An Immunohistochemical Study Of The Relationship Between Programmed Cell Death Ligand-1 (PD-L1) And Clinicopathological Characteristics Of Malignant Melanoma In General Hospital Haji Adam Malik Medan

Febri Susanto*, Ibnu Alferraly*, Delyuzar*

Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.*Corresponding Author

DOI: 10.29322/IJSRP.12.04.2022.p124XX
http://dx.doi.org/10.29322/IJSRP.12.04.2022.p124XX

Paper Received Date: 10th March 2022
Paper Acceptance Date: 26th March 2022
Paper Publication Date: 6th April 2022

Abstract: Malignant melanoma is a potentially aggressive and lethal malignancy deriving from melanocytic cells. Although it comprises only 3% of all cutaneous malignancies diagnosed each year, malignant melanoma contributes to 75% of all skin cancer deaths. In the last decades, there have been advances in immune checkpoint inhibitor (ICPI) development in treating melanoma metastases. ICPI such as programmed cell death ligand-1 (PD-L1) is primary membrane-bound protein expressed in dendritic cells and monocytes. Programmed cell death protein- 1 (PD-1) inhibitors or PD-L1 inhibitors will clinically improve treatment response and overall survival rates in many types of tumors. Nowadays, PD-L1 is being intensively used in cancer patient management revolution, especially in melanoma and non small cell lung cancer. Therefore, the researchers were interested in assessing the relationship between PD-L1 immunohistochemical expression and clinicopathology of malignant melanoma patients in General Hospital Haji Adam Malik Medan. Objective: To analyse the relationship between PD-L1 immunohistochemical expression and clinicopathology characteristics of malignant melanoma in General Hospital Haji Adam Malik Medan. Materials and Methods: A cross-sectional analytic study was performed using formalin-fixed tissue paraffin blocks from 18 skin cancer patients histopathologically diagnosed as malignant melanoma. Clinical data of patients (age, gender, tumor location) was acquired from medical record. Each slide was double blindly reevaluated by researcher and two pathologist. Tumor depth, tumor invasion and histopathological subtypes was evaluated. After that, each slide was stained with PD-L1 immunohistochemistry. Then, the relationship between PD-L1 immunohistochemical expression and clinicopathology characteristics of malignant melanoma was assessed and analyzed with statistical software by using the chi-square or Fisher's exact test. Results and Discussion: In this study found that there are statistically significant relationship between the expression of PD-L1 with Breslow and tumor location in malignant melanoma (p value 0.045 and 0.013, respectively). Gadiot et al also discovered that there is significant correlation between PD-L1 expression with Breslow. Massi et al. stated that PD-L1 is a prognostic marker in melanoma. Based on multivariat analyses, found that Breslow-thickness is an independent risk factors for melanoma-specific death. High PD-L1 expression is often correlated with prognosis of malignant melanoma, and this prognosis is related with stage.

Keywords: malignant melanoma, PD-L1, PD-1, cutaneous

I. INTRODUCTION

Malignant melanoma is a potentially aggressive and lethal malignancy deriving from melanocytic cells. Although it comprises only 3% of all cutaneous malignancies diagnosed each year, malignant melanoma contributes to 75% of all skin cancer deaths.[1] Based on World Health Organization (WHO), number cases of malignant melanoma worldwide has rapidly increased compared to other cancers.[2] In Indonesia, according to Global Burden of Cancer (GLOBOCAN) data for the past 5 years, there were 1,609 new cases and 699 cases ended with death.[3] There was 13 malignant melanoma patients found in General Hospital Haji Adam Malik Medan in 2011-2015.[4] Malignant melanoma is carcinoma derived from cells producing melanocytic pigments, locating in skin, ear, digestive system, eye, mouth, and genital mucosa.[5,6]

