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Original Research Article

Clinical, hematological, and flow cytometric correlation of acute leukemia in children: a study from a tertiary pediatric care facility in Hyderabad, Telangana

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ABSTRACT

Background: Acute leukemia is the most common malignancy in children, accounting for approximately 30-40% of all childhood cancers. In India, pediatric acute leukemia presents a significant public health challenge due to its rising incidence, diagnostic delays, and variable access to specialized care. clinical features, hematological parameters (including smear), flow cytometry are essential for diagnosis, risk stratification and prognosis of the disease.
Methods: The study was conducted on 77 patients' children aged 1 month to 12 years who were diagnosed with acute leukemia.
Results: The majority of participants were between 1 to 5 years (42.9%) with definite male predominance (57.1%). Common clinical features were fever (87%), Bleeding manifestations (40.3%), joint pain (15.6%). Splenomegaly and hepatosplenomegaly were observed in 29.9% and 48.1% of the participants. B-cell acute lymphoblastic leukaemia (ALL) was most common (70.1%). Among acute myeloid leukemia subtypes, AML M2 was predominant, and T-cell ALL was relatively rare (3.9%). On flow cytometry analysis of B-ALL, B-cell markers were exclusively expressed and CD34 was positive in 44.2% of the cases. CD10, CD19, and CD79 were exclusively expressed in B-ALL, while MPO, CD13, CD33, and CD117 were strongly associated with AML. T-ALL was characterized by the expression of CD3, CD2, and CD5. Distinct expression patterns of markers such as CD41 and CD61 observed in AML M7 subtype.
Conclusions: Overall, the integration of clinical, hematological, and flow cytometric findings will enable accurate diagnosis and characterization of pediatric leukemia and in further management.

Keywords: Leukemia, Flow cytometry, Prognostication

INTRODUCTION

Acute leukaemia is the predominant malignancy in children, constituting roughly one-third of all paediatric malignancies.[1] It is marked by the unregulated expansion of immature haematopoietic cells in the bone marrow, resulting in disrupted normal haematopoiesis and clinical symptoms include anaemia, thrombocytopenia, and leukocytosis. The Illness manifests as broad array of clinical signs, from nonspecific symptoms such as fever, pallor, and weariness to more severe consequences including haemorrhage, infections, organomegaly, and bone pain. Timely diagnosis and subtype classification are essential for commencing suitable therapy and enhancing survival rates.[2,3]

Paediatric acute leukaemia is primarily categorised as ALL and acute myeloid leukaemia (AML), with ALL being the commonest subtype among children.[4] Each subtype exhibits unique clinical characteristics, hematological metrics, and prognoses. B-cell ALL is the most prevalent kind, especially in children aged 1 to 5 years, but T-cell ALL and AML are comparatively rarer but linked to worse prognoses and more aggressive disease trajectories. Accurate categorisation of leukaemia subtypes is crucial, since it informs treatment strategies and aids in prognostic predictions.[5,6]

The diagnosis of acute leukaemia has conventionally relied on clinical presentation, peripheral smear analysis, and bone marrow aspiration examinations. Morphology offers first insights but frequently proves inadequate for precise differentiation across subtypes. This has resulted in a growing dependence on new diagnostic methods, especially flow cytometric immunophenotyping, which has transformed the diagnosis and categorisation of acute leukaemias.[7-9]

Flow cytometry facilitates the detection of particular cell surface and cytoplasmic markers that delineate the lineage and maturity of leukemic blasts. It is essential for verifying the diagnosis, distinguishing between lymphoid and myeloid leukaemias, and identifying mixed phenotype acute leukaemia (MPAL) patients. The detection of anomalous marker expressions with flow cytometry facilitates precise subtyping and offers prognostic insights that may influence treatment choices.[10-12]

Down syndrome is associated with a heightened risk of leukaemia, particularly AML, due to inherent genetic predisposition. Likewise, certain clinical manifestations such as splenomegaly, lymphadenopathy, and bone pain may be associated with distinct subtypes of leukaemia.[7,9,13] Comprehending these relationships can assist doctors in predicting difficulties and optimising the diagnostic procedure. Furthermore, hematological measures such as total leukocyte count, blast percentage, and differential counts possess prognostic significance and can inform therapy intensity.

