Original Article

Prognostic Impact of CD200 Expression in Pediatric Acute Lymphoblastic Leukaemia

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ABSTRACT

Background and study Aim: Acute lymphoblastic leukemia (ALL) is heterogeneous disease where immature lymphoid cells grow predominantly in the peripheral blood, bone marrow, and other tissues. The expression of CD200 has several diagnostic and potentially prognostic implications in the flow-cytometric assessment of lymphoid malignancies. The objective of this research is to evaluate the expression level of CD200 in de-novo ALL pediatric cases and its correlation with cytogenetic studies and response to induction of chemotherapy in them.

Materials and Methods: This prospective cohort research had been performed on 95 newly diagnosed ALL cases who attended the Hematology Oncology Unit of Ain Shams University Hospitals from September 2022 to February 2023.

Results: Regarding demographic and clinical data, CD200 expression was associated with splenomegaly and younger age. Regarding laboratory data, our study revealed a significant difference between CD200 expression and serum uric acid (p= 0.007) and platelets count (p= 0.001) being low in CD200 positive patients. Regarding cytogenetic risk stratification, the unfavorable chromosomal aberrations were higher in cases with higher CD200 expression >90% compared to patients with lower CD200 expression <90%. Regarding outcome of the studied patients and CD200% expression, a high statistically significant correlation was observed between CD200% and response to induction of chemotherapy.

Conclusion: From this study, we conclude that CD200 is a bad prognostic factor and associated with poor response to chemotherapy, since CD200 positive patients are more liable to bad response to treatment.

Key Words: Depression, fibromyalgia, interns, medical students, physical function.

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INTRODUCTION

ALL is a malignant disorder which originates from the proliferation of immature lymphoid cells in a single B- or T-lymphocyte progenitor cell as a result of consecutive somatic mutations in a single lymphoid progenitor cell at any stage of normal lymphoid development[1].

ALL is the most frequent type of cancer and most frequent subtype of leukemia in children. The global incidence is increasing at a rate of 1.4 percent per year. The largest prevalence was seen between the ages of one and four years, with a slight male predominance over females. The annual frequency in Egypt is approximately 4 cases per 100,000 kids, at Cairo University's National Cancer Institute (NCI)[2].

CD200 is a cell surface transmembrane glycoprotein expressed by a gene on chromosome 3q12. It is also known as MOX-2 and member of the type-I immunoglobulin superfamily. T and B lymphocytes, endothelial cells, and neurons are all known to express this protein[3].

CD200-CD200R interactions play role in cell-mediated immunity with lymphocytic involvement, and myeloid differentiation regulation. The change of cytokine profiles from Th1 to Th2 and the suppression of tumor-specific T-cell response were also defined[4]. Nevertheless, CD200 plays a critical role in immune tolerance, which protects other critical tissues and stem cells from immune damage[5].

Flow cytometry can be used to facilitate categorization and the diagnosis of T-ALL and B-ALL lineages in pediatric ALL. It can also be used to predict the disease's prognosis and track its progression[6].

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The current research aimed to evaluate the expression level of CD200 in de-novo ALL pediatric cases and its correlation with cytogenetic studies and response to induction of chemotherapy in them.

SUBJECTS AND METHODS

Subjects:

This prospective cohort research had been performed on 95 pediatric newly diagnosed ALL patients who attended the Hematology Oncology Unit of Ain Shams university hospitals during the period between September 2022 to February 2023. Only de novo pediatric ALL patients whose age less than 18 years were included in our study.

Ethical approval:

Faculty of Medicine Ain Shams University Research Ethical committee (REC) approval was granted under number FWA 000017585 (FMASU MS 576/2022). Informed consents were taken from the legal guardians of patients.

Study Methods:

All cases have been subjected to the following: Full history taking, clinical examination (laying stress on: organomegaly, lymphadenopathy, bleeding tendency), Complete Blood Count (CBC) using peripheral venous blood samples performed by XN-1000 (Sysmex, Japan) and examination of Leishman- stained blood film, Bone marrow (BM) aspiration and examination of Leishman- stained marrow smear, flowcytometric immunophenotyping on BM samples using an extended panel of monoclonal antibodies (Mo Abs) on 6 color Navios Beckman flowcytometer, Cytogenetic analysis by FISH technique in some cases and measurement of CD200 level using anti CD200 Mo Abs (PE) by 6 color Navios Beckman flowcytometer.

Patients received induction therapy (according to Ain Shams University protocol)[7]. A uniform treatment protocol was followed in all patients, this involved induction of remission phase with steroids for 28 days and Adriamycin (25 mg/m^2) and vincristine (1.5 mg/m^2) on day 0 and day 15, asparginase for 6 doses (10,000 IU/m^2) followed by cyclophosphamide on day 21 (1000 mg/m^2) and cytarabine (75 mg/m^2) for 8 days with 6 mercaptopurine (60 mg/m^2) for 14 days.

