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REVIEW ARTICLE

The Correlation of Hepcidin with Hemoglobin and Iron Parameters in Iraqi Patients with Beta-Thalassemia Majors

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Abstract

Background: Thalassemia is characterized by genetic abnormalities in the synthesis of hemoglobin, leading to a decrease or missing production of one or more in the globin chains. Consequently, this disrupts the synthesis of hemoglobin molecules, resulting in anemia, which is a prominent manifestation of thalassemia. Iron is an essential element for cellular health and is involved in various functions, including oxygen transportation, biomolecule synthesis, respiration, and homeostasis. Hepcidin, a low molecular weight peptide produced in the liver, plays a crucial role in regulating iron homeostasis.

Objectives: The current study aims to inspect the correlation of Hepcidin with Hemoglobin, Ferritin, and Iron Parameters in Patients with Beta-Thalassemia Major.

Methods: The serum ferritin of all the subjects was measured by the ELFA technique (Enzyme Linked Fluorescent Assay). The Serum Hepcidin was measured by the ELISA kit, and Iron, UIBC, and TIBC were measured via colorimetric methods.

Results: The {mean ± SD} of ages and genders among the patients and healthy groups were not significant. The {mean ± SD} of Hb and serum levels of Iron, UIBC, TIBC, Hepcidin concentration, and BMI between the patients and healthy groups were statistically significant. The study results of the correlation between Hepcidin and other markers in the beta-thalassemia patients group showed a non-significant negative correlation of Hepcidin with TIBC and UIBC. While there was a non-significant weak correlation between Hepcidin with Hb and Ferritin. The results also showed a non-significant positive correlation between Hepcidin and Iron.

Conclusion: This study showed a marked reduction in hemoglobin production and high levels of Iron and Ferritin concentrations, while the concentrations of TIBC and UIBC were observed to decrease in the individuals with this disease compared to the healthy individuals. This study showed low Hepcidin concentration in the β-TM major patients compared to the healthy subjects.

Keywords: Beta-Thalassemia Major, Hepcidin, Hemoglobin, Iron parameter.

Introduction:

Thalassemia is characterized by genetic hemoglobin synthesis abnormalities in which the production of one or more hemoglobin is reduced or missing or more globin chains, which might result in aberrant hemoglobin synthesis molecules, as a result, induces anemia, a defining sign of the thalassemia (1). The synthesis of the alpha- or beta-globin subunits of hemoglobin A is abnormally slowed down in thalassemia. Two alpha and two beta globin subunits make up this hemoglobin A. The genes that produce α-globin are located on chromosome 16, whereas the genes that produce β-globin are located on chromosome 11(2). The most prevalent blood abnormality is β -thalassemia, which affects a large region that includes Melanesia, the Pacific Islands, Southeast Asia, the Indian subcontinent, the Middle East, portions of Africa, and the Mediterranean region. The frequency range of β-Thalassemia carriers in these places is (1–20%)(3). Beta-thalassemia is a set of genetic blood disorders diseases caused by a lack of beta-globin chains (β -globin) or their inadequate production resulting in too many alpha chains. The damage caused by thalassemia to the circulating RBCs, as well as bone marrow both, contain precursors to erythropoiesis(4). The two genes on chromosome 11 which control the production of beta

globin (β-globin) in human hemoglobin (Hb) are responsible for this (5).

The liver-expressed antimicrobial peptide which is known as Hepcidin was discovered in 2000 (6). Hepatocytes, which are strategically positioned near portal veins and carry dietary iron, as well as Kupffer cells, which sense pathogens and recycle erythrocytes, are the main producers of hepcidin. Adipocytes and macrophages both produce a small amount of Hepcidin (7,8). The Hepcidin antimicrobial peptide (HAMP) gene is responsible for encoding Hepcidin. 84 amino acid pre-pro-hepcidin is first created during its early synthesis. After that, it is transformed into a pro-hepcidin with 60 amino acids before being cut into a mature C-terminal (9). In Beta-thalassemia, Hepcidin suppression enhances the severity of iron overload by increasing iron absorption in cases of iron overload with inadequate erythropoiesis, This extra iron collects in non-hematopoietic tissue's parenchyma. Because the capacity of transferrin to carry iron is finally exceeded by increased iron absorption, suppressed hepcidin causes the synthesis of non-transferrin-bound iron, which is not useful for erythropoiesis (10).

