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THE RELATIONSHIP OF HEPCIDIN AND SOME BIOCHEMICAL IN PATIENTS WITH B-THALASSEMIA

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THE RELATION OF HEPCIDIN AND SOME BIOCHEMICAL IN PATIENTS WITH B-THALASSEMIA

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May 2023

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ABSTRACT

THE RELATION OF HEPCIDIN AND SOME BIOCHEMICAL IN PATIENTS WITH B-THALASSEMIA

Mohammed Yasir Hasan ALMUSAWI

Master of Science in Chemistry

Advisor: Assoc. Prof. Dr. Saliha ALYAR

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May 2023

This case-control study was conducted in March-July 2022 at the Thalassemia and Genetic Blood Diseases Center in Thi-Qar Governorate on 55 patients with beta-thalassemia in addition to 55 healthy-appearing controls as a control group. hepcidin, ferritin and iron were evaluated. Hemoglobin, cultured cell size, aspartate aminotransferase, alanine aminotransferase, hepcidin hormone to determine the nature of the relationship between albumin and copper, and an indicator of the time of diagnosis in these producer thalassemia cells and the disease of this consumption. The current study revealed that the life expectancy and gender of beta-thalassemia patients differed significantly from the life expectancy of the control groups. The results revealed that the two groups tested showed hepcidin levels in their serum blood. The relationship between the ferritin bed in serum blood and β-thalassemia disease showed that the ferritin elevation spreads by drinking the patient environments relative to the control group. The findings revealed that the patient group was significantly superior in serum blood iron level when faced with the control group, with the patient group recording 203.13 and the control group 86.20. The results of the weight between the hemoglobin levels in the blood of the two groups tested (control group and patient group) showed a significant level of patient-desired hemoglobin elevation compared to the control group; the patient group recorded 7.88% when they were the control organs. 13.42% registered group. The results of the measurements between the ALT gate and β-thalassemia recorded that the ALT gate in the patient group was excel compared to the control group, patient groups 41.17 U/L, control cells 14.50 U/L. The results showed

that the patient group was superior to the control group in AST levels, the patient group recorded 44.14 U/L and the control group recorded 23.67 U/L. The extreme results between albumin level and thalassemia disease showed that the tested parameter (albumin) showed in our study in the mean of the two groups. The results of the copper elevations in the blood of the two tested groups (control and patient group) showed that the patient group had a significant generation in copper clusters compared to the control group, and the patient group recorded 12,7125 μmol/. L recorded 18.0845 μmol/L in group control. Results showed that hepcidin gate in blood did not measure overall with ferritin and iron (P = < 0.001**, r = -0.899), (P = < 0.001 ** , r = -0.823), respectively. A strong positive test was found between hepcidin and hemoglobin levels in the blood (P = 0.001**, r = 0.843). These results also showed positive evidence between serum hepcidin and albumin levels (P = 0.001**, r = 0.356**). In addition, there was a significant weight between the serum presence of hepcidin patients and the presence of blood copper (P=0.001, r=0.693). This study found no association between serum levels of hepcidin, aspartate aminotransferase and alanine aminotransferase (P<0.001**, r = -0.862**), (P<0.001**, r = -0.752), respectively.

2023, 72 pages

Keywords: β-Thalassemia, Hepcidin, Ferritin, Iron, Hemoglobin, Packed cell volume

ÖZET

B-TALASEMİLİ HASTALARDA HEPSİDİN VE BAZI BİYOKİMYASALLAR ARASINDAKİ İLİŞKİ

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Mayıs 2023

Bu vaka kontrol çalışması Mart-Temmuz 2022'de Thi-Qar Valiliği'ndeki Talasemi ve Genetik Kan Hastalıkları Merkezi'nde 55 beta-talasemili hasta ve kontrol grubu olarak 55 sağlıklı görünen kontrol üzerinde yapılmıştır. hepsidin, ferritin ve demir değerlendirildi. Hemoglobin, kültürlenmiş hücre boyutu, aspartat aminotransferaz, alanin aminotransferaz, hepsidin hormonu albümin ve bakır arasındaki ilişkinin doğasını belirlemek için ve bu üreticiler talasemi hücrelerinde teşhis zamanı ve bu hücrelerin hastalığın bir göstergesidir. Mevcut çalışma, beta-talasemi hastalarının yaşam beklentisi ve cinsiyetinin, kontrol gruplarının yaşam beklentisinden önemli ölçüde farklı olduğunu ortaya koydu. Sonuçlar, test edilen iki grubun serum kanlarında hepsidin seviyeleri gösterdiğini ortaya koydu. Serum kanındaki ferritin yatağı ile β-talasemi hastalığı arasındaki ilişki, ferritin yüksekliğinin kontrol grubuna göre hasta ortamlarında içilerek yayıldığını göstermiştir. Bulgular, hasta grubunun 203.13 ve kontrol grubu 86.20 ile kontrol grubu ile karşı karşıya kaldığında serum kan demir düzeyinde anlamlı olarak üstün olduğunu ortaya koydu. Test edilen iki grubun (kontrol grubu ve hasta grubu) kanındaki hemoglobin düzeyleri arasındaki ağırlık sonuçları, kontrol grubuyla karşılaştırıldığında önemli düzeyde hasta tarafından istenen hemoglobin artışı gösterdi; hasta grubu kontrol organı olduklarında %7,88 olarak kayıt yapmıştır. %13,42 kayıtlı grup. ALT kapısı ile β-talasemi arasındaki ölçüm sonuçları, hasta grubunda ALT kapısının kontrol grubuna göre üstün olduğunu kaydetti, hasta grupları 41,17 U/L, kontrol hücreleri 14,50 U/L. Sonuçlar, hasta grubunun AST düzeylerinde kontrol

grubuna göre üstün olduğunu, hasta grubunun 44,14 U/L ve kontrol grubunun 23,67 U/L kaydettiğini gösterdi. Albümin düzeyi ile talasemi hastalığı arasındaki uç sonuçlar, test edilen parametrenin (albumin) çalışmamızda iki grubun ortalamasını gösterdiğini gösterdi. Test edilen iki grubun (kontrol ve hasta grubu) kanlarındaki bakır yükselmelerinin sonuçları, hasta grubunun kontrol grubuna kıyasla bakır kümelerinde önemli bir jenerasyona sahip olduğunu ve hasta grubunun 12,7125 µmol/ kaydettiğini gösterdi. L, grup kontrolünde 18.0845 µmol/L kaydetti. Sonuçlar kandaki hepsidin kapısının genel olarak ferritin ve demir ile ölçüm yapmadığını gösterdi (P = < 0.001**, r = -0.899), (P = < 0.001 ** , r = -0.823). Kandaki hepsidin ve hemoglobin seviyeleri arasında güçlü bir pozitif test bulundu (P = 0.001**, r = 0.843). Bu sonuçlar ayrıca serum hepsidin ve albümin seviyeleri arasında pozitif kanıt gösterdi (P = 0.001**, r = 0.356**). Ayrıca hepsidin hastalarının serum varlığı ile kan bakır varlığı arasında anlamlı bir ağırlık vardı (P=0.001, r=0.693). Bu çalışma, sırasıyla hepsidin, aspartat aminotransferaz ve alanin aminotransferaz (P<0.001**, r = -0.862**), (P<0.001**, r = -0.752) serum seviyeleri arasında bir ilişki bulmadı.

2023, 72 sayfa

Anahtar Kelimeler: β-Talasemi, Hepsidin, Ferritin, Demir, Hemoglobin, Paketlenmiş hücre hacmi

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Mohammed Yasir Hasan ALMUSAWI
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CONTENTS

LIST OF SYMBOLS

-	Minus
%	Percent
**	Significant
/	Divide
+	Plus
<	Greater than
=	Equal
>	Less than
±	Plus minus
≤	Greater or equal to
≥	Less or equal to
Ca	Calsium
Cu	Copper
dL	Deciliter
g	Gram
kg	Kilogram
L	Liter
m^2	Square meter
µg	Microgram
Mg	Magnesium
mg	Milligram
mIU	Milli-international units
min	Minute
mL	Milliliter
mmol	Millimole
mol	Mole
ng	Nanogram
nm	Nanometer
NS	Non-significant
rpm	Revolutions per minute
Se	Selenium
Zn	Zinc
µL	Microliter

LIST OF ABBREVIATIONS

ALT	Alanine transaminase
AST	Aspartate aminotransferase
BTM	β-thalassemia major
CAB	Chromazurol B
CBC	Complate lood count
CMIA	Chemiflex immunoassay
CTMA	Cetyltrimethylammonium-bromide
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
Hb	Hemoglobin
HSCT	Hematopoietic stem cell transplantation
LD	Lactate dehydrogenase
MDH	Malate dehydrogenase
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide dehydrogenase
OD	Measure the absorbance
PCR	Polymerase chain reaction
PCV	Packed cell volume
Rb	Reagent blank
RBC	Red blood cells
RLUs	Relative light units
ROS	Reactive oxygen species
SGOT	Serum glutamate- oxaloacetate transaminase
SGPT	Serum glutamate-pyruvate transaminase
SGPT	Glutamate-pyruvate transaminase
SOD	Superoxide dismutase enzyme
STD	Standard
TM	Thalassemia major
D.W	Distilled water

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1. INTRODUCTION

The β-thalassemias are a collection of genetic illnesses with various degrees of hemoglobin production that are recessively inherited (Sardar *et al.* 2021). The distinctive characteristic of patients with -thalassemia is globin chains are produced unevenly, as the chain is synthesized at a normal rate offset by a lack of the manufacture of the –chain, and this results in an increase in the alpha chain. A hereditary condition that affects the production of hemoglobin called thalassemia that is widespread throughout the world, especially in the Mediterranean, tropical and subtropical regions, the Caucasus region, Central Asia, and Southeast Asia (Swee 2018).

Of the two various types, of -thalassemia beta & Alph, B-thalassemia, is the most common type, that results in severe anemia. This is due to a loss of balance in the produce of globin chains, the synthesis of inactive RBCS, and hemolysis, which results in varying degrees of anemia blood (Galanello and Origa 2010).

