Association of Programmed Death Ligand1(PDL1)Immunoexpression with Histopathology Grade of Rhabdomyosarcoma At The Unit Anatomical Pathology of Adam Malik Hospital In 2016-2018

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***Abstract*-** Rhabdomyosarcoma (RMS) is a malignancy in the mesenchyme that shows differentiationof skeletal muscles, most commonly found in childhood to old age and can occur in any location throughout the body. PDL1 is an inhibiting molecule that causes disruption of the immune response against tumor cells. Revealed expression of PDL1 is associated with a poor prognosis. This study used tissue samples of rhabdomyosarcoma tumors to assess the association between immunohistochemical expressions of PDL1 and histopathology grade among rhabdomyosarcoma patients. Formalin-fixed paraffin-embedded tissue blocks of 29 rhabdomyosarcoma patients were immunohistochemically studied for PDL1 expression. The basic characteristics of the samples were obtained through medical records or pathology archieves. The association between PDL1 expression and grade were anylized using SPSS 22 version. PDL1 was expressed in 17,2% of the rhabdomyosarcoma specimens. PDL1 positive expression was no significantly associated with histopathological grade ($p > 0.05$). This not can be a prognostic indicator and possible target therapy for these neoplasms.

***IndexTerms*:**PDL1,rhabdomyosarcoma,grade,immunohistochemistry.

I. Introduction

Rhabdomyosarcoma is a soft tissue sarcoma most commonly found in childhood to old age.[1] The grade of histopathology of rhabdomyosarcoma currently used is based on FNCLCC. Consists of score differentiation, mitotic and necrosis.[2] Depending on subtype histopatology and grade, treatment for rhabdomyosarcoma includes surgery and postoperative radiation therapy. Rhabdomyosarcoma are generally aggressive neoplasm and higher grades, they tend to be progressive and recurrent.[3] New molecular target therapy was tested in the 2010. Recently, the possibility of targeted PD1 and PDL1 pathway therapies has been widely studied in various types of malignancies, and can be used as new prognostic indicators for soft tissue sarcoma.Can also be used as a selection criteria for immunotherapy.[4,5] Many researchers have significantly related to clinicopathological parameters such as clinical stage, presence of metastasis, poor tumor differentiation and tumor necrosis.[6,7]

Programmed cell death protein 1 (PD1), a receptor expressed on T and B cells, NK cells, and monocytes, is included in the CD28 family that acts as a negative regulator of the immune system.[8] This PD1 binds two ligands namely PDL1 and PDL2. PDL1 acts as an inhibitor of T cell function in peripheral tissues, then PDL2 supreessed T cell activation in lymphoid organs.[9] To protect normal tissue by inducing immune tolerance, where the interaction between these two proteins in tumors will affect the anti tumor immune response by causing fatigue (exhaustion) and dysfunction of T cells, so that tumor cells can avoid the immune system, proliferate and metastasis.[10]

Where as PDL1 generally experiences an increase in regulation on the surface of tumor cells. And very high expression in the lungs, breast, large intestine and bladder. Tumor cells express PDL1 by increasing T cell apoptosis and to inhibit CD4+ and CD8 T cell activation.[11] Therefore, this study aimed to evaluate the immunohistochemical expression of PDL1 in rhabdomyosarcoma from the tumor tissue and analyze its association with histopathology grade of rhabdomyosarcoma.

II. Material and Methods

Sample selection

This cross sectional study was conducted in Department of Anatomical Pathology, Universitas Sumatera Utara/ H. Adam Malik General Hospital, Medan and includes 29 cases of rhabdomyosarcoma. All samples were obtained through surgical procedure. Inclusion criteria were rhabdomyosarcoma cases with adequate clinical data, available and undamaged formalin-fixed paraffin embedded tissue block with sufficient tumor tissue. Detailed clinical data were obtained from medical records or pathology archives consisting of age, sex, location of the tumor. and histological type were determined independently by researchers through hematoxylin and eosin stained slides examination.

Immunohistochemistry protocol and interpretation

The tissue sections were deparaffinized and rehydrated before pretreatment. Endogenous peroxidase was blocked with hydrogen peroxide followed by antigen retrieval. PDL1 (medaysis) mouse monoclonal antibodies was used as primary antibody. Diagnostic BioSystems (Diagnostic BioSystems, Pleasanton, CA, USA) polymer kit was used for detection. The reaction was visualized with diaminobenzidine and counterstained with Mayer's hematoxylin followed by dehydration, clearing, and mounting. Positive control was plasenta. PDL1 expressions were determined independently by researchers. The expression in cytoplasm and membrane was analyzed. Immunostaining of PDL1 was evaluated in terms of staining intensity of tumor cells. Staining intensity was evaluated as negative score <10%, and as positive staining score > 10%.

