



## Assessment of Serum Ferritin Levels in Sudanese Patients with Acute Lymphoblastic Leukemia

Mosab N. M Hamad<sup>1\*</sup>, Mogdad Kamal<sup>2</sup>, Mohamed A Saeed<sup>3</sup> and Mazin A Suliman<sup>4</sup>

<sup>1</sup> Department of Medical Laboratory Sciences, Elsheikh Abdallah Elbadri University, River Nile, Sudan

<sup>2</sup> Department of Hematology, Alkhalil Pediatric Hospital, Khartoum, Sudan

<sup>3</sup> Al-Arbaeen Specialist Hospital, Khartoum, Sudan

<sup>4</sup> Department of Hematology, Fedail Hospital, Khartoum, Sudan

\*Corresponding e-mail: [musab.noor13@gmail.com](mailto:musab.noor13@gmail.com)

### ABSTRACT

**Background:** Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. Although there are known increase in serum ferritin with many forms of malignancy, the pattern of elevation with different stages of ALL have not yet been elucidated. **Objectives:** To assess serum ferritin, uric acid, and LDH in Sudanese patients with acute lymphoblastic leukemia. **Materials and Methods:** This is a case-control study conducted in Khartoum state, Sudan during the period from May 2018 to November 2018. A total of 160 subjects were enrolled in this study, 80 patients of ALL as cases; and gender-matched, 80 healthy subjects as controls. All tests result were statistically analyzed using statistical package for social sciences (SPSS) version 20.0. **Results:** This study included 160 subjects, 80 patients with ALL among which 42 subjects (52.5%) were males and the reminder 38 subjects (47.5%) were females. The mean age of the cases group was  $17.6 \pm 5.6$  years old. The control group included 80 healthy subjects which were matched in age ( $17.8 \pm 12.1$ ) and gender (52.5% males, 47.5% females) with the cases group. Among ALL patients, statistically significant positive correlations were observed between number of blast cells serum ferritin level ( $r=0.735$ ,  $p<0.0001$ ), uric acid ( $r=0.618$ ,  $p<0.0001$ ) and LDH ( $r=0.570$ ,  $p<0.0001$ ). **Conclusions:** ALL was associated with elevated serum ferritin levels. The level of serum ferritin was significantly correlated with various stages of the disease and had a positive linear correlation with serum uric acid, LDH, and blast count, therefore determination of serum ferritin could be used as ALL prognostic markers.

**Keywords:** Serum ferritin, Acute lymphoblastic leukemia, Blood, Sudan

### INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most widespread childhood malignancy, happening with an incidence of about 2-4 per 100 children below 15 years of age. In the past 40 years, there has been remarkable development in the outcome of patients with ALL. Prior to the introduction of effective antineoplastic chemotherapy approximately 40 years ago, ALL was uniformly fatal, most children surviving only 2-3 months from diagnosis. Currently, though, approximately 60% of children with this disease are in continuous complete remission 5 years following their initial diagnosis; the majority of these children are considered to be cured [1].

The clinical onset of ALL is most often acute, although a small percentage of cases may evolve insidiously over several months [2]. The presenting symptoms and signs correlate with the leukemic cell burden and the degree of marrow replacement, leading to cytopenia. The most common symptoms include fever (caused by leukemia or a secondary infection secondary to neutropenia), fatigue and lethargy (as a result of anemia), bone and joint pain, and a bleeding diathesis (related to thrombocytopenia). Patients with precursor T-cell ALL/LBL often present with a mediastinal mass with or without associated pleural effusions, which may lead to respiratory distress and other signs of superior vena cava syndrome. Common extramedullary sites of involvement include lymph nodes, liver, spleen, and meninges, whereas less commonly, ALL may infiltrate orbital tissues, testes, tonsils, and adenoids. Rare

patients presenting with B-LBL may show skin lesions of lymphadenopathy in the head and neck area or discrete bone lesions [3]. The most common laboratory abnormalities in ALL include anemia, thrombocytopenia, neutropenia, and leucopenia or leukocytosis, with hyperleukocytosis ( $>100 \times 10^9/L$ ) present in approximately 15% of the pediatric patients. Other common laboratory abnormalities include elevated serum uric acid and lactose dehydrogenase levels, correlating with the tumor burden and degree of tumor lysis [2].

