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Flow cytometric evaluation of the CD34 marker as a prognostic factor in pediatric acute lymphoblastic leukemia, Baghdad 2021

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Abstract
Background: Leukemia is a heterogeneous group of hematological malignancies. It represents over one-third of all cancers affecting young children. CD34 marker is a cell surface glycoprotein that functions as a marker for identifying and isolating certain types of cells, particularly hematopoietic stem and progenitor cells. The absence of CD34 expression is highly suggestive of acute promyelocytic leukemia. This study aimed to evaluate the expression of CD34 in pediatric acute lymphoblastic leukemia and its correlation with clinical outcomes.
Methods: A cross-sectional study was conducted in the Oncology Department of Children's Welfare Teaching Hospital in Medical City/ Baghdad for 6 months, in 2021. A total of 50 children aged ≤ 12 years diagnosed with ALL were recruited. A structured questionnaire was designed for this study, including symptoms, physical signs, and hematological parameters. A blood film, bone marrow aspiration, and flow cytometry were done for all recruited children.
Results: The rate of positive CD34 was significantly higher among children aged < 5 years (75.7%, P=0.035), children who were classified with standard risk for ALL (64.9%, P=0.033), those who had B-ALL (89.2%, P=0.006), and children who had no CNS involvement (100%, P=0.001). The mean WBC count was significantly lower in children who had CD34 positive ALL than that in CD34 negative ALL. On the other hand, there was no significant association between CD34 expression and patients' gender, FAB classification, and response to the induction therapy.
Conclusions: The rate of positive CD34 was significantly higher among children aged < 5 years, children who were classified with standard risk for ALL, and those who had B-ALL. Further, children with CD34 positive ALL had a significantly lower mean WBC count compared to those with CD34 negative ALL. Evaluation of the co-expression of CD34 with CD10, growth fraction, multi-drug resistance, DNA index, and cytogenetic abnormalities with CD34 expression are recommended.

Keywords: Leukemia, CD34, oncology, flow Cytometry

Introductions

Leukemia is a heterogeneous group of hematological malignancies. It results from the proliferation of uncontrolled immature hematopoietic cells [1]. It represents over one-third of all cancers affecting young children. Depending on the cell type involved, leukemia can be divided into different subtypes that differ in prevalence, treatment strategy, and outcome. The most common leukemia in children is acute lymphoblastic leukemia (ALL), which accounts for nearly 70% of cases. It is characterized by high cure rates and good treatment outcomes [2, 3].

Attempts have been made to classify ALL cells in a way that correlates with clinical characteristics such as disease progression and response to therapy. According to the French American British (FAB) classification system, ALL is classified based on morphological criteria into three subgroups: L1, L2, and L3 [4]. For a long time, morphological and cytochemical evaluation have been considered the principal diagnostic methods, and the FAB classification has been generally accepted. However, this classification does not give information about the lineage or maturation stage of the cells from which the leukemia arises.

Alternatively, the WHO classification of all is generally based on cell lineage and maturation stages, and recent updates have emphasized the importance of specific cytogenetic and molecular data in the classification of ALL [5]. Leukemic cells from patients with ALL express a variety of surface differentiation antigens that are also found on normal lymphocyte precursors at discrete stages of maturation [6].

Flow cytometric immunophenotyping (FCI) is a useful tool in diagnostic hematopathology**.** FCI of childhood ALL plays an important role not only in the diagnosis and classification of B and T cell lineages but also in predicting the outcome [7]. Most leukocyte antigens lack lineage specificity, hence, a panel of antibodies is needed to establish the diagnosis and to distinguish among the different immunologic subclasses of leukemic cells. In general, the panel includes antibodies to at least one very sensitive marker for each hematopoietic and lymphoid lineage (CD19 for B-lineage cells, CD7 for T-lineage cells, and CD13 or CD33 for myeloid cells) and antibodies to a relatively specific marker (cytoplasmic CD79a and CD22 for B-lineage cells, cytoplasmic CD3 for T-lineage cells, and CD20 and surface immunoglobulin for mature B cells**)** [8].