Evaluation was done at the end of induction phase (day 28) to assess response to therapy by BM and CBC and to stratify the patients into good responders and bad responders.

Complete remission was achieved if no lymphadenopathy or organomegaly detected, CBC returned to normal ranges, disappearance of blast cells from PB, the BM blasts <5% and minimal residual disease less than 0.01%[8].

Immunophenotyping:

Reagent

- Phosphate buffered saline (PBS) (8.0 gram per liter NaCl, 0.2 gram per litre KCL, 1.44 gram per liter NaH2PO4 and 0.2-gram kH2PO4) added to 100 milliliters of distilled water with pH adjusted at 7.3 ± 0.2, stored at 4˚C and used as long as no visible contamination was present.
- Lysing solution (1.5 mmol/L NH4Cl, 100 mmol/L KHCO3 and 10 mmol/L tetra Na-EDTA) constituted of one liter with distilled water, pH corrected to 7.2.
- Negative isotypic control (appropriately labelled regarding the MoAbs utilized) for identifying the non-specific binding of MoAbs.
- Monoclonal antibodies provided by Beckman Coulter, France.

Cells were stained with different antibody combinations using either fluorescein isothiocyanate (FITC), phycoerythrin (PE), PC5 or PC7 conjugated MoAbs for diagnosis of ALL[9].

The acute leukemia panel included:

Lymphoid markers:

- B cell markers: CD19, CD20, CD10
- T cell markers: CD2, CD5, CD7

Myeloid markers

- CD13, CD33, CD14, intracellular MPO.

Progenitor markers

- CD34, HLA-DR, CD10.
- PE labeled MoAb for identification of CD200.

- Procedure.

BM samples were preferred to be within 6 hours of collection, otherwise samples were left overnight at room temperature (25^{o} C).

Surface marker analysis

1. Blood was diluted with PBS so that total leukocytic count (TLC) was adjusted between 5 and 10 x 10^3/mm^3.
2. For each sample, sets of tubes were labeled for all the MoAbs to be used, including 1 tube for the appropriate negative isotypic matched control MoAb.
3. 50 µL of diluted samples were delivered in each tube.
4. 5 µL of each MoAb and of the isotypic negative control MoAbs were added to the respective tubes.
5. The tubes were vortexed and incubated in the dark at room temperature for 15 minutes.
6. 1.5 mL of lysing solution was added to each tube.
7. The tubes were vortexed and incubated for 5-10 minutes in the dark at room temperature.

If the tubes weren't processed during two hours, 0.5 ml of fixative (2 g paraformaldehyde in 100 milliliters phosphate buffered saline with 0.1% Na azide, pH 7.4 ± 0.2) was added and the tubes were kept at 4^{o}C till it is analyzed on FCM during 24 hours.

At least 5000 events were examined. Gating has been performed on the blast cell population for ALL patients using CD45/ SSC gating strategy and CD200 was assessed.

Data interpretation: The percentage of blast cells positive for the relevant examined marker has been identified as a percentage from the gated blast cells population. The negative isotypic control was established at two percent.

Cells were considered positive for a specific marker if more than or equal 20% of cells expressed it including our marker CD200, except for intracellular MPO and CD34 in which expression by 10% of cells was sufficient to confer positivity[10].

Statistical analysis:

The collected data have been tabulated, revised, introduced to a PC and coded utilizing Statistical package for Social Science (SPSS 25). Quantitative data were shown as median and interquartile range, while qualitative data were displayed as number and percentages and the comparison between groups was carried by using Chi-square test and/or Fisher exact test. The comparison between two independent groups with quantitative data and parametric distribution was settled using Independent t-test while with non-parametric distribution was settled by using Mann-Whitney test. The correlation between two quantitative parameters in the same group was assessed following the Spearman correlation coefficients.

■ **Power of significance was evaluated as follows:**

The confidence interval was set to ninety-five percent and the margin of error accepted was set to five percent. So, the *p-valu*e considered significant.

- Probability level (*P-value*) ≥ 0.05 = Non significant (NS).
- *P-value* < 0.05 = Significant (S).
- *P-value* < 0.01 = highly significant (HS).

RESULTS

Total number of 95 newly diagnosed pediatric ALL cases were enrolled in our study. The diagnosis was based on immunophenotyping (IPT) criteria, cytochemical and standard morphologic criteria.

1. Demographic and clinical data:

Regarding the demographic data, the examined cases involved 66 males (69.5%) and 29 females (30.5%) with M: F ratio 2.2:1. Ages varied from 100 days to 18 years with a median of 10 years. Regarding the clinical data, 25 patients (26.3%) had hepatomegaly, 29 patients (30.5%) had splenomegaly and 53 patients (55.8%) had lymphadenopathy.

