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Role of serum hepcidin as a biochemical marker in children with Beta-thalassemia of iron status in Wasit Province Iraq

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Abstract— β -thalassemia is characterized by anemia and depending on the severity of the anemia. Hepcidin is a 25-amino-acid peptide generated by the liver, based on hypoxia, anemia, and iron storage. controls blood iron concentration and tissue distribution and regulates the body's iron hemostasis. To evaluate the usefulness of Serum Hepcidin level as a biochemical marker of iron status in children with β thalassemia major, β thalassemia Intermedia and its relation with serum ferritin level. A cross-section study which will involve 90 included 50 Thalassemia Major (TM) and 40 Thalassemia Intermedia (TI). Patients will be collected from the thalassemia center in Kut Hospital for Gynecology and Pediatrics, were randomly selected for this study. All patients child in both gender from (5-17 years). Enzyme-linked immunosorbent assay [ELISA] used for determination of Hepcidin, Abbott Architect C4000 And cobas c 111 analyzer used for the determination of Ferritin, Iron and Hemoglobin was measured by Sysmex XP-300. The study included both gender 62.2% were male and 37.8% were females, regarding male gender 57.1% had β TM and 42.9% had β TI while female gender 52.9% had B-thalassemia major and 47.1% had β -thalassemia Intermedia. thalassemia major patients had statistically significant higher mean $\pm$ SD level of Hb, serum ferritin and iron (8.77 ± 0.48 g/dl, 4016.54 ± 2500.81 ng/ml, 168.20 ± 28.91 mcg/dl) in compare to β -thalassemia intermedia mean $\pm$ SD level (8.23 ± 0.62 g/dl, 1629.48 ± 1235.33 ng/ml, 133.85 ± 33.92 mcg/dl), p-value 0.000, 0.000 and 0.000. β -thalassemia major patients had a statistical higher mean $\pm$ SD hepcidin (7.71 ± 2.74) in compare to β -thalassemia intermedia (6.53 ± 1.8), p-value 0.02. Serum hepcidin had no a statistical significant correlation with Serum Ferritin ($r = -$

0.04) in B-thalassemia major group, p-value 0.754; Also, serum hepcidin had no a statistical significant correlation with Serum Ferritin ($r= 0.08$) in B-thalassemia intermedia group, p-value 0.58. Hepcidin levels may be helpful in determining the diagnosis and prognosis of thalassemia syndromes.

Keywords---hepcidin, ferritin, thalassemia intermedia, thalassemia major.

Introduction

Thalassemia is an inherited disorder in which one or more globin chains are not synthesized properly, resulting in deficient hemoglobin (Hb) production and premature red blood cell destruction [1]. β -thalassemia is one of the most common types of thalassemia in which the production of hemoglobin B-chain is quantitatively impaired. β -thalassemia is characterized by anemia and based on anemia's severity [2]. There are 3 main types of β thalassemia, Thalassemia minor, thalassemia intermediate and thalassemia major[3]. A multitude of factors, including race and interaction with other inherited erythrocytic disorders, determine the severity of these kinds of thalassemia [4]. The clinical signs of thalassemia intermedia are medium between thalassemia minor and major. It included splenomegaly due to the large damaged amount of blood cells [5]. The thalassemia intermedia is associated with bone abnormalities patient and poor growth patient [6]. The infected thalassemia Patients need a periodical cure because of it very dangerous and have many wide clinical signs[7].

Thalassemia major, also known as Cooley's anemia and Mediterranean anemia, is the most severe form of B-thalassemia [8]. Thalassemia major (TM) patients are transfusion dependent, requiring more than eight red blood cell (RBC) transfusions per year, whereas Thalassemia intermedia (TI) patients are not transfusion reliant and require no or infrequent transfusions. Although blood transfusion is the most common treatment for these individuals, it has a number of side effects, including iron overload, which, if left untreated, can cause tissue and organ damage as well as increase death. [9]. Blood transfusion is an important factor in thalassemia patients' survival. Transfusions, on the other hand, can cause serious problems, increasing the morbidity and mortality of thalassemia patients [10].

Ferritin is a 450 kDa soluble protein. It's a protein that stores iron found in every cell of the organism. A small amount of iron can be present in serum and plasma, which represents iron reserves in healthy people. A low serum ferritin concentration is generally considered to be an indication of iron deficiency. Despite the fact that ferritin is increased in iron overload states and inflammation, interpreting normal or elevated serum ferritin values in the presence of acute or chronic inflammatory processes is difficult [11]. The level of plasma ferritin reflects the body's iron stores. The measurement of serum ferritin is used as a standard approach to assess iron overload and other thalassemia complications such as growth impairment[12]. Hepcidin is a 25-amino-acid peptide generated by the liver [13], based on hypoxia, anemia, and iron storage.

controls blood iron concentration and tissue distribution and regulates the body's iron hemostasis. [14].