CD34 is a surface glycol phosphoprotein expressed on developmentally early lymphohematopoietic stem and progenitor cells, small-vessel endothelial cells, and embryonic fibroblasts. It is an adhesion molecule that is found on hematopoietic precursors, in high concentrations in umbilical cord blood, capillary endothelium, and embryonic fibroblasts. Although assessment of the number of cells that express the antigen CD34 provides valuable data for diagnostic and prognostic purposes, the percentage of CD34 cells should not be considered a substitute for a blast count from the smears or an estimate from the bone marrow biopsy [9]. Although CD34 hematopoietic cells generally are blasts, not all blasts express CD34, and not all CD34-positive cells are blasts as early erythroblasts can express CD34. In acute leukemia, the main use of CD34 is to define the blast population. The absence of CD34 expression is highly suggestive of acute promyelocytic leukemia [10]**.**

Expression of the CD34 antigen is a positive prognostic factor in childhood ALL. In adult ALL the presence of this marker on leukemic cells does not seem to influence the clinical outcome of these patients. The survival rate of children with positive CD34 B-ALL was significantly better than those with negative CD34 expression so, CD34 expression may have prognostic significance and is associated with favorable clinical outcomes in children (11). The age of the patients with pediatric ALL ranges from 0 to 14 years, adolescents from 15 to 19 years, young adults from 20 to 39 years, adults from 40 to 60 years; and elderly patients include those beyond the age of 65 years (12). In this study, we have selected the age group of ≤ 12 years.

Objectives

- To evaluate the expression of CD34 in pediatric acute lymphoblastic leukemia.
- To investigate the correlation between CD34 expression and clinical characteristics in pediatric acute lymphoblastic leukemia.

Patients and Methods

Study Design and Setting: A cross-sectional study was conducted in the Oncology Department of Children's Welfare Teaching Hospital in Medical City/ Baghdad from November 2013 to April 2014. A total of 50 children aged ≤ 12 years diagnosed with ALL and who were not receiving any chemotherapy before the time of collecting blood samples were recruited in this study. The diagnosis was based on the morphology and cytochemistry of the PB and/or BMA samples by consultant hematologists in the laboratory of the mentioned Hospital.

Data Collection Methods: Data were obtained for each patient using a questionnaire that included symptoms, physical signs especially hepatosplenomegaly, lymphadenopathy, CNS involvement and mediastinal mass, and hematological parameters such as HB, PCV, WBC count, platelet count, and S.LDH were obtained from the case file of the patients where they were done by automated hematology analyzer (Cell-DYN, RUBY). Patients who had any of the following conditions were excluded from the study. ALL L3, those who died before starting treatment, those who received chemotherapy in other hospitals either before referral or after finishing induction, those whose parents' refused treatment, and those with a history of steroid intake more than one week.

Sample Collection and processing for all patients: Immediately after the patients had arrived at the hematology ward, 2.5 ml of peripheral blood sample and at least 0.5 ml of bone marrow aspirate were collected in ethylene diamine tetra-acetic acid (EDTA) tube. A peripheral blood and bone marrow films stained with Leishman stain. In addition to cytochemical stain (with SBB staining and periodic acid stain) done to BM films. Both peripheral blood film and bone marrow aspirate were reviewed by a consultant hematologist and then revised by a consultant hematologist to confirm the diagnosis and sub-classification of patients. ALL Patients were classified according to FAB classification for correlation purposes. The classification was based on the morphological features of leukemia.

Materials and Methods

For every patient at least 0.3 ml of EDTA anti-coagulated bone marrow or peripheral blood sample was collected for flow cytometry test. BMA is either aspirated and tested on the same day, or stored, if required, in a refrigerator (2-8◦ C), for a short period, less than 48 hours, and transported within a cold container so that the sample will not be in direct contact with the sunlight than transferred to a Private Lab. For newly diagnosed ALL patients one cytoplasmic marker CD3 and two surface markers, CD34 and CD19 were tested in the bone marrow aspirate. FSC\SSC used for gating on blast window, CD19 and CD3 were used to differentiate between T and B-ALL in which B-ALL is (CD19 positive, CD3 negative) and T-ALL is (CD19 negative, CD3 positive) and CD34 to study its expression pattern blasts.

