DOI: https://doi.org/10.54393/pjhs.v4i09.1012

PAKISTAN JOURNAL OF HEALTH SCIENCES

https://thejas.com.pk/index.php/pjhs

Volume 4, Issue 9 (September 2023)

Original Article

Immunophenotyping of Acute Leukemia in Pediatric Patients: Tertiary Care Centre Experience from Lahore

Farhana Shahzad[1*], Umaira Ahsan[2] and Aqsa Anam[1]

[1]Department of Immunology, University of Child Health Sciences & Children's Hospital, Lahore, Pakistan
[2]Department of Microbiology, Al-Aleem Medical College, Gulab Devi Hospital, Lahore, Pakistan

ARTICLE INFO

Key Words:
ALL, AML, Immunophenotyping, Flowcytometry, CD markers

How to Cite:
Shahzad, F., Ahsan, U., & Anam, A. (2023). Immunophenotyping of Acute Leukemia in Pediatric Patients: Tertiary Care Centre Experience from Lahore: Immunophenotyping of Acute Leukemia in Pediatric Patients. Pakistan Journal of Health Sciences, 4(09). https://doi.org/10.54393/pjhs.v4i09.1012

***Corresponding Author:**
Farhana Shahzad
Department of Immunology, University of child health sciences & children's hospital, Lahore
farhanashehzad@gmail.com

Received Date: 27th August, 2023
Acceptance Date: 16th September, 2023
Published Date: 30th September, 2023

ABSTRACT

Acute Leukemia is the most prevalent malignancy of childhood, globally. Immunophenotyping by flowcytometry has developed as an important technique for its outstanding contributions in diagnosis and management of leukemia patients. **Objective:** To determine the frequency and immunophenotyping by flowcytometry of Acute leukemia in pediatric age group. **Methods:** The study was conducted at The University of Child health sciences and Children's Hospital, Lahore from July-2021 to Feb-2022. Data were gathered from 101 consecutive patients, of age from 0.5 to 15 years, that had acute leukemia by immunophenotyping using flowcytometry. **Results:** Flowcytometric immunophenotyping of 101 leukemia patients over 8-month period showed that 67.3% were males and 33.6% were females respectively. AML, T-ALL, and B-ALL prevalence was highest in patients with 5 to 10 years of age. Out of 101 leukemia patients, 16 (15.8%) and 85 (84.1%) had AML and ALL diagnosis respectively. Of these 85 ALL cases, 72 (84.7%) were B-ALL and 13 (15.2%) were T-ALL. Study founded that most frequent CD markers in B-ALL, were CD79 and CD19 (100%) whereas CD3 and CD5 (100%) in in T-ALL and CD13 (93.8%) and CD34 (87.5%) in AML. CD34 was a common marker among B-ALL, T-ALL and AML. Among B-ALL and T-ALL, AntiTdt was a common CD marker whereas HLA-DR was common among AML and B-ALL. **Conclusions:** Results of Acute leukemia immunophenotyping were homologous to worldwide published research. For accurate leukemia lineage, immunophenotyping of AML and ALL is essential since, if therapy is started based solely on morphological diagnosis, approximately 25% of patients may not respond or recure.

INTRODUCTION

Leukaemia is a prevalent malignant disease globally, ranking fifteenth in 2018 and having a higher prevalence in industrialized nations. However, underdeveloped nations have a higher death rate [1]. According to the American Cancer Society, 178,520 Americans are anticipated to be diagnosed with leukaemia, lymphoma, or myeloma in 2020. Although both sexes are equally afflicted, men are more prone to get it [2]. The age-standardized incidence rates for leukaemia were 6.1 and 4.3 per 100,000 in the US in 2018 [1]. Malignant clonal diseases of the hematopoietic system known as acute leukaemia can affect one or many cell-lines. Due to the considerable replacement of bone marrow by undifferentiated, immature and aberrant hematopoietic cells in these diseases, there are less erythrocytes and platelets in the peripheral circulation. These illnesses can be classified as lymphoid, myeloid, mixed, or undifferentiated depending on the origin of the aberrant hematopoietic cells involved [3]. The etiology of these illnesses is still unclear, despite substantial advancements in acute leukemia therapy. A variety of environmental and genetic risk factors for the development of acute leukemia have been hypothesized, but none have been proven. A new "two-hit" theory suggests that acute lymphoblastic leukemia develops due to a combination of hereditary factors and infection exposure [4, 5]. Genetics play a significant role in leukemia development, with several

