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Original Article

Immunophenotypes in Acute Lymphoblastic Leukemia; association with Demographic Profile and Clinical Presentation

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Abstract

Objective: This study was carried out to determine the frequency of B lymphoblastic leukemia (B-ALL) and T lymphoblastic leukemia (T-ALL) in Acute Lymphoblastic Leukemia (ALL) patients in our population and to observe their association with the demographic profile and clinical features at presentation.
Study Design: Non randomized prospective study.
Place and duration of study: King Edward Medical University, Lahore and Hameed Latif Hospital, Lahore; from Feburary 2013 to March 2014.
Methodology: Fifty newly diagnosed and untreated patients of ALL were selected from Mayo Hospital and Hameed Latif Hospital. They included both genders and all age groups. Relevant history and findings of physical examination were recorded.
Immunophenotyping was done on bone marrow samples by 5 colour flowcytometery on Beck Man 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 4(22%) of the childhood and 10(31%) of the adult cases. Male: Female ratio was 4:1 in total patients, 2:1 in children and 7:1 in adults. A significant preponderance of T-ALL was seen in the age cohort of 15 to 25 years. No association was observed between ALL immunophenotypes and gender or the presenting clinical features.
Conclusion: The frequency of T-ALL is higher and the male predominance is greater in childhood as well as adult ALL in our population compared to the Western literature. Although T-ALL is significantly higher in the adolescents and young adults, it is not associated with male gender or organomegaly.
Key words: Acute lymphoblastic leukemia (ALL). B lymphoblastic leukemia (B-ALL). T lymphoblastic leukemia (T-ALL). Flowcytometry. Immunophenotypes.

Introduction

Acute lymphoblastic leukemia (ALL) is a neoplastic disorder of the hematopoietic system characterized by the proliferation of lymphoblasts. It is the commonest childhood malignancy and accounts for 25% of childhood cancers and less than 1% adult cancers.[1]

Immunophenotyping refers to the determination of the expression of antigenic markers on/in the cell population of interest. With exploration of an

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increasing number of monoclonal antibodies (MoAbs) and the improvements in immunofluorescence and flowcytometry; immunophenotyping is contributing to a better diagnosis and treatment of ALL.[2]

ALL immunophenotypes were classified as precursor B and precursor T cell lymphoblastic leukemia according to the 2001WHO classification. The term precursor was dropped and the nomenclature was changed to B and T lymphoblastic leukemia in the 2008 WHO classification.[3]

It is extremely important that the terminology 'B-ALL' is understood by the hematologists because in the past this abbreviation was used for the mature B phenotype. Mature B phenotype invariably represents the leukemic phase of Burkitt lymphoma and is no longer included in lymphoblastic leukemia. Mature B cell leukemia patients respond poorly to standard ALL therapy; hence its identification is important for the sake of exclusion.[4]

Approximately 75% of adult ALL cases are B lymphoblastic leukemia (B-ALL) and 25% are T lymphoblastic leukemia (T-ALL). Compared with adults, T lineage ALL is less common in children and constitutes about 15% of childhood ALL. [5]

The peak age incidence of ALL is between 2 to 3 years. In childhood ALL, the incidence is slightly higher in males, the male to female ratio being 1.3. In adult ALL the male predominance is much higher in whites (male to female ratio 1.6) as compared to blacks (male to female ratio 1.15).[6, 7]

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.[8]

Clinical signs due to leukemic infiltration of organs present with enlargement of lymph nodes spleen and liver. Patients with T-ALL are predominantly adolescent males and present with anterior mediastinal mass due to thymic enlargement which can lead to dyspnea.Interestingly T-ALL patients without thymic enlargement are reported to have a worse prognosis compared to those who have an enlarged thymus.[9]

Immunophenotypes have great prognostic significance in acute lymphoblastic leukemia. In this study immunophenotypic expression of a large number of surface and intracellular antigens in B-ALL and T-ALL in our region has been evaluated. The association of ALL immunophenotypes with age, gender and presenting clinical features in our patients has been analyzed. These findings will help in the prognostic stratification of patients in our setup and more rational risk-adapted therapeutic approach.

Methodology

This study was carried out after the approval of the ethical committee of KEMU.

Patients provisionally diagnosed with Acute Lymphoblastic Leukemia on the basis of CBC 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.

Bone marrow was aspirated from posterior superior iliac spine of the patients in EDTA vial. 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 Beck Man Coulter Navious Flow cytometer. The cases which did not conform to the diagnosis of ALL after immunophenotyping were excluded from the study. A panel of 24

fluorochrome conjugated antibodies (Abs) was used for the following antigens: Cytoplasmic CD3, CD3, CD5, CD2, CD7, CD16; CD19, CD79a, CD10, CD20, HLA-DR; TdT, CD34, CD45; cMPO, CD13, CD33, CD117, CD11b, CD11c, CD14, Kappa, Lambda. Fluorochromes conjugated to the Abs included FITC, PE, PC5, PC7, ECD and 7AAD.

