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Immunophenotypic Patterns of Childhood Acute Leukemia in Dhaka Medical College Hospital

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Abstract **Original Research Article**

Background: Leukemia is the most common malignancy at the age of under 15 years. Main subtypes of leukemia found in children are Acute Lymphoblastic Leukemia (ALL), with a percentage of 80%. Acute lymphoblastic leukemia (ALL) is one of the most common malignancies of childhood. ***Aim of the study:*** The aim of this study was to investigate the immunophenotyping profiles of childhood acute leukemia in Dhaka Medical College Hospital, Dhaka. ***Methods:*** This Cross-sectional study was conducted in the department of paediatrics in Dhaka Medical College Hospital, Dhaka, Bangladesh. A total 80 patients were selected as the study subjects. Immunophenotyping allowed classification into acute myeloid leukemia (AML) and ALL (B-lineage and T-lineage ALL). ***Result:*** Among of 80 samples, where we found maximum 51(63.75%) were Male and 29(36.25%) were Female. In age distribution, where maximum 38(47.5%) were (0-5) years of age, 32(40%) were (6-11) years of age, 10(12.5%) were (12-15) years of age, respectively. We have found, 57(71.25%) were B lineage ALL, then 10(12.50%) were AML, 9(11.25%) were T Cell ALL, 2(2.50%) was CML, 1(1.25%) was Mixed, 1(1.25%) was Aplastic. ***Conclusion:*** Immunophenotyping in a cross-sectional study proved feasible and appears particularly important for prognostic assessment of childhood leukemia in low-income countries such as Bangladesh.

Keywords: Leukemia; Childhood acute leukemia; Immunophenotyping; Age patterns.

INTRODUCTION

Leukemia is the most common malignancy at the age of under 15 years of children. Main subtypes of leukemia found in children are Acute Lymphoblastic Leukemia (ALL), with a percentage of 80%. Acute Lymphoblastic Leukemia (ALL) is one of the most common malignancies of childhood. The peak incidence occurs at age of 2-5 years and the average incidence of children under the age of 15 is 4-4.5/100,000 per year. The reasons why ALL is so common in childhood are not clear, but could be related to the proliferation of relatively undifferentiated cells which have remained dormant since embryonic and fetal life, in a manner analogous to neuroblastoma. But why these once-dormant rests should proliferate in some patients and not others are entirely unknown [1-3]. The diagnosis of acute leukemia is based on morphological and cytochemical investigations of bone marrow samples and/or peripheral blood smears. It also requires the integration of hematopathological diagnosis depending on studies of cell morphology, application of immunophenotyping and cytogenetic flow cytometry according to WHO 2016 classification [3-6]. The rationale for the clinical use of immunophenotypic techniques was based on the need for more objective criteria to support the morphological diagnosis and classification of ALL. The underlying hypothesis was that neoplastic cells from patients with these hematological malignancies corresponded to the leukemic counterpart of normal hematopoietic cells usually committed into one, or less frequently more than one, cell lineages, blocked at a specific maturation stage [7]. Refinement in classification of acute leukemias is accomplished by immunophenotyping. Differences in expression of surface membrane antigens or cytoplasmic components are used to identify and classify cell of origin and stage of differentiation. Immunophenotyping improves both accuracy and reproducibility of acute leukemia classification [8, 9]. It can be used to identify the lineage of acute leukemia and classify it into T-ALL, B-ALL, or AML (myeloid

lineage). This is important because the management and prognosis of the diseases are different. Immunophenotypic analysis is essential for accurately determining the lineage of the malignant clone of leukemic blasts besides the light microscopic diagnosis of childhood leukemia [10]. From this more general impression, a further hypothesis has been developed that suggests that the environment is a major determinant of the immunological sub-type of ALL. If this was true, we may be able to observe differences between the phenotypic character of the ALL blasts in Bangladeshi patients and those of patients in Western countries. Data in Bangladesh regarding the usage of immunophenotyping in acute leukemia as diagnostic instruments remain limited. For this reason, this study aimed to determine the immunophenotyping pattern of pediatric patients with acute leukemia[11]. The aim of the study was to investigate the immunophenotyping profiles of childhood acute leukemia in Dhaka Medical College Hospital, Dhaka.

