Exploring the Role of Hepcidin and Some Biochemical Indicators in β-thalassemia Major Patients with Negative and Positive *Entamoeba histolytica* / Original study

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استكشاف دور الهيبسيدين وبعض المؤشرات البيوكيميائية لدى مرضى الثلاسيميا الكبرى المصابين وغير المصابين بطفيلي الزحار الاميبي

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Abstract

Thalassemia syndromes encompass a wide range of hemoglobin disorders characterized by either the absence or diminished production of normal globin chains. Hepcidin, a peptide produced by the liver with antimicrobial properties, is mainly synthesized by hepatocytes situated near the portal veins, which are responsible for transporting dietary iron, as well as by Kupffer cells. Before blood transfusions, increased erythropoietic activity leads to a reduction in hepcidin, resulting in lower hepcidin levels that enhance the absorption of dietary iron. Parasite protozoa depend on iron for their survival, as it plays a crucial role in the growth and enzymatic functions of *E. histolytica* by participating in the synthesis of the parasite's enzymes. An elevation in hepcidin levels may serve as a host defense mechanism aimed at limiting iron availability to microbes during inflammatory responses; however, this mechanism may prove ineffective in non-infectious inflammatory conditions that lead to anemia. Aims: to identify the hepcidin hormone along with various biochemical indicators that can predict outcomes in Iraqi patients with β-thalassemia major, specifically those who have tested either positive or negative for *Entamoeba histolytica*. **Methods**: A total of 200 (150 with β-thalassemia patients and 50 healthy subjects) were involved in this study during their attendance at AL-Karama hospital and Ibn AL-Baladi hospital in Baghdad. The study was conducted during the period from January 2024 to May 2024. The age range of all patients were from (15–35) years and the necessary information's were taken from all patients. Stool specimens were collected from patients with blood and /or mucus diarrhea and five ml disposable syringe was used to collect venous blood by vein puncture. blood was transferred to 10 ml sterile serum separator tubes (gel

tube used for detection of: Hepcidin by ELISA ALT, AST, Iron and TIBC by using biochemical specific auto-analyzer instrument. **Results**: The results of biochemical indicators showed an elevated levels in liver the enzymes (ALT, AST) and (Iron and TIBC) was significantly higher than that observed in the control (P<0.0001). According to serum concentration of Hepcidin showed a lower for β-thalassemia patients' group (P<0.0001) (80.83 ± 77.7) as compared with control (320.9 ± 88.1). In the present study, no statistically significant difference (P < 0.9042) was observed in the levels of Hepcidin between patients with β-thalassemia and those infected with *Entamoeba histolytica*. Also, the results showed significant decreases (P < 0.0001) in Iron and TIBC levels, but a significant increases (P < 0.0001) in ALT and AST was observed when comparing to patients with β-thalassemia those infected with *Entamoeba histolytica*. **Conclusion**: Patients with β-thalassemia who are infected with *Entamoeba histolytica* exhibited a notable reduction in hepcidin levels, along with a significant difference in certain biochemical indicators.

Keywords: β-thalassemia, Entamoeba histolytica; Hepcidin, ALT, AST, Iron and TIBC.

