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Immunophenotypic Pattern in Pediatric Acute Lymphoblastic Leukemiain a Tertiary care Hospital in Bangladesh

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Key words:
Acute Lymphoblastic Leukemia, Immunophenotypic

Abstract

Background: *Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. Diagnosis of ALL is made on the basis of morphology, cytochemistry, immunophenotype and cytogenetics. Immunophenotyping is essential for WHO classification of acute leukemia. It is important for diagnosis, management and prognosis. The aim of the study was to evaluate the immunophenotypic pattern of pediatric ALL.*

Methods: *This cross-sectional study was conducted in the Department of Pediatric Hematology and Oncology in Sir Salimullah Medical College Mitford Hospital, Dhaka, Bangladesh from January 2022 to December 2023. A total of 54 children diagnosed with ALL were included in this study.*

Results: *This study found that out of 54 patients 47 (87%) were male and 7 (13%) were female. B-lineage ALL was found in 49 (90.7%) of the patients and T-lineage ALL was found in 5 (9.3%) patients. This study also found that B-lineage ALL patients were younger with median age six years in comparison to T-lineage ALL. According to French-American-British (FAB) classification L2 subtypes of ALL are more common in T-lineage ALL. Compared with B-lineage ALL higher leukocyte count, CNS involvement and mediastinal mass seem to be the typical features of T-lineage ALL. CD19, CD10, CD20, cCD79a, cCD22 markers were expressed in B-lineage ALL and CD3, CD4, CD5, CD7, CD8 were expressed in T-lineage ALL.*

Conclusion: *ALL is more common in male child. B-lineage ALL is more frequent than T-lineage ALL. T-lineage ALL is associated with FAB subtypes L2 variety, higher leukocyte count, mediastinal and CNS involvement. CD19, CD10, CD20, cCD79a, cCD22 markers were expressed in B-lineage ALL and CD3, CD4, CD5, CD7, CD8 were expressed in T-lineage ALL.*

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Introduction:

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. It accounts for 72% of all cases of childhood leukemia.[1] In the United States, approximately 4900 children are diagnosed with ALL annually with an incidence of 3 to 4 cases per 100,000 white children. The peak incidence is between ages of two to five years.[2] Diagnosis of ALL is made on the basis of morphology, cytochemistry, immunophenotype and cytogenetics. Morphological bone marrow examination is the first step for the primary diagnosis of ALL with an accuracy of 86.8% leaving a diagnostic dilemma.[3]

Leukemic cell of different type express characteristic nuclear, cytoplasmic and cell surface antigen which is known as the immunophenotype of the cell. Characterizing of the immunophenotype is referred to as immunophenotyping and is achieved by means of labeled antibodies that recognize specific cellular antigens by flowcytometry.[4-5]Immunophenotyping is crucial for ALL diagnosis because it definitively classifies the leukemia's lineage (B-cell or T-cell), distinguishes it from other leukemias like acute myeloid leukemia (AML), and identifies specific subtypes with prognostic significance. Immunophenotyping is essential for the identification of mixed phenotype acute leukemia. Detecting an aberrant immunophenotype that can be used for monitoring minimal residual disease. [6,7]

B-lineage ALL is positive for the following B cell markers CD19, CD22, TdT, cytoplasmic CD79a, CD20 and CD10. B-lineage ALL has been subclassified according to maturation stage into: early pre B (pro-B), pre-B, transitional (or late) pre-B and mature B-ALL.T-lineage ALL can also be categorized into phenotypic subgroups, correlating to differentiation stages of thymic T cells. T cell markers are cytoplasmic CD3, surface CD3, CD2, CD4, CD5, CD7, CD8. This lineage can be further subdivided into early, mid or late thymocyte differentiation. The World Health Organization (WHO) classification divides ALL into two main groups only, i.e., B-lineage and T-lineage ALL, without further categorization.[8-10]Analysis of the incidence of leukemia sub type across the world has revealed similar pattern. In Tamil Nadu, India a study revealed that 90.3% patients were ALL, 85.7% were identified as B-cell and 14.3% as T-cell. Of the B-ALL, 87.5% was pre-B, 8.3% pro-B and 4.2% mature B ALL. Myeloid antigen co-expression was seen in 35.7%.[11,12]

In a cross-sectional study conducted in Iran found that 23 children (44.23%) had B-ALL-ProB (pro-B) cell immunotyping features, 26 (50%) had B-ALL-PreB (pre-B) cell immunotyping features and 3 (5.7%) had T-cell immunotyping features. The ages of T-cell group children were higher than those of B-cell group children.[13]