Clinically, beta-thalassemia is divided into three categories specifically depends definitely on the intensity of the situation: The three types of thalassemia are major, which demands monthly blood transfusions for the rest of one's life, intermediate, which also causes anemia but is less harmful, and minor, or trait, which is thought to be symptomatic (Israa Imad Jabbar 2021). Hemoglobin is made up of four protein molecules, pair of which are chains and the others are -chains. The gene for -globin is found on chromosome 16, not chromosome 11, as is the case with the gene for -globin (Mehdi and Al Dahmash 2011).

The molecular makeup of beta-thalassemias varies. So far, more than 200 mutations that cause disease have been found. Single nucleotide substitutions, oligonucleotide deletions, and insertions that cause frameshift make up the majority of mutations. Gross gene loss only very infrequently causes beta-thalassemias (Galanello and Origa 2010).

The amount of B -globin chains synthesized is decreased in, B-thalassemia, which causes, an increase in alpha-chain production. As a results of the increases in, the production of hemoglobin without beta chains, the free alpha chains remain in the form of alpha-type (alpha4) tetramers, which causes their deposition in RBCS, and an early dissolution of RBCS (Sarnaik 2005).

Iron overload is a serious complication that affects thalassemia patients and can be brought on by the disease itself or by recurrent blood transfusions (Hasoon *et al.* 2020).

Beta-thalassemia patients have been shown to have elevated levels of various liver enzyme markers, which suggests a problem with liver function. In people with thalassemia, an excess of iron first affects the liver (Wanachiwanawin *et al.* 2003).

Hepcidin controls the iron homeostasis process, a hepatic peptides- hormone. Hepcidin regulate- the distribution of iron in tissues, (Fe) absorption from the diet and the level of (Fe) in plasma. In iron-loading anemias like -thalassemia, Iron excess is primarily or partially caused by a lack of hepcidin. Hepcidin deficit is caused by the high erythropoietic activity in -thalassemia patients (Nemeth *et al.* 2004).

1.1 The Aims of Thesis

1. Accurately measure the concentration of hepcidin, ferritin, Iron, hemoglobin, packed cell volume (PCV), Copper, and liver functions.
2. Identification of the potential association that exists between hepcidin and each of the characteristics that were evaluated.

2. LITERATURE REVIEW

2.1 B -Thalassemia

B -Thalassemia is a blood disease that results from a genetic flaw that prevents the hemoglobin molecule from developing properly. Common symptoms include anemia, problems with blood transfusion, liver or cardiac problems, and psychological impacts (Sardar *et al.* 2021).

There have been 350 reports of -thalassemia mutations so far. Gene (-globin) mutations are the most frequent source of genetic diseases in people (De Sanctis *et al.* 2017). The problems that it produces have a negative impact not only on the general health, academic performance, and mental health status of individuals who are afflicted by it, but also on the quality of life, relationships, and other aspects of their lives (Ansari *et al.* 2014).

Since thalassemia is a genetic condition, at least one of a patient's parents must carry the disease-causing gene. A mutated gene or the removal of a few essential gene segments is likely to be the underlying cause of this condition. The removal of the alpha-globin gene is what causes alpha thalassemia, which in turn leads to the development of fewer or no α-globin chains in affected individuals. Point mutations in the beta-globin gene are responsible for the inheritance of beta-thalassemia (Nashwan *et al.* 2018).

2.1.1 Thalassemia forms

People with thalassemia can receive treatment based on the severity of their illness. Thalassemia is a hereditary condition in which the production of hemoglobin is incorrect. Thalassemia mostly comes in two forms, which are further divided into subtypes. While some moderate types of thalassemia may go undiagnosed, causing only mild anemia and iron deficiency in people, other, thalassemia in more severe kinds can be fatal (Helmi *et al.* 2017). Thalassemia is divided into and – thalassemia depending on

the underlying genetic mutation, and affected globin-chain components within the Hb tetramer. (Piel and Weatherall 2014).

2.1.2 α-thalassemia

The body makes less alpha-globin due to the inherited blood disorder alpha thalassemia. Alpha-globin is a component of hemoglobin. Hemoglobin, a component of red blood cells (RBC), carries oxygen throughout the body. The body doesn't have enough RBCs due to the drop in alpha globin, which might also create various health issues. Anemia can range in severity from very mild to extremely severe, depending on the kind of alpha thalassemia (Corinna 2022).

2.1.3 β-thalassemia

A deficiency in the formation of the globin-chain is the root cause of several hereditary blood disorders. There is a wide range of phenotypes, from those with no symptoms to those with severe anemia (Galanello and Origa 2010).

Due to a defect in the manufacturing process of α-globin, buildup of chains that combine to create identical tetramers occurs. homotetramers that are prone to instability and have a detrimental effect on the formation of Hb and RBC_S subunits. In addition, these chains are laid down (Israa Imad Jabbar 2021).

2.1.4 Kinds of beta-thalassemia

1- Silent

The beta type of thalassemia, which is considered to be the most common kind, may be passed down via asymmetric marriages. This form of the disease is difficult to distinguish from other forms of anemia because the patients does not exhibit any clinical signs (Galanello and Origa 2010).

2- Minor

The inheritance of a non-beta gene results in the mildest type of beta-thalassemia. Patients with homozygous globin deficiency, which causes a little decrease in globin production, do not usually need treatment for their clinical symptoms (Weatherall and Clegg 2008).

3 - Beta intermedia

High that it causes quite extreme anemia and serious, health issues for the person, including spleen enlargement and bone abnormalities. At this point, there are many different symptoms (3-5). It may be difficult to distinguish between intermediate thalassemia and major or big thalassemia due to a modest change in symptoms. The patient is classified as having the main thalassemia group because of their need for blood transfusions. A blood transfusion is necessary for intermediate thalassemia patients to enhance their quality of life rather than to ensure their survival (Fathi, A *et al.* 2019).

4- Beta major

The most difficult form of thalassemia. Nemia arises as a result of a lack of -globin synthesis, which prevents the formation of large levels of HbA. Afterward, extra unpaired alpha-globin chains precipitate inside RBCs, damaging their plasma membranes and causing intravascular hemolysis. Early erythroid precursor death, apoptosis, lysis, necrosis further reduces the RBC count. Erythropoietin (EPO), which is produced as a result of severe anemia, triggers increased medullary hematopoiesis as a result of the hypoxia it causes (Cunningham 2010).

During delivery, the baby with severe thalassemia seemed to be in good health. The newborn's gamma to beta globulin changes, anemia appears a few months after birth, the child's growth is stunted, and he or she frequently has problems (due to the body's poor ability to absorb oxygen when there is major anemia), fever episodes (due to severe illness), sluggish bowel movements, and other digestive disorders that, if untreated, can lead to serious complications). Before the age of twenty, the sickness results in death (Hay and Weatherall 2017).

2.1.5 Distribution of β-thalassemia

Thalassemias, which are genetic defects that slow down the synthesis of hemoglobin's(Hb) -globin chains, are extremely common in the nations of the Eastern Mediterranean, including Iraq. In different regions of Iraq, Variables can affect the -thalassemia (-thal) carrier status predominance between 3.7 and 4.6%, and -thalassemia major (-TM) places a significant strain on the already underfunded health services. The latter makes starting a preventive program a need rather than an option because of it as well as the pain of the patients and their families (Fathi *et al.* 2019). One of the requirements for starting such a program is to identify the molecular causes of this illness in various regions of the nation. While numerous researchers have looked into the genetic causes of –that among Kurds in Northern Iraq (Al-Allawi *et al.* 2013).

Figure 2.1 The prevalence of -thalassemia in the worlds, according to geographic location. The thalassemia- prevalent countries are indicated by black spots

2.1.6 Pathophysiology of β-thalassemia

Due to the underlying genetic abnormality that causes beta-thalassemia, there is either no (o) or inadequate (+) beta chain synthesis; nevertheless, the formulation of the alpha chain happens at a normal rate. The reduced creation of -chains in adults results in a lower production of hemoglobin (HbA:2 2); this is the first consequence. Another result is something called imbalanced globin chain formation, which may take place even when chain synthesis is proceeding at the typical pace. In this particular instance, the erythrocytes have an excessive amount of chain. Large intracellular inclusions are formed as a consequence of the precipitation of unstable excess chains in the red cell progenitors that are found in the bone marrow. These inclusions prevent red cells from maturing, functioning normally, and living (Weatherall 2000, Elizabeth and Ann 2010).

Because these intracellular inclusions prevent red cells from maturing normally, this leads to the intramedullary death of red cell progenitors, which in turn hinders the operation of the erythropoiesis process. Adult red blood cells that leave the bloodstream have chain inclusion that stop them from, moving through the microcirculations, particularly in the spleen, red cell extramedullary elimination has become the norm as a result of this phenomenon. Therefore, the anemia associated with - thalassemias is caused not only by insufficient erythropoiesis but also by a lower RBC survival rate (Weatherall and Hatton 2010, Ginzburg and Rivella 2011).