Abbreviations

ALT: Alanine transaminase

AST: Aspartate aminotransferase

TIBC: Total Iron Biding Capacity

ELISA: Enzyme Linked Immune Sorbent Assay

BTM: B Thalassemia Major

المستخلص

المقدمة: تشمل متلازمات الثلاسيميا مجموعة واسعة من اضطرابات الهيموجلوبين التي تتميز إما بغياب أو انخفاض إنتاج سلاسل الجلوبين الطبيعية. الهيبسيدين، وهو ببتيد ينتجه الكبد وله خصائص مضادة للميكروبات، يتم تصنيعه بشكل أساسي بواسطة خلايا الكبد الموجودة بالقرب من الأوردة البابية، المسؤولة عن نقل الحديد الغذائي، وكذلك بواسطة خلايا كوبفر. قبل عمليات نقل الدم، تؤدي زيادة نشاط تكوين الكريات الحمر إلى انخفاض الهيبسيدين، مما يؤدي إلى انخفاض مستويات الهيبسيدين التي تعزز امتصاص الحديد الغذائي. تعتمد الأوليات الطفيلية على الحديد من أجل بقائها على قيد الحياة، لأنه يلعب دورًا حاسمًا في النمو والوظائف الأنزيمية لــلزحار الاميبي من خلال المشاركة في تخليق إنزيمات الطفيلي. قد يكون الارتفاع في مستويات الهيبسيدين بمثابة آلية دفاع مضيفة تهدف إلى الحد من توافر الحديد للميكروبات أثناء الاستجابات الالتهابية. ومع ذلك، قد تكون هذه الآلية غير فعالة في الحالات الالتهابية غير المعدية التي تؤدي إلى فقر الدم. **الهدف**: للتعرف على هرمون الهيبسيدين مع العديد من المؤشرات البيوكيميائية التي يمكنها التنبؤ بالنتائج لدى المرضى العراقيين المصابين بالثلاسيميا الكبرى، وتحديداً أولئك المصابين او غير المصابين بمرض الزحار الاميبي. **طريقة العمل**: شارك في هذه الدراسة 200 شخص (150 مريض بمرض الثلاسيميا الكبرى و 50 من الأصحاء) أثناء حضورهم إلى مستشفى الكرامة و مستشفى ابن بلدي في بغداد. أجريت الدراسة خلال الفترة من يناير 2024 إلى مايو 2024. وتراوحت الفئة العمرية لجميع المرضى من (15-35) سنة. أخذت المعلومات اللازمة من جميع المرضى فضلا عن جمع عينات البراز من المرضى الذين يعانون من الإسهال الدموي و/أو المخاط وتم استخدام حقنة سعة 5 مل لجمع الدم الوريدي عن طريق ثقب الوريد. نقل الدم إلى أنابيب فاصل مصل معقمة سعة 10 مل (أنبوب هلامي يستخدم للكشف عن : الهبسدين بواسطة الاليزا ، الالنين ترانس امينيز , اسبارتيت ترانسفيريز , الحديد , اجمالي سعة ارتباط الحديد) باستخدام جهاز التحليل الذاتي المخصص للتحاليل البيوكيميائية المحددة. **النتائج** : أظهرت نتائج المؤشرات الكيموحيوية في الكبد ارتفاع مستويات إنزيمات (الالنين ترانس امينيز ,

اسبارتيت ترانسفيريز) و (الحديد و اجمالي سعة ارتباط الحديد) مقارنة مع السيطرة (P<0.0001). وفقاً لتركيز الهيبسيدين في المصل فقد أظهر انخفاضاً في مجموعة مرضى الثلاسيميا بيتا (77.7 ± 80.83) (P<0.0001) مقارنة مع مجموعة السيطرة (320.9 ± 88.1). في هذه الدراسة لم يلاحظ أي فرق ذي دلالة إحصائية (P <0.9042) في مستويات الهيبسيدين بين المرضى الذين يعانون من الثلاسيميا بيتا والمصابين بالمتحولة الحالة للنسج. كما لوحظ وجود انخفاض معنوي (P <0.0001) في مستويات الحديد و اجمالي سعة ارتباط الحديد ، بينما لوحظ زيادة معنوية (P <0.0001) في الالنين ترانس امينيز و اسبارتيت ترانسفيريز عند المقارنة مع مرضى الثلاسيميا بيتا المصابين بالزحار الاميبي **الاستنتاجات** : أظهر المرضى الذين يعانون من الثلاسيميا بيتا والمصابين بالزحار الاميبي انخفاضًا ملحوظًا في مستويات الهيبسيدين، إلى جانب اختلاف كبير في بعض المؤشرات البيوكيميائية.

الكلمات المفتاحية : بيتا الثلاسيميا ، الزحار الاميبي ؛ هيبسيدين ، الالنين ترانس امينيز ، اسبارتيت ترانسفيريز ، الحديد و اجمالي سعة ارتباط الحديد .

1. Introduction

Thalassemia syndromes encompass a wide range of hemoglobin disorders resulting from the absence or diminished production of normal globin chains. It is estimated that between 1% and 5% of the global population carries a hereditary thalassemia mutation, making it the most common recessive disorder (Brancaleoni *et al.,* 2016). Thalassemia is characterized by several molecular irregularities, such as early termination of the protein chain, instability of mRNA, substitutions or deletions in the gene, and complications in the initiation of chain synthesis (*Nang, 2016; Sharma et al., 2017*). The word "thalassemia" originates from the Greek terms "thalassa," meaning sea, and "haema," meaning blood. This condition arises from the abnormal production of hemoglobin (Hb) subunits, which are inherited as pathological alleles of one or more globin genes located on chromosomes 11 (β) and 16 (α) (Rachmilewitz *et al.*, 2011). Thalassemia occurs in two types: α-thalassemia and β-thalassemia (Minor, Intermediate, and Major) (Moafi *et al.,* 2010). Similar to sickle cell anemia and β-thalassemia, α-thalassemia is more common in tropical and subtropical regions (Goh *et al*., 2020). Beta-thalassemia is an autosomal-recessive hereditary anemia with a lack or reduction in β globin chain synthesis. The excess of unbound α globin chains in the bone marrow leads to early mortality of erythroid precursors and inefficient erythropoiesis. The severity of β-thalassemia phenotypes ranges from severe, transfusion-dependent thalassemia major to moderate, intermedial forms (Danjou *et al.,* 2011). In a previous study, Gupta *et al.,* discovered that patients with beta-thalassemia major had an intestinal parasite infection rate of 21.9%. This study shows that individuals with beta-thalassemia major frequently have intestinal parasites. *Entamoeba histolytica*

