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Evaluation of serum level of lymphoid enhancer-binding factor-1 and its relation with clinico-hematological and prognostic parameters in pediatric patients with acute lymphoblastic leukemia

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Abstract:

BACKGROUND: Acute lymphoblastic leukemia (ALL) is a heterogeneous disorder characterized by the proliferation of immature lymphoid cells that accumulate in the bone marrow, peripheral blood, and extramedullary sites, causing the clinical manifestations of the disease. Lymphoid enhancer-binding factor-1 (LEF1) is a target gene and central mediator for the Wingless-type signaling pathway, and it has an important role in normal hematopoiesis. High LEF1 expression was reported as a prognostic marker in many types of hematological and nonhematological malignancies.

AIM OF THE STUDY: To evaluate the serum level of LEF1 in pediatric patients with ALL and its correlation with other hematological and clinical prognostic factors (white blood cells [WBC] count, age, gender, central nervous system involvement, and response to treatment).

PATIENTS, MATERIALS, AND METHODS: This cross-sectional study was conducted on 60 children; 20 patients with newly diagnosed ALL before starting induction therapy, 20 patients with ALL during remission (postinduction), and 20 healthy controls. Measurement of serum LEF1 level was done by enzyme-linked immunosorbent assay.

RESULTS: Serum level of LEF1 was higher in newly diagnosed patients than in either patients at remission or controls with highly significant differences. There is a significant positive correlation with total WBC count and no significant correlation between LEF1 level and other hematological and clinical parameters or with immunophenotypic subtypes. There was no significant correlation between LEF1 serum level and response to remission induction.

CONCLUSION: A high serum concentration of LEF1 is found in newly diagnosed patients with ALL and showed a significant positive correlation with total WBC count.

Keywords:

Acute lymphoblastic leukemia, ELISA, Lymphoid enhancer-binding factor-1 (LEF1)

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Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder that originates in a single B- or T-lymphocyte progenitor. Proliferation and accumulation of clonal blast cells in the marrow result in the suppression of hematopoiesis, leading to anemia, thrombocytopenia, and neutropenia.

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Lymphoblasts can accumulate in extramedullary sites, especially the meninges, gonads, thymus, liver, spleen, and lymph nodes.[1]

In children, ALL is the most common form of cancer (25%–30%) and the predominant subtype of leukemia (75%–80%). The disease has considerable phenotypic and genotypic heterogeneity, which is of diagnostic and prognostic importance.[2]

This heterogeneity reflects the fact that leukemic lymphoblast may develop at any point during the multiple stages of differentiation; thus, morphologic, immunologic, cytogenetic, and molecular genetic characterizations are essential to establish or exclude the diagnosis of ALL and to categorize its subtypes. Moreover, this biological heterogeneity has determined an increasing need to stratify patients into risk groups and provide risk-adapted therapy.[3]

Both B-cell and T-cell ALLs comprise multiple subtypes harboring distinct constellations of somatic structural DNA rearrangements and sequence mutations that commonly perturb lymphoid development, cytokine receptors, kinase, and Ras signaling, tumor suppression, and chromatin modification. Recent studies have helped understand the genetic basis of clonal evolution and relapse and the role of inherited genetic variants in leukemogenesis.[4]

Lymphoid enhancer-binding factor-1 (LEF1) is a member of the LEF/T-cell factor family of transcription factors and a key mediator of the canonical Wingless-type (Wnt) pathway.[5] It mediates Wnt signals through recruiting B-catenin and its co-activators to Wnt response elements of target genes and plays crucial roles during development, including normal hematopoiesis.[6]

In normal hematopoiesis, LEF1 plays a crucial role in developing B- and T-lymphocytes as well as neutrophil granulocytes.[7,8]

In different hematologic malignancies, including lymphomas, chronic lymphocytic leukemia, ALL, and acute myeloid leukemia, LEF1 was highly expressed.[9-14]

Aim of the study

- To assess the serum level of LEF1 in pediatric patients with ALL and correlate the serum level of LEF1 with other hematological and clinical prognostic factors (white blood cells [WBC] count, age, gender, central nervous system [CNS] involvement, and response to treatment).

Patients, Materials, and Methods

This cross-sectional study was conducted from January 2020 to September 2020 in the Central Teaching Hospital of Pediatrics, designed to include 40 pediatric patients (20 with newly diagnosed ALL and 20 with postinduction remission).

