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Evaluation of FOXP3 and IL10 as immunosuppressant markers in pediatric acute lymphocytic leukemia patients in Iraq

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ABSTRACT

Background. Increased FOXP3+ Treg levels in the TME were positively correlated with a worse prognosis in certain cancer patients. FOXP3 was significantly overexpressed in pediatric B-ALL patients, and this overexpression was associated with a worse prognosis and increased risk of disease relapse. It is unknown if children that have the condition would have different prenatal immune development. This study investigates the relationship between IL10, and immunological development in pediatric ALL.

Methods. The 70 blood samples from children between the ages of 2 and 14 for both sexes were obtained from patients with Acute lymphoblastic leukemia. Furthermore, this study comprised 54 healthy controls. Included in this study, FOXP3 expression on leukemic blast cells were assessed using flow cytometry. And IL10 were assessed using ELISA test.

Results. Increase of FOXP3 cells was noticed in children with ALL compared to healthy individuals with significant increase of FOXP3 (mean ± SD, 91.156 ± 12.255 vs. 14.88 ± 7.897pg/ml ($p < 0.05$). In light of these findings, significant differences in the levels of FOXP3, which was higher in consolidation than induction stage of chemotherapy (mean ± SD, 91.24± 11.078vs. 86.35±5. 16.66) pg/ml ($p < 0.05$). Additionally, it was found that the prevalence of lymphoblast that express FOXP3 was notably higher in the HR group in contrast to the SR group (mean ± SD, 95.52± 5.79 vs. 42.88± 31.38) pg/ml ($p < 0.05$). Following these findings, significant differences in the levels of FOXP3 was higher in relapse than new diagnosis chemotherapy stage (mean ± SD, 95.66± 5.167 vs. 91.035±5. 13.177) pg/ml ($p < 0.05$). On the other hand, children with ALL were also found to have significantly higher levels of IL10 in the current study when compared to healthy controls (mean ± SD, 0.146 ± 0.145 vs. 0.076 ± 0.10 pg/ml ($P < 0.05$). Following these findings, significant differences in the levels of IL10 was higher in consolidation than induction chemotherapy stage (mean ± SD, 0.165± 0.084 vs.0 .0720±.018) pg/ml ($p < 0.05$), the serum levels of IL-10 were evaluated in the two groups: new diagnosis and relapse cases. As in the description profile, serum levels are higher in the relapse group with a highly significant difference. For the parameter IL10 ($P \leq 0.05$). As for he mean level, it was as follows: of IL10 (0.130) pg/ml and (0.216) pg/ml, with (P-value =0.0001) in new diagnosis and relapse receptivity.

Conclusions. According to our findings, B-ALL patients have significant amounts of both FOXP3 and IL10. This pilot study provides a novel way to look into the process mediating the appearance of these markers in a greater number of B-ALL patients at various stages of their treatment.

Keywords: FOXP3, IL10, Acute lymphoblastic leukemia, chemotherapy

INTRODUCTION

Leukemia is a broad class of hematologic malignancies that arises from the aberrant proliferation of developing leukocytes [1], which is used to describe a broad spectrum of hematological malignancies that are currently categorized based on molecular, morphological, cytogenetic, clinical characteristics, immunophenotype, and immunological factors, with the excess of white blood cells that are seen in the bloodstream of these disease patients [2].

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In children and adolescents worldwide, leukemia is the most common malignancy, followed by diseases of the brain and central nervous system [3]. It is classified as either acute or chronic according to the stage of cell development, and the myeloid and lymphoid based on cell type [4]. In acute leukemias, there are usually immature, abnormally differentiated leukocytes (blasts), which can be either lymphoblasts or myeloblasts, the cells that are cancerous. Because of these blasts' capacity for clonal multiplication and proliferation, normal blood products' growth and functionality may be disrupted, leading to the replacement of healthy blood cells with cancerous ones and the manifestation of clinical signs [1]. The most common kind of treatment is chemotherapy. This activity highlights the role of the interprofessional group in leukemia diagnosis and treatment, as well as leukemia evaluation and therapy [1].

