ORIGINAL ARTICLE

Antigen Expression on Blast Cells and Hematological Parameters at Presentation in Acute Lymphoblastic Leukemia Patients

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Abstract

Objective: To analyze the expression of various antigens on the leukemic blasts and to determine the hematological parameters, in Acute Lymphoblastic Leukemia (ALL) patients at presentation.

Study Design: Observational study.

Place and Duration of Study: King Edward Medical University, Lahore and Hameed Latif Hospital, Lahore, from February 2013 to March 2014.

Methodology: A total of 50 newly diagnosed and untreated patients of ALL were selected from Mayo Hospital and Hameed Latif Hospital. These patients included both genders and all age groups. Hemoglobin, total leukocyte count and platelet count were determined on hematology analyser-Sysmex-Kx-2I. Blast cell percentage was estimated on Giemsa stained blood smears. Immunophenotyping was done on bone marrow samples by 5 colour flowcytometery on Beckman Coulter Navious Flowcytometer. An acute leukemia panel of 23 antibodies was used. The data was entered and analyzed in SPSS version 22.

Results: Of the 50 ALL patients, 36 (72%) were B-ALL and 14 (28%) T-ALL. There were 18 (36%) children and 32 (64%) adults. T-ALL included 22% of the childhood and 31% of the adult cases. Immunophenotypic analysis showed that CD19, CD79a and CD20 were B-lineage specific markers whereas cCD3, CD3 and CD5 were T-lineage specific. CD10 was the most sensitive marker for B-ALL and CD7 was the most sensitive marker of T-ALL. TdT was expressed in 92% B-ALL and 71% T-ALL cases, CD34 in 58% and 43% cases and CD45 in 83% and 100% respectively. High leukocyte count (> 50 x 10^9/L) was present in 58% cases. Hemoglobin was < 10 g/dl in 74% patients and platelet count was below 20 x 10^9/L in 12% patients. Leukocyte count, hemoglobin, platelet count and blast cell % did not show a significant difference in the two ALL immunotypes.

Conclusion: The frequency of T-ALL is higher in childhood as well as adult ALL in our population compared to the Western literature. Antigenic expression of the blast cells also shows some interesting differences. A large number of our patients present with high leukocyte count which is a known factor associated with poor prognosis.

Key Words: *Acute lymphoblastic leukemia (ALL). B-lymphoblastic leukemia (B-ALL). T-lymphoblastic leukemia (T-ALL). Flowcytometry. Immunophenotypes. Hematological parameters.*

INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is characterized by neoplastic proliferation of lymphoblasts primarily in the bone marrow. ALL is the commonest malignancy among children. It represents 25 - 30% of all cancers in children and < 1% in adults. In the United States, it accounts for almost 80% of all childhood leukemias and 20% of adult leukemias.[1]

With the discovery of an increasing number of Monoclonal Antibodies (MoAbs) and the improvements in the technique of flowcytometry; immunophenotyping is contributing to a better diagnosis and management of ALL patients.[2]

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ALL is classified into B-lymphoblastic leukemia (B-ALL) and T-lymphoblastic leukemia (T-ALL) immunophenotypes according to the 2008 WHO classification.[3]

The vast majority, i.e. about 85% of childhood and 75% of adult ALL cases are B-ALL and consequently 15% and 25% are T-ALL in children and adults respectively.[4]

ALL generally presents with a sudden clinical onset. Bone marrow failure is the cause of the presenting clinical complaints. These include clinical features of anemia like pallor and fatigue, petechial hemorrhages as a result of thrombocytopenia and infectious complications due to neutropenia.[5]

Childhood ALL, excluding infantile ALL has better prognosis than adult ALL and age group > 1 - 9.99 years has the best prognosis. TLC at presentation is a highly significant prognostic variable in B-ALL, both in children and adults. TLC > 50 x 10^9/L defines high risk category in childhood B-ALL.[6]

In children T-ALL generally has a higher risk than B-ALL. Conversely, in adults, T-ALL may have a better prognosis than B-ALL with modern treatment protocols.[3,7]

The aim of this study was to determine the antigenic expression on the leukemic blasts and the hematological parameters comparison between the ALL immunophenotypes.

METHODOLOGY

This study was carried out from February 2013 to March 2014, after obtaining the approval from the Institutional Review Board of King Edward Medical University, Lahore. Patients provisionally diagnosed with ALL on the basis of hematology in Mayo Hospital and Hameed Latif Hospital were included in the study after taking their informed consent.