Massive cell death and nuclear breakdown of malignant colon generate large quantities of the nucleic acid of these the purines (adenine and guanine) are converted to uric acid via the purine degradation pathway result in hyperuricemia [4,5].

An increase in LDH is typically seen in tumor lysis syndrome, probably because of anaerobic glucose metabolism. The serum level of LDH is commonly elevated in lymphoproliferative disorders, in patients with non-Hodgkin lymphoma LDH value have a prognostic value and are commonly used to assess treatment response and monitor for tumor recurrence [6].

While iron is an essential micronutrient for DNA synthesis in addition to respiratory and oxidative cell metabolism, its pro-oxidative properties can render it carcinogenic. Free iron can catalyze the formation of mutagenic hydroxyl radicals that, in turn, can cause increased oxidative stress, DNA damage, and oncogene activation. Iron also suppresses host defenses, thereby permitting cancer cell proliferation, and acts as a nutrient for unrestricted tumor cell multiplication. Iron has been carcinogenic in animal models, and in several studies, iron stores were positively associated with risks of certain human cancers, including colorectal and liver. Heme iron is of particular concern given that the body continues to absorb it even if stores are adequate [7].

Patients with a variety of hematologic malignant neoplasms were studied by Patel, et al., [8], to determine the relation between changes in serum ferritin concentration and the clinical status of the patients. Patients with Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, the blastic crisis of chronic myelocytic leukemia, acute myeloblastic leukemia and ALL were found to have significantly elevated serum ferritin levels. The serum ferritin level reflects acute phase reactions and is usually associated with iron storage. Other recent studies have suggested that ferritin is a surrogate for advanced disease and has an impact on relapse because elevated serum ferritin predicts overall survival and relapse-free survival following autologous stem cell transplantation for lymphomas [9,10].

In untreated patients with ALL the mean serum ferritin concentration was about 15 times higher than normal for that age group. During chemotherapy circulating ferritin levels were higher than during the pretreatment period. There was no correlation between ferritin concentration and length of remission in patients still on chemotherapy. The increase in circulating ferritin during chemotherapy could be due to an increased release from damaged leukemic cells. Possibly also chemotherapy damages other ferritin containing cells but the lack of correlation with serum transaminase activity makes it unlikely that liver parenchymal cells are an important source. Similarly, the lack of correlation between raised serum ferritin concentration and the amount of blood transfused, in contrast to aplastic anemia, makes it unlikely that this originates from increased stores. The low serum ferritin concentrations found in long-term survivors of ALL suggest that ferritin concentration may be a useful index for the prediction of relapse and as a prognostic sign [11].

Acute lymphoblastic leukemia is the most common malignancy among children. The treatment includes the induction of chemotherapy for a long period of time. And there were increased demands for prognosis during the treatment to guarantee the complete elimination of the malignant clone and to avoid an excess of chemotherapy. It is known that the cytogenetic means were so expensive so we analyze the serum ferritin as a prognostic factor and its association with severity of malignancy by its correlation to the percent of blast and serum level of uric acid and lactate dehydrogenase.

To the best of our knowledge, there are few studies in this type of malignancy; the aim of the current study is to provide more evidence about the association between serum ferritin and ALL.

#### **MATERIALS AND METHODS**

This is a case-control study, conducted in Khartoum state, Sudan during the period from May 2018 to November 2018. A total of 160 subjects were enrolled in this study, 80 patients were diagnosed and confirmed to have ALL according to WHO criteria admitted to Fedail Specialist Hospital, Alkhalil Pediatric Hospital, or Radiation and Isotopes Center, Khartoum, and 80 patients were age and gender-matched apparently healthy individuals as controls collected from Fedail Specialist Hospital, Alkhalil Pediatric Hospital, and Al-Arbaeen Specialist Hospital, sample size was calculated according to sample size basic formula. Subjects who were on iron supplementation or in drugs

affecting iron metabolism were excluded from this study. Verbal consent from all subjects or their guardians were taken before enrolment in the study.

Venous blood sample (5 ml) was collected from each participant with aseptic precautions from the antecubital vein. Of which, 2.5 ml was dispensed in EDTA tube and used immediately for blood counting, preparation of blood smear and estimation of serum ferritin. The other 2.5 ml was placed into a heparinized tube and used for the estimation of serum uric acid and LDH.

