Value of *FoxO3a* Gene Expression in Pediatric Acute Lymphoblastic Leukemia

Temerik DF[1], Abdou MAA[2], Ayoub MR[1], Bakry RM[1]

[1] Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
[2] Clinical Pathology Department, Faulty of Medicine, Assiut University, Egypt

Abstract:

Background: In children, acute lymphoblastic leukemia (ALL) serves as the most prevalent blood malignancy. Based on the American Cancer Society, there were 1580 fatalities and 5690 reported as new cases of ALL in adults and children in the United States (US) in 2021.

Aim of the Work: The objective of this research is to examine the expression levels of the *FoxO3a* gene in bone marrow samples of pediatric patients with ALL using real-time polymerase chain reaction (PCR), in order to develop a newly targeted treatment for the disease and determine whether there is a relationship between the expression levels of *FoxO3a* gene, the clinical presentation and laboratory data of these patients.

Subjects and Methods: This research was performed on fifty-three recently diagnosed pediatric ALL cases, according to the 2016 World Health Organization (WHO) categorization (36 were B-acute lymphoblastic leukemia (B-ALL) versus 17were T-acute lymphoblastic leukemia (T-ALL)) and 30 healthy participants age and sex matched to cases as a control group. These individuals were admitted at the South Egypt Cancer Institute (SECI), Assiut University in the period from June 2020 to December 2021.

Results: *FoxO3a* gene was significantly downregulated in ALL patients. Statistically significant correlations among downregulation of *FoxO3a* gene, hepatosplenomegaly, and the higher percentage of peripheral (P.B) blast cells were recorded.

Conclusion: *FoxO3a* gene acts as tumor suppressor gene, thus rendering *FoxO3a* gene as potential target for treating ALL as one of hematopoietic malignancies.

Keywords: Acute Lymphoblastic Leukemia - *FoxO3a* Gene

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Authors Information:

Doaa F. Temerik
Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
email: doaa_temerik@aun.edu.eg

Madleen Adel Attia Abdou
Clinical Pathology Department, Faulty of Medicine, Assiut University, Egypt
email: Madleen2001@yahoo.com

Madonna Refaat Ayoub
Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
email: madonnarefaat87@aun.edu .eg

Rania M. Bakry
Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
email: Rbakry.md@aun.edu.eg

Corresponding Author:
Madonna Refaat Ayoub Eshak
Clinical Pathology Department, South Egypt Cancer Institute, Assiut University, Egypt
email: madonnarefaat87@aun.edu .eg

Introduction:

Developing arrest occurs at several phases of differentiation in ALL. There is a malignant clonal proliferation of lymphoid hematopoietic progenitors [1]. B-ALL and T-ALL are lymphatic tumours that develop from B- or T-lineage progenitors [2].

Forkhead box O3, also named *FoxO3a* is one of the isoforms of the forkhead box (FoxO) group, which includes FoxO1, FoxO4, *FoxO3a*, and FoxO6. The FoxO group regulates a variety of physiological processes that occur in cells, including apoptosis [3], cell cycle progression [4], DNA damage [5], proliferation [6], and response to oxidative stress. This transcription factor has numerous activities; therefore, dysregulation of its expression is linked to a variety of diseases, particularly malignancies [7].

As a tumour suppressor, degradation of *FoxO3a* promotes cell transition, proliferation and reduction of apoptosis. Consequently, controlling of its production and location is essential for preserving the hemostasis of the body [8]. *FoxO3a* gene can be regulated by the Phosphatidyl inositol-4,5-bisphosphate 3-kinase-protein kinase B (PI3K-AKT) pathway which is hyperactivated in various types of cancers and leukemia [9]. As a result of this hyperactivation, *FoxO3a* is translocated from the nucleus to the cytoplasm, where it combines with 14-3-3 proteins, resulting in its degradation by proteasomal activity and loss of its function [10].

Aim of the Work: The objective of this research is to examine the expression levels of *FoxO3a* gene in bone

marrow samples of patients with ALL by using real-time PCR and determine if there is a relationship between the expression levels of *FoxO3a* gene, the clinical presentation and laboratory data of these patients.

Patients and Methods:

Patients:

This research was performed on fifty-three recently diagnosed pediatric ALL cases, according to the 2016 World Health Organization (WHO) categorization (36 were B-acute lymphoblastic leukemia (B-ALL) versus 17 were T-acute lymphoblastic leukemia (T-ALL)) and 30 healthy participants age and sex matched to cases as a control group. These patients were admitted at the South Egypt Cancer Institute (SECI), Assiut University between June 2020 and December 2021. The Assiut University Faculty of Medicine's medical ethical committee gave the research the thumbs up. After receiving the parents' expressed agreement, all participants were included. The patients in this study were children aged up to 17 years newly diagnosed ALL. Individuals with other hematological cancers, under radiation or chemotherapy or older than 17 years old were excluded from the study.

