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10.4103/ijh.ijh_29_25

Evaluation of the plasma level of programmed death-ligand 1 and its relation with clinical and hematological parameters in pediatric patients with acute lymphoblastic leukemia

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Abstract:

BACKGROUND: Acute lymphoblastic leukemia is a diverse illness characterized by the development of immature lymphoid cells. Programmed death-ligand 1 (PDL-1) is a protein 33-kDa glycoprotein encoded by the CD274 gene on chromosome 9p24. It plays a crucial role in immune modulation and serves as a checkpoint regulator.

OBJECTIVE: To evaluate the plasma level of PDL-1 in pediatric patients with ALL and to correlate the plasma level PDL-1 with some hematological and clinical parameters.

MATERIALS AND METHODS: This study was conducted on 63 pediatric patients, <14 years old (newly diagnosed) at the central teaching hospital of pediatrics, and 27 healthy children were included in this study as a control group. Plasma PDL-1 level measuring by enzyme-linked immunosorbent assay.

RESULTS: PDL-1 was significantly higher in newly diagnosed patients with ALL 33.8 ng/ml for B-ALL and 43.5 ng/ml for T-ALL than controls (31.6 ng/ml), with $P = 0.024$. A substantial positive association was seen at the 0.05 level between PDL-1 and total white blood cell (WBC) count, pallor, and lymphadenopathy, whereas no significant correlation was found with other hematological and clinical parameters.

CONCLUSIONS: Plasma level of PDL-1 was significantly higher in newly diagnosed patients with ALL than controls. Furthermore, there was significant positive correlation between PDL-1 with total WBC count, pallor, and lymphadenopathy, while there was no significant correlation with other hematological and clinical parameters.

Keywords:

Acute lymphoblastic leukemia, enzyme-linked immunosorbent assay, hemoglobin, platelet, programmed death liganed-1, white blood cell

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Submission: 12-04-2025
Revised: 19-06-2025
Accepted: 20-06-2025
Published: 13-09-2025

Introduction

Acute lymphoblastic leukemia (ALL) is the clonal growth of immature lymphocytes or lymphoid progenitors, typically of B-cell or T-cell lineage. The proliferation and accumulation of clonal blast cells in the marrow lead to the inhibition of hematopoiesis, resulting in anemia, thrombocytopenia, and neutropenia. Lymphoblast's may aggregate in multiple extramedullary locations, particularly the meninges, gonads, thymus, liver, spleen, and lymph nodes.[1] Acute lymphoblastic leukemia is the most prevalent type of malignancy (25%–30%) and the most

How to cite this article: Farhan SK, Al Wash MM. Evaluation of the plasma level of programmed death-ligand 1 and its relation with clinical and hematological parameters in pediatric patients with acute lymphoblastic leukemia. Iraqi J Hematol 2025;14:172-8.

common subtype of leukemia (75%–80%) in pediatric populations. However, childhood ALL is not a singular illness and exhibits significant phenotypic and genotypic variation, which holds diagnostic and prognostic significance.[2] The heterogeneity indicates that leukemic lymphoblasts can arise at various stages of differentiation; therefore, morphological, immunological, cytogenetic, and molecular genetic assessments are crucial for diagnosing or ruling out ALL and for classifying its subtypes. This biological heterogeneity has necessitated the stratification of patients into risk groups and the provision of risk-adapted therapy.[3] There are several subtypes of B-cell and T-cell allogeneic lymphoma (ALL), each of which harbors a unique constellation of somatic structural DNA rearrangements and sequence mutations. These mutations frequently disrupt lymphoid development, cytokine receptors, kinase and Ras signaling, tumor suppression, and chromatin modification. Recent research has contributed to a better understanding of the genetic framework underlying clonal evolution and relapse, as well as the role that inherited genetic variants play in the process of leukemogenesis.[4]