Flow cytometry: All flow cytometric analysis in this study was done by Cyflow® cube 6 flow cytometers from Partec Company (Germany). CyFlow® Cube 6 is an ultra-compact and uniquely designed, stand-alone flow Cytometer for all applications in cell analysis and absolute counting. CyFlow® Cube 6, high-performance accurate, and cost-effective flow cytometry employing 2 lasers (red and blue), 6 optical parameters, and 4 colors offered: Forward Scatter

(FSC), Side Scatter (SSC) in combination with 4 fluorescence channels (FL1-FL4).
Sample processing for Partec Cube6 flow cytometer using a technique with the principle of Stain–Lyse–No Wash. Procedure that involved the addition of surface and cytoplasmic antibodies:

10µ of each antibody was added to 100µ of well-mixed EDTA-anticoagulated blood or BM into labeled tubes and incubated in the dark at room temperature for 15 minutes.

- Then 100 µ Fix and Perm reagent was added to each tube, vortexed, and incubated for 15 minutes in the dark. Then the solution was re-suspended by vigorous mixing and shaking, and data were acquired on the flow cytometer.
- Then 100 µ of the fixative solution was added and incubated in the dark at room temperature for 10 minutes.
- Then 2.5 ml of lysis solution was added and incubated in the dark for 20 minutes.
- Then the solution was re-suspended by vigorous mixing and shaking, and data were acquired on the flow cytometer.

Cytoplasmic markers

- Add 2.5 mL of lysing solution to 100 µl of whole blood. Shake well and incubate for 20 minutes at room temperature in the dark.
- Centrifuge at 500 x g for 5 minutes; decant the supernatant.
- Add 0.5 mL of permeabilizing solution 2. Vortex and incubate for 10 minutes at room temperature.
- Add the appropriate volume of CD3 fluorescent conjugated monoclonal antibody to the tube. Vortex and incubate for 30 minutes at room temperature in the dark.
- Add 0.5 ml of BD Cell Fix solution and mix well.
- Store at 2-8 ºC until analyzed.
- For newly diagnosed ALL patients: Two tubes were used, one for the surface markers CD34 and CD19. Another tube was used for the cytoplasmic marker CD3.

Gating strategy: The dominant population of leukemic blasts was identified in the forward -versus side scatter histogram to isolate the blast population then the gating of CD19 versus CD34 to determine the B linage of ALL and to assess the expression pattern of CD34 and CD3 /SSC plots to determine the T linage of ALL. A 20% cut-off value was put to indicate the positivity of surface markers CD34 and CD19 while the cut-off value for the cytoplasmic marker CD3 was 10% [6].

Statistical Analysis

The data analysis was conducted using Statistical Package for Social Sciences (SPSS) version 25. Descriptive statistics, including means, standard deviations, and ranges, were utilized to summarize the characteristics of the participants, while frequencies and percentages were used to present categorical data. To explore the relationships between categorical variables, chi-square tests were applied, and t-tests were employed to compare continuous variables. A p-value of less than 0.05 was considered indicative of statistical significance.

Ethical considerations and official approvals

Official approval for the research was granted by the Medical City Directorate and the Iraqi Ministry of Health. Data collection involved obtaining detailed information from the case files of the patients under study. To ensure ethical standards, all collected data was treated with strict confidentiality and privacy. The information was used exclusively for the purposes of the research study, with no disclosure or utilization beyond the specified scope of the investigation.

Table 1: Distribution of the study group according to sociodemographic & related characteristics and CD34 expression

Variable		CD34 Marker		Total No. (%)	P-Value*
		Positive (N=37) No. (%)	Negative (N=13) No. (%)		
Age Group (Years)	< 5	28 (75.7)	5 (38.4)	33 (66.0)	0.035
	5 – 10	5 (13.5)	3 (23.2)	8 (16.0)	
	> 10	4 (10.8)	5 (38.4)	9 (18.0)	
Gender	Male	24 (64.9)	6 (46.2)	30 (60.0)	0.236
	Female	13 (35.1)	7 (53.8)	20 (40.0)	
FAB Classification	L1	6 (16.2)	4 (30.8)	10 (20.0)	0.259
	L2	31 (83.8)	9 (69.2)	40 (80.0)	
Risk Group	Standard	24 (64.9)	5 (38.4)	29 (58.0)	0.033
	High	13 (35.1)	8 (61.6)	21 (42.0)	
Immunological Classification	B-ALL	33 (89.2)	7 (53.8)	40 (80.0)	0.006
	T-ALL	4 (10.8)	6 (46.2)	10 (20.0)	
Response to Therapy	Yes	34 (91.9)	11 (84.6)	45 (90.0)	0.452
	No	3 (8.1)	2 (15.4)	5 (10.0)	

*Significant difference between percentages using Pearson Chi-square test at 0.05 level.