Relevant history and findings of physical examination were recorded on a proforma. Hb, TLC and platelet count were determined on Hematology Analyser Sysmex-Kx-2I. Blast cell % was estimated on Giemsa stained blood smears.

Bone marrow was aspirated from posterior superior iliac spine of the patients and transferred to EDTA vial for flowcytometry. Smears were also made from the aspirate and stained with Giemsa and Sudan Black stains for morphology and cytochemistry. Immunophenotyping was done in the pathology laboratory of Shaukat Khanum Memorial Cancer Hospital. Five colour flow cytometry of bone marrow aspirates was done on Beckman Coulter Navious Flow Cytometer. The cases which were not diagnosed as ALL after immunophenotyping were excluded from the study. A panel of 23 fluorochrome conjugated Antibodies (Abs) was used for the following antigens: predominantly B-Cell markers-- CD19, CD79a, CD10, CD20, HLA-DR, kappa, lambda; predominantly T-Cell markers- cCD3, CD3, CD5, CD2, CD7, CD16; non-lineage specific antigens-- TdT, CD34, CD45 and Myeloid markers-cMPO, CD13, CD33, CD117, CD11b, CD11c, CD14.

Data was entered and analyzed on SPSS version 22. Quantitative variables like Hb, TLC, platelet count, blast cell percentage were analyzed by mean ± standard deviation. Qualitative variables i.e. gender and immunophenotypes were presented as frequencies and percentages.

One sample Kolmogorov-Smirnov test was applied to check the assumption of normality. Hb, TLC and platelet count showed a non-normal distribution of data whereas Blast % showed a normal distribution. Mann-Whiteny U-test was used to compare non-normal quantitative data (Hb, TLC and platelet count) in B-ALL and T-ALL. Blast % was compared in the two groups (B-ALL and T-ALL) using independent sample t-test as it was normally distributed. P-value ≤ 0.05 was taken as significant.

RESULTS

This study included 50 patients of ALL. Immunophenotyping revealed 72% B-ALL and 28% T-ALL patients. There were 18 children (upto 15 years) and 32 adult patients. The distribution of immunophenotypes in children and adults is shown in Table I.

Table I: Frequency of B-ALL and T-ALL in children (upto15 years), adults (> 15 years) and total patients.

Age group		Type		Total
		B- ALL	T-ALL	
Children	No.	14	4	18
	%	77.8%	22.2%	100.0%
Adults	No.	22	10	32
	%	65.6%	31.2%	100.0%
Total	No.	36	14	50
	%	72.0%	28.0%	100.0%

Forty (80%) of the total patients were males with male to female ratio (M:F) 4:1. The M:F ratio was 2: 1 in children and 7:1 in adults; it was 3.5:1 in B-ALL and 6:1 in T-ALL.

On analysis of antigen expression; CD19, CD79a and CD20 showed B-lineage specificity and were not expressed in any of the T-ALL cases. CD10 was the most sensitive marker for B-ALL and was expressed in 35 of the 36 cases, however, it was also expressed in one case of T-ALL. HLA DR was not a sensitive marker for B-ALL as it was positive in only 25% of the cases. It was also expressed in 21% T-ALL (Table II).

Cytoplasmic cCD3, surface CD3 and CD5 were T-lineage specific as they were not expressed in B-ALL. CD7 was the most sensitive marker of T-ALL being positive in 13 of the 14 patients. However, it was also expressed in 2 B-ALL cases. CD2 was neither sensitive (expressed in 43% T-ALL), nor specific (positive in one case of B-ALL) for T-ALL (Table II).

TdT expression was seen in 92% B-ALL and 71% T-ALL cases, CD34 in 58% and 43% and CD45 in 83% and 100% cases respectively (Table II). There was no case of myeloid antigen positive ALL.

Hb ranged from 4.2 to 13.7 g/dl. Majority of the patients were anemic as 74% had Hb < 10 g/dl. Hb levels were not statistically different in B-ALL as compared to T-ALL, p-value = 0.071 (Table III).

Although higher values of TLC were observed in T-ALL versus B-ALL, this difference was not statistically significant, p-value = 0.247 (Table III). Almost half of the total patients had TLC between 50 and 100 x 10^9/L. Hyperleukocytosis (TLC > 100 x 10^9/L) was seen in 3 patients and one of these 3 had a count above 200 x 10^9/L (Table IV).