In the last decades, there have been advances in immune checkpoint inhibitor (ICPI) development in treating melanoma metastases. ICPI such as programmed cell death ligand-1 (PD-L1) is primary membrane-bound protein expressed in dendritic cells and monocytes. These programmed cell death protein- 1 (PD-1) receptors are expressed in T and B cells, dendritic cells, and monocytes. PD-1 inhibitors or PD-L1 inhibitors will clinically improve treatment response and overall survival rates in many types of tumors.[7]

Nowadays, PD-L1 is being intensively used in cancer patient management revolution, especially in melanoma and non small cell lung cancer.[8] In advanced melanoma, an objective

response was found about 40%, meanwhile in non small cell lung cancer, response level of Nivolumab was 20%.[9] Blockade of PD-1 produced higher response level than Ipilimumab.[10]

This study aimed to analyse the relationship between PD-L1 immunohistochemical expression and clinicopathology characteristics of malignant melanoma in General Hospital Haji Adam Malik Medan. Clinical data of patients (age, gender, tumor location) was acquired from medical record. Each slide was double blindly reevaluated by researcher and two pathologist. Tumor depth, tumor invasion and histopathological subtypes was evaluated. After that, each slide was stained with PD-L1 immunohistochemistry. Then, the relationship between PD-L1 immunohistochemical expression and clinicopathology characteristics of malignant melanoma was assessed and analyzed with statistical software by using the chi-square or Fisher's exact test..

II. MATERIAL AND METHODS

Sample selection

This analytic research with cross-sectional approach was conducted in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara and Anatomic Pathology Unit, General Hospital Haji Adam Malik Medan. This study was done from March 2021 until December 2021, after receiving approval from Health Research Ethics Committee, Medical Faculty, Universitas Sumatera Utara.

These study samples were all histopathologically diagnosed as malignant melanoma fulfilling inclusion and exclusion criteria. Samples were gathered by using total sampling technique The inclusion criteria were all adequate slides or paraffin blocks from malignant melanoma cases as well as having medical record data such as age, gender, tumor location, and tumor invasion. Exclusion criteria for this study was diminished or damaged paraffin blocks which can't be cut back and also incomplete medical record data. Each sample was stained with hematoxylin-eosin and immunohistochemistry PD-L1 antibody, clone MD21R (Medaysis, CA), ready to use using BOND-MAX Fully Automated IHC (Leica Biosystems). This immunohistochemistry was stained in membrane cytoplasms. Placenta tissue was used as positive control.[11]

PD-L1 expression was defined as percentages of partially or completely viable tumor cells stained in membrane cytoplasms (≥1+) relative to all tumour cells found in the samples (positive and negative). They were interpreted as low (negative) if found only partial or complete cell membrane staining (≥1+) in <1% or 1-49% of viable tumor cells. On the contrary, they were defined as high (positive) if found partial or complete cell membrane staining (≥1+) in ≥50% of viable tumor cells.

Data analysis

Data collected in this research were processed by using statistical software and presented in tables. Statistical tests used in this study were chi-square or Fisher exact test.

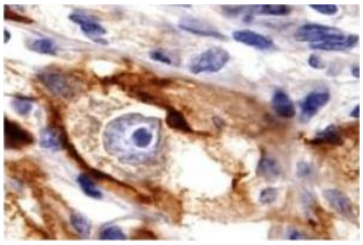

Figure 1. FFPE human RCC stained with anti-PD-L1 using DAB.[12]

III. RESULTS

In this study, 18 samples with malignant melanoma histopathological diagnosis in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara and Anatomic Pathology Unit, General Hospital Haji Adam Malik Medan were obtained. In this study, analysis about the relationships between PD-L1 immunohistochemical expression and clinicopathology characteristics of malignant melanoma was done. Statistical tests showed that there are statistically significant relationship between the expression of PD-L1 with Breslow and tumor location in malignant melanoma (p value 0.045 and 0.013, respectively) (Table 1). On the contrary, there are no statistically significant relationship between expression of PD-L1 with other clinicopathological characteristics in malignant melanoma (p value >0.05).