The diagnosis of ALL was based on a routine morphological assessment of the stained peripheral blood (PB) and bone marrow (BM) smears according to the standard FAB criteria and confirmed by cytochemical stains in the Laboratory of Central Teaching Hospital of Pediatrics by an expert hematopathologist. Flow cytometric immunophenotyping using a panel of well-characterized monoclonal antibodies (MPO, Ccd79a, CD19, CD20, CD7, CD3, CD34, CD45, CD11b, CD13, CD10, HLA-DR, CD33, CD117, TdT, SIgm, CD38, and CD66) was done at Flowcytometry Department in Medical City, Baghdad, for further confirmation and characterization of the cases.

For the assessment of remission induction, patients were evaluated for the achievement of complete remission at the end of the induction phase (day 28) by morphological evaluation of the PB and BM smears, which should reveal BM blast <5%.[15]

This study, approved by Ethics Committee of Iraqi council for medical specialization and was conducted in concordance with the Declaration of Helsinki and Informed written consents were obtained from all of the patients who participated in the study.

Data by a questionnaire include the main symptoms and physical signs, especially the presence of extramedullary features, which include lymphadenopathy, splenomegaly, hepatomegaly mediastinal widening, and CNS involvement besides hematological parameters were obtained from each patient.

The included patients were newly diagnosed patients with ALL and patients with postinduction remission of both B- and T-ALL subtypes who were aged <15 years and randomly collected concerning gender.

The control group of 20 healthy children was included in this study. The age ranged between 2 and 14 years and they were 10 males and 10 females.

Blood sample collection and preparation

Two and a half milliliters of venous blood samples was taken from each patient and control under completely aseptic technique, and serum was stored at −80°C and then used for measuring serum LEF1 level by double-sandwich enzyme-linked immunosorbent

assay (ELISA) technique using LEF1 ELISA kit from MYBIOSOURCE.[16]

Results

Demographic characteristics of the study population

The mean age of the newly diagnosed patients was 6.27 ± 3.41 years (range 2.0–12.0 years) which did not differ significantly from that of the remission group (mean = 6.39 ± 2.77 years, range 2.0–12 years) or control group (mean = 7.6 ± 3.22 years, range 2.0–13.0 years).

The frequency of males in the newly diagnosed, remission, and control group was 12 (60%), 13 (65%), and 10 (50%), respectively, with no significant differences [Figure 1].

Hematological and clinical characteristics of the newly diagnosed patients

The mean hemoglobin (Hb) concentration was 7.93 ± 2.2 g/dL (range 4.5–13 g/dL). The majority of the patients (90%) were anemic, with those having Hb <7 mg/dL accounting for 40% of the patients. The total WBC count was 132.91 ± 166.63 × 10^9/L as a mean (range 0.7–556.4 × 10^9/L). Exactly half of the patients had WBC count >50 × 10^9/L, while the other half had WBC count ≤50 × 10^9/L. The mean platelet count was 87.7 ± 83.02 × 10^9/L (range 14–374 × 10^9/L). The majority of the patients (75%) demonstrated thrombocytopenia (platelets count <100 × 10^9/L). The mean blast percentage was 87.15% ± 12.62% (range 50%–98%). More than two-thirds of patients (70%) demonstrated a blast percentage over 80%. T-ALL type was encountered in about one-third of the patients, while the other two-thirds were B-ALL type. The vast majority of the patients (90%) achieved remission, while only two patients (10%) did not achieve remission [Table 1].

Fever was the most common manifestation of the newly diagnosed patients and was present in 80% of them, followed by hepatosplenomegaly (75%) and then pallor (70%). In contrast, bone pain was the least common manifestation encountered in nine patients (45%).

Serum concentration of lymphoid enhancer-binding factor-1

Data regarding LEF1 concentration in three groups were found to be nonnormally distributed (according to the Shapiro–Wilk test). Therefore, a nonparametric Kruskal–Wallis test compared the medians between the three groups. Newly diagnosed patients showed higher serum concentration of LEF1 (median 0.582 ng/mL, range 0.034–4.642 ng/mL) than either patients at remission (median = 0.056 ng/mL, range 0.01–2.76 ng/mL) or controls (median = 0.032 ng/mL, range 0.01–0.28 ng/mL) with highly significant differences. Of note, there was no significant difference between the remission group and controls [Figure 2].