The expression of several cosignaling molecules on T cells, such as programmed death-1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4, facilitates inhibitory signaling that coincides with T cell fatigue, characterized by diminished T-cell effector activity.[5] The primary ligand for PD-1 is programmed death-ligand 1 (PDL 1). This immune-regulating receptor and its ligand play a pivotal role in autoimmune, infectious immunity, transplantation immunology, and tumor immunotherapy.[6] Programmed cell death-ligand 1, also known as PDL 1, is a glycoprotein that is produced on antigen-presenting cells and contains immunomodulatory properties. The suppression of cytokine production, T-cell proliferation, and T-cell adhesion is one of the key roles that the binding of PDL 1 to the PD-1 receptor, which is found on the surface of T cells, plays in the regulation of effector T-cell responses.[7] PDL1 (CD274, B7-H1) and PD-L2 (CD273, B7-DC) are two ligands from the B7 family that are expressed on antigen-presenting cells (macrophages and dendritic cells), a subset of activated B lymphocytes, and microvascular endothelial cells. The PD-1 receptor is responsible for binding these two ligands.[8] The objective of this study as to evaluate the plasma level of PDL-1 in pediatric patients with ALL and to correlate the plasma level PDL-1 with some hematological and clinical parameters.

Materials and Methods

Sixty-three patients (male and female) were diagnosed with ALL with age <14 years. Collected from hematology unit in Central Teaching Hospital of Pediatric and pediatric welfare hospital in Baghdad. Verbal consent from parents was taken after they were told about the aim and possible advantages of this study. The diagnosis of cases was based on the clinical examination of consultant physicians in the hematology unit confirmative diagnosis and classification were performed by the hematologists based on routine morphological assessment of the stained peripheral blood (PB) and bone marrow (BM) smears according to the standard FAB criteria (confirmed by cytochemical stains), by an expert hematopathologists.

Flowcytometric immunophenotyping using a panel of well-characterized by monoclonal antibody (cMPO, cCD79a, CD19, CD20, CD7, CD3, CD34, CD45, CD11b, CD13, CD10, HLADR, CD33, CD117, TdT, sIgm, CD38, CD66) was done at flowcytometry department in Medical City, Baghdad, for further confirmation and characterization of the cases. For each patient and control, a verbal consent from parents was obtained for accepting to take PB sample. Data were collected from each patient via a questionnaire, focusing on primary symptoms and physical signs, particularly the presence of extramedullary features such as lymphadenopathy, splenomegaly, hepatomegaly, mediastinal widening, and central nervous system involvement, in addition to hematological parameters obtained from the patient case file.

Inclusion criteria

Patients were less than 14 years old and randomly collected in relation to sex, newly diagnosed with ALL by morphology, and immunophenotyping (B-ALL and T-ALL subtypes).

Exclusion criteria

Patient's adult (older than 14 years) and patients with other types of blood cancers such as acute myeloid leukemia, hemophilia, and sickle cell anemia.

Healthy control samples

Blood samples were collected from healthy twenty-seven controls (27) aged <14 years from hospital attendees for patient have elective surgery as control group.

Ethical approval

Approval for the research was obtained from the Scientific Committee of the Department of Pathology, Iraqi Board for Medical Specialization. Issue Number: path 49 dated April 2, 2024. Informed consent was secured from the parents of all participants before the commencement of data collection, following an explanation of the study's objectives and a guarantee of anonymity.

Estimation programmed death-ligand 1 plasma level

The plasma was separated from the blood after centrifuging for 20 min at 2000–3000 rpm. Plasma was collected in

Eppendorf tubes in 2 aliquots for each sample and then stored at –80°C at Molecular Department in Medical City. Estimation of PDL-1 plasma level was conducted using enzyme-linked immunosorbent assay (ELISA) and the human PDL-1 ELISA Kit (Mindray\China). The results were derived by averaging the absorbance readings of each pair of duplicate standards, and the standard curve was constructed by graphing the mean absorbance of each standard tube against its human PDL-1 concentration on the X-axis. Microsoft Excel was used to create a table of two columns: the first column for absorbance (OD) and the second for concentration (ng/ml), after which a curve was drawn where the absorbance column was chosen to be the Y-axis while the concentration column was the X-axis, and the option of display equation on chart was activated in the format trendline to add the curve line equation to the chart. The X value for the samples (concentration) was calculated from the curve equation.