By analyzing the clinical presentation, hematological parameters, and immunophenotypic patterns, this study aims to enhance understanding of pediatric leukemia subtypes and contribute to early diagnosis and better risk stratification.

Ultimately, the goal of this study is to strengthen the diagnostic approach to pediatric acute leukemia, enabling timely initiation of appropriate treatment protocols and improving survival outcomes. The integration of clinical assessment, hematology, and flow cytometry will provide a robust framework for accurate diagnosis, prognostication, and management of childhood leukemia, paving the way for future research and therapeutic advancements in pediatric oncology.

METHODS

The present prospective study included 77 patients included children aged 1 month to 12 years diagnosed with acute leukemia presenting to Niloufer hospital, Hyderabad from June 2023-December 2024.

The sample size was calculated based on the study by Shuchismita et al where the proportion of clinical manifestations (fever) among children with leukemia was 53%; with 95% confidence interval, 15% absolute precision and with 10% excess sampling to account for non- response, sample size was derived using the formula.[1]

$n=Z^2\times PQ/d^2$

Prior to the initiation of the study, approval was obtained from institutional ethical committee.

Patients who received the initial treatment elsewhere or presented with relapse for the first time to our institution, children diagnosed with chronic leukemia or who did not undergo flow cytometry were excluded from the study. Patients diagnosed with acute leukemia were admitted to the hospital and thorough clinical evaluation was done along with investigations like hematological profile, bone marrow morphology, immunophenotyping by flow cytometry were performed.

Data was analysed using SPSS software analysis.

RESULTS

Demography

Of 77 children, 44 (57.1%) were boys and 33 (42.9%) were girls. 42.8% of the participants were between 1-5 years of age, minimum age was 15 days of life and maximum age was 13 years (Figure 1 and 2).

Clinical signs and symptoms

Fever was the most predominant symptom seen (87%), followed by bleeding manifestation (40.3%) and joint pain (15.6%). The 19 participants had bone pain while 8 participants had weight loss. Some uncommon symptoms were cellulitis (1.3%), testicular swelling (2.6%) and joint swelling (2.6%).

The 51 patients had pallor, and 23 (30%) of the patients presented with splenomegaly while 37 (48%) patients had hepatosplenomegaly. Generalized lymphadenopathy was observed in 9 patients. Figure 3 depicts the clinical presentation.

Mean blood values among the participants

The mean red blood cell (RBC) count was 3.02±0.90 million/µl, while the hemoglobin (HB) level was

7.61±2.56 g/dl. The mean corpuscular volume (MCV) was 80.04±14.90 fL, the mean corpuscular hemoglobin (MCH) was 26.83±3.97 pg, and the mean corpuscular hemoglobin concentration (MCHC) was 31.86±3.71 g/dL. Regarding white blood cell (WBC) parameters, the total leukocyte count (TLC) was 50055.84±12052.58/mm³.

The differential leukocyte count showed that neutrophils comprised 13.96±18.14%, lymphocytes 69.73±22.68%, monocytes 14.40±6.25%, eosinophils 1.33±0.77%, and basophils 0.68±0.17%. The mean platelet count was 56412.99±15159.46/mm³, indicating significant variation within the study population. Table 1 shows the hematological parameters of the study

Type of leukemia

Among the participants, the majority were diagnosed with B-ALL (54 participants, 70.1%). Among acute myeloid leukemia (AML) subtypes, AML M2 was the most common, observed in 7 participants (9.1%), followed by AML M3 (5 participants, 6.5%), AML M4 (3 participants, 3.9%), AML M7 (3 participants, 3.9%), and AML with aberrant CD7 (2 participants, 2.6%). T-ALL was present in 3 participants (3.9%). Table 2 shows the distribution of leukemia in the study group.