DOI: https://doi.org/10.54393/pjhs.v4i09.1012

proposed genetic influences. Increased leukemia risk is related to the processes of leukemogenesis as well as genetic variables in normal hemopoiesis and the development of acute leukemia [6, 7]. Similarly, leukemia development is linked to various environmental factors, including exposure to cancer-causing substances like chemicals, infections, radiation, poisons, hydrocarbons, pesticides, alcohol, tobacco, and illegal drugs [8]. The most prevalent form of cancer in children is acute lymphoblastic leukaemia (ALL), which makes up roughly 25–30% of all cancer diagnoses in this age group. Between the ages of 0 and 14, ALL has a yearly incidence of around 4.6 cases per 100,000 persons in the United States, with a peak incidence at 2 to 5 years. Females are somewhat more likely than males to have ALL in the first year of birth [3]. The peak periods for acute myeloblastic leukaemia (AML) are early infancy and later in life. Approximately AML affected 7.7 children between the ages of 0 and 14 years during 2005 and 2009. The first year of life in pediatric age group is the time of its greatest incidence rate, which subsequently steadily drops until the age of four. Under one year of age, children encounter the disease at a rate of 18.4 per million [9]. Leukemia is diagnosed and categorized using a variety of methods, such as cytomorphology, histomorphology, cytochemistry, multi-parameter flow cytometry, chromosomal analysis, FISH, and molecular methods. The primary approach for diagnosing, categorizing, staging, and tracking the development of the disease and its response to treatment is immunophenotyping by flowcytometry [10]. Considering that each subtype has a different treatment option and prognosis, flow cytometry's ability to discriminate between them is crucial [11].

METHODS

The Ethical Committee of the School of Allied Health Sciences, The University of Child Health sciences, The Children's Hospital, Lahore approved this descriptive study design, based on total of 101 pediatric patients. One hundred and one requests sent to the Department of Immunology during the period of July 2021 to February 2022 for the immunophenotyping of hematological malignancies by flow cytometery were included in this study. Using data from Narang *et al.*, (2015), the sample size was estimated while retaining the power of study at 90% and its level of significance at 5% [12]. The samples were sent from patients ranging in age from 6 months to 15 years, of both genders. All the patients were divided into three groups of <5 years, 5-10 years and 11-15years. On peripheral blood or bone marrow aspirate immunophenotyping was carried out using a dual laser BD FACS Canto II flow cytometer (BD Biosciences, California USA). Whole blood was lysed using BD FACS lysing solution to produce mononuclear cells. Monoclonal antibodies (MoAbs) that have been fluorescein-conjugated (BD Biosciences USA) have been absorbed on monocyte, myeloid cell, T & B cells, or immature precursor cell antigens in various combinations. These MoAbs included phycoerythrin (PE), allophycocyanine (APC), peridinin chlorophyll protein (PerCP), and fluorescein isothiocyanate (FITC). Samples containing 20% or more blast cells reacting with a specific antibody were deemed positive. Data from the study were analyzed using the Statistical Package for Social Sciences (SPSS) version 26.0 for Windows. Percentages and frequencies were used to express the data. All statistical tests were regarded as two-sided and statistical significance was defined as a p-value of equal to or less than 0.05.

RESULTS

Data of one hundred and one patients for the immunophenotyping by flowcytometry was analyzed in the immunology department. The age distribution of acute leukemia is depicted in Figure 1. B-ALL was the most common leukemia in all age groups with 45 (56.9%) in the age group of <5 years, 23(31.9%) in 5-10 years, and 8(11.1%) in the age group of 11-15 years. T-ALL and AML were most common 7(53.8%) %) and 7 (43.8%) respectively in the age group of 5-10 years.

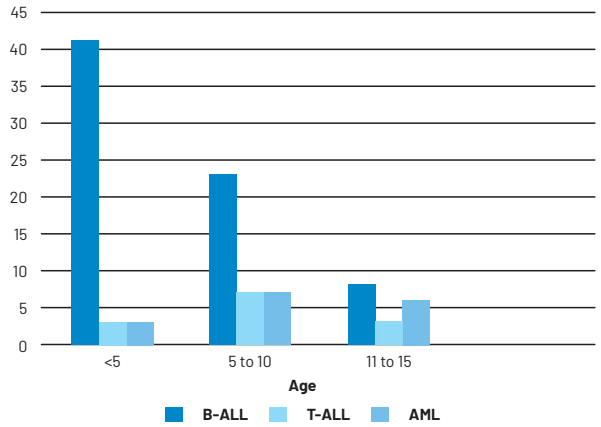

Figure 1: The age distribution of acute leukemia in pediatric population. B-ALL was the most prevalent leukaemia in all age groups, T-ALL and AML were same in the age groups other than 11-15 years in which AML was more frequently present

Figure 2 shows the gender distribution of acute leukemia. Out of 101 patients, 67 (66.3%) were male and 34 (33.6%) were female. On further analysis of gender distribution in each type of leukemia males were more as compared to females.

