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HIGH INTERLEUKIN-6, LOW CD4$^+$ AND CD8$^+$ T-LYMPHOCYTES EXPRESSIONS AS RISK FACTORS OF CERVICAL CARSINOMA INFECTED BY HUMAN PAPILLOMA VIRUS TYPE-52

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ABSTRACT

In Indonesia cervical carcinoma is the most common cancer in women and one of the leading cause of mortality. High risk human papillomavirus (HPV) is the major risk factor of cervical cancer. This study aims to know the role of IL-6, CD4$^+$ and CD8$^+$ T-lymphocyte for the risk of cervical carcinoma infected by HPV52. This study was a case control study, specimens of cervical carcinoma patients infected by HPV type-52 as the case group and HPV type-16 or 18 as the control group. HPV genotyping used SPF10 primer and type specific E7 primer by LiPA. Immunohistochemistry method was used to know expression of IL-6, CD4$^+$ and CD8$^+$ T lymphocyte. Pearson's χ^2 test was applied with statistical significance was set at the 2-sided 0.05 level. The odds ratios (OR) were calculated for the risk, with 95% confidence intervals on SPSS 16.0 for windows. PCR examination was performed in 185 paraffin-embedded tissue. The risk of high IL-6 expression in cervical carcinoma infected by HPV type-52 was statistically significant 6-fold higher compare with cervical carcinoma infected by HPV type 16 (OR = 6.00 ; CI 95% = 1.13-31.99; p = 0.03; p < 0.05) and HPV type 18 (OR = 6.00 ; CI 95% = 1.13-31.99; p = 0.03; p < 0.05). The risk of low CD4$^+$ T lymphocyte expression in cervical carcinoma infected by HPV type 52 was statistically significant 6-fold higher and 7.43-fold higher respectively compare with cervical carcinoma infected by HPV type 16 (OR = 6.00 ; CI 95% = 1.003-35.91; p = 0.04; p < 0.05) and HPV type 18 (OR = 7.43 ; CI 95% = 1.23-45.01; p = 0.02; p < 0.05). The risk of low CD8$^+$ T lymphocyte expression in cervical carcinoma infected by HPV type 52 was statistically significant 13.5-fold higher and 11-fold higher respectively compare with cervical carcinoma infected by HPV type 16 (OR = 13.50 ; CI 95% = 1.42-128.26; p = 0.01; p < 0.05) and HPV type 18 (OR = 11.00 ; CI 95% = 1.16-103.94; p = 0.02; p < 0.05). No significance different between cases and controls group in mean-age, parity and sexual activity (p > 0.05). In conclusion, this study found that high IL-6 expression, low CD4$^+$ and CD8$^+$ T lymphocyte expression were the risk factors of cervical carcinoma infected by HPV type 52.

Keywords: IL-6, CD4$^+$ T-lymphocyte, CD8$^+$ T-lymphocyte, cervical carcinoma, HPV type-52

INTRODUCTION

Cervical cancer is the second most common cancer in women in developing countries. It accounts for 80-90% of all cancer cases.[1,2,3] In Indonesia cervical carcinoma is the most common cancer in women and one of the leading cause of mortality. In Bali cervical cancer is the second most common after breast cancer, it accounts for 27.5%.[4,5,6] Many attempts have been carried out, however, many obstacle and could not decrease the incidence and mortality rate of cervical cancer were faced. High risk oncogenic human papilloma virus (HPV) is the major risk factor of cervical cancer. It can be detected in about 90% of all cervical cancers.[5,7,8] The two predominant high-risk HPV types are HPV-16 and 18 associated with more than 90% of cervical cancers worldwide.[5,8,9] In Bali HPV type-52 is the most common type found in cervical cancer patients, account for 18%.[10] The main pathogenesis of cervical carcinoma infected by HPV type-16 and 18 have been well established and widely accepted worldwide through tumor suppressor gene p53 and pRb. Further study is needed to know the

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pathogenesis of cervical carcinoma infected by other high risk HPV. [8,11,12] The aim of this study was to know the role of immunologic factor, especially IL-6, $CD4^+$ T-lymphocyte and $CD8^+$ T- lymphocyte for the risk of cervical carcinoma infected by HPV type-52, because HPV type-52 is the most common type in Bali. The results of this study were expected to be used for new input in pathogenesis of cervical cancer infected by HPV type-52, for early diagnosis and as predictor factors.