Hemoglobin, an oxygen-binding protein found in erythrocytes, is in charge of transporting oxygen from the lungs to tissues. Each hemoglobin molecule has a tetramer structure comprised of four polypeptide globin chains. Each globin subunit consists of a heme moiety, which is composed of an organic protoporphyrin ring and a core iron ion in the ferrous state (Fe+2). The iron molecule found in each heme moiety can bind and unbind oxygen, allowing the organism to transfer oxygen. The most common type of hemoglobin in adults is HbA, which is made up of two alpha- and two beta-globin subunits. Different globin genes encode different types of globin subunits (11). Because there are either too few beta-globin chains (β+) or none at all (β0) in the bone marrow of people with beta-thalassemia major, an excess of unbound alpha globin chains precipitates in erythroid precursors, leading to early death and inefficient erythropoiesis. Thalassemia major is peripheral hemolysis, which contributes to anemia and happens when insoluble alpha globin chains rupture the membranes of peripheral erythrocytes (12).

Iron is necessary for the health of cells. It is found in proteins that carry out several functions such as oxygen transport, biomolecule synthesis, respiration, and homeostasis. Numerous proteins involved in cell cycle development, nucleic acid metabolism, and repair require iron as a vital component(13). Because iron is so important in anatomy and physiology and because it has a limited bioavailability, the body's iron stores are strictly controlled to promote conservation and reduce toxicity (14). Transferrin, a binding protein, transports serum iron. The ability of the blood to bind iron with the transferrin is called total iron binding capacity. The serum iron is deducted from the TIBC to determine the UIBC(15). Transfusion-dependent thalassemia patients usually have more rapid iron loading due to the high iron concentration of transfused cells. Accumulation of iron in the liver, heart, and endocrine organs is the primary cause of the majority of serious disorders. Iron overload in beta-thalassemia patients is the main cause of death from heart disease (16).

The Ferritin protein, which contains iron, is very symmetrical and stable. It was crystallized, given the name Ferritin, and discovered in 1937. It is known as the major iron storage protein because it has a large cavity that can store a lot of iron (17). In Beta-thalassemia, Iron transporters interact with transferrin after absorbing iron from various parts of the small intestine. Transferrin is then stored in the reticuloendothelial cells of the spleen, liver, and bone marrow, where it binds with hemosiderin and ferritin. This explains the high level of Ferritin in patients with thalassemia major, which is considered one of the diagnostic signs of thalassemia major (18).

The Materials and Methods

The Subjects

The study was conducted on patients who were clinically, and laboratory diagnosed with Beta-thalassemia Major, and who attended the Thalassemia Unit of Al-Suwaira General Hospital/ Wasit Health Department in Wasit Governorate/Iraq. The samples and all information were taken from the patients, as well as healthy people were selected for the study. Laboratory tests were carried out in the laboratories of the Clinical Biochemistry Branch in the College of Medicine at the University of Al-Qadisiyah. Some laboratory tests were conducted in the Clinical Chemistry Unit / Laboratory Division of AL-Suwaira General Hospital. Ninety individuals participated in the study between November 2022 and March 2023 (for sample collection) and were divided into two groups. The first group G1 included Forty-five people who were patients with Beta-thalassemia Major disease and were selected from the thalassemia ward at Suwaira General Hospital after confirming their clinical and laboratory diagnoses. The second group G2 included Forty-five healthy people who did not have any disease and they were confirmed after conducting all the required laboratory analyses.

The Blood Samples Collection

Five milliliters of blood were extracted from each patient's vein and put into two test tubes, one milliliter in the EDTA for Gene polymorphism and four milliliters in the Gel tube for biochemical analysis. The whole blood was processed and subjected to the necessary analyses directly. While the blood samples in gel tubes had been centrifuged for ten minutes at a force of 3000 x g to obtain a sample (serum), which was then kept in three separate Eppendorf tubes at -20 C in the freezer until the time of the analysis (19).

