RESEARCH ARTICLE

INDONESIAN JOURNAL OF CANCER

The Association of Programmed Death Ligand 1 (PD-L1) and Cluster of Differentiation 95 Ligand (CD95L) Immunoexpression with Chemotherapy Response in Classical Hodgkin Lymphoma

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ARTICLE INFO

Received : 14 August 2021
Reviewed : 14 September 2021
Accepted : 07 December 2021

Keywords:
CD95L, chemotherapy response, Classical Hodgkin lymphoma, PD-L1

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ABSTRACT

Background: Programmed Death Ligand (PD-L1) and Cluster of Differentiation 95 (CD95L) are influenced by oncogenes and function in the anti-apoptosis process which is thought to play a role in chemotherapy resistance. This study aimed to analyze the association of PD-L1 and CD95L immunoexpression with chemotherapy response in Classical Hodgkin Lymphoma (CHL).

Methods: This study involved 40 cases of histopathologically diagnosed CHL treated with doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) chemotherapy. PD-L1 and CD95L immunohistochemical staining were performed in selected paraffin-embedded tissue blocks of all cases. The chemotherapy response status of the patients was taken from the medical record.

Results: High PDL-1 immunoexpression was evident in 19 (47.5%) cases while positive CD95L immunoexpression was found in 14 (35%) CHL cases. High PD-L1 immunoexpression was significantly associated with the Non-Responsive (NR) group (78.9%) with p-value = 0.0001. Positive CD95L immunoexpression was greater in the NR group (71.4%) with p-value = 0.37.

Conclusions: High PD-L1 immunoexpression indicated an unfavorable response to ABVD chemotherapy in CHL. CD95L immunoexpression was not associated with ABVD chemotherapy response in CHL.

INTRODUCTION

Classical Hodgkin lymphoma (CHL) is considered a lymphoid malignancy characterized by the presence of HRS (Hodgkin Reed Sternberg) cells [1]. CHL patients respond well to therapy, but the number of refractory patients or relapses is increasing. About 80% of patients have experienced complete remission after primary therapy with ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine), but up to 40% relapse and 10–25% are refractory or non-responsive and require additional therapy [2]. Through recent studies in CHL therapy, the role of the tumor microenvironment (TME) is starting to attract a lot of attention [3]. Currently, new therapies are being developed for relapsed and progressive CHL patients with immune checkpoint inhibitors. One of the therapies that affect TME is immune checkpoint inhibitors targeting the programmed death receptor pathway [4].

PD-L1 is a type I transmembrane protein and has a role in inhibiting T cell function [5]. Studies are showing that PD-L1 is strongly expressed in solid tumors, including colorectal cancer, lung cancer, pancreatic carcinoma, hepatocellular carcinoma, gastric cancer, ovarian cancer, endometrial cancer, and cervical cancer [6–8]. Increased binding of PD-L1 and PD-1 can also increase resistance to conventional chemotherapy in breast cancer cells, prostate cancer cells, myeloma, and diffuse large B cell lymphoma (DLBCL) [7-9]. PD-L1 is also expressed in HRS cells. By expressing PD-L1 on its surface, HRS cells can attenuate the immune response, allowing tumor cells to develop [10].

The cluster of differentiation 95 ligand (CD95L), also known as FasL, is a ligand for CD95 (Cluster of Differentiation

95) known as Fas, which plays a role in the apoptosis process [11]. Expression of CD95L on HRS can lead to activation of CD95/CD95L bonds and result in a "counter-attack" in the body's immune system, thereby increasing apoptosis in involved Th1 cells and cytotoxic T-cells [12,13].

Research on PD-L1 and CD95L related to chemotherapy response in CHL is limited. This study aimed to analyze the association of PD-L1 and CD95L immunoexpression with chemotherapy response in Classical Hodgkin Lymphoma (CHL).

