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LOW MOLECULAR MASS POLYPEPTIDE AND TRANSPORTER ANTIGEN PEPTIDE GENES POLYMORPHISM AS THE RISK FACTORS OF CERVICAL CANCER WHICH CAUSED BY HUMAN PAPILLOMAVIRUS TYPE-16 INFECTION IN BALI

I Nyoman Bayu Mahendra

Obstetrics and Gynaecology Department,
Faculty of Medicine, Udayana University/Sanglah General Hospital, Bali-Indonesia

Background: Until recently, cervical cancer is one of the major problem in women's health issue related to its high incidence and mortality rate. The etiology of cervical cancer is the high risk oncogenic group of Human Papillomavirus (HPV), especially HPV-16 and 18 and its phylogenies. Meanwhile in Bali, more than 50% of infection are caused by HPV-16 infection. The main objective of this study was to investigate the role of LMP-2, LMP-7, TAP-1 and TAP-2 gene polymorphism as the risk factor in the cervical cancer carcinogenesis that is caused by HPV-16 infection. **Method:** A nested non-paired case-control study was conducted at Obstetric and Gynecologic Department Sanglah General Hospital, Bali-Indonesia from March 1 until August 31, 2013. Laboratory testing was carried out at Laboratory of Histopathology Leiden University Medical Centre, Netherlands,. **Results:** A total of 40 samples were collected, consist of 20epithelial cervical cancer patients with positive HPV-16 infection as the case group and 20 non-cervical cancer patients with positive HPV-16 infection as the control group. Women infected by HPV-16 with LMP-7 gene polymorphism had a higher risk (OR=7.36, CI 95%=1.38-40.55, *p*=0.013) to be diagnosed with cervical cancer. Balinese women who were infected by HPV-16 with TAP-2 gene polymorphism had a higher risk (OR= 9.33, CI 95%=2.18-39.96, *p*=0.001) to be diagnosed with cervical cancer. Meanwhile, Balinese women who were infected by HPV-16 with LMP-7 and TAP-2 genes polymorphism had a higher risk (OR=12.67, CI 95%=1.40-114.42, *p*=0.020) to be diagnosed with cervical cancer. As the result, it was shown that both of this gene polymorphism was working synergistically. **Conclusion:** TAP-2 and LMP-7 genes polymorphism play a role in the carcinogenesis mechanism of cervical cancer that is caused by HPV-16 infection in Bali. Meanwhile, LMP-2 and TAP-1 genes polymorphism were not found to play a role in the immunology pathway of cervical cancer that is caused by HPV-16 infection.

Keywords: cervical; cancer; HPV-16; LMP and TAP; genes polymorphism.

INTRODUCTION

The annual incidence of cervical cancer is about 496,000 new cases, where 75-80% occurs in the developing countries; including Indonesia.[1] In 2002, the incidence of cervical cancer in South East Asia was 42.58 cases with 22.59 mortality cases.[2] In Indonesia, the prevalence of cervical cancer is 27% amongst all type of female cancer and considered as the first rank for three decades. Meanwhile in Bali, the incidence of cervical cancer is 0.98%, or about 150/100,000 women are having the risk. Besides, there is an increasing tendency of cervical cancer incidence throughout the world, from 2.7‰ in 1972 to 3.0‰ in 1989, and 4.1‰ in 1998.[1,2]

Mortality rate of cervical cancer varies throughout the world and depends on the staging when the diagnosis was made. In Europe and America, cervical cancer the second most common cases after breast cancer, and the sixth leading cause of death.[2] In Indonesia, every hour another person dies from cervical cancer, while in Bali there is one death case in every two days. Cervical cancer is the first leading causes of mortality related to the delayed diagnosis.[3]

The etiology of cervical cancer is the infection from the high risk oncogenic group of Human Papilloma Virus (HPV), especially HPV-16 and 18.[4] In Bali, the prevalence of HPV-16 holds the

