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PD-L1 Expression and Immune Response in Squamous Cervical Lesion and Squamous Cell Carcinoma Cervix

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Abstract

Aim : To explore the possibility of using anti-PD-L1 immunotherapy in low-grade intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL), and squamous cell carcinoma (SCC) of the cervix. Material and methods: PD-L1 expression was analyzed by an immunohistochemical technique using an anti-PD-L1 antibody. Tumour infiltrating lymphocytes (TILs) were analyzed by comparing the area occupied by lymphocytes with the stromal area between tumors. Result: there was a statistical difference between PD-L1 expression and TILs ($p < 0.001$). PD-L1 expression appeared higher in cervical intraepithelial lesion than squamous cell carcinoma, but there was no statistical difference ($p = 0.781$). Conclusion: It is possible to use anti-PD-L1 in cervical intraepithelial squamous lesions and cervical squamous cell carcinoma.

Keywords: PD-L1; TILs; cervical intraepithelial lesion; cervical squamous cell carcinoma.

1. Introduction

Worldwide, cervical cancer is the fourth most common cancer, 7.9% of all cancers experienced by women. The death rate from cervical cancer is 7.5% of all deaths caused by malignancy.[1] PD-L1 is encoded by the CD274 gene located on human chromosome 9 in the p24 band. The interaction of PD-L1 on effector T cells with PD-1 will inhibit TCR signal transduction, causing inhibition of T cell cytotoxic activity.

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Reference [2] Anti PD-L1 is used for immunotherapy in head and neck squamous cell carcinoma, colorectal malignancy, and lung malignancies [3–5]. Another study in cervical malignancy showed that the E7 protein produced by HPV 16 and 18 decreased t cell CD8+ response [6–8]. This is due to the increased intensity of PD-L1 in premalignant lesions and cervical cancer [9].

2. Material and Method

This study used 45 samples collected from the Anatomical Pathology Laboratory of Dr. Wahidin Sudirohusodo Makassar Hospital, Hasanuddin University Hospital Makassar, and Sentra Diagnostik Patologia Makassar for the 2017-2019 period. All samples were taken from patients who underwent hysterectomy with a diagnosis of squamous intraepithelial lesion or cervical squamous cell carcinoma assessed by two gynecological pathologists. The ethics committee has approved all samples of Hasanuddin University, Makassar.

The immunohistochemical procedures were performed based on the manufacturer's protocols. Histological sections of 3 mm were taken from each sample. Positive controls for primary antibodies use lymph nodes tissue. The tissue section was incubated with phosphate buffer saline instead of the primary antibody as negative controls. The primary antibodies were proceeded with working dilution 1:40 (Monoclonal Rabbit Anti-PD-L1, Clone 28-8, Dako). The positive expression will appear brown stained on the membrane and cytoplasm of tumor cells.

Calculation of PD-L1 expression was carried out by assessing tumor cells stained brown on the membrane or cytoplasm. Score 0 (Negative) if there were no stained cells; score 1(Low) if weakly stained <33% tumor cells or strongly stained <10% tumor cells; score 2(Moderate) if weakly stained >33% of tumor cells or strongly stained in 10-33% of tumor cells; score 3 (High) if strongly stained more than 33% of tumor cells. (Figure 1)

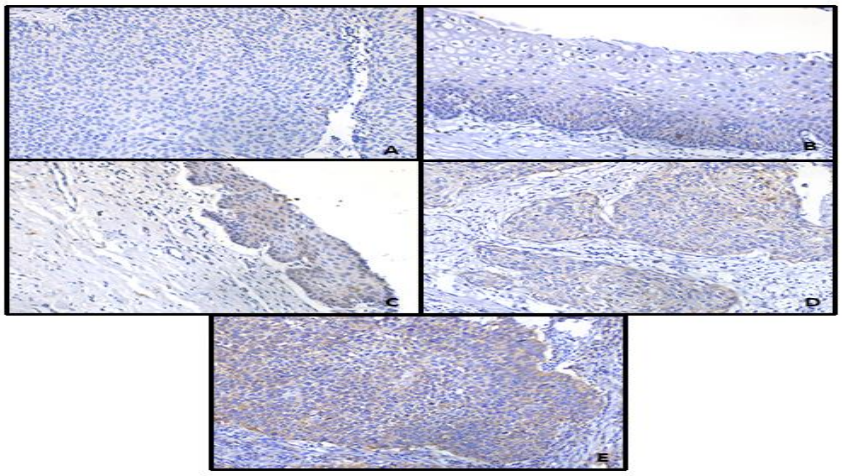

Figure 1: A. PD-L1 negative; B. PD-L1 Score 1. Weakly stained <33% intraepithelial squamous lesions; C. PD-L1 Score 2. Weakly stained >33% intraepithelial squamous lesions; D. PD-L1 Score 2. Weakly stained >33% squamous cell carcinoma; E. PD-L1 Score 3. Strongly stained >33% squamous cell carcinoma. (DAB stain with PD-L1 antibody).