METHODS

Patients selection

The study materials were paraffin blocks of 40 patients aged 13–76 years, with 2 patients < 18 years. The samples have been diagnosed histopathologically as CHL from 2014 to 2019 in the Department of Anatomical Pathology, Hasan Sadikin General Hospital Bandung, Indonesia. All patients that received only ABVD chemotherapy for at least 4 cycles, without or before administration of radiotherapy, and had complete medical records were included in this study. This study used a cross-sectional design. The research sample was taken by consecutive sampling. Statistical calculations were used to determine the sample size. Through hypothesis testing between two populations from Hosmer and Lemeshow's 2.0 sample size program, the total sample size for the 2 groups was 40 patients.

Clinical Response Criteria

Clinical response criteria in this study were based on Response Evaluation Criteria in Solid Tumors (RECIST), divided into two categories: non-responsive and responsive patients. Non-responsive patients have stable disease or progressive disease with an increased lesion size of ≥ 20% of the total longest diameter of the target lesion and increased absolute lesion size of ≥ 5 mm or ≥ 1 new lesion. Responsive patients have complete response/partial response, loss of all target lesions, or reduction of the lesion size of ≥ 30% from the total longest diameter of the target lesion [14].

The patient response was evaluated with radiological modalities using CT scan at the tumor site. Evaluation of response with CT scan should be performed at least for four cycles of chemotherapy ABVD and without or before administration of radiotherapy.

Immunohistochemical Staining

Immunohistochemical staining was performed using the labeled streptavidin-biotin immunoperoxidase complex method with the One Step Neopoly Detection Kit (Biogear Scientific). The primary antibodies included were rabbit monoclonal anti-PD-L1 (clone SP142, Abcam) with 1: 300 dilution and mouse monoclonal anti-FAS-L (NOK-1, Santa Cruz) with 1:50 dilution.

The procedure used for immunohistochemistry was as follows: the sections were cut 4μ thick on 0.01% poly -L-lysine coated glass slides, heated on a hotplate, and stored in an incubator at 38°C overnight. Sections were dewaxed in xylene and treated with three changes of ethanol and alcohol before being washed under running water. Sections were subjected to heat-induced antigen retrieval in a decloaking chamber in EDTA for 20 minutes at 96°C for PD-L1 and 30 minutes at 100°C for CD95L. Cooling at room temperature for 20 minutes followed this. Sections were then treated to block endogenous peroxidase, stained with primary antibodies, and incubated for 1 hour at room temperature for PD-L1 (incubate overnight for CD95L). Detection was done by horseradish peroxidase polymer-based detection system, diaminobenzidine chromogen, and counterstain with hematoxylin.

Immunohistochemical analysis and interpretation

The immunoreactivity of PDL-1 was identified by the presence of membranous and/or cytoplasm brown staining of HRS using Olympus CX21 light microscope. The proportion was calculated by dividing the number of PD-L1 positive HRS cells by the total number of HRS cells. Positive PD-L1 immunoexpression was rated high when the proportion was ≥ 90% and low when it was < 90% (**Figure 1**) [15].

The immunoreactivity of CD95L was identified by the presence of membranous and/or cytoplasm brown staining of HRS cells. The percentage of positive (PP) HRS cells and the staining intensity in at least two different high-power fields (400x) were evaluated by Olympus CX21 light microscope. The PP cells were assigned as score 1 (≤ 10%), score 2 (10–50%), and score 3 (≥ 50%). The intensity (IS) was rated as negative (0), weak (1), moderate (2), and strong (3), as seen in **Figure 2**. The final score for each case was calculated by multiplying PP by IS. The results were scored as positive (> 3) and negative (≤ 3) [16].

Statistical Analysis

The association of PDL-1 and CD95L immunoexpression with chemotherapy response was evaluated using the Chi-square test. The significance of the data was obtained if the p-value < 0.05**.** Statistical tests were performed using Statistical Package for Social Science (SPSS) version 24.0 for Windows.