Hepcidin is synthesized as prohepcidin and transformed to its bioactive form by hepatocytes before being released into the bloodstream and eliminated by the kidney. Hepcidin is also produced by other tissues and organs, such as adipose tissue, macrophages, monocytes, and the kidney, but at a lesser level than the liver, and the function of hepcidin in these cell types is unknown [15]. Elevated blood iron levels increase hepcidin production, which is decreased by erythropoietic activity and increased by inflammation. Furthermore, anemia and hypoxia can significantly reduce hepcidin synthesis. Increased hepcidin levels reduce iron absorption, while lower levels enhance iron release from enterocytes and macrophages [16]. Hepcidin levels in β -thalassemia are variable. Hepcidin deficiency will occur if anemia and strong erythropoietic activity predominate, whereas hepcidin expression will be raised if iron overload is present [16].

Hepcidin synthesis is stimulated by increased plasma and stored iron, which prevents dietary iron absorption and thus iron loading. In contrast, in iron deficiency, hepcidin is inhibited. Chronically transfused patients' hepcidin concentration is significantly greater than non-transfused patients', likely due to iron overload [17]. Both Non transfusion dependent (NTDT) and Transfusion dependent (TDT) patients have an excess of iron and require long-term iron chelation therapy to avoid serious consequences like liver and heart failure [18], [19]. The objective of this study is to evaluate the usefulness of Serum Hepcidin level as a biochemical marker of iron status in children with β thalassemia major, β thalassemia Intermedia and its relation with serum ferritin level.

Methodology

A cross-section study which will involve 90 patients included 50 Thalassemia major (TM) and 40 Thalassemia Intermedia (TI). Patients will be collected from the thalassemia centre in Al- Kut Hospital for Gynecology and Paediatrics, were randomly selected for this study. This study was conducted in Kut city during the period of November 2020 to January 2021. All patients child in both gender from (5-17 years) criteria β thalassemia, Iraq. The diagnosis of β -thalassemia was based on conventional clinical and hematological criteria.

Blood Samples

In this study, approximately 5 ml of blood was drawn from the veins of β thalassemia patients. Each blood samples divided into two parts:

- The first section 2 ml of blood samples added into EDTA tube for Hb measurements which was quickly carried out. The Hemoglobincyanide (HiCN) Method was used to measure hemoglobin using the Sysmex XP-300 Automated Hematology Analyzer. It is based on the addition of ferricyanide and potassium cyanide to convert hemoglobin to cyanmethemoglobin.
- The second section 3 mL of blood were left at room temperature for 30 minutes to allow samples to clot in a plain tube. Sera were separated after

coagulation by centrifugation at 3000 rpm for 10 minutes. Sera were aspirated and separated into two aliquots in Eppendorf tubes for : -

- Aliquot 1: The ferritin assay was measured using an automated method using an Abbott Architect c4000 analyzer.
- Aliquot 2: The remaining samples were kept at (-20 to -80 C) until they were tested for Hepcidin measu.by using enzyme-linked immunosorbent assay (ELISA).

The study has an approval from the ethical committee at the College of medicine, Baghdad University. An informed consent was attained from each participant.

Results

The current study is cross sectional study included 50 patient of β TM and 40 patients of β TI. The study included both gender 62.2% were male and 37.8% were females, regarding male gender 57.1% had β TM and 42.9% had β TI while female gender 52.9% had β TM and 47.1% had β TI, no statistical significant association between gender and development of specific type of β -thalassemia, p-value 0.69. Two chelating agents were used in which 7.8% of all patients use Desferal, 86.7% use X Jade and 5.5% without any chelation agent who all of them had β TM. Patients who were used Desferal, 71.4% had β TM and 28.6% had β TI while those who use X Jade, 57.7% had β -thalassemia major and 42.3% had β TI, a statistical significant association between need to use chelating agent and β TM, p-value 0.02.

The frequency of blood transfusion is changeable, 4.4% need blood transfusion every week, 70% needed every 2-3 weeks, 17.8% every 4-8 weeks and 7.8% needed in duration > 9 weeks. Those who needed transfusion in less than a week 50% of them had β -thalassemia major and 50% had β -thalassemia intermedia, 74.6% of those who need transfusion every 2-3 weeks are β -thalassemia major and 25.4% are β -thalassemia intermedia, all those who needed transfusion every 4-8 weeks are β -thalassemia intermedia, and 85.6% of those who needed in duration > 9 weeks are also β -thalassemia intermedia, a statistical significant association between β -thalassemia major and increase frequency of blood transfusion, p-value 0.000, as presented in table 1.

