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ORIGINAL ARTICLE

Immunophenotypic Characterization of Childhood Acute Leukaemia in A Tertiary Care Center of Bangladesh

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Abstract

Background: *Leukemia is one of the most common tumors in children. Childhood acute leukaemia (AL) is a heterogenous disease. Immunophenotyping is an essential part of the modern diagnostic workup/for typing and subtyping and prognostic stratification of AL and thus for an appropriate treatment of these complex and heterogeneous diseases.*

Objectives: *Objective of this study was to find immunophenotypic charectarization of childhood acute leukemia in children of Bangladesh. There is very limited study done on this subject in our country.*

Methods: *This is a retrospective observational study done in children with acute leukemia under 15 years of age, treated in two tertiary care centers for Paediatric Oncology [Combined Military Hospital Dhaka and Ahsania Mission Cancer Hospital, Mirpur, Dhaka]. Data were collected from hospital registry from 2014 to 2020 and then analyzed.*

Results: *Total study population were 82; among them male 55%, female 45% and M:F 1:0.82. Most common age group was <5 years age with 55% patients. Disease distribution showed 77% patients had ALL and 23% AML. Among ALL, subtype distribution showed B-cell type 90.5% T-cell type 9.5%. A good number of patients did Immuno-phenotyping analaysis before starting chemotherapy, 68 out of 82 acute leukemia patients (83%). In case of B-ALL highest expression of antigen was CD19 (90%) followed by CD10 (76%), HLADR (76%), CD22 (74%), CD79a (68%), TdT (56%) and CD34 (48%). co-expression of CD10/19was seen in 38% cases. Even in 13% cases, expression of myeloid marker CD13 (14%) and T cell marker CD5 (2%) were seen. In case of T-ALL there was 100% expression of CD3. Expression of other antigen CD4, CD5, CD7, CD45, TdT was 33.33% in each. Expression of CD10, CD1a, CD2 and TCRAb also found 33.33% in each. In case of AML highest expression was MPO (93.24%) followed by CD33 (86.58%), CD13 (79.92%), CD117 (73.26%), CD45 (66%), HLADR (46.62%) and CD64 (46.62%). There was 6.66% aberrant expression of B cell marker CD19 and and T-cell marker CD3 (6.66%), CD5 (6.66%) and CD7 (6.66%).*

Conclusion: *In this study we 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.*

Keywords*: Childhood acute leukaemia, immunophenotyping.*

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Introduction

Acute leukemias (AL) are hematological neoplasms featured by altered proliferation and/or differentiation of hematopoietic progenitors, leading to accumulation of immature cells in bone marrow (BM) and peripheral blood (PB).[1] It is a heterogenous disease, presents with varying clinical, morphological, immunological and molecular characteristics.[2] It is very highly curable if diagnosed and treated properly. Dramatic improvements in survival have been achieved in children and adolescents with cancer. Between 1975 and 2010, childhood cancer mortality decreased by more than 50%. For ALL, the 5-year survival rate has increased approximately 90% for children & AML 68% for children younger than 15 years.[3,4] For detail typing and subtyping of acute leukemia immuno-phenotypingis crucial.[5] It is an essential part of the modern diagnostic workup and prognostic stratification of AL and thus for an appropriate treatment of these complex and heterogeneous diseases. To do precise classification and identification of aberrant antigen expression among neoplastic population immunophenotyping is the only way.[6] It is also important for selection of treatment, to predict prognosis and to see minimal residual disease (MRD). There is very limited study on immunophenotyping of childhood acute leukaemiain Bangladesh because of many limitations.

The objective of this study was to see the type, subtype andimmunophenotypic characterization of childhood acute leukemia presented in the children of Bangladesh.