RESULT

This study included 40 patients with an age range between 13 and 76 years and a mean age of 39 years. Patient characteristics are shown in **Table 1**. There were more subjects under 45 than 45 years old. Thirty-two patients were male, and 28 were female. Most histopathology subtypes were Mixed Cellularity Classical

P-ISSN: 1978-3744 E-ISSN: 2355-6811

Hodgkin Lymphoma (CHL) (37.5%). Most of the patients were at stage II (35%). Study subjects who responded to chemotherapy were 21 patients (52.5%), and 19 patients (47.5%) did not respond [17].

Table 1. Patient Characteristics

Variable	N=40
Age (years)	
Median	39.5
Range (min-max)	13–76
Age category (year)	
< 45	26 (65%)
≥ 45	14 (35%)
Sex	
Male	22 (55%)
Female	18 (45%)
Histological subtype	
Mixed cellularity	13 (32.5%)
Nodular sclerosis	15 (37.5%)
Lymphocyte Rich	8 (20%)
Lymphocyte depleted	4 (10%)
Stage	
I	4 (10%)
II	14 (35%)
III	12 (30%)
IV	10 (25%)
Chemotherapy response	
NR	19 (47.5%)
CR/PR	21 (52.5%)

NR: Non-response, CR/PR : Complete response/ Partial response [17].

In this study, PD-L1 immunoexpression was evaluated in all cases. The results showed that 21 cases (52.5%) expressed low PD-L1. Most of the non-responded patients (78.9%) showed high expression of PDL-1 while patients with complete/partial response showed low expression of PDL-1 as seen in **Table 2**. High PD-L1 immunoexpression was significantly associated with the NR group (78.9%) with a p-value = 0.0001. It can be concluded that the probability of patients with low PDL-1 immunoexpression to experience non-response to chemotherapy is 0.063 times compared to patients with high PDL-1 immunoexpression with a confidence interval of 0.013–0.296.

CD95L immunoexpression was evaluated in all cases. Twenty-six cases (65%) did not express CD95L. Positive CD95L immunoexpression was greater in the NR group (71.4%) as seen in **Table 3**. CD95L immunoexpression was not significantly associated with the chemotherapy response with a p-value = 0.37. The negative CD95L immunoexpression for non-response to chemotherapy is 0.550 times with a confidence interval of 0.148–2.046.

DISCUSSION

In this study, based on patient characteristics, the percentage of responsive cases (complete response and partial response) to the chemotherapy regimen was 52.5%. This result is quite different in percentage terms from the study according to Dominguez et al. [18] (95 samples) who stated that after 6 cycles of chemotherapy (ABVD), 94% of patients experienced a complete response, and 4% experienced a partial response. Canellos et al. [19] (361 samples) showed that the complete response was 82% of cases, the partial response was 15%, and 2% did not respond. Gordon

Table 2. Association of PD-L1 immunoexpression with chemotherapy response

PD-L1 immunoexpression	Chemotherapy Response Group			OR CI (95%)	p-value
	NR N=19	CR/PR N=21	Total 40		
Low	4(21.1%)	17(81%)	21(52.5%)	0.063 (0.013-0.296)	**0,0001****
High	15(78.9%)	4(19%)	19(47.5%)		

PD-L1, programmed death-ligand 1; NR, non-response; CR/PR, complete response/partial response; OR, odds ratio; CI, confidence interval.
The ** sign indicates p value ≤0.05 means significant or statistically significant

Table 3. Association of CD95L immunoexpression with chemotherapy response

CD95L immunoexpression	Chemotherapy Response Group			OR CI (95%)	p-value
	NR N=19	CR/PR N=21	Total 40		
Negative	11(57.9%)	15(71.4%)	26(65%)	0.063 (0.148-2.046)	**0.37**
Positive	8(42.1%)	6(28.6%)	14(35%)		

CD95L, cluster of differentiation 95; NR, non-response; CR/PR, complete response/partial response; OR, odds ratio; CI, confidence interval.
The ** sign indicates p value ≤0.05 means significant or statistically significant

P-ISSN: 1978-3744 E-ISSN: 2355-6811

et al. [20] (47 samples) obtained a complete response of 72.7%, partial response of 7.6%, stable 8.4%, and progression of 0.3%. The difference in these results can be related to the difference in the sample number. Another factor that can influence the differences in results is explained by Dominguez et al. [18] and Gordon et al. [20]. Some cases received additional radiotherapy treatment, which may have an effect on the successful response to therapy.