Flow cytometry

The expression of B-cell lineage markers was observed exclusively in B-ALL cases, with CD10, CD19 and CD79 being significantly associated with B-ALL. Additionally, CD34 (67.6%) and CD45 (67.9%) were predominantly expressed in B-ALL cases.

Markers associated with AML included MPO, CD13, CD33, CD117, CD64 and CD56, indicating their strong association with myeloid leukaemia. CD7 was present in both AML (62.5%) and T-ALL (37.5%) cases, suggesting partial overlap in marker expression.

Markers exclusive to T-ALL included CD3, CD2, and CD5, reinforcing their role as T-lineage-specific markers.

TdT was found only in B-ALL cases, while HLA DR was expressed primarily in AML cases with some presence in B-ALL.

Immature Markers such as CD34, CD45 was predominantly expressed in B-ALL. CD34 Expression is associated with poor therapy response. These findings demonstrate significant marker-leukemia associations, with the clear lineage differentiation between AML, B-ALL, and T-ALL based on the immunophenotypic expression.

Table 3 shows the correlation of flow cytometry markers with corresponding leukemia.

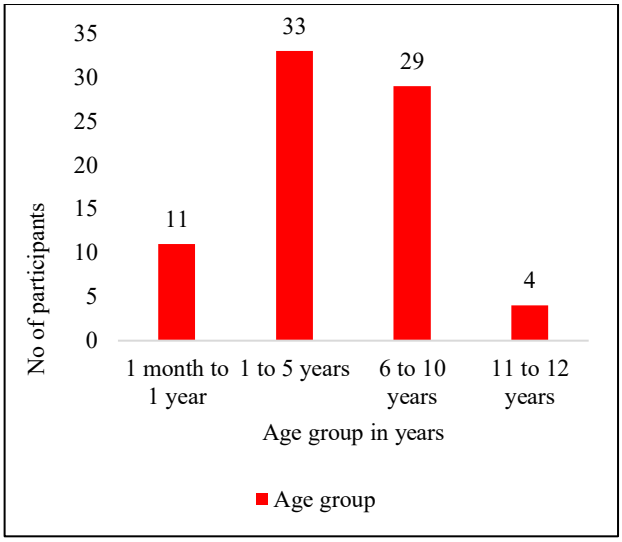

Figure 1: Age of the participants.

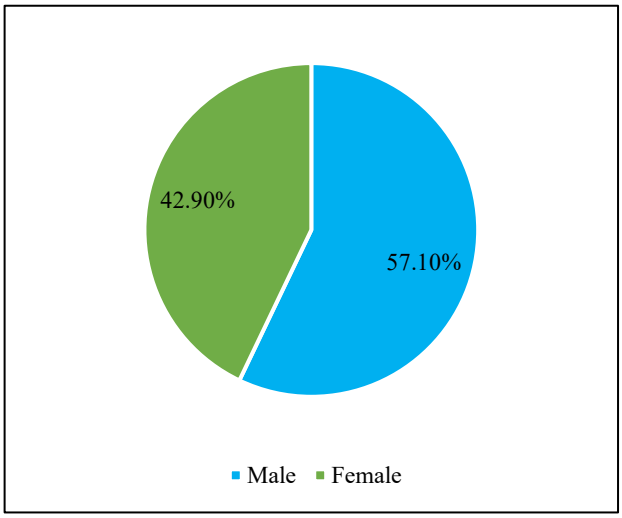

Figure 2: Gender of the participants.