Calculation of the degree of TILs using preparations stained with hematoxylin-eosin stain. Furthermore, the degree of TILs was calculated by assessing the extent of the stroma occupied by mononuclear inflammatory cells following the recommendations of the International TILs working group, 2014. TILs were categorized into three groups, score 1 = low (<10% TILs), score 2 = moderate (10-39% TILs), score 3 = high (>39% TILs). In intra-epithelial lesions, the area of the stroma assessed was 2mm from the basement membrane (Figure. 2).

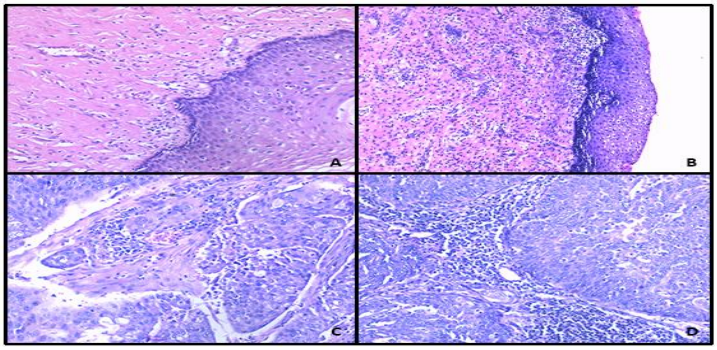

Figure 2: A. TILs <10% in squamous intraepithelial lesions; B. TILs 10-39% in squamous intraepithelial lesions; C. TILs 10-39% in squamous cell carcinoma. D. TILs >40% in squamous cell carcinoma. (Hematoxylin-Eosin stain).

The univariate analysis described the primary data in sample frequency and age. Chi-square was used to see the relationship between the expression of PD-L1 with histopathological features and PD-L1 with the area of TILs.

3. Result and Discussion

The sample in this study had an age range of 36 to 94 years. The mean age of the samples was diagnosed with low-grade and high-grade intraepithelial lesions, respectively 69.84 years (SD 12.22) and 68.2 years (SD 8.71). the highest incidence of squamous intraepithelial lesions is found in young adults, which is 80% in the second decade and decreases to only about 5% in the sixth decade. [10]. The difference in mean age in this sample is because this study took samples from patients who had hysterectomy. Sample with cervical squamous cell carcinoma had a mean age of 52.7 years (SD 13.99). (Figure 3). In another study, the average age of squamous cell carcinoma is in the sixth decade, with a median of 51 years [11].

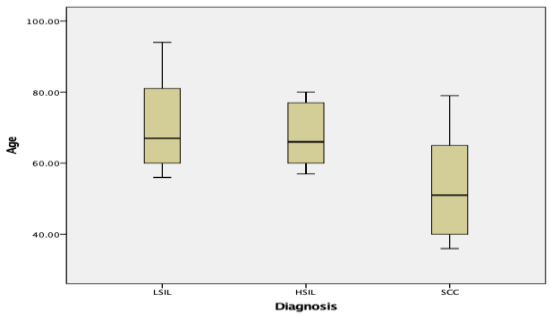

Figure 3: Relationship between age and diagnosis.

PD-L1 works by inhibiting the T cell response by inhibiting activation, proliferation, or causing apoptosis [12]. In several previous studies using cervical samples, the reduced number of lymphocyte cells can be assessed based on the stromal area occupied by lymphocytes [13,14].

Table 1: Sample characteristic.