The Detection of Ferritin, Iron, UIBC, TIBC, Hemoglobin, and Hepcidin Concentrations

The Serum Ferritin of all the subjects was measured by the ELFA technique (Enzyme Linked Fluorescent Assay). The serum Iron and TIBC were measured by using a spectrophotometer and UIBC was calculated by the equation Unsaturated iron binding capacity = Total iron binding capacity – Total Iron(20). Hb was measured by a Hematology analyzer (Complete blood count analysis). The Serum Hepcidin levels were measured via sandwich ELISA.

The Statistical Analysis

The statistical analysis was performed using version 25 of the Statistical Package for the Social Sciences (SPSS) from IBM on Windows® platform. Continuous variables were represented by mean and standard deviation. The comparison between the Beta thalassemia major patients group and the healthy group was conducted using the analysis of variance student T-test, and a P-value of ≤ 0.05 indicated a statistical significance. The strength and direction of the correlation were measured by the Pearson correlation coefficient (r) value, with significant association indicating the direction of the correlation. The Pearson correlation coefficient (r) measured the strength and direction of the association between two variables. A weak correlation was indicated by an R-value less than 0.5, a moderate correlation was indicated by an R-value between 0.4 and 0.7, and a strong correlation was indicated by an R-value greater than 0.7.

The Results

The Results of Biochemical Markers

The results revealed that the (mean ± SD) of ages and genders among the participants in G1 and G2 were not significant at (P. value >0.05). The (mean ± SD) of Hb and serum levels of Iron, UIBC, TIBC, Hepcidin concentration, and BMI for G1 and G2 were statistically significant at (P. value ≤ 0.05) this is illustrated

in Table (1).

The Correlation of Hepcidin Levels with Some Biochemical Markers in G1 and G2

This correlation is illustrated in Table (2) where *in G1 the results of the correlation showed a non-significant negative correlation of Hepcidin with TIBC and UIBC. while there was a non-significant weak correlation between Hepcidin with Hb and Ferritin. The results also showed a non-significant positive correlation between Hepcidin and Iron as in Figure (1).

*In G2, the results of the correlation showed a highly significant weak correlation of Hepcidin with Ferritin at ($P \leq 0.01$). However there was no significant negative correlation between Hepcidin with Iron, and the results showed a non-significant weak correlation between Hepcidin and Hb, TIBC, and UIBC as in Figure (2).

The Discussion

It is important to ensure that there was no significant difference in the patients' age and gender between the Beta-thalassemia patients and the healthy group. This is because any such difference could lead to variations in the results. However, after analyzing the data, it was found that the mean age and variance in gender between the two groups were not statistically significant with a P-value greater than 0.05.

In this study, the body mass index (BMI) had decreased in G1 compared to G2. The reasons for the low BMI in the patients with Beta-thalassemia Major were due to several reasons, of which Malabsorption, many people with Beta-thalassemia Major had an enlarged spleen, which can lead to malabsorption of nutrients from food. This could result in a reduced intake of calories and nutrients, leading to a low BMI (21). Moreover, the increased energy expenditure, where people with Beta-thalassemia Major have an increased energy expenditure due to the increased production of red blood cells in the bone marrow. This can result in a higher caloric requirement, which can be difficult to meet, especially if there is malabsorption (22,23).

In this study, the hemoglobin level had decreased in G1 compared to G2. In a previous study, it was found that the reduced hemoglobin production was due to the production of hemoglobin being impaired in Beta-thalassemia Major patients due to the lack of beta-globin chains. This leads to a reduced number of functional red blood cells and, therefore, a decrease in the total amount of hemoglobin in the blood (24). A previous research also indicated that hemolysis has a clear effect on a decrease in hemoglobin levels which hemolysis refers to the breakdown of red blood cells. In Beta-thalassemia Major patients, the abnormal red blood cells are destroyed at a faster rate than normal, leading to a condition called hemolytic anemia. This results in a decrease in hemoglobin levels as well (25,26). Studies also showed that iron overload has a clear effect on the level of hemoglobin in the blood in patients with Beta-thalassemia Major since the body is not able to use the excess iron in the absence of beta-globin chains, it accumulates in the body and can cause organ damage and this can lead to a decrease in hemoglobin levels as well (27).