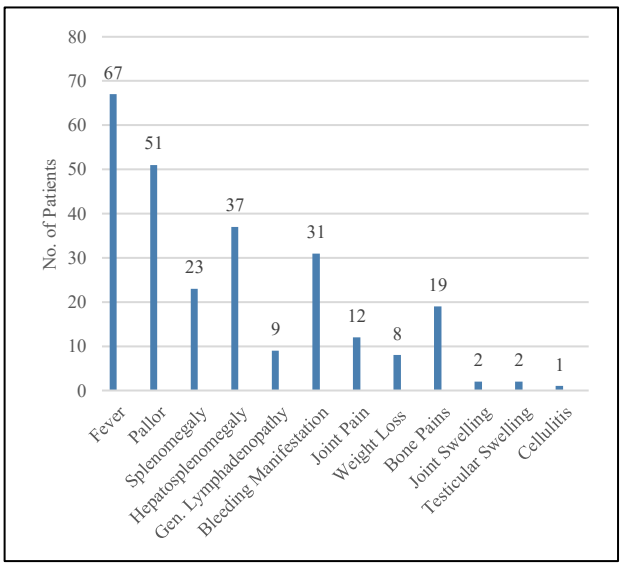

Figure 3: Clinical presentation.

Table 1: Hematological parameters.

Parameters	Mean±SD
RBC (million/µL)	3.02±0.90
HB (g/dL)	7.61±2.56
MCV (fL)	80.04±14.90
MCH (pg)	26.83±3.97
MCHC (g/dL)	31.86±3.71
TLC (/mm³)	50055.84±12052.58
Neutrophils (%)	13.96±18.14
Lymphocytes (%)	69.73±22.68
Monocytes (%)	14.40±6.25
Eosinophils (%)	1.33±0.77
Basophils (%)	0.68±0.17
Platelets (/mm³)	56412.99±15159.46

Table 2. Leukemia type among the participants.

Leukemia type	N	Percent (%)
AML M2	7	9.1
AML M3	5	6.5
AML M4	3	3.9
AML M7	3	3.9
AML with aberrant CD7	2	2.6
B-ALL	54	70.1
T-ALL	3	3.9
Total	77	100.0

Table 3: Correlation of cytometry markers with leukemia.

CD markers	B-ALL	AML	T-ALL
B cell markers (CD10, CD 19, CD 79A)	54 (100%)	0	0
Myeloid markers (MPO, CD13, CD33, CD117, CD64, CD56)	0	20 (100%)	0
T cell markers (CD3, CD2, CD5, CD7)	0	CD7-5	3 (100%)
CD34	23 (67.6%)	8 (23.5%)	3 (8.3%)
CD45	38 (67.9%)	15 (26.8%)	3 (5.4%)
Tdt	2 (100%)	0	0
HLA DR	3 (33.3%)	6 (66.7%)	

DISCUSSION

Acute leukemia remains one of the most common malignancies in the pediatric age group, characterized by diverse clinical presentations, hematological abnormalities, and varying immunophenotypic profiles. The accurate diagnosis and classification of leukemia subtypes are crucial, as they directly influence treatment strategies and prognosis. Clinical features such as fever, bleeding manifestations, lymphadenopathy, and organomegaly often serve as initial indicators, but overlap between subtypes necessitates the use of advanced diagnostic modalities. In recent years, flow cytometric immunophenotyping has emerged as an essential tool in refining leukemia classification by identifying lineage-specific markers and detecting aberrant antigen expressions, thereby enhancing diagnostic precision. This cross-sectional study aimed to analyze the clinico-hematological profile of children with acute leukemia and correlate these findings with flow cytometric results. The study provides valuable insights into age and gender distribution, presenting symptoms, hematological parameters, and distinct immunophenotypic patterns associated with various leukemia subtypes.