Address of corresponding author:
I Nyoman Bayu Mahendra
Obstetrics and Gynaecology Department, Faculty of Medicine Udayana University/Sanglah General Hospitals, Bali-Indonesia
Email: bayu.mahendra.nyoman@gmail.com

first position, which is about 50-61% amongst the whole high risk oncogenic HPV group. The risk of woman to be infected by HPV is 80% throughout their life.[4]Most infection occur transiently and directly cleared up by human immune system within 1-2 years. However, the humoral immune responsetowardsnatural HPV infection is low, because HPV does not enter the blood vessel.[3,4]

Until recently, the study of cervical cancer carcinogenesis mechanism is more focusing on the immune response, tumor supressor gene, and association of the related protein.[5] The low immune response towards natural HPV infectionhas not been able to be explained yet, whereas it is suspected that the genetic factor plays an important.Research evidences show that there was genetic predisposition towards cervical neoplasia.[4,5] Variations at several gene loci that coding the APM component is the hypothesis that related with the gene predisposition and susceptibility of the oncogenic HPV infection. In this condition, there might be any information disparity of the cervical epithelial cell in form of Antigen Processing Machinery (APM) failure which affect the optimalization of the Antigen Presenting Cell (APC) function. The role of APM involves Low Moleculer Mass Polypeptide (LMP) and Transporter Antigen Peptide (TAP). The inability of APC to relay the information towards the cytotoxic T-lymphocyte through Major Histocompatibility Complex (MHC) cause the low immune response.[6] This is the main point in the detecting the infected cell.[7]

Proteasomal components in form of LMP-2 and LMP-7 increase the proteolytic production of certain peptide. On the other hand, TAP-1 and TAP-2 are heterodimer and functions as pumping the antigen peptides towards the endoplasmic reticulum lumen. The decreasing expression of TAP-1, TAP-2, LMP-2 and LMP-7 are known to suppress the expression of MHC-I surface molecule. The decreasing expression of LMP and TAP have been detected in many cancer cells and tissues. Recently, two single nucleotide polymorphisms (SNPs) on TAP-2 and LMP-7 genes seem to be correlated with the increasing risk of esophageal cancer related to HPV infection.[9-11]

METHODS

This was a non-paired nested case-control study conducted in Obstetrics and Gynaecology Department, Histopathology Laboratory, Molecular Biology Laboratory, Faculty of Medicine, Udayana University/Sanglah General Hospital, Bali-Indonesia and Laboratorium of Histopathology Leiden University Medical Centre (LUMC) Netherlands. This study was conducted from March 1st, 2013 until August 31st 2013. Samples were the cervical cancer and non-cervical cancer patients with positive HPV-16 infection, treated at Oncology Gynaecology outpatient Clinics of Obstetrics and Gynaecology Department of Sanglah General Hospital, Bali-Indonesia with Balinese background, and participated voluntarily in this study and had been selected consecutively from the population. Paraffin blocks from cervical biopsy were used as sample preparations in this research. The exclusion criteria were the cervical cancer patients with HPV infection other than type 16 and broken paraffin blocks that could not be used in this study. The total samples were 40 samples, consisted of 20 samples cervical cancer patients with HPV-16 infection as the case group and 20 samples of non-cervical cancer with positive HPV-16 infection as control group.

The diagnosis of cervical cancer and non-cervical cancer were based on the results of cervical biopsy histopathology preparation, which had been evaluated in the Histopathology Laboratory, Faculty of Medicine Udayana University/Sanglah General Hospital, Bali-Indonesia.

Detection of HPV-16 was conducted through molecular biology with PCR technique, using specific primer commercial kit HPV INNO-LiPA HPV Genotyping from INNOGENETICS® Company, which was carried out in the Biomolecular Laboratory, Faculty of Medicine, Udayana University Bali-Indonesia.

Sample preparation that had previously obtained positive for HPV type 16 infections were then detected for LMP-2 LMP-7, TAP-1, and TAP-2 genes polymorphism using Taqman SNP Assay Kit in Laboratory of Histopathology LUMC in the Netherlands. Data from this study was analyzed using Normality Test with Shapiro Wilk, Homogenity Test with Levene Test and Chi Square Test to find the odds ratio.

