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Original Research Paper

Pathology

CLINICO-HEMATOLOGICAL STUDY OF ACUTE LYMPHOBLASTIC LEUKEMIA AND THEIR CORRELATION WITH INFLAMMATORY MARKERS IN SERUM.

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ABSTRACT

SUMMARY: Acute lymphoblastic leukemia (ALL) is early childhood hematological malignancies. In present scenario immunophenotyping became an important tool for subtyping of ALL into B-ALL and T-ALL. In order to understand the mechanism of development of leukemia it is important to study the cytokine environment of malignant cells.

OBJECTIVE: Aim of the present study was to evaluate clinical and hematological features in ALL and correlate serum levels of IL6 and IL-10 expression in ALL patients and their subtypes.

MATERIALS & METHODS: A total of 68 ALL cases along with 20 healthy controls were included in the study between periods of 2015 to 2017. About 4 mL blood samples were collected from all cases for immunophenotyping and serum studies. Levels of IL6 and IL10 were determined in all cases by ELISA.

RESULT: In the present study immunphenotyping was done in all cases of ALL, which showed 52 cases (76.5%) of B-ALL and 16 cases (23.5%) of T-ALL. T-ALL was mostly found in higher aged children than B-ALL. A male predominance was seen in all cases. No significant differences in hemoglobin levels and platelet counts were found between T-ALL and B-ALL. A significantly high percentage of T-ALL cases were having more than 50000 cells per microliter than B-ALL (56.2% vs. 23.1%). Almost similar clinical features were found in both subgroups, only bleeding manifestation was found significantly higher in T-ALL than B-ALL (31.2% vs.11.5%). Acute lymphoblastic leukemia (ALL) patients were associated with significantly elevated serum IL6 and IL-10 level than the healthy control group. Mean levels of serum IL6 were 167.9±306.46 pg/mL in ALL, and 6.51 ± 2.27 pg/mL in healthy control group. Mean IL10 levels were 70.56±111.48 pg/mL in ALL and 29.39 ± 4.27 pg/mL in control group. There were no significant differences found in IL-6 and IL-10 serum levels between T-ALL and B-ALL.

CONCLUSION: Present study found elevated level of IL-6 and IL-10 in ALL patients which suggest possible role of these cytokines in disease transformation. Detection of IL-6 and IL-10 in newly diagnosed patient may predict disease outcome and possibly poor prognosis in patients.

KEYWORDS : Acute lymphoblastic leukemia (ALL), IL-6, IL-10, B-ALL, T-ALL, Immunophenotyping, Cytokines

INTRODUCTION:

Acute leukemia is a heterogeneous group of leukemia with diverse clinical, morphological and molecular features. ALL was the most common childhood cancer comprising approximately 25% of cancers and 80% of overall leukemia "–(1). WHO, 2008 classification combined the importance of morphological, immunophenotyping and genetic studies for diagnosis of acute lymphoblastic leukemia. Although for subtyping of ALL, molecular and genetic features are important, but morphological study and immunophenotyping are main modality for initial lineage definition which is the first step of therapy initiation. At present flow cytometry is most widely accepted method of diagnosing and immuno phenotyping of acute lymphoblastic leukemia. In flow cytometry monoclonal antibodies directed against surface or cytoplasmic antigens of specific cells are used for diagnosis or deciding therapy against a particular disease. It is also important in prognosis and as well as for the disease monitoring during the treatment. The Europian group for immunological classification (EGIL) has proposed importance of atleast 20% positivity of blast cell for specific monoclonal antibodies or CD markers except for MPO, CD3 and CD79a for which the criteria is 10%.(2) The present study was done to establish flow cytometry in our department for diagnosis of acute leukemia and to evaluate the use of immunophenotyping for subtyping of ALL .Also comparison between different subtypes of ALL was done in the present study.

In order to understand the mechanism of disruption of hematopoiesis in hematological malignancies many researches are going on to investigate role of cytokines. Among all IL-6 and IL-10 is most widely studied by different groups and regarded as prominent target for clinical intervention.(3)(4) –(5) Interleukin 6 (IL-6) is a pleiotropic cytokine, secreted by all stromal cells and cells of the immune system in response to several types of stimuli such as acute phase response, hematopoiesis, and immune reaction (6). Its gene is situated on chromosome 7p21.(7) Two types of signaling mechanisms by IL-6 –membrane bound IL-6

receptor (mbIL6R) and IL6-soluble IL-6 receptor (sIL6R) are known as classic and trans-signaling pathways respectively. IL-6 classic signaling is responsible for the synthesis of acute-phase proteins which contribute to anti-inflammatory properties whereas trans-signaling pathways are found to be involved in a proinflammatory activity such as in inflammation, cancer, and autoimmune disorders. (8) Several studies had suggested that an abnormal level of IL-6 serum level is associated with the pathogenesis of leukemia and a low level is associated with a favorable prognosis, but still, it is hard to understand. Further investigation is needed to evaluate the exact role of IL-6 in signaling pathways of leukemia. (9)