/ *dispar* was the most commonly found intestinal protozoan, followed by *Blastocystis hominis* and, *Ascaris lumbricoides* infestation, and *Ancylostoma duodenale* infestation were all revealed to be helminthic infections (*Gupta et al.,2016*). Furthermore, parasite infection stimulates catecholamine release, which can mobilize red blood cells from the spleen or cause red blood cell enlargement due to fluid shifting into the intracellular compartment (*Thachil & Bates 2017*). Hepcidin, identified as the liver-expressed antimicrobial peptide, is primarily produced by hepatocytes located near portal veins (which transport dietary iron) and Kupffer cells (which sense bacteria and recycle erythrocytes). Furthermore, adipocytes and macrophages both produce a small amount of hepcidin (*Ganz and Nemeth, 2012*). Hepatocytes produce more hepcidin when iron is abundant because iron regulates this process; when iron is scarce, hepatocytes produce little or no hepcidin, allowing more iron to reach the plasma (Ramos *et al*., 2011). During beta-thalassemia, the suppressive and stimulating effects of erythropoiesis work together to regulate hepcidin production. Prior to transfusion, erythropoietic activity lowers hepcidin. It causes a low hepcidin level, which boosts dietary iron absorption. After the transfusion, inefficient erythropoiesis partly subsides, increasing hepcidin levels (*Nemeth, 2013*). Parasite protozoa require iron for survival and it is necessary for *E. histolytica* growth and enzymatic activities because iron enters the synthesis of parasite enzymes (*Frederick et al., 2011, Nevitt, 2011*). Increased hepcidin may be a host defense mechanism to reduce iron availability for microbes during inflammation, but this may not be effective in non-infection inflammatory disorders that cause anemia (*Elzen et al., 2009, Hernandez et al.,2014*). Infection with *Entamoeba histolytica* has been linked to increased levels of

some biochemical indicators and changes in hepcidin level in patients suffering from β-thalassemia major. Therefore, the current study was conducted to guess cut off values of hepcidin and biochemical indicators (ALT, AST, iron and TIBC) and clarify their correlation with beta-thalassemia who positive and negative for *Entamoeba histolytica*.

2. Materials and methods

2.1. Populations studied

The current study involved 200 (150 with β-thalassemia patients and 50 healthy subjects) during their attendance at AL-Karama hospital and Ibn AL-Baladi hospital in Baghdad. The study was conducted during the period from January 2024 to May 2024. The patients had suffered from β-thalassemia major from different period of time. The age range of all patients were from (15–35) years and the necessary information's were taken from all patients after taking their permission depending on the letter of approved by the Research Ethical Committee and Scientific Committee designated by Middle Technical University, the College of Health and Medical Techniques, and the Department of Medical Laboratory Techniques (No. 172/3) on January 08, 2024.

2.2. Laboratory tests

Stool specimens were collected from patients with blood and /or mucus diarrhea and five ml disposable syringe was used to collect venous blood by vein puncture. blood was transferred to 10 ml sterile serum separator tubes (gel tube), centrifuged, and the serum was then divided into

several 0.5 ml aliquots and immediately frozen at -20°C until used for detection of: Hepcidin and biochemical indicators (ALT, AST, iron and TIBC).

The blood samples (*Gel tube*) were centrifuged for 10 minutes at 6000 rpm to obtain serum which is used for the determination of alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). Serum concentrations of Hepcidin were evaluated by using the technique enzyme-linked immunosorbent assay (ELISA). While, the ALT, AST and iron were evaluated by using electro-chemiluminescence immunoassay, Roche Cobas Integra 400 plus (Roche Diagnostics GmbH, Mannheim, Germany) serum TIBC were evaluated by spectrophotometer, Serum samples for all subjects were applied to the instrument then the concentration of biochemical parameters were automatically calculated.

2.3. Statistical analysis

The statistical analysis was performed using GraphPad Prism version 9.2 (GraphPad Software Inc., LaJolla, CA). Student's *t*-test was used to determine whether group variance was significant or not. Pearson coefficient r value was employed to assess correlation. Receiver operating characteristic (ROC) curve analysis was performed to determine area under curve (AUC) and the optimum cut-off value of markers best prediction. Quantitative parametric data were subjected to Shapiro-Wilk test to confirm the normal distribution and were expressed as mean ± SD and statistical differences were defined as $p < 0.05$ and $p < 0.01$ iss significant .