Blood mixed with EDTA was used for full blood count by automated hematology analyzer (Sysmex XT-2000I-Japan) within 1 hour of collection to minimize variations due to sample aging. For examination of PBP 2 thin blood films stained: one with rapid Diff Quick stain from RAL and the other with MGG, also another smear was made and stained with SBB. The remaining of blood in EDTA was centrifuged at 3200 rpm for 3 minutes to obtain plasma, plasma obtained was then used for estimation of serum ferritin by Immunoassay based on Electrochemiluminescence method using Cobas e411 automated clinical chemistry analyzer (Roche-Germany).

The heparinized blood was centrifuged at 3200 rpm for 3 minutes and serum was collected in 1.5 ml Eppendorf tubes and stored at (2-8°C) for a maximum of 7 days. Serum uric acid and LDH were then measured by Cobas c311 automated clinical chemistry analyzer (Roche-Germany).

Statistical analysis was performed using statistical package for social sciences (SPSS) version 20.0. Descriptive statistics were used to summarize study population characteristics. Mean and the standard deviation was calculated for numerical variables (age, blood count parameters, blast count, uric acid concentration, serum levels of ferritin and LDH). Independent sample T-test was applied to compare serum ferritin levels between cases and controls. Pearson's correlation was applied to test correlations between serum ferritin and the other variables (uric acid, LDH, and blast count). Analysis of variance was applied to compare serum ferritin level between patients group according to the stage of ALL by one-way ANOVA test. For all tests  $p < 0.05$  were considered statistically significant.

## RESULTS

This study included 160 subjects, 80 patients with ALL among which 42 subjects (52.5%) was male and 38 subjects (47.5%) was female with mean age  $17.6 \pm 5.6$  years old, and other 80 healthy subject statistically matched in age ( $17.8 \pm 12.1$ ) and gender (52.5% male, 47.5% female) as control group.

Hematological parameters in case group show a significant decrease in hemoglobin, red cells count and red cells indices, and thrombocytopenia when compared with the control group. While the total white cells counts were dramatically increased with predominant of blast cells indicate the acute phase of the disease (Table 1).

**Table 1 Mean value of blood count parameters**

Variables	RBCs ( $\times 10^{12}/l$ )	Hb (g/dl)	PCV (%)	PLTs ( $\times 10^9/l$ )	WBCS ( $\times 10^9/l$ )	Blast count ( $\times 10^9/l$ )	Blast (%)
Case Group	2.9	8.2	25.8	54.4	157.1	151.4	87.6%
Control Group	4.9	13.4	40.0	268.2	7.0	0.0	0.0%

Mean value of serum uric acid, LDH and ferritin levels was elevated in case of the group when compared with the control group (Table 2), and correlation of blast to ferritin, uric acid, and LDH show positive linear correlation using Pearson correlation coefficient and  $p < 0.05$  (Table 3).

**Table 2 Mean value of uric acid, LDH and serum ferritin**

Variables	Uric Acid (mg/dl)	LDH (U/L)	Ferritin (mg/l)
Case Group	7.4	699.0	510
Control Group	4.5	94.2	245

**Table 3 Correlation of blast cells count with serum uric acid, LDH and ferritin levels**

Variables	Mean value	Blast cells count ( $\times 10^9/l$ )	Pearson correlation (r)	p-value
Uric Acid (mg/dl)	7.4	151.4	0.604	<0.0001
LDH (U/L)	699.0	151.4	0.783	<0.0001
Ferritin ( $\mu g/l$ )	510.0	151.4	0.735	<0.0001

Correlation of serum ferritin levels between cases and controls show a significant difference when statistically analyzed using independent sample T-test ( $p < 0.05$ ) (Table 4).

**Table 4 Correlation of serum ferritin levels between cases and controls**

Variables	Case Group	Control Group	p-value
Serum Ferritin ( $\mu\text{g/l}$ )	510	245	<0.05

Serum levels of ferritin show positive correlation with serum levels of uric acid ( $r = 0.618$ ,  $p < 0.0001$ ) and LDH ( $r = 0.570$ ,  $p < 0.0001$ ) (Table 5).