Interleukin 10 (IL-10) is an anti-inflammatory cytokine that plays a central role in limiting immune response against infection, hence the host cell damage is prevented and tissue homeostasis is restored (10). It is encoded by the IL-10 gene present on chromosome 1, at 1q31-32. IL-10 was first described as a cytokine derived by T-helper2 cells, But it is produced by almost all leukocyte cells mainly T-helper cells, monocytes, macrophages, dendritic cells, and in some conditions B-cell, cytotoxic T-cell, NK cells, Mast cells, and granulocyte cells. (11) IL-10 activates JAK1 and tyrosine kinase2 which induce activation of STAT1, STAT3, and in some cases STAT5 which finally leads to anti-inflammatory and proliferative response of IL-10. (12) One study showed that low birth level of IL-10 are associated with twenty five percent increased risc of developing B-ALL and IL-10 play an important role in neonatal immune response to infection.(13)

The present study is designed to evaluate clinical and hematological features in ALL and their subtypes and to investigate correlation of serum level of IL-6 and IL-10.

MATERIAL AND METHOD:

This case control study was conducted in the Department of Pathology. All cases were newly diagnosed including 68 ALL cases and 20 healthy control cases. All ALL cases were collected from Medicine OPD and Pediatric OPD. Median age for ALL patients was 7 years (Range: 0.5 yrs – 18 yrs) including 42 male (61.8%) and 26 female patients (38.2%). Healthy control group included 9 male (45%), 11 female (55%) and median age was 8 years (Range: 4 yrs - 12 yrs). Detailed clinical history as age, sex, peripheral blood count, bone marrow aspiration studies, presence of hepatomegaly and splenomegaly studies were recorded.

Sample size was determined on the basis of feasibility and availability of the samples during the period of 2015-2017.

About 4 mL blood samples were collected from the subject, 2 mL in plain vials for serum marker study and 2 mL in EDTA vial for immunophenotyping. Written informed consents were collected from each patient as per approval of the institutional ethical committee of the Institute of Medical sciences, Banaras Hindu University. Serum was separated and stored at -80°C until needed. All patients who satisfied WHO criteria for acute lymphoblastic leukemia, confirmed by peripheral blood examination and or bone marrow examination were included in the study. All treated cases of ALL in first instance were excluded.

Morphological studies

In ALL cases peripheral blood smear and bone marrow aspiration studies were done after staining with Leishman stain and Sudan black B stain.

Flow cytometry

All CD markers in the form of mononuclear antibody were obtained from Becton Dickinson (San José, California, United States). Six tubes were prepared for immunophenotyping. In each tube, 100 μl of whole blood (around one million cells) and a combination of four different fluorochrome conjugated CD markers were added and staining protocol as per the instruction manual was followed.

	Tube1	Tube2	Tube3	Tube4	Tube5	Tube6 (cyto)
FITC	-	CD 3	CD34	CD7	Cd5	CD3 cyto
PE	-	CD8	CD10	CD117	CD13	MPO cyto
PerCP	CD45	CD45	CD45	CD45	CD45	CD45 surface
APC	-	CD4	CD19	HLA DR	CD33	CD 79a cyto

After the staining procedure acquisition of the samples was done with BD Accuri C6 (Becton Dickinson, San Josè, CA). Analysis was performed with specific BD Accuri C6 software. Samples were acquired at a low sample rate (14 □l min-1) to ensure that the event rate was lower than 2,000 events s-1. For each sample, a minimum of 20,000 cells was acquired. Light-scatter and fluorescence signals were resulted from 20 mW laser illumination at 488 nm and 640nm. BD Accuri C6 Software is set to a primary threshold of channel 80,000 on FSC-H.. Cells were visualized at first in a FSC-A versus FSC-H morphological dot plot, and a gate (R1) was set to exclude Doublet cells. R1 cells were then visualized based on SSC and CD45 expression. Further analyses were performed including only R1 CD45, dim positive cells i.e. possible blast cells. The lineage detection was achieved according to the markers expressed by the blast cells and EGIL guideline. The positivity of the markers was determined on the basis of internal controls. But final decision was made possible only after incorporating morphological studies i.e. gross blood picture and bone marrow aspiration studies.

Serum Studies:

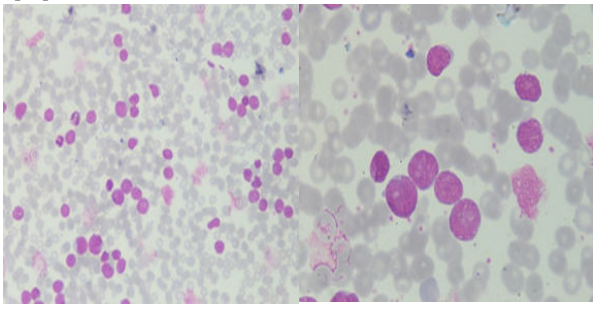
However the serum studies were done only in 43 ALL cases. Level of IL6 and IL-10 was determined in all cases using ELISA Kit (DIAsource immunoassay SA Belgium) which was a quantitative sandwich ELISA kit. Test was done according to the manual instruction.

Statistical Analysis:

Data of present study were analysed using Statistical Package for Social Sciences (SPSS, Chicago, Illinos, USA), trial version 16. Chi-square test has been used to compare differences between the frequencies as per the requirement. Kruskal Wallis and Mann Whitney tests were done to compare the mean values of serum markers in different patient groups because the data were not distributed normally. p-values less than 0.05 was taken as significant at two tailed test for all analysis.

