smear samples from healthy individuals (control). The HPV gene identification was done using polymerase chain reaction (PCR) technique. The clinical and pathologic data was obtained by the medical record, questionnaire, and histopathologic test result.
Results: There were significant associations between HPV 18 gene, contraception, and cervical carcinoma.
Conclusion: The HPV gene identification from the blood is still needed in individual who is claimed normal from the pap smear results and is expected to be the method of HPV infection detection..

***Index Terms*-:** Polymerase Chain Reaction, HPV 18, cervical cancer.

I. Introduction

Cervical cancer is the fourth most frequent cancer in women with an estimated 570,000 new cases and resulting in 266,000 deaths in 2012.[1] Most cervical cancer cases occurred in developing countries. In USA, cervical cancer is the third most common malignancy. However, mortality and incidence rates have declined in developed countries due to routine papaniculou smear screening test (pap test).[2]

The risk factors of cervical cancer are generally associated with the exposure of Human Papillomavirus (HPV), smoking, and immune suppression. The infection of HPV occurs in 99.7% of all cervical cancer cases. Human Papillomavirus 16 is the predominant type (±50%), followed by HPV 18, 45, and 31. Other causes include having sexual intercourse at young age multiple sexual partners, history of sexual transmitted disease, smoking, high parity, and hormonal contraception.[2-6]

Infection of HPV is mostly transmitted sexually. Accordingly, most of the studies on HPV infection are focusing on sexually exposed women. However, HPV infection can also be transmitted non-sexually. These non-sexual modes are differentiated into vertical and horizontal transmissions.[7]

The studies of HPV DNA detection in the blood have often been done, but only few that have been conducted in healthy individuals. Considering the conditions mentioned above, the authors were interested in detecting the presence of HPV DNA (especially HPV 18) in the blood of cervical cancer patients and healthy individuals. This study was aimed to assess the association of HPV 18 in the blood, contraception, age of menarche, and parity with cervical carcinoma, which involving a group of cervical cancer patients and a group of control individuals.

II. Material and Product

This is an observational analytic study, using a cross sectional approach. The study was conducted at the Oncologic Gynaecology polyclinic and ward, Department of Clinical Pathology, Department of Anatomical Pathology in Haji Adam Malik Medan General Hospital. The research was held from September 2018 until March 2019, after approved by the University of North Sumatera and Haji Adam Malik General Hospital Health Research Ethics Committee.

The sample was divided into two groups. Case group was patients who were diagnosed with cervical carcinoma based on histopathological examination with HPV DNA positive from previous study stored preparation. Control group were social service participants whose pap smear test was normal. Each group was consisted of 46 participants and written informed consent was obtained from all participants. Participants were recruited using consecutive sampling technique. For control group, blood 3 ml sample was taken and DNA isolation was done. Blood sample was stored until pap smear cytology test was obtained.

The analysis of DNA was done in the integrated laboratory of University of North Sumatera. First step was DNA isolation (Wizard Genomic DNA Purification Kit), continued with PCR Mix, and last step was gel electrophoresis. For the stored DNA samples for each group, amplification (PCR) was done using Go

Taq® PCR master Mix from Promega. Applied BiosystemVeriti 96 was used for thermal cycler. Specific primer for HPV 18 were 5'-TCG TTT TCT TCC TCT GAG TCG CCT-3' (Forward) and 5'-CCG AGG ACG ACA GGA ACG ACT-3' (Reverse). A tube of 25 µl mixed solution, which consisted of 12.5 µl PCR mix solution (Go Taq® PCR master Mix); 1 µl forward primer 10 pmol; 1 µl reverse primer 10 pmol; 6.5 µl Nuclease-free water; 4 µl DNA template, was prepared for spindown. The mixture was then inserted to the PCR machine for HPV 18 primer amplification. The initiation was set in 95°C for 5 minutes, continued with 40 other cycles comprised of the process of denaturation (95°C, 60 seconds), annealing (59°C, 60 seconds), and extension (72°C, 120 seconds), and last extension (72°C, 5 minutes). After PCR, the process was continued with electrophoresis. The electrophoresis was done in 2% agarose gel, dyed with 2 µl Ethidium Bromide for 70 minutes in 80 V, and was visualised using UV transluminator 2 (Uvitec Cambridge). The result was determined HPV 18 positive if electrophoresis band was seen as many as 173 base pairs. Data was analysed with statistic software program and the results were shown in the table of frequency.

