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CORRELATION BETWEEN PDL-1 AND P16 EXPRESSION IN CERVICAL SQUAMOUS CELL CARCINOGENESIS

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ABSTRACT : Human papillomavirus (HPV) infection caused a morphological change known as Low-grade squamous intraepithelial lesion (LSIL). Certain type of HPV infection could become persistent and progress to High-grade squamous intraepithelial lesion (HSIL), which are high risk of becoming invasive cancer, Squamous cell carcinoma (SCC). Immunohistochemical staining with p16 known as the marker on cervical lesion caused by HPV infection, p16 strongly expressed on HSIL and SCC, but very weak to none on LSIL or HPV negative. Programmed Death Ligand 1 (PDL-1) pathway has an important role in decreasing T cell response and promoting T cell tolerance on chronic viral infection. Analyzing correlation between PDL-1 and p16 expressions with LSIL, HSIL and SCC of the cervix. Analytical observational conducted on paraffin block samples of the LSIL, HSIL and SCC of the cervix, at the Anatomical Pathology laboratory, Dr. Soetomo Academic General Hospital, Surabaya. Immunohistochemical staining is performed to detect expression of PDL-1 and p16. The result was statistically analyzed using Chi-Square correlation test. There are marked positive correlation between P16 and PDL-1 with LSIL, HSIL, SCC ($p<0.05$) with positive correlative coefficient of 0.647. There is a marked positive correlation between P16 and PDL-1 with LSIL, HSIL, SCC.

Key words : LSIL, HSIL, Squamous Cell Carcinoma, cervix, PDL-1, p16.

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INTRODUCTION

Cervical cancer is the fourth most common malignancy in women worldwide and is one of the three most common cancers in women aged over 45 years in 140 countries. The incidence of cervical cancer worldwide shows an increase from year to year. Based on estimates from the International Agency for Research on Cancer (IARC) there were approximately 311,000 deaths and 570,000 cases in 2018 (Lyon, 2020). Incidence rates very widely, from less than 4 per 100,000 women per year in Europe to more than 40 per 100,000 women in some East African countries. Cervical cancer is also the leading cause of cancer-related deaths in Sub-Saharan Africa (Lyon, 2020).

GLOBOCAN Indonesian 2020 about cervical cancer showed a cancer with the second highest prevalence in Indonesia in 2020 which was 9.2% new cases after breast cancer and was 17.2% in female. The mortality of cervical cancer was 14.4%. The total number of LSIL cases examined in the Anatomical Pathology laboratory of RSUD Dr. Soetomo in the period January 2015 - December 2019 was around 300 cases, HSIL around 200 cases and SCC around 1100 cases.

Almost all cases of cervical cancer are caused by persistent HPV infection. There are more than 180 types of HPV have been identified. HPV is grouped into high-risk HPV (HPV-HR) and low risk HPV (HPV-LR). Cervical cancer carcinogenesis is divided into the acquisition of HPV, persistent HPV infection, progression to cervical cancer precursors and progressive to invasive cervical cancer (Frumovitz, 2007).

P16 protein levels in cells are usually low, but have been shown to be elevated in HPV-infected cervical cells due to inactivation of the Rb complex by the HPV E7 oncoprotein (Keating *et al,* 2001). The E7 protein interferes with the retinoblastoma protein binding to the transcription factor E2F and therefore increases cell cycle progression. HPV-infected cells produce oncoprotein E7

overexpressing P16 to counteract irregular cell cycle activation, however, because E2F is no longer released via CDK4/6 action, P16 expression has no effect on cell cycle activation and accumulates in cells over time. Consequently, P16 can be used as a surrogate marker against active HPV-HR infection. Overexpression of P16 has been seen in CIN and cervical cancer, and increases with increasing degree of cancer differentiation (Keating *et al*, 2001 and Ca *et al*, 2012).