Both adults and children can be affected with acute lymphoblastic leukemia (ALL), also referred to as lymphocytic leukemia. In the bone marrow, blood, and extramedullary areas, lymphocyte precursor cells proliferate and undergo malignant transformation [5].

The immune system is a multifaceted, dynamic biological system that protects host organisms against the formation of malignancies and invasion by pathogens. Moreover, it actively contributes to organ regeneration and tissue homeostasis [6]. It comprises a wide variety of immune cell types and immunological mediators, which combine to form the innate and adaptive immune systems. It is common knowledge that the body's primary defense mechanism consists of innate immune cells. Dendritic cells (DCs), macrophages, and mast cells release immune mediators in response to an attack on the body. These cells also perform immunological surveillance [7].

Immune system function is critical for both preventing and treating hematological malignancies and cancer [8]. Tregs have the ability to suppress antitumor immunity, which speeds up the progression of malignancies. Furthermore, there is a substantial correlation between their presence in the tumor microenvironment and a bad prognosis [9].

The Forkhead box (FOX) protein superfamily of transcriptional regulators is involved in a variety of processes, including cell division, proliferation, and survival as well as embryonic development and adult tissue homeostasis [10]. Regulatory T cells, or Tregs, are the most important immunosuppressive cells because they express the transcription factor FOXP3 (forkhead box protein). During development, the transcription factor FOXP3 determines a cell's lineage and is necessary for Treg maintenance and function [11].

Cytokines mediate important interactions between immunological and non-immune cells inside the tumor microenvironment. (TME) [12]. The highly expressed anti-inflammatory cytokine IL10 was found when Treg cytokines were studied [13]. Multifunctional immune-regulatory cytokine interleukin IL10 has anti-angiogenic and immunosuppressive properties [14].

A variety of immune cells release IL-10, a strong regulatory cytokine that has the ability to either directly or indirectly reduce inflammation by inhibiting T cells or antigen-presenting cells (APCs) [15].

Regulatory T-cell subsets, specifically T-regulatory type 1 cells (TR1) and FOXP3+-regulatory T cells (Treg cells), have been well-documented as sources of IL-10 [16]. Furthermore, IL-10 directly inhibits memory Th17 and Th2 cells while promoting the survival and functionality of FOXP3 + regulatory T cells (Tregs) [17] IL10 may have pleiotropic effects in a variety of illnesses and may be linked to the emergence of ALL [18].

METHODS

Patients and samples

A total number of 70 patients (25 newly diagnosed, 12 relapse and 21 during induction and 12 during consolidation chemotherapy) were enrolled, aged 2 years to14 years, along with 54 healthy controls who were the same age and gender as the study. We included in this study FOXP3 expression on leukemic blast cells that were assessed using flow cytometry and IL10 were assessed using ELISA test.

Blood samples were drawn from 70 patients in 3 cohorts of pediatric patients hospitalized between July 2022 and December 2023 as follows:

(G1): contained 25 samples of children with newly diagnosed ALL who had not yet received therapy (steroids or chemotherapy).

(G2) comprised 21 samples from ALL patients receiving induction chemotherapy.

(G3) 11 samples from ALL patients receiving consolidation chemotherapy were included.

(G4) 12 patients who had achieved full remission before experiencing a relapse. Moreover, the age range was 2 to 14 years. The current study also included a control group of 54 boys and girls in good health, ages 2 to 14.

Flow Cytometric Determination of FOXP3

For FOXP3 analysis, for immunophenotyping, cells were stained with monoclonal antibodies conjugated to phycoerythrin (PE), which consist of a processing method with three steps, /lyse/wash method was applied.

First step (processing): 1-5 ml of FOXP3 -PE were added to 100 µl of (EDTA) whole blood tube.

2. Vortex and incubated for 15 to 30 minutes in the dark at room temperature.

3. Added 2 ml of 1x BD FACS lysing solution mixed, and left for 15 minutes in the dark, centrifuge at 300g for 5 minutes.

4. Vortex and incubated for 10 minutes in dark at room temperature.

5. Centrifuge at 300g for 5 minutes.

6. Added 2 to 3 ml of BD cell WASH solution and centrifuge for 5 minutes.

After that, data analysis was carried out using a flow cytometer (FACSCanto flow cytometer with Cell Quest software; Becton Dickinson) and FlowJo X 10.0.7 software (FlowJo, Ashland, OR, USA).