Materials and Methods

It was a retrospective observational study. The study was done in department of paediatric oncology of Combined Military Hospital, Dhaka and Ahsania Mission Cancer Hospital, Mirpur, Dhaka. All diagnosed cases of childhood acute leukemia treated in these two centres from 2014 to 2020 were included in this study. Their age range was less than 15 years. There were total 82 cases of children with acute leukaemia were analysed, their types and subtypes were evaluated. Immunophenotyping were advised and only 68 cases were available for analysis. It was about 83% of total study population. Immuno-phenotyping was done by flowcytometry of bone marrow aspiration and in few cases by immunohisto-chemistry of bone marrow trephine biopsy also done. In earlier years, this test is not widely available in Bangladesh, for this some patient did that from neighbouring countries most lyIndia. All data were collected from patient registry, scrutinized and then analyzed with the help of MS-Excel 10. Variables that have been collected were age, sex, types and subtypes of leukemia and their immunophenotypic findings.

Results

From these two centers, total study population we collected 82 of acute leukemia patients. Gender distribution of them found, male 55%, female 45% and male female ratio 1:0.82 (Fig.-1). Age group distribution showed most common age group was <5 years age with 55% patients (Fig.-2). Other age groups like 5-10 years age group were 33% and >10 years age group were 12%.

Disease distribution analysis showed 77% patients had ALL and 23% AML (Fig.-3). In case of ALL, morphologically L1 were commonest (62%) and then L2 (38%). Among AML patients, both M0 and M3types were commonest found in 26.31% each, next common groups were M1 (21.06%), M4 (10.53%) and undifferentiated type (15.79%) (Table I). Among all the 82 patients, immunophenotyping were done in 68 patients (83%), and 14 patients (17%) were unable to do it (Fig.-4). Subtype distribution among ALL patients showed, among the 63 patients, 53 did immunophenotyping prior starting their chemotherapy. Of them B-cell type were 90.5% T-cell type were 9.5%**.** Among the AML patient, 15 out of 19 patients did immunophenotyping, of them Non APML was 73.33%, APML was 26.67% (Table II).

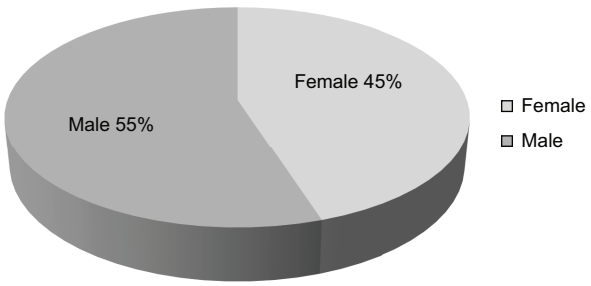

Fig.-1: *Distribution of patients as per gender (N= 82)*

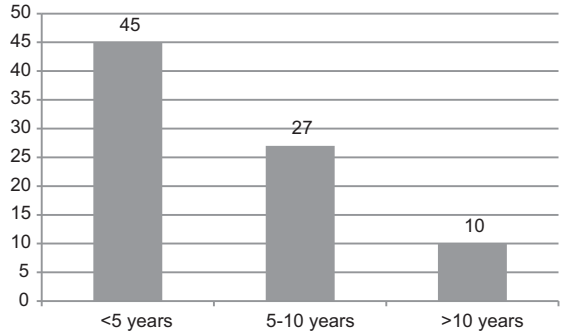

Fig.-2 *Distribution of patients as per age (N=82)*

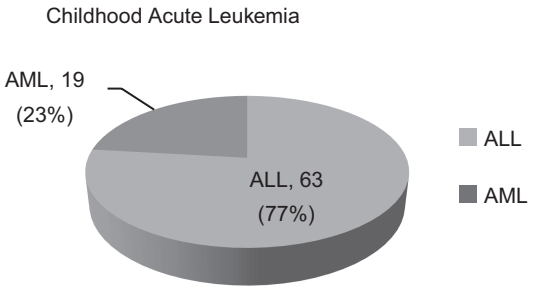

Fig.-3 *Disease distribution among childhood leukemia patient*

Table I
Distribution of patients as per types and subtypes of Leukemia (N=82)

Types of Leukemia	Number	Subtype	Number	%
ALL	63 (76.83%)	L_1	39	61.90
		L_2	24	38.09
		L_3	0	0
		B - ALL	57	90.47
		T - ALL	6	9.53
AML	19 (23.17%)	M_0	5	26.31
		M_1	4	21.06
		M_2	-	-
		M_3	5	26.31
		M_4	2	10.53
		Undifferentiated	3	15.79