Table 1: Hematological characteristics of the newly diagnosed

Variables	Frequency (%)
Hemoglobin (g/dl)	
<7	8 (40)
7-11	10 (50)
>11	2 (10)
WBC (×10^9/L)	
≤50	10 (50)
>50	10 (50)
Platelets (×10^9/L)	
≤100	15 (75)
>100	5 (25)
Type	
T-ALL	7 (35)
B-ALL	13 (65)
Remission	
Yes	18 (90)
No	2 (10)

WBC=White blood cells, ALL=Acute lymphoblastic leukemia

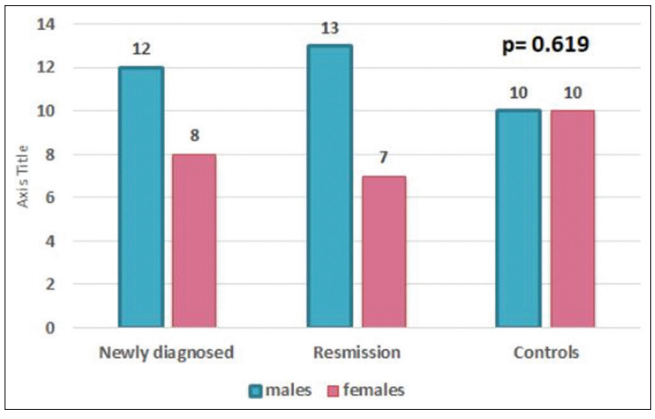

Figure 1: Sex distribution in acute lymphoblastic leukemia patients and control

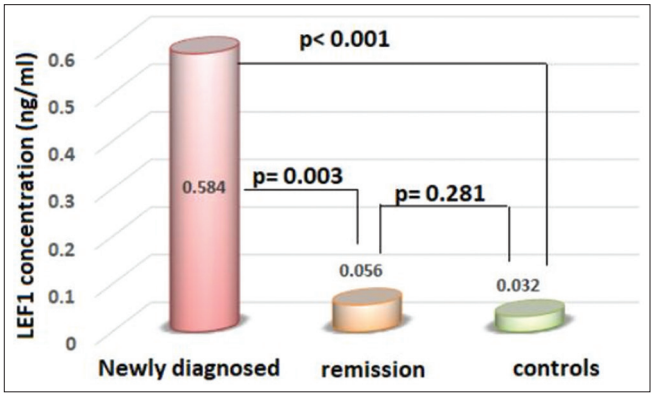

Figure 2: Median serum concentration of lymphoid enhancer-binding factor-1 in the three groups

Correlation between lymphoid enhancer-binding factor-1 and other variables in newly diagnosed patients

Pearson's correlation test explored the possible correlation between LEF1 and age, Hb, platelets, WBCs, and blast in newly diagnosed ALL patients. LEF1 showed a significant positive correlation with total WBC count (r = 0.471, P = 0.036) as shown in Table 2 and Figure 3.

Association of lymphoid enhancer-binding factor-1 with sex, all type, remission, and clinical features

Generally, LEF-1 showed no significant association with sex, remission, ALL subtypes, or clinical features of the disease.

Newly diagnosed patients were subdivided into two categories according to the National Cancer Institute (NCI)/Rome criteria: higher risk and standard risk. Accordingly, eight patients (40%) had standard-risk ALL while 12 patients (60%) had high-risk ALL.

Demographic and clinical characteristics of patients with standard- and high-risk acute lymphoblastic leukemia

Among nine included factors (age, gender, HB, WBC count, platelet, blast%, ALL type, remission, and LEF1 concentration), only three were significantly associated with high-risk ALL. Interestingly, the serum level of LEF1 was comparable between the two groups with no significant difference [Table 3].

Discussion

In this study, the patients were randomly selected concerning gender, and the male-to-female ratio was 1.5:1. Similarly, many other studies revealed that males predominate in pediatric ALL.[17-19]

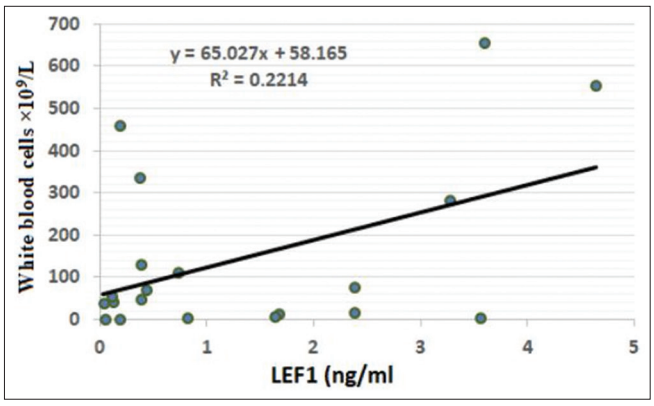

Figure 3: Regression line between lymphoid enhancer-binding factor-1 and total white blood cells count

Out of the 20 pediatric patients with ALL included in this study, 13 were of B-ALL subtype (65%) and 7 were of T-ALL subtype (35%). These findings were close with Noronha *et al.* in Brazil,[20] Supriyadi *et al.* in Indonesia,[21] Bachir *et al.* in Morocco,[22] and Abbasi *et al.* in Jordan.[23]

The majority of patients presented with fever, pallor, and hepatosplenomegaly; this was comparable with AlMulla *et al.*'s study[24] while it was not in line with Jaime-Pérez *et al.*'s[25] study, which showed a different distribution of the clinical signs and symptoms; many factors may play a role of this differences such as racial, genetic, and environmental factors.