Methods:

All patients were subjected to complete clinical examination for assessment of lymphadenopathy, splenomegaly, hepatomegaly, and manifestations of anemia and thrombocytopenia. Laboratory investigations as complete blood count, bone marrow examination, immunophenotyping and cytogenetic and molecular testing were done.

Sampling:

FoxO3a gene expression was done by using Ethylene Diamine Tetra Acetic Acid (EDTA) vacutainer tubes to collect peripheral blood samples and bone marrow specimens from these patients under complete aseptic conditions.

Steps of measuring FoxO3a gene expression level:

A - Purification of total cellular RNA was done by using the QIAamp RNA Blood Mini Kit, complete cellular RNA was purified from human whole blood (Germany, cat. No.52304).

Qubit 4 fluorometer was used to detect the RNA concentration within every specimen (Singapore, serial number: 232260124908). Up to 5 micrograms of total RNA can be used for cDNA synthesis in the reverse transcription reaction.

B - Reverse transcription and quantification was done Using Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit (K1622, Lithuania).

C- Detection of the *FoxO3* gene by real time PCR was done by using the Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X) (K0251) and Applied Biosystem QuantStudio5 Real-Time PCR System (Indian, SN: 272527907). The primer sequences were as follows: Forward, 5-CGGACAAACGGCTC ACTCT-3 and reverse,

5-GGACCCGCATGAATCG ACTAT-3 for *FoxO3a* gene; and forward, 5-CCCGAAACGCCGAATATAAT-3 and reverse, 5-CTGGACTGTTCTTCAC TCTTG-3 for TBP gene. The PCR cycling began with an initial step of 95˚C for 15 min followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min as presented in Table 1.

Table (1): The cycling condition for real-time PCR for *FoxO3a* gene expression

Step	Time	Temperature
Initial denaturation step:	10 min	95 °C
Cycling steps:		
▪ Denaturation	15 sec	95 °C
▪ Annealing/ Extension	1 min	60 °C
* Cycle number = 40 cycles		

D- Analysis of the data:

Results interpretation:

The fractional cycling frequency where the fluorescence exceeded the specified threshold was used to calculate the cycling threshold (Ct). A widely used method to present the relative expression of a gene is the 2-ΔΔct method. Then the equation 2-ΔΔct for each patient sample was applied (If more than 1 → Upregulated and If less than 1 → Downregulated) [11]. Different groups of ALL patients were compared with healthy control group and tested for statistical significance using mean ± SD of fold changes.

Statistical analysis:

SPSS (statistical package for the social sciences; SPSS Inc., Chicago, IL, USA) version 22 was used for all statistics. When applicable, percentages (number of instances) and frequency distributions (percentages) were used to statistically describe the data along with means, standard deviations (SD), medians, and ranges when the statistics were not normally dispersed. Since the data were not normally distributed, the Mann Whitney U test was used to compare the quantitative variables. An analysis using the Chi square ($\chi 2$) analysis was done to compare categorical variables. The Pearson correlation test was used to determine the correlation between different variables. P-value is always two-tailed and set to 0.05 threshold of significance.

Results:

This case-control study has been conducted to assess the expression of *FoxO3a* gene in patients of newly diagnosed ALL (n=53); 36 (67.9%) patients were B-ALL and 17(32.1%) were T-ALL respectively. The median age of the studied ALL cases was 10 years and ranged from 7 months up to 17 years old. Out of 53 studied ALL cases; 29 (54.7%) were males versus 24 (45.3%) were females, with male: female ratio of 1.2:1.

There was no significant difference in age or sex between patients and controls as regard to demographic data of the studied participants (age, and sex), P=0.069 and 0.903 respectively as presented in Table 2.

According to the clinical presentation of ALL patients; the majority of the studied cases were presented with lymphadenopathy documented in 79.2%, followed by hepatosplenomegaly in 64.2%, fever in 56.6%, bleeding tendency in eight cases (15.1%), seven cases (13.2%) were presented with anemia, two cases (3.8%) with bone pain, abdominal pain, pancytopenia, and abdominal distension were documented in one case only (1.9%).

By comparing the clinical presentation of ALL cases according to the flowcytometric results, there was no significant difference between B-ALL and T-ALL cases, except for hepato-splenomegaly which was significantly frequent in B-ALL patients (80.6%) than T-ALL patients (29.4%) as presented in Table 3 and Figure 1.

