

ASSOCIATION BETWEEN SERUM FERRITIN LEVEL AND LIVER FUNCTION TESTS IN CHILDREN WITH BETA-THALASSEMIA IN THALASSEMIA CENTER OF KUT HOSPITAL**Hawraa Fadhil Jabbar**

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Abstract: Background:Thalassemia represents a heterogeneous autosomal recessive hereditary anemia.Ferritin is a 450 KDa soluble protein. It can be found in all of the body's cells, but it's particularly abundant in marrow macrophages, spleen, and liver.

Objective:In patients with β thalassemia major and β thalassemia Intermedia, serum ferritin levels are measured, and their correlation with bilirubin levels, liver enzymes AST (aspartate transaminase) and ALT (alanine transaminase), in beta-thalassemia major and β thalassemia Intermedia.

Patients and Methods:A cross-section study that involved 90 patientsincluded 50 Thalassemia Major (TM) and 40 Thalassemia intermedia(TI). Patients who were randomly chosen for this study would be gathered from the Kut Hospital Thalassemia Center. Every patient's are kid, aged 5 to 17, regardless of gender. Abbott C4000 Architect Additionally, the TSB, ALT, AST, and serum ferritin were determined using the Cobas c 111 analyzer.

Results:The studies include both genders 52.9% of the females had B-thalassemia major and 47.1% had β -thalassemia intermedia, whereas 57.1% of the men had β TM and 42.9% had β TI. There were 37.8% of females and 62.2% of males in the sample.Patients with severe thalassemia exhibited a statistically significant increase in the mean \pm SD of serum ferritin (4016.54 ± 2500.81 ng/ml) compared to the mean \pm SD of β -thalassemia intermedia (1629.48 ± 1235.33 ng/ml), with a p-value of 0.000. Patients with major β -thalassemia had a mean TSB level that was statistically significantly lower (1.32 ± 0.69 mg/dl) than the mean level of β -thalassemia intermedia (2.10 ± 0.86 mg/dl), with a p-value of 0.000. In the B-thalassemia major group, serum hepcidin did not exhibit a statistically significant association with serum ferritin ($r=-0.04$), while in the B-thalassemia intermedia group, serum ferritin exhibited a statistically significant moderate negative correlation with TSB ($r=-0.38$),

p-value. 0.01. Conclusions: Ferritin levels may be useful in thalassemia syndrome diagnosis and prognosis determination

Keywords: Ferritin; Thalassemia Major; Thalassemia Intermedia, Alanine Transaminase, Aspartate Transaminase, Total Serum Bilirubin



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Introduction

The heterogeneous autosomal recessive hereditary anemia known as thalassemia is typified by either decreased or missing α and/or β -globin chain production [1]. It was first characterized in 1927 as a type of severe anemia accompanied by splenomegaly and deformities of the bones [2]. Thalassemia is split into two types: alpha thalassemia and beta thalassemia, based on the decreased or missing synthesis of the α -globin chain or the β -globin chain of hemoglobin, respectively [3]. It is classified into subtypes depending on the location of the defect. The two pairs of genes encode the α -chains to chromosome 16, while the pair of genes encoded the β -chains to chromosome 11. For this reason, thalassemia is a more common β type for the lack of dominant genes [4], [5]. There are 3 main types of β thalassemia, While the homozygous state is referred to as thalassemia major, the heterozygous state is known as thalassemia minor or trait, and thalassemia intermedia. Numerous factors, such as race and how these kinds of thalassemia interact with other hereditary erythrocytic illnesses, affect how severe these types of thalassemia are [6]. The World Health Organization (WHO) stated in 2018 that 2.7 out of every 1,000 pregnancies were impacted, 1.1% of couples globally were at risk of producing children with a hemoglobin disease, and at least 5.2% of people were thalassemia carriers. Whereas children in impoverished nations pass away before turning five, the majority of thalassemia-affected newborns in high-income countries live with a chronic illness [7].

With a high frequency of 37.1/100,000 people, thalassemia is the most prevalent genetic hemoglobinopathy in Iraq. The two most prevalent types of this illness among Iraqi patients were β -thalassemia major (TM) and β -thalassemia intermedia (TI) [1]. Several elements contribute to the evolving β -thalassemia epidemiology. These variables include increased survival rates, the introduction of β -thalassemia preventive measures, and migration [8].