Statistical analysis

Statistical analysis was performed using SPSS software package version 22.0 (SPSS Inc., Chicago) with 95% confidence interval and Microsoft Excel 2010. Categorical variables were presented in frequency and percentage. The association between PDL1 expressions with histopathology grade of rhabdomyosarcoma. The p-values < 0.05 were considered significant.

III. RESULT

Patients' characteristics

The mean age for rhabdomyosarcoma patients was 39,17 (±20,13) years. The most common in >50 years age group. Nineteen patients (65,5%) were males, only 10 patients (34,5%) were females. All the tumors were located in extremity was the predominance. The histological subtypes of rhabdomyosarcoma varied pleomorphic rhabdomyosarcoma was the majority of this case. Clinical basic characteristic of rhabdomyosarcoma patients were summarized in table 1. Representative H&E sections are shown in figure 1.

Table 1. Characteristic of rhabdomyosarcoma patients

Characteristics	Number of cases	Percentage (%)
Age, mean ± SD, years	39.17	± 20.13
< 20 years	6	20.7
21-30 years	3	10.3
31-40 years	6	31.3
41-50 years	12	20.7
51-60 years	7	24.1
61-70 years	4	13.8
Sex		
Female	19	65.5
Male	10	34.5
Location		
Extremity	24	82.8
Head & Neck	2	6.9
Others	3	10.3
Subtype		
Pleomorphic Rhabdomyosarcoma	14	48.3
Alveolar Rhabdomyosarcoma	10	34.5
Embryonal Rhabdomyosarcoma	5	17.2
Spindle cell Rhabdomyosarcoma	-	-

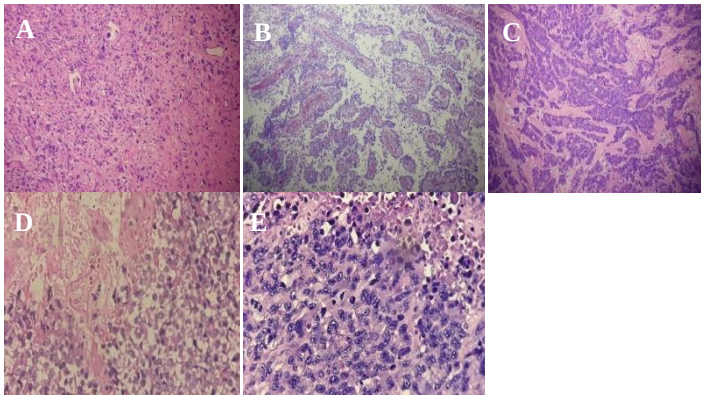

Figure 1. Histological type and grade. A, Pleomorphic rhabdomyosarcoma. B, Alveolar rhabdomyosarcoma C, embryonal rhabdomyosarcoma. D, Grade 2. E, Grade 3.

PDL1 expression

Twenty-four of 29 (51.7%) rhabdomyosarcoma cases were negative while positive expression was seen in five cases (17.2%) (table 2). The intensity of PDL1 expression in cytoplasm and membrane are shown in figure 2.

Table 2. Rhabdomyosarcoma cases based on PDL1 expression

PDL1 expression	Number of cases	Percentage (%)
Negative	24	51.7
Positive	5	17.2

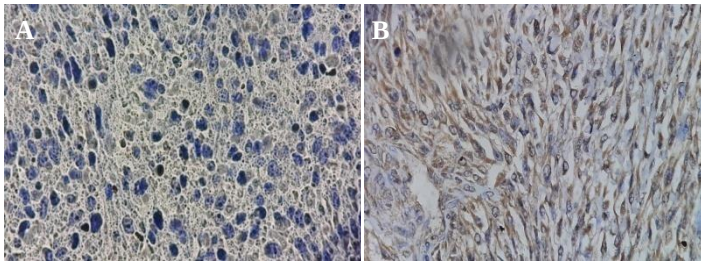

Figure 2. Immunohistochemical PDL1 expression. A, Negative intensity. B, Positive intensity.

Association between PDL1 expression and grade

The number of cases for positive PDL1 expression was found more in grade 2 rhabdomyosarcoma (10.3%) while negative expression was found more in grade 3 (41.3%) and no expression found in grade 1. This difference was no significant (p=0.076) (table 3).