Results

This study included 50 patients diagnosed with ALL. The age range was 2 months to 12 years with a mean of 5.1±0.8 years. More than two-thirds of patients aged < 5 years 33 patients (66%), 8 patients (16%) aged 5-10 years, and 9 patients (18%) aged > 10 years. There were 30 males (60%) and 20 females (40%) with a male-to-female ratio of 1.5:1. Regarding FAB classification, L2 subtype was the dominant morphological subtype among the studied patients 40 (80%) while 10 patients (20%) were with L1. According to the National Cancer Institute (NCI), 29 (58%) cases were classified as standard risk group (1 year to 10 years and

WBC count less than 50 x 10 9/L), and the remaining 21 (42%) were classified as a high-risk group (< 1 year or > 10 years or WBC count more than 50 x 10 9/L). Concerning immunological classification, 40 children (80%) had B-ALL and the remaining 10 (20%) had T-ALL. The distribution of the studied children according to CD34 marker positivity was as follows: 37 children (74%) had a positive CD34 marker while 13 (26%) had a negative marker.

This study found a significant association between CD34 expression and patients' age, risk group, and immunological classification of ALL. The rate of positive CD34 was significantly higher among children aged < 5 years (75.7%, P=0.035), children who were classified with standard risk for ALL (64.9%, P=0.033), and those who had B-ALL (89.2%, P=0.006). On the other hand, there was no significant association between CD34 expression and patients' gender, FAB classification, and response to the induction therapy (p≥0.05). As illustrated in (Table 1).

The distribution of clinical parameters across the CD34 categories revealed that no CNS involvement in all CD34 positive ALL while CNS involvement was found in 5 patients (38.4%) of CD34 negative ALL, with a significant association between the CD34 positive and the absence of CNS involvement, P-value =0.034. The rate of signs and symptoms were higher in CD34 negative ALL compared to CD34 positive ALL: pallor (76.9% vs 54%), Bruising (38.4% vs 18.9%), hepatosplenomegaly (61.6% vs 46%), fever (76.9% vs 51.3%), lymphadenopathy (69.2% vs 56.7%), and mediastinal mass (23.1% vs 5.4%), but these differences were not statistically significant (p≥0.05).

Table 3.2: Distribution of the study group according to clinical presentation and CD34 expression

Clinical Parameter		CD34 Marker		P-Value*
		Positive (N=37) No. (%)	Negative (N=13) No. (%)	
Pallor	Present	20 (54.0)	10 (76.9)	0.147
	Absent	17 (46.0)	3 (23.1)	
Bruising	Present	7 (18.9)	5 (38.4)	0.156
	Absent	30 (81.1)	8 (61.6)	
Hepatosplenomegaly	Present	17 (46.0)	8 (61.6)	0.334
	Absent	20 (54.0)	5 (38.4)	
Fever	Present	19 (51.3)	10 (76.9)	0.107
	Absent	18 (48.7)	3 (23.1)	
Lymphadenopathy	Present	21 (56.7)	9 (69.2)	0.429
	Absent	16 (43.7)	4 (20.8)	
Mediastinal mass	Present	2 (5.4)	3 (23.1)	0.067
	Absent	35 (94.6)	10 (76.9)	
CNS Involvement	Present	0 (0)	5 (38.4)	0.001
	Absent	37 (100.0)	8 (61.6)	

* Significant difference between percentages using Pearson Chi-square test at 0.05 level.

The comparison of laboratory parameters according to CD34 expression revealed that the mean level of WBCs was significantly lower in patients who had CD34 positive ALL compared to those with CD34 negative ALL (42.2 x 10^9/L vs 127.3 x 10^9/L, P=0.001). No significant differences were found in the mean levels of HCT%, hemoglobin, platelets, and blast cells %. The frequency of serum lactate dehydrogenase level ≥ 400 U/L was higher in CD34 negative ALL as compared to the CD34 positive ALL (38.4% and 21.7%), but the difference was not significant (P=0.233). As shown in (Table 3.3).