In our research, IPT demonstrated that 74/95 (77.9%) were diagnosed as B-ALL while 21/95 (22.1%) were diagnosed as TALL as shown in (Table 1).

Table 1: Demographic data, clinical features and diagnosis of the examined cases:

		Total no.=95
Age (years)	Median(IQR)	10 (5-14)
	Range	0.29 – 18
Sex	Female	29 (30.5%)
	Male	66 (69.5%)
Fever	No	6 (6.3%)
	Yes	89 (93.7%)
Lymphadenopathy	No	42 (44.2%)
	Yes	53 (55.8%)
Hepatomegaly	No	70 (73.7%)
	Yes	25 (26.3%)
Splenomegaly	No	66 (69.5%)
	Yes	29 (30.5%)
Bone ache	No	49 (51.6%)
	Yes	46 (48.4%)
CNS infiltration	No	95 (100%)
	Yes	0 (0.0%)
Diagnosis	PreB-ALL	59 (62.1%)
	ProB-ALL	5 (5.3%)
	B-ALL	10 (10.5%)
	T-ALL	21 (22.1%)

2. Laboratory data:

The WBC count ranged from 0.7 to 700 x10^9/L (Median= 9 x10^9/L), hemoglobin level ranged from 2.8 to 13.5 g/dl (mean 8.4 ± 2.18), and platelets count ranged from 6 to 308 x10^9/L (Median= 40 x10^9/L).The range of blasts in PB was from 0% to 98% (Median= 43%), while that of blasts in BM was from 37% to 98% (mean 87.58 ± 13.15). Serum uric acid ranged from 1.4-23 mg/dl (Median = 3.5 mg/dl), while serum LDH ranged from 139 to 8000 mg/dl (Median = 450 mg/dl) as shown in (Table 2).

Table 2: Laboratory data of the examined cases.

WBCS (x 10^9/L)	Median(IQR)	9 (4.9-35)
	Range	0.7 – 700
HB (g/dl)	Mean±SD	8.4 ± 2.18
	Range	2.8 - 13.5
PLT (x 10^9/L)	Median(IQR)	40 (25-60)
	Range	6 – 308
P.B blasts%	Median(IQR)	43 (0-80)
	Range	0 – 98
CSF blasts %	No	95 (100%)
BM blasts %	Mean±SD	87.58 ± 13.15
	Range	37 – 98
URIC ACID (mg/dl)	Median(IQR)	3.5 (2.5-5.5)
	Range	1.4 – 23
LDH (mg/dl)	Median(IQR)	450 (300-750)
	Range	139 – 8000

WBCS: white blood cells; HB: hemoglobin; PLT: platelets; P.B blasts: peripheral blood blasts; CSF blasts: cerebrospinal fluid blasts; BM blasts: bone marrow blasts; LDH: lactate dehydrogenase.

3. Cytogenetic features:

Confirmed cytogenetic data were available for 35/95 patients (36.8 %), of which 10/95 (10.5%) with t (9:22), 7/95 (7.4%) with t (1; 19), 7/95 (7.4%) with t (12:21) and 11/95 (11.5 %) with MLL rearrangement. The other 60(63.2 %) patients were tested for most of the recurrent cytogenetic abnormalities and they revealed negative results as shown in (Table 3).

According to cytogenetic risk groups, 7 out of 35 patients (20%) had good risk, 7(20%) had intermediate risk and 21 (60%) had poor risk as shown in (Table 3).

Table 3: Cytogenetic results of the examined cases.

		No. = 95
BCR/ABL FUSION GENE	Negative	76 (80.0%)
	Positive	10 (10.5%)
	Not done	9 (9.5%)
MLL REARRANGEMENT	Negative	36 (37.9%)
	Positive	11 (11.6%)
	Not done	48 (50.5%)
t(1:19)	Negative	39 (41.1%)
	Positive	7 (7.4%)
	Not done	49 (51.6%)
t(12:21)	Negative	26 (27.4%)
	Positive	7 (7.4%)
	Not done	62 (65.3%)
t(9:22)	Negative	76 (80.0%)
	Positive	10 (10.5%)
	Not done	9 (9.5%)
Cytogenetic risk (no. =35 (36.8%)	Poor	21(60.0%)
	Intermediate	7 (20.0%)
	Good	7 (20.0%)

4. Response to induction of chemotherapy:

Regarding clinical response, out of the 95 newly diagnosed patients, 46(48.4 %) patients achieved complete remission at the 28th day from the beginning of the induction treatment, they were regarded as having good prognosis, while those with partial remission or died following the 28th day from the beginning of the induction treatment, were 49 cases (51.6%), they were considered to have poor prognosis, 27(28.4%) of them died as shown in (Table 4).