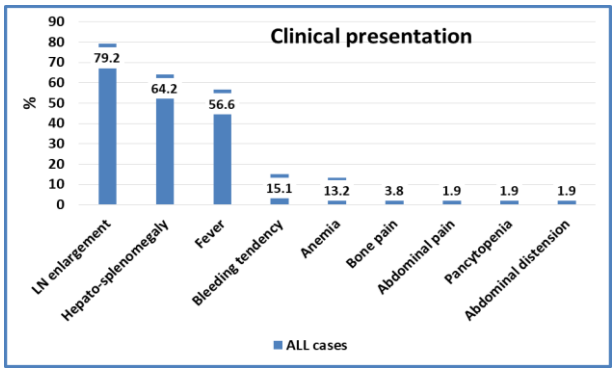

Figure (1): Clinical presentation of ALL cases

By comparing the laboratory data between B-ALL and T-ALL patients, the count of leucocytes was significantly higher in T-ALL patients (Median (range) = 93.8(0.8-366) than in B-ALL patients (median (range) =13.3 (0.9-150).

T-ALL cases were presented with higher platelets count (103/ul) (Median (range) =140 (19- 414) versus (Median (range) =22 (4 -98) in B-ALL cases. Also, T-ALL participants suffered from higher peripheral blasts (%); (Median (range) = 77.0 (5.0 – 97.0) in T-ALL cases versus 50.0 (2.0 – 95.0) in B-ALL cases as presented in Table 4.

According to cytogenetic results of ALL patients, BCR/ABL (t (9; 22)) was positive in two cases (3.8%), negative in 47 cases (88.7%), not done in four cases (7.5%). ETV6-RUNX1 (t (12; 21)) was negative in nine cases (17.0%), not done in 44 cases (83.0%). MLL (t (4; 11)) was positive in two cases (3.8%), negative in 11 cases (20.8%), not done in 40 cases (75.5%). CRLF2 was negative in seven cases (13.2%), not done in 46 cases (86.8%). TCF3 (t (1; 19)) was positive in three cases (5.7%), negative in 30 cases (56.6%), not done in 20 cases (37.7%) as presented in Table 5.

The relative quantification method (RQ) was obtained and interpreted as up- or downregulated in the ALL cases (n=53) compared with expression in controls (n=30). The RQ of healthy controls were calculated as one and RQ of acute leukemia patients were calculated to be greater (upregulated) or lesser (downregulated) than one. The level of expression of the *FoxO3a* gene was significantly lower in ALL cases as compared to controls (median (range) 0.33 (0.01 – 513.81) versus 1 (1 – 1) in both studied groups respectively as presented in Table 6 and Figure 2.

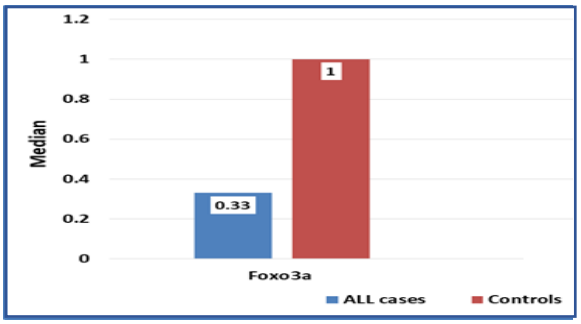

Figure (2): Bar graph showing difference in *FoxO3a* gene expression in both studied groups

Both studied groups showed no significant difference for studied gene (*FoxO3a*), however the expression analysis of *FoxO3a* was close to significant value (P=0.066) and more downregulated in B-ALL cases than T-ALL cases as shown in Table 7.

By comparing the expression analysis of *FoxO3a* among ALL patients according to age, *FoxO3a* gene showed no significant difference according to the age groups of the studied participants (≤ 5 years old versus > 5 years old), P=0.748 in both studied groups respectively.

By comparing the expression analysis of *FoxO3a* among ALL patients according to sex, the studied gene (*FoxO3a*) showed no significant difference according to gender of the studied participants (P=0.883) in both studied groups respectively.

Expression analysis of *FoxO3a* gene according to the clinical presentation of the ALL patients showed that there was no significant difference for all mentioned clinical presentation, except for hepatosplenomegaly which was more observed in cases with down regulation (73.0%) than in cases with up regulation (43.8%) (P=0.042) as shows in Table 8.

By comparing the expression analysis of *FoxO3a* gene according to the laboratory data in the ALL patients, there was no significant difference for all laboratory data namely (leucocytic count, hemoglobin level, platelets count, bone marrow and peripheral blast (%), (P>0.05 for all) as shown in Table 9.