IV. DISCUSSION

Programmed cell death 1, also known as PD-1 and CD279 is a protein found in cell surface having a role in regulating the immune system's response to cells of human body by down-regulating immune system and promoting self-tolerance by suppressing inflammatory activity of T cells.[13] PD-1 is an immune checkpoint and bodyguard against autoimmune through two mechanisms. First, by apoptosis (programmed cell death) of antigen-specific-T cells in lymph node. Second, by decreasing apoptosis in regulatory T cells (anti-inflammatory, suppressive T cells). PD-1 has two ligands (PD-L1 and PD-L2), which are members of B7 family. The expression of PD-L1 on tumour cells hinders anti-tumor activity by binding of PD-1 to effector T cells. Monoclonal antibodies targeting PD-1 that can improve immune system are nowadays being developed for treatment of cancer.[14]

In the last decades, there have been advances in ICPI development in treating melanoma metastases. ICPI such as PD-L1 is primary membrane-bound protein expressed in dendritic cells and monocytes. These PD-1 receptors are expressed in T and B cells, dendritic cells, and monocytes. PD-1 inhibitors or PD-L1 inhibitors will clinically improve treatment response and overall survival rates in many types of tumors.[7] In this study, found that most common PD-L1 expression was high PD-L1

(>50%). This study was in line with Akiyama et al and Sumi et al.[15,16]

Table 1 The relationships between clinicopathological characteristics of malignant melanoma samples and PD-L1

Clinicopathological characteristics	PD-L1 Expression				P value	PR
	High		Low			
	n	%	n	%		
Age (years old)						-
• <30	1	100	0	0	0.836	
• 30-50	5	83.33	1	16.67		
• >50	10	90.91	1	9.09		
Gender						-
• Male	9	81.82	2	18.18	0.231	
• Female	7	100	0	0		
Tumor location						
• Extremity	11	91.6	1	8.33	0.013	0.73
• Head and neck	5	100	0	0		
• Back	0	0	1	100		
Breslow						
• Breslow I	1	50	1	50	0.045	0.533
• Breslow II	2	66.67	1	33.33		
• Breslow III	13	100	0	0		
Clark level						
• Clark level 3	8	100	0	0	0.245	-
• Clark level 4	2	100	0	0		
• Clark level 5	6	75	2	25		
Histopathological subtypes						
• Superficial spreading melanoma	14	87.5	2	12.5	0.569	
• Nodular melanoma	2	100	0	0		
• Lentigo melanoma	0	0	0	0		
• Acral melanoma	0	0	0	0		

In this study, the mean age for malignant melanoma was 59.5 years old (± 13,44) with range of 27-80 years old. This study also found that there is no significant relationship between *PD-L1* expression and age in malignant melanoma patients. From previous studies and based on this research result, it could be concluded that mean age of melanoma is about sixth decade and relative older age. This study supported researches from Yang et al.[17] But, Gadiot et al didn't find any significant correlation between PD-L1 expression and age.[18]

In this study, malignant melanoma was more found in males than females. Researchers didn't find any significant relationship between gender and PD-L1 expression because malignant melanoma can occur in all gender. In this study, males and females have no different in terms of sun exposure. This study was in line with Akiyama et al., Kluger et al, and Berghoff et al.[12,19,20]

In this study, most commonly found was in extremity (66,67%), head and neck (27,78%), and back (5,56%). This study revealed that there is significant relationship between PD-L1 expression and tumor location where extremity has 0.73 higher risk than other location to express PD-L1. Lower extremity is more common found in this study (thigh and calf) due to often exposed to the sun. Most studies also found that more melanoma in acral, which followed by non-acral.[12,15] But other researches didn't discover any significant correlation between tumor location and PD-L1.