Table 4: Response to induction of chemotherapy and percent of mortality.

		Total no.=95
Response to induction of chemotherapy	Poor (incomplete remission, non-remission and death)	49 (51.6%)
	Good	46 (48.4%)
Died patients	No	68 (71.6%)
	Yes	27 (28.4%)

5. Immunophenotyping:

On comparing B-ALL patients and T-ALL patients regarding CD200 expression, CD200 was expressed in 72 (97.3%) B-ALL patients while 13 (61.9%) of T-ALL patients expressed CD200. CD200% was found to be significantly higher in B-ALL patients (Median (IQR) =95(90-97.5)) than T-ALL (Median (IQR) =30(15-88.9)) as shown in (Table 5).

Table 5: Comparison between B-ALL and T-ALL groups regarding flow cytometric analysis.

		B-ALL	T-ALL	Testvalue	*P value*	Sig.
		No.=74	No.=21			
CD200 expression	Median (IQR) Range	95(90– 97.5) 4.8 – 99.6	30(15– 88.9) 1.9 – 96	6.937•	0.000	HS
	Negative Positive	2 (2.7%) 72 (97.3%)	8 (38.1%) 13 (61.9%)	21.756	0.000	HS

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant ;*: Chi-square test; ‡: Mann-Whitney test.

Correlation between CD200 expression and other studied parameters:

There was a statistically significant negative correlation observed between CD200 expression and age (years) (p= 0.008), uric acid (p= 0.007) and platelets count (p= 0.001) while no significant correlation was observed between CD200 expression and the other laboratory parameters. (Table 6).

Table 6: Correlation among CD200 expression and the other examined parameters among ALL cases.

	CD200 expression	
	R	*P-value*
Age (years)	-0.271**	0.008
WBCS (x 10^9/L)	-0.134	0.197
HB (g/dl)	0.093	0.369
PLT (x 10^9/L)	-0.326**	0.001
P.B blasts%	-0.036	0.726
BM blasts %	0.055	0.597
Uric acid (mg/dl)	-0.277**	0.007
LDH (mg/dl)	0.007	0.945

R: Spearman correlation coefficients; P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant WBCS: white blood cells; HB: hemoglobin; PLT: Platelets; P.B blasts: peripheral blood blasts; CSF blasts: cerebrospinal fluid blasts; BM blasts: bone marrow blasts; LDH: lactate dehydrogenase

Relation between CD200 expression, clinical and demographic data:

A statistically significant relation was observed among CD200 expression and splenomegaly (p= 0.041). (Table 7).

Relation between CD200 expression, cytogenetic analysis and response to induction of chemotherapy:

As regards cytogenetic studies, a high statistically significant relation was observed between MLL rearrangement and CD200 expression (p= 0.000), where CD200 expression was found to be higher with MLL rearrangement positive patients. Additionally, a high statistically significant relation was observed among CD200 expression and t (12:21) (p= 0.004), where CD200 expression was lower with t(12:21) positive patients. (Table 7).

As regards response to induction of chemotherapy, a high statistically significant correlation among clinical response and CD200 % (p= 0.000). (Table 7).

Table 7: Relation between CD200 expression and the other examined parameters among ALL cases.

		CD200 expression				
		Median (IQR)	Range	Test-value•	*P-value*	Sig.
Sex	Female	95.6 (90 – 97.5)	4.8 – 98.3	1.872	0.064	NS
	Male	90 (66.6 – 96.1)	1.9 – 99.6			
Fever	No	96.55 (80 – 97.8)	71.7 – 98	0.928	0.356	NS
	Yes	91.3 (80 – 96.3)	1.9 – 99.6			
Lymphadenopathy	No	95.85 (90 – 97.7)	4.8 – 99.3	1.951	0.054	NS
	Yes	90 (62.2 – 95)	1.9 – 99.6			
Hepatomegaly	No	90.55 (71.7 – 96.1)	1.9 – 99.6	-1.617	0.109	NS
	Yes	95 (88.9 – 97.6)	30 – 99.3			
Splenomegaly	No	90 (70 – 96)	1.9 – 99.6	-2.077	0.041	S
	Yes	95 (90 – 97.6)	30 – 99.3			
Bone ache	No	93.9 (88.9 – 97)	10 – 99.6	0.943	0.348	NS
	Yes	90.9 (66.6 – 95.9)	1.9 – 99.4			
Diagnosis	PreB-ALL	95 (90 – 97.6)	57.8 – 99.6	20.923*	0.000	HS
	ProB-ALL	90 (5 – 95.8)	4.8 – 98			
	B-ALL	95.95 (93.1 – 97)	92.1 – 98			
	T-ALL	30 (15 – 88.9)	1.9 – 96			
BCR/ABL FUSION GENE	Negative	90.9 (70 – 96.2)	1.9 – 99.6	1.517	0.133	NS
	Positive	93.7 (90 – 97.6)	80 – 98			
MLL rearrangement	Negative	90 (75.85 - 95.15)	1.9 – 98.3	4.029	0.000	HS
	Positive	98 (97.5 - 99.2)	90 – 99.4			
t(1:19)	Negative	91 (80 97.5)	1.9 – 99.3	0.735	0.462	NS
	Positive	70 (16 98.3)	14 – 99			
t(12:21)	Negative	92.95 (90 - 97.5)	5 – 99.3	3.528	0.000	HS
	Positive	13.9 (4.8 – 62.2)	1.9 – 70			
t(9:22)	Negative	90.9 (70 – 96.2)	1.9 – 99.6	1.517	0.133	NS
	Positive	93.7 (90 – 97.6)	80 – 98			
Response to induction of chemotherapy	Good	80.0 (30- 90)	1.9 – 98	7.299	0.000	HS
	Poor	96.1 (93.9- 98.0)	80.0 – 99.6			
Died patients	No	90.55 (70.85 – 96.55)	1.9 – 99.6	1.197	0.234	NS
	Yes	95 (90 – 97)	4.8 – 99.4			
Cytogenetic risk group	Poor	97.6 (92.1 98)	80 – 99.4	16.620	0.000	HS
	Intermediate	70 (16 98.3)	14 – 99			
	Good	13.9 (4.8 62.2)	1.9 – 70			