Serological tests

Determination level of Human IL10 by ELISA

All serum samples were examined to detect IL10 Determination was performed by using ELISA according to (Elabscience kit, USA).

Statistical analysis

Version 26 of the (SPSS) was utilized to examine the information. Quantitative data were expressed as mean±standard deviation and median with lowest and maximum values, whereas there was expression of qualitative data as numbers and percentages. We employed the Shapiro, Smirnoff, and Kolmogorov Will examinations to evaluate the normality of the quantitative data distribution. The chi2 test was developed to look at relationships between two or more qualitative variables. The Kruskal Wallis and Mann Whitney U tests were employed to examine differences between quantitative data. A p-value of less than 0.05 indicated that a statistical association was significant.

RESULTS

The present study showed that the highest percentage of ALL patients was in the age group (2-5) years (54.3%) followed by age group (6-12) years (41.4%) whereas the lowest percentage was in patients older than 12 years (4.3%) (P-value = 0.802).

Three age categories were created within the study group: 2-5 years, 6-12 years, and <12 years. Between the cases and control group, there was no noticeable difference (P = 0.802). Additionally, there is no discernible variation in the two groups' sex distribution (P = 0.538) (Tables 1, 2).

Flow Cytometer study

Higher percentages of FOXP3 marker were shown in ALL children compared to healthy controls with significantly increased percentage of FOXP3 (mean±SD, 91.156 ± 12.255 vs. 14.88 ± 7.897pg/ml, (P<0.05). In light of these results, notable variations in the FOXP3 levels in consolation was higher than induction chemotherapy stage (mean±SD, 91.24±11.078 vs. 86.35± 5. 16.66) pg/ml, (p<0.05). Following these findings, there were significant differences in the levels of FOXP3, which was higher in relapse than new diagnosis chemotherapy stage (mean±SD, 95.66± 5.167 vs. 91.035±5. 13.177) pg/ml, (p<0.05). Another observation was that the frequency of FOXP3 was significantly higher in the HR than in the SR group (mean±SD, 95.52±5.79 vs. 42.88±31.38) pg/ml (p<0.05) (Tables 3 and 4) (Figure 1, 2, 3 and 7).

TABLE 1. Distribution of age between studied and control groups

Age groups	Group		Total	P-value
	Patient	Control		
2 to 5 years	38	29	67	0.802
	54.3%	53.7%	54.0%	
6 to 12 years	29	24	53	
	41.4%	44.4%	42.7%	
12-18 years	3	1	4	
	4.3%	1.9%	3.2%	
Total	70	54	124	
	100.0%	100.0%	100.0%	

TABLE 2. Distribution of sex between studied and control groups

Sex	Group		Total	P-value
	Patient	Control		
Male	44	31	75	0.538
	62.9%	57.4%	60.5%	
Female	26	23	49	
	37.1%	42.6%	39.5%	
Total	70	54	124	
	100.0%	100.0%	100.0%	

Serological tests

On the other hand, increased percentages of IL10 were noticed in children with ALL compared to healthy individuals, with the significantly increased percentage of IL10 (mean±SD, 0.146 ± 0.145 vs. 0.076 ± 0.10 pg/ml (P<0.05). In light of these findings, notable variations in the IL10 levels were higher in the consolidation stage than in the induction stage of chemotherapy (mean±SD, 0.165±0.084 vs. 0.0720± 0.018) pg/ml (p<0.05). Serum levels of IL10 in risk groups, HR and SR, are comparable with No significant differences, with p=.1655 (Table 5).

