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Original Article

Characterization of Acute Leukemia Types and Subtypes using Flowcytometer at a Tertiary care Hospital of South Punjab

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ABSTRACT

Introduction: Acute leukaemia is a common disorder among children having age 2 to 5 years and fever, progressive pallor, fatigue, bleeding and easy bruising are common complaints of patients. Flow cytometric immunophenotyping is the test that is used for the diagnosis of acute leukaemia and its sub-classification. This susceptible and specific test gives an insight into bone marrow cell maturation and shows multidimensional radar plots.

Objective: To characterize acute leukaemia, its types and subtypes using a flow cytometer in children

Material and Methods: This descriptive Cross Sectional study was conducted at the department of Hematology in Children's Hospital and Institute of Child Health, Multan for a duration of one year from 1st February 2021 to 30th January 2022. It was performed after written informed consent in the Pathology department of Children's Hospital & the Institute of Child Health Multan. Immunophenotyping was performed using a flow cytometer of all study participants.

Results: A total of ninety-three children aged 1-15 years with acute leukaemia on peripheral blood or bone marrow aspirate smears were enrolled in the current study. We analyzed that fever, pallor and body aches were the common (80%) clinical presentations in our patients. The median age of study participants was 7.1 ± 3.98 years. Male to female ratio was 2.4:1. Majority of patients were found to have pre-B cell ALL (73.1%) followed by T cell ALL (15%), AML (7.5%) and phenotypic acute leleukaemia4.3%). We divide our patients into three age groups. Pre B cell ALL was commonly found in age group 1-5 years, T cell ALL in 10-15 years and AML in 5-10 years. CD 10 marker showed maximum positivity in pre-B cell ALL. Among B cell markers CD 79a showed the highest positivity (96.7%) followed by CD19 (92.1%) while in T cell ALL 100% positivity was seen in CD 3.

Conclusion: Flowcytometer is an essential tool for the characterization of acute leukaemia, its types and subtypes

Key Words: Immunophenotyping, Flowcytometer, Acute leukaemia

Introduction

Acute leukaemia is a clonal disorder of hemopoietic stem cell. Immature cells (blast cells) are increased in peripheral blood and bone marrow[1].

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Acute lymphoblastic leukaemia is a type of acute leukaemia which is a malignant disorder of lymphoid progenitor cells. It is common in children[2]. Its peak incidence occurs in ages between two to five years[3]. The common presenting complaints in these children are fever, progressive pallor, fatigue, bleeding and easy bruising. Hepatosplenomegaly and lymphadenopathy are commonly found on physical examination[4]. Precursor B cell (pre-B cell ALL) and thymic (T cell ALL) are two subtypes of ALL. Flow cytometric immunophenotyping is the test that is used for the diagnosis of acute leukaemia and its subclassification. This susceptible and specific test

gives an insight into bone marrow cell maturation and shows multidimensional radar plots [5,6]. Expert analysis is required in the flow cytometer process and the interpretation of its results[7]. Flow cytometer demonstrates immunophenotypic abnormalities and is specific for diagnosing acute leukaemia [8]. The principle of its working is based on the fact that leukemic cells in peripheral blood and bone marrow expressed immunophenotypes which are identified by flow cytometry using cluster deviation (CD markers)[9]. Due to frequent diagnoses of acute leukaemia on peripheral blood at The Children's Hospital & Institute of Child Health, Multan, the current study was performed to characterize acute leukaemia, its types and subtypes using a flow cytometer in children. A flow cytometer is better to diagnose acute leukaemia and its subtypes and also to evaluate the pattern of causes responsible for pancytopenia in children of our region.

Material and Methods

This descriptive cross-sectional study was conducted in the Pathology department of The Children's Hospital & Institute of Child Health, Multan for the period of one year from 1st February 2021 to 30th January 2022 after taking permission from the Ethical review committee of the hospital. After taking informed consent from attendants of patients with acute leukaemia, we enrolled ninety-three patients aged 1-15 years with 20% or more blast cells on peripheral film or bone marrow aspirate smear. A detailed history was taken from the parents and a physical examination of the patient was done. Samples of 2-3 ml blood or marrow aspirate were collected in EDTA vials. Laboratory investigations included complete blood count (RBC, Hemoglobin, TLC, DLC, platelet count) peripheral blood and marrow aspiration smears were made using Giemsa stain and their morphologies were studied under the light microscope. Immunophenotyping was performed on a flow cytometer (Navious Beckman Coulter) 05 colours using fluorochromes Fitch, PE, ECD, PC5 and PC7. The panel of antibodies used was HLA DR, CD45, CD34, CD10,CD19,CD20, CD79a, CD5,CD7,CD3, CD117,MPO,Tdt. Leukemic samples were considered positive when at least 20% of blast cells expressed the marker at fluorescence intensity above the cutoff established using the corresponding type-matched control antibody. These above-mentioned blood tests were noted on the Excell sheet for further analysis.

Data Analysis: All the data were analyzed by using SPSS version 20.0. The frequency distribution of quantitative variables was presented in tables and the Mean, median and SD of quantitative variables were evaluated.

Results

The total of ninety-three patients having acute leukaemia was included in this study. Fever, pallor and body aches were the common (80%) clinical presentations in our patients. Out of 93 patients 10% of patients presented with petechial haemorrhage, 4% with bleeding gums and 2% with epistaxis. The median age of our patients was 7.1 ± 3.98 years. There were 66 males (70.9%) and 27 females (29.1%). Male to female ratio was 2.4:1. immunophenotypic analysis on flow cytometer showed pre-B-ALL in 68 patients (73.1%),14 patients (15.%) showed T-ALL, while AML in 07 patients (7.5%) and bi-phenotypic acute leukaemia in 04 patients (4.3%).Expression of different markers were shown in table 1.