RESULT

All the ALL cases was reported according to WHO classification (>20%) blast in peripheral smear and Bone marrow smears. Blast range was 22-95% in ALL patients. Blast were large with high N:C ratio, opened up nuclear chromatin, 2-3 prominent nucleoli and scant to moderate amount of cytoplasm.



A B

Figure 1: Photomicrograph of peripheral blood smear of ALL patients showing blast with few smudge cells. A. Leishman stain, 40X, B. Leishman stain, 100X

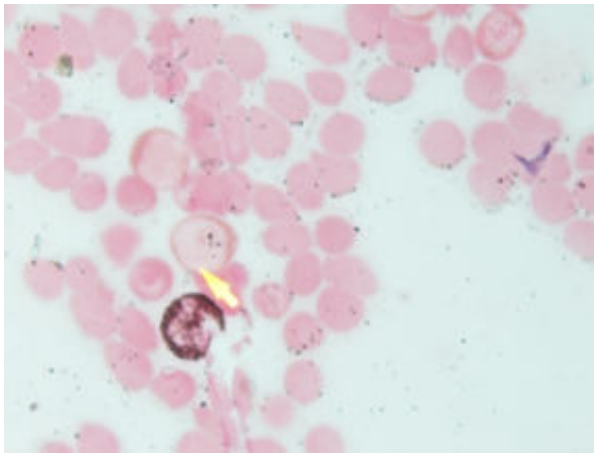
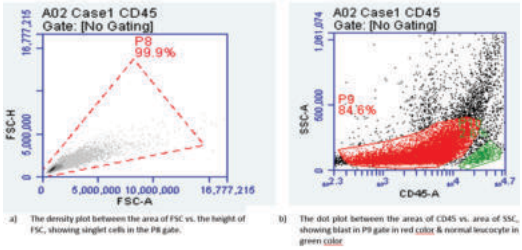


Figure 2: Photomicrograph of peripheral blood smear of ALL patients showing Sudan B Black negative stained blast (Sudan B Black stain, 100X).

Table1: Showing Blast percentage in ALL and subtypes of ALL based on morphology

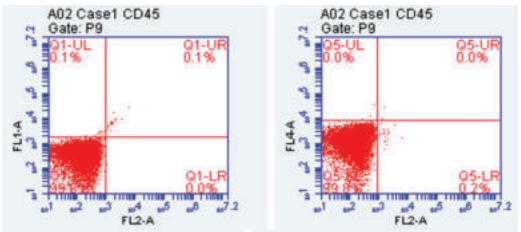
Groups with total no. of cases	No. of Patients (%)	Blast percent (Mean ± SD)	Range of Blast	Median
ALL	68 (100%)	76.24± 19.36	22-95	88
B-ALL	52(76.5%)	78.08±19.40	22-95	90
T-ALL	16(23.5%)	70.25±18.55	77	35-92

Tube 1: Unstained Tube for B-ALL case



a) The density plot between the area of FSC vs. the height of FSC, showing singlet cells in the P8 gate.

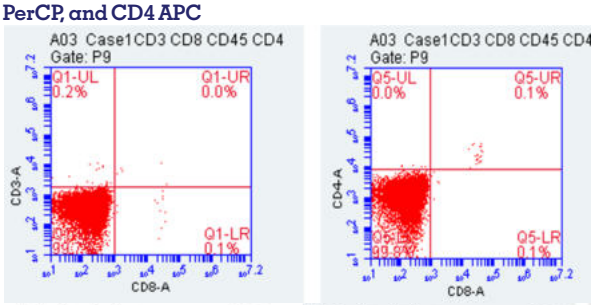
b) The dot plot between the areas of CD45 vs. area of SSC, showing blast in P9 gate in red color & normal leucocyte in green color



c) The dot plot between the areas of FL2 vs. FL1 showing unstained blast cells in lower left (LL) quadrant. The quadrant was set keeping unstained cells in the lower-left region and the setting was applied to all tubes.

d) The dot plot between the areas of FL2 vs. FL4 showing unstained blast cells in lower left quadrant. The quadrant was set keeping unstained cells in the lower-left region and the setting was applied to all tubes.

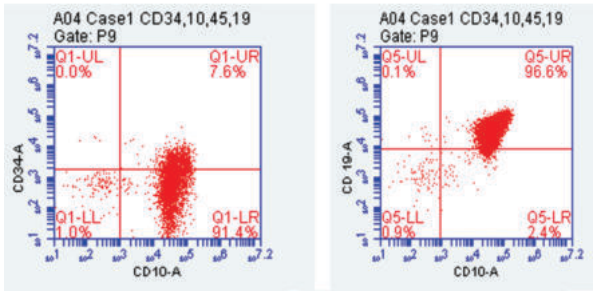
Tube 2 for B-ALL stained with CD3 FITC, CD8 PE, CD45 PerCP, and CD4 APC



e) The dot plot between areas of CD8 vs. CD3, showing all blast cells negative for CD 8 and CD3 markers in the lower left quadrant.

f) The dot plot between areas of CD8 vs. CD4, showing all blast cells negative for CD 8 and CD4 markers in the lower left quadrant.