Studying the correlation between the RQ values of *FoxO3a* gene and age of the patients, leucocytic count, hemoglobin level, percentage of bone marrow and peripheral blast revealed that there was a significant negative correlation between the RQ of *FoxO3a* and peripheral blast (%) of the studied patients (r= - 0.284, P = 0.048) as shown in Table 10 and Figure 3 and also another significant negative correlation between the RQ of *FoxO3a* and bone marrow blast (%) of the studied patients (r= - 0.428, P = 0.001) as shown in Table 10 and Figure 4 .

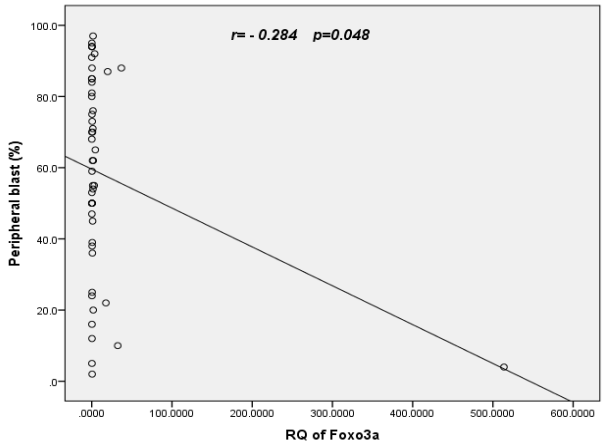

Figure (3): Scatter plot diagram showing the correlation between *FoxO3a* gene and Peripheral blast (%) in ALL cases

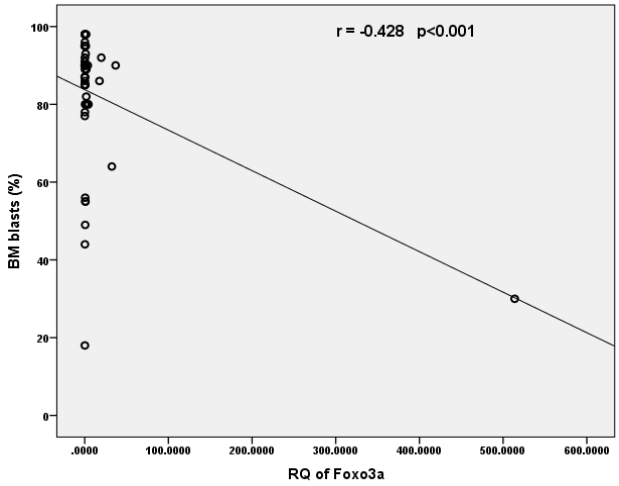

Figure (4) Scatter plot diagram showing the correlation between *FoxO3a* gene and BM blast (%) in ALL studied cases.

Table (2): Demographic data between ALL cases and controls

Variable name	ALL cases (n=53)	Controls (n=30)	P value
Age			
▪ Mean ± SD	8.90 ± 4.78	6.87 ± 3.12	0.069
▪ Median (range)	10 (7 mon – 17 yrs)	6 (3 – 14)	
Sex			
▪ Male	29 (54.7)	16 (53.3)	0.903
▪ Female	24 (45.3)	14 (46.7)	

Quantitative data are presented as mean ± SD and median (range), qualitative data are presented as number (percentage). P value is significant ≤0.05.

Table (3): Clinical presentation of ALL cases

Clinical presentation	Total cases (group1) (n=53)	B-ALL (n=36)	T-ALL (n=17)	P value
LN enlargement	42 (79.2)	30 (83.3)	12 (70.6)	0.301
Hepatosplenomegaly	34 (64.2)	29 (80.6)	5 (29.4)	**0.000***
Fever	30 (56.6)	21 (58.3)	9 (25.0)	0.712
Bleeding tendency	8 (15.1)	6 (16.7)	2 (11.8)	1
Anemia	7 (13.2)	5 (13.9)	2 (28.6)	1
Bone pain	2 (3.8)	1 (2.8)	1 (5.9)	0.543
Abdominal pain	1 (1.9)	0 (0.0)	1 (5.9)	0.321
Pancytopenia	1 (1.9)	1 (2.8)	0 (0.0)	1
Abdominal distension	1 (1.9)	0 (0.0)	1 (5.9)	0.321

Qualitative data are presented as number (percentage). P value is significant ≤0.05.