The IL-10 serum level was assessed in two groups: those with a new diagnosis and those experiencing a relapse. Serum levels are higher in the relapse group with a highly significant difference, as indicated by the descriptive profile. Regarding

TABLE 3. Level of FOXP3 in patient among study groups and control

Marker	Control (n=54)				Patient (n=70)				P value
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	
Foxp3	14.88	7.89	2.04	36.84	91.15	12.25	17.51	100.00	0.0001
	New (n=25)				Relapse (N=12)				
	91.03	13.17	17.51	99.51	95.66	5.16	87.27	100.00	0.0001
	Induction (n=12)				Consolidation (n=21)				
	86.35	16.66	53.97	100.00	91.24	11.078	60	100.00	0.0001

* Kruskal Wallis Test

TABLE 4. The level of FOXP3 in risk groups

Risk group	N	Foxp3			
		Mean	SD	Minimum	Maximum
High RG	19	95.52	5.79	77.78	99.51
Stander RG	6	42.88	31.38	17.51	95.5
P-value	0.0001				

* Mann-Whitney U Test

the IL10 parameter. The mean level of IL10 (0.130) pg/ml and (0.216) pg/ml, with (P-value =0.0001) in new diagnosis and relapse receptivity (Table 4), IL-10 serum levels in the two risk groups HR and SR, are comparable with no discernible differences p=.1655 (Table 6) (Figures 3-5).