Platelet count ranged from 7.0 to 207 x 10^9/L. Thrombocytopenia < 20 x 10^9/L was present in 12% of the total patients. In majority (78%) of the patients platelet count was between 20 x 10^9/L and 100 x 10^9/L. Count above 100 x 10^9/L was seen in only 10% of the patients. The difference in platelet count between B -ALL and T-ALL was not statistically significant, p-value= 0.364 (Table III).

Table II: Antigen expression of the blast cells in B-ALL and T-ALL.

Antigen	B-ALL		T-ALL		Total	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Predominantly B-cell antigens						
CD19	34 (94%)	02 (6%)	0	14 (100%)	34 (68%)	16 (32%)
CD79a	29 (81%)	07 (19%)	0	14 (100%)	29 (58%)	21 (42%)
CD10	35 (97%)	01 (3%)	01 (7%)	13 (93%)	36 (72%)	14 (28%)
CD 20	09 (25%)	27 (75%)	0	14 (100%)	09 (18%)	41 (82%)
HLA-DR	33 (92%)	03 (8%)	03 (21%)	1 (79%)	36 (72%)	14 (28%)
Predominantly T-cell antigens						
cCD3	0	36 (100%)	09 (64%)	05 (36%)	13 (26%)	37 (74%)
CD3	0	36 (100%)	06 (43%)	08 (57%)	06 (12%)	44 (88%)
CD7	2 (6%)	34 (94%)	13 (93%)	01 (7%)	15 (30%)	35 (70%)
CD5	0	36 (100%)	06 (43%)	08 (57%)	06 (12%)	44 (88%)
CD2	1 (3%)	35 (97%)	06 (43%)	08 (57%)	07 (14%)	43 (86%)
Non-lineage specific antigens						
TdT	33 (92%)	03 (8%)	10 (71%)	04 (29%)	43 (86%)	07 (14%)
CD34	21 (58%)	15 (42%)	06 (43%)	08 (57%)	27 (54%)	23 (46%)
CD45	30 (83%)	06 (17%)	14 (100%)	0	44 (88%)	06 (12%)

Table III: Comparison of hemoglobin (Hb), total leukocyte count (TLC) and platelet count in B-ALL and T-ALL patients .

	No.	Median	IQR	Range	p-value
Hb (g/dl)					
B- ALL	36	9.46	0.775	4.20 - 13.00	0.071
T- ALL	14	9.75	2.66	7.70 - 13.70	
TLC ($x10^9$/L)					
B- ALL	36	43.9	34.07	1.40 - 172.00	0.247
T- ALL	14	52.5	44.73	4.00 - 218.00	
Platelet count ($x10^9$/L)					
B- ALL	36	51.83	26	7.00 - 207.00	0.364
T- ALL	14	48.00	23.08	10.00 - 80.00	

IQR: Inter quartile range.

Table IV: Distribution of total leukocyte count (TLC) in B-ALL, T-ALL and total patients.

Immunotype	TLC x 10^9/L					Total
	Upto 10	10 - 50	50 - 100	100 - 200	> 200	
B-ALL	4	12	19	1	0	36
T-ALL	1	4	7	1	1	14
Total patients	5	16	26	2	1	50

Table V: Comparison of Blast % in B-ALL and T-ALL patients.

Blast %	N	Mean	Std. Deviation	Range	p-value
B-ALL	36	72.40	16.287	20.00 - 98.00	0.238
T-ALL	14	66.09	17.976	28.00 - 90.00	

Percentage of blast cells in blood smear ranged from 20% to 98%. The difference in blast % between B-ALL and T-ALL was not statistically significant, p-value= 0.238 (Table V).

DISCUSSION

The present study revealed 36 (72%) B-ALL and 14 (28%) T-ALL patients. The frequency of T-ALL in this study is much higher both in children (23.2%) as well as adults (31.2%) than that reported in the West (B-ALL 85% and T-ALL 14%), China (B-ALL 88% and T-ALL 12%) and Karachi, Pakistan (T-ALL14% in children and 22% in adults).[8-10]

Majority (64%) of the patients in this study were adults, although ALL is the most common malignancy among children and is rare in adults.[1] A greater number of adult ALL patients present in these centers because Shaukat Khanum Memorial Cancer Hospital and Children Hospital, Lahore, cater for the pediatric ALL cases only.