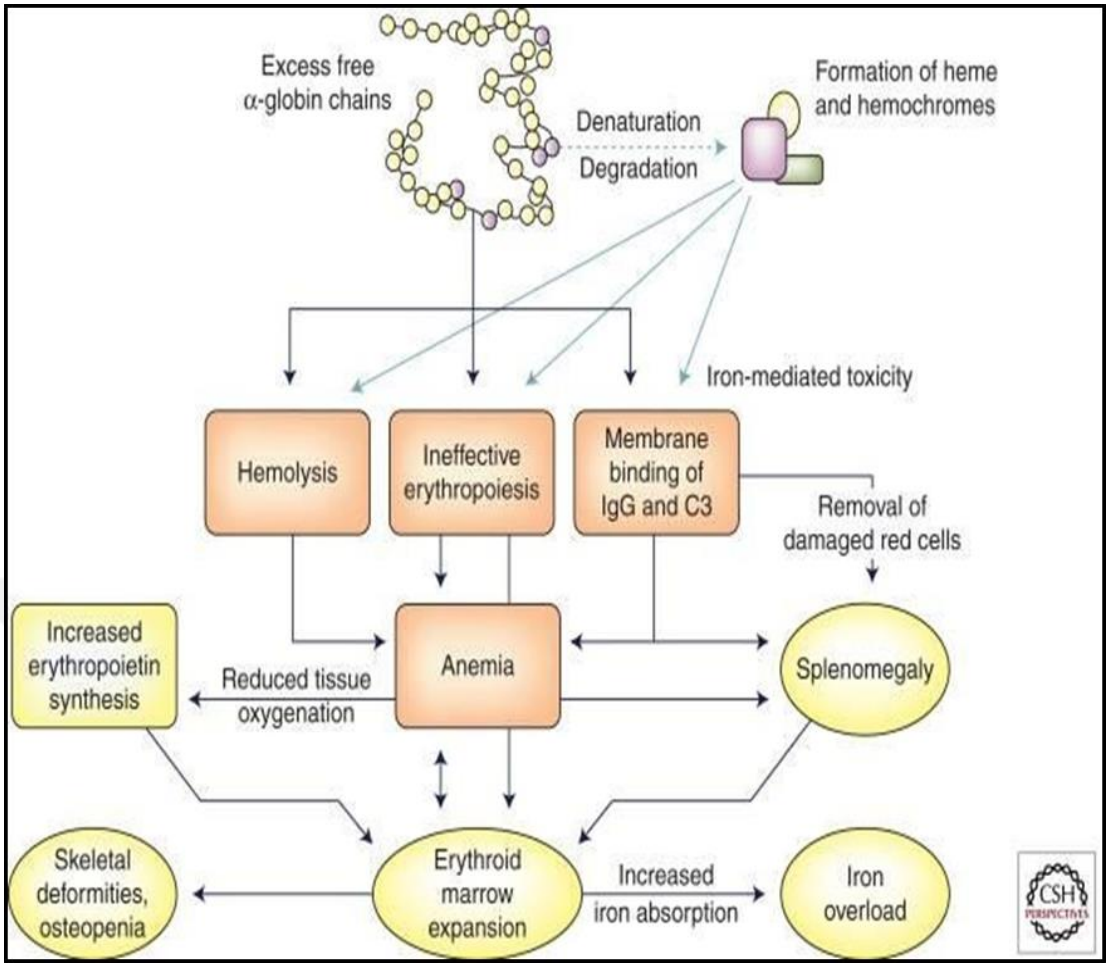

Figure 2.2 β-thalassemia pathophysiology

2.1.7 Complications

The following are some of the difficulties of beta thalassemia, particularly thalassemia major: The danger of injury and organ rupture is increased with spleen enlargement, which can be fatal. When the spleen becomes big and starts to press against other important organs, a splenectomy may be necessary to remove it. A splenectomy may raise the risk of infection because the spleen is crucial in preventing infection. Hepatitis, an enlarged liver, and liver scarring are among liver problems (cirrhosis). Joint discomfort, osteoporosis, and fracture susceptibility are all risks brought on by compromised bone marrow and bony abnormalities. Other concerns include sepsis following splenectomy, thromboembolic problems, and pulmonary hypertension (HBP in the lungs' blood vessels) (Taher and Cappellini 2018).

2.1.8 Diagnosis of β-thalassemia

Thalassemia is diagnosed by hemoglobin electrophoresis, which often reveals elevated serum iron levels, increased HbF and HbA2 of more than (3.5%) as well as elevated serum ferritin levels. The normal range for HbA2 is between (1.5 & 3.0%) (Eleftheriou 2008).

2.1.9 Clinical diagnosis

Thalassemia major is typically identified when children under the age of two present with severe anemia, mild jaundice, and enlarged liver and spleen. The clinical symptoms of thalassemia intermedia, which appear later in life and have similar but fewer signs, might cause moderate anemia in some persons (Galanello and Origa 2010).

2.1.10 Peripheral blood smear

Blood smears of affected patients show a change in the size and shape of RBC_S, known as anisocytosis, which can be small, large, hypochromic, or poikilocytosis, which can be teardrop-shaped or elongated. Red blood cell numbers are significantly increased after having your spleen removed, and this increase is inversely proportionate to the degree of anemia that you have (Galanello and Origa 2010).

2.1.11 Hematologic diagnosis

Patients with thalassemia are identified through a full blood count because their Hb levels are less than 7 g/dL (Galanello and Origa 2010).

2.1.12 Molecular diagnosis

It is feasible to use polymer reaction technology; one of the most recent methods for discovering thalassemia involves utilizing polymerase chain reaction (PCR) to

categorize variations in the gene for beta-globin, one of the most recent methods for locating the genes that cause thalassemia (Old *et al.* 2005).

2.1.13 Management

1- Protection

Since these disorders are more prevalent in parents' consanguinity, especially at the 1 degree level, who have acquired genetics and undergone prenatal testing, raising public awareness is essential for halting the spread of thalassemia disease in society. Examples of such measures include carrier detection scans, genetic counseling, and avoiding marriages with relatives (Peters *et al.* 2012).

2- Transfusions of blood

B -thalassemia(major) patients need to have frequent, blood transfusions once every two to three weeks for the remainder of their life (9.5 g/dL) to keep their bodies growing normally and to keep their hemoglobin levels above the normal range (Al Haddad 2012).

3- Transplantation of bone marrow

Transplanting hematopoietic stem cells is the only treatment for beta-thalassemia that has been proven effective. The major's long-term outcomes have been excellent. However, restricted bone marrow transplantation occurs frequently due to a lack of suitable donors and high costs (Gaziev *et al.* 2010).

4- Chelation treatment for iron

Thalassemia patients are those who have the condition, and use procedures to get rid of extra iron from their bodies, with blood transfusions as a result. Three categories of iron-repellent treatments are distinguished: Deferiprone, Deferoxamine, and Deferasirox (Saliba *et al.* 2015).

5- Splenectomy

Among the most dangerous side effects of increased RBCS breakdown is an enlarged spleen, which is why more than half of thalassemia patients (54-55%) have their spleens removed to decrease the requirement for blood transfusions and avoid a high rate of iron accumulation (Kurtoglu *et al.* 2012).

2.2 Hepcidin

2.2.1 Definition

It is a twenty five amino acid peptide-hormone that prevents the release of stored (Fe) from hepatocytes, recycled (Fe) from macrophage, and meal absorption in the duodenum. These three processes are the 3 main sources of (Fe) that are prevented from entering the plasma compartment (Ganz and Nemeth 2012).

2.2.2 Hepcidin's function in people with thalassemia

Iron-loading anemias that do not need periodic blood transfusions, including thalassemia intermedia, have much lower amounts of hepcidin in the urine and serum than other types of anemia (Nemeth, and Ganz 2006, Origa *et al.* 2007).

It seems that the quantity of iron content in many other organs and the pace at which iron is absorbed are connected. This condition has been connected to a rapid buildup of iron, which is comparable to what is found in extreme cases of hepcidin insufficiency. This condition has been connected to a significant accumulation of iron in the heart as well as in a number of the endocrine organs. Hepatocytes are quite efficient in absorbing iron as long as it is not coupled to ferritin. Because a lack of hepcidin allows for increased iron absorption, the most- common form of excess (Fe) in patients with TM is obtained via transfusions rather than through the absorption of iron from their food. Chronically transfused patients had noticeably higher hepcidin concentrations than non-transfused patients because of an increased iron load and enhanced erythropoiesis (Kearney *et al.* 2007).

2.2.3 Hepcidin regulates iron homeostasis

Hepatocytes secrete a little peptide hormone called hepcidin, which circulates in blood plasma and is eliminated through urination (Park *et al.* 2001).

It controls the amount of (Fe) in the plasma and how it is distributed across various tissues. Many iron problems are caused by dysregulation of the hepcidin production process. Hepcidin overproduction results in iron-restricted anemia over time (Roy *et al.* 2007).

Whereas (Fe) excess with iron accumulation in the liver and other tissues originates from a hepcidin deficit (Roetto *et al.* 2003).

Its receptor, ferroportin, which regulates intestinal (Fe) absorption, plasma iron concentrations, and tissue (Fe) distribution, is degraded by hepcidin. Extracellular and intracellular iron concentrations affect transcriptional regulation of hepatic hepcidin synthesis via a molecular complex of bone morphogenetic protein receptors. The need for iron in erythroid precursors for the manufacture of hemoglobin also controls the homeostatic regulation of hepcidin. Hepcidin production is controlled by inflammation, which is consistent with the role of hepcidin mediated (Fe) redistribution in host defense. In hereditary hemochromatosis, a lack of hepcidin results in (Fe) overload and inefficient erythropoiesis (Ganz and Nemeth 2012).

Hepcidin binds to ferroportin, causing internal processing and degradation of the receptor. When ferroportin is taken out of the membrane, cellular iron export is stopped, which reduces the amount of iron available to the plasma, on the other hand, when the quantity of hepcidin is low and ferroportin remains on the cell surface, macrophages have a greater capacity for absorbing iron and exporting it. If measures are not taken to prevent it, increased iron absorption will inevitably result in iron overload (Nemeth *et al.* 2004).

2.2.4 Iron

For the survival and development of humans, iron is essential. Even though the daily iron requirement for the average person is 13–18 mg, only (1-2mg) is absorbed. In the duodenum, where it then reaches the blood and is disseminated to other areas of the body for a variety of metabolic reasons, iron absorption happens largely. This is true regardless of the kind of iron that is taken. Dietary iron comes in two varieties: hem iron, which seems present in meats, and non-hem, which is primarily ferric (Fe3+) iron and is found in nuts, vegetables, and greens. Hem iron is the more common kind (Andrews 2000, Miret *et al.* 2003).

An iron storage protein called ferritin deposits iron in a safe region, stores it in an inert state, and then transfer it to where it is needed. The body's ability to store iron is directly inversely associated with serum ferritin level, which is required for RBC formation (Chrysohoou *et al.* 2006).

Ferritin levels in patients with iron excess are significantly greater, helping to identify Patients with thalassemia and those with iron insufficiency both have low RBCS counts (Ashena *et al.* 2007).