Data was entered and analyzed on SPSS version 22. Kruskal Wallis H test has been used to see the average difference of different immunophenotypes with age. Chi-Square test has been applied to see the association with qualitative variables (gender and organomegaly). P-value ≤0.05 has been taken as significant.

Results

This study included 50 patients of Acute Lymphoblastic Leukemia (ALL). Immunophenotyping revealed 36 (72%) B-lymphoblastic leukemia (B-ALL) and 14 (28%) T- Lymphoblastic leukemia (T-ALL) patients .

B-ALL markers were CD19, CD79a, CD10, CD20 and HLA-DR; T-ALL markers included cCD3, CD3, CD7, CD5 and CD2; while non lineage specific markers were TdT, CD34, CD45. Neagtive reaction with anti kappa and lamda MoAbs excluded mature B (Burkitt cell leukemia). Absence of following myeloid lineage markers; cMPO, CD13, CD33, CD117, CD11b, CD11c and CD14 excluded Acute Myeloid Leukemia.

The descriptive statistics for age are shown in Table 1 There were 18 (36%) children (age up to 15 years) and 32 (64%) adult patients (age above 15 years).An interesting finding was that there was no T-ALL case above the age of 25 years. Hence when the age groups were further stratified as children (up to 15 years), adolescents and young adults (> 15 to 25 years), and adults (> 25 years); majority i.e. 10 (71.4%) of the total T-ALL patients were revealed as adolescents and young adults. This difference was statistically significant (Table 2).

Table 1. Descriptive statistics for Age in B-ALL (n=36) and T-ALL (n=14)

		N	Mean	Std. Deviation	Minimum	Maximum	p-value
Age (years)	B-ALL	36	23.000	16.42	3.0	65.0	0.610
	T-ALL	14	18.750	5.34	5.5	25.0	
	Total	50	21.690	14.10	3.0	65.0	

Table 2. Distribution of different Age groups in T-ALL and B-ALL

			Type		
			B-ALL	T-ALL	Total
Age group (Years)	Upto 15	Count	14	4	18
		% within Type	40.0%	28.6%	36.0%
	15-25	Count	10	10	20
		% within Type	28.6%	71.4%	40.0%
	> 25	Count	12	0	12
		% within Type	34.3%	.0%	24.0%
Total		Count	36	14	50
		% within Type	100.0%	100.0 %	100.0 %

Note: The distribution of different Age groups in T-ALL and B-ALL was statistically significant (p=0.017)

Majority (80%) of the total patients were males with a male to female ratio (M:F) 4:1. Among the 18 childhood ALL patients there were 12 males and 6 females (M:F 2:1). Of the 32 adults 28 were males and 4 females (M:F 7:1). Although male dominance was much higher in adults the difference was not statistically significant (p=0.077). It remained insignificant even after further categorizing the patients into 3 age groups (Table 3).

Gender distribution in B-ALL and T-ALL is shown in Figire 2. The male predominance in T-ALL versus B-ALL was not statistically significant.

Table 3. Gender distribution in different Age groups (n=50)					
			Sex		
			Male	Female	Total
Age (Years)	Upto 15	Count	12	6	18
		%within age	66.7%	33.3%	100.0%
	15-25	Count	16	4	20
		%within age	80.0%	20.0%	100.0%
	> 25	Count	12	0	12
		%within age	100.0%	.0%	100.0%
Total		Count	40	10	50
		%within age	80.0%	20.0%	100.0%

Note: The gender distribution in different age groups was not statistically significant p=0.082

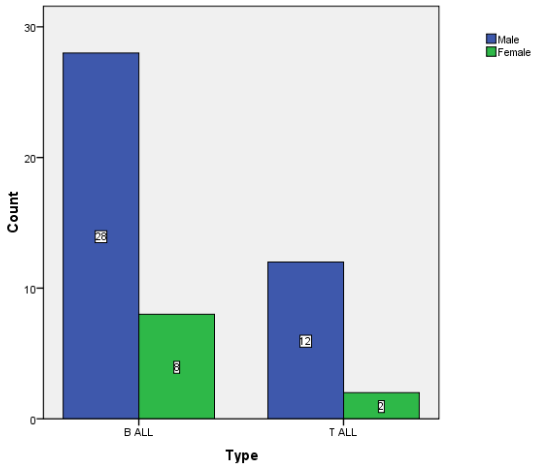

Figure 2 : Gender distribution in B-ALL (n=36) and T-ALL (n=14)

Note: The gender distribution in B-ALL and T-ALL was statistically insignificant (p=0.7)

Fever was seen in 42(84%) patients, bleeding manifestations in 13(26%), palpable lymph nodes in 34(68%), hepatomegaly in 25(50%) and splenomegaly in 18(36%) patients at the time of presentation. The frequency of these clinical features in B-ALL and T-ALL patients is shown in Table 4. No association was observed between any of the clinical features and the two ALL immunophenotypes after statistical analysis (Table 4).