In this study, the iron levels had been increased in G1 compared to G2. The ineffective erythropoiesis that occurred in Beta-thalassemia Major led to increased absorption of iron from the gastrointestinal tract due to the increased expression of the iron transport protein, divalent metal transporter 1 (DMT1), in the duodenum. A previous study showed that increased expression of DMT1 in the duodenum of Beta-thalassemia Major patients was mediated by the transcription factor, hypoxia-inducible factor 2α (HIF-2α). HIF-2α is activated in beta-thalassemia major due to chronic hypoxia caused by ineffective erythropoiesis. HIF-2α induces the expression of DMT1, which leads to increased iron absorption from the diet and increased iron levels in the body (28). Additionally, the frequent blood transfusions that are required to manage the anemia in Beta-thalassemia Major patients can lead to iron overload, Beta-thalassemia Major are also at risk for iron overload due to the frequent blood transfusions they require. Blood transfusions contain iron, which can accumulate in the body over time and lead to organ damage (29).

In this study, TIBC and UIBC levels had decreased in G1 compared to G2. Transferrin is a protein that binds to iron and transports it throughout the body. Total Iron Binding Capacity (TIBC) and Unbound Iron Binding Capacity (UIBC) are measures of the body's ability to bind and transport iron through transferrin (30). Studies have shown that in beta-thalassemia major, there is an increased demand for iron due to the production of new red blood cells. However, the decreased production of beta-globin chains leads to ineffective utilization of the absorbed iron, resulting in an excess of unbound iron in the blood. This excess iron can cause tissue damage and other complications (31). To counteract this excess iron, the body produces more transferrin, which binds to the iron and transports it to the tissues. As a result, TIBC and UIBC levels can decrease in Beta-thalassemia Major, as there is less unbound iron available for transferrin to bind. This decrease in TIBC and UIBC is a compensatory mechanism to prevent tissue damage that is caused by excess iron (32).

In this study, the Ferritin levels had been increased in G1 compared to G2. Ferritin is an intracellular protein that plays a critical role in iron homeostasis by storing excess iron in a non-toxic form. The concentration of ferritin in the blood is directly proportional to the amount of iron stored in the body. In Beta-thalassemia Major, the excessive accumulation of iron in the body results in high ferritin levels (33,34). The studies showed that serum ferritin levels correlate with the severity of iron overload in Beta-thalassemia patients. The high levels of ferritin in these patients can lead to organ damage, particularly in the heart, liver, and endocrine systems, which can significantly affect their overall health and quality of life(35,36).

In this study, the Hepcidin level had decreased in G1 compared to G2. The decrease in hepcidin level in the Beta-thalassemia Major can be attributed to several factors. Ineffective erythropoiesis, which is a hallmark of Beta thalassemia Major, leads to the release of large amounts of erythroferrone (ERFE) into circulation. ERFE is a hormone that suppresses hepcidin expression in the liver, leading to increased iron absorption and release from macrophages to support erythropoiesis (37). The iron overload that occurs in the Beta-thalassemia Major can also play a role in the decrease in Hepcidin level. Iron overload leads to increased expression of transferrin receptor 2 (TfR2) on hepatocytes, which enhances hepcidin expression. However, this effect is blunted in Beta-thalassemia Major due to the increased expression of erythroferrone (38,39). Another study showed that the ERFE binds to the bone morphogenetic protein (BMP) co-receptor hemojuvelin (HJV) and prevents BMP signaling, which is necessary for hepcidin expression (40).