2.2.5 Iron's functions

The creation of the heme molecule, which is required to create hemoglobin, is iron's main role in the body. The body typically contains 4 to 5 g of iron, which is distributed as follows and is necessary for the synthesis of other vital substances including: myoglobin, and cytochromes. Hemoglobin makes up 65% of the entire amount. Heme molecules make up 1% of the total, which stimulate internal cell oxidation 4 percent of the total is made up of myoglobin. Transferrin protein in blood plasma is combined with 0.1%. Between 15% to 30% is retained in cells, primarily as ferritin; the liver parenchymal cells and reticuloendothelial system are the main storage locations. These shops might employ people (Miret *et al.* 2003).

2.2.6 The distribution and absorption of iron

The three primary cell types that are involved in iron metabolism are the duodenal enterocytes, which are responsible for iron absorption, the reticule endothelium monocytes, which are responsible for iron recycling, and the liver hepatocytes, which are responsible for iron storage. The first step in the process of iron metabolism is the reduction of ferric oxyhydroxide (Fe^{3+}) to ferric hydroxide (Fe^{2+}) by an enzyme termed duodenal cytochrome b, which is located on the anterior membrane of enterocytes (Dcyt b). The acidic brush border membrane. The protein ferritin, which may contain up to (4500) iron atoms per molecule, stores extra iron in the cell, where it may serve as an iron reserve for several physiological functions (Miret *et al.* 2003, Andrews and Schmidt 2007).

2.2.7 Metabolism of iron

Hepcidin the master (Fe) regulator, is predominantly produced in, and secreted from the liver, which is also the primary organ responsible for its release. Iron consumption, iron retention, bone metabolism, hypoxia, and inflammation all play a role in its development (Nicolas *et al.* 2001, Muckenthaler *et al.* 2017).

Once it has reached the circulation, the molecule that is produced as hepcidin interacts with ferroportin, which is the only (Fe) exporter and is a membrane-bound protein, and is subsequently absorbed and degraded by lysosomes; preventing iron from the inside of cells from leaking out into the outside of cells (Nemeth *et al.* 2004, Muckenthaler *et al.* 2017).

Enterocytes, parenchymal liver cells, and macrophages all contain the protein known as ferroportin. Macrophages also contain ferroportin. The amounts of hepcidin in circulation and the quantity of transferrin on external membranes are what govern the absorption of dietary (Fe) in the duodenum, the storage of (Fe) in the liver and the recycling of (Fe) in macrophages (Muckenthaler *et al.* 2017).

It should not come as a surprise that organisms have developed complicated mechanisms for the intake of (Fe) the recycling of (Fe), and the effective distribution of (Fe) throughout their tissues. There are relatively few forms of iron that can be employed for biological activity. Although iron is necessary for life, an excess of it may lead to the development of very harmful. Reactive oxygen species (ROS). These (ROS) are capable of causing damage to (DNA), enzymes, and membrane proteins, which can ultimately end in organ failure (Papanikolaou and Pantopoulos 2005, Arezes and Nemeth 2015, Oikonomidou *et al.* 2016).

2.2.8 Overabundance of Iron

Thalassemia is characterized by an inadequate amount of erythropoiesis and an increased amount of iron in the body. This condition is caused by genetic abnormalities in either the globin-genes coding on (chromosome 16) or the -globin gene clusters on (chromosome 11), respectively. Chronic hemolytic anemia results from the early death of growing nucleated erythroid cells with hematopoietic growth as an attempt for possible compensation when the relative numbers of -globin and -globin chains are out of balance. Inadequate erythropoiesis is a symptom of increased intestinal iron absorption, which can also be a side effect of frequent transfusions. The degree of iron buildup in various organs may vary between individuals as a result of these factors (Taher and Saliba 2017).

Iron overload in the heart is the leading reason for death in individuals with TM who are dependent on regular blood transfusions. (Fe) overload is the most common cause of sickness, and mortality in those who have TM (Ayatollahi *et al.* 2020).

2.2.9 Liver disease

The liver serves as the body's main iron- storing organ. Moreover, it is the only location where transferrin and ferritin are produced (Soliman *et al.* 2014).

Liver disease is one of the most prevalent issues among individuals with thalassemia major. Iron excess is the main factor contributing to liver damage, along with hepatitis C and hepatitis B infections acquired through frequent blood transfusions (Hasoon *et al.* 2020).

2.2.10 Alanine transaminase (ALT)

Another term for it is serum glutamate-pyruvate transaminase (SGPT).In the liver, ALT is present in high amounts (Pesce *et al.* 1987). As a biomarker of hepatocellular injury, as well as to a lesser extent in skeletal muscle, the kidney, and the heart, serum ALT activity is measured (Milk 1996).

It is an enzyme component that is used to determine the possibility of liver damage (Pesce *et al.* 1987).

2.2.11 Aspartate transaminase (AST)

This enzyme also referred to as (glutamate oxaloacetate transaminase) serum (SGOT), is abundant in the heart, liver, skeletal muscle, kidney, and erythrocytes. Plasma AST levels may increase as a result of any of these tissues (Zilva *et al.* 1988). The most typical diagnostic uses for AST activity are testing for myocardial infarction, liver disease, and muscle disease (Milk 1996).

2.2.12 Albumin

Albumin is the most widely distributed circulating protein in plasma. It comprises between 3.5 to 5 g/dL, or 50%, of the total protein in plasma in healthy human patients. To assess serum albumin, employ common serum laboratory assays, Albumin is another colloid fluid that is given to patients who need fluid resuscitation, particularly when there has been trauma (i.e., hypovolemic shock) or when a large volume paracentesis has been performed (Rajat N *et al.* 2013). The ability of a patient's liver to produce

proteins and other materials necessary for total body homeostasis can be determined by the serum albumin level, which can be measured by clinical specialists (Ayatollahi *et al.* 2020).

2.3 Human Hemoglobins

The molecule called hemoglobin (Hb), which is found in red blood cells, is in charge of carrying oxygen to the tissues. A suitable hemoglobin level must be kept to provide appropriate tissue oxygenation. In whole blood, the amount of hemoglobin is measured in grams per deciliter (g/dL). Males should have a Hb level of 14 to 18 g/dL, while females should have 12 to 16 g/dL. The patient gets anemia if the hemoglobin level is low. Too many red blood cells lead to erythrocytosis, which raises hemoglobin levels above normal (Henny 1990).

2.3.1 Structure of hemoglobin

Hemoglobin is made up of four polypeptide chains and heme groups that are attached to four different subunits. The iron protoporphyrin IX prosthetic heme group, which is joined to a polypeptide chain with residues of (141 alpha), and (146 beta) amino acids, is a component of all hemoglobins. The heme's ferrous ion is connected to a histidine's N by a histidine. The porphyrin ring's polypeptide chain phenylalanine pushes it into its pocket. While the two types of polypeptide chains that make up adult hemoglobin, the alpha and beta chains, differ in amino acid sequences, they are both roughly the same length. The identical alpha chain is present in both adult and embryonic human hemoglobin. The beta chain of healthy adult hemoglobin (22), the gamma chain of fetal hemoglobin (22), and other non-alpha chains are examples of these. Non-alpha chains include the gamma chain of fetal hemoglobin (HbA2), the delta chain of adult healthy hemoglobin (HbA2), and the beta chain. In rare cases, the duplication of the gamma genes results in the production of two distinct types of gamma chains (Marengo-Rowe 2006).

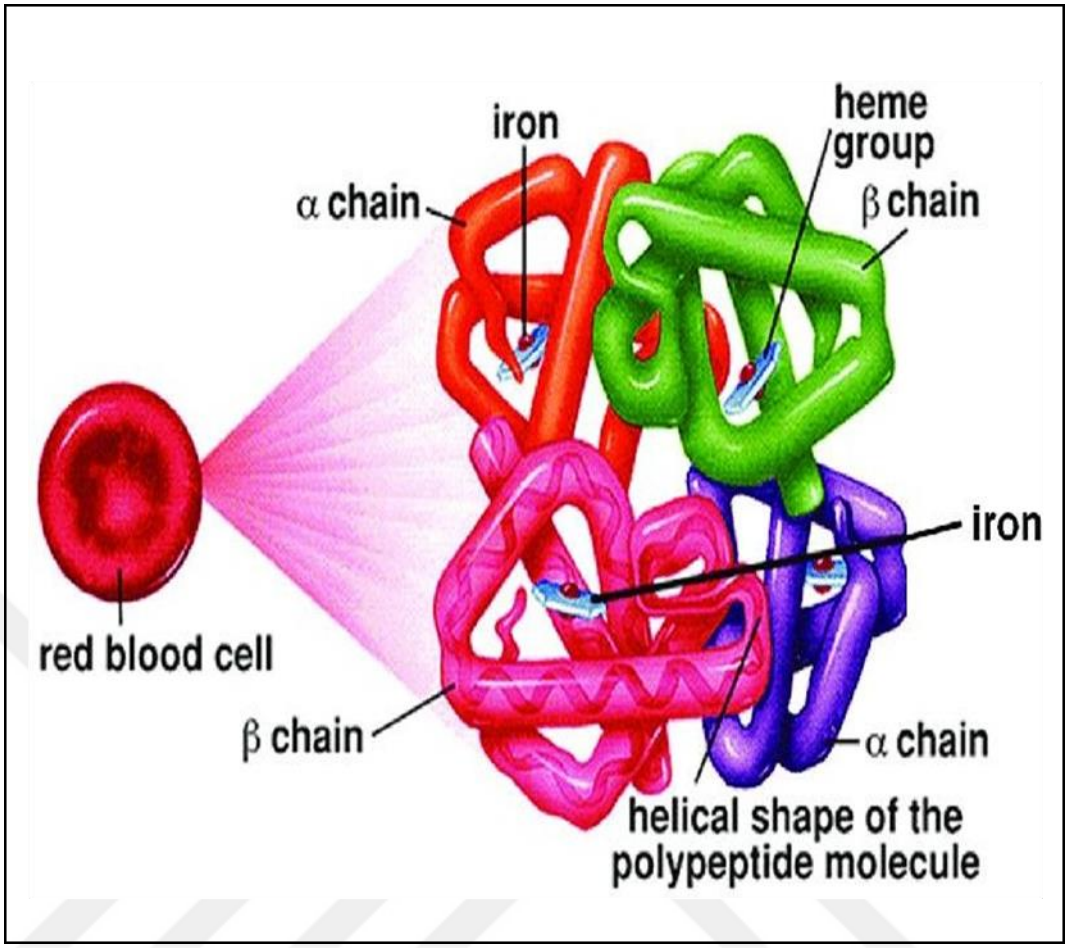

Figure 2.3 Structure of hemoglobin protein

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Medical tools

The tools and materials utilized in this work are covered in this chapter. To get incredibly precise outcomes, devices and materials that are state-of-the-art internationally are used.