DOI: https://doi.org/10.54393/pjhs.v4i09.1012

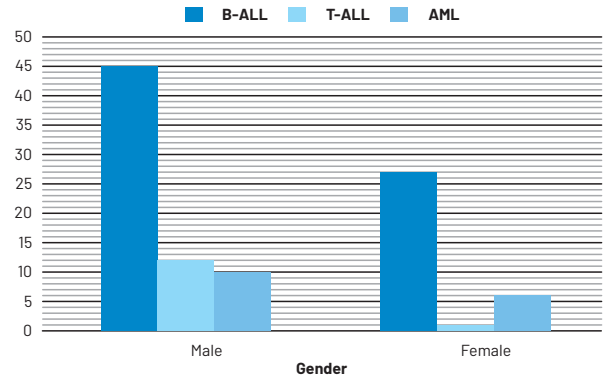

Figure 2: Gender distribution of acute leukemia in pediatric population. All types of acute leukemia were more frequent in males as compare to females

From 101 cases of acute leukemia that underwent immunophenotyping by flow cytometric analysis, 85 (84.1%) were ALL, and of those 85 cases, 72 (84.7%) were of B cell phenotypes and 13 (15.2%) were of T cell phenotypes. From total of 101 leukemia patients, 16 (15.8%) were found to be AML (Table 1).

Table 1: Frequency of acute leukemia on the basis of Immunophenotyping by flowcytometry

Type of acute leukemia	Frequency (%)
B-ALL	72(84.7%)
T-ALL	13(15.2%)
AML	16(15.8%)

Most frequent type of acute leukemia is B-ALL in pediatric age group followed by AML and T-ALL

Immunophenotypic profile of B-ALL showed positivity of cytoplasmic CD79a (cCD79a) and CD19 in 72 (100%) cases, CD10 in 71(98.6%) cases, CD34 in 50 (69.4%), AntiTdt in 66 (88.9%), HLA-DR in 39 (54.2%) and cytoplasmic CD22 (cCD22) was positive in 4 (5.6%) cases. In T-ALL, immunophenotypic profile presented a positive expression of CD2 in 11 (84.6%) cases, cytoplasmic CD3 (cCD3) in 13 (100%), CD5 in 13 (100%), CD7 in 10 (76.9%), CD34 in 7 (53.8%) and AntiTdt was positive in 12 (92.3%) cases. Immunophenotyping for AML showed that AntiMPO was positive in 12 (75%) cases, CD13 in 15 (93.8%) cases, CD34 in 14 (87.5%) cases, HLA-DR 13 (81.3%) and CD11c was positive in 8 (50%) cases. The most common CD markers in B-ALL were cCD79 and CD19 (100%) whereas the most common CD markers in T-ALL were CD3 and CD5 (100%) and in AML were CD13 (93.8%) and CD34 (87.5%). A common marker for B-ALL, T-ALL, and AML was CD34. B-ALL and T-ALL shared the CD marker antiTdt whereas AML and B-ALL shared the CD marker HLA-DR (Table 2).

Table 2: Immunophenotypic profile of acute leukemia in pediatric population

CD Markers	AML (n=16)	B-ALL (n=72)	T-ALL(n=13)
CD10	N/A5	71(98.6%)	N/A
CD191	N/A	72(100%)	N/A
CD343,4	14(87.5%)	50(69.4%)	7(53.8%)
cCD79a1	N/A	72(100%)	N/A
Anti Tdt4	N/A	66(88.9%)	12(92.3%)
HLADR4	13(81.3%)	39(54.2%)	N/A
CD2	N/A	N/A	11(84.6%)
cCD32	N/A	N/A	13(100%)
CD52	N/A	N/A	13(100%)
CD7	N/A	N/A	10(76.9%)
CD11c	8 (50%)	N/A	N/A
CD133	15(93.8%)	N/A	N/A
CD14	5(31.3%)	N/A	N/A
CD117	9(56.3%)	N/A	N/A
Anti MPO	12(75%)	N/A	N/A
Cd33	13(81.3%)	N/A	N/A

In B-ALL, cCD79a, CD10, CD19, CD34, and Anti-Tdt were present with more than 50% of expression. In T-ALL, CD2, CD3, CD5, CD7, CD34, and AntiTdt were positive with more than 50% of expression. In AML, AntiMPO, CD13, CD34, and HLA-DR was positive with more than 50% of expression.