Table 1
Association between type of thalassemia with gender, use of chelating agent and frequency of blood transfusion

		β -thalassemia major (No.50)	β -thalassemia Intermedia (No.40)	Total	p-value
Gender	Male	32 (57.1%)	24 (42.9%)	56 (62.2%)	0.69
	Female	18 (52.9%)	16 (47.1%)	34 (37.8%)	
type of chelating agent	Desferal	5 (71.4%)	2 (28.6%)	7 (7.8%)	0.02*
	X Jade	45 (57.7%)	33 (42.3%)	78 (86.7%)	
	no chelating therapy	0 (0%)	5 (100%)	5 (5.5%)	

frequency of blood transfusion	< 1 week	2 (50%)	2 (50%)	4 (4.4%)	0.000**
	2-3 weeks	47 (74.6%)	16 (25.4%)	63 (70%)	
	4-8 weeks	0 (0%)	16 (100%)	16 (17.8%)	
	>9 weeks	1 (14.3%)	6 (85.7%)	7 (7.8%)	
Total		50	40	90	

Anova:

* p-value $\leq$ 0.05 Significant** p-value $\leq$ 0.01 highly

Significant

In table 2, all patients were evaluated by measuring Hb, serum hepcidin, serum ferritin and iron. β -thalassemia major patients had statistically significant higher mean $\pm$ SD level of Hb, serum ferritin and iron (8.77 ± 0.48 g/dl, 4016.54 ± 2500.81 ng/ml, 168.20 ± 28.91 mcg/dl) in compare to β -thalassemia intermedia mean $\pm$ SD level (8.23 ± 0.62 g/dl, 1629.48 ± 1235.33 ng/ml, 133.85 ± 33.92 mcg/dl), p-value 0.000, 0.000 and 0.000. β -thalassemia major patients had a statistical higher mean $\pm$ SD hepcidin (7.71 ± 2.74) in compare to β -thalassemia intermedia (6.53 ± 1.8), p-value 0.02 as presented in table 2

Table 2

Mean difference in mean level of serum hepcidin, serum ferritin, Hb and iron

Variables	Diagnosis	Mean $\pm$ SD	P-value
Hb (g/dl)	β -thalassemia major	8.77 ± 0.48	0.000**
	β -thalassemia Intermedia	8.23 ± 0.62	
serum hepcidin (ng/ml)	β -thalassemia major	7.71 ± 2.74	0.02*
	β -thalassemia Intermedia	6.53 ± 1.8	
serum ferritin (ng/ml)	β -thalassemia major	4016.54 ± 2500.81	0.000**
	β -thalassemia Intermedia	1629.48 ± 1235.33	
Iron (mcg/dl)	β -thalassemia major	168.20 ± 28.91	0.000**
	β -thalassemia Intermedia	133.85 ± 33.92	

t-test:

* p-value $\leq$ 0.05 Significant** p-value $\leq$ 0.01 highly

Significant

In Table 3 Serum hepcidin had no significant correlation with Serum Ferritin ($r = -0.04$), Hb ($r = -0.26$) and Iron ($r = -0.25$) in B-thalassemia major group, p-value 0.754, 0.066 and 0.079; Also, serum hepcidin had no significant correlation with Serum Ferritin ($r = 0.08$), HB ($r = 0.007$) and Iron ($r = -0.007$) in B-thalassemia intermedia group, p-value 0.58, 0.96 and 0.96.

Table 3

Correlation Between Hepcidin and Variables (S. Ferritin, Hb and Iron) in Patients With β -Thalassemia Major and β -thalassemia Intermedia

Group	Serum Ferritin (ng/ml)		Hb (g/dl)		Iron (mcg/dl)	
	Pearson Correlation	P Value	Pearson Correlation	P Value	Pearson Correlation	P Value
β TM patients	-0.04	0.754	-0.26	0.066	-0.25	0.079
β TI patients	0.08	0.58	0.007	0.96	-0.007	0.96

In Table 4 The mean level of serum hepcidin according to gender, show no statistical significant difference in mean level of hepcidin in male (7.53 ± 2.48) in compare to female (7.45 ± 2.44), p-value 0.88

Table 4
Mean level of serum hepcidin according to gender

	Gender	N	Mean $\pm$ SD	p-value
serum hepcidin	Male	56	7.53 ± 2.48	0.886
	Female	34	7.45 ± 2.44	

In table 5, To test the validity of hepcidin, ROC test was used, when diagnosis B-thalassemia major, it has AUC 0.939 and cut off value 5.65, Sensitivity 86% and Specificity 90%. When diagnosis B-thalassemia intermedia, it has AUC 0.769 and Cut off value 5.43, Sensitivity 70% and Specificity 80%.