In the present study Hb below 10 g/dl was observed in 73% patients. Severe thrombocytopenia (< 20 x 10^9/L) was present in only 12% of the total patients. In the vast majority of the cases (78%), platelet count was between 20 x 10^9/L and 100 x 10^9/L. High TLC above 50 x 10^9/L, a known factor associated with poor prognosis in ALL, was seen in more than half, 29 (58%) of the total patients.

An earlier study done in 1999 on 67 adult ALL patients in Mayo Hospital, Lahore, showed differences from the present findings. Anemia (Hb < 10 g/dl) was more frequent (92.5%), and TLC > 50 x 10^9/L was less frequent (24%) as compared to the present study. Platelet count < 25 x 10^9/L was present in 22% of the patients.[11] Another study from the same institution and in the same year, which included 257 children and 57 adults of ALL, reported TLC > 50 x 10^9/L in 17.5% cases only.[12]

This interesting finding may represent a changing disease pattern with an evolvement into an ALL with a less favorable prognosis. A factor to be considered, however, is that the present study included a larger proportion of patients from the private sector hence the socioeconomical status of these patients was higher than the patients in the previous studies.

In a study from Karachi on 611 cases of childhood ALL, Hb < 7 g/dl was reported in 54%, platelet count < 20 x

10^9/L in 33% and TLC above 50 x 10^9/L in 34% of the patients.[13] Another study on 58 adult ALL cases from Karachi showed Hb < 10 g/dl in 69% patients and TLC > 50 x 10^9/L in 31%.[14]

This study included 3 cases with hyper-leukocytosis (TLC > 100 x 10^9/L) and one of these had a count above 200 x 10^9/L. The estimated 4-year event-free survival for patients with hyper-leukocytosis was reported to be significantly less than that for patients with lower count and the prognosis was even worse in those with counts greater than 200 x 10^9/L.[15]

B-ALL patients had lower Hb levels as compared to T-ALL patients though this difference was not found to be significant on statistical evaluation. Previous studies have reported lower Hb in B-ALL.[8,16]

Contrary to the previous reports, platelet counts were lower in T-ALL compared to B-ALL in this study although the difference was not statistically significant. Previous studies have reported significantly lower platelet counts in B-ALL.[8]

Although higher values of TLC were observed in T-ALL. In this study, this difference was not statistically significant. High WBC counts (> 50 x 10^9/L) have been reported to be more frequent in T-ALL compared to B-ALL patients, irrespective of age.[8]

The immunophenotypic analysis in this study showed that HLA DR was not a sensitive marker for B-ALL, it was positive in only 25% of the cases. It was also expressed in 21% of T-ALL, showing that it is a poor discriminator of B from T lineage. It is reported that HLA-DR is present in most of the cases of B-ALL, irrespective of the stage of development of the blast cells. In T-ALL, the expression of HLA-DR is associated with an immature immunophenotype.[17]

Among T-ALL cases, the finding of 64% positivity of cCD13 in this study is different from previous reports where cCD3 is the most sensitive and specific marker of T-ALL.[18]

TdT identifies precursor lymphoid cells, therefore, it is extremely useful in differentiating lymphoblastic leukemia/lymphoma from peripheral lymphoid neoplasms. In the present study, its expression in B-ALL was greater (92%) than T-ALL (71%). TdT is reported to be usually positive in pro B-ALL and common B-ALL but may be negative in pre B-ALL.[17] Zhou *et al*. reported that absence of TdT expression in T-ALL identifies a high-risk subset.[19]

CD45, the common leukocyte antigen, was positive in 83% B-ALL and all of the present T-ALL cases but 6 (17%) of B-ALL cases did not express this antigen. In previous reports one-third or more childhood ALL cases failed to express CD45. This must be kept in mind if the gating protocol of flowcytometry uses CD45.[17] In a recent report Cario *et al*. reported that high CD45 surface expression is associated with increased relapse risk in childhood ALL and suggested the addition of CD45 expression in the stratification scheme of the therapeutic protocols.[20]

CONCLUSION

The frequency of T-ALL was higher in childhood as well as adult ALL in the studied population compared to the Western literature. Antigenic expression of the blast cells also shows some interesting differences. A large number of our patients present with high leukocyte count which is a known factor associated with poor prognosis.

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