Table (4): Laboratory data of ALL cases

Laboratory data	Total case	B-ALL	T-ALL	P value
Leucocytes (10^3/ul)				
▪ Mean ± SD	63.85 ± 87.29	30.54 ± 37.19	134.38 ± 118.14	**0.001***
▪ Median (range)	23 (0.8 –366.0)	13.3 (0.9 -150.0)	93.8 (0.8 -366.0)	
Hemoglobin (g/dl)				
▪ Mean ± SD	9.04 ± 1.97	8.82 ± 1.93	9.48 ± 2.04	0.345
▪ Median (range)	8.7 (4 – 13)	8.6 (4.0 – 12.1)	9.1 (6.4 – 12.9)	
Platelets (10^3/ul)				
▪ Mean ± SD	67.13 ± 81.78	27.03 ± 19.92	152.06 ± 97.92	**0.000***
▪ Median (range)	31 (4 – 414)	22 (4 – 98)	140 (19 – 414)	
Bone marrow blast (%)				
▪ Mean ± SD	82.52 ± 17.17	82.00 ± 16.63	83.53 ± 18.75	**0.619**
▪ Median (range)	89 (18 – 98.0)	87 (30.0 – 98.0)	90 (18 – 98.0)	
Peripheral blast (%)				
▪ Mean ± SD	58.12 ± 28.19	52.49 ± 26.95	69.75 ± 27.94	
▪ Median (range)	62.0 (2.0 -97.0)	50.0 (2.0 – 95.0)	77.0 (5.0 – 97.0)	**0.021***

Quantitative data are presented as mean ± SD and median (range). P value is significant ≤0.05.

Table (5): Cytogenetic results of ALL patients

Cytogenetic		Total cases (n=53)	B-ALL (n=36)	T-ALL (n=17)
t (9;22) BCR –ABL	▪ Negative	47 (88.7)	31 (86.1)	16 (94.1)
	▪ Positive	2 (3.8)	2 (5.6)	0 (0.0)
t (12;21) ETV6-RUNX1	▪ Negative	9 (17.0)	8 (22.2)	1 (5.9)
t (4;11) MLL	▪ Negative	11 (20.8)	9 (25.0)	2 (11.8)
	▪ Positive	2 (3.8)	2 (5.6)	0 (0.0)
CRLF2	▪ Negative	7 (13.2)	7 (19.4)	0 (0.0)
t (1;19) TCF3	▪ Negative	30 (56.6)	23 (63.9)	7 (41.2)
	▪ Positive	3 (5.7)	3 (8.3)	0 (0.0)

Qualitative data are presented as number (percentage).
Abbreviations: BCR-ABL: Break point cluster- Abelson tyrosine kinase gene, ETV6-RUNX1: ETS Variant Transcription Factor 6- Runt-related transcription factor-1, CRLF2: Cytokine receptor-like factor 2, TCF3: Transcription factor 3 and MLL: Mixed Lineage Leukemia

Table (6): Comparison of Expression Analysis of *FoxO3a* Gene between both studied groups

Variable name	ALL cases (n=53)	Controls (n=30)	P value
FoxO3a			**0.002***
▪ Median (range)	0.33 (0.01 – 513.81)	1 (1 – 1)	

Quantitative data are presented as mean ± SD and median (range). p value is significant ≤0.05.
Abbreviations: *FoxO3a*: Forkhead box O.

Table (7): Comparison of Expression Analysis of *FoxO3a* Gene between B-ALL patients and T-ALL patients

Variable name	B-ALL (n=36)	T-ALL (n=17)	P value
FoxO3a			
▪ Up regulation	8 (22.2)	8 (47.1)	0.066
▪ Down regulation	28 (77.8)	9 (52.9)	

Qualitative data are presented as number (percentage). P value is significant ≤0.05.
Abbreviations: *FoxO3a*: Forkhead box O.

Table (8): Comparison of Expression Analysis of *FoxO3a* Gene according to the clinical presentation of the ALL cases

Clinical presentation	*FoxO3a*		P value
	Up regulation (n=16)	Down regulation (n=37)	
LN enlargement			
▪ Absent	5 (31.3)	6 (16.2)	0.275
▪ Present	11 (68.8)	31 (83.8)	
Hepato-splenomegaly			
▪ Absent	9 (56.3)	10 (27.0)	**0.042***
▪ Present	7 (43.8)	27 (73.0)	
Fever			
▪ Absent	9 (56.3)	14 (37.8)	0.214
▪ Present	7 (43.8)	23 (62.2)	
Bleeding tendency			
▪ Absent	12 (75.0)	33 (89.2)	0.224
▪ Present	4 (25.0)	4 (10.8)	
Anemia			
▪ Absent	16 (100.0)	30 (81.1)	0.088
▪ Present	0 (0.0)	7 (18.9)	
Bone pain			
▪ Absent	16 (100.0)	35 (94.6)	1
▪ Present	0 (0.0)	2 (5.4)	
Abdominal pain			
▪ Absent	15 (93.8)	37 (100.0)	0.302
▪ Present	1 (6.3)	0 (0.0)	
Pancytopenia			
▪ Absent	16 (100.0)	36 (97.3)	1
▪ Present	0 (0.0)	1 (2.7)	
Abdominal distension			
▪ Absent	16 (100.0)	36 (97.3)	1
▪ Present	0 (0.0)	1 (2.7)	