Ferritin is a 450 kDa soluble protein. It can be found in all of the body's cells, but it's particularly abundant in marrow macrophages, spleen, and liver. It stores bioavailable iron in a healthy and easily accessible form inside the cell. It protects cells from toxicity caused by iron-mediated free radical formation, such as the Fenton reaction between iron and hydrogen peroxide [9].

The two subunit kinds that make up intracellular ferritin are H (heavy chain) and L (light chain). Twenty-four subunits unite to produce a shell-like molecule with a cavity that can hold up to 4,500 iron atoms [10].

Serum ferritin, which represents the ferritin content of macrophages, is a sign of iron overload/inflammation when its level is elevated and iron insufficiency when it is low in the clinical context. Serum ferritin's origin and function, however, are still mostly unknown. One theory is that when iron release from macrophages is restricted in inflammation, for example, the produced ferritin may be reabsorbed by cells as an alternate mode of iron recycling [11].

The enzymes alanine transaminase (ALT) and aspartate transaminase (AST) are formed primarily in the liver and are typically tested in liver function tests. However these enzymes are essential for human survival and are involved in the metabolism of amino acids, high levels of these enzymes can be a sign of inflammation or damage to the liver or, less frequently, other organs involved in its development, such as the heart, kidney, brain, or muscles [12].

This study's goal was to estimate serum ferritin levels in patients with β thalassemia major and β thalassemia Intermedia, as well as to determine how these levels correlated with bilirubin levels and the liver enzymes ALT (alanine transaminase) and AST (aspartate transaminase) in patients with beta-thalassemia major and β thalassemia Intermedia.

Methods

There will be 90 individuals in cross-sectional research, 50 with Thalassemia major (TM) and 40 with Thalassemia intermediate (TI). The study will involve the random selection of patients from the Thalassemia Center at Al-Kut Hospital. From November 2023 to January 2024, this study was carried out at Kut City. Iraqi youngsters aged 5-17 years, both male and female, met the criteria for β thalassemia.

Blood Samples:

A volume of around 3 milliliters was extracted from the veins of individuals suffering from beta-thalassemia for this research. For samples to clot in a plain tube, they are kept at room temperature for thirty minutes. spun at 3000 rpm for ten minutes to separate. The Cobas c111 analyzer was used to measure the ALT, AST, and TSB assays, and the Abbott Architect c4000 analyzer was used to assess the serum ferritin assay.

Result and Discussion

This cross-sectional research comprised 40 individuals with β TI and 50 patients with β TM. In this study, 62.2% of the participants were men and 37.8% were women. Serum ferritin, ALT, AST, and TSB levels were measured for each patient.

In Table; The mean level of serum ferritin was significantly greater in β -thalassemia major patients (4016.54 ± 2500.81 ng/ml) compared to the intermedia mean level (1629.48 ± 1235.33 ng/ml), with a p-value of 0.000. Compared to β -thalassemia intermedia mean levels (28.70 ± 12.46 , 22.43 ± 13.64 U/L), β -thalassemia major patients showed statistically higher mean levels of ALT and AST (38.50 ± 22.87 , 43.04 ± 33.11 U/L), with p-values of 0.017 and 0.000. Major patients with β -thalassemia had a significantly lower mean TSB level (1.32 ± 0.69 mg/dl) compared to the intermedia mean level (2.10 ± 0.86 mg/dl), with a p-value of 0.000, as presented in table 1.