The failure of local cellular immunity in the cervix contributes to the persistence of HPV-RT infection. The PD-1 and PDL-1 pathways have an important role in attenuating T-cell responses and increasing T-cell tolerance in chronic viral infections. Programmed Death Ligand 1 (PDL-1) is a protein that has the ability to strongly modulate the adaptive immune system. PDL-1 can bind to its ligands, PD-1 and CD80, and reduce the proliferative ability and activity of the CD8 cytotoxic T cell response to viruses and cancer associate antigens (Mezache *et al*, 2015). Increased expression of PDL-1 may cause the virus to evade immune surveillance (Yang *et al*, 2013 and Kansy *et al*, 2017).

MATERIALS AND METHODS

This research used analytical observation method, Samples are consisted of 56 paraffin blocks from surgical tissue and biopsies of patients with LSIL, HSIL and cervical SCC diagnoses from 2015-2019. Ethical approval for this investigation was issued by Dr. Soetomo General Hospital with number of 0453/LOE/301.4.2/IV/2021.

Immunohistochemical examination

Immunohistochemistry was conducted using monoclonal antibody PDL-1 (1:200 dilution, ABCAM) and P16 monoclonal antibody (1:100 dilution, Santa Cruz Biotechnology). PDL-1 was positively expressed on the cell membrane and cytoplasm. P16 is positively expressed in the nucleus and/or cytoplasm of cells. Immunohistochemistry was evaluated by 2 pathologist. The slides were observed used binocular and CX31 Olympus microscopes. Expression of immunohistochemistry was semiqualitative measured and statistically analyzed.

Statistical analysis

Data were analyzed using statistical tests. The differences in the expression of PDL-1 and p16 in LSIL, HSIL and SCC was analyzed by the Mann-Whitney statistical test. The correlation between variables in this study were carried out by the Spearman correlation statistical test and the Chi-Square correlation test. Statistical test was confirmed when p value < 0.05.

RESULTS

Samples were 56 paraffin blocks, consisting of 21 LSIL, 15 HSIL and 20 SCC. The median age of the sample in this study was 45.5 years. The youngest age at the time of diagnosis was 28 years, while the oldest age was 65 years. The distribution of samples by age group was divided into 8 groups with a span of 5 years. The highest number of samples was found in the 46-50 year age group with 13 samples (23.2%), followed by the 40-45 year age group with 12 samples (21.7%).

Expression of P16 in research is found in LSIL expressed positive 28.6% and negative 71.4% in HSIL positive 60.0% and negative 40.0%, while the SCC positive 90.0% and negative 10.0%. Kolmogorov-Smirnov test showed that the expression of P16 in LSIL, HSIL and SCC was normally distributed.

Table 1 : Distribution of samples

	Total
Age (years)	
26-30	4 /56
31-35	6 /56
36-40	6 /56
41-45	12 /56
46-50	13 /56
51-55	8 /56
56-60	4 /56
61-65	3 /56
Histology	
LSIL	21/56
HSIL	15/56
SCC	20/ 56
P16 expression	
LSIL	6/21
HSIL	9/15
SCC	18/20
PDL-1 expression	
LSIL	5/21
HSIL	10/15
SCC	13/20

Differences in P16 expression in LSIL, HSIL and SCC were tested using the Mann-Whitney Test, showing no significant difference between P16 expression in LSIL and HSIL with a p value of 0.063, but showing a significant difference in LSIL and SCC with a p value of 0.001, as well as on HSIL and SCC with a p value of 0.039 ($p<0.05$).

Spearman correlation test showed a significant correlation between cervical epithelial lesions with P16 expression ($p<0.05$) with a positive correlation coefficient of 0.534. These results indicate that P16 expression is in