III. RESULT

The HPV 18 gene was found positive in 37 (80.4%) patients from case group. Some of the electrophoresis results of the HPV 18 gene of case group can be seen in Figure 1.

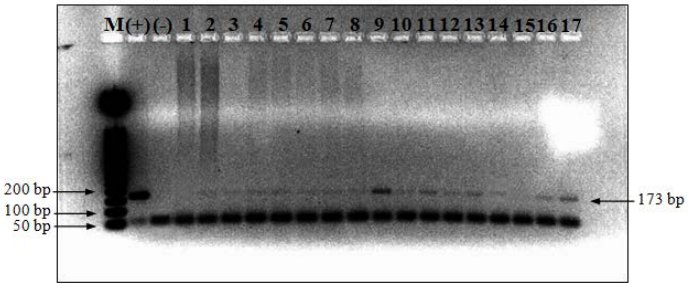

Figure 1. The electrophoresis results of HPV 18 gene PCR product in 2% agarose gel 20 well, case group: Marker of 50 bp (line 1), positive control (line 2), negative control (line 3), sample 1 - 17 (line 4 - 20).

None of the control group showed positive HPV 18 gene. Some of the electrophoresis results of the HPV 18 gene of control group can be seen in Figure 2.

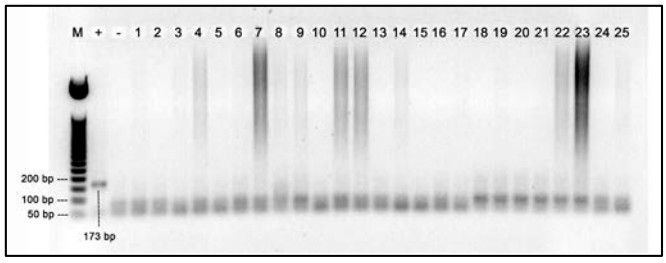

Figure 2. The electrophoresis results of HPV 18 gene PCR product in 2% agarose gel 20 well, control group: Marker of 50 bp (line 1), positive control (line 2), negative control (line 3), sample 1 - 17 (line 4 - 28).

In this study, analysis of association of HPV 18 gene in the blood, contraception, age of menarche, and parity with cervical carcinoma was done, comparing the case and control groups. The variables were HPV 18 gene, contraception, age of menarche, and parity (Table 1). (See below).

Nine (16.4%) participants without HPV 18 in blood serum had cervical cancer, while 37 (100%) participants with serum HPV 18 positive had cervical cancer. Statistical test showed p value = 0.001, concluded that there was a significant relationship between HPV 18 and cervical cancer.

For the association of contraception and cervical cancer, there were 12 (30%) participants who did not use contraception, 26 (78.8%) participants who used contraceptive pill, and 8 (42.1%) participants who used contraceptive injection had cervical cancer. Statistics showed p value = 0.001, thus there was a significant association between contraception and cervical cancer.

As many as 39 (54.9%) participants who had menarche at 13 – 19 years old and 7 (33.3%) participant who had menarche at 11 – 12 years old, had cervical cancer. Statistic test resulted in p value = 0.136, which meant there was no significant association between age of menarche and cervical cancer.

Last, the analysis of parity and cervical cancer showed that 10 (58.8%) participants who had 5 – 12 children and 36 (48%) participants who had 0 – 4 children had cervical cancer. Statistical analysis showed p value = 0.591, hence there was no significant relationship between parity and cervical cancer.

IV. DISCUSSION

It is well established that the most common cause of cervical cancer was HPV infection. Human papillomavirus 18 is one of the high-risk type of HPV that can cause cervical cancer.[2] Nevertheless, human papillomavirus is not the only causal factor of cervical cancer, other factors might contribute as well. In this study, there was a significant association between HPV 18 and cervical cancer (p = 0.001). Arias, *et al.* (2010) reported that HPV 18 was found in 100% cervical cancer cases.[8] Former study conducted by Pornthanakasem, *et al.* (2001) also found that the prevalence of HPV 18 was as much as 8%.[9] Asmiati, *et al.* (2018) also showed that HPV 18 was detected in 80.4% cervical cancer patients.[10] However, those studies did not analyse the relationship between HPV 18 and increased risk of cervical cancer, and the difference of number of the cervical cancer patients and control. In fact, several studies did not involve control group in investigating HPV 18 detection.