3.1.2 Plan

The current investigation was conducted between the end of March 2022 and the beginning of July 2021 at the Thalassemia Center and Hereditary Blood Diseases in the Iraqi Dhi Qar Governorate and the Central Private Laboratory. In Figure 3.1, all the patient control groups that were part of this investigation are detailed. Note that only β-thalassemia major (BTM) patients were sampled due to the unavailability of beta thalassemia minor or intermediate samples at the Thalassemia and Genetic Blood Disease Center.

3.1.3 Group of patients

Fifty-five people with beta-thalassemia major who require transfusions. The samples, which come from the Thalassemia and Genetic Blood Diseases Center in the Iraqi region of Dhi Qar, are made up of people of various ages and genders who regularly receive blood transfusions.

3.1.4 The control group

Randomly chosen, fifty-five apparently healthy people of various ages and genders weredrawn from various populations. Description of all studied groups in figure 3.1

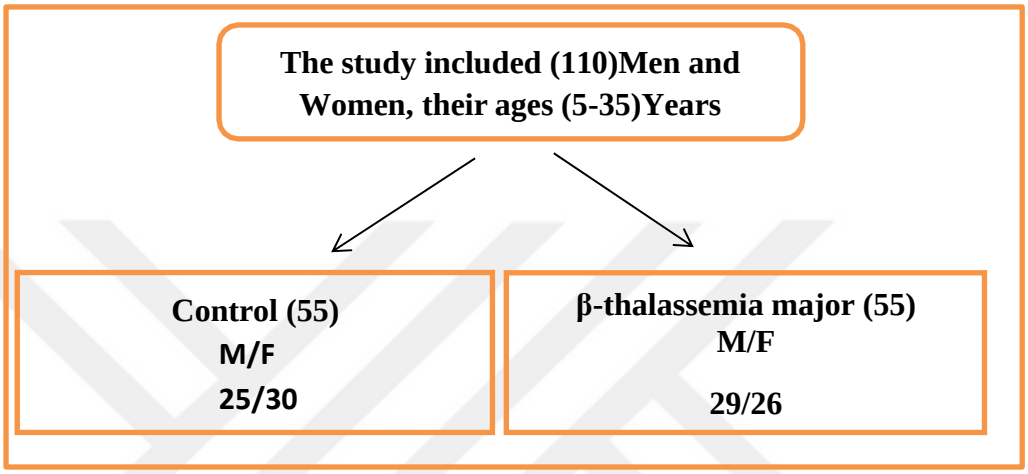

Figure 3.1 Description of all studied groups

3.1.5 Collection of a blood sample

Both patients diagnosed with thalassemia and members of the control group had needles inserted into their veins to draw 5 milliliters of blood, which was then divided into the following categories:

1. Two milliliters of the blood sample were put in tubes that contained EDTA so that Hb and PCV could be calculated.
2. Three milliliters are withdrawn from the sample, placed in a centrifuge tube and allowed to coagulate at room temperature for (10 min) before being spun at a speed of three thousand revolutions per minute.
3. If the serum samples were not utilized immediately, they were withdrawn and kept at a temperature of -20 degrees Celsius for use in subsequent studies of the biochemical parameters.
4. The clinical list for this study's empirical parameters is described in an appendix.

3.1.6 Kits and other chemical compounds

Table 3.1 shows, the list of the sources of the compounds used in this investigation along with the substances themselves. Table 3.2 shows list the manufacturers of the used equipment.

Table 3.1 Products and suppliers of chemicals

NO.	Chemical	Supplied Company
1-	Ferritin Kit	Abbott i1000. USA
2-	Iron Kit	Human. Germany
3-	Copper Kit	LTA. Italia
4-	ALT Kit	Abbott C4000. USA
5-	AST Kit	Abbott C4000. USA
6-	Albumin Kit	Human. Germany
7-	Hepcidin ELISA Kit	YH Biosearch. China

Table 3.2 The manufacturers of used equipment

No	Equipment	Manufacturers
1	Centrifuge	Hettich, Germany
2	Centrifuge	DLAB DM0408, China
3	Hematology analyzer (CBC)	Abbott CEEL-DYN Ruby, USA
4	Hormone analyzer	Abbott ARCHITECT PLUS i1000 sr, USA
5	Chemical analyzer	Abbott ARCHITECT PLUS C4000, USA
6	Spectrophotometer	Biosystems, SPAIN
7	Vortex mixer	Model KMC-1300V, Korea
8	Water bath	Model No. HH-S1, China
9	Roller mixer	Huma Roller, Germany

3.2 Methods

3.2.1 Albumin

Bromocresol green form, a colored compound with albumin in the citrate buffer. This complex's absorbance varies in direct proportion to the sample's albumin concentration. The reagents used in the current experiment and their concentrations are illustrated in

Table 3.3, and the lists of the quantity of the reagent for the standard and the blank are illustrated in Table 3.4. For calculation of the albumin concentration, see (3.1) and (3.2).

Table 3.3 The reagents used in the current experiment and their concentrations

Reagent	**Contents**	**Concentration**
Buffer	Citrate buffer(pH 4.2)	30 mmol/L
Enzymes	Bromocresol -green	260 µmol/L
STD	Albumin Sodium azide	4g/dL or 40g/L 0.095%

Table 3.4 lists the quantity of the reagent for the standard and the blank

Pipette into cuvettes	**Rb**	**Sample or STD**
Sample /STD Reagent	----- 1000 µL	10 µL 1000 µL
Mix, incubate for 5 min. at 20-25C. Measure the absorbance of the sample and the standard against the reagent blank within 30 min.(ΔA).		

$$C = 4 \times \frac{\Delta A Sample}{\Delta A STD} \quad (g/dl) \qquad (3.1)$$

$$C = 40 \times \frac{\Delta A sample}{\Delta A STD} \quad (g/l) \qquad (3.2)$$

3.2.2 Alanine aminotransferase (ALT)

Procedures based on principle

As long as ALT is found, it is capable of catalyzing the transition of the amino group from L alanine to -ketoglutarate, which will ultimately result in the production of pyruvate and L-glutamate. Pyruvate undergoes a chemical transformation that results in the production of L-lactate when NADH and lactate dehydrogenase are present (LD). During this procedure, NADH is converted into NAD. As a reaction indicator, the rate at which the absorbance at 340 nm decreases as a result of NADH being converted to NAD is measured.

Table 3.5 The lists of the reagents used in the investigation and their concentrations

Reagent	Ingredient	Concentration
R1	B-NADH Lactate-Dehydrogenase L-Alanine	0.16 mg/ml 2.57 U/ml 392 mmol/l
R2	α-Ketoglutarate L-Alanine	77 mmol/l 1000 mmol/l

3.2.3 Aspartate aminotransferase (AST)

Procedures based on principles

When the amino group of L aspartate is transformed to -ketoglutarate in the presence of AST, oxaloacetate and L-glutamate are produced as a byproduct of this reaction. In the presence of oxaloacetate, NADH and malate dehydrogenase catalyze the transformation of oxaloacetate into L-malate (MDH). During this process, NADH will be converted to NAD. To maintain track of the reaction, the ratio at which NADH is being transferred to NAD is monitored.

Table 3.6 The reagents used in the investigation and their concentrations

Reagent	Ingredient	Concentration
R1	B-NADH Malate-Dehydrogenase Lactat- Dehydrogenase L-Aspartate	0.16 mg/ML 0.64 U/mL 0.64 U/mL 232 mmol/L
R2	α -Ketoglutarate L -Apartate	51.3 mmol/L 100 mmol/L

3.2.4 Iron

Principles

When iron (III) reacts with chromazurol B (CAB) and cetyltrimethylammonium-bromide, a colorful ternary complex with maximum absorption at 623 nm is produced

(CTMA). There is a clear correlation between the amount of iron in the sample and the intensity of the color produced. Table 3.6 shows the list of the reagents used in the investigation and their concentrations. Table 3.7 shows the list of the quantity of the reagent for the standard and the blank.

Table 3.7 The reagents used in the investigation and their concentrations

Solutions	Ingredient	Concentration
Reagent	CAM CTMA Guanidinium-chloride Sodium acetate buffer (pH 47)	0.18 mmol/l 2.2 mmol/l 2.6 mmol/l 45 mmol/l
STD	iron (ionised) Or	100 µg/dl 17.9 µmol/l

Table 3.8 The quantity of the reagent for the standard and the blank

Pipette into cuvettes	(Rb)	Sample / Standard
Sample / STD	-----	50 µl
Distilled water	50 µl	-----
Reagent	1000 µl	1000 µl
Mix well, incubate for 15 minutes at 20-25°C. Measure the absorbance of the sample (ΔA_{Sample}) and the standard($\Delta A_{Standard}$) against the reagent blank within 60 minutes.		

$$C = 100 \times = \frac{\Delta A\ Sample}{\Delta\ ASTD} \quad (\mu g/dl) \tag{3.3}$$

$$C = 100 \times = \frac{\Delta A\ Sample}{\Delta A\ STD} \quad (\mu mol/l) \tag{3.4}$$

3.2.5 Ferritin

Biological principles of the procedure

The ARCHER: The ferritin assay, also known as the Chemiflex assay, is a two-step immunoassay that uses CMIA technology to detect ferritin in human serum and plasma. Using a:

1-Sample in conjunction with tiny paramagnetic particles that have been treated with anti-ferritin. Tiny particles that have been coated with anti-ferritin can bind to ferritin that is already present in the sample.