Statistical analysis

Statistical analysis was conducted utilizing Statistical Package for the Social Sciences (SPSS) (version 26.0.0. IBM SPSS Statistics for Windows Armonk, NY: IBM Corp.; 2019). Analytic statistics were conducted using a *t*-test, with $P < 0.05$ being significant. Descriptive statistics were presented in tables, including the mean and standard deviation. Pearson correlation analysis was conducted to evaluate the association among various variables (r).

Results

Table 1 shows a demographic comparison between two groups of participants: the patient group ($n = 63$) and the control group ($n = 27$). The results show that the mean age of the patient group was 5 years, while the mean age of the control group was 7 years. The sex ratio was 1.6:1 in the patient group and 1.5:1 in the control group, indicating a slight male predominance in both groups. The results indicate that the patient group and the control group were demographically homogeneous in terms of age and sex, as no statistically significant differences were recorded between them. Both groups of ALL (B and T) had a median age of 5, indicating no significant difference in age between the two groups. Regarding gender, 64% of B-ALL patients were male, while 54% of T-ALL patients were male. Indicating a significant difference in sex distribution between the two groups (B-ALL and T-ALL) as shown in Figure 1.

The clinical characteristics of the patients and controls were analyzed. A statistically significant difference was observed in the lymphadenopathy, fever, pallor, and hepatosplenomegaly between the two groups, as shown in Table 2.

Both groups showed high percentage of lymphadenopathy and fever, with 86% and 90% of B-ALL patients, respectively, and 92% of T-ALL patients presenting with these symptoms. Pallor was universal in B-ALL patients (100%) and present in 85% of T-ALL patients. Hepatosplenomegaly was observed in 60% of B-ALL patients and 69% of T-ALL patients, as shown in Figure 2.

Significant differences were observed in hematological parameters. Patients had a highly significantly differences in median of white blood cell (WBC)

Table 1: Demographic characteristics of study participants

Characteristics	Patients' group (*n*=63)		Control (*n*=27)	*P*
	B-ALL (*n*=50)	T-ALL (*n*=13)		
Age, median (IQR)	5.0 (5)	5.0 (6)	7.0 (7)	0.432
Male, *n* (%)	32 (64)	7 (54)	16 (59.3)	0.512
Female, *n* (%)	18 (36)	6 (46)	11 (40.7)	
Ratio	1.6:1		1.5:1	

P-value significant at the 0.05 level. ALL=Acute lymphoblastic leukemia, IQR=Interquartile range

Table 2: Clinical characteristics of acute lymphoblastic leukemia patients' groups

Characteristics	B-ALL (*n*=50), *n* (%)	T-ALL (*n*=13), *n* (%)	ALL patients group (*n*=63), *n* (%)	*P*
Pallor				
Yes	50 (100)	11 (85)	61 (96.8)	<0.001
No	0	2 (15)	2 (3.2)	
Fever				
Yes	45 (90)	12 (92)	57 (90.4)	<0.001
No	5 (10)	1 (8)	6 (9.6)	
Lymphadenopathy				
Yes	43 (86)	12 (92)	55 (87.3)	<0.001
No	7 (14)	1 (8)	8 (12.7)	
Hepatosplenomegaly				
Yes	30 (60)	9 (69)	39 (62)	<0.001
No	20 (40)	4 (31)	24 (38)	