Table II
Distribution of sub types of Acute leukemia according to IPT report (N=68)

Types of Leukaemia	Subtype	Number	%
ALL	IPT done	53	
	B - ALL	50	90.50
	T - ALL	3	9.50
AML	IPT done	15	
	Non APML	11	73.33
	APML	4	26.67

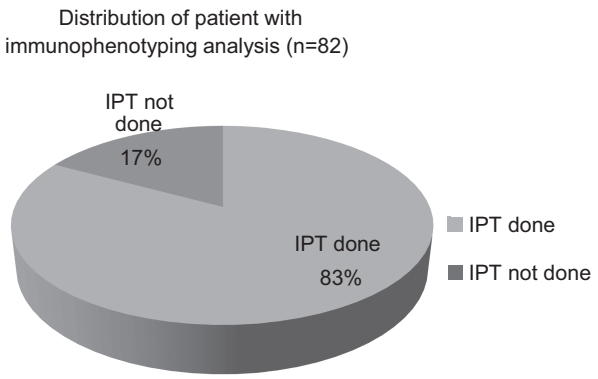

Fig.-4 *Distribution of patient with immunophenotyping analysis (N=82)*

In case of B-ALL highest expression of antigen was CD19 (90%) followed by CD10 (76%), HLADR (76%), CD22 (74%), CD79a (68%), TdT (56%) and CD34 (48%). co-expression of CD10/19 was seen in 38% cases. Even in 13% cases, expression of myeloid marker CD13 (14%) and T cell marker CD5 (2%) were seen. In case of T-ALL there was 100% expression of CD3. Expression of other antigen CD4, CD5, CD7, CD45, TdT was 33.33% in each. Expression of CD10, CD1a, CD2 and TCR Ab also found 33.33% in each. In case of AML highest expression was MPO (93.24%) followed by CD33 (86.58%), CD13 (79.92%), CD117 (73.26%), CD45 (66%), HLADR (46.62%) and CD64 (46.62%). There was 6.66% aberrant expression of B-ALL marker CD19 and T-ALL marker CD3, CD4, CD5, CD7 also (Table III).

Table III
Antigen expression of B-ALL, T-ALL and AML

Antigen	Expression					
	B- ALL (n=50)		T- ALL (n=3)		AML(n=15)	
	Number	%	Number	%	Number	%
CD19	45	90	-	-	1	6.66
CD20	12	24	-	-	-	-
CD22	37	74	-	-	-	-
CD79a	34	68	-	-	-	-
Tdt	28	56	1	33.33	-	-
CD34	24	48	-	-	6	39.96
CD38	2	4	-	-	2	13.62
CD117	1	2	-	-	11	73.26
CD10	38	76	1	33.33	1	6.66
CD 10/19coexpression	19	38	-	-	-	-
CD3	-	-	3	100	1	6.66
CD4	-	-	1	33.33	1	6.66
CD5	1	2	1	33.33	1	6.66
CD7	-	-	1	33.33	1	6.66
CD8	-	-	1	33.33	-	-
CD36	-	-	-	-	2	13.62
MPO	-	-	-	-	14	93.24
CD13	7	14	-	-	12	79.92
CD14	-	-	-	-	3	19.98
CD15	1	2	-	-	6	39.96
CD33	2	4	-	-	13	86.58
HLADR	38	76	-	-	7	46.62
CD64	-	-	-	-	7	46.62
CD58	2	4	-	-	-	-
CD9	1	2	-	-	-	-
CD99	1	2	-	-	-	-
CD11b	-	-	-	-	3	19.98
IgM	3	6	-	-	-	-
CD1a	-	-	1	33.33	-	-
CD2	-	-	1	33.33	-	
TCRab	-	-	1	33.33	-	-