MATERIAL AND METHODS

Specimen collection and histopathological diagnosis

This study was a case control study. The sample consisted of biopsy or operation specimens of cervical carcinoma patients infected by HPV type-52 as the case group and biopsy or operation specimens of cervical carcinoma patients infected by HPV type-16 or 18 as the control group. The study was conducted during the years 2011-2012 at Department of Obstetrics and Gynecology Faculty of Medicine Udayana University/Sanglah Hospital Denpasar for the biopsy or cervical operation. And then, these specimens were processed at Department of Anatomical Pathology Faculty of Medicine Udayana University/Sanglah Hospital Denpasar for histopathology diagnosis.

HPV DNA detection and typing

The paraffin-embedded tissue which have been diagnosed as cervical carcinoma were sent for detection and genotyping of HPV DNA by PCR-based methods at Department of Pathology, Leiden University Medical Centre, the Netherland and Molecular Biology Unit, Faculty of Medicine, Udayana University. HPV DNA was detected in 2 steps. In the first step, the SPF10 primer at several dilution were used to amplified the DNA. In the second step, the positive products was performed using specific probes type-specific E7 primer by LiPA which could detected 25 HPV types to know the evidence of HPV type 52, 16 and 18.

Immunohistochemistry examination

Ten specimens with HPV type 52 positive were used as cases. The specimen of HPV type 16 or 18 positive, consist of 20 specimens each as controls. Immunohistochemistry examination was carried out to all of these 50 specimens for examination of IL-6, $CD4^+$ T-lymphocyte and $CD8^+$ T-lymphocyte expression at Department of Anatomical Pathology Faculty of Medicine Gajah Mada University/ Dr. Sardjito Hospital Yogyakarta.

Statistical analysis

The results of this study was analyzed on SPSS 16.0 for windows, i.e. Shapiro-Wilk test for normality of data, Levene's test for homogeneity, logistic regression for ratio odd (RO), and Pearson's chi-square test for significance. Statistical significance for these tests was set at the 2-sided 0.05 level with 95% confidence intervals (CIs).

RESULTS

The case group in this study consist of 10 specimens of cervical carcinoma infected by HPV type-52, the control group consist of 20 specimens of cervical carcinoma infected by HPV type 16 and 20 specimens of cervical carcinoma infected by HPV type 18. Age, parity and sexual activity were controlled by analysis. The results of this study were displayed in Table 1-3 and Figure 1-4.

Table 1
IL-6, $CD4^+$ and $CD8^+$ T-lymphocyte expression in Cervical Carcinoma Infected by HPV Tipe-52 and 16

		HPV Type		RO	*p*	CI 95%
		52	16			
IL-6	high	6	4	6.00	0.03	1.13-31.99
	low	4	16			
$CD4^+$	high	1	12	6.00	0.04	1.00-35.91
	low	8	8			
$CD8^+$	high	1	12	13.50	0.01	1.42-128.26
	low	9	8			

Significant at $p < 0.05$

The risk of high IL-6 expression in cervical carcinoma infected by HPV type 52 was statistically significant 6-fold higher compare with cervical carcinoma infected by HPV type 16 (RO = 6.00; CI 95% = 1.13-31.99; p = 0.03; $p < 0.05$). The risk of low $CD4^+$ T-lymphocyte expression in cervical carcinoma infected by HPV type 52 was statistically significant 6-fold higher compare with cervical carcinoma infected by HPV type 16 (RO = 6.00 ; CI 95% = 1.003-35.91; p = 0.04; p < 0.05). The risk of low $CD8^+$ T lymphocyte expression in cervical carcinoma infected by HPV type 52 was statistically significant 13.5-fold higher compare with cervical carcinoma infected by HPV type 16 (RO = 13.50 ; CI 95% = 1.42-128.26; p = 0.01; $p < 0.05$).