P-value is significant at the 0.05 level. ALL=Acute lymphoblastic leukemia

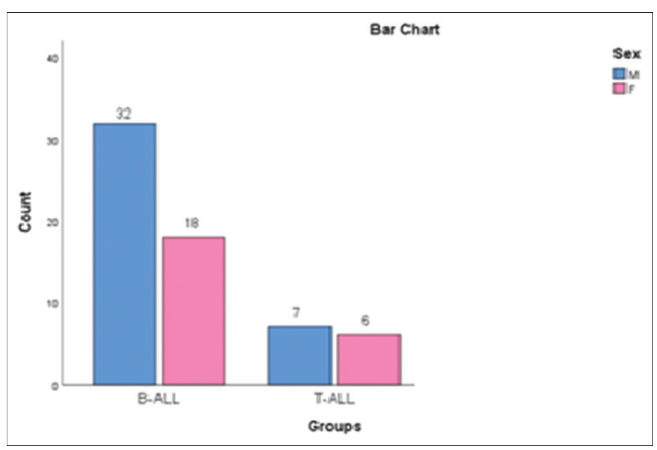

Figure 1: Sex distribution in ALL patients and controls

count, hemoglobin (Hb) level, and platelets count of 21.9×10^9/L, 7.5 g/dl, and 50×10^9/L, respectively, in B-ALL group and 61.52×10^9/L, 8.1 g/dl, and 27×10^9/L in T-ALL group compared to the control group's median of 8.9×10^9/L, 12.0×10^9/L, and 304×10^9/L, respectively ($P < 0.001$). Furthermore, ALL pediatric patients had a significantly higher median percentage of blast cells, in B-ALL (63%) and T-ALL (62%), compared to the control group's 0% ($P < 0.001$), as shown in Table 3 and Figure 3.

The median PDL-1 level was significantly higher in the patient group T-ALL (43.5) than B-ALL (33.8) and control group (31.6), with $P = 0.024$, as shown in Table 4 and Figure 4.

PDL-1 level and lymphadenopathy were shown to be significantly positively correlated ($r = 0.258$, $P = 0.041$). Indicating that higher PDL-1 level is associated with the presence of lymphadenopathy .In addition, a significant positive correlation was observed between PDL-1 level and pallor ($r = 0.324$, $P = 0.010$), suggesting a link between increased PDL-1 level and a pale appearance. No significant correlations were found between PDL-1 level and fever or hepatosplenomegaly, as shown in Table 5.

A significant positive correlation was observed between PDL-1 and WBC at the 0.05 level ($r = 0.059$, $P = 0.659$). However, no significant correlations were observed between PDL-1 and Hb ($r = 0$, $P = 0.999$), platelet count ($r = -0.123$, $P = 0.337$), or blast percentage ($r = 0.233$, $P = 0.066$), as shown in Table 6.

Table 3: Hematological characteristics distinguishing B-acute lymphoblastic leukemia and T-acute lymphoblastic leukemia patients from the control group

Characteristics	Patients' group (*n*=63)		Control (*n*=27), median (IQR)	*P*
	B-ALL (*n*=50), median (IQR)	T-ALL (*n*=13), median (IQR)		
WBC $\times 10^9$/L	21.9 (30.9)	61.5 (259)	8.9 (3.6)	<0.001
Hb g/dL	7.5 (3.18)	8.1 (4.2)	12.0 (1.7)	<0.001
Platelets $\times 10^9$/L	50 (66.2)	27 (64.2)	304 (124)	<0.001
Blast cells	63 (49)	62 (34)	0	<0.001

P-value is significant at the 0.05 level. ALL=Acute lymphoblastic leukemia, IQR=Interquartile range, WBC=White blood cell, Hb=Hemoglobin

Table 4: Programmed death liganed-1 expression levels in B-acute lymphoblastic leukemia and T-acute lymphoblastic leukemia patients compared to controls

Parameters	Patients' group (*n*=63)		Control (*n*=27), median (IQR)	*P*
	B-ALL (*n*=50), median (IQR)	T-ALL (*n*=13), median (IQR)		
PDL-1	33.8 (9.85)	43.5 (22.8)	31.6 (23.6)	0.024

P-value is significant at the 0.05 level. ALL=Acute lymphoblastic leukemia, IQR=Interquartile range, PDL-1=Programmed death liganed-1