2-Following the step of washing, an anti-ferritin acridinium-labeled associates conjugate is added to produce a reaction mixture. After a further cycle in the washing machine.

3-The reaction mixture is next subjected to the addition of both the Pre-Trigger and the Trigger solutions.

4-The chemiluminescent response that occurs thereafter is evaluated based on the number of light units (RLUs). The ferritin content of the sample has a direct correlation to the relative light units (RLUs) that are measured through ARCHITECT I System optics. The concentrations of each Reagent that were used in the current investigation are detailed in Table 3.8.

Table 3.9 Reagent that used in the present study and its concentrations

Reagent	Ingredient	Concentration	Preservatives
Microparticles	Anti-Ferritin(mouse. monoclonal)coated microparticles in TRIS butter with protein (mouse and bovine)stabilizers	Minimum concentration- O.125 % solids	Antimicrobial-agent
Conjugate	Anti-Ferritin (rabbit. polyclonal) acridinium labeled Conjugate in MES butter with protein (bovine) stabilizers.	Minimum concentration 75 ng/ml	antimicrobial - agent
Pre Trigger Solution	hydrogen peroxide	1.32 % (w/v)	------
Trigger-Solution	sodium hydroxide.	0.35 N	---------
Wash buffer	phosphate buffered saline solution	---------	antimicrobial - agent

3.2.6 Copper

Principles

The amount of blue-violet compound produced by the reaction of chromogen 3,5- Di-Br-PAESA with cupric ions is related to the amount of copper present in the sample. Both the serum and the blank sample don't need to be deproteinized for the approach to work. Table 3.10 shows the Reagents utilized in the investigation at hand and their concentrations, Table 3.11 shows the procedures.

Table 3.10 Reagents utilized in the investigation at hand and their concentrations

Reagent/ STD	Ingredient	Concentration
R A	Acetate- buffer	0.1 m pH 4.9
R B	**3**,S-Di8r-Paesa	------
STD	Ion copper	200µg/dl

Table 3.5 Procedures

Reagent	Blank	(STD)	Sample
Work reagent	1ml	1ml	1ml
D.W	66µl	-----	-----
(STD)	-----	66µl	-----
Sample	-----	-----	66µl
Mix & wait, for 10 min then read the absorbance, against the blank at 580 nm. The colour is stable for 30 min.			

3.2.7 Hepcidin

Principles

For the determination of human hepcidin, this kit employs an ELISA that is formulated on the principle of the biotin twin antibody sandwich (HEPC). Following the addition of hepcidin (HEPC) to wells that had been coated with hepcidin (HEPC) monoclonal antibody, the immunological complex was formed by incubating biotin-labeled anti-HEPC antibodies with streptavidin-HRP and then mixing the resulting mixture. After culture, cleaning, and the introduction of substrates A and B, it is necessary to remove any enzymes that have not been bound to a substrate. Because acid is present, the color of the liquid will transform from blue to yellow as a result of this alteration. The correlation between the concentration of human hepcidin (HEPC) and the color of the solution is a favorable one. The assay procedures are the calculation of the hepcidin illustrated in Table 3.12.

Procedure:

a) Standard original concentration dilution, this package includes one standard original concentration. Following Figure 3.2 below, users can independently dilute in small tubes.

2400pg/ml	Standard No.5	120 µL Original Standard + 120 µL Standard diluents
1200 pg/ml	STD No.4	120 µl STD No.5 + 120 µL STD diluent
600 pg/ml	STD No.3	120 µl STD No.4 + 120 µL STD diluent
300 pg/ml	STD No.2	120 µl STD No.3 + 120 µL STD diluent
150 pg/ml	STD No.1	120 µl STD No.2 + 120 µL STD diluent

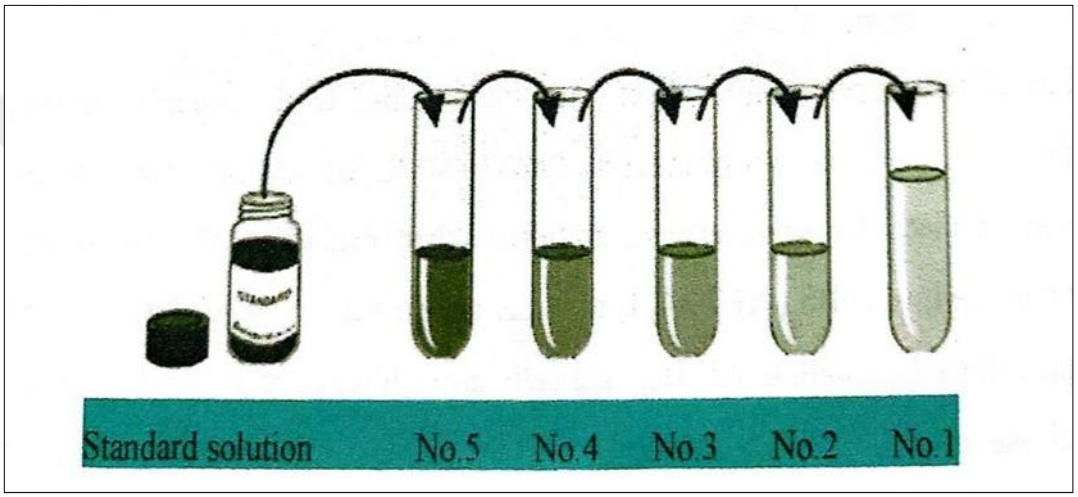

Figure 3.2 The standard solution

b) The number of samples that will be analyzed, as well as the standards that will be utilized to determine how many stripes are needed. Each standard solution and each blank well should be separated using as many wells as is practical.

c) Injecting the specimen: 1) Blank space Without adding the sample, add chromogen reagents A and B, stop solution, and an anti-HEPC antibody that has been biotin- and streptavidin-HRP-labeled. The remaining step operations are identical. 2) A dependable response of 50 µl of the standard should be added along with 50 µl of the streptomycin-HRP (the standard contains biotin antibodies that have already joined; extra biotin antibodies are not required). 3) A test well sample: It is necessary to add 40 µl of material, 10 µl of HEPC antibodies, and 50 µl of streptavidin-HRP. It is then covered by a membrane seal plate. Shake to blend gently. At 37 °C, incubate for 60 minutes.

d) Make the washing solution by diluting the washing concentration by a factor of thirty with sterile water so that it may be used in the future.

e) Cleaning: The washing process is carefully removing the membrane from the seal plate, allowing the liquid to drain, and then shaking off any residue that remains. After adding cleaning liquid to each well, wait 30 seconds for it to rest, and then drain the wells. Following the completion of this process a total of five repetitions, the plate has to be blotted.

f) To get the desired color result, pour 50 µl of chromogen reagent A and 50 µl of chromogen reagent B into each well. Mixing requires gentle shaking. Incubate the sample at 37 degrees Celsius and out of the light for 10 minutes to allow the color to develop.

g) Put a stop to the process by pouring 50 µl of stop solution into each well.

h) Measure the optical density (OD) of each well at a wavelength of 450 nm using a blank well as a reference following the addition of the stop solution, this procedure has to be carried out within the next 10 minutes.

i) Applying the contents and OD values of the standards, you can calculate the linear regression equation of the standard curve. Next, as a function of the optical density values of the samples, calculate the amount of the sample that is connected. In addition to that, statistical analysis tools could be used.

Calculate

The optical density value (OD) ought to be the ordinate, while the concentration of the standards ought to be the abscissa. Create the standard curve by following the instructions in Figure 3.3.

Find the concentration that corresponds to the optical density values associated with the samples, or determine the regression equation of the standard curve by using standard concentration and OD value. Alternatively, find the concentration that relates to the OD value of the sample. After that, substitute the OD value of the sample for x to get the sample's concentration.

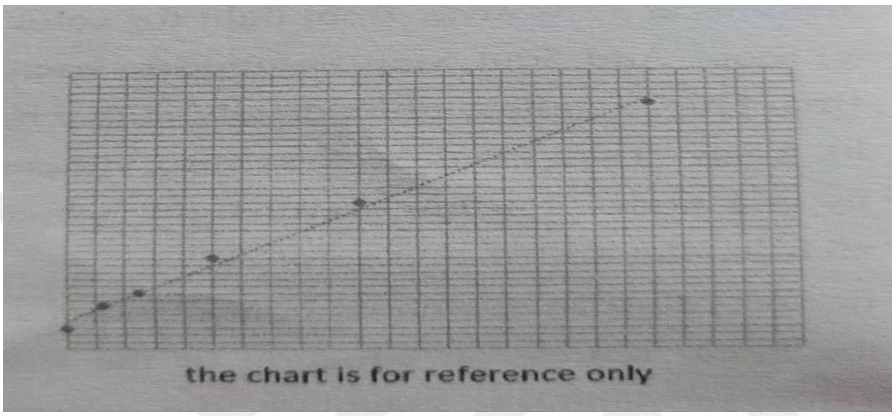

Figure 3.3 The chart for drawing the standard curve

RESULTS AND DISCUSSION

This study, was conducted in order to evaluate the relationship between hepcidin and some biochemical parameters (ferritin, Iron, hemoglobin (Hb), packed cell volume (PCV), AST, ALT, albumin, Copper) in patients with thalassemia as a predicative and diagnostic indicator of thalassemia disease in patients in experimental tests.

4.1 The age and β-Thalassemia Disease

The results of Table 4.1, and Figure 4.1 showed that the mean age of β- thalassemia patients were no significant as compared to healthy persons (control) which recorded 25.53 years as compared to the healthy group, which recorded (27.75 years).