This study and other studies showed that most common found cases have included in higher clark level. Most common was more than clark level 3, where tumour mass has infiltrated between papillary dermis and reticular dermis, just like in Kakavand et al.[16]

Melanoma clinical stage is related to T (tumor size), N (lymph node) and M (metastases). T or tumor size follows size based on Breslow and presence or absence of ulcer. This study discovered that there is significant relationship between high Breslow and PD-L1 expression. Breslow III has 0.53 higher risk to expressed PD-L1 than other Breslow. Gadiot et al also revealed the same result with this study.[18] Massi et al stated that PD-L1 is a prognostic marker in melanoma. Based on multivariate analysis, Breslow-thickness is an independent risk factor for melanoma-specific death.[21] High PD-L1 expression often correlates with prognostic of malignant melanoma, and this prognostic alone associates with stage (based on thickness or tumor size, ulcer, nodule in lymph node, and metastasis).[22] Even though this study didn't evaluate nodules or metastasis, we can concluded that high tumor thickness can be used as a reference to assess prognosis of melanoma.

For histopathological subtypes, most commonly found in this study were superficial spreading melanoma and nodular melanoma, which was in line with Gadiot et al but on contrary with Sumi et al, Kaunitz et al.[15,18,23] According to theory, superficial spreading melanoma dan nodular most often occurred in patients directly exposed to sun. Zhou et al identified 4 subtypes of melanoma and found that all types express PD-L1.[24] Namikawa et al. also discovered that positive PD-L1 expression varies in all melanoma subtypes. But, this study showed no significant relationship between PD-L1 expression and histopathological subtypes.

V. CONCLUSION

In this study, some conclusion could be emphasized. There were statistically significant relationship between the expression of PD-L1 with Breslow and tumor location in malignant melanoma (p value 0.045 and 0.013, respectively).

VI. COMPETING INTERESTS

The author has no financial interests relevant to the product or company described in this article.

VI. ACKNOWLEDGMENT

We would like to thank all staff of the Department of Anatomical Pathology, University of North Sumatra, and Anatomic Pathology Unit, General Hospital Haji Adam Malik Medan in Medan, Indonesia for all their assistance and cooperation.

VII. ETHICAL APPROVAL

Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia approved this study.

REFERENCES

[1] Sharma K, Mohanti BK, Rath GK. Malignant melanoma: A retrospective series from a regional cancer center in India. J Cancer Res Ther. 2009; 5:173-80.

[2] Lens MB, Dawes M. Global perspectives of contemporary epidemiological trends of cutaneous malignant melanoma. Br J Dermatol. 2004; 150:179-85.

[3] Indonesia Globocan source 2020. Available at: https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf. accessed on 12 Feb 2021.

[4] Razi F. Profil tumor infiltrating lymphocytes dan mitotic index pada melanoma malignan di RSUP Haji Adam Malik Medan tahun 2011-2015. Available at: http:/repository.usu.ac.id. accessed on 12 feb 2021.

[5] Wu E, Golitz LE. Primary noncutaneous melanoma. Clin Lab Med. 2000; 20:731-44.

[6] McCourt C, Dolaé O, Gormley G. Malignant melanoma: A pictorial review. Ulster Med J. 2014; 83(2):103-110.

[7] Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, *et al*. Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. The New England Journal of Medicine. 2012; 366:2443–2454.

[8] Elder DE, Barnhill RL, Bastian BC. Cook MG, De la Fouchardiere A, Gerami P, et al. Melanocytic tumours. In: Elder DE, Massi D, Scolyer RA, Willemze R, editors. WHO Classification of tumours of skin tumors. Lyon: IARC Press, 2018; p. 65-152.

[9] Kaunitz GJ, Cottrell TR, Lilo M, Melanoma subtypes demonstrate distinct PD- L1 expression profiles. Lab Investig J Tech Methods Pathol. 2017; 97(9):1063– 1071.

[10] Larkin J, Hodi FS, Wolchok JD. Combined Nivolumab and Ipilimumab or monotherapy in untreated melanoma. The New England Journal of Medicine. 2015; 373:1270–1271.