CD19, cCD79a[1]= Most frequent markers in B-ALL

cCD3, CD5[2]= Most frequent markers in T-ALL

CD34, CD13[3]= Most frequent markers in AML

CD34, HLA-DR, Anti-Tdt[4]= Most common CD markers in acute leukemia

N/A[5]= Not applicable

DISCUSSION

In Pakistani pediatric population B-ALL was the most common leukemia in all age groups, where as T-ALL and AML were most frequently present in the age group of 11-15 years. These findings are in accordance with the international data such as in a study B-cell ALL is much more common in all age groups than T-cell disease, accounting for approximately 80% of cases of ALL [13]. Similarly, AML was more frequently present with incidence peak between the ages of 10-14 years [14]. According to the American cancer society AML is significantly more prevalent in the first two years of life and during adolescence, whereas ALL is most prevalent in early infancy, peaking between the ages of 2 and 5 years [15]. According to our findings acute leukemia was more common in boys compared to girls. These results are in favor of another study showing boys were four times at more risk to develop ALL than girls [16]. Similarly, in another study out of 100 acute leukemia patients there were 76 males and 24 females [17]. These results are in line with another study from Pakistan, where males were more likely to have acute leukemia (60%) than females (40%) [18]. In pediatric population of this study ALL was more common than AML. Among ALL, B-ALL was more in children as compared to T-ALL. These findings are in favor of recent Indian data depicting that ALL, constitute 75-80% of childhood acute leukemia [19]. One more study of pediatric

DOI: https://doi.org/10.54393/pjhs.v4i09.1012

acute leukemia showed 56% of B-ALL, 32% T-ALL and 12% of AML [17]. According to another study AML is less prevalent than ALL, accounting for approximately 18% of children leukemias augmenting findings of this study showing 15% of AML [9]. In this study immunophenotyping revealed CD34 as a common marker for B-ALL, T-ALL, and AML. B-ALL and T-ALL shared the CD marker antiTdt whereas AML and B-ALL shared the CD marker HLA-DR. Moreover, according to this study in B-ALL, cCD79a, CD10, CD19, CD34, and Anti-Tdt were present with more than 50% of expression with most frequent expression of CD19 and cCD79a whereas in T-ALL, CD2, cCD3, CD5, CD7, CD34, and AntiTdt showed more than 50% of positive expression and cCD3, CD5 were the most frequent markers. ALL of B cell lineage is identified if the B-cell markers CD19, cCD79a, and CD22 are expressed together, while T-ALL is identified by the presence of cCD3 and co-expression of CD5 in all blasts. In a similar manner, according to a different research CD19, CD22, and cCD79a were expressed in almost all B-ALL patients, while cCD3 and CD5 were found in nearly all T-ALL cases [18]. Another different study by Salem *et al.*, the most sensitive markers for T-ALL were cCD3 and CD5, whereas the most sensitive markers for B-ALL were cCD79a and CD19 [19]. According to the present study in AML, AntiMPO, CD13, CD34, and HLA-DR were positive with more than 50% of expression and CD34 and CD13 were the markers of highest expression. CD117, CD13, CD33, and MPO were regarded as markers of myeloid lineage, while HLADR and CD34 showed markers of immature cells [14]. The markers for hematopoietic progenitor cells HLA-DR (87%), CD117 (73%), and CD34 (68%), as well as the myeloid lineage antigens CD13 (95%), CD33 (91%), and MPO (73%) were consistently positive in a study by Legrand *et al.*, as well [20].

CONCLUSIONS

The present study evaluated the age, gender, and immunophenotypic distribution of ALL in the pediatric population highlighting the frequency of acute leukemia in children. It also reflects the significance of flow cytometry in the diagnosis of leukemia because in order to accurately diagnose leukemia and determine lineage, it is crucial.

Authors Contribution

Conceptualization: FS
Methodology: FS, AA
Formal analysis: FS
Writing-review and editing: FS, UA, AA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca: A Cancer Journal for Clinicians. 2018 Nov; 68(6): 394-424. doi: 10.3322/caac.21492.

[2] Bispo JA, Pinheiro PS, Kobetz EK. Epidemiology and etiology of leukemia and lymphoma. Cold Spring Harbor perspectives in medicine. 2020 Jun; 10(6): a034819. doi: 10.1101/cshperspect.a034819.

[3] Tebbi CK. Etiology of acute leukemia: A review. Cancers. 2021 May; 13(9): 2256. doi: 10.3390/cancers13092256.

[4] Terwilliger T and Abdul-Hay MJ. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer Journal. 2017 Jun; 7(6): e577. doi: 10.1038/bcj.2017.53.