OBJECTIVE

General objective

1. To investigate the immunophenotyping profiles of childhood acute leukemia in Dhaka Medical College Hospital, Dhaka.

Specific Objective

1. To illustrate the immunophenotypic pattern of the children diagnosed acute leukemia.
2. To determine the feasibility of incorporating the results of the study children.

MATERIALS AND METHODOLOGY

This cross-sectional study was conducted in the department of paediatrics in Dhaka Medical College Hospital, Dhaka, Bangladesh. This study was conducted from 1 July 2017 to 30 June 2018. A total 80 patients were selected purposively for the purpose of this study. A written consent was taken from the parents of the children. In addition to routine evaluation by complete blood counts, the patients were evaluated with peripheral blood films, bone marrow aspirate & trephine biopsy. Bone marrow aspirate and/or peripheral blood samples collected from all the patients were processed, stained with 4 colors combination of antibody with standardized “stain-lyse-wash” technique and acquired on recalibrated 2 laser 6 color BD FACS Canto II flow cytometer. The calibration was done using 7 Color set up beads and Cytometer Setup and tracking beads. The immunophenotyping by flow cytometry with a panel of Monoclonal Antibodies specific to acute leukemias usually used (29) as: CD3, CD5, CD7, CD10, CD13, CD14, CD19, CD20, CD33, CD34, CD117, CD 45, CD46, HLA-DR, Cy-MPO and TdT. T-cell ALL: cytoplasmic (cy) CD3, CD5, CD7 For B-cell ALL: CD19, CD10 and CD20. Myeloid cells: CD13, CD33, CD117, CD14, CD64 and cytoplasmic myeloperoxidase (Cy-MPO). Pan leukocyte marker: CD45. Precursor markers: CD34, TdT, HLA-DR. Any antigenic marker was considered positive if 20% or more of the blast cells reacted with a particular antibody. We included all patients both sexes if his/her age up to 15 years old with diagnosis of acute leukemia proved clinically, cyto-morphology and done flow cytometric immunophenotyping were included in this study. Patients without complete data and who refused to participate were excluded from this study.

RESULTS

A total 80 patients were selected as the study subjects. Of them, gender was distributed of the study people in table-1. Where maximum 51(63.75%) were Male and 29(36.25%) were Female respectively. In the age distribution maximum 38(47.5%) were (0-5) years of age, 32(40%) were (6-10) years of age, 10(12.5%) were (11-15) years of age, respectively. In this study, we found 57(71.25%) were B Cell ALL, then 10(12.50%) were AML, 9(11.25%) were T Cell ALL, 2(2.50%) were CML, 1(1.25%) was Mixed, 1(1.25%) was Aplastic above respectively (Table-1). The highest patients were followed in 3-5 years of age in B Cell ALL then second highest patients were followed in 8 years of age. In T Cell ALL highest patients found in 10-12 years of age. AML patients found most 4 years, 7 years and 11 years of age respectively (Figure-1). Regarding ALL 66 cases, we found in B Lineage ALL positive for CD19(52.50%), CD10 (51.25%), CD79a(35.00%), HLADR(50.00%), CD45(2.50%), CD13(5.00%), CD33(3.75%), CD34(32.50%), TdT (2.50%); T–ALL were positive for CD3(11.25%), CD5(11.25%), CD7(11.25%), HLADR(5.00%), CD79a(2.50%), CD15(1.25%), CD4(2.50%), CD34(5.00%); Myeloid markers were positive for CD13(12.50%), CD33(12.50%), CD34(5.00%), MPO(10.00%), CD117(8.75%), CD64(1.25%), CD56(1.25%), HLADR(5.00%), TdT(1.25%), CD7(3.75%); and others like mixed acute leukemia found positive (1.25%) in HLADR, CD45, CD3, CD7, CD13, CD33 respectively (Table-2).