As can be seen from Table 2, the risk of high IL-6 expression in cervical carcinoma infected by HPV type-52 was statistically significant 6-fold higher compare with cervical carcinoma infected by HPV type

18 (RO = 6.00 ; CI 95% = 1.13-31.99; p = 0.03; p < 0.05). The risk of low $CD4^+$ T lymphocyte expression in cervical carcinoma infected by HPV type 52 was statistically significant 7.43-fold higher compare with cervical carcinoma infected by HPV type 18 (OR = 7.43 ; CI 95% = 1.23-45.01; p = 0.02; p < 0.05).

Table 2
IL-6, $CD4^+$ and $CD8^+$ T-lymphocyte expression in Cervical Carcinoma Infected by HPV Tipe-52 and 18

	HPV Type		RO	*p*	CI 95%
	52	18			
high IL-6	6	4	6.00	0.03	1.13-31.99
low	4	16			
high $CD4^+$	2	13	7.43	0.02	1.23-45.01
low	8	7			
high $CD8^+$	1	11	11.00	0.02	1.16-103.94
low	9	9			

Significant at $p < 0.05$

The risk of low $CD8^+$ T-lymphocyte expression in cervical carcinoma infected by HPV type-52 was statistically significant 11-fold higher compare with cervical carcinoma infected by HPV type 18 (RO = 11.00 ; CI 95% = 1.16-103.94; $p = 0.02$; $p < 0.05$).

Table 3
Mean-age, Parity and Sexual Activity between Case and Control Group

Variable	Case	Control	*p*
	HPV-52	HPV-16	
Age (years)	53.40±13.10	48.20±10.52	0.24
Parity	3.40±1.35	2.80±1.11	0.23
Sexual Activiy	2.40±3.18	23.40±2.76	0.54
Variable	Case	Control	*p*
	HPV-52	HPV-18	
Age (years)	53.40±13.10	50.80±10.24	0.69
Parity	3.40±1.35	3.70±1.49	0.64
Sexual Activiy	2.40±3.18	22.05±2.70	0.11

Significant at $p < 0.05$

No significance different between case and controls group in mean-age, parity and sexual activity ($p > 0.05$).

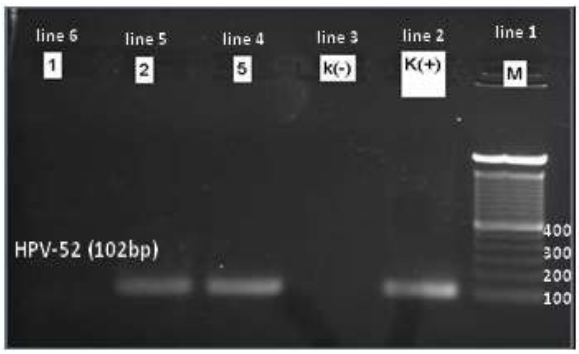

Figure 1
The product of PCR amplification on HPV DNA type-52
Remarks:
bp: base pair, Line 1: Standard DNA, Line 2: Positive control (102 bp), Line 3: Negative control, Line 4 and 5: Positive result (102 bp), Line 6: Negative result.