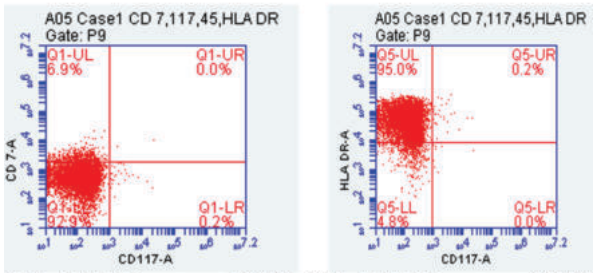
Tube 3 For T-ALL stained with CD34 FITC, CD10 PE, CD45 PerCP, and CD19APC



g) The dot plot between areas of CD10 vs. CD34, showing all blast cells positive for CD 10 in lower right & upper right quadrants (UR) and very dim positivity for CD34 in UR.

h) The dot plot between areas of CD10 vs. CD19, showing all blast cells positive for both markers CD 10 and CD19 in the upper right quadrant.

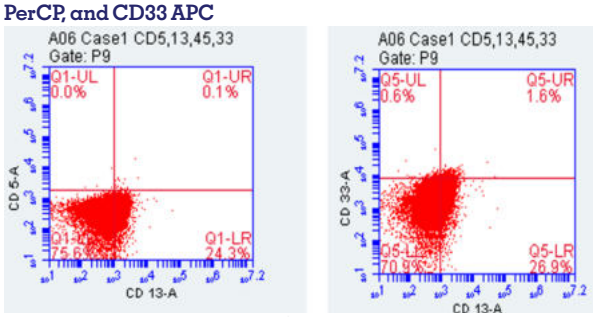
Tube 4 For B-ALL stained with CD7 FITC, CD117 PE, CD45 PerCP, and HLA-DR APC



i) The dot plot between areas of CD117 vs. CD7, showing all blast cells negative for both markers in the lower left quadrant.

j) The dot plot between areas of CD117 vs. HLA DR, showing all blast cells negative for CD 117 and positive for HLA DR in the upper left quadrant.

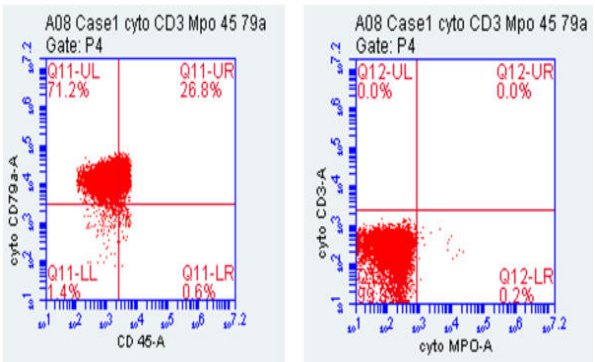
Tube 5 For B-ALL stained with CD5 FITC, CD13 PE, CD45 PerCP, and CD33 APC



k) The dot plot between areas of CD13 vs. CD5, showing all blast cells negative for CD5 and dim positive for CD13.

l) The dot plot between areas of CD13 vs. CD33, showing all blast cells negative for CD 33 and dim positivity for CD13.

Tube 6 for B-ALL stained with cytoCD3 FITC, cytoMPO PE, CD45 PerCP, and cytoCD79α APC

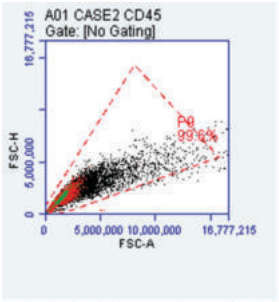


m) The dot plot between areas of CD45 vs. cytoCD79, showing all blast cells positive for cytoCD79 and dim

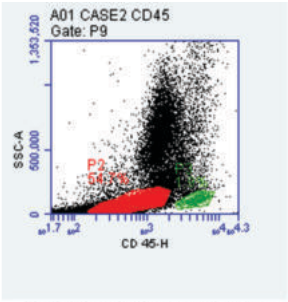
n) The dot plot between areas of cytoplasmic MPO vs.CD3 showing all

Figure 3: Results of immunophenotyping for B-ALL case showing dot plots for six stained tubes along with unstained tubes.

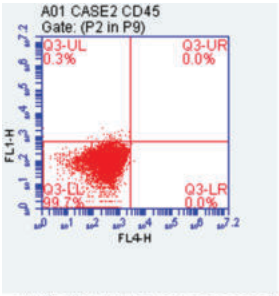
Tube 1: Unstained Tube for T-ALL



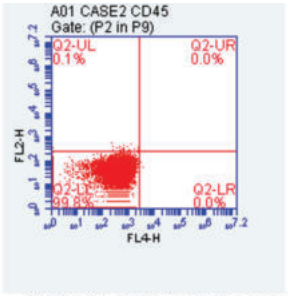
a) The dot plot between the area of FSC vs. the height of FSC, showing singlet cells in the P9 gate.



b) The dot plot between the areas of CD45 vs. area of SSC, showing blast/leukemic cells in P2 gate in red color and normal lymphocytes in green color.

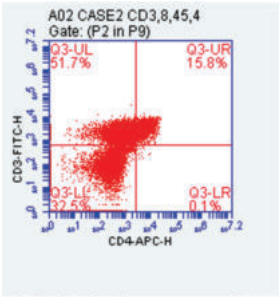


c) The dot plot between the areas of FL4 vs. FL1 showing unstained blast cells in lower left (LL) quadrant. The quadrant was set keeping unstained cells in lower left region and the setting were applied to the all tubes.