Table-1: Demographic and immunophenotypic characteristics of the study subjects

Variable	n	%
Gender		
Male	51	63.75
Female	29	36.25
Age		
0-5	38	47.5
6-10	32	40
11-15	10	12.5
Mean±SD	5.79±3.18	
Immunophenotyping Patterns		
ALL	66	82.50
B Cell ALL	57	71.25
T Cell ALL	9	11.25
AML	10	12.50
CML	2	2.50
Mixed	1	1.25
Aplastic	1	1.25

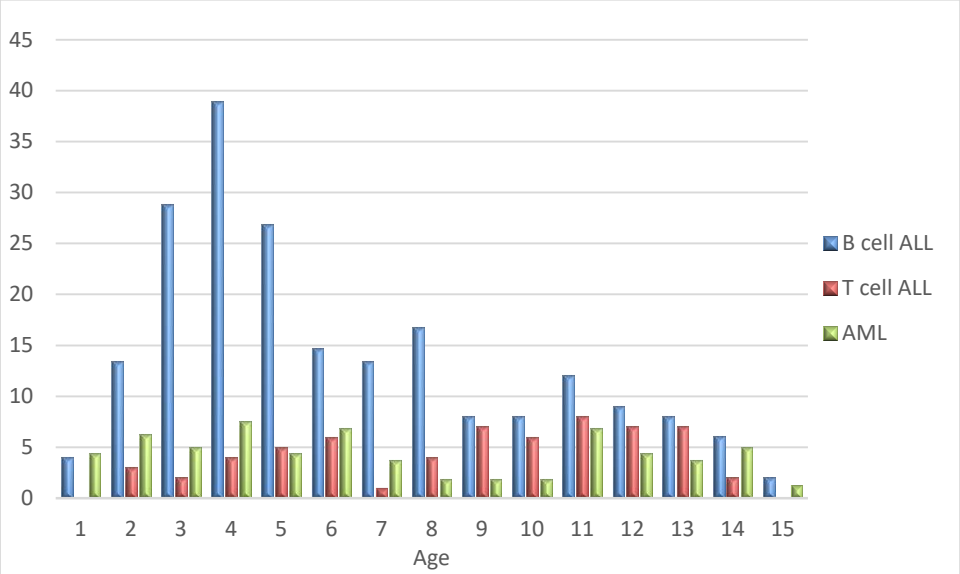

Figure-1: Age distribution of the different subtypes of childhood acute leukemia diagnosed by immunophenotyping

Table-2: Immunophenotypic profile using immunohistology examination in 80 cases of acute leukemia

Subtype	ALL (n=66)		AML (n=10)	Mixed (n=1)
	B cell (n=57)	T cell (n=9)		
CD19	42(52.50%)			
CD10	41(51.25%)			
CD79a	28(35.00%)	2(2.50%)		
HLADR	40(50.00%)	4(5.00%)	4(5.00%)	1(1.25%)
CD34	26(32.50%)	4(5.00%)	4(5.00%)	
TdT	2(2.50%)		1(1.25%)	
CD45	2(2.50%)			1(1.25%)
CD3		9(11.25%)		1(1.25%)
CD4		2(2.50%)		
CD5		9(11.25%)		
CD7		9(11.25%)	3(3.75%)	1(1.25%)
CD15		1(1.25%)		
CD13	4(5.00%)		10(12.50%)	1(1.25%)
CD33	3(3.75%)		10(12.50%)	1(1.25%)
MPO			8(10.00%)	
CD117			7(8.75%)	
CD64			1(1.25%)	
CD56			1(1.25%)	

DISCUSSION

Acute leukemias account for 352,000 new cases and 265,000 deaths worldwide in 2012 [12, 13]. The incidence is also on rise in North India with Punjab emerging as cancer capital of the country. Considering the poor prognosis early and accurate diagnosis of leukemia can reduce the morbidity and mortality rate in developing countries like India. The immunophenotyping has emerged as important tool in diagnosing acute leukemia. In our PCU, immunophenotyping on suspected leukemia patients started since March 2017-18, besides morphological and cytochemical methods. A total 80 patients were selected as the study subjects. Where maximum 51(63.75%) were Male and 29(36.25%) were Female respectively. Study done by Khan AH *et al.*, on Acute Lympmphoblastic Leukemia of northern India also showed two-fold male predominance, with 27 females and 48 males of total 75 cases [14].