Our current study showed that the higher the PD-L1 expression, the higher the possibility of chemotherapy resistance. This is consistent with a study of non-small-cell lung carcinoma (NSCLC) patients that showed positive PD-L1 expression of 73.9% in chemoresistance NSCLC patients [21]. Similar results were obtained in the study of Bianchini et al. [22] who reported that high PD-L1 expression in breast carcinoma was associated with a lower pCR (pathologic complete response) to chemotherapy. In another study, it was reported that chemotherapy on breast cancer cells can induce PD-L1 on tumor surface cells, thereby leading to increased PD-L1-mediated T cell apoptosis [23].

In CHL, there is an amplification of the 9p24.1 locus in nearly one-third of cases. PD-L1 expression is associated with the number of copies of the PD-L1 gene locus present on the arm of chromosome 9p24.1. High amplification is associated with high expression of the PD-L1 protein on the surface of tumor cells. The 9p24.1 amplification is also associated with Janus kinase 2 (JAK2) which further regulates PD-L1 expression through activation of JAK/STAT signaling. More than 90% of CHL contain genetic changes that can activate JAK/STAT signals. The most common occurrences are JAK2 and STAT6, which in turn can increase PDL1 expression. Epstein Barr virus (EBV), present in HRS cells in 30–40% of CHL cases, can also increase PD-L1 expression. EBV induces PD-L1 expression via activation of the transcription factor pathway AP1 and increases in c-Jun and JunB [24].

PD-L1 expression is also associated with the ability of tumor cells to avoid chemotherapy-induced apoptosis [9]. Wu et al. [25] reported that PD-L1 overexpression might lead to tumor cell survival to increased activation of ERK through an association with the catalytic subunit of the DNA-dependent serine/threonine-protein kinase (DNA-PKcs). PD-L1 deficiency has been proven to decrease p38/MAPK activation which affects the downregulation of Bcl-2. Bcl-2 is an anti-apoptotic factor for tumor cells. The increase of Bcl-2 may result in the ability of the tumor cells to avoid the apoptotic process. Both of these mechanisms are thought to contribute to chemoresistance.

This study indicated that patients with low or even negative CD95L immunoexpression tended to respond well to chemotherapy although it was not statistically significant ($p = 0.37$). A similar study was conducted by Sproll et al. [26] regarding CD95L immunoexpression in squamous cell carcinoma of the head and neck; it was found that there was no significant correlation with therapeutic response although there was an increase in CD95L expression. Different results were obtained in a study conducted by Zheng et al. [27]. It was said that CD95L overexpression supported chemoresistance through activation of ERK1/2-MAPK and increased the expression of P-glycoprotein (P-gp). The results stated that CD95L overexpression can increase the activation of P-glycoprotein which is coded as the Multi-Drug Resistance 1 (MDR1) gene. Proteins that are part of ATP-binding cassette (ABC) transporters can actively cause drug efflux or chemotherapy drugs from tumor cells.

HRS cells expressing CD95L can carry out a strong counterattack against effector T cells as an antitumor [12]. According to Chapell et al. [28], in this phase, there is a perforin/granzymes pathway mediated by T cells. This pathway can remove tumor cells before T cells die by a CD95L-mediated process. T cells that can survive can eventually resume their function in the apoptosis of tumor cells. The effects of chemotherapy can eventually kill tumor cells again. Based on research by Kim et al. [29], it was suggested that CD95L expression was low in HRS cells. According to Maggio et al. [30], low Fas mutations were found in HRS cells so that they did not play too much role in the process against apoptosis. The possibility of low CD95L expression in HRS is probably the same as in this study, causing its role in inhibiting chemotherapy to be suboptimal. Because of these many factors, the CD95L used in this study became less significant in helping predict chemotherapy response.