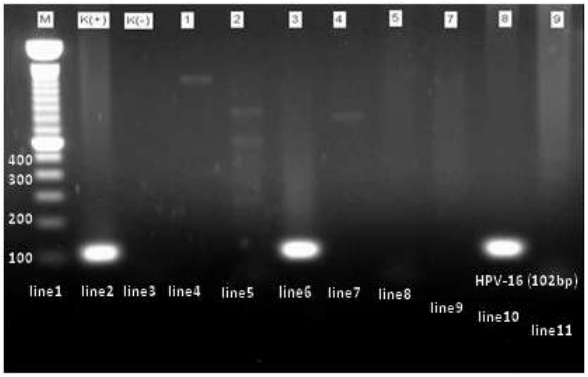

Figure 2
The product of PCR amplification on HPV DNA type-16
Remarks:
bp: base pair, Line 1: Standard DNA, Line 2: Positive control (102 bp), Line 3: Negative control, Line 6 and 10: Positive result (102 bp), Line 4, 5, 7, 8, 9 and 11: Negative results.

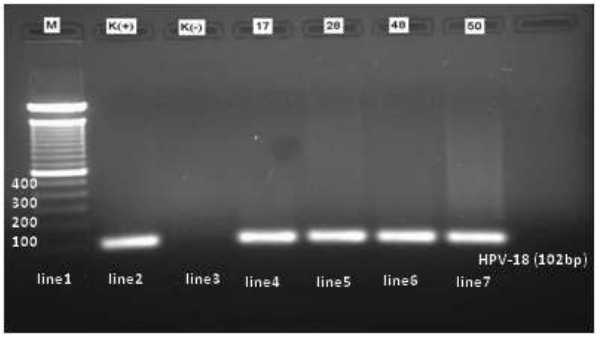

Figure 3
The product of PCR amplification on HPV DNA type-18
Remarks:
Bp: base pair Line 1: Standard DNA, Line 2: Positive control (102 bp), Line 3: Negative control, Line 4, 5, 6 and 7: Positive result (102 bp).

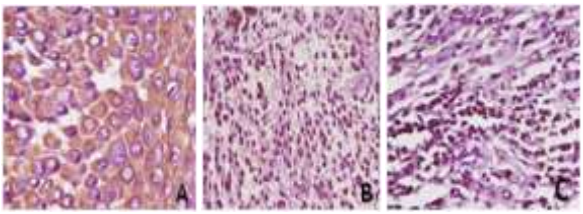

Figure 4
Immunohistochemistry Staining

Remarks:
A. Strong positive expression of IL-6 (450x)
B. Strong positive expression of $CD4^+$ T Lymphocyte (450x)
C. Strong positive expression of $CD8^+$ T Lymphocyte (450x)

DISCUSSION

A total of 185 cervical carcinoma specimens were examined in this study for genotyping of HPV by PCR. The positive HPV prevalence was 108 (58.38%). A total of 12 different high risk HPV were detected in 106 (98.15%) specimens i.e type 16, 18, 33, 35, 39, 45, 51, 52, 53, 58, 59, 66, but 2 specimens (1.85%) of unknown type. The three most common type were 43 (39.8%) HPV type 16, 22 (20.4%) HPV type 18 and 11 (10.2%) HPV type 52. Multiple infections were 12 (11.1%). Study at Dr. Cipto Mangunkusumo Hospital, Jakarta in 74 cervical cancer specimen also found these three most common HPVs prevalence were 44% HPV type-16, 39% HPV type-18 dan 14% HPV type-52.[4] Population-base study for HPV genotyping in Bali found the three most common HPV type were 18% HPV type 52, 15% HPV type-16 dan 12% HPV type-18.[10]

Immunologic factor, genetic, environment and behavior have been implicated in development of HPV infection.[13] Persistent HPV infection is require for disease progression because it lead to genomic instability and resulting in cervical cancer formation.[12,14,15] Dysfunctional immune response is likely increase this persistence.[16] Several studies found that immune responses vary by HPV type.[17-19]