Discussion

Leukemia is the most common childhood cancer with the incidence of 25-30%.[7] Children usually suffer from Acute leukemia (97%) is a heterogeneous group of neoplastic diseases and is categorized into two main subgroups: acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML).[8] ALL is a heterogeneous disease with abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow, peripheral blood and lymphoid tissues. ALL patients are subdivided into three morphological subsets including L1, L2 and L3.[9,10] AML is an aggressive malignancy same as ALL and it is characterized by accumulation of immature myeloid progenitors in the bone marrow.[11] It is the most common childhood malignancy in Bangladesh also.[12] The relative survival rate is also very high in case of childhood acute leukaemia. From 2009 to 2015 for children younger than 15 years of age, the 5-year relative survival rates were 91.9% in ALL and 68.7% in AML.[13] Though we are facing high number of childhood oncology cases there is no centralized cancer registry in our country.[14-16] Despite being the most common malignancy the number of studies on immunophenotyping of childhood acute leukaemia is very limited in our country.This data is one of the largest in Bangladesh. This data demonstrating demography, types, subtypes and immunophenotypic characterization of childhood acute leukaemia. Incidence of childhood leukemia occur in 1-10 year age group.[17] ALL is more common in 2-5 years of age and AML in <2 years and teenage age group.[18] In this study maximum patients were in the <5 years age group'. A study conducted by Amna et al[19] from Pakistan reported 83.3% ALL, two studies from India reported 76.9% and 77.84%.[20,21] Khasru AA et al[22] from Bangladesh also reported 58% cases of ALL. In our study we found 76.83% cases of ALL is which is similar to data of neighboring countries. Amna et al[19] also reported 14.7% AML cases, and incidence was slightly higher in Indian studies.[20,21] Khasru AA et al[22] found incidence of AML was 10% in Bangladesh. In our study, AML was 23.17% which is higher may be due to small sample size. In this study B-ALL is 79.36% which is similar to Pakistani studies (78.5-87%)[23,24] as well as an Indian study by Madhumathi et al.[25] However studies from West reported between 72.9 and 91% frequency.[26] Studies from Pakistan reported T-ALL 13-23%[27,28] while similar distribution was reported from India.[20,21] In this study T-ALL is only 4.76% which is lower than others may be due to small sample size. Belurkar et al[29], Bhattacharyya et al[30] reported 'CD19' as the most sensitive marker for diagnosis of B-ALL. We also found similar result in our study, for B-ALL expression of CD19 is 90% which is consistent with other studies. Tong et al[26] reported CD79a as the most often expressed antigen. We found expression of CD79a for B-ALL is 68% which is lower than other studies. A wide range of aberrant expression of myeloid antigens including CD13 and CD33 in B-ALL and T-ALL cases reported in different literature.[31,32] There is 14% aberrant expression of CD13 and 4% aberrant expression of CD33 in case of B-ALL in this study which is consistent with a study done in Srilanka.[33] In this study there is 76% negativity of CD20 for B-ALL which is high in contrast to other study. Negativity for CD20, as B-cell specific marker was observed in 61.8% of B-ALL cases reported by Tong et al.[26] Absence of CD10 expression was in 5% to 18.8% in eastern and western studies[26,32] and in this study, we found it 24% which is higher. It is an established fact that CD3 is the best marker for T-ALL, we found it 100% cases of T-ALL similar like other studies[33,34] but CD5 (33%) and CD7(33%) expressions found lower in this study may be due to small sample size. In this study, in case of AML maximum positivity was MPO (93.24%) followed by CD33 (86.58%), CD13 (79.92%) and CD117 (73.26%) but study from Pakistan and India shows that maximum positivity is for CD33 & CD13.[34,35] Also there is 6.66% aberrant expression of B-cell marker CD19 and T-cell marker CD3 (6.66%), CD5 (6.66%) and CD7 (6.66%) found in our study. Tien HF et al reported that in B-cell marker (7.5% cases) and T-cell marker (16.8% cases) may be present in case of AML.[36] This study has got some limitations. Some cases immunophenotyping was not performed. Correlation between morphologic subtype and immunophenotypical subtype was not done.

Conclusion

In this study we 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.

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