Qualitative data are presented as number (percentage). P value is significant ≤0.05.
Abbreviations: *FoxO3a*: Forkhead box O

Table (9): Comparison of Expression Analysis of *FoxO3a* Gene in ALL cases according to laboratory data

Clinical presentation	*FoxO3a*		P value
	Up regulation (n=16)	Down regulation (n=37)	
Leucocytes (10^3/ul)	32 (1.3 – 366.0)	23 (0.8 – 293.0)	0.555
Hemoglobin (g/dl)	10.1 (6.4 – 12.9)	8.4 (4.0 – 12.7)	0.068
Platelets (10^3/ul)	44 (11 – 248)	29.0 (4 – 414)	0.323
Bone marrow blast (%)	89.0 (30.0 – 98.0)	87.0 (18.0 – 98.0)	0.899
Peripheral blast (%)	58.5 (4.0 – 97.0)	62.0 (2.0 – 95.0)	0.881

Quantitative data are presented as median (range). P value is significant ≤0.05.
Abbreviations: *FoxO3a*: Forkhead box 3a

Table (10): The correlation between RQ of *FoxO3a* gene and laboratory data of ALL studied cases

Variable name	RQ of *FoxO3a*	
	r value	P value
Age (years)	0.043	0.760
Leucocytes (10^3/ul)	-0.065	0.642
Hemoglobin (g/dl)	0.100	0.476
Platelets (10^3/ul)	-0.032	0.819
Bone marrow blast (%)	-0.428	0.001*
Peripheral blast (%)	-0.284	**0.048***

* Significance defined by p < 0.05, r=correlation coefficient, RQ: relative quantification
Abbreviations: FoxO3a: Forkhead box O

Discussion:

According to Chen et al. [3] and Wang et al. (2022) [12], Apoptosis, proliferation, cell cycle progression and DNA damage are some of the pathological and physiological functions that *FoxO3a* regulates. It acts as a relevant tumor suppressor gene. Deregulation of *FoxO3a* activities or expression may result in a number of illnesses, including cancers [13].

The present study aims at studying the expression levels of *FoxO3a* gene in BM samples of ALL patients by real-time PCR, its correlation with clinical presentation and laboratory data of these patients, and discovering if there is a relation between the expression levels of *FoxO3a* gene to achieve a new target therapy for the disease.

In this study, the number of B-ALL cases (67.9%) was more than T-ALL cases (32.1%). This is consistent with Sayınalp et al. (2020) [14] and Chiaretti et al. (2013) [15] as they recorded that there is an overall predominance of B-ALL in the whole cohort, while T-ALL is much less frequent. As well, Kakaje et al., (2020) [16] studied 203 ALL patients in Syria and found that the number of B-ALL cases (79.8%) was more than T-ALL cases (20.2%). However, Mukhopadhyay et al. (2013) [17], studied 500 ALL patients and recorded the predominance of T-ALL in Indian adolescent patients. They reported that T-ALL cases (50.4%) were more than B-ALL cases (47.6%). This discrepancy is due to the socioeconomic and environmental factors that can play a major role in the determination of pediatric ALL type as said [18].

1. Demographic characteristics of ALL patients:

Our study's median age of ALL patients was 10 years (the age range was from 7 months to 17 years). The number of male patients with ALL was 29 (54.7%) which is more than that of female patients which reached 24(45.3%), with male: female ratio: 1.2:1.

A study done in Syria on 203 patients with de novo ALL who aged 0–14 years with a median age of 5-9 years with a male predominance (60.9%) and these results agree with our results [16]. Similarly, another study was done in Poland on 84 newly diagnosed children with ALL (median age 5 years; range 3–10; the study had 58% male and 42% female patients [19]. In agreement with our study, Chiaretti et al. (2013) [15] retrospectively studied the clinical-biological characteristics of 5202 ALL individuals, 2889 males and 2313 females, with a male-to-female ratio of 1.25, who participated in Italian multicenter procedures. According to certain research, men appear to be more susceptible to developing any type of leukemia since they are subjected to environmental and occupational dangers on a relative basis more often [20].

2. Clinical presentation of ALL patients:

The study revealed that lymphadenopathy (79.2%), hepatosplenomegaly (64.2%) and fever (56.6%) were the most frequent clinical presentation in ALL patients. Clarke et al. (2016) [21], Gurbuxani et al., (2021) [22] and Kakaje et al. (2020) [16] who studied 203 patients with de novo ALL and reported that Lymphadenopathy (82.9%), hepatosplenomegaly (73.2%) were the main presenting symptoms, agree with the study in this finding.