3. Results

3.1 Age and Biochemical Parameters Value in β-Thalassemia Major Patients with or without *Entamoeba histolytica* and Control

The result of this study showed that there was no significant (*P* value = 0.0737) difference in the age between the patients with β-thalassemia major (22.91 ± 5.6) years and controls (24.54 ± 5.5) years as shown in Table (1) and Figure (1). from the same Table and the accompanying Figure indicate a highly significant increase in serum ALT, AST, and iron and TIBC levels in the patient group when compared to the control group. The mean values for ALT, AST, iron and TIBC in patients with β-thalassemia were found to be significantly elevated (P<0.0001), with values of 94.28 ± 63.7, 77.33 ± 55.9, 93.08 ± 35.7 ng/mL and 499.9 ± 94.4 ng/mL, respectively, in contrast to the control group, which exhibited mean values of 28.58 ± 8.8, 30.34 ± 9.3, 21.22 ± 6.84 ng/mL and 317.7 ± 38.9 ng/L respectively.

Table (1). Age and Biochemical Parameters Value in β-Thalassemia Major Patients with or without *Entamoeba histolytica E. histolytica* and Control

Parameter	Mean ± SD		Sig.	*p* Value
	Control	Patients		
Age	24.54 ± 5.5	22.91 ± 5.6	NS	0.0737
ALT	28.58 ± 8.8	94.28 ± 63.7	HS	<0.0001**
AST	30.34 ± 9.3	77.33 ± 55.9	HS	<0.0001**
Iron	21.22 ± 6.84	93.08 ± 35.7	HS	<0.0001**
TIBC	317.7 ± 38.9	499.9 ± 94.4	HS	<0.0001**

By using independent T-test S: Significant , HS: Highly significant ,NS: Not significant.

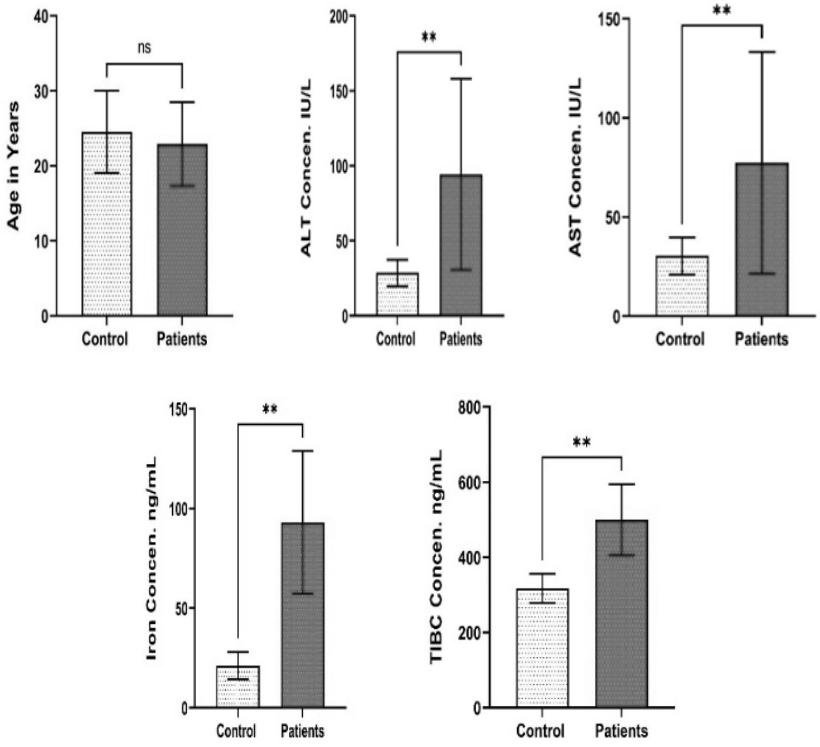

Figure (1). Age and some biochemical markers of β-thalassemia major in patients and control

The current study found significant decreases ($P < 0.0001$) in iron and TIBC levels, but a significant increases ($P < 0.0001$) in ALT and AST was observed when comparing patients with β-thalassemia to those infected with *Entamoeba histolytica*, as shown in the Table (2) and Figure (2).