Cytokines are pleiotropic glycoproteins for cell survival, proliferation and cell activation. IL-6 is one of cytokines that has role in both innate and adaptive immunity.[16,20] In this study the risk of high IL-6 expression in cervical carcinoma infected by HPV type-52 was statistically significant 6-fold higher compare with cervical carcinoma infected by HPV type 16 (RO = 6.00 ; CI 95% = 1.13-31.99; p = 0.03; $p < 0.05$) and HPV type 18 (RO = 6.00 ; CI 95% = 1.13-31.99; $p = 0.03$; $p < 0.05$). IL-6 increases the regulation of *vascular endothelial growth factor-A* (VEGF-A) protein, which plays role in tumor angiogenesis so increase tumor development. IL-6 is a tumor angiogenesis modulator.[21,22] IL-6 increases cells tumor invasion by affecting endothelial cell permeability, cause by decrease in endothel gap junction endothel, rearrangement of actin fibers, resulting in changes in cell shape so malignant cells easier to invasion.[23] IL-6 also has role as antiapoptotic through increases the expression of Mcl-1, the family of Bcl-2 via PI3-K/Akt pathway (Wei dkk., 2001a),[24] increases tumor cell motility and decreases intercellular adhesion.[25,26]

Cell-mediated immunity (CMI) especially $CD4^+$ and $CD8^+$ T lymphocytes play important role in adaptive immunity.[27,28] Tumor infiltrating lymphocyte (TIL) in cervical carcinoma, the ability of persistence and progression were vary depend on HPV type.[18,29] HPV immunity is genotype specific.[30] In this study the risk of low $CD4^+$ T lymphocyte expression in cervical carcinoma infected by HPV type-52 was statistically significant 6-fold higher and 7.43 fold higher respectively compare to cervical carcinoma infected by HPV type16 (RO = 6.00 ; CI 95% = 1.003-35.91; $p = 0.04$; $p < 0.05$) and HPV type 18 (RO = 7.43 ; CI 95% = 1.23-45.01; p = 0.02; $p < 0.05$). The risk of low $CD8^+$ T-lymphocyte expression in cervical carcinoma infected by HPV type-52 was statistically significant 13.5 fold higher and 11 fold higher, respectively compare to cervical carcinoma infected by HPV type 16 (RO = 13.50; CI 95% = 1.42-128.26; p = 0.01; $p < 0.05$) and HPV type 18 (RO = 11.00 ; CI 95% = 1.16-103.94; $p = 0.02$; $p < 0.05$). The role of this immunologic factor may occur in various mechanisms.

HPV may evade cellular immune responses by decreases expression of MHC I and II.[27] MHC class II polymorphisms have positive correlation with cervical cancer. This polymorphisms cause genetic susceptibility and increase HPV infection persistence and evade the tumor cells from host immune recognition.[13,31] Normal formation and presentation of HLA-I at cell surface for normal response of T cell antiviral need various components of antigen processing machinary (APM).[32] Genetic variation in APM components are also significantly correlated with risk of cervical carcinoma.[33] To be full activated, T cells require costimulation with nonspecific molecule. Without this molecule T-cell may be come anergic and tolerant to HPV.[16] Effective immune response also limited cause by late expression of protein viral that occur in terminal differentiation of squamous epithelial cells.[27] The role of viral protein E6 and E7 have been shown to be the main role in development of cervical carcinoma infected by HPV type 16 and 18 by inactivation of tumor suppressor gene p53 and pRb.[8,11,12] Cervical carcinoma infected by HPV type-52 has different E2 disruption profile from HPV type-16. In HPV type 52 the disruption most common occur at

N-terminal region, whereas in HPV type 16 at H region. This E2 gene disruption pattern of HPV type 52 could be type specific that occurred early in the course of infection and may be as initiators of oncogenic progression.[34]

High risk HPV infection is the major risk factor for developing cervical carcinoma but other minor risk factors or co-factors also contributes for disease progression, such as age, parity, sexual activity, race, sexually transmitted infection, multiple sexual partner, alcohol, smoking, contraception, low socio economic, etc.[10,16,35,36] In this study no significance different between cases and controls group in mean-age, parity and sexual activity ($p > 0.05$). It means that this control variable not influence the result of IL-6, $CD4^+$ and $CD8^+$ - lymphocytes expression.

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