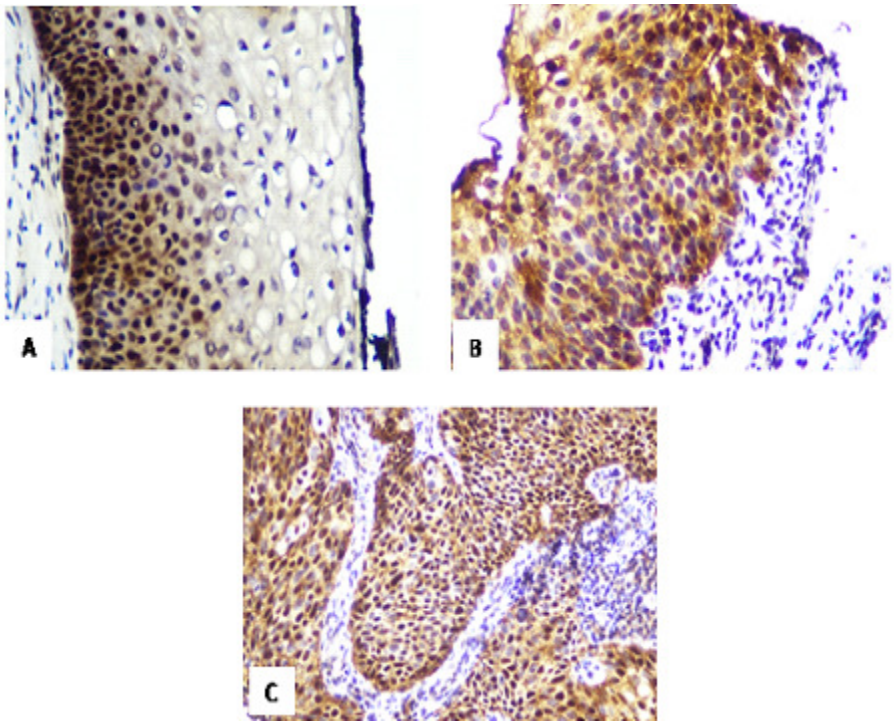

Fig. 1 : P16 expression in LSIL. HSIL, SCC, expressed in the nucleus and cytoplasm of cells, magnification 200x. A. P16 positive on LSIL, B. P16 positive on HSIL, C. P16 on SCC.

Table 2 : Spearman correlation test results of cervical epithelial lesions with P16 expression.

		P16 expression
Cervical epithelial lesions	r	0.534
	p	0.01
	n	56

Table 3 : Spearman correlation test of cervical epithelial lesions with PDL-1 expression.

		PDL-1 expression
Cervical epithelial lesions	R	0.357
	P	0.01
	N	56

Table 4 : Chi Square test between the expression of P16 and the expression of PDL-1.

		PDL-1 expression
P16 expression	r	0, 647
	p	0,001
	n	56

line with the increase in cervical epithelial lesions ranging from precursors to malignancies.

In this study, the expression of PDL-1 was positive 23.8% in LSIL, 66.7% in HSIL and 65.0% in SCC. Differences in the expression of PDL-1 in LSIL, HSIL and SCC were tested using the Mann-Whitney Test, showed no significant differences the expression of PDL-1 between neither on LSIL and HSIL (p-value 0.063) nor on HSIL and SCC (p-value 0.543). However, there was significant difference between LSIL and SCC with p value of 0.009 ($p < 0.05$).

Spearman correlation test showed the significant correlation between lesions of epithelial cervical with the expression of PDL-1 ($P < 0.05$) with the value of the coefficient of correlation were positive by 0.357. The results are showing that the expression of PDL-1 in line with the increase in lesions of epithelial cervical start precursors to malignancy.

The correlation between P16 expression and PDL-1 expression in LSIL, HSIL and SCC was tested using the Chi-Square test. The results of the Chi-Square correlation test showed a significant relationship between the expression of P16 and the expression of PDL-1 ($p<0.05$) with a positive correlation coefficient of 0.647. These results indicate that the expression of P16 is in line with the expression of PDL-1.

DISCUSSION

This study used PDL-1 antibody as a marker of cervical squamous epithelial cell carcinogenesis in LSIL, HSIL and cervical SCC in corellation with P16 expression, as a marker of HPV infection. In this study, the youngest age for the LSIL category was 28 years and the oldest 55 years, with the highest number in the age range of 26-40 years. For HSIL the youngest age is 29 years and the oldest is 65 years, with the most in the 35-50 year age range. In SCC, the youngest age is 39 years and the