Table 3. Association of PDL1 expressions with histopathology grade

Grade	Negative		Positive		p
	n	%	n	%	
Grade 1	-	-	-	-	0.076*
Grade 2 and 3	12	41.3	3	10.3	

IV. DISCUSSION

After the discovery of PD1 in 1992, PD1 and PDL1 have been shown to have a function as regulators of the immune system. Anti PD1 therapy has been proven for cases of melanoma, NSCLC, and various other solid tumors. Also in the case of sarcomas, several previous studied have reported that more than 50% of proven efficacy for sarcomas, including leiomyosarcoma, liposarcoma, osteosarcoma and other sarcomas.[12]

During this period there was a recent development of immunotherapy including immunology checkpoint blockade targeting cytotoxic T- Lymphocyte Protein-4 (CTLA-4) and programmed cell death protein 1 (PD1).[13] Research on the development of sarcoma in immunosupressed patients also support and association with the immune system. In an 8191 study of transplanted patients, of the 8724 malignancies occurring and 7.4% of them were most in Kaposi Sarcoma, 1.7% in other sarcomas including MFH, leiomyosarcoma, fibrosarcoma, rhabdomyosarcoma, hemangiopericytoma with incident 0.5% of cases.[13]

Activation of PD1 and PDL1 which leads to inhibition of specific antigens and causes the disruption of the immune response against tumor cells. T cell proliferation induces apoptosis. The role of PD1 and PDL1 as physiological immune to T cell responses and prevent autoimmunity. The expressions of PDL1 on soft tissue has the same expressions as other malignancies and is associated with poor prognosis. PD1/PDL1 inhibitors work differently than most cancer immmunotherapy strategies in clinical trials, which are usually intended to stimulate T cell response to antigen.[6,12]

Results clinical trials of pembrolizumab (SARCO28) only 7 of 40 (18%) soft tissue patients and 2 of 40 (%%) bone sarcomas showed clinical response. In SARCO28 patients with undifferentiated pleomorphic sarcoma showed a high response to anti PD1 therapy, 4 out of 10 (40%) responding to pembrolizumab.[6]

Monoclonal antibodies called “checkpoint inhibitors” are an efficient way to maintain T cell function by inhibiting the interaction of PD1 with PDL1. Clinical research has proven that antibodies that inhibit PD1 and PDL1 have a good effect on various malignancies. PD1 inhibitors are immunotherapy, which works through the immune system. T cells are the body’s natural defense system that can kill cells that have infections and cells that do not function properly as in cancer cells. T cells have two signals that can be activated and deactivated. When the immune system is active, cells called antigens activate two inhibitors on T cells, a small protein derived from T cells and a molecule called PDL1 that binds to tumor cells.[12]

This study used an immunohistochemical technique PDL1 in rhabdomyosarcoma cases. Kim RJ et al. reported PDL1 expressions based on histopathologicaltype that is obtained with the most cases in alveolar rhabdomyosarcoma and embryonal rhabdomyosarcoma.[4]

In Paydas et al. is study revealed expression on PD1/PDL1 can be used as a prognostic value. PDL1 Expression can be seen in the majority of cases of dedifferentiated liposarcoma, alveolar rhabdomyosarcoma, and pleomorphic rhabdomyosarcoma. In this study also reported that there was no relationship between PDL1 expression with clinical features and survival rates.[5]

Zhang C et al. research states that there is a significant association of PDL1 expressions for soft tissue sarcoma with poor survival rates and shows that there is no significant relationship with histopathological subtypes as well as clinicopathologicl grade, and location of the tumor. So it was assessed that PDL1 expressions could be useful as a predictive factor for prognosis for soft tissue sarcomas.[14]

Zhu Z et al. who analyzed the relation ship between PDL1 expression with clinicopathological parameters and survival rates in soft tissue sarcoma patients, showed that there was a relationship between PDL1 and survival rates whereas in the clinicopathology parameters showed no significant correlation between age, sex, location and grade of histopathology.[15]

Kim RJ et al. reported for soft tissue sarcoma that PDL1 expression was also significantly associated with histopathological subtypes, survival rate with p value 0.001 and clinicopathologi parameters such as age, sex, tumor location and histopathological grade with p value 0.002 where at grade 1 the number of cases 22 (63%) and grade 3 the number of cases 39 (81%). [4]

The expression of PDL1 in soft tissue sarcoma appears to show a tendency towards histopathological type, clinicopathological parameters and survival rates that are useful as prognostic factors.

V. CONCLUSION

There is no significant association between PDL1 expression and histopatholgy grade of rhabdomyosarcoma.

COMPETING INTERESTS

The authors have no relevant financial interest in the products or companies described in this article.

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ETHICAL APPROVAL

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

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