**Table 5 Correlation of serum levels of ferritin with serum levels of uric acid and LDH**

Variables	Mean value	Serum Ferritin ( $\mu\text{g/l}$ )	Pearson correlation (r)	p-value
Uric Acid (mg/dl)	7.4	510	0.618	<0.0001
LDH (U/L)	818.0	510	0.570	<0.0001

Out of 80 patients with ALL, there were 52 patients (65%) in early stage, they are newly diagnosed with acute lymphoblastic leukemia and have increased TWBCs count and blast cells than others stage and have the highest level of serum uric acid, LDH, and ferritin. And there were 21 patients (26.3%) in the recurrent stage show a moderate increase in TWBCs count, blast cells, uric acid, LDH, and serum ferritin. The remaining 7 patients (8.8%) were in remission stage, clinical status and hematological parameters show normal or near-normal value (Table 6).

**Table 6 Frequency and the mean value for (TWBCs, blast, uric acid, LDH, and ferritin) within the different stage of the disease**

Stage	Frequency (%)	TWBCs ( $\times 10^9/\text{l}$ )	Blast ( $\times 10^9/\text{l}$ )	Uric acid (mg/dl)	LDH (U/L)	Ferritin ( $\mu\text{g/l}$ )
Early	52 (65.0%)	432.7	216.4	10.3	1280.0	834.6
Recurrent	21 (26.2%)	48.4	39.2	7.6	624.0	497.8
Remission	7 (8.8%)	11.6	5.2	4.4	194.1	200.0

Serum ferritin levels was significantly increased in patients at early stage (mean=834.6  $\mu\text{g/l}$ ) when compared with recurrent (mean=497.8  $\mu\text{g/l}$ ) and remission (mean=200  $\mu\text{g/l}$ ), using one-way ANOVA test ( $p < 0.05$ ) (Table 7).

**Table 7 Difference in serum ferritin levels within three ALL groups**

Variables	Early	Recurrent	Remission	p-value
Serum Ferritin ( $\mu\text{g/l}$ )	834.6	497.8	200	<0.05

## DISCUSSION

Acute lymphoblastic leukemia is the cancer of childhood and it accounts for more than 50% of leukemia in this age group. In recent years there is an increased focus on iron status and iron chelator in leukemic malignancies such as the cytotoxic effect of anti-proliferating agents and induced cells apoptosis suppose that iron deprivation could control the proliferation of various tumor cells and induce apoptosis [12-14].

In this study, there is a significant increase in serum ferritin levels among patients with acute lymphoblastic leukemia when compared with normal subjects and this agrees with the previous study done by Jain, et al., they evaluate 260 patients with ALL, the med ferritin was 1081 with a significantly higher prevalence of LDH [15]. And other study done by Zhang, et al., in which they reported several cases with different type of malignancies had increased serum ferritin level due to increased transferrin receptors on malignant clone of leukemic cells, also the increase of cells destruction rate make it exposed its carriage of ferritin and increase the serum levels [12], this findings were also in agreement with the study done by Aulbert and Schmidt [13]. Even so, there is disagreement with a study conducted by Chua, et al., who reported that serum ferritin was not associated with cancer risk or cancer death, these differences may be due to differences in race, sample size and methods used for measuring serum ferritin.

Also our study show variation in serum ferritin levels with various stage of disease with peak incidence of elevation within patients at early stage and normal levels within patients at clinical remission also had a direct positive correlation with serum uric acid, LDH, WBCs count and blast cells, the study was done by Jain, et al., and Zhang, et al., also agree with our results [12,15], although there are disagreements with the study done by Ahlawat, et al., which found that there was no correlation between the serum ferritin concentration and the total white cell count and the peripheral

blood blast count and this disagreement may be due to differences in race, sample size and method used for measuring serum ferritin [14].

### CONCLUSION AND RECOMMENDATIONS

Serum ferritin levels in patients with acute lymphoblastic leukemia at an early stage and recurrent stage are significantly increased, while its fall to the normal value in patients at clinical remission. So measurement and surveillance of changes in serum ferritin levels could be helpful for easy and simple assessing and prognosis of the disease in these patients.

### DECLARATIONS

#### Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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