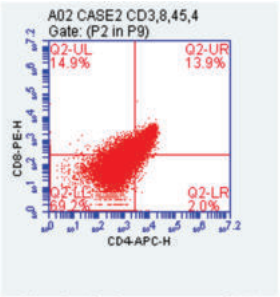


d) The dot plot between the areas of FL4 vs. FL2 showing unstained blast cells in lower left quadrant. The quadrant were set keeping unstained cells in lower left region and the setting were applied to the all tubes.

Tube 2 for T-ALL stained with CD3 FITC, CD8 PE, CD45 PerCP, and CD4 APC

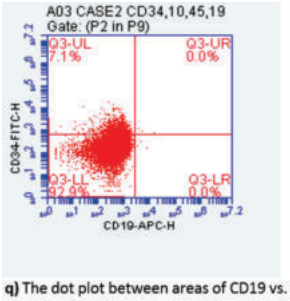


o) The dot plot between areas of CD4 vs. CD3, showing all blast cells positive for CD 3 and dim positivity for CD4 marker

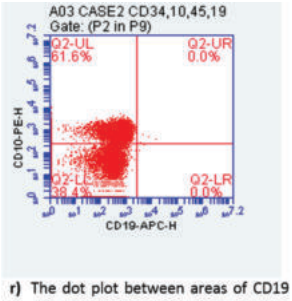


p) The dot plot between areas of CD4 vs. CD8, showing all blast cells positive for CD 8 and CD4 markers.

Tube 3 for T-ALL stained with CD34 FITC, CD10 PE, CD45 PerCP, and CD19APC

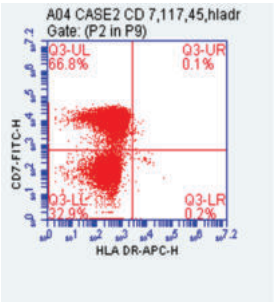


q) The dot plot between areas of CD19 vs. CD34, showing all blast cells negative for CD 19 & very dim positivity for CD34 in UR.

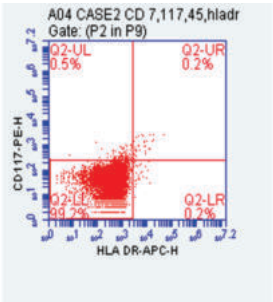


r) The dot plot between areas of CD19 vs. CD10, showing blast cells positive for CD 10 and negative for CD19 in the upper left quadrant.

Tube 4 For T-ALL stained with CD7 FITC, CD117 PE, CD45 PerCP, and HLA-DR APC

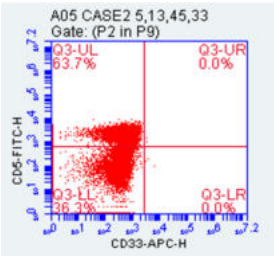


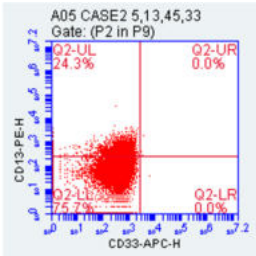
s) The dot plot between areas of HLA-DR vs. CD7, showing blast cells positive for CD7 in the upper left quadrant and negative for HLA-DR



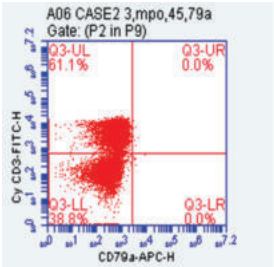
t) The dot plot between areas of HLA DR vs.CD117, showing all blast cells negative for both markers.

Tube 5 For T-ALL stained with CD5 FITC, CD13 PE, CD45 PerCP, and CD33 APC

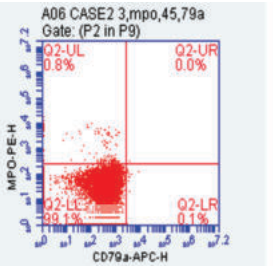




Tube 6 for T-ALLstained with cytoCD3 FITC, cytoMPO PE, CD45 PerCP, and cytoCD79a APC



u) The dot plot between areas of cytoCD79a vs. cytoCD3, showing blast cells positive for cytoCD3 in UL quardrant and negative for CD79a



v) The dot plot between areas of cyto CD79a vs.cytoMPO showing all blast cells negative for both markers in lower left quadrant.

Figure 4: Results of immunophenotyping for T-ALL case showing dot plots for six stained tubes along with unstained tubes.

Immunophenotyping showed, the blast cells in B-ALL were negative to dim positive for CD45, showing occasional positivity for CD34. These blast cells showed positivity for B cell markers i.e. CD10, CD19, HLA DR, and cytoplasmic CD79a sometimes showing occasional positivity for CD13 and CD33. The blast cells in T-ALL showed positivity for T cell markers i.e surface CD3, CD4, CD8, CD5, CD7, and cytoplasmic CD3 with occasional positivity for CD10 and CD117. Immunophenotypic analysis showed 52 cases (76.5%) of B-ALL and 16 cases (23.5%) of T-ALL. The patient characteristics are summarised in the Table 2.