RESULTS

In this case control study, Levent test was conducted towards the mean age, parity, and coitarche age variables. As seen in Table 1, the *p* value for mean age, parity, and coitarche age in both groups was >0.05, which means that there was no significance difference amongst the two groups.

Data of polymorphism and incidence were listed in Table 2. As shown in Table 2, there was significance difference of LMP-7 and TAP-2 genes polymorphism with the incidence of cervical cancer ($p<0.05$). On the other hand, the polymorphism of LMP-2 and TAP-1 genes was having no significance difference ($p<0.05$).

In order to evaluate the role of LMP-7 and TAP-2 genes polymorphism towards cervical cancer risk with HPV-16 infection, Chi-square test was conducted. LMP-7 gene polymorphism odd ratio (Table 3) to cause cervical cancer with HPV-16 infection was 7.36 (CI 95% = 1.34-40.55; $p=0.013$). Meanwhile, the odd ratio of risk factor

towards TAP-2 gene polymorphism (Table 4) was 9.33 (CI 95% = 2.18 – 39.96; *p*=0.001).

Table 1
Prevalence Distribution of Age, Parity, and Coitarche Age

Risk factor	Case n = 20	Control n = 20	*p*
Age (year)	38.75 (5.92)	40.85 (4.15)	0.202
Parity	2.40 (1.05)	2.00 (1.08)	0.241
Sexual activity	20.15 (1.93)	20.30 (2.56)	0.835

Significant at $p < 0.05$
(…) standard deviation.

Table 2
Prevalence Distribution of LMP and TAP Genes Polymorphism

Polymorphism	Case (n=20)	Control (n=20)	*p*
LPM-2			
positive	13 (65%)	12 (80%)	0.744
negative	7 (35%)	8 (40%)	
LPM-7			
positive	9 (45%)	2 (10%)	0.013
negative	11 (55%)	18 (90%)	
TAP-1			
positive	9 (45%)	10 (50%)	0.752
negative	11 (55%)	10 (50%)	
TAP-2			
positive	14 (70%)	4 (20%)	0.001
negative	6 (30%)	16 (80%)	

Significant at $p < 0.05$

Correlation test between LMP-7 and TAP-2 genes with having cervical cancer risk was also conducted using regression test as seen in Table 5. It was found that the odd ratio of combination of TAP-2 and LMP-7 genes polymorphism in cervical cancer that caused by HPV-16 was 12.67 (CI 95% = 1.40-114.42; *p*=0.02).

DISCUSSION

In this study, it was found that LMP-7 and TAP-2 genes polymorphism difference in the case and control group was significantly different ($p<0.05$). Moreover, the combination of LMP-7 and TAP-2 genes polymorphism was increasing the risk even more to cause cervical cancer that was caused by HPV-16. As the result, higher confidence value was obtained for both of those genes polymorphism in causing cervical cancer.

Table 3
LMP-7 Gene Polymorphism with Risk of Cervical Cancer caused by HPV-16 Infection

Groups	LPM-7 Polymorphism	
	positive	negative
Case	9	11
Control	2	18
OR	7.36	
CI 95%	1.34-40.55	
p	0.013	

Significant at $p < 0.05$

Table 4
TAP-2 Gene Polymorphism with Risk of Cervical Cancer caused by HPV-16 Infection

Groups	TAP-2 Polymorphism	
	positive	negative
Case	14	6
Control	4	16
OR	9.33	
CI 95%	2.18-39.96	
p	0.001	

Significant at $p < 0.05$

Table 5
LMP-7 and TAP-2 Genes Polymorphism with Risk of Cervical Cancer caused by HPV-16 Infection

Groups	LMP-7 and TAP-2 Polymorphism	
	positive	negative
Case	8	12
Control	1	19
OR	12.67	
CI 95%	1.40-114.42	
p	0.02	