Table 4.1 The age and β-Thalassemia disease

Age	**Thalassemia**	**Control**
Mean	25.53	27.75
Standard Deviation	17.715	14.672
Median	20.00	27.00
Percentile 50	20.00	27.00
Percentile 95	55.00	53.20
P value	0.476 ^{NS}	

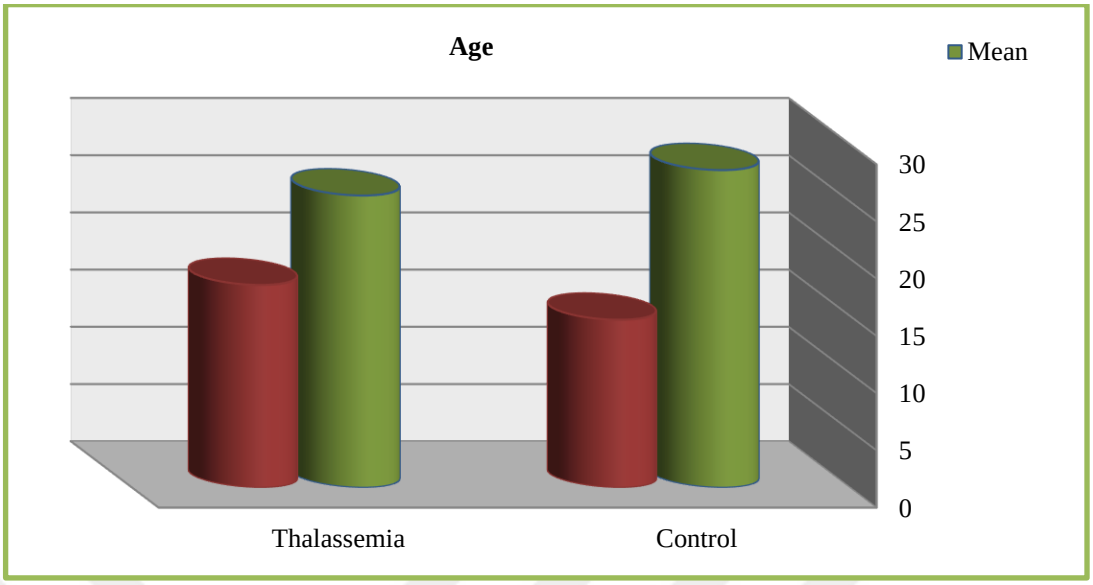

Figure 4.1 The age and β-Thalassemia disease

4.2 The Gender and β- Thalassemia Disease

The results of the gender (male or female) parameter and - thalassemia disease indicated that there was no significant difference between the mean of male & female - thalassemia patients and the mean of the control group, in the patient group there were 29 males with a percentage of 52.30 percent and 26 females with a percentage of 47.70 percent, whereas the control group had 20 males with a percentage of 36.40 percent and 35 females with a percentage of 63.60 percent (Table 4.2).

Table 4.2 Statistics group of sex parameter

Sex group	Study groups		
	Thalassemia	**Control**	**Total**
Female	26	35	61
%	47.7%	63.6%	55.5%
Male	29	20	49
%	52.3%	36.4%	44.5%
Total	55	55	110
%	100.0%	100.0%	100.0%
P value	0.084 ^{NS}		

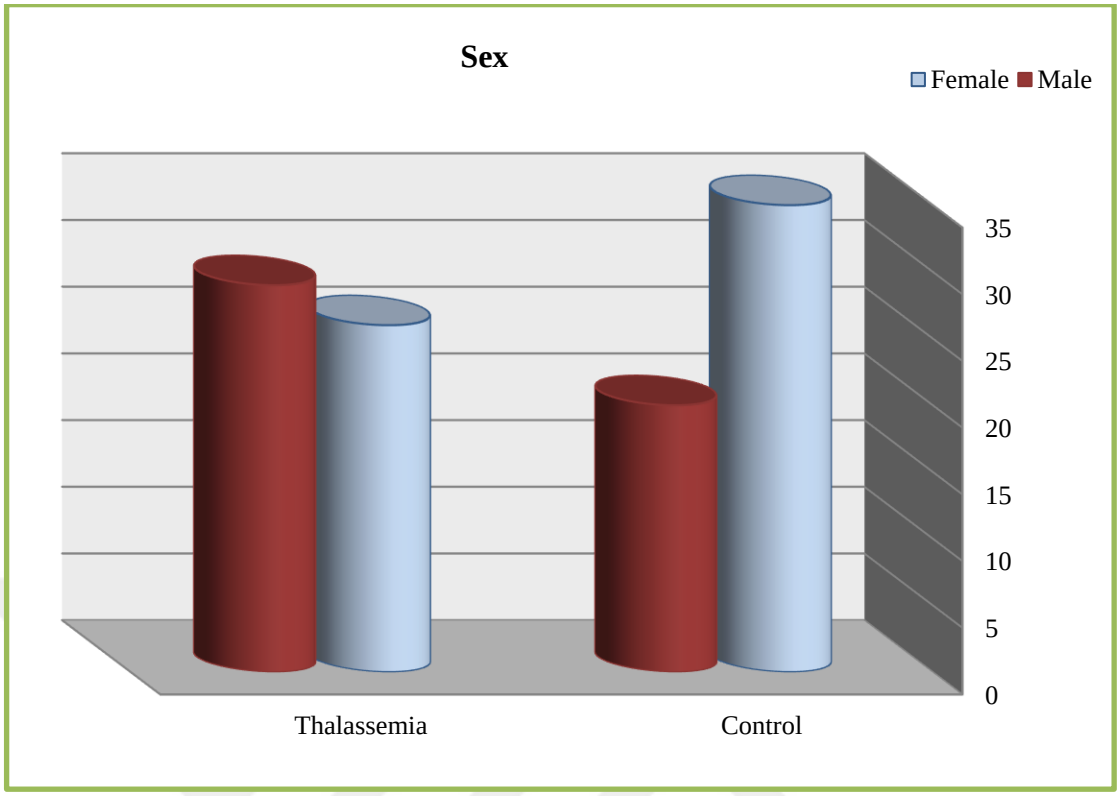

Figure 4.2 Correlation between the gender and thalassemia

4.3 The Hepcidin Hormone and Thalassemia Disease

As can be seen in table 4.3 and figure 4.3. The two groups that were examined, the control group and the patient group, had significantly different concentrations of the hormone hepcidin in their serum blood. With a P value of 0.001**, the patient group had a substantially lower mean hepcidin level (47.81 pg/mL) than the control group (152.88 pg/mL), demonstrating a strong link between hepcidin level and -thalassemia illness.

The control group had a significantly higher hepcidin level (Table 4.3). In individuals diagnosed with beta-thalassemia, the patient group had a standard deviation of 8.69 pg/mL for hepcidin levels in serum blood, but the control group had a standard deviation of 30.04 pg/ml. Between the two groups, there was a statistically significant difference (P 0.001). Table (4.3).

These results are in good agreement with the results (of Salah Hassan Al-Zuhairi *et al.* 2021). Which revealed that the level of hepcidin in the blood of patients with a beta-thalassemia major was significantly lower when compared to controls.

This hormone deficit is either the major cause of iron overload or one of the contributing factors in cases of iron-loading anemias like beta-thalassemia. A high level of erythropoietic activity has a significant, and (negative impact) on the expression of hepcidin, which leads to a lack of this hormone in the body. Even though iron contributes lesser to the overall iron hardship in hemoglobinopathies major patients than transfusions do, the main culprits of systemic (Fe) overload in semi-thalassemia patients are (low hepcidin), and the arising hyperabsorption of dietary (Fe). This is because low hepcidin causes hyperabsorption of dietary iron (Elizabeth Nemeth 2010).

The reduction of hepcidin in this study may be because of the exuberant erythropoiesis, the synthesis of hepcidin is suppressed by erythropoiesis and increases the absorption of Iron (Nemeth 2013).

Table 4.3 Statistics group of Hepcidin parameter

	Study group	**Mean**	**Std. Deviation**	**P value**
Hepcidin	Thalassemia	47.8122	8.69520	<0.001**
	Control	152.8896	30.04205	
	Total	100.3509	57.18584	

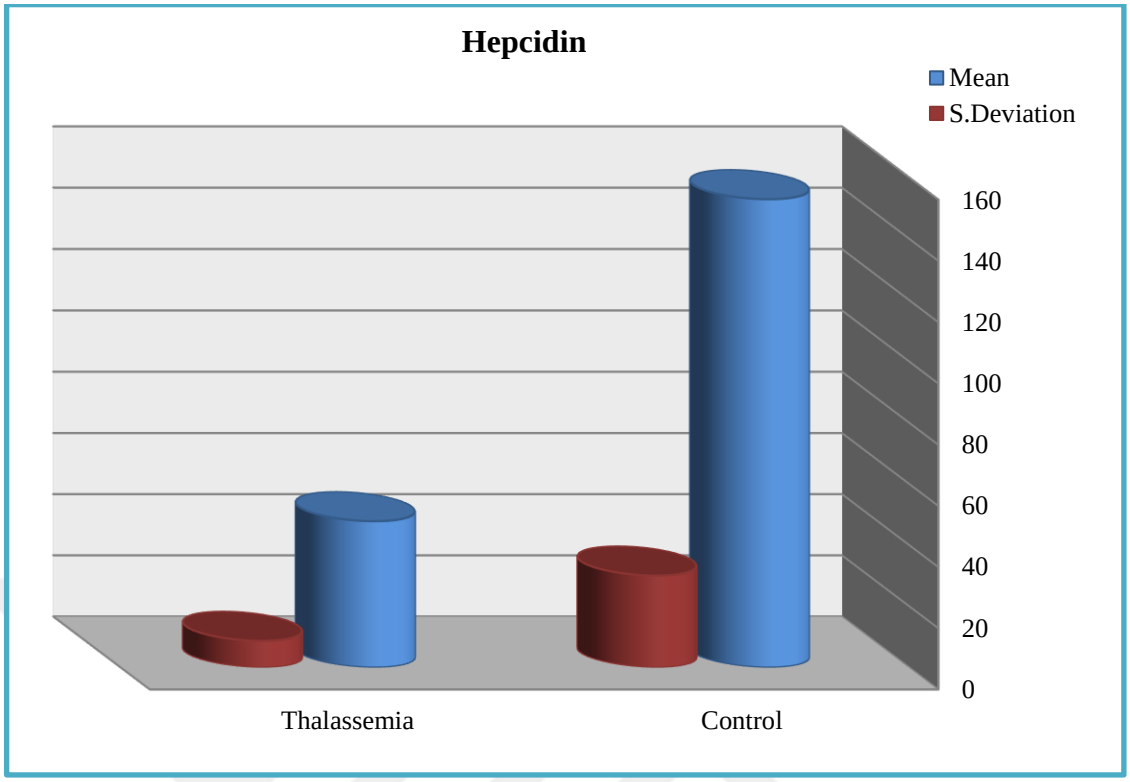

Figure 4.3 Correlation between the hepcidin and β-thalassemia

4.4 The Ferritin Level and β-Thalassemia Disease

The results of correlation between ferritin levels in serum blood and β-thalassemia disease showed that ferritin levels were increased significantly in (patient group) as compared to (control group) Table 4.4, the patient group had 4313.32ng/mL, while the control group registered 59.58ng/mL.

