

Prognostic Impact of CD200 Expression in Pediatric B-cell Acute Lymphoblastic Leukemia

Mustafa Jassim Alwan, Subh Salim Al-Mudallal¹

Department of Pathology, Laboratories of Al-Khadmia Teaching Hospital, ¹Pathology Department, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous disease in which immature lymphoid cells proliferate mostly in bone marrow, peripheral blood, and other organs. Flow-cytometric immunophenotyping in childhood ALL assists in the diagnosis and subclassification of B- and T-lineages, as well as predicting outcomes of the disease. CD200 expression has many diagnostic and potentially prognostic implications in the flow-cytometric evaluation of lymphoid malignancies. **Aim of Study:** The aim of this study was to evaluate the expression of CD200, correlate its expression with hematological and clinical parameters, and assess patient's response to induction of chemotherapy in the newly diagnosed *de novo* pediatric B-cell ALL. **Materials and Methods:** This prospective cross-sectional study was conducted on 30 pediatric patients (<15 years) with newly diagnosed *de novo* B-cell ALL. Morphology, cytochemistry, and flow cytometry (FCM) of the peripheral blood and/or bone marrow were performed for all patients and the patients were re-evaluated morphologically at day 28 from the start of chemotherapy for assessment of complete remission achievement. **Results:** Majority of the patients (80%) had a positive expression of CD200. All patients were in high-risk group and had positive CD200 expression and majority of the them were responded to induction therapy. **Conclusion:** CD200 is frequently expressed and closely related to the high-risk groups; however, it cannot be considered as an independent poor prognostic marker as not all cases with positive CD200 had low response to induction therapy.

Keywords: Acute lymphoblastic leukemia, flow cytometry, high risk

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disorder that develops first from proliferation of immature lymphoid cells in a single B- or T-lymphocyte progenitor cell as a result of sequential somatic mutations in a single lymphoid progenitor cell during any step of normal lymphoid development.^[1] ALL is the most common type of cancer and most common subtype of leukemia in children. On the contrary, childhood ALL is not a single disorder with a wide range of phenotypic and genotypic heterogeneity. Furthermore, because of this biological heterogeneity, there is an increasing need to classify patients into risk groups and to provide risk-adapted treatment.^[2]

The use of flow cytometry for immunophenotyping of childhood ALL aids in the diagnosis and classification of B-ALL and T-ALL lineages. It is also helpful in predicting the disease's outcome and monitoring its

progression. Children with ALL show a wide range of surface differentiation antigens on leukemic lymphoblasts that may also be present on normal lymphoid precursors at various stages of maturation.^[3]

CD200 (OX-2 antigen) is a type I immunoglobulin superfamily transmembrane cell surface glycoprotein encoded by a gene on chromosome 3 (OX-2 gene). It is found in a variety of cell types, like B cells, T cells, dendritic cells, endothelial cells, and peripheral and central nervous system cells.^[4,5]

Address for correspondence: Dr. Mustafa Jassim Alwan,
Department of Pathology, Laboratories of Al-Khadmia Teaching Hospital,
Baghdad, Iraq.
E-mail: mja89hmp@yahoo.com

Submission: 12-July-2021 **Accepted:** 24-Aug-2021 **Published:** 17-Dec-2021

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Jassim Alwan M, Al-Mudallal SS. Prognostic impact of CD200 expression in pediatric B-cell acute lymphoblastic leukemia. Med J Babylon 2021;18:358-63.

Access this article online

Quick Response Code:



Website:
www.medjbabylon.org

DOI:
10.4103/MJBL.MJBL_50_21

CD200 interacts with CD200 receptor (CD200R), a type I membrane glycoprotein and an inhibitory receptor of the immunoglobulin superfamily that suppresses T-cell-mediated immune responses.^[6,7] Although the distribution of CD200 expression is widely distributed, CD200R is mainly expressed in myeloid and lymphoid cells.^[6,8]

CD200–CD200R interactions are involved in modulated cell-mediated immunity, delivering a signal that is inhibitory to the immune system and cells of the hematopoietic lineage, especially myeloid cells, as well as fetal loss, transplant rejection, autoimmunity, and inhibition of tumor-specific T-cell immunity.^[7,9,10]