The present study indicated that there was a weak correlation between hecidin protein levels with hemoglobin and Ferritin levels in G1. Hepcidin is a key regulator of iron metabolism, and its deficiency could lead to increased iron absorption from the gastrointestinal tract and decreased release of iron from macrophages. This could contribute to the development of iron overload and anemia in patients with Beta-thalassemia Major. This study showed a positive correlation between hepcidin with Iron in G1. Patients with this disease require regular blood transfusions to manage their anemia, which can lead to iron overload in the body (41). In Beta-thalassemia Major, chronic

transfusion therapy leads to increased iron absorption in the gut and deposition of iron in various organs of the body, including the liver, heart, and endocrine glands (42). However, the patients in this study also had low levels of Hepcidin in their blood. The results of this study showed a negative correlation of hepcidin with TIBC and UIBC in G1. The results of the correlation showed a weak correlation between Hepcidin and Ferritin in G2. In the healthy individuals, there was a correlation between hepcidin and ferritin levels. Hepcidin levels are usually high when iron stores are sufficient or elevated, and low when iron stores are depleted this is because hepcidin regulates the release of iron from ferritin in response to the body's iron needs. When iron stores are low, the body produces less hepcidin. Conversely, when iron stores are high, the body produces more hepcidin (43,44). This corresponds with the results of the current study which revealed a negative correlation between hepcidin with Iron in G2. Hepcidin plays a crucial role in maintaining iron homeostasis in healthy individuals, ensuring that iron levels are maintained within a narrow range compatible with optimal body function(45). In healthy individuals, the relationship between hepcidin and iron is one of balance. In the present study, the results demonstrated a weak correlation between hepcidin and Hb, TIBC, and UIBC in G1. In healthy individuals, the regulation of hepcidin, Hb, TIBC, and UIBC is tightly controlled to maintain iron homeostasis. Hepcidin levels are influenced by various factors, including iron stores, erythropoietic activity, inflammation, and hypoxia (46,47). In healthy individuals, the regulation of Hepcidin and Iron metabolism is finely tuned to ensure that Iron is available for essential physiological processes while preventing iron overload or deficiency (48). The correlation between Hepcidin and Hb, TIBC, and UIBC in healthy individuals is therefore dynamic and subject to the changing needs of the body for Iron. The results of the present study were in correspondence with what was mentioned above.

The Conclusions

The study investigated the impact of Beta-thalassemia Major, a genetic condition characterized by reduced or absent beta-globin chains in hemoglobin resulting in severe anemia. The results showed a marked reduction in hemoglobin production and an increase in Iron and Ferritin levels, while the concentrations of TIBC and UIBC were observed to be decreased in the individuals with this disease compared to the healthy individuals. The study results revealed a low hepcidin concentration in the Beta-thalassemia Major patients compared to the healthy subjects. In this study, the results of the correlation between Hepcidin and other markers in the Beta-thalassemia patients group showed a non-significant negative correlation of Hepcidin with TIBC and UIBC, while there was a non-significant weak correlation between Hepcidin with Hb and Ferritin. Moreover, there was a non-significant positive correlation between Hepcidin and Iron.

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Tables

Table 1: The Demographic Characteristics and Biochemical markers Between G1 and G2

Biochemical Parameters	G1 No. 45 (Mean ±SD)	G2 No. 45 (Mean ±SD)	P-value
Age	13.47 ± 8.31	13.79 ± 9.69	P = 0.865
Gender	1.44 ± 0.5	1.53 ± 0.5	P = 0.405
BMI(Kg/m^2)	18.38 ± 2.75	19.93 ± 3.80	P = 0.029*
Hemoglobin(g/dl)	8.31±1.19	12.85±1.02	P = 0. 0001**
Ferritin(ng/ml)	2963.36±2401.94	52.96±21.89	P = 0. 0001**
Iron (mmol/L)	35.37±9.14	14.59±3.91	P = 0. 0001**
TIBC (mmol/L)	52.53±5.83	63.72±9.52	P = 0. 0001**
UIBC (mmol/L)	17.53±9.99	49.44±8.52	P = 0. 0001**
Hepcidin (pg/ml)	1146.20±202.17	1668.87±460.18	P = 0. 0001**