Table 2: Characteristics of ALL Patients

Features	All patients (N=68)	B-ALL (N=52)	T-ALL (N=16)	(Chisquare test) p-value
Age				
mean	7.9±4.2	7.1±3.7	10.3±4.9	0.03*
median	7	7	11	
Range	0.5-18	1.5-15	0.5-18	

0-9	45	38 (73.1%)	7 (43.75%)	
10-19	23	14 (26.92%)	9 (56.25%)	
Gender				
M	42	32	10	0.945
F	26	20	6	
Sex Ratio (M:F)	1.6:1	1.6:1	1.7:1	

(Note: *significant = p-value <0.05)

There was a significant difference between the ages of different subgroups of acute lymphoblastic leukemia. The mean age was found 7.13 years and 10.25 years in B-ALL and T-ALL respectively. ALL was found mostly in children groups but in B-ALL, it was limited to the lower aged children (0-9 years) and T-ALL was found in children of higher age and adolescent (10-19 years). This difference was found significant when compared statistically.. Sexwise distribution showed male predominance in all subtypes of ALL and there were no specific pattern found in different subtypes of ALL (Table: 2)

Table 3: Hematological features in patients

Features	ALL patients (N=68)	B-ALL (N=52)	T-ALL (N=16)	p-value (□2 test)
Hemoglobin (gm/dl)				
mean	7.0±2.2	6.9±2.3	7.5±2.2	0.74
median	7	6.8	7.8	
Range	2.7-12.4	2.7-12.4	2.7-11.9	
<5	15 (22.1%)	12 (23.1%)	3 (18.8%)	
5-10	46 (67.7%)	34 (65.4%)	12 (75%)	
>10	7 (10.3%)	6 (11.5%)	1 (6.2%)	
Platelet (cells/μL)				
mean	46366.2± 46043	50980.8± 50643.5	31368.8± 20861.6	0.566
median	30000	30000	25000	
Range	4000-201000	4000-201000	13000-95000	
≤150000	64 (94.1%)	48 (92.3%)	16 (100%)	
151000-450000	4 (5.88%)	4 (7.7%)	0	
TLC (cells/μL)				
mean	57166.5± 81655.4	463769.0± 57549.3	110885± 120400	0.027*
median	23040	19100	59000	
Range	1630-400000	1630-315600	3300-400000	
≤50000	47 (69.1%)	40 (76.9%)	7 (43.8%)	
>50000	21 (30.9%)	12 (23.1%)	9 (56.2%)	

(Note: *significant = p-value <0.05)

The mean Hb was found 6.87±2.24 gm/dl in B-ALL (median 6.8 gm/dl, range 2.7-12.4gm/dl) and 7.46±2.2 gm /dl in T-ALL (median 7.8 gm/dl, range 2.7-11.7gm/dl). Hb less than 5% was seen in 23% of B-ALL followed by 10% of T-ALL. Hb ranging 5-10 gm/dl was observed in 75% of T-ALL and 65% of B-ALL. However more than10 gm/dl was seen only in 6 cases of B-ALL (12%) and 1 case of T-ALL (6%). There were no significant differences found between subtypes of ALL for Hb distribution. (Table: 3)

The mean and median platelet count was found 50980.8±50643.5 cells/μL and 30000cells/μL (range 4000-201000 cells/μL) in B-ALL, higher than T-ALL, where it was found 31368.8±20861.61cells/μL and 25000 (range: 13000-95000 cells/μL) (Table3), although this difference was not significant. The platelet count distribution showed that all 16 cases of T-ALL (100%) whereas 48 cases of B-ALL (92.3 %), were suffering from thrombocytopenia (<150000/μL). Although the difference for platelet count were not significant between B-ALL and T-ALL. No subtypes of ALL were found to be associated with higher platelet count. (Table 3)

No significant difference for leukocyte count was found between B-ALL and T-ALL, although leucocytosis was more common in T-ALL than B-ALL. Our study had demonstrated a higher mean and median TLC in T-ALL (110885±120400/μL, 59000μL) than B-ALL (40637.7±57549.3/μLμL, 19100μL). A WBC counts more than 50000/μL was more frequently recorded in T-ALL than in B-ALL patients (56.2% vs 23.1). Leucocytopenia (<4000/μL) was recorded more in B-ALL than T-ALL (13.5% vs 6.2).

Table 4: Showing clinical features in different subtypes of ALL

Clinical sign	B-ALL (N=52)		T-ALL (N=16)		Comparison b/n groups	
	No.	%	No.	%	Pearson Chi square	P-value
Fever	24	46.2	5	31.5	1.159	0.56
Pallor	20	38.5	4	25	1.405	0.495
Petechiae	6	11.5	5	31.2	9.405	0.009*
Joint Swelling	5	9.6	1	6.2	1.405	0.495
Lymphadenopathy	23	44.2	5	31.2	1.736	0.42
Hepatomegaly	19	36.5	5	31.2	0.999	0.607
Spleenomegaly	18	34.6	6	37.5	2.851	0.24

(Note: *significant = p-value <0.05)

Table 4 is showing clinical features in B-ALL and T-ALL. Only Bleeding manifestation was found significantly higher in T-ALL than B-ALL i.e 31.2% and 11.5% respectively.