Discussion

The results of this study show that the median age of newly diagnosed ALL patients was 5.0 years old. This was in agreement with the study of Ghanavat *et al.*[9] who showed that the most common age group was 2–10 years (71.4%), as well as Iraqi study of Ahmed and Ahmed[10] found that most of the cases (75%) were between the ages of 1–10 and only 25% were older than 10 years old. Mohammad *et al.*[11] reported that ALL

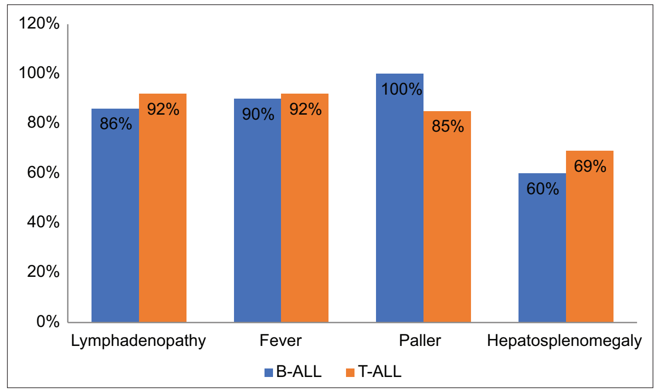

Figure 2: Symptom frequency in B-ALL and T-ALL patient groups

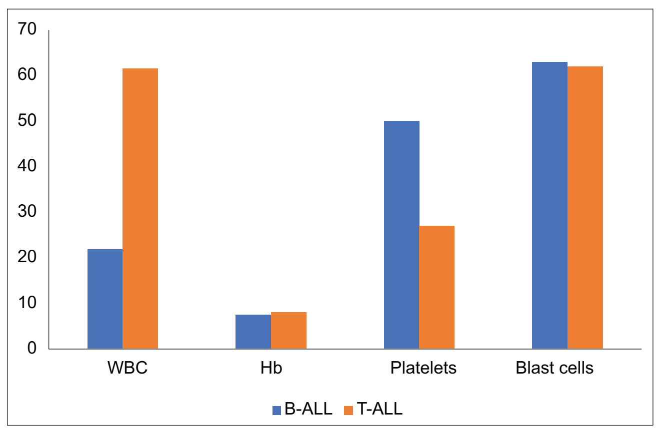

Figure 3: Hematological parameters in study groups

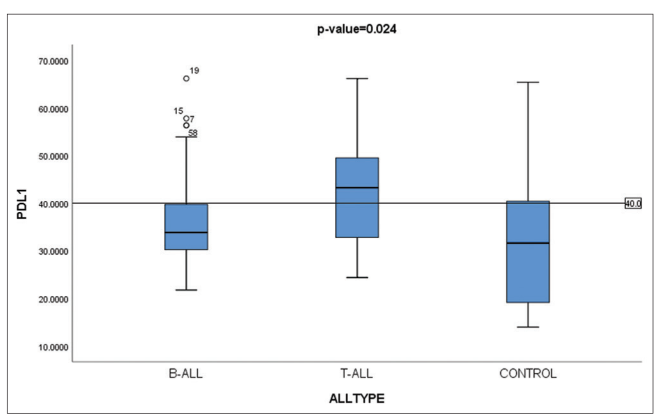

Figure 4: Programmed death-ligand 1 level in ALL groups and control

Table 5: Correlations among programmed death-ligand 1 and clinical feature

PDL-1	Lymphadenopathy	Fever	Pallor	Hepatosplenomegaly
Pearson correlation	0.258*	−0.062	0.324**	−0.144
P	0.041	0.629	0.010	0.259

*Correlation is significant at the 0.05 level (two-tailed), **Correlation is significant at the 0.01 level (two-tailed). PDL-1=Programmed death liganed-1

Table 6: Correlation of hematological parameters and programmed death liganed-1 level

Parameters	WBC	Hb	Platelets	Blasts
PDL-1				
Pearson Correlation (*r*)	0.059*	0	−0.123	0.233
P	0.659	0.999	0.337	0.066