As a result, CD200 has been linked to tumor progression in a number of carcinomas and is a poor prognostic marker in many hematological malignancies.^[5] However, CD200 plays a critical role in immune tolerance, which aims to protect stem cells and other vital tissues from immune attack.^[11] Many hematolymphoid neoplasms express CD200 in varying degrees, so its expression has many diagnostic and potentially prognostic implications in the flow-cytometric evaluation of lymphoid malignancies.^[12]

MATERIALS AND METHODS

A prospective cross-sectional study was conducted from October 2018 to July 2019 in the Central Teaching Hospital of Pediatrics, designed to include 30 patients aged less than 15 years with newly diagnosed *de novo* B-cell ALL.

The diagnosis of ALL was based on routine morphological assessment of the stained peripheral blood (PB) and bone marrow (BM) smears and confirmed by cytochemical stains in the Laboratory of Central Teaching Hospital of Pediatrics. For further confirmation and characterization of the cases, flow-cytometric immunophenotyping using a panel of well-characterized monoclonal antibodies was done at Flow cytometry Unit in Medical City and at Private Laboratory, Baghdad. By using a questionnaire

form, data of the main signs and symptoms as well as hematological parameters were obtained from patient's case file. The inclusion criteria of the study included the following:

1. Patients who were *de novo* newly diagnosed with ALL.
2. Patients with B-ALL.
3. Patients who were less than 15 years old.
4. Patients who were randomly collected in relation to gender.

From each patient included in this study, 2 mL of PB sample and at least 1 mL of BM aspirate were both collected in two ethylene diamine tetra-acetic acid (EDTA) tubes, and examined for PB and BM smears after staining with Leishman stain and special cytochemical stains including Periodic Acid Schiff (PAS) and Sudan Black B (SBB) stains. At least, 1 mL from the aspirated anticoagulated marrow or PB sample was sent to flow-cytometry unit of the Medical City for confirmation of the diagnosis and subtyping of ALL patients into B- or T-lineage ALL. Then fresh or left over sample from established B-ALL cases was collected for flow-cytometry study in a private laboratory to examine the expression of CD200 surface marker on B-lymphoblast and this should be performed within 6–8 h of collecting the sample.

For the assessment of remission induction, first of all the patients were categorized into high-risk (HR) and standard-risk (SR) groups according to their age and initial WBC count at presentation (risk stratification). All patients were re-evaluated morphologically for achievement of complete remission at the end of the induction phase (day 28).

For statistical analysis, data were summarized, analyzed, and presented using two software programs; SPSSR Software (version 23.0 for LinuxR) and Microsoft Office Excel 2010 were used to perform the statistical analysis for this study. Qualitative data were represented as numbers and percentages, whereas numerical data were represented