A cross-sectional study conducted in Department of Pediatric Hematology and Oncology of DMCH by Khan et al. found that immunophenotyping is important for prognostic assessmentof childhood leukemiain low-income countries such as Bangladesh.[14]In a retrospective observational study conducted in Combined Military Hospital Dhaka and Ahsania Mission Cancer Hospital, Mirpur, Dhaka. In this study they found in case of B-ALL there was maximum expression was CD19 (90%), 2% aberrant expression of T-ALL marker CD5 and 14% aberrant expression of myeloid marker CD13 were present. In case of T-ALL maximum expression was CD3 (100%). In case of AML there was maximum expression of MPO (93%) and CD33 (87%) along with aberrant expression of B cell marker CD19 (6.66%)and 6.66% of each T cell marker CD3, CD5 and CD7 were present. [15]

Immunophenotype is necessary for accurate diagnosis of leukemia. Immunophenotypic characterization of acute lymphoblastic leukemia in childhood is important for directing therapy and predicting outcome. As there is paucity of information from developing countries, this studycertainly help as a baseline information for clinicians and other research workers.[9,14]So, the aim of this study was to evaluate the immunophenotypic pattern of pediatric ALL.

Methods:

This cross-sectional study was conducted in the Department of Pediatric Hematology and Oncology in Sir Salimullah Medical College Mitford Hospital, Dhaka, Bangladesh. This study was conducted from January 2022 to December 2023. Purpusive sampling was done. Newly diagnosed fifty four admitted cases of ALL of both sexes age eighteen years or less who received induction phase of

chemotherapy were included in this study.Newly diagnosed cases of ALL age less than one year or more than eighteen years were excluded from this study.All the information regarding the study was collected in a structured questionnaire after taking informed written consent.

Diagnosis of ALL was done on the basis of history, physical examination and morphological examination of PBF and bone marrow aspirate. Written informed consent was obtained before enrollment in the study from a parents or guardian. Data were collected using a preformed data collection sheet. Demographic data regarding age, sex had been collected from guardian or parents. Medical data regarding initial presentation at diagnosis, treatment received before admission were compiled. Clinical information about pallor, temperature, pulse, blood pressure, respiratory rate, bleeding manifestation, bony tenderness, lymphadenopathy and other systemic clinical parameter had been taken.

In suspected cases of leukemia, bone marrow aspirates were taken from iliac crest of patients at least two years of age and from tibial tuberosity less than two years of age. Immunophenotyping from aspirate marrow samples were done in a special hematology laboratory at department of Pediatric Hematology & Oncology, BSMMU using essential antibody panel. Immunophenotyping was performed on CYTOMICS FC-500 flowcytometer using CXP software. The cells were analyzed with the most appropriate blast gate using the combination of forward and side scatters. An antigen was considered positive when the expression is at least 20% of the gated cells. Prior to initiation of therapy routine baseline investigations were done which includes serum electrolytes, uric acid, inorganic phosphate, calcium, SGPT, creatinine, LDH, PT, APTT and Chest x-ray. Cytospin examination of CSF was done to determine the CNS status. Proper hydration and alkalization was done 24 hours before the start of chemotherapy and continued for 5-7 days through induction remission period. General supportive management like allopurinol, phosphate binder, oral care, anal care were given in all patients. All patients of ALL were treated with modified UKALL 2003 protocol, regimen 'A' or 'B' according to risk stratification. Regimen-A was given to patients aged 1-9 years and initial total WBC count <50000/mm^3. Regimen-B was given to patients aged 10 years or above and initial total WBC >50000/mm^3. Remission of induction therapy in ALL had been given for 35 days.

B-lineage markers are CD19, CD79a, CD22, CD20, CD10 (>20% of total cells). Among B-ALL, if CD10+, it is pre-B cell and if negative, it is pro-B cell. If IgM (surface) +, it is mature B-cell. T-lineage markers include CD2, CD3, CD4, CD5, CD7, CD8. Multipotent stem cell markers are CD34, CD45, TdT, HLA-DR. Any antigenic marker was considered positive if 20% or more of the blast cells reacted with a particular antibody.[3]

Statistical analyses were performed using IBM SPSS Statistics 26. Statistical significance is considered at a 95% confidence level with a 5% margin of error. Differences is considered significant at the $P < 0.05$ level for all these tests. The data is expressed as means ±SD for continuous variables and as frequencies (%) for categorical variables. Chi-square test is usedtoassessthe correlation between two qualitative variables.

Results:

A total of 54 patients were included, 41 (75.9%) were 1 to 10 years of age and 13 (24.1%) were more than 10 years of age. Among them 47 (87%) were male and 7(13%) were female(TableI).