Table 5
The area under operator characteristic (ROC) curve for prediction of B-thalassemia major and Intermedia patients

Diagnosis	AUC	Cut off value	p-value	Sensitivity	Specificity
B-thalassemia major	0.939	5.65	0.000	86 %	90%
B-thalassemia Intermedia	0.769	5.43	0.00	70%	80%

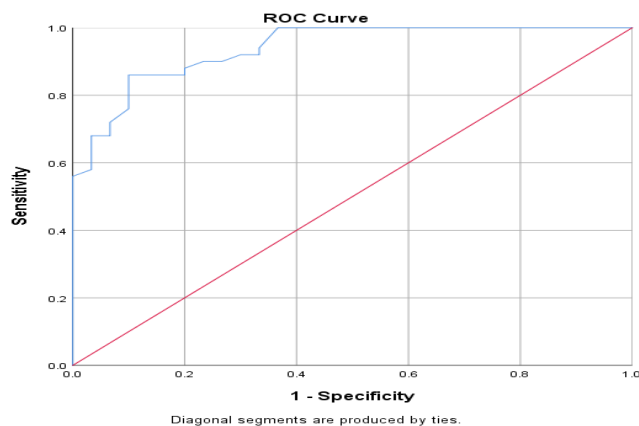


Figure 1. ROC test of B-thalassemia major group

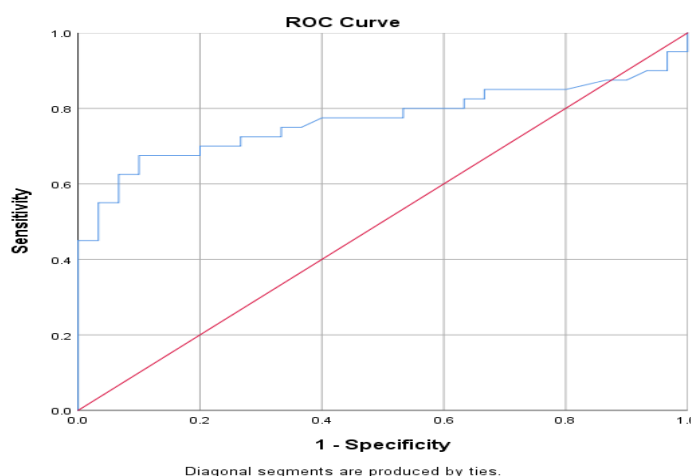


Figure 2. ROC test of B-thalassemia Intermedia group

Discussion

This data showed no statistical significant association between gender and development of specific type of β -thalassemia, p-value 0.69 as shown table (1) because the β -thalassemia gene is coded on chromosome 11(somatic chromosome) and its non-sex-linked disorder. This was consistent with previous study [17]. A statistical significant association between need to use chelating agent and β -thalassemia major, p-value 0.02. as shown table (1). Iron overload develops rapidly and β -TM patients have more problems than non-transfusion-dependent -TI patient. Chelation therapy, is a type of treatment which is used in accordance with the degree of iron overload and iron accumulation, iron-related complications can be easily controlled [20]. The current results were similar to the findings from previous studies [15]. A statistical significant association between β -thalassemia major and increase frequency of blood transfusion, p-value 0.000, as shown table (1). Blood transfusion is needed to maintain pre-transfusion Hb levels of at least 9–10.5 g/dL as life saving for patients. [21], this was in agreement with previous studies [16].

β -thalassemia major patients had statistically significant higher mean $\pm$ SD level of Hb (8.77 ± 0.48 g/dl) in compare to β -thalassemia Intermedia mean $\pm$ SD level (8.23 ± 0.62 g/dl) p-value 0.000. as shown table (2). which could be related to the fact that anemia is caused by ineffective erythropoiesis rather than hemolysis. This result supports evidence from previous observations by [22]. In β -thalassemia patients, hepcidin has a complicated regulation. Its production is controlled by opposing effects from erythropoiesis, anaemia and iron overload. Elevated bodily iron levels, infection, and inflammation up regulate hepcidin, while anemia, hypoxia, iron deficiency, inefficient erythropoiesis, and increased erythropoietin levels down regulate it [23],[17]. Other factors, including genetic or laboratory variables, could influence hepcidin production in patients with β -thalassemia [23],[24].