Contraception was reported to have a significant relationship with the risk of cervical cancer.[6] This was in line with the finding of this study where contraceptive pill and injection had a significant association with cervical cancer (p = 0.001). Similar result was reported by Paramita, *et al.* (2010), in which estrogen and progestin had a high association with the risk of cervical cancer (p = 0.001), increasing the risk of cervical cancer 4.21 times.[11] Baudu, *et al.* (2014) also concluded that pill contraception is very related to increased risk of cervical cancer (p = 0.012).[12] In contrast, Parija, *et al.* (2017) reported that women who did not use contraception significantly has high risk of cervical cancer, as much as 89.3% (p = 0.001).[13] The difference in study results might be the fact that some of those

studies included the length of contraception use, whereas our study did not. Other limitation in this study was that we did not explore the type of contraception, whether it contained estrogen or progesteron or the combination of both.

The age of menarche is very related with the risk of cervical cancer.[14] Da Silva, *et al.* (2017) reported that in 18 – 30 years old women who had their menarche in the age of 12 years old were 1.95 times more susceptible to CIN 2–3 or cervical cancer (CI = 1.17 – 3.25).[15] The study by Sharma, *et al*. (2018) stated that women who had menarche at 13 – 14 years old were 2.91 times more risky of having cervical cancer (p = 0.02).[16] On the contrary, Paramita, *et al.* (2010) argued that age of menarche was not associated with the risk of cervical cancer (p = 0.074), while women who menarche in 13 – 19 years old were 0.39 more risky to have cervical cancer.[11] This study also reported no significant relationship between age of menarche and cervical cancer. Previously, study by Fujita. *et al*, (2008) concluded that age of menarche had no role in causing cervical cancer (p= 0,33).[17] The discrepancy among these results may be due to the diverse age of menarche range categories in each study, some even did not limit the age of the first menstrual cycle. Difficulty in remembering the exact age of menarche is an issue in this study. Some samples only remember the grade of primary school when they experienced their first period. Thus, we have to convert it into an approximate age based on the grade of Indonesian primary school in general.

Parity was also strongly associated with the risk of cervical cancer, where Sharma. *et al,* (2018) reported that women who had 3-5 children significantly would be 3,16 times at risk for cervical cancer (p= 0,029).[6,16] In line with Parija. *et al*, (2017), high parity (>5) was strongly associated with the risk of cervical cancer, accounted for 57,1%, with p=0,05.[13] Paramita. *et al,* (2010) found no significant association between parity and cervical cancer (p=0,073).[11] However, they concluded that women who had 5-12 children had 2,62-fold risk of cervical cancer. Our study also found no significant association between parity and cervical cancer, which is in accordance with Fujita. et al (2008).[17] We consider that the similarity of these results might be related with the similarity of ethnicity and geographic region, despite one of the studies had a large number of participants in control group compared to the case group. Furthermore, we concluded that the society has the tendency of having few children these days due to the governmental program in limiting the rate of birth through the family planning program.

V. CONCLUSION

After conducted this study, which involving to cervical cancer patients (case group) and healthy individuals (control group), we highlighted several points in the following :

1. Each group, case group and control group, was consisted of 46 participants, in which most participants in case group were HPV 18 infected whereas none of control group was infected by HPV 18.
2. There were significant associations between HPV 18, methods of contraception and cervical cancer.
3. Detection of HPV in the blood of healthy individuals can be implemented and is expected to be one of the alternative options for screening and diagnostic tests of cervical cancer.

REFERENCES

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M,et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359–E386. doi: 10.1002/ijc.29210 PMID: 25220842.

[2] Daaz-montes TP, Goodrich K. Cervical Cancer. in: Fortner KB, Szymanski LM, Fox HE, Wallach EE. Eds: The John Hopkins Manual of Gynecology and Obstetrics. 3rd Ed. Maryland:Lippincott William & Wilkins; 2007.p. 481-7.