Table 2: Pearson's correlation between lymphoid enhancer-binding factor-1 and other variables in newly diagnosed acute lymphoblastic leukemia patients

Variable	Newly diagnosed patients	
	R	*P*
Age	−0.133	0.320
Hemoglobin	−0.053	0.824
Platelets	−0.143	0.547
WBC	0.471	0.036
Blast	−0.346	0.135

WBC=White blood cells

Table 3: Demographic and clinical characteristics of patients with standard- and high-risk acute lymphoblastic leukemia

Variables	Standard-risk ALL (*n*=8), *n* (%)	High-risk ALL (*n*=12), *n* (%)	*P*
Age (years)			
1-10	8 (100)	2 (16.67)	0.035
>10	0	10 (83.33)	
Gender			
Male	4 (50)	8 (66.67)	0.546
Female	4 (50)	4 (33.33)	
Hemoglobin (g/dL)			
<7	3 (37.5)	5 (41.67)	0.179
7-11	3 (37.5)	7 (58.33)	
>11	2 (25)	0	
WBC (×10⁹/L)			
≤50	8 (100)	2 (16.67)	<0.001
>50	0	10 (83.33)	
Platelets (×10⁹/L)			
≤100	5 (62.5)	10 (83.33)	0.292
>100	3 (37.5)	2 (16.67)	
Type			
T-ALL	0	7 (58.33)	0.007
B-ALL	8 (100)	5 (41.67)	
Remission			
Yes	8 (100)	10 (83.33)	0.224
No	0	2 (16.67)	
LEF1 (ng/mL)			
Median	1.226	1.206	0.521†
Range	0.05-3.6	0.13-4.64	

†Mann-Whitney test. ALL=Acute lymphoblastic leukemia, LEF1=Lymphoid enhancer-binding factor-1, WBC=White blood cells

The majority of ALL patients included in this study had high WBCs counts, anemia, and thrombocytopenia, and this goes with many other studies.[13,15,17]

The newly diagnosed patients showed higher level of LEF1 than either patients at remission or controls with highly significant differences, which is in agreement with many studies.[26,27]

This is expected as the LEF1 mRNA levels in patients with ALL are significantly higher than those of normal controls, and the LEF1 levels are dramatically decreased following induction therapy.[27]

A significant positive correlation between serum level of LEF1 and total WBC count ($r = 0.471$, $P = 0.036$), and this result is in agreement with Guo *et al.*'s study,[25] which showed higher median WBC counts in patients with high LEF1 level. This can be explained by increased cellular proliferation by the effect of LEF-1 but not in agreement with Jia *et al*.'s study[26] and ElSourdy *et al.*'s study.[28]

There is no significant correlation between serum LEF1 level and another hematological and clinical parameter or with immunophenotypic subtypes, and this goes with many studies.[26,27]

Newly diagnosed patients were subdivided into two categories according to the NCI/Rome criteria: higher risk and standard risk. Accordingly, eight patients (40%) had standard-risk ALL while 12 patients (60%) had high-risk ALL. All T-ALL patients are within a high-risk group and thus considered as poor prognostic parameters.[21]

Regarding the correlation between LEF1 level and NCI risk groups, there was no significant correlation between them, and this is in agreement with many studies.[26,27]

In the present study, there was no significant correlation between LEF1 and the response to induction therapy, and this goes with the study by ElSourdy *et al*.[28]

Conclusion

1. High serum concentration of LEF1 is found in newly diagnosed patients with ALL
2. LEF1 showed a significant positive correlation with the total WBC count.

Recommendation

1. Further studies with large numbers of patients, other methods for detection of LEF1, and longer time of follow-up to determine the significance of LEF1 in ALL are needed
2. Evaluation of specific molecular and cytogenetic abnormalities (BCR - ABL fusion gene) and correlate them with serum level of LEF1.

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Conflicts of interest

There are no conflicts of interest.

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