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant •: Mann-Whitney test; *: Kruskall-Wallis test

Assessment of CD200 expression in relation to standard prognostic factors in ALL:

ROC curve has been used to evaluate relationship among CD200 expression and prognostic factors, this study revealed that: CD200 expression value of (90) is the best cut off value with specificity (86.96%), sensitivity (93.88%), positive predictive value (PV) of (88.5%), negative predictive value of (93%) and area under the curve (AUC) of (0.861). This cut off value could discriminate among high (>90) and low (<90) expression of CD200 in ALL cases as shown in (Table 8).

Table 8: Prognostic characteristics of CD200 expression in ALL cases using ROC curve.

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
>90	0.861	93.88	86.96	88.5	93.0

AUC: Area under the curve, PV: predictive value

- Demographic and clinical data: age was found higher in cases with low CD200 expression (<90%) than cases with high CD200 expression (>90%) (p=0.013). A statistically significant difference was observed among cases with low CD200 expression (<90%) and cases with high CD200 expression (>90%) regarding lymphadenopathy being more presented in cases with low CD200 expression (<90%) (p=0.038). (Table 9).
- Laboratory data: A statistically significant difference was observed between patients with low CD200 expression (<90%) and patients with high CD200 expression (>90%) regarding platelets count and serum uric acid, where platelets count and serum uric acid were found to be higher in patients with low CD200 expression (<90%. Bone marrow blasts % was higher in cases with high CD200 expression (>90%) (Mean 90 ± 9.52) compared to patients with low CD200 expression (<90%) (Mean 84.65 ± 16.16) (p=0.048). (Table 10).
- Cytogenetic analysis and cytogenetic risk: Regarding cytogenetics, MLL rearrangement was expressed in 10/52 (38.5%) patients with high CD200 expression (>90%) while expressed in 1/43 (4.8%) patients with low CD200 expression (<90%) (p=0.007). Meanwhile, t(12:21) was expressed in 0/52 (0%) patients with high CD200 expression (>90%) while expressed in 7/43 (41.2%) cases with low CD200 expression (<90%) (p=0.004). (Table 11).
- Response to induction of chemotherapy: good responders in patients with

CD200% <90 were higher than patients with CD200% >90 (p=0.002). (Table 11).

Table 9: Relation of CD200 expression to standard prognostic factors in ALL.

		CD200% < 90 No. = 43 (45.3%)	CD200% > 90 No. = 52 (54.7%)	Test-value•	*P-value*	Sig.
Age (years)	Median (IQR)	12 (7 – 14)	7.59 (4 – 12.5)	-2.477≠	0.013	S
	Range	3 – 17	0.29 – 18			
Sex	Female	9 (20.9%)	20 (38.5%)	3.411*	0.065	NS
	Male	34 (79.1%)	32 (61.5%)			
Fever	No	2 (4.7%)	4 (7.7%)	0.368*	0.544	NS
	Yes	41 (95.3%)	48 (92.3%)			
Lymphadenopathy	No	14 (32.6%)	28 (53.8%)	4.325*	0.038	S
	Yes	29 (67.4%)	24 (46.2%)			
Hepatomegaly	No	34 (79.1%)	36 (69.2%)	1.175*	0.278	NS
	Yes	9 (20.9%)	16 (30.8%)			
Splenomegaly	No	34 (79.1%)	32 (61.5%)	3.411*	0.065	NS
	Yes	9 (20.9%)	20 (38.5%)			
Bone ache	No	21 (48.8%)	28 (53.8%)	0.236*	0.627	NS
	Yes	22 (51.2%)	24 (46.2%)			
CNS infiltration	No	43 (100.0%)	52 (100.0%)	–	–	–

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant *: Chi-square test; ≠: Mann-Whitney test

Table 10: Relation of CD200 expression to standard prognostic factors in ALL.