Discussion

In this study 32(64%) patients were adults, although ALL is the most common malignancy among children and is rare in adults. The most likely reason for a greater number of adult ALL patients in our study is that Shaukat Khanum Memorial Cancer Hospital and Children Hospital Lahore, treat childhood ALL only. Therefore the adult ALL patients seek treatment in other private and government hospitals which have an oncology department.

In an Italian multicenter study, 5202 patients were analysed in nine age cohorts. The highest prevalence of acute lymphoblastic leukemia was seen in children.[7]

The vast majority i.e. 40 (80%) of the total patients in the present study were males with a male to female ratio 4. This male predominance is much higher than reported in previous studies. Wang et

Table 4. Clinical Features at Presentation in Total, B-ALL and T-ALL Patients													
Clinical Features	Total Patients (n=50)				B-ALL Patients (n=36)				T-ALL Patients (n=14)				P Value B-ALL vs T-ALL
	Present		Absent		Present		Absent		Present		Absent		
	n	%	n	%	n	%	n	%	n	%	n	%	
Fever	42	84%	08	16%	31	86%	05	14%	11	79%	03	21%	0.514
Bleeding	13	26%	37	74%	09	25%	27	75%	04	29%	10	71%	0.796
Palpable L.Ns.	34	68%	16	32%	23	63%	13	37%	11	79%	03	21%	0.446
Hepatomegaly	25	50%	25	50%	19	54%	17	46%	06	43%	08	57%	0.462
Splenomegaly	18	36%	32	64%	14	39%	22	61%	04	29%	10	71%	0.565

al reported a male to female (M:F) ratio 1.38 in adult ALL cases and Chiaretti et al observed a ratio 1.25 in all patients.[7, 10] Studies from Karachi, Pakistan reported M:F ratio 1.7 in children and 3.0 in adults.[11, 12]

Immunophenotyping revealed 36 (72%) B-ALL and 14 (28%) T-ALL patients in the present study. The frequency of T-ALL in our study is much higher both in children (23.2%) as well as adults (31.2%) than that reported in the West. An Italian multicenter study revealed B-ALL in 85.8% of patients and T-ALL in 14.2%.[7] While Tong et al reported 88.4% B-ALL and 11.6% as T-ALL. [13] In a recent study from China on 110 adult patients, 21.8% were identified as T-ALL and 78.2% as B-ALL.[14] Similar findings were reported from Karachi Pakistan, the proportion of T-ALL being 14.2% in children and 22% in adults.[15]

In our study majority i.e. 10 (71.4%) of the total 14 T-ALL patients were adolescents and young adults. This finding was statistically significant. T-ALL was reported to occur more frequently in males younger than 30 years of age and was associated with a high WBC count and tumor mass at diagnosis.[16]

There was male predominance in B-ALL (77.8% males) as well as in T-ALL (85.7% males) in the present study. Although the result was slightly higher in T-ALL it was not significant hence there was no association between the gender and immunophenotypes in our study. On the other hand the previous studies show an association of male predominance with T-ALL [7]

Majority (84%) of our patients were febrile at presentation whereas bleeding manifestations were present in 26%. Although a greater percentage (78.6%) of T-ALL patients had enlarged lymph nodes compared to B-ALL (63.9%), the difference was not statistically significant. Interestingly, contrary to previous reports, splenomegaly and hepatomegaly were more frequent in B-ALL (40% and 54.3% respectively) compared to T-ALL (28.6% and 42.9% respectively). However this difference was not statistically significant.

A study on 611 childhood ALL cases from Karachi, reported fever and pallor as the commonest presenting features; lymphadenopathy (75%), hepatomegaly (67%) and splenomegaly (58%) were frequent findings.[11] Another study from Karachi on adult ALL showed lymphadenopathy in 17.2%, hepatomegaly in 32.7% and splenomegaly in 62% patients.[12] In a recent study from India fever, pallor and weakness were the most common clinical presentations of ALL. Lymphadenopathy was observed in 63% of the patients, hepatomegaly in 56.7% and splenomegaly was also present in 56.7%.[17]

In previous studies, T-ALL has been reported to be more frequently associated with lymph node enlargement, hepatomegaly and splenomegaly. Greaves et al reported lymph node enlargement in 80% T-ALL childhood patients compared to 69% in B-ALL.[18] In a study by Alves et al correlation analysis between immunophenotypic and clinical features of 126 ALL patients showed that T-ALL was more frequently associated with lymphadenopathy, hepatomegaly, splenomegaly and CNS leukemic infiltration.[19]

Adult patients comprise 64% of our patients, hence the findings of this study conform more to adult ALL rather than childhood ALL.

Conclusion

The frequency of T-ALL is higher and the male predominance is greater in childhood as well as adult ALL in our population compared to the Western literature. Although T-ALL is significantly higher in the adolescents and young adults, it is not associated with male gender or organomegaly.

Conflict of interest: The authors have no conflict of interest.

Authorship: Samina Naeem was the principal researcher and prepared the first draft of this article from her PhD Thesis and **Mulazim Hussain Bukhari** was supervisor.

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