Table (2). Concentration of ALT, AST and LDH in β-thalassemia major patients with negative and positive *Entamoeba histolytica*

Parameter	Mean ± SD		Sig.	*p* Value
	+ve	-ve		
ALT	118.3 ± 51.9	74.74 ± 64.43	HS	<0.0001**
AST	107.0 ± 52.77	49.17 ± 42.83	HS	<0.0001**
Iron	77.99 ± 27.01	109.0 ± 37.05	HS	<0.0001**
TIBC	450.3 ± 50.30	552.2 ± 101.9	HS	<0.0001**

By using independent T-test S: Significant, HS: Highly significant ,NS: Not significant

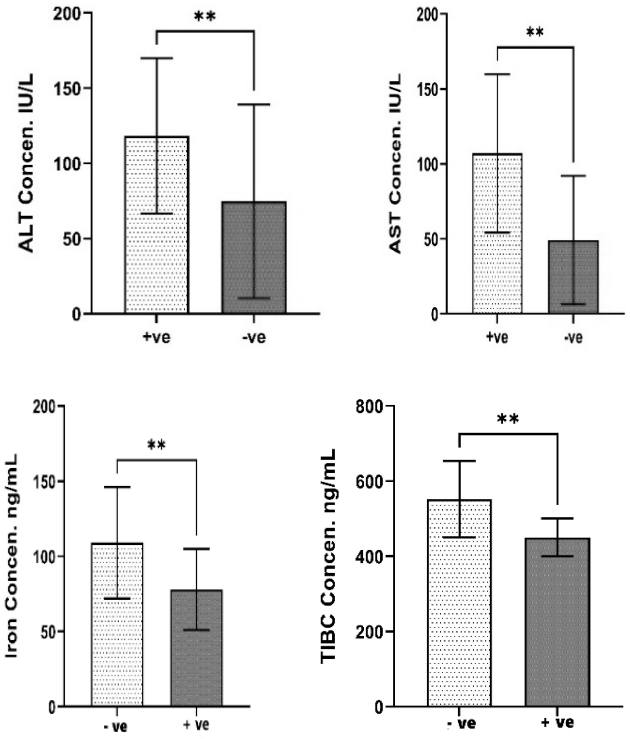

Figure (2). ALT, AST, iron and TIBC in β-thalassemia major patients with negative and positive *Entamoeba histolytica*

Concentration of Hepcidin in β- thalassemia major patients with or without *Entamoeba histolytica* and Control.

The concentration of Hepcidin showed a highly significant (P<0.0001) decrease in patients with β-thalassemia (1.57 ± 1.12) as compared with control (4.16 ± 1.34) as shown in Figure (3). In the present study, no statistically significant difference (P < 0.9042) was observed in the levels of Hepcidin between patients with β-thalassemia and those infected with *Entamoeba histolytica*, as illustrated in the Table (3).

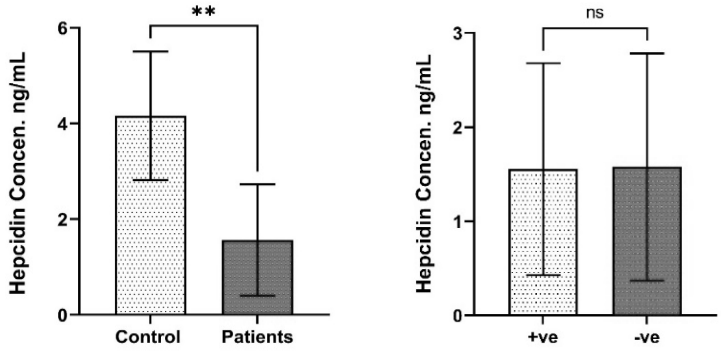

Figure (3). Hepcidin levels in β-thalassemia patients and β-thalassemia major patients with negative and positive *Entamoeba histolytica* were compared to controls.

Table (3). Concentration of GSH in β-thalassemia major patients and with negative and positive *Entamoeba histolytica*

Parameter	Mean ± SD		Sig.	*p* Value
	Control	Patients		
Hepcidin	4.16 ± 1.34	1.57 ± 1.12	HS	<0.0001
Parameter	Mean ± SD		Sig.	*p* Value
	+ve	-ve		
Hepcidin	1.553 ± 1.126	1.576 ± 1.208	NS	0.9042
Concentration of GSH in β-thalassemia major patients with negative and positive *Entamoeba histolytica*.				

To evaluate the effectiveness of various biochemical markers in predicting β-thalassemia among patients both positive and negative for *Entamoeba histolytica*, Receiver Operating Characteristics (ROC) Curve analysis was utilized. This method involves plotting the true positive rate (sensitivity) against the false positive rate (1 - specificity). The area under the ROC curve (AUC) was computed, serving as an indicator of the test's validity in forecasting the outcome. As interpretation, AUC of less than 0.600 indicates failure of a test as predictor, 0.600 to 0.700 is sufficient predictor, 0.700-0.800 good, 0.800 -0.900 very good and > 0.900-1.00 indicates excellent predictor test, according to this cutoff value of AUC, S. ALT and S. AST were good predictors of the disease with AUC 0.7946 and 7595 respectively, , while hepcidin, iron and TIBC were excellent predictors of disease with AUC 0.9481, 0.9969, and 0.9973 respectively Figure (4).