[3] Lacey JV, Frisch M, Brinton LA, Abbas FM, Barnes WA, Gravitt PE et al. Associations between smoking and adenocarcinomas and squamous cell carcinomas of the uterine cervix (United States). Cancer Causes & Control. 2001;12: Proquest. p. 153.

[4] Chen Y, Williams V, Filippora M, Filoppov V, Duerksen-Hughes P. Viral carcinogenesis: factors inducing DNA damage and virus integration. Cancer. 2014;6:2155-2186; doi: 10.3390/cancers6042155.

[5] Erickson BK, Alvarez RD, Huh WK. Human papillomavirus: what every provider should know. Am J Obstet Gynecol. 2013;208(3):169-175. Doi:10.1016/j.ajog.2012.09.007.

[6] Einstein MH, Schiller JT, Viscidi RP, Strickler HD, Coursaget P, Tan T. Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis. 2009;9:347.

[7] Sabeena S, Bhat P, Kamath V, Arunkumar G. possible non-sexual modes of transmission of human papillomavirus. J. Obstet. Gynaechol. Res. 2017 (43);3:429-35.

[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.

[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] Asmiati TP, Delyuzar, Hidayat. Human Papilloma Virus Type 16 & 18 Gene Identification in Plasma of Cervical Squamous Cell Carcinoma and Adenocarcinoma Patients at Haji Adam Malik General Hospital Medan. International Journal of ChemTech Research. 2018;11(11):379-85.

[11] Paramita S, Soewarto S, Widodo MA, Sumitro SB. High parity and hormonal contraception use as risk factors for cervical in East Kalimantan. Med J Indones. 2010;19:268-72.

[12] 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.

[13] Parija J, Mohapatra J, Padhy A. Manuscript of risk factors for cancer cervix: an analysis of 1000 cases. Int J Res Med Sci. 2017;8:3364-7.

[14] Plummer M, Peto J, Franceschi S. Time since first sexual intercourse and the risk of cervical cancer. Int J Cancer. 2012;130 (11): 2638-44.

[15] da Silva IF, Koifman RJ, Parreira VAG, Soares S, Koifman S. Risk factors for cervical cancer in a sample comprising three generations of Brazilian women. Journal of Cancer Therapy. 2017;8:12-25.

[16] Sharma P, Pattanshetty SM. Astudy on risk factors of cervical cancer among patients attending a tertiary care hospital: A case-control study. Clinical Epidemiologi and Global Health. 2018;6:83-87.

[17] Fujita M, Tase T, Kakugawa Y, Hoshi S, Nishino Y, Nagase S *et al.* Smoking, earlier menarche and low parity as independent risk factors for gynecologic cancer in Japanese: A case-control study. Tohoku J. Exp.Med. 2008;216:297-307.

AUTHORS

First Author – dr. Tri Puji Asmiati, Resident of Department of Anatomical Pathology, Faculty of Medicine, Universitas

Sumatera Utara, medan, Indonesia, **email ID:** tripujiasmiati@gmail.com
Second Author – Dr. dr. Delyuzar, M.Ked(PA), Sp.PA(K), Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
Third Author – dr. Hidayat, M.Biomed, Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Correspondence Author – dr. Tri Puji Asmiati, Resident of Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, **email ID:**tripujiasmiati@gmail.com

Table 1. Association betwen *HPV* 18 gene in the blood, contraception, age of menarche and parity with cervical carcinoma.

Variable	Cervical cancer				Total	P
	Yes	%	No	%		
HPV 18						
• Positive	37	100	-	-	37	0,001
• Negative	9	16,4	46	83,6	55	
Contraception						
• Pill	26	78,8	7	21,2	33	0,001
• Injection	8	42,1	11	57,9	19	
• Other methods/ None	12	30	28	70	40	
Menarche (years)						
• 11-12	7	33,3	14	66,7	21	0,136
• 13-19	39	54,9	32	45,1	71	
Parity						
• 0-4 births	36	48	39	52,0	75	0,591
• 5-12 births	10	58,8	7	41,2	17	
Total	46	50	46	50	92	