[11] Rabbit Anti-PD-L1/PDCD1-L1/CD274 [MD21R]: RM0324, RM0324RTU7. [Product Data Sheet]. Medaysis. 2017.

[12] Akiyama M, Matsuda Y, Arai T and Saeki H. PD_l1 expression in malignant melanomas of the skin and gastrointestinal tract.Oncology letters 19: 2481-2488, 2020.

[13] Yamazaki T, Akiba H, Iwai H. Expression of programmed death 1 ligands by murine T cells and APC. J Immunol. 2002; 169:5538–45.

[14] Freeman GJ, Long AJ, Iwai Y. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family members lead to negative regulation of lymphocyte activation. J Exp Med. 2000; 192:1027–34.

[15] Yun S, Park Y, Moon S, Ahn S, Lee K, Park HJ *et al*,.Clinicopathological and prognostic significance of programmed death ligand 1 expression in Korean melanoma patients. Journal of cancer 2019;10(13): 3070-3078. Doi: 10.7150/jca.30573

[16] Kakavand H, Vilain RE, Wilmott JS, Burke H, Yearley JH, Thompson JF *et al*,.Tumor PD-L1 expression, immune cell correlates and PD-1+ lymphocytes in sentinel lymph node melanoma metastases. Modern pathology (2015) 28, 1535-1544.

[17] Yang J, Dong M, Shui Y, Zhang Y, Zhang Z, Mi Y et al,.A pooled analysis of the prognostic value of PD-L1 in melanoma: evidence from 1062 patients. Cancet cell int (2020) 20:96.

[18] Gadiot J, Hooijkaas AI, Kaiser ADM. Overall survival and PD-L1 expression in metastasized malignant melanoma. Cancer / Volume 117, Issue 10/ p.2192-2201. https://doi.org/10.1002/cncr.25747

[19] Kluger HM, Zito CR, Barr ML, Barr ML, Baine MK, Chiang VLS et al,. Characterization of PD-L1 expression and associated T cell infiltrates in metastatic melanoma samples from variable anatomic sites. Accjournals DOI: 10.1158/1078-0432. 2015.

[20] Berghof AS, Ricken G, Widhalm G, Rajky O, Djeckmann K, Birner P *et al*,.Tumour-infiltrating lymphocytes and expression of programmed death ligand 1 (PD-L1) in melanoma brain metastases. Histopathology 2014 DOI: 10.1111/his.12537

[21] Massi D, Brusa D, Merelli B, Ciano M, Audrito V, Serra S *et al*,.PD-L1 marks a subset of melanomas with a shorter overall survival and distinct genetic and morphological characteristics. Annals of oncology 25: 2433-2442, 2014.

[22] De Vries E, Bray F, Coebergh JW. Melanocytic tumours. WHO classification pathology and genetics of tumours of the skin. IARC press. Lyon. 2006.

[23] Kaunitz GJ, Cottrell TR, Lilo M, Muthappan V, Esandrio J, Berry S *et al*,. Melanoma subtypes demonstrate distinct PD-L1 expression profiles. Laboratory investigation (2017) 97, 1063-1071.

[24] Zou J, Mahoney KM, Gobbie-Hueder A. Soluble PD-L1 as a biomarker in malignant melanoma treated with checkpoint blockade. Cancer Immunol Res. 2017; 5(6): 480–492.

AUTHORS

First author- dr. Febri Susanto, Resident of the Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, **email ID:** febrisusanto1982@gmail.com

Second Author- Dr. dr. Ibnu Alferraly, M.Ked (PA), Sp.PA, D. Bioeth. Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
The third author- Dr. dr. Delyuzar, M.Ked (PA), Sp.PA (K), Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Correspondence Author- Dr. dr. Ibnu Alferraly, M.Ked (PA), Sp.PA, D. Bioeth. Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, **email ID:** tia_mdn2004@yahoo.com