*The correlation is significant at the 0.05 level, Correlation is significant at the 0.01 level. PDL-1=Programmed death liganed-1, Hb=Hemoglobin, WBC=White blood cell

represents about 56.8% of all cases with a peak incidence at age ≤5 year. In another study by Mohammed *et al*.,[12] the age group of 2–5 years old had the highest percentage of ALL patients (54.3%), followed by the age group of 6–12 years old (41.4%), while the age group of patients older than 12 years old had the lowest percentage (4.3%). Other result of childhood leukemia by age group in period 2000–2019 Iraqi distribution for Al-Hashimi[13] showed that the higher group was 0–4 years (41%) then age group 5–9 years (33%) while the lowest percentage was in group 11–14 years (25%).

In this study, the patients were randomly selected in relation to sex, and the male-to-female ratio was 1.6:1; males have a higher incidence of acute lymphoblastic leukemia and a worse prognosis than females. Studies evaluating the impact of sex on ALL are rare.[14] The results of the univariable analysis of Rafieemehr *et al*.[15] indicated that boys were more likely to develop ALL, it is yet unknown whether the correlation between sex and the development of ALL could be due to the influence of sexual hormones because the relationship has not been well studied. The results of Abdulmenem Arosi[16] indicated that the percentage of boys with ALL was 61.25%, whereas the percentage of girls with ALL was 38.75%.

The majority of the study's cases had no family history of cancer which represents about 90.4% in 57 from the total 63 patients. Like other cancers, ALL appears to be multifactorial disorder in which hereditary and environmental variables combine to influence the disease's occurrence; it appears that environmental factors may have an impact on the occurrence of ALL.[17] The result of Rafieemehr *et al*.[15] agreed with the recent investigation that did not find any significant correlation between it and a history of cancer in first- and second-degree relatives. According to Iraqi study of Mohammed *et al*.[18] who investigated the relation between ALL and family history, it was found that the disease's familial history account for just five (20.8%) leukemic children in Iraqi study who had a positive family history of leukemia. The majority of patients exhibited fever, pallor, lymphadenopathy, and hepatosplenomegaly, which was consistent with the findings of Al-Mulla *et al*.[19] Although it did not align with the study by Jaime-Pérez *et al*.,[14] which demonstrated a divergent distribution of clinical signs and symptoms, other factors, including racial, genetic, and environmental influences, may contribute to these discrepancies.

Anemia in leukemia can result from various conditions that either destroying or preventing the body's production of healthy red blood cells due to BM infiltration, cytokine-mediated suppression, hemolysis, and blood loss. Iraqi study of Al-Doski[20] demonstrated that 81% of the patients had hemoglobin levels below 9g/dl, with a mean hemoglobin concentration of 8.2 ± 4.9 g/dl. Iraqi investigation of Ahmed and Ahmed[10] noted that the average Hb concentration was 7.93 ± 2.2 g/dl as well as anemia affected 90% of the individuals. Study of Ahmad *et al*.[21] agreed with the current result when discovered that the average hemoglobin level was 7.41 ± 2.57 g/dl. The result of the current study observed decrease in platelet count in patients compared to controls, and this was compatible with many studies as Dai *et al*.[22] when they were studied platelet count in children with ALL who indicated to predict the prognosis in children with acute lymphoblastic leukemia, and the study conducted by Cornelissen *et al*.[23] examined thrombocytopenia in patients with acute leukemia, quantifying the correlation between diminished platelet counts and the occurrence of intracranial hemorrhage. Prolonged durations of thrombocytopenia were linked to an increased risk. The study by Al Khafaji *et al*.[24] predicted indicators of thrombocytopenia linked to acute lymphoblastic leukemia and acute myeloid leukemia. The HBG levels and WBC count in T-ALL are more than in B-ALL, B-ALL, and T-ALL and may have different immunological responses to leukemic cells, which might have an impact on the development and survival of platelets and red blood cells. Leukemic cells have significantly entered the BM, in B-ALL, affecting the normal synthesis of platelets and red blood cells.