Table 5: Comparison for serum level of IL-6

Groups	Total no. of cases	≤ 11 pg/mL (Value of IL6)	(Value of IL6)	>11 pg/mL		Comparison Between Groups		
		No.	%	No	%	Groups	χ2 Value	p-value
Control	20	20	100%	0	0.00 %	Control vs. ALL	32.233	0.000*
ALL	43	10	23.26 %	33	76. 74%			
B-ALL	34	7	20.6	27	79.4	B-ALL vs.T-ALL	0.648	0.421
T-ALL	9	3	33.3	6	66.7			

Note: *significant = p-value <0.05)

Our study had shown that all cases of the control group were associated with the normal level of IL-6, whereas 76.74 % of ALL patient's serum were found to be associated with an elevated level of IL-6 (>11.0 pg/mL). There was a significantly elevation in IL-6 level in leukemia patients shown in Table 4. (p value= 0.000). Different subgroups of ALL were compared for IL-6 levels have shown in Table-4. Although a high number of B-ALL patients showed a positive value of IL-6 (79.4%) than T-ALL (66.7%). It was not statistically significant.

Table 6: Comparison of mean serum level of IL-6

Groups	No. of Patients	Range (pg/mL)	Median (pg/mL)	Mean ± SD (pg/mL)	Mann -Whitney test		
					Groups	Z value	P value
Control	20	2.42 - 10.0	6.86	6.51 ± 2.27	Control vs ALL	5.044	0.000*
ALL	43	3.53 - 1196.92	48.65	167.86 306.46			
B-ALL	34	5.01- 1196.92	77.78	195.50 337.79	B-ALL vs T-ALL	1.269	0.208

T-ALL	9	3.53-235.77	13.88	63.43 82.90			

(Note: *significant = p-value <0.05)

The mean IL-6 values were found significantly elevated in ALL than control group (p-value: 0.000). No significant differences were found between different subtypes of ALL in serum IL-6 level. Serum mean values were found 195.50 ± 337.79 pg/ml and 63.43±306.46 pg/mL in B-ALL and T-ALL respectively. (Table 6)

Table 7: Comparison of serum level of IL-10

Groups	Total no. of cases	≤ 39pg/mL (Value of IL10)		>39pg/mL (Value of IL10)		Comparison Between Groups		
		No.	%	No.	%	Groups	χ2 Value	p-value
Control	20	19	95.0	1	5.0	Control vs ALL	12.55	0.000*
ALL	43	21	48.8	22	51.2			
B-ALL	34	16	47.1	18	52.9	B-ALL vs T-ALL	0.206	0.65
T-ALL	9	5	55.6	4	44.4			

(Note: *significant = p-value <0.05)

The cut-off of IL-10 value was decided based on the control group mean±2SD. One case of the control group was found positive for IL-10 (3.1%), whereas 51.2% of ALL patient's serum IL-10 levels were found significantly elevated than.(p value= 0.000). Our results showed that a high number of B-ALL patients showed a positive value of IL-10 (52.9 %) than T-ALL (44.4 %) but it was not found statistically significant as shown in the Table 7.

Table 8: Comparison of mean serum level of IL-10

Groups	No. of Patients	Range (pg/mL)	Median (pg/mL)	Mean ± SD (pg/mL)	Mann -Whitney test		
					Groups	Z value	P value
Control	20	22.55–39.71	30.09	29.39 ± 4.272	Control vs. ALL	3.45	0.001*
ALL	43	21.69–620.61	40.53	70.56± 111.48			
B-ALL	34	21.69 - 620.6	41.42	77.10± 124.4	B-ALL vs. T-ALL	0.642	0.527
T-ALL	9	26.84 - 88.62	33.7	45.5± 23.34			

(Note: *significant = p-value <0.05)

Mean serum IL-10 value also showed significantly higher level In ALL patients than control group. But no significant difference in mean IL-10 serum values was found between B-ALL and T-ALL (77.1± 124.4 vs 45.5± 23.34 pg/ml).

DISCUSSION:

At present Immunophenotyping is the essential technique for accurate and confirmational diagnosis and prognosis of acute leukemias and, also for the subtyping of acute leukemias. In the current study, a total of 68 cases of acute lymphoblastic leukemia were successfully diagnosed and further subclassified into B-ALL and T-ALL, although further subtyping of T-ALL and B-ALL in pre, pro, and mature T or B ALL could not be done due to limited CD marker availability. The immunophenotypic study revealed 52 cases (76.47%) of B lineage and 16 cases were of (23.53%) of T lineage. Similar to our study previous reports had also shown B-ALL preponderance i.e. 21-50%. (14) (15) Contrary to it Mukhopadhyay et al., 2013 had reported high T-ALL proportion than B-ALL. (16) Rajalekshmy et al., 2011 had reported a decrease in the trend of T-ALL in twenty years. Their study elucidated that socioeconomic and environmental factors play a major role in the determination of immunophenotype in pediatric ALL. Also, developing countries have a relatively higher proportion of T-ALL than developed countries.(17)