In the present study, B-ALL was predominantly observed in the 1 to 5 years age group (53.7%), AML was more common in the 6 to 10 years age group (40%), and T-ALL was mostly seen in older children, particularly in the 11 to 12 years group (66.7%). A statistically significant association was observed between age and leukemia type (p=0.001), indicating that B-ALL is more common in younger children, while AML and T-ALL increase in prevalence as age advances. Similar findings were reported by Venkatesan et al who noted that acute lymphoblastic leukemia (59.45%) was the predominant leukemia among children, while AML was more common in adults (82.22%).[3] Ferdousi et al also highlighted that the most common age group affected was less than 5 years (55%), with B-ALL being the major subtype, which aligns with the age pattern seen in our study.[12]

In the current study, B-ALL showed a male predominance (57.4%), while AML was equally distributed among males and females (50% each). Interestingly, T-ALL was observed exclusively in males (100%). However, the association between gender and leukemia type was not statistically significant (p=0.26). Khairwa et al also observed a striking male-to-female ratio of 4.6:1, highlighting male predominance in pediatric acute leukemia.[5] Vemprala et al while studying AML, reported a higher male-to-female ratio (1.5:1), which aligns with the general trend of male preponderance in leukemias.[14]

Fever was a predominant symptom in the present study, found in 87% of B-ALL, 85% of AML, and 100% of T-ALL cases. However, no significant statistical difference was observed between the types of leukemia concerning fever (p=0.77), suggesting that fever is a universal symptom in pediatric acute leukemia regardless of subtype. Similarly, Siddaiahgari et al reported fever as the most frequent symptom in pediatric ALL, observed in 92.33% of patients.[15] Choudhury et al found fever to be the most common presenting symptom across all acute

leukemias, aligning with our study's findings.[16] Ferdousi et al while focusing on the immunophenotypic profile, also noted fever as a common presenting complaint, reflecting the significant role of fever as an initial symptom leading to diagnosis.[12]

In the present study, bleeding manifestations were observed in 42.6% of B-ALL cases and 40% of AML cases, while none of the T-ALL patients presented with bleeding. Venkatesan et al reported that anemia and thrombocytopenia were common hematological abnormalities, contributing to bleeding manifestations, although they did not detail bleeding prevalence by subtype.[3] Khairwa et al emphasized that poor prognostic immunophenotypes like AML and T-ALL had a higher risk of mortality, often linked with severe cytopenias and bleeding.[5] Choudhury et al also observed that anemia and thrombocytopenia were prevalent, leading to bleeding tendencies, especially in ALL cases.[16] Kumar et al similarly found hepatosplenomegaly and pallor to be common, with bleeding manifestations frequently seen in ALL, underscoring the importance of thrombocytopenia-driven bleeding in acute leukemia presentations.[15]

In the present study, B-cell lineage markers like CD10, CD19, and CD79 were exclusively expressed in B-ALL cases, with 100% expression rates (p=0.001), establishing their strong diagnostic correlation. CD34 and CD45 were also predominantly seen in B-ALL, supporting their role in disease identification. Ferdousi et al similarly reported that in B-ALL, CD19 (90%) was the most frequently expressed marker, followed by CD10 and CD79a, mirroring our study's findings.[12] Jamal et al documented CD79a positivity in 99.8% of B-ALL cases and noted aberrant expression of myeloid markers in some B-ALL cases.[4] Jha et al also found that the flow cytometric pattern of CD10, CD19, and CD79a expression effectively established B-ALL diagnosis.[17] Sukumaran et al stressed the importance of including cytoplasmic markers like CD79a and CD22 in flow panels to accurately diagnose B-ALL, which aligns with our methodology.[18] Signe Modvig et al reported that high CD34 expression was associated with poor therapy response.[19]