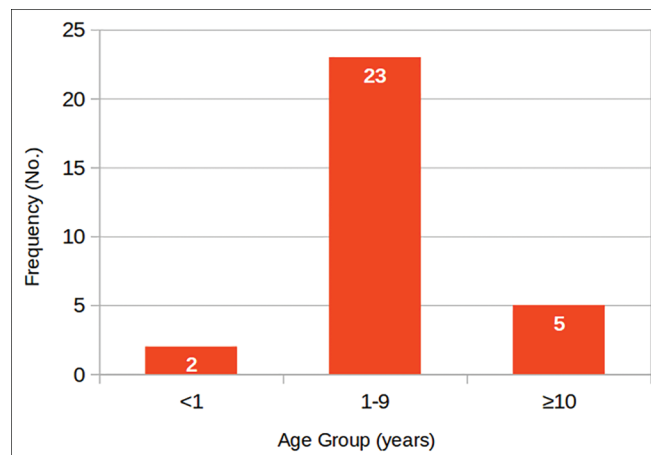


Figure 1: Age group distribution among study sample

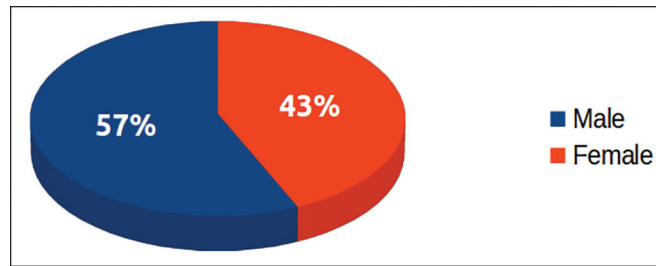


Figure 2: Gender distribution among study sample

Table 1: Physical signs and symptoms among study participants

Signs and symptoms	Frequency	Percentage (%)
Pallor	25	83.33
Fever	22	73.33
Fatigue	20	66.66
Hepatosplenomegaly	19	63.33
Lymphadenopathy	14	46.67
Bruising	11	36.66
Bone pain	7	23.33

*Patients had more than one sign and symptom

as mean $\pm$ standard deviation (SD). A value of $P < 0.05$ was considered statistically significant.

RESULTS

This study was conducted on 30 pediatric B-ALL patients, with majority of patient's age between (1–10 years) and a mean age of (5.63 ± 3.58) as shown in Figure 1.

Men comprised 17 of 30 cases (56.67%) of the study samples, whereas women comprised the remaining 13 cases (43.33%), with male-to-female (M:F) ratio of 1.3:1 as shown in Figure 2.

Pallor was the most common physical sign, which was present in more than 83% of the cases, followed by fever in 73% of cases. The remaining signs and symptoms are summarized in Table 1.

The majority of patients had an initial WBC count of less than $50 \times 10^9/L$, and this is an important marker in the risk group classification. The remaining hematological parameters are described in Table 2.

Highest proportion (73.33%) of patients had positive PAS stain. Approximately 43.33% of them had blocks and granular positivity, whereas 30% had positive findings with granules only. Approximately 26.66% of patients had negative PAS Stain. Majority of patients (86.67%) were CD10-positive precursor B-ALL; these were mostly categorized as common precursor B-ALL. Approximately two-thirds of the cases (60%) had SR, whereas the remaining (40%) had high risk (HR).

CD200 expression was positive in majority of patients (80%), whereas the remaining (20%) had a negative result.

[Figures 3 and 4] show an example of positive and negative flow-cytometric Dot Plot results, respectively.

Fisher exact test was used to assess the correlation between CD200 and certain variables. Regarding NCI risk group, the two-tailed Fisher exact P -value was insignificant although it was borderline ($P = 0.057$). However, one-tailed P -value was statistically significant ($P = 0.031$), as detailed in Table 3.

Regarding the assessment of remission to induction, majority of the patients (90%) were responded to induction therapy, and most of the respondents were expressed CD10 and had PAS stain positive. Only 3 of 30 cases (10%) did not respond to therapy; all of them were expressed CD200, as shown in Tables 4–6.