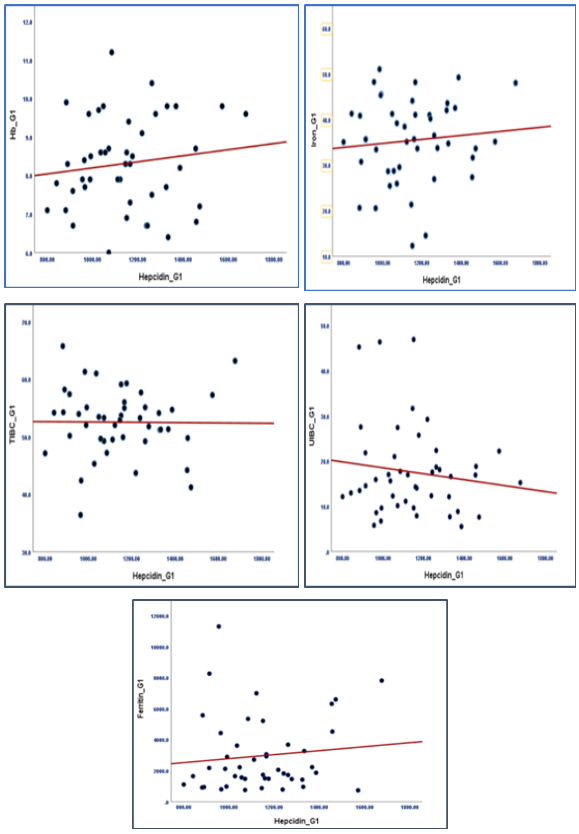
** highly significant (P.≤٠,٠١), *significant (P.≤٠,٠٥), SD (standard deviation), Independent T-test.

Table 2: The correlation of hepcidin level with some biochemical markers in G1 and G2

Biochemical markers	G1		G2	
	r-value	P-value	r-value	P-value
Hb	0.134	0.381	0.146	0.339
Iron	0.97	0.525	-0.081	0.595
TIBC	-0.10	0.950	0.136	0.372
UIBC	-0.133	0.384	0.084	0.585
Ferritin	0.108	0.479	0.395**	0.007

**At a (P ≤ ٠,٠١), a correlation is considered statistically highly significant (-٢tailed).

Figures



Figures (1): Correlation of hepcidin concentration with some Biomarkers in G1.

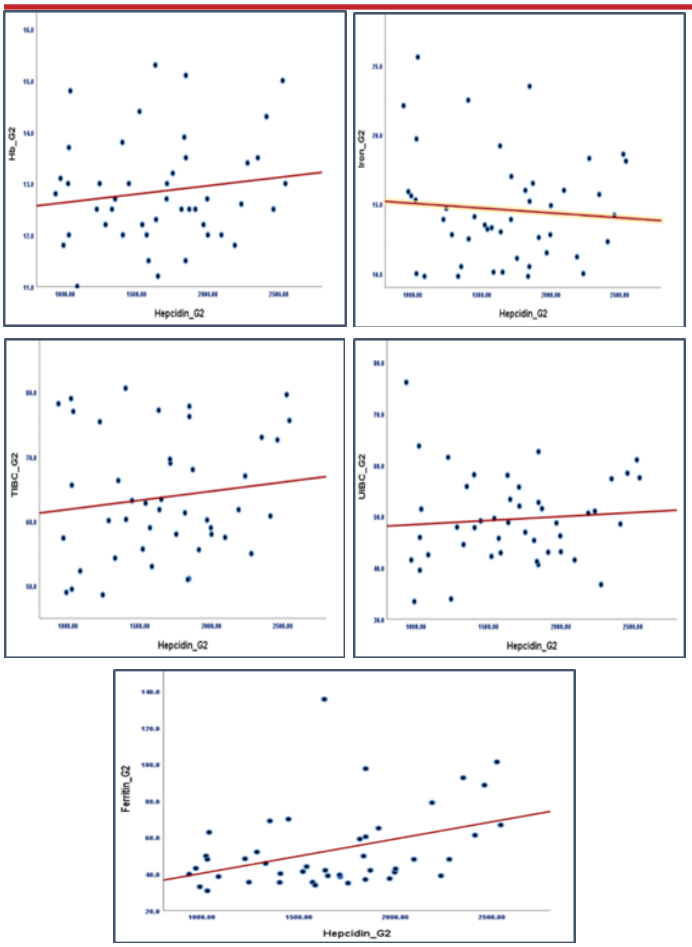


Figure (2): Correlation of hepcidin concentration with some Biomarkers in G2.

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