Table 1: mean difference in mean level of serum ferritin, ALT, AST and TSB

Variables	Diagnosis	Mean \pm SD	P-value
serum ferritin (ng/ml)	β -thalassemia major	4016.54 ± 2500.81	0.000**
	β -thalassemia Intermedia	1629.48 ± 1235.33	
ALT (U/L)	β -thalassemia major	38.50 ± 22.87	0.017*
	β -thalassemia Intermedia	28.70 ± 12.46	
AST (U/L)	β -thalassemia major	43.04 ± 33.11	0.000**
	β -thalassemia Intermedia	22.43 ± 13.64	
TSB (mg/dl)	β -thalassemia major	1.32 ± 0.69	0.000**
	β -thalassemia Intermedia	2.10 ± 0.86	

t-test: * p-value ≤ 0.05 Significant ** p-value ≤ 0.01 highly Significant

In Table 2 serum ferritin correlation with ALT, AST and TSB was evaluated using person correlation coefficient r. Serum ferritin level found weak insignificant positive correlations with ALT ($r = 0.207$) and AST ($r = 0.240$) in B-thalassemia major group, p-value 0.150 and p-value 0.09, Serum ferritin level found weak insignificant positive correlations with ALT ($r = 0.174$) and AST ($r = 0.12$) in B-thalassemia intermedia group, p-value 0.28 and p-value 0.42. Serum ferritin had a statistical insignificant weak positive correlation with TSB ($r = 0.074$) in B-thalassemia major group, p-value 0.6, Serum ferritin had a statistical significant moderate negative correlation with TSB ($r = -0.38$) in B-thalassemia intermedia group, p-value 0.01, as presented in table 2

Table 2: Correlation Between Serum Ferritin and Variables (ALT, AST, and TSB) in Patients With B-Thalassemia Major and B- Thalassemia Intermedia.

Group	ALT (U/L)		AST (U/L)		TSB (mg/dl)	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
BTM patients	0.207	0.15	0.24	0.09	0.074	0.6
BTI patients	0.174	0.28	0.12	0.42	-0.38*	0.01

In Table3, To test the validity of ferritin, the ROC test was used, when diagnosed with B-thalassemia major, it has an AUC 0.99, and Cut off value 122.5, Sensitivity 97.9% and Specificity 92.3%. When diagnosed with B-thalassemia intermedia, it has AUC 0.99 and Cut off value 132, Sensitivity 95.7%, and Specificity 94.9%

Table 3: The area under operator characteristic (ROC) test for serum ferritin in BTM and BTI

Diagnosis	AUC	Cut off value	Sensitivity	Specificity	p-value
B-thalassemia major	0.99	122.5	97.9%	92.3%	0.000
B-thalassemia Intermedia	0.99	132	95.7%	94.9%	0.00

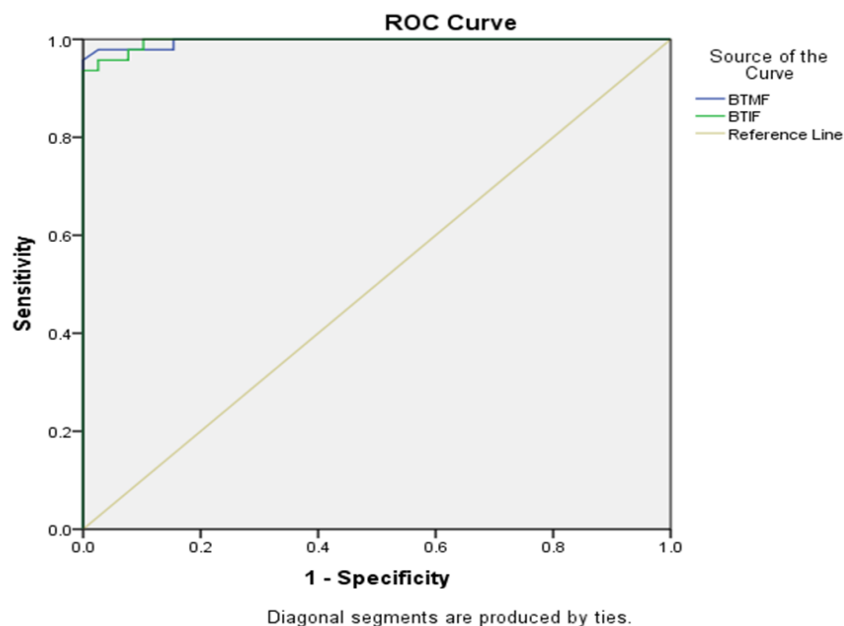


Figure 1: ROC test of B-thalassemia major group and of B-thalassemia Intermedia group.