Significant at $p < 0.05$

Our study result was having similar result with several previous studies, where there was significance difference of LMP-7 and TAP-2 genes polymorphisms found in cancer patients. Recent study from Cao et al., 2005 was investigating the LMP-2, LMP-7, TAP-1 and TAP-2 genes polymorphism towards esophageal cancer, and it was found that TAP-2 and LMP-7 genes polymorphisms were related with higher risk of esophageal cancer in correlation with HPV infection. That study supported the statement that the genetic of immune response play a role in the HPV infection tumorgenesis. Other investigator from Germany, which is Fellerhoff et al. in 2011 were investigating LMP-2, LMP-7, TAP-1 and TAP-2 genes polymorphism in colon cancer, and showed that LMP-7 polymorphism caused the production of immunoproteasomes was decreasing in the peptide processing, and followed by the

decreasing quality of HLA ability to present the antigen. Fellerhoff et al. also showed that the defect stated before was a crucial immunological factor in fighting the colon cancer. They also did not obtain LMP-2, TAP-1 and TAP-2 genes polymorphism in colon cancer.[10]

There was a similar study with our study that was conducted in Netherlands and also investigating about cervical cancer specifically. They found that LMP-7 and TAP-2 genes polymorphism was correlated significantly with cervical cancer incidence in Dutch women. In this study also suggested that LMP-7 and TAP-2 genes polymorphisms was correlated with the progress of the disease.[11]That study was not investigating the correlation with HPV-16 specifically.

Meanwhile for the significance analysis of LMP-2 and TAP-1 polymorphism as seen in Table 2 was having result of $p>0.05$. It means that there was no significant difference for LMP-2 and TAP-1 genes polymorphism for both groups.

In our study, no correlation of TAP-1 and LMP-2 with cervical carcinoma risk was causing an interesting phenomenon, because those gene were previously suggested having a strong correlation with the incidence and survival rate of some autoimmune disease and malignancy. Our study result was similar with the study of esophageal cancer and colon cancer, where they also did not find any correlation between TAP-1 and LMP-2 genes polymorphism with cancer incidence.[7,10]

A study in Netherlands that was similar with our study showed that the decreasing expression of TAP-1 did not having correlation with the survival rate cervical cancer patients.[11] In the study that was conducted to know the expression of TAP-1 and LMP-2 upon mice, found that the tumor incidence was not different from the control group. Even though LMP and TAP was already confirmed to play a role in detecting and presenting antigen towards HLA, but the specific role of each LMP-2, LMP-7, TAP-1 and TAP-2 genes had not been known yet due to inadequate study before.[7]

Up until now, it is still unclear about how far the cross reactivity of immune response amongst HPV variants. Current hypothesis is that there is gene polymorphism in cellular immunity response towards HPV-16 infection. Not only that, it is also suspected that the oncogenicity of several variants were correlated with the geographic and ethnicity of the samples' population.

This difference might be explained with the HLA molecule distribution that is very polymorphic, due to some HLA alleles that are more efficient in presenting HPV-16 strain peptides. As previously known, that this HLA is very important in determining immune response. It was suspected that the inefficient body response to present any viral infection that was supposed to be detected as foreign objects, causing the HPV infection cannot be eliminated naturally by immune system. As the result, the failure of immune system in presenting the foreign substances of HPV infection through MHC-I is the main factor of cervical neoplasm development.[12] Any disruption of MHC expression is suspected caused by the defect of LMP-2, LMP-7, TAP-1 and TAP-2 components. Disruption of those single or multiple components are causing defect in expression or function of MHC-I – peptide complex. The disruption of LMP-2, LMP-7, TAP-1 and TAP-2 components is considered caused by gene polymorphism that was causing any changes in protein expression, and also affect the final function of LMP and TAP components afterwards.[11,12]

CONCLUSION

Balinese women with HPV-16 infection and LMP-7 and TAP-2 gene polymorphism were having higher risk of cervical cancer compared to women with HPV-16 infection without LMP-7 and TAP-2 genes polymorphism. LMP-7 and TAP-2 genes polymorphism as APM components play a role in cervical cancer carcinogenesis mechanism that was caused by HPV-16 infection. Meanwhile, LMP-2 and TAP-1 genes polymorphism were not as risk factors in immunologic pathway of cervical cancer carcinogenesis that was caused by HPV-16 infection in Bali.

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