DISCUSSION

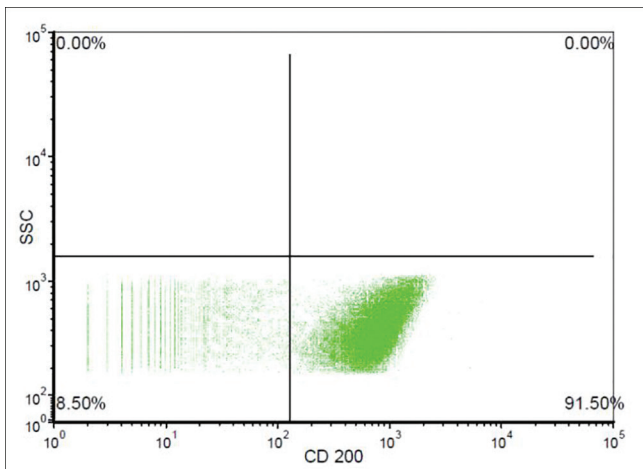
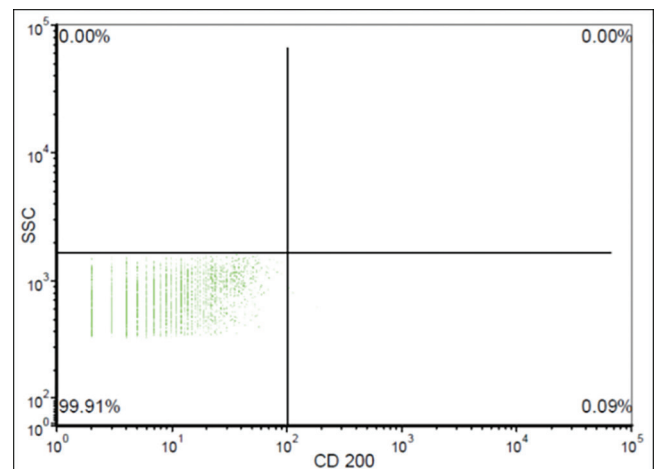
The mean age of all patients included in this study was 5.6 ± 3.58 SD. These results were comparable to Iraqi and non-Iraqi studies.^[13-15] Concerning the age interval, majority of patients (76 %) were within the age group 1–9 years. This was comparable with Iraqi and non-Iraqi studies,^[15-17] knowing that this age group was within favorable age group (< 10 years). Regarding the gender, men were more frequently affected than women with M:F ratio of 1.3:1. This ratio was close to many Iraqi and non-Iraqi studies.^[15,18-20]

Pallor and fever were the most common clinical presentation. These were in agreement with Al-Mudallal *et al.*^[15] and Al-Mulla *et al.*,^[21] whereas it was not in line with Jaime *et al.*^[17] and Harpani *et al.*^[19] studies, which showed a different distribution of the clinical signs and symptoms.

This study was in concordance with many studies that focus on the importance of initial WBC count as a continuous prognostic variable in determining the risk strategies in B-lineage ALL. In this study, 83% of patients had an initial WBC count of less than $50 \times 10^9/L$. This result clarifies that majority of B-ALLs patients had WBC counts ranging within favorable limits ($< 50 \times 10^9/L$). This finding was also reported by Al-Mudallal *et al.*^[15] and Supriyadi *et al.*,^[22] whereas it was not in line with Bachir *et al.*^[16] Moreover, the mean of total WBC count in this study was 27.35 ± 22.09 SD and was close to Abdalla *et al.*'s^[23] results.

Table 2: Hematological parameters of study participants

Parameters	Class	Frequency	Percentage (%)	Mean $\pm$ SD
Hb (g/dL)	<7	17	56.67	7.02 $\pm$ 1.56
	7–11	13	43.33	
Platelet count ($\times 10^3$ / μ L)	<100	24	80.00	69.99 $\pm$ 67.78
	≥ 100	6	20.00	
WBC ($\times 10^3$ / μ L)	<50	25	83.33	27.35 $\pm$ 22.09
	≥ 50	5	16.67	
PB blasts	≤ 25	4	13.33	60.63 $\pm$ 28.15
	26–50	6	20.00	
	51–75	7	23.33	
	76–100	13	43.33	
BM blasts	≤ 25	–	–	88.27 $\pm$ 11.47
	26–50	–	–	
	51–75	4	13.33	
	76–100	26	86.67	

**Figure 3: Positive CD200 expression****Figure 4: Negative CD200 expression****Table 3: CD200 relation with NCI risk group**

Risk group	CD200 status		Total	P Value	
	+ve	–ve		Two-tailed	One-tailed
Standard risk (SR)	12 (66.67%)	6 (33.33%)	18 (100%)	0.057	0.031*
High risk (HR)	12 (100%)	–	12 (100%)		
Total	24 (80.00%)	6 (20.00%)	30 (100%)		

*Significant at $P < 0.05$ **Table 4: Response to therapy in relation to CD10 expression**

Response to induction therapy	B-ALL		Total
	CD10 +ve	C10 –ve	
Response	25 (92.6%)	2 (7.4%)	27 (100%)
No response	1 (33.34)	2 (66.66%)	3 (100%)
Total	26 (86.7%)	4 (13.3%)	30 (100%)

The patients were classified according to NCI criteria, where they were stratified into SR and HR groups. Forty percent of patients were within HR group and 60% of patients were within SR; these results were in agreement with Uckun *et al.*'s^[24] study.