Discussion

An iron overload diagnosis can be made based on the ferritin level in plasma. According to the current investigation, both the TM and TI groups had abnormally high blood ferritin levels. (mean \pm SD level 4016.54 ± 2500.81 ng/ml and 1629.48 ± 1235.33 ng/ml, respectively). This study indicates that iron overload is a significant issue for individuals with TM and TI. Transfusions of blood might result in iron overload.[13] Ineffective erythropoiesis and extramedullary erythropoiesis [14], According to table (2), the current study revealed that patients with β -thalassemia major had a statistically significant higher mean levels

of serum ferritin (4016.54 ± 2500.81 ng/ml) than those with β -thalassemia intermedia (1629.48 ± 1235.33 ng/ml), with a p-value of 0.000. This difference was caused by more iron overload in the TM patients as opposed to the TI patients. Ferritin levels were much greater in the TM participants, as the current investigation found. The present investigations contradict with [15].

By assisting in the systemic management of iron and the storage of extra iron in the event of an iron overload, the liver is the primary organ responsible for maintaining iron homeostasis. Because the liver is the main organ for iron storage when liver iron concentration increases especially with chronic iron overload led to a decline in liver function and elevation of transaminases enzymes ALT and AST. Excess iron is largely stored in the liver hepatocytes [16],[17] as shown in table (1). Due to iron overload in thalassaemic patients receiving multiple transfusions, β -thalassemia major patients had statistically higher mean levels of ALT and AST (38.50 ± 22.87 , 43.04 ± 33.11 U/L) compared to β -thalassemia intermedia mean levels (28.70 ± 12.46 , 22.43 ± 13.64 U/L), with p-values of 0.000 and 0.017. The current study agreement with [18]

Patients with major β -thalassemia had a mean TSB level that was statistically significantly lower (1.32 ± 0.69 mg/dl) than the mean level of β -thalassemia intermedia (2.10 ± 0.86 mg/dl), with a p-value of 0.000. because Patients with thalassaemia experience elevated blood levels of bilirubin due to heightened erythrocyte lysis. This is the primary cause of hyperbilirubinemia, which has the adverse impact of causing damage to additional hepatic cells as a result of iron excess. The current study agreement with [19].

Serum ferritin had a statistically insignificant weak positive correlation with TSB ($r=0.074$) in B-thalassaemia major group, p-value 0.608, Jaundice and iron overload are common signs of thalassaemia. Serum ferritin and serum bilirubin, a measure of iron overload and jaundice, are associated in the current investigation. Yet, there was no statistically significant difference between these two parameters [20].

This data showed that serum ferritin level found weak insignificant positive correlations with ALT ($r=0.207$) and AST ($r=0.240$) in B-thalassaemia major group, p-value 0.150 and p-value 0.093 This agrees with the results [18] And disagreement with [21] demonstrated that liver dysfunction begins when serum ferritin levels rise and surpass 3000 ng/ml. Another study [22] demonstrated that liver dysfunction begins when serum ferritin levels rise beyond 1000 ng/ml and the number of blood transfusions exceeds 30. and the data showed that serum ferritin level found weak insignificant positive correlations with ALT ($r=0.174$) and AST ($r=0.42$) in B-thalassaemia intermedia group, p-value 0.28 and p-value 0.093).

ROC curve, specificity, and sensitivity for serum ferritin in iron overload prediction. Serum ferritin had a 97.9% sensitivity and a 92.3% specificity at the cut-off value of 122.5 ng/ml in the diagnosis of BTM. The area under the curve (AUC) for ferritin was 0.99 on the receiver operating characteristic (ROC) curve, indicating that the serum level of ferritin offered an accurate test for diagnosing the illness in BTM. A great test is shown by an area of 90–100 ($p < 0.000$; Figure 1).

Serum ferritin had a 95.7% sensitivity and 94.9% specificity at the 132 ng/ml threshold range for diagnosing BTI. as seen in table (3) The area under the curve (AUC) for ferritin was 0.99 on the receiver operating characteristic (ROC) curve, indicating that the serum level of ferritin offered an accurate diagnostic for diagnosing the illness in BTI..

Conclusion

Thalassaemia syndrome diagnosis and prognosis may be made with the use of ferritin levels

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