Table I. Demographic and immuno- phenotypic characteristics of the study subjects

Variable		Number of patients	Percentage (%)
Age	1-10 yrs	41	75.9
	>10-18 yrs	13	24.1
	Mean±SD	3.56 - 10.78	
Gender	Male	47	87.0
	Female	7	13.0
	M:F	6.7:1	

B-lineage ALL was found in 49 (90.7%) of the patients and T-lineage ALL was found in 5 (9.3%) patients(Figure I).

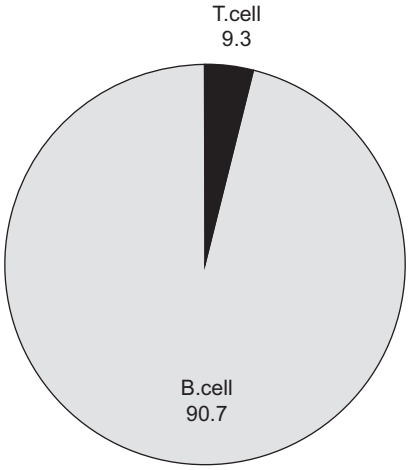

Figure 1: Immunophenotypic characteristics of the study subjects

The patient'us characteristics in relation to immunophenotyping are summarized in Table II. The age distribution of the different ALL subtypes shows that B-lineage ALL patients were younger with median age six years. There was a trend for patients with T-lineage ALL to have a more advanced age with median age twelve years which was statistically significant*(p = 0.002)*.FAB subtype L1 is more common in B-lineage ALL (91.8%) in comparison to T-lineage ALL (40.0%) and L2 is more common in T-lineage ALL (60.0%) in comparison to B-lineage ALL (8.2%) which was statistically significant (0.001). Compared with B-lineage ALL higher leukocyte count (*p= 0.004*) and mediastinal mass (*p<0.001*) seem to be the typical features of T-lineage ALL subtype.

Table II: Patients' characteristics

Features		B-ALL (n=49)	T-ALL (n=5)	All patients (n=54)	p-value
Age at diagnosis (y)	Median	6.0	12.0	6.0	
	Range	(2-15) yrs	(10-15) yrs	(2-15) yrs	
	1-10 yrs	40(81.6%)	1(20.0%)	41(75.9%)	0.002
	>10 yrs	9(18.4%)	4(80.0%)	13(24.1%)	
Gender	Male	43(87.8%)	4(80.0%)	47(87.0%)	0.623
	Female	6(12.2%)	1(20.0%)	7(13.0%)	
	Sex ratio (M/F)	7.2:1	4:1	6.7:1	
FAB subtype n (%)	L1	45(91.8%)	2(40.0%)	47(87.0%)	0.001
	L2	4(8.2%)	3(60.0%)	7(13.0%)	
	L3	0(0.0%)	0(0.0%)	0(0.0%)	
Leukocyte count ($\times 10^9$/L)	<50	39(79.6%)	1(20.0%)	40(74.1%)	0.004
	>50	10(20.4%)	4(80.0%)	14(25.9%)	
CNS status	CNS1	49(100.0%)	2(40.0%)	51(94.4%)	<0.001
	CNS2	0(0.0%)	2(40.0%)	2(3.7%)	
	CNS3	0(0.0%)	1(20.0%)	1(1.9%)	
Mediastinuminvolvement	Present	8(16.3%)	4(80.0%)	12(22.2%)	0.001
	Absent	41(83.7%)	1(20.0%)	42(77.8%)	

Table III: *Expression of CD markers in ALL patients through immunophenotyping (n=54)*

CD marker	B-ALL(n=49)	T-ALL(n=5)
CD 19	49(100.0%)	0(0.0%)
CD 10	42(85.7%)	0(0.0%)
CD20	26(53.1%)	1(20.0%)
cCD22	22(44.9%)	0(0.0%)
cCD79a	25(51.0%)	0(0.0%)
CD 2	0(0.0%)	2(40.0%)
CD 3	0(0.0%)	5(100.0%)
cyCD3	0(0.0%)	5(100.0%)
CD 4	0(0.0%)	4(80.0%)
CD 5	1(2.0%)	5(100.0%)
CD 7	0(0.0%)	3(60.0%)
CD 8	0(0.0%)	3(60.0%)
CD 38	41(83.7%)	0(0.0%)
HLA DR	49(100.0%)	0(0.0%)
NuTdT	30(61.2%)	2(40.0%)
CD 34	30(61.2%)	1(20.0%)
CD 117	1(2.0%)	0(0.0%)
CD 123	11(22.4%)	0(0.0%)
CD 81	14(28.6%)	0(0.0%)
CD 56	0(0.0%)	0(0.0%)
CD 58	40(81.6%)	0(0.0%)
CD 99	11(22.4%)	0(0.0%)
CD73	2(4.1%)	0(0.0%)
CD13	4(8.2%)	0(0.0%)
CD33	2(4.1%)	0(0.0%)
cMPO	0(0.0%)	0(0.0%)
CD14	0(0.0%)	0(0.0%)
CD64	0(0.0%)	0(0.0%)
CD73	10(20.4%)	0(0.0%)
CD66	18(36.7%)	0(0.0%)