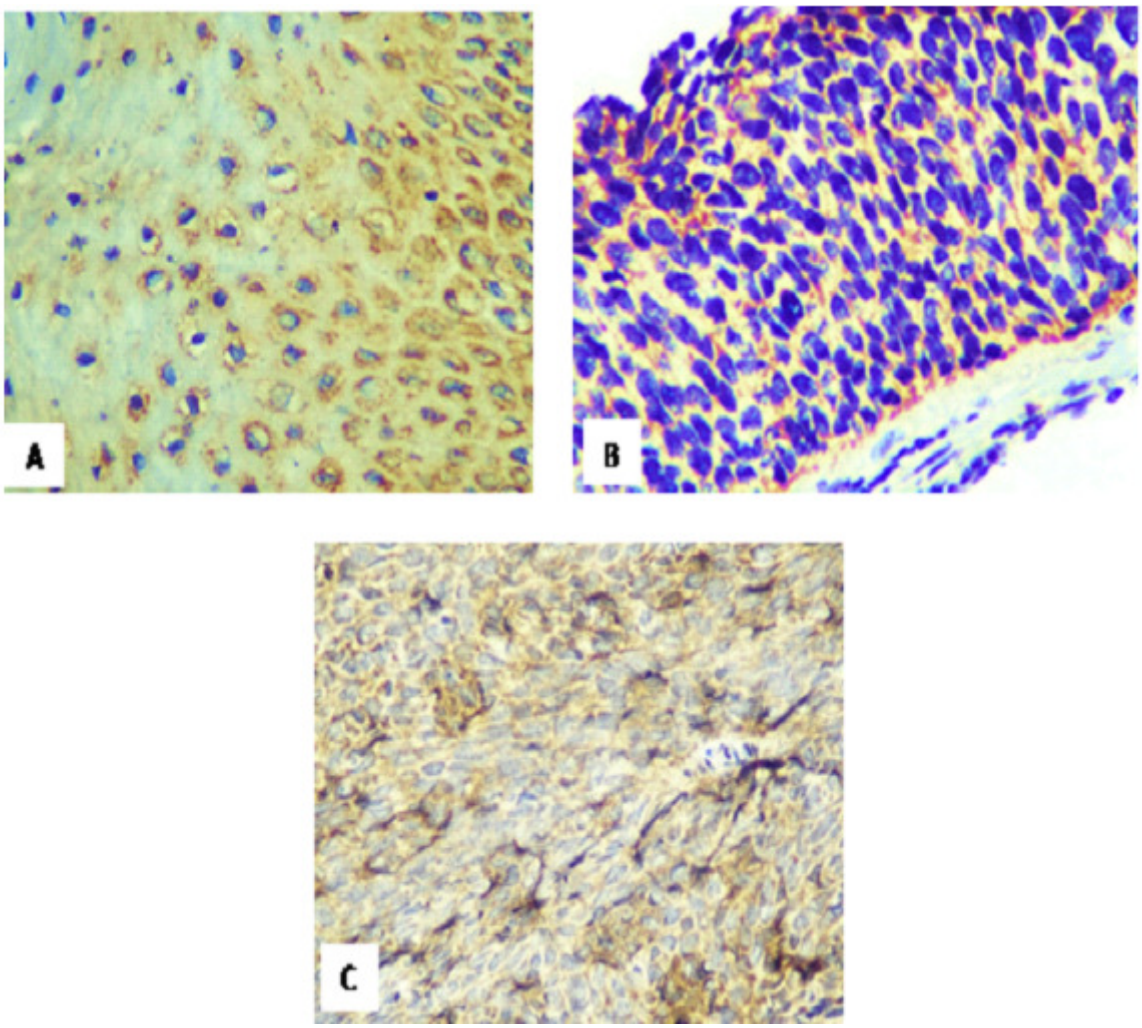

Fig. 2 : PDL-1 expression in LSIL. HSIL, SCC expressed on cell membranes, 200x magnification. A. PDL-1 positive on LSIL, B. PDL-1 positive on HSIL C. PDL-1 positive on SCC.

oldest age is 60 years, with the highest age being in the age range of 40-50 years. This is in accordance with Sznol *et al* (2013), who stated that LSIL was more often found at a younger age while HSIL and SCC were more common at an older age.