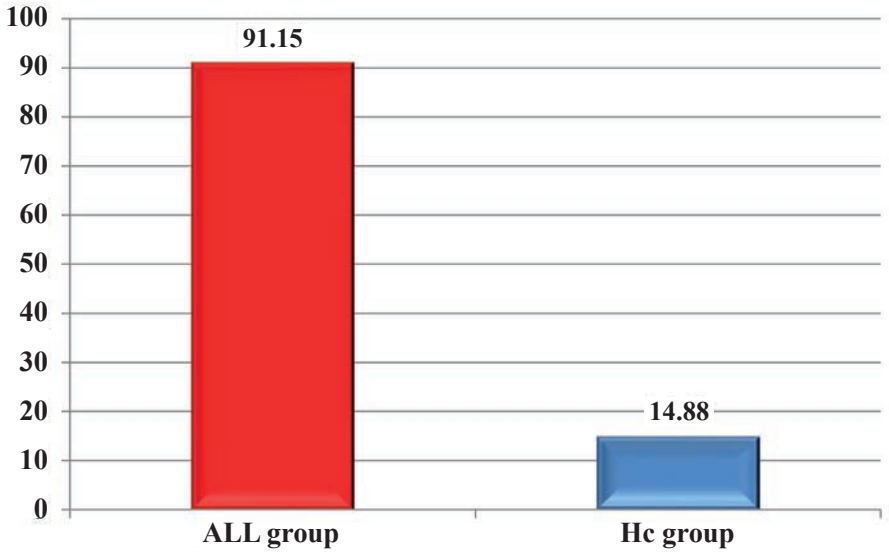


FIGURE 1. Level of FOXP3 between patient and control

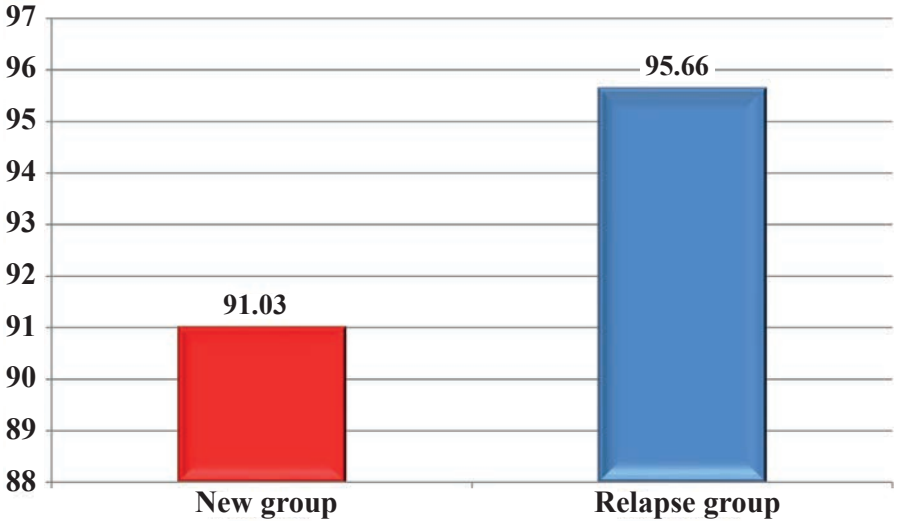


FIGURE 2. The level of FOXP3 between new and relapse groups

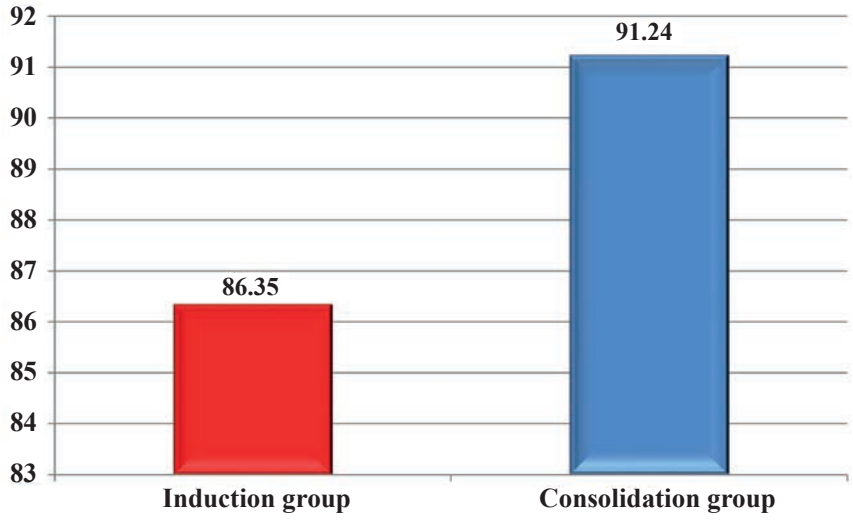


FIGURE 3. Level of FOXP3 between induction and consolidation groups

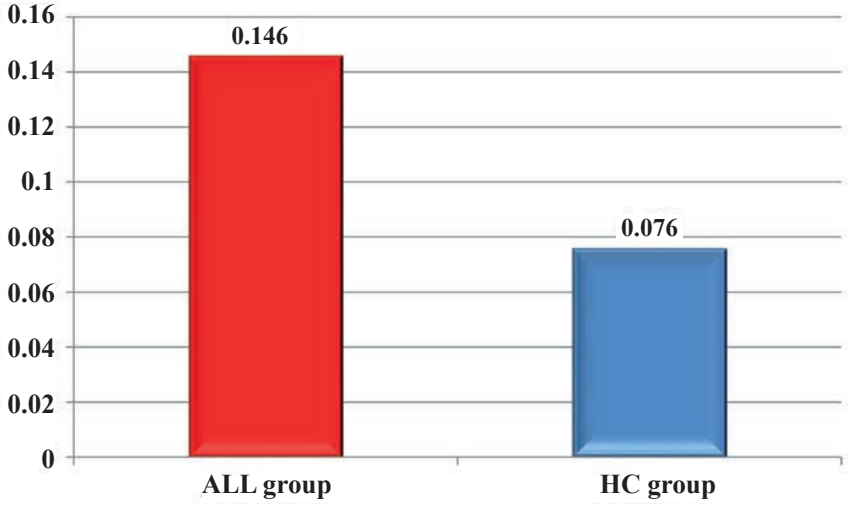


Figure 4. Serum level of IL10 between patient and Control groups

TABLE 5. Serum level of IL10 in patient, control, new diagnosis, relapse, induction and consolidation groups

IL	Control (n=54)				Patient (n=70)				P value
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum	
IL10	0.076	0.10	0.05	0.6	0.146	0.145	0.05	0.7	0.0001
	New (n=25)				Relapse (N=12)				
	0.130	0.160	0.05	0.68	0.216	.218	0.06	0.71	0.0001
	Induction (n=12)				Consolidation (n=21)				
	0.0720	0.018	0.06	0.13	0.165	0.084	0.07	0.37	0.216

* Kruskal Wallis Test

TABLE 6. Serum level of IL10 in risk groups

Risk group	N	IL10			
		Mean	SD	Min	Max
High RG	19	0.1222	0.143	0.0532	0.6781
Stander RG	6	0.1533	0.211	0.0666	0.6321
p-value	0.1655				