In this study, it has been proposed that the predictive value of PD-L1 expression may be more informative and useful to decide on therapy with a single checkpoint inhibitor. There are several limitations to our study, including the small number of samples, low availability of paraclinical data, and limitations in the use of radiological modalities such as PET scan to evaluate chemotherapy response.

CONCLUSIONS

The increased PD-L1 immunoexpression indicates a poor chemotherapy response to CHL so that the occurrence of chemoresistance could be higher. From the results of this study, it can be suggested to perform PD-L1 immunohistochemical examination in patients with classical Hodgkin's Lymphoma to predict chemotherapy response and as a consideration for giving anti-PD-L1 therapy. The use of adequate targeted therapy is expected to optimize the anticancer potential.

P-ISSN: 1978-3744 E-ISSN: 2355-6811

DECLARATIONS

Ethics Approval

Ethics approval was given by Hasan Sadikin General Hospital Ethics Committee (2019/1012).

Competing of Interest

The authors declare no competing interest in this study.

Acknowledgment

This work was partially supported by Academic Leadership Grant, Universitas Padjadjaran, Bandung, Indonesia.

REFERENCES

1. Swerdlow SH, Campo E, Haris NL, et al. Hodgkin Lymphoma. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues Lyon: International Agency for Research on Cancer Press. 2017;423–41.
2. Gerrie AS, Power MM, Shepherd JD, et al. Chemoresistance can be overcome with high-dose chemotherapy and autologous stem-cell transplantation for relapsed and refractory Hodgkin lymphoma. Ann Oncol. 2014;25(11):2218–23.
3. Jo Y, Choi N, Kim K, et al. Chemoresistance of Cancer Cells: Requirements of Tumor Microenvironment-mimicking In Vitro Models in Anti-Cancer Drug Development. Theranostics. 2018;8(19):5259–75.
4. De Goycoechea D, Stalder G, Martins F, Duchosal MA. Immune Checkpoint Inhibition in Classical Hodgkin Lymphoma: From Early Achievements towards New Perspectives. J Oncol. 2019;2019:9513701.
5. Riella LV, Paterson AM, Sharpe AH, Chandraker A. Role of the PD-1 pathway in the immune response. Am J of Transplant. 2012;12(10):2575–87.
6. Dong P, Xiong Y, Yue J, et al. Tumor-Intrinsic PD-L1 Signaling in Cancer Initiation, Development and Treatment: Beyond Immune Evasion. Front Oncol. 2018;8:386.
7. Liu S, Chen S, Yuan W, et al. PD-1/PD-L1 interaction up-regulates MDR1/P-gp expression in breast cancer cells via PI3K/AKT and MAPK/ERK pathways. Oncotarget. 2017;8(59):99901–12.
8. Liu J, Quan L, Zhang C, et al. Over-activated PD-1/PD-L1 axis facilitates the chemoresistance of diffuse large B-cell lymphoma cells to the CHOP regimen. Oncol Lett. 2018;15(3):3321–8.
9. Ishibashi M, Tamura H, Sunakawa M, et al. Myeloma Drug Resistance Induced by Binding of Myeloma B7-H1 (PD-L1) to PD-1. Cancer Immunol Res. 2016;4(9):779–88.
10. Villasboas JC, Ansell S. Checkpoint Inhibition: Programmed Cell Death 1 and Programmed Cell Death 1 Ligand Inhibitors in Hodgkin Lymphoma. Cancer J. 2016;22(1):17–22.
11. Lavrik IN, Krammer PH. Regulation of CD95/Fas signaling at the DISC. Cell Death Differ. 2012;19(1):36–41.
12. Houston A, O'Connell J. The Fas signalling pathway and its role in the pathogenesis of cancer. Curr Opin Pharmacol. 2004;4(4):321–6.
13. Peter ME, Hadji A, Murmann AE, et al. The role of CD95 and CD95 ligand in cancer. Cell Death Differ. 2015;22(5):885–6.
14. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228–47.
15. Hollander P, Kamper P, Smedby KE, et al. High proportions of PD-1(+) and PD-L1(+) leukocytes in classical Hodgkin lymphoma microenvironment are associated with inferior outcome. Blood Adv. 2017;1(18):1427–39.
16. de Carvalho-Neto PB, dos Santos M, de Carvalho MB, et al. FAS/FASL expression profile as a prognostic marker in squamous cell carcinoma of the oral cavity. PLoS One. 2013;8(7):e69024.
17. Aryanti, Hernowo B, Agustina H. Clinicopathologic Characteristics and Chemotherapy Response of Classic Hodgkin Lymphoma: A Study in Tertiary Teaching Hospital. Journal of Drug Delivery and Therapeutics. 2020;10:95–8.
18. Domínguez RA, Márquez A, Gumá J, et al. Treatment of stage I and II Hodgkin's lymphoma with ABVD chemotherapy: results after 7 years of a prospective study. Ann Oncol. 2004;15(12):1798–804.
19. Canellos GP, Anderson JR, Propert KJ, et al. Chemotherapy of advanced Hodgkin's disease with MOPP, ABVD, or MOPP alternating with ABVD. New Engl J Medicine. 1992;327(21):1478–84.
20. Gordon LI, Hong F, Fisher RI, et al. Randomized Phase III Trial of ABVD Versus Stanford V With or Without Radiation Therapy in Locally Extensive and Advanced-Stage Hodgkin Lymphoma: An Intergroup Study Coordinated by the Eastern Cooperative Oncology Group (E2496). J Clin Oncol. 2013;31(6):684–91.
21. Zhang P, Ma Y, Lv C, et al. Upregulation of programmed cell death ligand 1 promotes resistance response in non-small-cell lung cancer patients treated with neo-adjuvant chemotherapy. Cancer Sci. 2016;107(11):1563–71.
22. Bianchini G, Pusztai L, Pienkowski T, et al. Immune modulation of pathologic complete response after neoadjuvant HER2-directed therapies in the NeoSphere trial. Ann Oncol. 2015;26(12):2429–36.