Table 3.3: Comparison of laboratory parameters according to CD34 expression

Laboratory Parameters	CD34 Marker		P-Value
	Positive (N=37) Mean ± SD	Negative (N=37) Mean ± SD	
WBC count (× 10^9/L)	42.2±5.9	127.3±17	0.001
HCT (%)	27.5±3.1	26.4±3.3	0.284
Hemoglobin(g/dl)	9.1±3.8	6.9±2.2	0.287
Platelets (x 10^9/L)	53.5 ±9.3	52.2.0±11.3	0.753
Blast cells (%)	63.2±11.5	68.7±6.5	0.110
* Significant difference between two independent means using Students-t-test at 0.05 level.			
Serum Lactate Dehydrogenase Level			
< 400 (U/L)	29 (78.3)	8 (61.6)	0.233
≥ 400 (U/L)	8 (21.7)	5 (38.4)	

*Significant difference between percentages using Pearson Chi-square test at 0.05 level.

Discussion

A total of 50 patients diagnosed with ALL were recruited for this study with an age range between 2 months to 12 years and a mean of 5.1±0.8 years. The distribution of the studied children according to CD34 marker positivity revealed that 37 children (74%) had a positive CD34 marker while 13 (26%) had a negative marker. Expression of CD34 varies in different studies of ALL where it was between 48 to 86%. The possible reasons for this difference may be due to the immaturity of most blast cells that express CD34. Another plausible explanation for the differences in CD34 expression can be the different ethnicity and thereby different genetic backgrounds [14].

In the current study, the rate of positive CD34 was significantly higher among children aged < 5 years (84.8%, P=0.035) which agreed with other Iraqi studies and other international studies [15, 16]. However other national studies reported an older age of pediatric patients at presentation,

ranging between 7-9 years [17, 18]. In agreement with the current study, younger age at presentation was also observed in a hospital-based cancer registry study during the years 2004-2009, carried out in Basrah, Iraq [19]. In the present study, there were 30 males (60%) and 20 females (40%) with a male-to-female ratio of 1.5:1 which was in tune with Abid Salih *et al.*, 2013 [15] and other literature which cited a male predominance in pediatric ALL [18, 20].

In the present study, the most common presenting features for pediatric ALL were pallor and fever followed by lymphadenopathy, hepatosplenomegaly, and bruising while the least presenting features were CNS involvement and mediastinal mass. The same findings were reported in the other studies [15, 21].

In the current study, we found that the CD34 positive ALL was significantly associated with good risk factors (age 1-10 years) while CD34 negative ALL was associated with a poor risk factor (< 1 year or > 10 years) but there was no difference between CD34 positive and CD34 negative ALL regarding the sex and the ALL subtype. Similar findings were reported by Basso G *et al.*, 2001. We found that CD34-positive ALL was significantly characterized by no risk of CNS involvement while there was no difference in other clinical parameters. These results go in line with other studies [14, 22] and this may be due to the absence of cytogenetic abnormality in CD34-positive ALL which was associated with CNS involvement [23].

High WBC count in children was the most consistent difference between CD34 negative and CD34 positive ALL. Our results showed that the mean WBC count was 127.3 x 10^9/L in CD34 negative ALL vs 42.2 x 10^9/L in CD34 positive ALL, and this in agreement with Basso *et al.* 2001 [23] which showed that the mean WBC count was less than 50 x 10^9/L in CD34 positive ALL [24]. In Kamazani *et al.* there was no significant correlation with studied prognostic factors such as WBC numbers. The cytogenetic features are closely linked to the WBC and at least partly explain the prognostic of WBC count [25].

Concerning immunological classification, we found that 40 children (80%) had B-ALL and the remaining 10 (20%) had T-ALL, and CD34 expression was significantly higher in B- than T-lineage ALL. This result agreed with previous reports in childhood series. Expression of CD34 varies in different studies [14, 26, 27]. In these studies of B-lineage ALL, CD34 expression was 48%-86%. In T-lineage ALL less patients expressed this marker; CD34 was 10%-50%. The possible reasons for this difference may be due to the immaturity of the most blast cells that express. Another plausible explanation for the differences in CD34 expression can be the different ethnicities and thereby different genetic backgrounds.