This data showed that β -thalassemia major patients had a statistical higher mean $\pm$ SD hepcidin (7.71 ± 2.74 ng/ml) in compare to β -thalassemia Intermedia

(6.53 ± 1.8 ng/ml) p-value 0.02. as shown table (2) Due to regular transfusions, the rate of iron loading in β TM is higher than in β TI. Transfusions also suppress the erythropoietic drive; Both lead to a rise in hepcidin in β -TM patients [25], Our results were in agreement with those of previous studies [15]. Ferritin is a cellular protein that stores iron for a period of time before releasing it when it's needed. The level of ferritin in plasma can be used to diagnose iron overload. The current study indicated that serum ferritin levels were extremely high in both the β TM and β TI groups. (mean $\pm$ SD level 4016.54 ± 2500.81 ng/ml and 1629.48 ± 1235.33 , ng/ml respectively), Iron overload is a major problem in TM and TI patients, according to this study. A blood transfusion may cause iron excess. [26] Extramedullary erythropoiesis and inefficient erythropoiesis [27], The current study showed β -thalassemia major patients had statistically significant higher mean level of serum ferritin (4016.54 ± 2500.81 ng/ml) in compare to β -thalassemia intermedia mean level (1629.48 ± 1235.33 ng/ml), p-value 0.000 as shown table (2) due to more iron overload resulted from regular blood transfusion in the patients with TM compared to TI. As observed in the current study, ferritin levels were significantly higher in the subjects with TM. The current study disagreement with [28].

The current study the serum iron level was a highly significant increase in BTM patients when compared with BTI patients. This result that agreement with Kaddah,2011. who reported serum iron level in BTM which is significantly higher in BTM compared to BTI β -thalassemia major patients had statistically significant higher mean level of iron (168.20 ± 28.91 mcg/dl) in compare to β -thalassemia Intermedia mean level (133.85 ± 33.92 mcg/dl), p-value 0.000, as shown table (2) the blood transfusion of thalassemia patients leads to increase of serum iron. This data showed no significant association between serum hepcidin and ferritin levels as a measure of iron excess. in any of the BTM or BTI groups. as shown table (3) This data was similar to previous studies that demonstrate the dominant role of erythropoiesis compared to iron overload in regulation of hepcidin in patients with Thalassemia. [25]. In contrast to the results presented by [29].

According to the current study, there is no correlation between hepcidin and iron., as shown table (3) this result is matching with [25] where showed that hepcidin not correlate with indices of iron stores, such as, ferritin. In addition result of(17) compatible with present result were showed that no statistically significant correlation between the level of serum hepcidin and ferritin ,this result due to the dominant role of erythropoiesis compared to iron overload in regulation of hepcidin in patients with thalassemia (17).This study found that in children with β -thalassemia, serum hepcidin is unaffected by gender no statistical significant difference in mean $\pm$ SD level of hepcidin in male (7.53 ± 2.48 ng/ml) in compare to female (7.45 ± 2.44 ng/ml), p-value 0.88, as shown table (4). This was in agreement with previous studies [16].

Sensitivity, specificity, and ROC curve for serum Hepcidin in predicting iron overload. At the cut-off value of 5.65 ng/ml, serum Hepcidin had a sensitivity of 86% and a specificity of 90% in diagnosing BTM. as shown table (5). The receiver operating characteristic (ROC) curve indicated that the serum level of Hepcidin provided an accurate test for defining the disease in BTM as the area under the

curve (AUC) for Hepcidin was 0.939. An area of 90-100 represents an excellent test ($p < 0.000$; Figure 1). Sensitivity, specificity, and ROC curve for serum Hepcidin in predicting iron overload. At the cutoff value of 5.43 ng/ml, serum Hepcidin had a sensitivity of 70% and a specificity of 80% in diagnosing BTI, as shown in Table (5). The receiver operating characteristic (ROC) curve indicated that the serum level of Hepcidin provided a fair test for defining the disease in BTI as the area under the curve (AUC) for Hepcidin was 0.769. An area of 70-80 represents a fair test ($p < 0.00$; Figure 2).

Conclusions

Hepcidin levels may be helpful in determining the diagnosis and prognosis of thalassemia syndromes. Exogenous hepcidin could be used in the future to help patients with thalassemia intermedia restore normal iron homeostasis. Hepcidin and ferritin measurements may provide further information regarding the severity of iron overload and iron misdistribution in thalassemia in the future.

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