23. Zhang P, Su DM, Liang M, Fu J. Chemopreventive agents induce programmed death-1-ligand 1 (PD-L1) surface expression in breast cancer cells and promote PD-L1-mediated T cell apoptosis. Molecular immunology. 2008;45(5):1470–6.
24. Meti N, Esfahani K, Johnson NA. The Role of Immune Checkpoint Inhibitors in Classical Hodgkin Lymphoma. Cancers (Basel). 2018;10(6).
25. Wu X, Li Y, Liu X, et al. B7-H1(PD-L1) confers chemoresistance through ERK and p38 MAPK pathway in tumor cells. bioRxiv. 2018:308601.
26. Sproll KC, Balló H, Hoffmann TK, et al. Is there a role for the Fas-/Fas-Ligand pathway in chemoresistance of human squamous cell carcinomas of the head and neck (SCCHN)? Oral Oncology. 2009;45(1):69–84.
27. Zheng H, Liu Z, Liu T, et al. Fas signaling promotes chemoresistance in gastrointestinal cancer by up-regulating P-glycoprotein. Oncotarget. 2014;5(21):10763–77.
28. Chappell DB, Restifo NP. T cell-tumor cell: a fatal interaction? Cancer Immunol Immunother. 1998;47(2):65–71.
29. Kim LH, Eow GI, Peh SC, Poppema S. The role of CD30, CD40 and CD95 in the regulation of proliferation and apoptosis in classical Hodgkin's lymphoma. Pathology. 2003;35(5):428–35.
30. Maggio EM, Van Den Berg A, de Jong D, et al. Low frequency of FAS mutations in Reed-Sternberg cells of Hodgkin's lymphoma. The Am J Pathol. 2003;162(1):29–35.