In the present study, myeloid markers such as MPO, CD13, CD33, and CD117 were exclusively expressed in AML cases, with strong statistical significance (p=0.001). MPO was widely distributed across AML subtypes, most commonly in AML M2 (35%), while CD117 was predominantly expressed in AML M2 (54.5%) and AML M7 (27.3%). CD64 and CD56 also showed significant association with AML subtypes.CD56 was expressed in 40% of the cases, while CD7 was expressed in 25% of the participants. Ferdousi et al also reported high expression of MPO (93.24%), CD33 (86.58%), CD13 (79.92%), and CD117 (73.26%) in AML, closely aligning with our findings.[12] Jha et al found CD34, MPO, and CD13 critical for AML diagnosis and subtype differentiation, especially noting negative CD34 expression in APL variants.[17] Jamal et al highlighted aberrant myeloid marker expression even in lymphoid leukemias but confirmed these markers' strong association with AML.[4] Vemprala et al emphasized that cytogenetics and immunophenotyping, including myeloid marker profiling, were crucial for diagnosing AML and guiding treatment decisions.[14] Sandeep Rai et al conferred that expression of CD7 and CD56 was associated with higher relapse rate and death rate.[20]

In the current study, T-lineage-specific markers-CD3, CD2, and CD5-were expressed exclusively in T-ALL cases, with 100% positivity and high statistical significance (p=0.001). Additionally, CD7 was expressed in both T-ALL (37.5%) and AML (62.5%), reflecting lineage overlap. Ferdousi et al also found that in T-ALL, CD3 was universally expressed (100%), with additional expression of CD4, CD5, CD7, and TdT in subsets, similar to our observations.[12] Jamal et al reported that T-ALL accounted for 19.6% of lymphoblastic leukemias, with consistent expression of T-lineage markers.[4] Sukumaran et al observed that CD7 was the most common aberrantly expressed marker in AML, emphasizing the importance of careful interpretation.[18] Jahan et al found aberrant expression of myeloid markers in T-ALL, but CD3 remained lineage-specific, reinforcing its diagnostic specificity.[21]

The present study showed distinct immunophenotypic patterns within AML subtypes. AML M2 had the highest expression of CD34, MPO, CD13, and CD117. AML M3 showed strong CD64 expression, while CD41 and CD61 were exclusive to AML M7 (100%), reinforcing the diagnostic value of flow cytometry in distinguishing AML subtypes. Khairwa et al also noted that immunophenotypic profiling categorized AML into subtypes, with M2 and M4 being most frequent, consistent with our study.[5] Jha et al demonstrated that morphology and cytochemistry needed confirmation through flow cytometry for subtyping, especially distinguishing M2 from M3 and M4.[17] Vemprala et al highlighted the use of cytogenetics alongside immunophenotyping to understand subtype variability.[14] Ferdousi et al emphasized the high frequency of MPO and CD33 in AML M2, matching our findings and validating flow cytometry's crucial role.[12]

Limitation

This study is limited by its single-center design, which may affect the generalizability of the findings to other populations. Molecular and cytogenetic studies were not performed, restricting comprehensive risk stratification.

CONCLUSION

This study provides a comprehensive overview of the clinico-hematological profile and flow cytometric correlation in pediatric acute leukemia cases presenting to a tertiary care hospital. B-ALL was identified as the most

common subtype, predominantly affecting children between 1 to 5 years of age, while AML and T-ALL were more frequent in older age groups. Fever, pallor, and bleeding manifestations were the most common clinical presentations. Among the significant findings, age showed a strong association with the type of leukemia, with B-ALL common in younger children and T-ALL prevalent in older children. Flow cytometry revealed strong marker-subtype associations, such as CD10, CD19, and CD79 exclusively with B-ALL, and MPO, CD13, CD33, and CD117 with AML, emphasizing its indispensable role in diagnosis and classification.

Based on these findings, it is recommended that all suspected pediatric leukemia cases undergo detailed clinical evaluation, complete blood counts, and flow cytometric immunophenotyping for accurate subtype diagnosis. Integrating flow cytometry into routine diagnostic protocols and expanding access to molecular testing would further improve risk stratification, guide therapy, and ultimately enhance survival outcomes in pediatric acute leukemia. The study reinforces the importance of combining clinical evaluation, hematological parameters, and immunophenotyping for better diagnosis and management.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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