*Mann-Whitney U Test, p ≤0.05

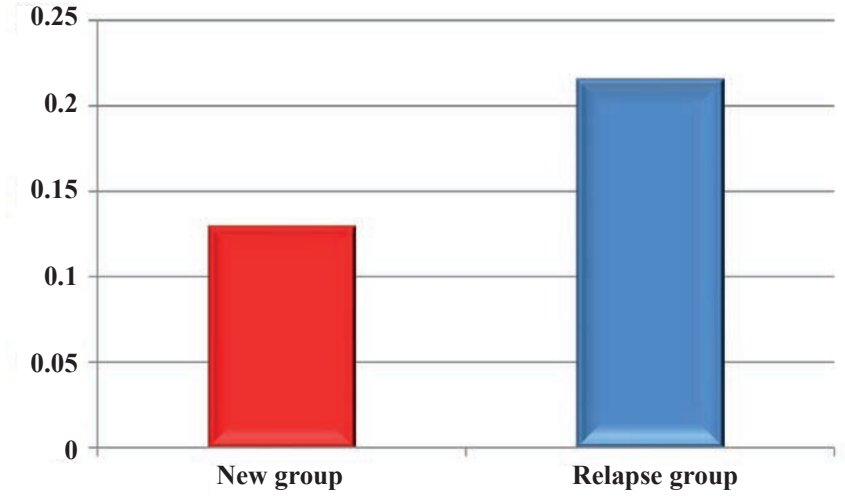


FIGURE 5. Serum level of IL10 between new diagnosis and relapse groups

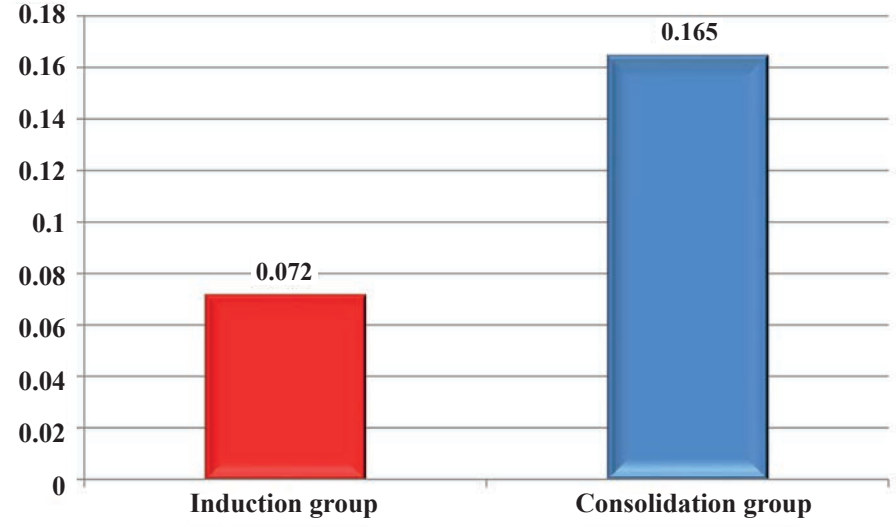
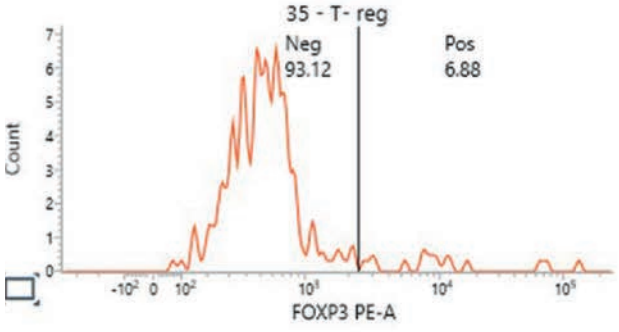
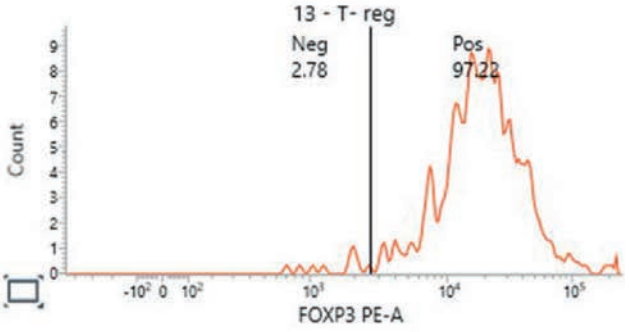