		CD200% < 90 No. = 43 (45.3%)	CD200% > 90 No. = 52 (54.7%)	Test-value•	*P-value*	Sig.
WBCS (x 10^9/L)	Median (IQR)	9 (4.8 – 40)	8.95 (5.3 – 29.7)	-0.097≠	0.923	NS
	Range	0.7 – 700	1.1 – 680			
HB(g/dl)	Mean ± SD	8.69 ± 2.28	8.17 ± 2.08	1.162•	0.248	NS
	Range	4 – 13.4	2.8 – 13.5			
PLT (x 10^9/L)	Median (IQR)	45 (35 – 135)	34.5 (21.5 – 57.5)	-2.402≠	0.016	S
	Range	8 – 300	6 – 308			
P.B blasts %	Median (IQR)	35 (0 – 80)	56 (2.5 – 79.5)	-0.976≠	0.329	NS
	Range	0 – 98	0 – 98			
CSF blasts%	No	43 (100.0%)	52 (100.0%)	–	–	–
BM blasts%	Mean ± SD	84.65 ± 16.16	90 ± 9.52	-2.004•	0.048	S
	Range	40 – 95	37 – 98			
URIC ACID (mg/dl)	Median (IQR)	4.5 (3.5 – 5.9)	3 (2.45 – 4.15)	-2.955≠	0.003	HS
	Range	1.4 – 23	1.5 – 15.5			
LDH (mg/dl)	Mean ± SD	400 (260 – 900)	499.5 (312 – 695)	-0.531≠	0.595	NS
	Range	139 – 6500	151 – 8000			

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant •: Independent t-test; ≠: Mann-Whitney test, WBCS: white blood cells, HB: hemoglobin, PLT: Platelets, P.B blasts: peripheral blood blasts, CSF blasts: cerebrospinal fluid blasts, BM blasts: bone marrow blasts, LDH: lactate dehydrogenase.

Table 11: Relation of CD200 expression to standard prognostic factors in ALL.

		CD200% < 90 No. = 43 (45.3%)	CD200% > 90 No. = 52 (54.7%)	Test-value•	*P-value*	Sig.
Diagnosis	B-ALL	25 (58.1%)	39 (75.0%)	3.044*	0.081	NS
	T-ALL	18 (41.9%)	13 (25.0%)			
BCR/ABL FUSION GENE	Negative	36 (90.0%)	40 (87.0%)	0.193*	0.661	NS
	Positive	4 (10.0%)	6 (13.0%)			
MLL rearrangement	Negative	20 (95.2%)	16 (61.5%)	7.359*	0.007	HS
	Positive	1 (4.8%)	10 (38.5%)			
t(1:19)	Negative	18 (81.8%)	21 (87.5%)	0.287*	0.592	NS
	Positive	4 (18.2%)	3 (12.5%)			
t(12:21)	Negative	10 (58.8%)	16 (100%)	8.362*	0.004	HS
	Positive	7 (41.2%)	0 (0.0%)			
t(9:22)	Negative	36 (90.0%)	40 (87.0%)	0.193*	0.661	NS
	Positive	4 (10.0%)	6 (13.0%)			
Cytogenetic risk group	Poor	5 (31.3%)	16 (84.2%)	12.741	0.002	HS
	Intermediate	4 (25.0%)	3 (15.8%)			
	Good	7 (43.8%)	0 (0.0%)			
Response to induction of chemotherapy	Good	40 (93.0%)	6 (11.5%)	62.574	0.000	HS
	Poor	3 (7.0%)	46 (88.5%)			

P-value > 0.05: Non significant; *P-value* < 0.05: Significant; *P-value* < 0.01: Highly significant *: Chisquare test

DISCUSSION

Acute lymphoblastic leukemia is defined as a clinically and biologically heterogeneous group of diseases characterized by the abnormal proliferation and accumulation of immature lymphoid cells in lymphoid tissues and bone marrow[11].

The results of pediatric ALL have significantly improved within the past 5 decades. Such improvements were made possible by the incorporation of new diagnostic technologies and the effective administration of conventional chemotherapeutic agents[12].