Age was distributed of the study people about maximum 38(47.5%) were (0-5) years of age. Then 32(40%) were (6-10) years of age, 10(12.5%) were (11-15) years of age, respectively. Mean age was found in this study was 5.79 years. In the study of GT Roberts *et al*., the mean age of the whole group was 6.2 years and the median 5.0. The oldest patient was 14 years [15]. A study conducted in Indonesia in 2006, they found the patients age in between 0-14 which is very similar to our study [16].

Patterns status was distributed of the study we found maximum 57(71.25%) were B Cell ALL, then 10(12.50%) were AML, 9(11.25%) were T Cell ALL, 2(2.50%) were CML, 1(1.25%) were Mixed, 1(1.25%) were Aplastic above respectively. The classification into B- and T-lineage ALL is important for risk stratification and therapy of the patients [17]. However, others studies reported that ALL was predominant finding. In this study the majority of acute leukemia cases were diagnosed by flow cytometry, was AML compared to ALL. This observation was reported before from the Middle East countries [18]. Regarding ALL 66 cases, we found in B cell ALL positive for CD19(52.50%), CD10 (51.25%), CD79a(35.00%), HLADR(50.00%), CD45(2.50%), CD13(5.00%), CD33(3.75%), CD34(32.50%), TdT (2.50%); T–ALL were positive for CD3(11.25%), CD5(11.25%), CD7(11.25%), HLADR(5.00%), CD79a(2.50%), CD15(1.25%), CD4(2.50%), CD34(5.00%); Myeloid markers were positive for CD13(12.50%), CD33(12.50%), CD34(5.00%), MPO(10.00%), CD117(8.75%), CD64(1.25%), CD56(1.25%), HLADR(5.00%), TdT(1.25%), CD7(3.75%); and others like mixed acute leukemia found positive (1.25%) in HLADR, CD45, CD3, CD7, CD13, CD33 respectively. This result coincides with studies done before, where sub type of B cell ALL was predominant finding. Among 57 B cell ALL 41 patients were CD10 positive B cell ALL. CD34 is normally expressed in immature haemopoietic cells or blasts so it is an excellent marker for monitoring blast population. However, variable expression rate of CD34 was found in different studies. Expression rate of CD13 and CD33 was found in all patients with AML. MPO was the second frequent 8(10%) recognized marker and CD117 third frequent 7(8.75%) markers in AML patients were found in our study. Similarly, the CD13 was the second frequent recognized marker found in (92.06%) of the patients. Nearly similar result was reported from other studies [19]. Khan AH *et al*., reported 72% cases with B cell phenotype while 28% case had T cell phenotype. B-ALL was predominantly seen in children while T-cell ALL was predominantly seen in adults [14]. Shrestha S *et al*., also highlighted that if CD22 or CD79a expression is found either cytoplasmic or on the cell surface with the expression of CD19 and HLA-DR and also B-ALL with CD10 positivity has better prognosis than B-ALL with CD10 negative [20].

LIMITATIONS OF THE STUDY

The limitations of this cross-sectional study, as this study was conducted only in a hospital with a small sample size which may not reflect the whole scenario.

CONCLUSION

More than two-thirds of children with ALL can now be cured. Because of the diverse nature of the disease, we favor risk-directed therapy for all patients based on the molecular characterization of their leukemic cells at diagnosis. Our future goals include the identification of new genetic subgroups of ALL and the development of new therapies to directly target the oncogenic products of ALL translocations.

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