Conclusion

CD34 expression was a frequent event in childhood ALL. The rate of positive CD34 was significantly higher among children aged < 5 years, children who were classified with standard risk for ALL, and those who had B-ALL. Further, children with CD34 positive ALL had a significantly lower mean WBC count compared to those with CD34 negative ALL. Evaluation of the co-expression of CD34 with CD10, growth fraction (percent of leukemic cells with nuclear positivity for the ki67 monoclonal antibody), multi-drug resistance, multi-drug resistance (by evaluation of P-170 glycoprotein), DNA index (which reflects chromosome ploidy), and cytogenetic abnormalities with CD34 expression are recommended.

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References

1. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: A comprehensive review and 2017 update. Blood Cancer J. 2017 Jun 30;7(6):e577. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5520400/
2. Inaba H, Pui CH. Advances in the Diagnosis and Treatment of Pediatric Acute Lymphoblastic Leukemia. J Clin. Med. 2021 Apr 29;10(9):1926. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8124693/
3. Abboud MR, Ghanem K, Muwakkit S. Acute lymphoblastic leukemia in low and middle-income countries: disease characteristics and treatment results. Current opinion in oncology. 2014 Nov 1;26(6):650-655. Available from: https://pubmed.ncbi.nlm.nih.gov/25202926/
4. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR. The morphological classification of acute lymphoblastic leukemia: concordance among observers and clinical correlations. British journal of hematology. 1981 Apr;47(4):553-61.
5. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2141.1981.tb02684.x
6. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, *et al*. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood, The Journal of the American Society of Hematology. 2016 May 19;127(20):2391-2405. Available from: https://ashpublications.org/blood/article/127/20/2391/35255/The-2016-revision-to-the-World-Health-Organization
7. Bain B. Leukemia diagnosis. 4th ed. Chichester: West Sussex; Hoboken, NJ: Wiley-Blackwell; c2010.
8. Iwamoto S, Deguchi T, Ohta H, *et al*. Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/Lymphoma Study Group. Int J Hematology 2011;94:185–192. Available from: https://link.springer.com/article/10.1007/s12185-011-0900-1
9. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond evolving concepts and practical applications. Blood, The Journal of the American Society of Hematology. 2011 May 12;117(19):5019-32. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3109529/
10. Ye D, Manero GG, Kantarjian HM, Xiao L, Raj VS, Fernandez MH, Nguyen MH, *et al*. Analysis of Aurora