Table 1: Expression of lineage-specific and differentiation markers of acute leukaemia

Pre B ALL n=68	**Positive**	**Negative**	**Percentage of positivity**
CD 45	62	6	91.1%
CD79a	67	1	98.5%
CD 20	49	19	72.0%
CD 10	60	8	88.2%
CD 34	52	16	76.4%
Tdt	61	5	89.7%
CD 19	62	4	91.4%
T-ALL n=14	Positive	Negative	Percentage of positivity
Cd 5	11	03	78.5%
Cd 3	12	02	85.7%
HLA DR	05	05	50%
cCD3	14	00	100%
CD 7	10	04	71.4%
AML n=04	Positive	Negative	Percentage of positivity
CD 117	04	00	100%
MPO	04	00	100%

We divide our patients into three groups according to age as presented in Table 2. Pre-B ALL was common in age group 1-5 years (52.4%), 23 males and 8 females. 29.5% of patients in the age group 5-10 years, 13 males and 5 females while 18% in group 10-15 years, 8 males and 3 females. While T-ALL is common

in the age group 10-15 years (54.5%) followed by 27.2% in patients with 5-10 years of age and least common in the age group 1-5 years (18.1%). AML is most common in the age group 10-15 years (57.1%) 1 male and 3 female.

Table 2 Subtype distribution of acute leukemia in pediatric age groups

Age Group Years	Pre B cell ALL n=68	T cell ALL n= 14	AML n=7	MPAL n=4
1-5	38 (55.8%)	02(14.2%)	02(28.5%)	02(50%)
5-10	19 (27.9%)	05(35.7%)	02(28.5%)	02(50%)
10-15	11 (16.1%)	07(50.1%)	03(42.8%)	-

CD 10 is common marker of Acute Lymphoblastic Leukemia expression was positive in patients with pre B cell ALL 88.2%, and 40.1 % in T-ALL. In pre B cell ALL, CD 79a showed highest positivity 96.7% followed by CD19 92.1% . while in T cell ALL 100% positivity seen with CD 3.figure 1.

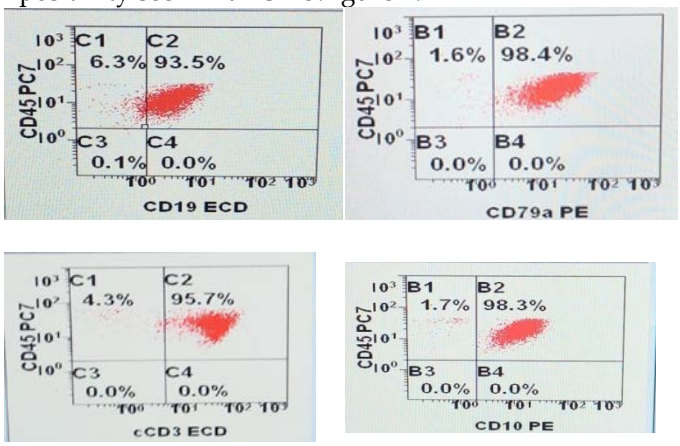

Figure-1: Immunophenotyping of pre B-ALL showing positivity for markers CD10, CD79a, CD 19 and of T-cell ALL showing positivity for Ccd3

Discussion

In our study immunophenotyping tests were performed on flowcytometer and showed that majority of patients had pre B cell ALL (86.7%) followed by T cell ALL (57.1%). These are comparable to other studies conducted on acute leukemia[10,11,12,13].However our results contradicts with a study conducted in Maranhao on paa ediatric patients that revealed T cell ALL as most commonly found subtype[14].

In current study pre B cell ALL was common in age group 1-5 years (55.8%) while T-cell ALL was commonly found in patients with 10-15 years of age (50.1%). Similar trend is seen in a study conducted in Morroco that showed patients with pre B cell ALL were younger (3-5 years) as compared to those with T-cell ALL[15].

In present study we found that ALL and its subtypes were common in male patients that is in accordance to a study conducted on pediatric patients in Brazil[14].

CD10 is a common marker of ALL and its expression has prognostic value in childhood leukemia. Our study showed CD10 expression to be 88.2 % in pre B cell ALL and 40.1 % in T cell ALL. In another study CD 10 expression reported as 91.2% in pre B cell ALL and 21.3% in T cell ALL [15].

In current study the myeloid marker CD 117 was co expressed with MPO, this is in accordance with a study conducted by Malutes et al[16].

The pattern of differentiation in MPAL is that multipotent progenitor stem cells differentiates into both lymphoid and myeloid lineages flow cytometric immuno phenotyping analysis in our study showed MPAL seen in 4% patients. It ranges from 2.2 -2.6% in a study but in that study adult patients were also included along with children[17]. It was found to be 2.2% in another study conducted in India by Nishi Gupta et al[1].

Our results revealed that among markers B-ALL, CD 79a showed highest positivity (96.7%) followed by CD19 (92.1%) while in T cell ALL 100% positivity seen in CD 3. Similar pattern was reported in other studies[14, 15] where ALL was more frequent in children (77.1%) and AML was more common in adults (77.3%). MPAL accounted for 2.9% of the cases of acute leukemia. These results are contrary to present study as we clearly mentioned that ALL was most frequently present.

Most of the studies on types and subtypes of acute leukemia, previously done in this region were retrospective and were carried out from patient's files and medical charts. Our work involves immunophenotyping test performed on flowcytometer and constitutes the first study to be carried out on pediatric patients in the region of South Punjab.

Conclusion

Flowcytometer is an essential tool for characterization of acute leukemia, its types and subtypes.

Conflict of interest: None

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