Compared to B-ALL cells, T-ALL cells typically multiply more quickly and aggressively. WBC counts in T-ALL patients may rise more as a result of this increased proliferation rate.[25] The present result was in agreement with the study of Verma *et al*.[26] when they found that there was different in hematological parameters in B-ALL and T-ALL and revealed that WBC = 43.76 ± 63.40, HBG = 6.62 ± 2.08, and PLT = 41.67 ± 53.37 for B-ALL versus WBC = 49.46 ± 57.22, HBG = 7.10 ± 2.29, and PLT = 31.40 ± 15.65 for T-ALL.

Newly diagnosed patients showed high plasma level of PDL-1 than controls, with highly significant differences. The function of PD-1/PD-L1 in hematologic malignancies is under investigation, with clinical trials now under progress. Research indicated that the PD-L/PD-1 pathway may be pivotal in tumor immunity and is upregulated due to immune-evading mechanisms employed by tumor cells. The significant immune checkpoint molecule, PD-L1, engages with T cells via its receptor, PD-1. The connection inhibits T-cell activation, proliferation, and cytokine production, leading to immune suppression. Nevertheless, there is a paucity of data regarding PD-L1 in the immunology of acute leukemia. Chen *et al*.[27] examined the expression of PD-L1 in human acute leukemia cells. The PD-1/PD-L1 pathway may play a role in the immune evasion mechanism of acute lymphoblastic leukemia (ALL), potentially serving as a novel therapeutic target for the condition.[26] The findings of the current study align with several investigations, including the research conducted by Ruan *et al*.,[28] which shown that PD-L1 levels were considerably elevated in children with ALL compared to the control group. Furthermore, the study result showed that PDL-1 level was higher in T-ALL than B-ALL due to that T-ALL has higher activation of oncogenic pathways that upregulate PD-L1; T-ALL cells experience more inflammatory cytokine exposure; in addition, T-cells are more prone to immune checkpoint evasion differences in the leukemic microenvironment and has genetic and epigenetic factors.[29] The study of Hamdan *et al*.[30] investigated that the expression of PD-L1 is common among newly diagnosed B-ALL and AML patients.

This was the first study who investigated that there was a positive correlation between PDL-1 and WBC only, and no significant correlations were observed between PDL-1 and Hb, platelet count, or blast percentage. The WBC levels and PD-L1 in ALL are still being studied, but some research such as Cao *et al*.[31] has indicated that higher WBC counts in ALL patients are linked to higher PD-L1 levels on leukemic blasts. It means that to avoid immune monitoring, leukemic cells with increased rates of proliferation may increase PD-L1 as well as lower PLT and HGB levels may be associated with higher PD-L1 expression, this could be due to the immunosuppressive environment created by leukemic cells, which can affect red blood cell and platelet production with PD-L1 expression. While the study of Abdel Hafeez *et al*.[32] in acute myeloid leukemia who investigated that there was no significant correlation between PDL1 level with various laboratory findings, including WBCs, Hb, and platelet count. In this study, there was a significant positive correlation found between PDL-1 level and lymphadenopathy. Lymphadenopathy may indicate the dispersion or severity of the disease, highlighting the significance of this mechanism in cancer progression and metastasis. No previous studies confirmed the relationship between PDL-1 expression and lymphadenopathy in acute leukemia.[33] Because leukemic cells infiltrate the BM in ALL, pallor is frequently linked to anemia. Anemia can be a sign of disease load and immunological dysregulation, which may be indirectly associated with increased PD-L1 expression, even if there is not any direct evidence connecting pallor to PD-L1 levels.[31]

Conclusions

High plasma concentration of PDL-1 is found in newly diagnosed patients with ALL compared to the control group, and the most prevalent clinical symptoms in patients are pallor and lymphadenopathy, while fever and hepatosplenomegaly are less prevalent. The PDL-1 had a substantial positive link with total WBC count, lymphadenopathy, and pallor, while showing no correlation with fever, hepatosplenomegaly, platelet count, or hemoglobin levels.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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