P16 expression in this study was found in positive 28.6% and negative 71.4% for LSIL, 60.0% positive and 40.0% negative HSIL, while 90.0% positive and 10.0% negative SCC. It was seen that P16 expression was found more and more as the severity of cervical epithelial lesions increased. However, based on the results of statistical tests using the Mann-Whitney Test, there was no significant difference between the expression of P16 in LSIL and HSIL but showed a significant difference in LSIL and SCC as well as in HSIL and SCC and based on statistical tests using the Spearman test, it showed a significant relationship. significant in the expression of P16 among various histopathological features of cervical epithelial lesions ($p<0.05$). This is in accordance with the existing literature where P16 expression is used as a marker of HPV infection which can be found in almost all stages of cervical cancer development from LSIL to SCC (Keating *et al*, 2001). This is also consistent with previous studies which stated that P16 protein has also been shown to be 100% expressed in HR-HPV-related HSIL and cervical SCC, which gives high P16 levels, while in non-dysplastic or low-grade SIL (LSIL) cervical epithelium, associated with low-risk HPV or HPV-negative, do not express P16 (Lim *et al*, 2016). Increased expression of P16 is not only used as a marker of HPV infection, but can also be used to determine the presence of active viral oncogene expression and the presence of cell cycle deregulation by the virus (Mezache *et al*, 2015). Increased expression of P16 also showed an association with cytological and histological severity of cervical lesions, leading to the involvement of P16 in the progression of epithelial lesions (Tsoumpou *et al*, 2009).

In this study, there were differences in the expression of PDL-1 in LSIL, HSIL and SCC. Where the LSIL is expressed positively 23.8% and negative 76.2%, the HSIL

is expressed positive 66.7% and negative 33.3%, while the SCC is expressed positive 65.0% and negative 35.0%. At first glance, it can be seen that there are significant differences in PDL-1 expression in LSIL, HSIL and SCC, however, based on statistical tests using the Mann-Whitney Test, show different results. The statistical test showed no significant difference between the expression of PDL-1 in LSIL and HSIL as well as in HSIL and SCC but showed a significant difference between LSIL and SCC ($p<0.05$). The results of this study are in accordance with the literature, that increased expression of PDL-1 can cause the virus to avoid the control of the immune system, resulting in the progression of squamous epithelial cell carcinogenesis to SCC (Carvalho *et al*, 2016).

This difference in PDL-1 expression is also in agreement with previous studies, where PDL-1 expression was found in 95% of dysplastic epithelial cells in CIN 1–2 lesions. Which was significantly increased when compared with squamous epithelial cells with negative CIN. Strong PDL-1 expression was also found in SCC in both tumor cells and mononuclear cells, especially in tumor areas that invaded the stroma (Mezache *et al,* 2015).

In a study by Mezache *et al* (2015), it was also found that there was a difference in PDL-1 expression in CIN 1–2 (LSIL) lesions compared to cervical SCC. These differences were found in the expression of PDL-1 both in epithelial cells and in mononuclear cells (Mezache *et al*, 2015).

An increase in PDL-1 was also found by Yang *et al* (2013), in cervical lesions associated with HPV infection. These findings suggest an association between increased PDL-1 expression in persistent HPV infection and the development of cervical epithelial lesions (Yang *et al*, 2013).

CONCLUSION

There was no significant difference between P16 expression in LSIL and HSIL. There was a significant difference between P16 expression in LSIL and SCC as well as in HSIL and SCC. There was no significant difference between PD-L1 expression in LSIL and HSIL as well as in HSIL and SCC. There was a significant difference between the expression of PD-L1 in LSIL and SCC. There was a significant relationship between PD-L1 expression and P16 expression in the LSIL, HSIL and cervical SCC groups.

Conflict of interest

The authors declare that no conflict of interest is found within this study.