kinase A expression in CD34(+) blast cells isolated from patients with myelodysplastic syndromes and acute myeloid leukemia. J Hematopathology. 2009 Mar;2(1):2-8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2713495/
11. Liang J, Wu D, Liu Y, Ma Q, Xue Y, Zhu M, *et al*. Clinical and laboratory features of patients with CD34(+) acute promyelocytic leukemia. Chinese Journal of Oncology. 2009;31:196-198. Available from: https://pubmed.ncbi.nlm.nih.gov/18953948/
12. Dakka N, Bellaoui H, Bouzid N, Khattab M, Bakri Y, Benjouad A. CD10 AND CD34 expression in childhood acute lymphoblastic leukemia in Morocco: clinical relevance and outcome. Pediatric hematology and oncology. 2009 Jan 1;26(4):216-231. Available from: https://pubmed.ncbi.nlm.nih.gov/19437324/
13. Soliman A, Yassin M, Al Yafei F, Al-Naimi L, Almarri N, Sabt A, De Sanctis V. Longitudinal study on liver functions in patients with thalassemia major before and after deferasirox (DFX) therapy. Mediterranean journal of hematology and Infectious diseases. 2014, 6(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4010606/
14. Lab Wrench. Cyflow® cube 6. Cited on October 10, 2019. Available from: http://www.labwrench.com/?equipment.view/equipmentNo/11369/Partec/CyFlow--Cube-6/
15. Supriyadi E, Veerman AJ, Sutaryo S, van de Ven PM, Cloos J. Detection of CD10, CD34 and their combined expression on childhood acute lymphoblastic leukemia and the association with clinical outcome in Indonesia. Journal of Cancer Therapeutics & Research. 2012;1:1-0.
16. Abid-Salih B. Evaluation of oncogene fusion transcripts [t (12; 21)/TEL-AML, t (1; 19)/E2A-PBX1, t (4; 11)/MLL-AF4, and t (9; 22)/BCRABL] in children with acute lymphoblastic leukemia by multiplex PCR analysis. Baghdad: Al-Nahrain University, College of Medicine; c2013.
17. Feller M, Adam M, Zwahlen M, Brazzola P, Niggli F, Kuehni C. Swiss Pediatric Oncology Group (SPOG) and the Swiss National Cohort (SNC). Family characteristics as risk factors for childhood acute lymphoblastic leukemia: A population-based case-control study. PLOS One. 2010 Oct 4;5(10):e13156. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0013156
18. Hassan MK, Ibrahim JJ, Abbas MS. The adverse effects of chemotherapeutic agents in Iraqi patients with acute lymphoblastic leukemia during the induction phase. IJABR. 2013;3(4):584-592.
19. Dhahir EK. The Significance of FLT3-ITD Mutation and Nucleophosm in Mutation in Acute Leukemia (Doctoral dissertation, A Thesis Submitted to the College of Medicine–Al Nahrain University in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy in Pathology (Hematology); c2012. Available from: https://nahrainuniv.edu.iq/en/node/1905
20. Alrudainy LA, Hassan JG, Salih HM, Abbas MK, Majeed AA. Time trends and geographical distribution of childhood leukemia in Basrah, Iraq, from 2004 to 2009. Sultan Qaboos University Medical Journal. 2011 May;11(2):215. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3121026/
21. Seegmiller AC, Kroft SH, Karandikar NJ, McKenna RW. Characterization of immunophenotypic aberrancies in 200 cases of B acute lymphoblastic leukemia. American journal of clinical pathology. 2009 Dec 1;132(6):940-949. Available from: https://academic.oup.com/ajcp/article/132/6/940/1761341
22. Pahloosye A, Hashemi AS, Mirmohammadi SJ, Atefi A. Presenting clinical and laboratory data of childhood acute lymphoblastic leukemia. Iranian Journal of Pediatric Hematology and Oncology. 2011 Sep 10;1(3):71-77. Available from: https://ijpho.ssu.ac.ir/article-1-37-en.html
23. Basso G, Lanza F, Orfao A, Moretti S, Castoldi G. Clinical and biological significance of CD34 expression in acute leukemia. Journal of biological regulators and homeostatic agents. 2001 Jan 1;15(1):68-78. Available from: https://pubmed.ncbi.nlm.nih.gov/11388747/
24. Greaves M. A causal mechanism for childhood acute lymphoblastic leukemia. Nature Reviews Cancer. 2018 Aug;18(8):471-484. Available from: https://www.nature.com/articles/s41568-018-0015-6#citeas
25. Buffler PA, Kwan ML, Reynolds P, Urayama KY. Environmental and genetic risk factors for childhood leukemia: appraising the evidence. Cancer investigation. 2005 Jan 1;23(1):60-75. Available from: https://www.tandfonline.com/doi/abs/10.1081/CNV-46402
26. Nava OJ, Gómez GY, Aguiar IB, Vázquez LMA. Survival of patients with acute lymphoblastic leukemia. Clinical epidemiology of acute lymphoblastic leukemia from the molecules to the clinic. New York: Intech; c2013 .p. 237-264. Available from: http://cdn.intechopen.com/pdfs-wm/44057.pdf
27. Dakka N, Bellaoui H, Bouzid N, Khattab M, Bakri Y, Benjouad A. CD10 AND CD34 expression in childhood acute lymphoblastic leukemia in Morocco: clinical relevance and outcome. Pediatric hematology and oncology. 2009 Jan 1;26(4):216-231. Available from: https://www.tandfonline.com/doi/abs/10.1080/07357900902897557
28. Bachir F, Bennani S, Lahjouji A, Cherkaoui S, Harif MH, Khattab M, *et al*. Characterization of acute lymphoblastic leukemia subtypes in Moroccan children. International Journal of Pediatrics. 2009;2009(1):674801. Available from: https://onlinelibrary.wiley.com/doi/full/10.1155/2009/674801