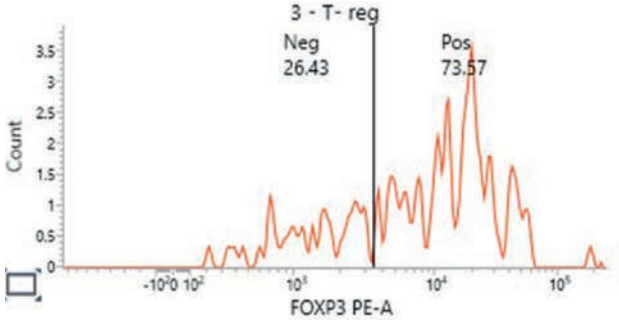
FIGURE 6. Serum level of IL10 between induction and consolidation groups



a. FOXP3 in control group



b. FOXP3 in induction group



c. FOXP3 in consolidation group

FIGURE 7. FOXP3 in different groups of the study

DISCUSSION

Since Tregs express fork head box protein 3 (FOXP3), a transcription factor essential for suppressive action and the production of immune regulatory molecules including the anti-inflammatory cytokine IL-10, FOXP3 is believed to be a distinct hallmark of Tregs [19,20].

According to the current study, the majority of pediatric cases of acute lymphoblastic leukemia fall into the 2-5 and 6-12-year age ranges, and this result was agreed with [21-25].

In this investigation, we discovered a distinct difference in the expression of FOXP3, which was considerably higher in patients with ALL than in the control group.

The mean level was as follows: of FOXP3 in patient (91.156) pg/ml and control (14.880) pg/ml, with P-value ≤0.05 About the current investigation's findings, other researchers corroborated as well [26-29].

Showed that the frequency of FOXP3 -expressing lymphoblast was significantly greater in the HR group than in the SR group. The mean HR (94.42) pg/ml was higher than the stander risk group's mean (42.79) with p-value ≤0.05. This outcome was accepted with [30].

In our investigation, it was discovered that the newly diagnosed patients had a noticeably high level of FOXP3 (91.03) pg/ml and induction stage, the mean (86.35) pg/ml and in consolidation (91.247) P-value <0.05 This finding is concurred with Salem et al. [31] and Idris et al. [32], in our investigation, we hypothesized that FOXP3 is connected to the suppression of cancer cell growth. This could be triggered by the effects of chemotherapy drugs.

The present study was used Enzyme-linked immunosorbent assay (ELISA) test to detection IL10.

As the current study has showed, the patient group had higher IL-10 concentrations than the control group. About the mean serum level, it was as follows: of IL10 in patients (0.146) pg/ml and control (0.076) with P-value <0.05. This outcome was accepted with [33,34].

Significant variations in the serum IL10 levels at the time of diagnosis and control are observed in our investigation. The mean serum level was as follows: of IL10 in the new diagnosis (0.130) pg/ml and control (0.076). This result was agreed with [33-35].

Following these findings, while the opposite effects with decreased IL-10 were seen after 3-month induction (P=0.001), the mean serum level of IL10 in 3-month was (0.072) pg/ml, due to corticosteroid effects, which alter metabolism and produce an increase in food intake [36,37]. The glucocorticoid receptor is a binding site for steroids, which inhibits the synthesis of cytokines [38].

Significant variations in serum IL-10 levels are observed in our study following three months of induction rather than consolidation, the mean serum level of IL10 in induction (0.072) pg/ml and consolidation (0.165) pg/ml (P=0.001). However, the opposite results were observed with decreased IL-10.

Through the investigation of our study, we find that the serum IL10 levels after relapse are significantly different from the initial diagnosis and the mean serum level of IL10 in new diagnosis (0.130) pg/ml and relapse (0.2160) pg/ml with P-value ≤0.05. This outcome was accepted by [39]. Compared to initial ALL, IL10 was higher in ALL patients with disease recurrence.

CONCLUSION

According to our findings, B-ALL patients have significant amounts of both FOXP3 and IL10. This pilot study provides a novel way to look into the process mediating the appearance of these markers in a greater number of B-ALL patients at various stages of their treatment.

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