However, Chiaretti et al. (2013) [15] declared and mentioned that spleen and liver enlargement were prevalently recorded in T-ALL more than in B-ALL cases. This is due to the smaller number of patients in the study (53 cases) compared to the previously mentioned study which was done retrospectively on a more significant number of ALL cases (5202 cases) with different clinical conditions of the patients.

3. Hematological data of ALL patients:

According to Leucocytic count in the present study, T-ALL patients were presented with significantly marked leukocytosis (Median: 93.8) than B-ALL patients (Median: 13.3) (P value: 0.001). Many previous studies stated that T-ALL patients are often presented with marked leukocytosis than B-ALL patients, i.e. Chiaretti et al. (2013) [15]; Sallman et al. (2020) [23]; and Kavyanjali Sharma et al. (2021) [24].

Regarding hemoglobin level, there was no statistically significant difference in hemoglobin levels between both types of ALL patients. This is consistent with Kavyanjali Sharma et al. (2021) [24] as they evaluated the clinical and hematological features in ALL patients (68 cases) and found that there were no significant difference in hemoglobin levels between T-ALL and B-ALL cases.

Contrary to the present study, Chiaretti et al, (2013) [15] found a significant difference in hemoglobin levels as they were <10g/dL in B-ALL patients (77.14%) compared to T-ALL patients (42.94%). The discrepancy between studies may result from the small number of patients in the present study (53 cases) compared to the previously mentioned study that was done retrospectively on a more significant number of ALL cases (5202 cases) with different clinical conditions of the patients.

According to platelets count in the present study, a significant increase in platelets count in T-ALL patients (Median: 140) compared to B-ALL patients (Median: 22) (P value: 0.000) was found. This result is consistent with Chiaretti et al. (2013) [14] who declared that cases with platelet count <100×109/L were observed in B-ALL cases (70.12%) compared to T-ALL cases (64.9%).

Regarding Peripheral and bone marrow blast cells, significant higher numbers of P.B blast cells were observed in T-ALL cases (Median: 77) than in B-ALL cases (Median: 50) (P value: 0.021) but no previous studies could be found to correlate these findings with them.

4. FoxO3a gene:

In the present study, a significant down-regulation of *FoxO3a* gene in ALL cases was recorded (Median (range): 0.33 (0.01 – 513.81)) compared to controls (Median (range): 1 (1 – 1)) (P value: 0.002). This result is similar to those of Mirzaie et al. (2019) [11]. This could be explained by overexpressing of *FoxO3a* in B

and T cell lines, which causes cell cycle arrest in the G1 phase and initiates apoptosis by upregulating the pro-apoptotic molecules like Fas ligand (FasL) and Bcl-2-like protein 11(Bim) as well as the cell cycle inhibitory proteins P27 [10].

In similarity to the present study, Ausserlechner et al. (2013) [10] also reported that therapy-resistant *FoxO3a* is localized in the cytoplasm of T-ALL individuals. Additionally, it appears that T-ALL cells in these individuals deactivate *FoxO3a* to avoid apoptosis-inducing effects of Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Phorbol-12-myristate-13-acetate-induced protein 1(PMAIP1) gene.

As well, Zheng et al. (2020) [25] identified that undesirable ALL subtypes, minimal residual disease (MRD) positivity, and recurrence were all observed to be substantially correlated with lower levels of FOXO1 transcription.

Different studies supported that *FoxO3a* acts as a tumor suppressor gene in different types of cancers and down-regulation of *FoxO3a* expression can lead to malignancy:

According to Yang et al. (2013) [26], the expression of *FoxO3a* mRNA was lower in cancerous tissues of gastric adenocarcinoma (p=0.03) compared with their adjacent non-tumorous tissues. Shou et al. (2012) [27] reported that *FoxO3a* was low expressed in nasopharyngeal carcinoma. Zhang et al. (2021) [28] stated that the expression of *FoxO3a* was lower in upper tract urothelial carcinoma patients than in normal tissues.

Contrary to the present study, Bandari et al., (2021) [29] evaluated using of real-time PCR to examine the expressions of oxidative stress resistant strains in 60 ALL participants, particularly Catalase, manganese superoxide dismutase (MnSOD), *FoxO3a*, and sirtuin-1 (SIRT1). The catalase, FoxO, MnSOD, and SIRT1 genes were demonstrated 4 times (p = 0.04), 4.5 times (p = 0.001), 2.2 times (p = 0.05), and 4.8 times (p = 0.01) greater than healthy subjects in the control group, respectively. They supposed that the levels of all studied genes were dramatically higher in ALL patients than in the control group. The age ranges of the participants may be the cause of this disparity (the age of the patients in that study ranged from 2-70 years).