	Sample	Percentage (%)
Diagnose		
LSIL	13	28.9
HSIL	15	33.3
SCC	17	37.8
Percentage TILs		
<10%	11	24.4
10-39%	14	31.1
>39%	20	44.4
PD-L1		
Negative	8	17.8
Low	25	55.6
Moderate	8	17.8
High	4	8.9

Table 2 shows that samples with low PD-L1 expression have a higher percentage of TILs area. Meanwhile, samples with high PD-L1 expression all have a low percentage of TILs area. In the Fisher test, the data obtained a strong relationship. Similar data results were obtained in another study using intraepithelial squamous lesion samples [14] and in studies using cervical squamous carcinoma samples [8].

Table 2 : Relationship between PD-L1 and diagnosis.

	Gambaran Histopatologi				
PD-L1	LSIL(%)	HSIL(%)	SCC(%)	Total(%)	p
Negatif	2 (25)	1 (13)	5 (63)	8 (100)	0.781
Low	7 (28)	9 (36)	9 (36)	25 (100)	
Moderate	3 (38)	3 (38)	2 (25)	8 (100)	
High	1 (25)	2 (50)	1 (25)	4 (100)	

PD-L1 after binding to PD-1 will activate SHP1 and SHP2 and inhibit PI3K/AKT pathway. SHP-1 can regulate CD8+ T cell activity and inhibit SHP-1. Inhibition of SHP1 will inhibit the interaction of T cells and dendritic cells by blocking TCR signal transduction. Blocked TCR signal transduction inhibits PI3K/Akt (Protein kinase B) and mitogen-activated protein kinase (MAPK) signaling. Inhibition of PI3K activation suppresses B-cell lymphoma-extra-large (Bcl-xl) expression and Akt activation that cause T Cell apoptotic [2]. The interaction between PD-L1 and PD-1 inhibits RAS activation and the MEK pathway that inhibits T cell proliferation [12]. Internal apoptotic activity in T cells and reduced proliferation of T cells leads to a reduction in the number of

inflammatory cells in the stroma. In addition, a decrease in AKT activity will cause a decrease in FAS, so that lymphocyte cells become inactive in causing apoptosis of tumor cells. Although there was no statistically significant relationship between PD-L1 expression and diagnosis (p 0.781), PD-L1 expression was present in all diagnoses. PD-L1 expression was higher in samples diagnosed with intra-epithelial lesions than squamous cell carcinoma (LSIL 84%; HSIL 93%; SCC 70%). (Table 3) Similar findings are found in another study where PD-L1 expression is found in 95% of cases of intraepithelial squamous lesions and 80% of cases of squamous cell carcinoma [15]. The percentage of positive PD-L1 expression was relatively higher in squamous intraepithelial lesions because of the association between E7 protein and PD-L1 [7]. Almost all intraepithelial squamous lesions are caused by infection with HPV that produces E7 protein, whereas not all squamous cell carcinomas are caused by HPV infection [16].

Table 3: Relationship between TILs and PD-L1 expression. *Fisher exact test**.**

	TILs			Total	p-value
	<10%	10-39%	>39%		
PD-L1 expression					
Negatif	0 (0)	1 (12.5)	7 (87.5)	8 (100)	0.001*
Low	3 (12)	10	12	25 (100)	
Moderate	4 (50)	3 (37.5)	1 (12.5)	8 (100)	
High	4 (100)	0 (0)	0 (0)	4 (100)	

Increased expression of PD-L1 gives a poor outcome in patients with cervical carcinoma [17]. Several studies show that PD-L1 expression has a significant relationship with the infiltration of TILs in several cancers. Anti-PD-L1 itself has been used as an adjunct therapy in cancer treatment [3–5]. The use of anti-PD-L1 in cervical carcinoma can increase the number and activity of T lymphocytes [18]. The use of anti-PD-L1 in cervical squamous carcinoma that has metastasized caused by HPV has been carried out and gives good results [19]. n this study, more than 84% of samples of intraepithelial squamous lesions and 70% of squamous cell carcinomas expressed PD-L1. In addition, this study also found a relationship between PD-L1 expression with the area of TILs, so it can be considered the use of anti-PD-L1 in patients with squamous intraepithelial lesions and squamous cell carcinoma.

4. Conclusion

PD-L1 has a significant relationship with the immune response. PD-L1 expression is relatively high in intraepithelial squamous lesions and cervical squamous cell carcinoma. Intraepithelial squamous lesions express PD-L1 more frequently than cervical squamous cell carcinomas. This allows anti-PD-L1 therapy in squamous intraepithelial lesions and cervical squamous cell carcinoma.

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We have no conflict of interest to declare.

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