The prognosis and treatment response are influenced by several clinical and biochemical factors that exist at the time of presentation. These prognostic markers involve immunological phenotype, white blood cell (WBC) count, extramedullary involvement (EMI), cytogenetic abnormalities, platelet count, number of blasts and age[13].

CD200 is a type-1 cell membrane glycoprotein of the immunoglobulin supergene family, found in both cells with myeloid/lymphoid origin and on epithelial cells and numerous cancer cells. Although CD200/CD200R can reduce inflammation, it can also, unfortunately, hinder the body's natural ability to fight malignancies[14].

Objective of the present study is to provide clarification of the significance of blast cell CD200 expression as a prognostic factor for ALL. The significance of CD200 expression was investigated in relation to various clinical, laboratory and standard prognostic factors and to response to chemotherapy.

The age of selected patients during the period of the study ranged from 100 days to 18 years. The patients in the present study were 66(69.5 %) males and 29(30.5%) females with male to female ratio 2.2:1. This was in concordance with previous studies by ***Salah et.al (2018)***, ***Alwan and Al-Mudallel (2021)*** & ***Khalil et al. (2023)*** who confirmed a male predominance in ALL patients[5,10,15].

Among the 95 newly diagnosed ALL cases; 25 cases (26.3%) had hepatomegaly and 29 cases (30.5%) had splenomegaly, likely a previous study done by ***Alwan and Al-Mudallel(2021)*** detected hepatosplenomegaly in 63.33% of their patients[5].

The present research detected lymphadenopathy in 53(55.8%) patients. This is inconsistent with a previous study done by by ***Alwan and Al-Mudallel (2021)*** which found that 14 (46.67%) patients were presented with lymphadenopathy[5].

Our study showed that 89 cases (93.7%) were presented with fever and 46 (48.4%) were presented with bone aches which was higher than the study done by ***Alwan and Al-Mudallel(2021)*** which showed that 22 (73.33%) patients were presented with fever and 7 (23.33%) patients were presented with bone aches[5].

Regarding CD200 expression, 85 (89.5%) of ALL patients were positive. ***Aref et al. (2017)*** found that 28 of 43 acute lymphoblastic leukemia cases (65%) demonstrated CD200 positive expression[16]. The study done by ***Alwan and Al-Mudallel (2021)*** showed that majority of patients (80%) were positive for CD200[5].

Regarding demographic and clinical data, we observed a statistically significant correlation between CD200 expression, age (years) (p= 0.008) and splenomegaly (p= 0.041) where statistically insignificant relation was observed among CD200 expression and other clinical data. In contrast, the research performed by ***Aref et al. (2017)*** demonstrated statistically insignificant correlation between CD200 expression and any of the clinical data[16].

Regarding laboratory data, our research revealed a significant variance among CD200% expression, serum uric acid (p= 0.007) and platelets count (p= 0.001) being low in CD200 positive patients, while statistically insignificant relation found among CD200% expression and other laboratory data. Likely the study done by ***Aref et al. (2017)*** showed that platelets count was less in CD200 positive cases than CD200 negative ones[16].

Regarding cytogenetic analysis, in our research, a high statistically significant correlation was observed between CD200% expression and MLL rearrangement (KMT2A) (11q23) being higher in percentage in MLL rearrangement positive cases with a median (IQR) 98% (97.5% - 99.2%) than MLL rearrangement negative patients with a median (IQR) 90% (75.85% - 95.15%). Also a high statistically significant correlation among CD200% and t(12:21) (ETV6-RUNX1) was found, being lower in percentage in t(12:21) positive cases with a median (IQR) 13.9% (4.8 %– 62.2%) than t(12:21) negative cases with a median (IQR) 92.95% (90% - 97.5%) (p= 0.000). On the contrary, the study done by ***Smith et al. (2011)*** showed that cases with t(12:21) (ETV6-RUNX1) were associated with higher prevalence of CD200 expression[17].

In our research, 43 cases showed lower CD200% expression (<90%), 16 patients of them had positive

cytogenetic analysis. Five out of sixteen had unfavorable chromosomal aberrations, 4 had intermediate chromosomal aberrations and the other 7 cases had favorable chromosomal aberrations. Higher CD200% expression (> 90%) was shown in 52 cases, 19 cases of them had positive cytogenetic analysis. Sixteen cases out of nineteen had unfavorable chromosomal aberrations and 3 cases had intermediate chromosomal aberrations while none of the cases had favorable chromosomal aberrations. So the unfavorable chromosomal aberrations were higher in cases with higher CD200 expression (>90%) than cases with lower CD200 expression (<90%). (p=0.002)

The research performed by ***Aref et al. (2017)*** demonstrated that the examined pediatric acute lymphoblastic leukemia cases group with CD200 expression was categorized into the following subgroups: 20 (58.8%) favorable risk; 12 (35.3%) intermediate risk, and 2 (5.9%) unfavorable risk. Despite of that no significant association was found between CD200 expression and cytogenetic categories in the cases studied, may be due to smaller sample size[16].