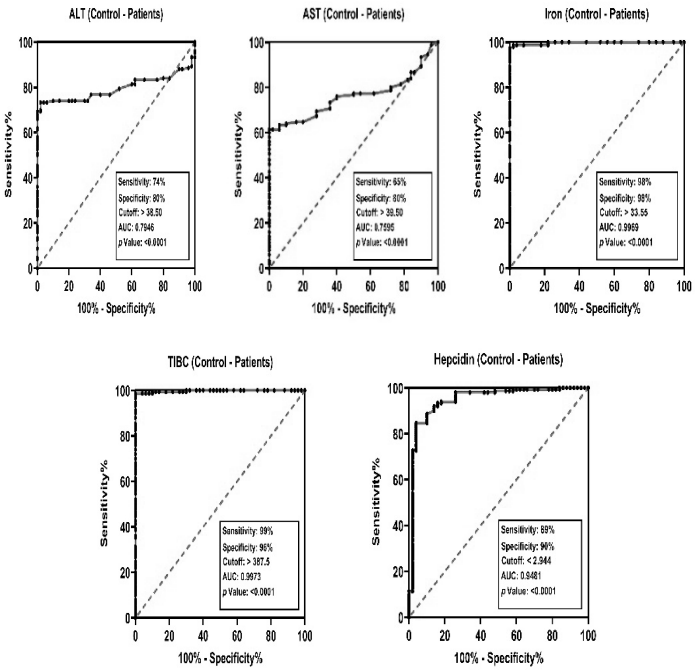

Figure (4). Receiver Operating Characteristics (ROC) curve for the validity of Hepcidin S. ALT, S. AST, S. Iron and S. TIBC in prediction of severity of disease.

4. Discussion

The age of the patients did not exhibit a statistically significant difference when compared to the control group. This outcome may be attributed to the sample selection process. Various studies have indicated differing average ages, with **Chirico** *et al.,* (2013) noting that the mean age of the patients was 34.4 years. **Ang** *et al.,* (2014) indicated that the average age of patients with β-thalassemia was 36 years, with a range from 18 to 59 years. Furthermore, **Poggi** *et al.,* (2016) reported a mean age of 39.9 years among β-thalassemia patients. The discrepancies in these age figures may stem from variations in selection criteria, patient demographics, and lifestyle factors. Additionally, it is conceivable that age may not play a crucial role in the onset of β-thalassemia, as suggested by **Rai & Stuart**, (2024). Further investigation is warranted to better understand the connection between age and β-thalassemia.

Hepcidin concentration showed a highly significant decrease ($P < 0.0001$) in β- thalassemia patients in both negative and positive for *Entamoeba histolytica* and no statistically significant difference ($P < 0.9042$) was observed in the levels of hepcidin patients with β-thalassemia and those infected with *Entamoeba histolytica*. Additionally, the ROC curve analysis illustrated in figure 3-4 demonstrated that serum hepcidin possess remarkable prognostic accuracy in patients with β-thalassemia major, regardless of their *Entamoeba histolytica* status, whether negative or positive.

The present research aligns with the findings of **Mohammadi** *et al.,* (2018), who demonstrated that the hepatic peptide hepcidin plays a crucial role in regulating the body's iron homeostasis by preventing iron from entering the plasma through various mechanisms. In cases of thalassemia,

inadequate erythropoiesis leads to a compensatory reduction in hepcidin levels, which in turn enhances the absorption of iron in the intestines. Furthermore, thalassemia major is characterized by a disruption in globin chain production, resulting in ineffective erythropoiesis, anemia, and an elevated production of erythropoietin. This, in turn, stimulates the synthesis of erythroferrone, a hormone derived from erythroid cells that inhibits the liver's capacity to produce hepcidin. Furthermore, **Ali** (2018) indicates that following blood transfusions, levels of hepcidin-25 decrease, facilitating iron absorption and resulting in an excess of iron. Dysregulation of ferroportin has been associated with hepcidin (HAMP) or related genetic disorders, such as hemochromatosis (HFE), transferrin receptor 2 (TFR2), and HJV, as well as conditions like intermediate β-thalassemia. These disorders are often correlated with heightened iron absorption and export, which can lead to elevated levels of free systemic iron and excessive iron accumulation in tissues, ultimately resulting in tissue damage (Liu *et al.,* 2016). In hereditary hemochromatosis, the accumulation of iron is associated with inadequate production of hepcidin due to mutations in either the hepcidin or HJV gene. Similarly, beta-thalassemia, marked by ineffective erythropoiesis and impaired beta-globin production, is also associated with increased absorption of dietary iron. This phenomenon is connected to the presence of hepcidin inhibitors such as ERFE (Agarwal and Yee, 2019).