[5] Hakkarainen M, Kaaja I, Douglas SP, Vulliamy T, Dokal I, Soulier J, *et al.* The clinical picture of ERCC6L2 disease: from bone marrow failure to acute leukemia. Blood, The Journal of the American Society of Hematology. 2023 Jun; 141(23): 2853-66. doi: 10.1182/blood.2022019425.

[6] Heuser M, Thol F, Ganser A. Clonal hematopoiesis of indeterminate potential: a risk factor for hematologic neoplasms. Deutsches Ärzteblatt International. 2016 May; 113(18): 317. doi: 10.3238/arztebl.2016.0317.

[7] Lagunas-Rangel FA, Chávez-Valencia V, Gómez-Guijosa MÁ, Cortes-Penagos C. Acute myeloid leukemia—genetic alterations and their clinical prognosis. International Journal of Hematology-Oncology and Stem Cell Research. 2017 Oct; 11(4): 328.

[8] Onyije FM, Olsson A, Baaken D, Erdmann F, Stanulla M, Wollschläger D, *et al.* Environmental risk factors for childhood acute lymphoblastic leukemia: an umbrella review. Cancers. 2022 Jan; 14(2): 382. doi: 10.3390/cancers14020382.

[9] Puumala SE, Ross JA, Aplenc R, Spector LG. Epidemiology of childhood acute myeloid leukemia. Pediatric Blood & Cancer. 2013 May; 60(5): 728-33. doi: 10.1002/pbc.24464.

[10] Hossain MG, Islam MN, Islam KM, Jakiyamani M, Chowdhury MA, Khan MR, *et al.* Frequency of Discrepancy between Bone Marrow Morphology and Immunophenotype in Acute Leukaemia. Haematology. Journal of Bangladesh. 2019; 3(01): 16-9. doi: 10.37545/haematoljbd201929.

DOI: https://doi.org/10.54393/pjhs.v4i09.1012

[11] Mahto SK and Prasad N. Utility of Flow Cytometry in Diagnosis of Acute Leukemias: A Study at RIMS, Ranchi, India. Journal of Family Medicine and Primary Care. 2022 Nov; 11(11): 7335. doi: 10.4103/jfmpc.jfmpc_1211_21.

[12] Narang V, Dhiman A, Garg B, Sood N, Kaur H. Immunophenotyping in Acute leukemias: First tertiary care centre experience from Punjab. Indian Journal of Pathology and Oncology. 2017 Apr; 4(2): 297-300.

[13] Zivarpour P, Hallajzadeh J, Asemi Z, Sadoughi F, Sharifi M. Chitosan as possible inhibitory agents and delivery systems in leukemia. Cancer Cell International. 2021 Dec; 21(1): 1-4. doi: 10.1186/s12935-021-02243-w.

[14] Tasian SK, Pollard JA, Aplenc R. Molecular therapeutic approaches for pediatric acute myeloid leukemia. Frontiers in Oncology. 2014 Mar; 4: 55. doi: 10.3389/fonc.2014.00055.

[15] American Cancer Society. Key Statistics for Childhood Leukemia. 2023. [Last cited: 12th Jan 2023]. Available at: https://www.cancer.org/cancer/types/leukemia-in-children/about/key-statistics.html.

[16] Forsythe A, Breland T, Majumdar S, Elkin TD, Johnson D, Megason G. Gender differences in incidence rates of childhood B-precursor acute lymphocytic leukemia in Mississippi. Journal of Pediatric Oncology Nursing. 2010 May; 27(3): 164-7. doi: 10.1177/1043454209357919.

[17] Shahab F and Raziq F. Clinical presentations of acute leukemia. Journal of College of Physicians and Surgoens Pakistan. 2014 Jul; 24(7): 472-6.

[18] Arora RS and Arora B. Acute leukemia in children: A review of the current Indian data. South Asian Journal of Cancer. 2016 Jul; 5(03): 155-60. doi: 10.4103/2278-330X.187591.

[19] Shrestha S, Shrestha J, Pun CB, Pathak T, Bastola S, Bhatta R. Immunophenotypic study of acute leukemia by flow cytometry at BPKMCH. Journal of Pathology of Nepal. 2013 Mar; 3(5): 345-50. doi: 10.3126/jpn.v3i5.7856.

[20] Legrand O, Perrot JY, Baudard M, Cordier A, Lautier R, Simonin G, *et al.* The immunophenotype of 177 adults with acute myeloid leukemia: proposal of a prognostic score. Blood, The Journal of the American Society of Hematology. 2000 Aug; 96(3): 870-7. doi: 10.1182/blood.V96.3.870.015k16_870_877.