CD10 (CALLA) status

Majority of patients were CALLA (CD10) positive precursor B-ALL. These were mostly categorized as common precursor B-ALL. CD10 expression was shown in 86% of the cases. This result was comparable

Table 5: Response to induction therapy relation with PAS stain

PAS stain	Induction therapy		Total	P Value
	Response	No response		
Negative	5 (62.50%)	3 (37.50%)	8 (100%)	0.014*
Positive	22 (100%)	–	22 (100%)	
Total	27 (90.00%)	3 (10.00%)	30 (100%)	

*Significant at $P < 0.05$ **Table 6: Response to induction therapy relation with CD200 expression**

Response to induction therapy	CD200 status		Total	P Value
	+ve	–ve		
Response	21 (77.78%)	6 (22.22%)	27 (100%)	0.999
No response	3 (100%)	–	3 (100%)	
Total	24 (80.00%)	6 (20.00%)	30 (100%)	

with Abbasi *et al.*^[25] and Bachir *et al.*,^[16] which showed 90% and 91.2% of B-ALL cases expressed CD10, respectively.

CD200 expression

Concerning the aberrant expression of CD200, majority of patients (80%) were positive for CD200, which is in agreement with Awad *et al.*'s^[26] study that revealed 80.3% of their results were CD200 positive. However, Aref *et al.*'s^[27] study revealed that CD200 expressed in 65.1% of pediatric B-ALL group and it was much less expressed in Themhare *et al.*'s^[28] study that showed only 28.9% of B-cell precursor ALL expressed CD200.

Regarding the correlation between CD200 expression and NCI risk groups, there was a significant correlation between them as all the patients who were in HR group express CD200 marker.

In this study, no significant correlation was found between CD200 expression and the response to induction therapy; however, the only three patients who failed response to remission therapy were CD200 positive and this concept is close to Aref *et al.*'s^[27] study, which showed that 9 of 10 cases who did not respond to remission therapy were CD200 positive.

Periodic acid schiff stain

In this study, 73% of patients showed positive PAS stain. This result was approximately similar to Lilleyman *et al.*'s^[29] study, which showed 75% positivity for PAS stain, and it is also in line with Belurkar *et al.*'s^[30] study, which showed 66.6% positivity for PAS stain.

Assessment of the relation between PAS stain and response to induction therapy revealed a good association, as all cases with positive PAS reaction had

complete remission induction therapy. Furthermore, the only three cases who did not respond to induction therapy were PAS negative.

About the relation of PAS stain with the CD10 (CALLA) expression status, there was a significant relation between them. Majority of the cases (95 %) with positive PAS stain had positive CD10 expression.

Knowing that some of studies concluded that strong PAS reaction particularly with block positivity is a feature of hyperdiploidy and common ALL, and predicts better response to current treatment in children with B-ALL, but not independently of other cell characteristics.^[31,32] Similar studies concluded that CD10 (CALLA)-positive expression was associated with several favorable presenting features but also not an independent prognostic factor.^[33,34] Thus, we may propose that CD10 expression and PAS positivity may serve as surrogate parameters in childhood B-ALL.