Hepcidin is an important regulator of iron homeostasis and participates in a variety of iron metabolism pathways. Specific polymorphisms in the HAMP promoter region have been shown to reduce this hormone's expression, resulting in increased serum iron (Zarghamian *et al.,* 2020).

Hepcidin, a hormone synthesized in the liver, regulates iron absorption in the intestine, influences iron release from the reticuloendothelial system, and is also involved in placental iron transfer. The extracellular parasite *E. histolytica*, which causes intestinal and hepatic diseases known as amoebiasis, has a significant impact on iron levels in the body. Its presence leads to increased iron consumption and a subsequent reduction in overall iron levels. Given that the liver is the site of hepcidin production, the infection results in markedly decreased levels of this hormone, indicating that the parasitic infection adversely affects the regulation of iron within the body. In β-thalassemia, higher erythropoiesis suppresses hepcidin synthesis, leading to elevated iron absorption through the gastrointestinal tract. Blood transfusions provide additional iron for β-thalassemia patients (Jones *et al.,* 2015).

The findings of the present study indicated a statistically significant elevation in the levels of AST and ALT enzymes among thalassemia patients, both those uninfected and infected with the *Entamoeba histolytica*, compared to the control group as shown in in table 3.2 and figure 3.2. Iron overload, indicated by high serum ferritin levels, is known as a cause of liver dysfunction which is reflected by elevated ALT and AST levels. Our findings regarding highly liver enzymes among thalassemia patients were agreement with previous reports from Iraq (Mohammad, 2012). According to Hashim *et al,* reported in Maysan research, the difference in the concentration of Transferases compared to healthy individuals is due to excessive hemolysis (Hashim *et al.,* 2020).

Excessive red blood cell breakage (hemolysis) or the need for peptide chain synthesis may cause a difference in transportable enzyme concentrations compared to healthy individuals. These enzymes are effective in transmitting

amine groups in amino acids (Al-Khashli and Al-Shawi, 2013). ALT and AST are common in many tissues of the human body. AST enzymes are more effective than ALT enzymes. It is more prevalent in the heart, liver, skeletal muscles, and kidneys. The liver contains a large amount of ALT, as do the kidney, heart, and skeletal muscles. Increased enzyme effectiveness in patients may be due to elevated iron levels in their serum, which can lead to fat breakdown in certain organ cells as a result of the free radical's formation that attacks all vital molecules such as lipids, proteins, and cell DNA. (Hashim, *et al.,* 2020; Bushra, *et al.,* 2013, Ellis, 2010). Sateriale and his colleagues (2011) discovered that approximately 50 million infections of the *E. histolytica* parasite affect the liver each year. Amoebic liver abscesses are caused by eating food or water contaminated with human feces. However, the most common symptomatic infection is amoebic dysentery. In rare cases, trophozoites can invade the intestinal mucosa and spread blood, resulting in extra-intestinal disease. Aside from the intestine, the most common symptom is liver abscess. The trophozoites enter the liver through the portal venous circulation and cause significant damage (Tharmaratnam *et al.,* 2020). The current findings align with previous research indicating increased levels of liver enzymes in the serum of individuals diagnosed with amebiasis (Morán, *et al.,* 2023; Pluta and Pluta , 2008). Moreover, ALT and AST were good predictors of the disease with AUC 0.7946 and 7595 respectively.

In the present study the patients with β-thalassemia exhibited elevated iron levels in comparison to the control group. This observation aligns with the findings of Abdulla (2022), who similarly reported increased iron levels in cases of β-thalassemia major. Furthermore, the results of the current investigation corroborate the conclusions drawn by Kuppusamy and Tan

(2011). Thalassemia management is primarily concerned with iron levels and issues related to excess or scarcity (Needs *et al*., 2018). Iraqi patients had significantly elevated serum iron levels, indicating an existing iron excess, with an elevated iron level being a significant risk factor for myocardial infarction. Frequent blood transfusions in β- thalassemia without chelation therapy can cause iron overload, leading to splenomegaly and other complications.

The liver's functionality is also influenced, leading to an increased likelihood of additional complications (Abdulla, 2018). According to **Mishra and Tiwari** (2013), all patients with β-thalassemia major exhibited elevated serum iron, ferritin levels, and total iron-binding capacity (TIBC), which could suggest iron overload. This phenomenon may occur due to enhanced iron absorption stemming from disrupted erythropoiesis or continuous blood transfusions. The accumulation of iron in patients with thalassemia can surpass the storage and detoxification capacity of ferritin, resulting in the complete saturation of transferrin and the presence of free iron in the bloodstream and tissues. This highly reactive free iron can generate hydroxyl radicals that target lipids, leading to the formation of lipid peroxides, which in turn contribute to oxidative stress. Consequently, this process results in the production of extremely harmful compounds, such as hydroxyl radicals (Prabhu, *et al.,* 2009).