Forty nine children with B-lineage ALL were studied. All tested B-lineage ALL expressed CD19. Almost all cases had CD10 (85.7%) positive.More than half of the cases expressed CD20 (53.1%), cCD79a (51.0%). HLA DR, CD 38, CD 58, terminal deoxynucleotidyl Transferase (NuTdT), CD 34 were detectable in 100.0%, 83.7%, 81.6%, 61.2%, 61.2% of cases respectively. cCD22 is also positives in 44.9% of cases.

Five children with T-lineage ALL were studied. Based on their reactivity with various anti-T-cell monoclonal antibodies (Table III), all tested T-cell ALL cells had cytoplasmic CD3, surface CD3. CD5 and CD4 were found positive in 80% of cases. Both CD7 and CD8 were present in 60% of cases, NuTdT were present in 40% of cases.

Discussion

Immunophenotyping has become an indispensable diagnostic tool for identification of cell lineage of leukemia, disease classification, patient management and for disease monitoring of acute leukemia.[16,17] During the study, a total 54 patients of ALL in the Department of Pediatric Hematology and Oncology, Sir Salimullah Medical College, Mitford Hospital were studied to see the pattern of immunophenotyping.

In this study most of the patients were in 1 – 10 years of age and male predominance. Mean age was found in this study 7.17 years. In a study conducted in Syria found that 48.8% patients age ranging from 5-9 years with a male predominance (60.9%).[18]In a study done by Khan AH et al. on acute lymphoblastic leukemia of northern India also showed two-fold male predominance, with 27 females and 48 males of total 75 cases.[19]In the present study B-lineageALL was found to be more prevalent than T-lineageALL.

In a Pakistani study Moizza S. found that B-lineage ALL was more common than T-lineage ALL.[20]In a prospective study conducted in northern India found that 72% patients with B-lineage ALL and 28% patients with T-lineage ALL.[15]Different study found that percentage of T lineage ALL varies from 9.7% to 25.5%.[21,22]

We found that there is significant relationship among T-cell phenotype with >10 years of age, L2 variety ALL, leukocyte count >50 $\times 10^9$/L, CNS and mediastinum involvement. Different study found that the incidence of T-lineage ALL is more common in older age, male sex, L2 variety. Mediastinal and CNS involvement are more common in T-lineage ALL.[23-25]

In our study all cases of B lineage ALL expressed CD19. Many of the cases expressed CD10, CD20, cCD79a, HLA DR, CD38, CD58, NuTdT. All tested T-lineage ALL expressed cytoplasmic CD3, surface CD3. Many of these are positive for CD4, CD5, CD7 and CD8. In a retrospective study conducted by Zulfania et al. in Morocco found that CD19, CD22, and CD79a expression were found in all cases. Most of the T- lineage ALL express cytoplasmic CD3, CD5, and CD7.[26]

Another study done on immunophenotyping of acute leukaemia reported all cases of B-lineage

ALL to be positive for CD19, 90% to be CD10 positive while CD79a positivity was reported to be in 92.5% cases. CD20 was reported to be positive in 42.5% cases in the same study.[27]

Studies of the prognostic significance of CD10 expression in ALL have showed the CD10 expression in childhood B lineage ALL is associated with several favorable presenting features but is not an independent prognostic factor.[28-29]

Santoset al. also highlighted that if CD22 or CD79a expression is found either cytoplasmic or on the cell surface with the expression of CD19, HLADR and also B-ALL with CD10 positivity has better prognosis than B-ALL with CD10 negative.[30]

Conclusion:

ALL is more common in male child. B-lineage ALL is more frequent than T-lineage ALL. FAB subtypes L1 varietyis more common in B-lineage ALL and L2 varietyis more common in T-lineage ALL. Compared with B-lineage ALL higher leukocyte count, mediastinal and CNS involvement seem to be the typical features of T-lineage ALL subtype. CD19, CD10, CD20, cCD79a, cCD22 markers were expressed in B-lineage ALL patients and cCD3, surface CD3, CD4, CD5, CD7 and CD8 were expressed in patients of T-lineage ALL.

Limitations:

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.

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Conflict of interest:

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Fundiung: Self

Ethical Clearance :

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board of the Sir Salimullah Medical College Mitford Hospital, Dhaka. Written informed consent was taken from all the patients before taking part of the study.

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