CONCLUSIONS

CD200 marker is frequently expressed in pediatric B-ALLs and its expression was closely related to the HR groups according to NCI criteria. Although all cases with low remission rate were CD200 positive, CD200 expression cannot be considered as an independent poor prognostic marker as not all cases with positive CD200 had low response to induction therapy. However, CD10 expression and positivity results of PAS stain could be of auxiliary prognostic parameters in pediatric B-ALL.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Larson RA. Acute lymphoblastic leukemia. In: Kaushansky K, *et al* editor. Williams Hematology. 9th ed. China: The McGraw-Hill Companies; 2016. p. 1505-27.
- Conter V, Rizzari C, Sala A, Chiesa R, Citterio M, Biondi A. Acute lymphoblastic leukemia. Orphanet Encyclopedia 2004;14:1-13.
- Iwamoto S, Deguchi T, Ohta H, Kiyokawa N, Tsurusawa M, Yamada T, *et al.* Flow cytometric analysis of *de novo* acute lymphoblastic leukemia in childhood: Report from the Japanese pediatric leukemia/lymphoma study group. Int J Hematol 2011;94:185-92.
- McCaughan GW, Clark MJ, Hurst J, Grosveld F, Barclay AN. The gene for MRC OX-2 membrane glycoprotein is localized on human chromosome 3. Immunogenetics 1987;25:133-5.
- Dorfman DM, Shahsafaei A. CD200 (OX-2 membrane glycoprotein) expression in b cell-derived neoplasms. Am J Clin Pathol 2010;134:726-33.
- Minas K, Liversidge J. Is the CD200/CD200 receptor interaction more than just a myeloid cell inhibitory signal? Crit Rev Immunol 2006;26:213-30.