In our study, regarding outcome of the examined cases and CD200% expression, A high statistically significant correlation was observed among CD200% and response to induction of chemotherapy, where higher CD200 % was associated with poorer response to induction of chemotherapy (p=0.000).

In contrast, the study done by ***Alwan and Al-Mudallel (2021)*** showed insignificant correlation between CD200 expression and the response to induction therapy, might be because of smaller sample size and inclusion of B-ALL patients only in their study, while ***Aref et al. (2017)*** declared that CD200 positive expression in acute lymphoblastic leukemia at diagnosis is poor prognostic biomarker and is correlated with less remission rate[5,16].

CONCLUSION AND RECOMMENDATIONS

From this study, we conclude that CD200 is a bad prognostic factor, and for the prediction of poor response to chemotherapy, since CD200 positive patients are more liable to achieve poor response to therapy.

We recommend:

- Further studies on a wide scale of ALL patients for accurate assessment of incidence and prognostic value of CD200 expression.

Therefore, routine screening of CD200 using flow cytometry should be done at diagnosis of ALL to detect possible prognosis & disease outcome.

DECLARATION OF INTEREST AND FUNDING INFORMATION

The authors report no conflicts of interest.

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There are no financial disclosure.

AUTHOR CONTRIBUTION

We declare that all listed authors have made substantial contributions to all of the following three parts of the manuscript:

- Research design, or acquisition, analysis or interpretation of data.
- Drafting the paper or revising it critically.
- Approving the submitted version.

We also declare that no-one who qualifies for authorship has been excluded from the list of authors.

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الاثار التنبؤية لوجود سي دي ٢٠٠ في سرطان الدم الليمفاوي الحاد لدي الاطفال

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الخلفية وهدف الدراسة: إن سرطان الدم الليمفاوي الحاد هو اضطراب خبيث للخلايا الليمفاوية البدائية التي تتكاثر وتحل محل خلايا الدم الطبيعية في النخاع العظمي مما يؤدي إلي انخفاض ملحوظ في انتاج خلايا الدم الطبيعية. سي دي ٢٠٠ هو غلايكوبروتين مرسل على سطح الخلية يوجد علي سطح الخلايا الليمفاوية و النقوية بالاضافة إلي الخلايا البطانية و الكثير من الخلايا السرطانية. تهدف الدراسة الحالية إلى توضيح اهمية وجود سي دي ٢٠٠ علي سطح الخلايا السرطانية كعامل تنبؤي او استشرافي لسرطان الدم الليمفاوي الحاد.

المواد والطرق: تم إجراء هذه الدراسة على ٩٥ من المرضى الاطفال الذين شُخِّصوا حديثاً بسرطان الدم الليمفاوي الحاد بوحدة أمراض الدم بمستشفيات جامعة عين شمس، في الفترة من سبتمبر ٢٠٢٢ إلى فبراير ٢٠٢٣.

النتائج: وفيما يتعلق بالبيانات الديمغرافية والإكلينيكية، اقترن تعبير و وجود سي دي ٢٠٠ بصغر السن و تضخم الطحال. وفيما يتعلق بالبيانات المختبرية، كشفت دراستنا فرقاً كبيراً بين نسبة سي دي ٢٠٠ وعدد الصفائح الدموية بالاضافة الي مستوي حامض اليوريك في الدم، حيث انهم وجدو بمستوي منخفض في المرضى الإيجابيين سي دي ٢٠٠.وفيما يتعلق بالتصنيف الطبقي للمخاطر السيتوجينية، كانت الاضطرابات الكروموسومية الغير مواتية أعلى في المرضى الذين لديهم نسبة أعلى من سي دي ٢٠٠ (>٩٠٪) بالمقارنة مع المرضى الذين لديهم نسبة منخفضة من سي دي ٢٠٠ (<٩٠٪). وفيما يتعلق بنتائج المرضي الذين شملتهم الدراسة و نسبة وجود سي دي ٢٠٠، ارتبطت النسبة الاعلي لسي دي ٢٠٠ بالاستجابة الضعيفة للعلاج الكيماوي.

الخلاصة: واخيرا تشير هذة الاستنتاجات الي ان التعبير الايجابي ل سي دي ٢٠٠ يعطي تشخيصا ضعيفا لمرضي سرطان الدم الليمفاوي الحاد. و نستنتج من هذة الدراسة ان سي دي ٢٠٠ عامل تشخيصي سيء و ايضا للتنبؤ بالاستجابة الضعيفة للعلاج الكيماوي، حيث ان المرضي الايجابيين لسي دي ٢٠٠ اكثر عرضة للاستجابة الضعيفة للعلاج.