Additionally, iron overload and tissue injury resulting from excessive iron due to frequent blood transfusions or heightened intestinal iron absorption, along with early hemolysis and ineffective erythropoiesis, represent two prevalent and serious complications of thalassemia. Consequently, iron chelators have been employed to prevent iron accumulation in patients with thalassemia (Saeidnia, *et al.,* 2022).

The findings of this study indicated an increase in Total Iron Binding Capacity (TIBC) among patients with β-thalassemia when compared to the control group. This observation aligns with the results reported by Hesham *et al.,* (2018) and Guimaraes *et al.,* (2015), who also identified elevated TIBC levels alongside increased ferritin and iron concentrations. Furthermore, the present study corroborates the conclusions of Karim *et al.,* (2016), who demonstrated a significant rise in TIBC levels in individuals diagnosed with β-thalassemia. Furthermore, Ali (2018) indicated a positive correlation between Total Iron Binding Capacity (TIBC) and increased serum ferritin and iron levels. The quantity of iron stored in the body is influenced by the levels of TIBC and ferritin; as the iron content rises, so do the levels of TIBC and ferritin (Badiee *et al.,* 2015).

the current investigation, a statistically significant decrease in iron levels and total iron-binding capacity (TIBC) was observed ($P < 0.0001$) when comparing patients with β-thalassemia to those infected with *Entamoeba histolytica*. The reduction of iron levels in patients infected with *E. histolytica* is linked to the pathogenicity of this organism, which is influenced by the relationship between iron concentration and the adhesion of *E. histolytica* to epithelial cells (Al-Hadraawy *et al.,* 2015). The findings align with those of Hernandez-Cuevas *et al.,* (2014), which demonstrated a reduction in total iron-binding capacity (T.I.B.C) among individuals infected with the *E. histolytica* parasite. This suggests that the parasite utilizes ferritin, the protein responsible for iron storage, to support its survival and reproductive processes. Also, this finding aligns with Weinberg's research, which indicated that *E. histolytica* employs serum ferritin as a source of iron. Consequently, this interaction results in decreased ferritin levels in humans infected with

the parasite when compared to healthy individuals (Weinberg, 1999). A study conducted by Oguntibeju (2003) indicated a correlation between parasitic infestations and alterations in hematological parameters, which can lead to anemia. Additional evidence from community research has highlighted the involvement of *E. histolytica* and *G. lamblia* infections in cases of iron deficiency anemia, as reported in other studies (Juomaa, 2006; Al-Naemi *et al.,* 2011). The liver contains many iron sources, so the parasite's ability to use these sources may be related to its survival in the liver and the formation of liver abscesses, The parasite *Entamoeba histolytica* uses iron as a cofactor for its enzyme alcohol dehydrogenase 2 (EhADH2) (Hernandez-Cuevas *et al.,* 2014). This enzyme serves a dual function, operating as both Alcohol dehydrogenase (ADH), which removes hydrogen atoms from alcohol molecules, and Aldehyde dehydrogenase (ALDH), which extracts hydrogen atoms from aldehyde molecules within the glycolysis pathway of *Entamoeba histolytica* (Espinosa *et al.,* 2001).

Since *Entamoeba histolytica* does not possess mitochondria, it derives the energy required for essential functions through fermentation. The glucose fermentation pathway culminates in the conversion of Acetyl-coA to Ethanol, facilitated by the activation of Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes. Consequently, the enzyme EhADH2 is essential for the survival of this parasite (Espinosa *et al.,* 2004). Numerous studies have indicated that iron plays a crucial role in the growth of parasites and the activation of enzymes. These studies have also underscored the significance of iron in the pathogenesis of parasitic infections and proposed the use of iron chelation as a potential chemotherapy strategy, contingent upon the immune response of mammals. The isolation of iron is

considered a primary defense mechanism. Furthermore, various investigations have demonstrated the impact of iron on the enzymatic activities of two specific enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), as well as on the survival of the feeding phase of the parasite. The objective of reducing or eliminating iron is to explore its potential as a treatment option in the future (Espinosa *et al.,* 2004; Espinosa *et al.,* 2009). The reticuloendothelial system (RES) cells play a crucial role in the metabolism of iron. The primary storage locations for iron include aged red blood cells and the liver parenchyma (Edison *et al.,* 2008).

Entamoeba histolytica is recognized as an intestinal parasite that necessitates iron for its metabolic activities. Consequently, it utilizes the iron found in the liver and the lining of the large intestine, where the parasite initially manifests. Research conducted by López-Soto *et al.,* (2009) revealed that *E. histolytica*, during its trophozoite stage, attaches to ferritin via specific proteins, thereby utilizing it as a vital iron source during this phase. This interaction leads to a notable reduction in the iron reserves of individuals infected with this parasite.

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