7. Walker DG, Lue LF. Understanding the neurobiology of CD200 and the CD200 receptor: A therapeutic target for controlling inflammation in human brains?. *Future Neuro* 2013;8:321-32.
8. Holmannová D, Koláčková M, Kondělková K, Kunes P, Krejsek J, Andrýs C. CD200/CD200R paired potent inhibitory molecules regulating immune and inflammatory responses; part I: CD200/CD200R structure, activation, and function. *Acta Medica (Hradec Kralove)* 2012;55:12-7.
9. Gorczynski RM, Chen Z, Hu J, Kai Y, Lei J. Evidence of a role for CD200 in regulation of immune rejection of leukaemic tumour cells in C57BL/6 mice. *Clin Exp Immunol* 2001;126:220-9.
10. Coles SJ, Wang EC, Man S, Hills RK, Burnett AK, Tonks A, *et al.* CD200 expression suppresses natural killer cell function and directly inhibits patient anti-tumor response in acute myeloid leukemia. *Leukemia* 2011;25:792-9.
11. Wright GJ, Jones M, Puklavec MJ, Brown MH, Barclay AN. The unusual distribution of the neuronal/lymphoid cell surface CD200 (OX2) glycoprotein is conserved in humans. *Immunology* 2001;102:173-9.
12. Alapat D, Coviello-Malle J, Owens R, Qu P, Barlogie B, Shaughnessy JD, *et al.* Diagnostic usefulness and prognostic impact of CD200 expression in lymphoid malignancies and plasma cell myeloma. *Am J Clin Pathol* 2012;137:93-100.
13. Barazanchi ZA, Al-Sami AK, Naema NF. Hematological and cytomorphological study of Acute Lymphoblastic Leukemia (ALL). *Bahrain Med Bull* 2005;27:175-9.
14. Kostić G, Đurić Z, Bunjevački G. Bone changes, mineral homeostasis in childhood acute lymphoblastic leukemia. *Med Biol* 2004;11:123-6.
15. Al-Mudallal SS, Shakir H, Dede H. Assessment of expression and prognostic significance of interleukin 3 receptor alpha subunit (CD123) in childhood acute lymphoblastic leukemia. *Int J Med Res Prof* 2016;2:247-54.
16. Bachir F, Bennani S, Lahjouji A, Cherkaoui S, Harif M, Khattab M, *et al.* Characterization of acute lymphoblastic leukemia subtypes in Moroccan children. *Int J Pediatr* 2009;2009:674801.
17. Jaime-Pérez JC, García-Arellano G, Herrera-Garza JL, Marfil-Rivera LJ, Gómez-Almaguer D. Revisiting the complete blood count and clinical findings at diagnosis of childhood acute lymphoblastic leukemia: 10-year experience at a single center. *Hematol Transfus Cell Ther* 2019;41:57-61.
18. Kashmoola MA, Abdul-Ameer SJ, Gzeer LF. Chromosomal Changes in childhood acute lymphoblastic leukemia in Mosul. *J Med J* 2011;45:190-4.
19. Harpani PT, Parmar BJ, Makwana AM. Clinicopathological profile of acute leukemia in children. *J Nepal Paediatr Soc* 2012;32:95-8.
20. Frost BM, Forestier E, Gustafsson G, Nygren P, Hellebostad M, Jonsson OG, *et al.* Translocation t(12;21) is related to *in vitro* cellular drug sensitivity to doxorubicin and etoposide in childhood acute lymphoblastic leukemia. *Blood* 2004;104:2452-7.
21. Al-Mulla NA, Chandra P, Khattab M, Madanat F, Vossough P, Torfa E, *et al.* Childhood acute lymphoblastic leukemia in the middle east and neighboring countries: A prospective multi-institutional international collaborative study (CALLME1) by the middle east childhood cancer alliance (MECCA). *Pediatr Blood Cancer* 2014;61:1403-10.
22. Supriyadi E, Veerman A J, Sutaryo I. Detection of CD10, CD34 and their combined expression on Childhood Acute Lymphoblastic Leukemia and the association with clinical outcome in Indonesia. *J Cancer Ther Res* 2012;1:1-10.
23. AbdAlla HA, Humeida AA, Abbass E, Altayeb OA, Marghani GM. The role of kappa and lambda in subclassification of B cell lymphoblastic leukemia in Sudanese patients using flow cytometry. *J Blood Dis* 2016;6:44.
24. Uckun FM, Sensel MG, Sun L, Steiner PG, Trigg ME, Heerema NA, *et al.* Biology and treatment of childhood T-lineage acute lymphoblastic leukemia. *Blood* 1998;91:735-46.
25. Abbasi N, Kamal N, AL-Kaisi N. Immunophenotypic profile of acute leukemia cases using multicolor flow cytometry: Three year experience at King Hussein Medical Center. *JRMS* 2015;22: 53-8.
26. Adnan Awad S, Kamel MM, Ayoub MA, Kamel AM, Elnoshokaty EH, El Hifnawi N. Immunophenotypic characterization of cytogenetic subgroups in Egyptian pediatric patients with B-cell acute lymphoblastic leukemia. *Clin Lymphoma Myeloma Leuk* 2016;16 Suppl:S19-S24.e1.
27. Aref S, Azmy E, El-Bakry K, Ibrahim L, Abdel Aziz S. Prognostic impact of CD200 and CD56 expression in pediatric B-cell acute lymphoblastic leukemia patients. *Pediatr Hematol Oncol* 2017;34:275-85.
28. Tembhare PR, Ghogale S, Ghatwai N, Badrinath Y, Kunder N, Patkar NV, *et al.* Evaluation of new markers for minimal residual disease monitoring in B-cell precursor acute lymphoblastic leukemia: CD73 and CD86 are the most relevant new markers to increase the efficacy of MRD 2016; 00B: 000-000. *Cytometry B Clin Cytom* 2018;94:100-11.
29. Lilleyman JS, Britton JA, Anderson LM, Richards SM, Bailey CC, Chessells JM. Periodic acid Schiff reaction in childhood lymphoblastic leukaemia. *J Clin Pathol* 1994;47:689-92.
30. Belurkar S, Mantravadi H, Manohar C, Kurien A. Correlation of morphologic and cytochemical diagnosis with flowcytometric analysis in acute leukemia. *J Cancer Res Ther* 2013;9:71-9.
31. Lilleyman JS, Scott CS. PAS and acid phosphatase cytochemistry in acute lymphoblastic leukaemia. In: Scott CS, editor. *Leukaemia Cytochemistry: Principles and Practices*. Chichester: Ellis Horwood; 1989. p. 103-20.
32. Hann IM, Evans DIK, Palmer MK, Morris-Jones PJ, Haworth C. The prognostic significance of morphological features in childhood lymphoblastic leukaemia. *Clin Lab Haematol* 1979;1: 215-26.
33. Consolini R, Legitimo A, Rondelli R, Guguelmi C, Barisone E, Lippi A, *et al.* Clinical relevance of CD10 expression in childhood ALL: The Italian Association for Pediatric Hematology and Oncology (AIEOP). *Haematologica* 1998;83:967-73.
34. Pui CH, Evans WE. Acute lymphoblastic leukemia. *N Engl J Med* 1998;339:605-15.