Table 9: Showing proportion of subtypes of ALL reported by different studies

Author	B-ALL %	T-ALL %
Arya et al., 2011 (18)	69.8	30.2
Mukhopadhyay et al., 2013 (16)	47.6	50.4
Chiaretti et al.,2013 (19)	85.8	14.2
Siddaiahgari, 2015 (14)	84.5	15.5
Arora & Arora, 2016 (15)	79-50	21-50
Gupta et al., 2019 (20)	81.7	18.3
Our study	76.5	23.5

A significant age difference was found between both subtypes of ALL. T-ALL was found in children of higher age and adolescent people. In our study in B-ALL mean age was found 7.13±3.73 years and in T-ALL, 10.25±4.95 years. Study by Arya et al., 2011 (18), Rajalekshmy et al., 2011 (17), Bachir et al., 2009(21) also supported our study. Similar to our study, other studies also found male preponderance in leukemia patients.(22)(18)(21). However, the reason behind the higher male predominance and occurrence of T-ALL in older children is still unknown, which might be attributed by genetic factors as T-ALL is a more complex disease than B-ALL. One study enlightened the mechanism of leukemia development and showed an association of inactivating somatic mutation and deletion of X-linked PHF6 gene (plant homeodomain (PHD) finger protein 6 gene) with the development of T-ALL. So it affects more in male patients as they have only a single copy of this gene (23). However it is clearly not understood but one more explanation can be more susceptibility of males to infectious diseases such as relative immunodeficiency (24). Previous studies had shown that T-ALL is more commonly linked to the low socioeconomic status which may be associated with an increased frequency of viral infections.(18) The mean Hb was found to be 6.87±2.24 gm/dl in B-ALL and 7.46±2.2 gm /dl in T-ALL. Similar to our study Noronha et al., 2011 had also reported no significant difference in Hb level between subtypes of leukemia.(25) Contrary to this Chiaretti et al., 2013 had found a significant difference. (19) Our study had demonstrated a higher mean TLC in T-ALL (110885±120400 cells/μL) than B-ALL (40637.7±57549.3 cells/μL, 19100 cells/μL). A WBC count more than 50000 cells/μL was more frequently recorded in T-ALL than in B-ALL patients (56.2% vs 23.1%). Other reports supported our study.(21)(18)(19) Similar to our study, Noronha et al., 2011 had reported that all subtypes of ALL were associated with thrombocytopenia with a median value less than 50000 cells/μL.(25) Contrary to our study Chiaretti et al., 2013 had reported a higher percentage of B-ALL patients having less than 100000 cells/μL platelet count than T-ALL. (70.12% vs 64.94%). (19) One of the studies had shown T- ALL have a higher platelet count than B-ALL. (23).

We found only one study evaluating serum IL-6 levels in ALL cases at the time of diagnosis. Pérez-Figueroa et al., 2016 had reported higher serum IL-6 levels in ALL i.e 8.79 pg/ml (range, 0.00-615 pg/ml) than control group i.e. 3.20 pg/ml (range, 0.00-87.85 pg/ml). They also found no significant difference in IL-6 levels in ALL subgroups i. with fever and without fever at the time of presentation and suggested IL-6 to be an important pro-inflammatory markers in patients with ALL at diagnosis in the absence of clinically apparent infection. (26) One study had reported a significantly higher intracellular level of IL-6 detected by flowcytometry in ALL children at the time of disease presentation than the control group. (81.74±9.31vs 5.67 ± 0.96)(P < 0.01) and suggested role of IL-6 in ALL patients for monitoring response. (27)

Drabko et al., 2008 had reported a significantly higher serum

IL-10 level in Childhood ALL cases at the time of diagnosis (28.16 pg/ml) than control cases (11.32 pg/ml) and ALL patients during remission phase (16.67 pg/ml). They had also showed a significant positive correlation of IL-10 with total antioxidant status and malonyldialdehyde (MDA) at the time of diagnosis.(28) Bien et al., 2009 had also reported a higher serum IL-10 level in ALL patients than control group (18.3±9.6 vs 6.33±2.60 pg/mL). The pretreatment significant higher level of IL-10 was associated with poor response to therapy and relapse due to disease progression.(29) Similar to our study one report found plasma IL-10 level was significantly elevated at the time of diagnosis of ALL patients than control group. (9.71±3.7 vs 6.1±1.85 pg/mL). Also the level was found significant higher in diagnosis group (9.71 ± 3.7) than treatment group (3.48 pg/mL ± 1.3; p = 0.01) and remission group (0.12 pg/mL ± 0.1; p = 0.0001). The authors had also shown no association between IL-10 polymorphism and plasma level in childhood ALL patients. (30) Similar to our study Pérez-Figueroa et al., 2016 had found a higher circulating IL-10 levels in ALL patients (median 91.81 pg/mL) than the control group (Median 5.15 pg/ml).(26) Mohammed, 2020 had reported a significant decrease in total serum IL-10 level after chemotherapy in childhood ALL cases and suggested the role of serum IL-10 as a marker for response to therapy.(31) Liu et al., 2020 had found the gene polymorphism rs3024489 and rs3024493 of IL-10 were significantly correlated with susceptibility and pathogenesis of childhood ALL.(32)

In conclusion IL-6 and IL-10 were found elevated in ALL patients which may play a significant role in prognosis and disease monitoring in ALL patients. But further study is needed to elucidate exact role in ALL.

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