In the present study, there was no significant difference in *FoxO3a* gene expression between B-ALL and T-ALL cases. The studies of Mirzaei et al., (2016) [30] and Mirzaie et al., (2019) [11], which studied the expression levels of the *FoxO3a* gene in 70 children with different stages of ALL patients by real-time PCR, agree with the current study in this finding. They found also that *FoxO3a* mRNA expression was decreased in recently diagnosed ALL individuals in comparison to the controls, maintenance, and relapsed cohorts (P <0.0001, P = 0.0342, and P = 0.0006, respectively).

According to age, no statistical significance between age and *FoxO3a* gene expression was found. This agrees with Zhou et al. (2019) [31] who studied the expression of Foxo3 in 122 de novo AML patients by real-time quantitative PCR.

Regarding sex, there was no statistically significant correlation between sex and *FoxO3a* gene expression. This result agrees with Zhou et al. (2019) [31] but his study was done on acute myeloid leukemia (AML) patients. To the best of our knowledge, no other studies detected sex relation to *FoxO3a* expression levels in ALL patients.

In the present study, a significant correlation between the down-regulation of *FoxO3a* gene expression and hepatosplenomegaly was recorded (P value: 0.042), but a correlation with this result did not be examined in previous studies.

There was no statistically significant correlation among white blood cells (WBC), hemoglobin, platelets, BM blast percentage and *FoxO3a* gene expression. This agrees with Zhou et al. (2019) [31] who studied the expression of Foxo3 and circ-Foxo3 in 122 de novo AML patients by real-time quantitative PCR.

In the present study, the lower expressions of the *FoxO3a* gene were significantly related to the higher percentage of P.B and BM blast cells (r value: -0.284, P value: 0.048) for PB blast cells and (r value: -0.428, P value: 0.001) for BM blast cells. Kornblau et al., (2010) [32] agree with the current study in that the higher levels of inactivated phosphorylated *FoxO3a* are associated with higher P.B and BM blast cells, but differ in that the higher levels of inactivated phosphorylated *FoxO3a* are associated with higher WBC count but our study did not find any significant correlations among *FoxO3a* expression and WBC count. This discrepancy may be due to that study was done on AML patients and statistical method difference.

Conclusion:

FoxO3a gene was significantly downregulated in ALL patients. Statistically significant correlations among downregulation of the *FoxO3a* gene, hepatosplenomegaly, and the higher percentage of peripheral blast cells were recorded. According to the report's findings, *FoxO3a* gene functions as tumour suppressor gene, making it a possible target for treating ALL as one of hematological malignancies.

List of abbreviations:

AKT	Protein kinase B
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
B-ALL	B-acute lymphoblastic leukemia
BCR-ABL	Break point cluster- Abelson tyrosine kinase gene
BIM	Bcl-2-like protein 11
BM	Bone marrow
CRLF2	Cytokine receptor-like factor 2
CT	Cycling threshold
EDTA	Ethylene Diamine Tetra Acetic Acid
ETV6-RUNX1	ETS Variant Transcription Factor 6-Runt-related transcription factor-1
FasL	Fas ligand
FoxO3a	Forkhead box O3
FOXP1	Forkhead box protein P1
MLL	Mixed Lineage Leukemia

MnSOD	Manganese superoxide dismutase
MRD	Minimal residual disease
PMAIP1	Phorbol-12-myristate-13-acetate-induced protein 1
PCR	Polymerase chain reaction
PI3K	Phosphatidyl inositol-4,5-bisphosphate 3
r value	Correlation coefficient
RQ	Relative quantification
RT	Reverse Transcription
SD	Standard deviations
SECI	South Egypt Cancer Institute
SIRT1	Sirtuin-1
SPSS	Statistical package for the social sciences
T-ALL	T-acute lymphoblastic leukemia
TCF3	Transcription factor 3
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
US	United States
WBC	White blood cells
WHO	World Health Organization

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions:

H.B.H.: Conceptualization, Supervision, Investigation, Writing-review & editing. E.M.N.: Supervision, Investigation, Writing -review & editing. M.B.M.: Methodology, Investigation, Data curation, Writing- original draft. M.G.E.: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing- original draft, Supervision. All authors have read and approved the manuscript.

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Ethics approval and consent to participate:

The protocol was approved by the Institutional Review Board of Faculty of Medicine, Assiut University (approval number: 17100901, 13 January 2020 date of approval). Our study conformed to all requirements as governed by the declaration of Helsinki.

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