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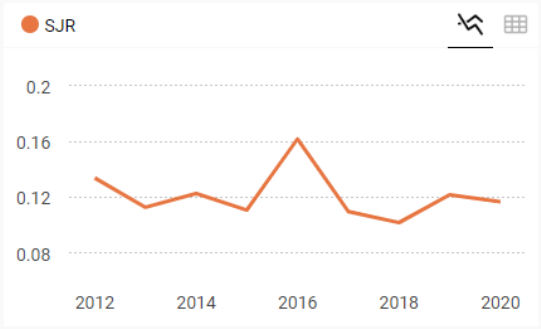

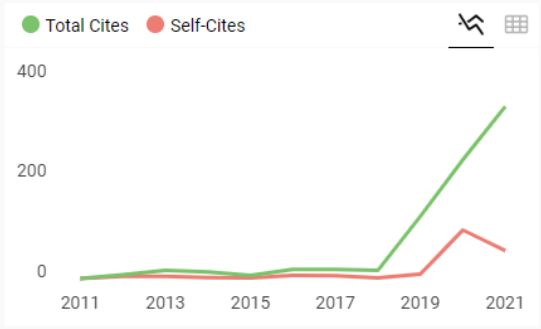

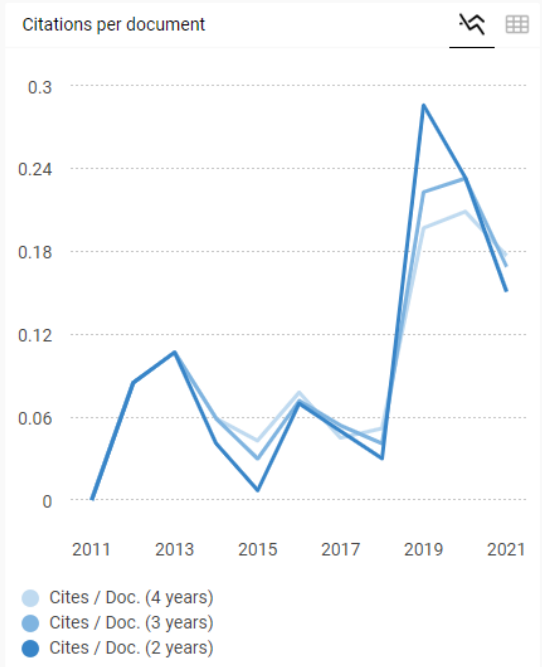

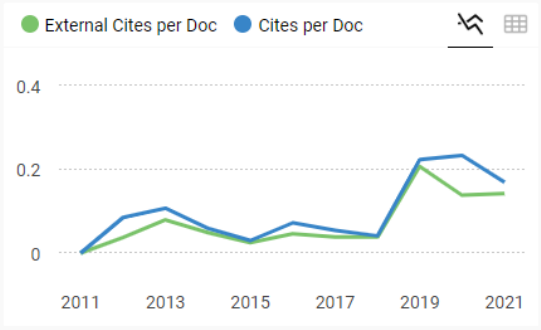

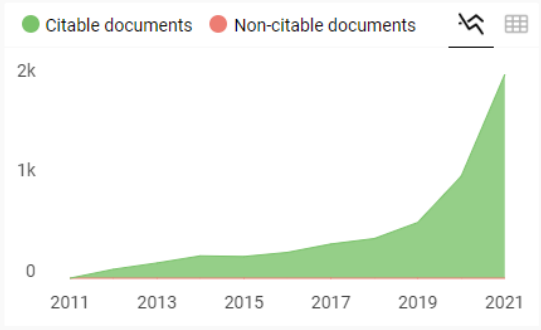

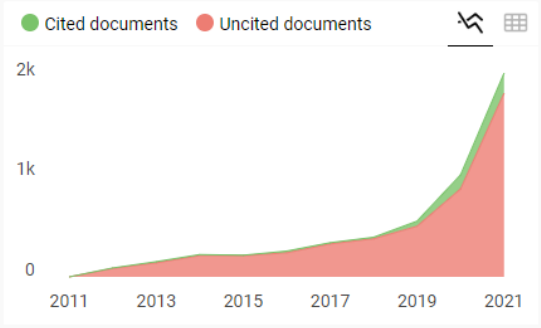

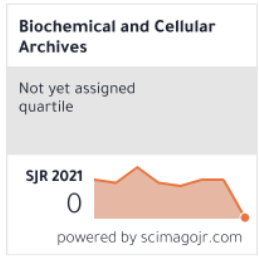

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