These results, were consistent with findings (Pootrakul *et al.* 1981) which indicated that higher serum ferritin levels resulted from external gastrointestinal absorption rather than from short-term iron supplementation.

These results of ferritin levels in serum blood of thalassemia may be used as a predictor of -thalassemia in individuals or healthy individuals who do not exhibit signs of thalassemia disease.

Table 4.4 Statistics group of ferritin parameter

	Study group	Mean	S. Deviation	P value
Ferritin	β-Thalassemia	4313.3273	743.80732	<0.001**
	Control	59.5851	13.73219	
	Total	2186.4562	2199.83218	

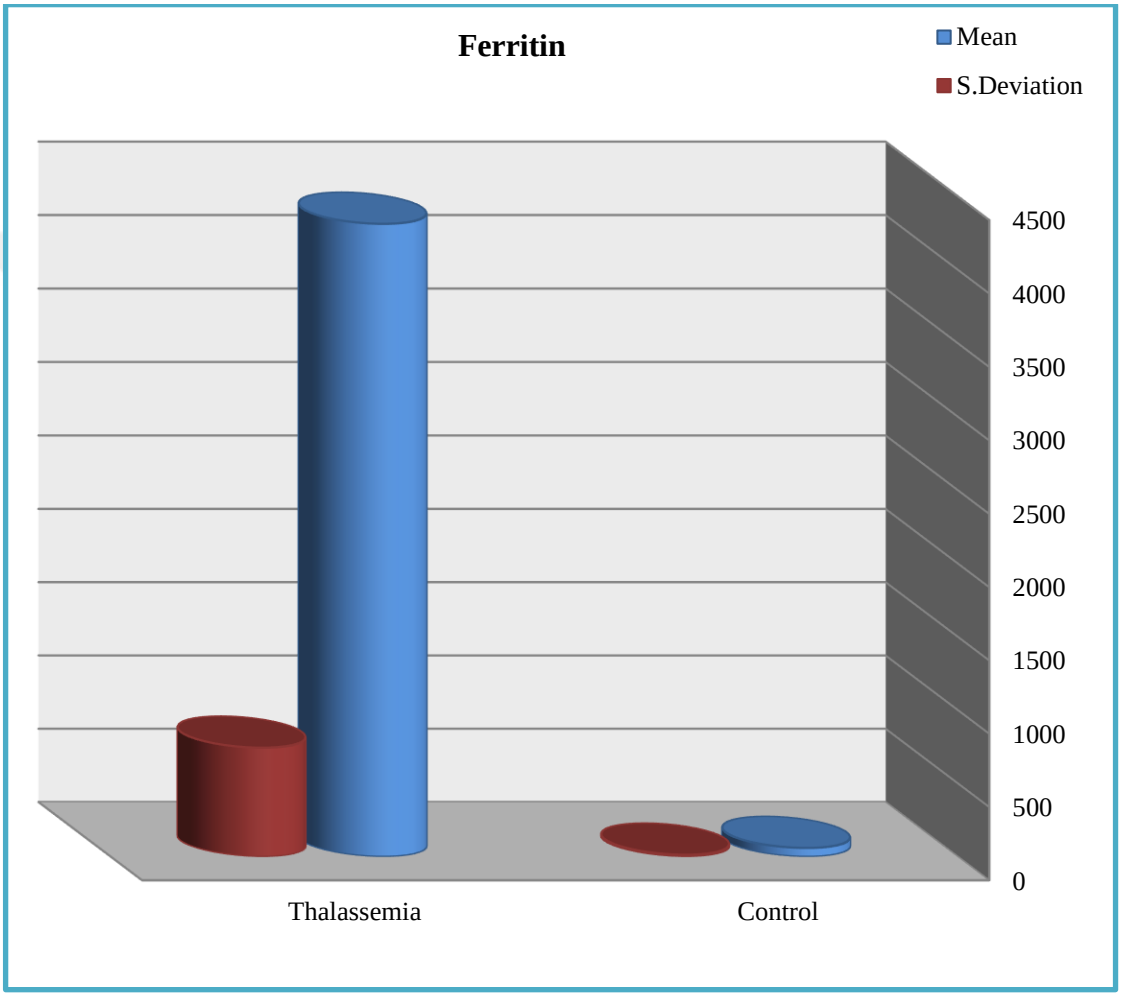

Figure 4.4 Diagram of correlation between ferritin and β- thalassemia

4.5 The Iron Concentration and β-Thalassemia

According to Table 4.5, the ill group had iron levels in their serum blood that were significantly, greater than those of the (control group), the patient group had 203.13g/dL of iron while the control group had 86.20g/dL. The sick group's serum blood levels of

iron were noticeably greater, than those of the (control group). In comparison to the control group, the patient groups standard deviation was 38.58 g/dl, whereas that of the (control group) was 21.28 g/dl (See Figure 4.5).

The accumulating of extra iron in the body as a result of either -thalassemia or frequent blood transfusions, which causes an increase in iron levels (Mishra and Tiwari 2013). Iron increment is caused by red blood cells (RBC) count being cemented by monthly blood carting, hemolysis and boosted iron absorption from duodenum and proximal jejunum (Hendy *et al.* 2010).

Table 4.5 Statistics group of iron parameter and β-thalassemia

	Study group	**Mean**	**S. Deviation**	**P value**
Iron	Thalassemia	203.13	38.589	<0.001**
	Control	86.29	21.289	
	Total	144.71	66.379	

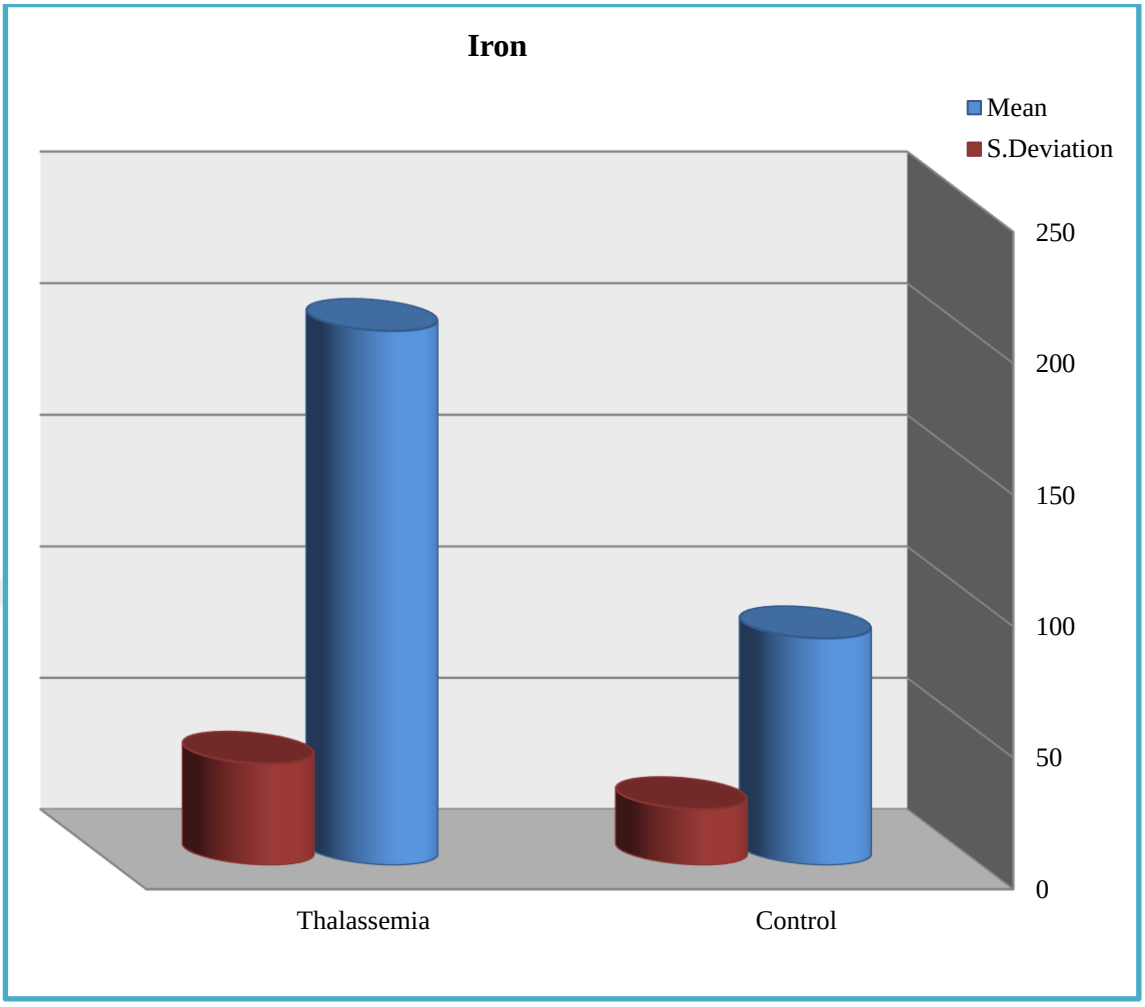

Figure 4.5 Statistics group of Iron parameter and β-thalassemia

4.6 The Hemoglobin Level and Thalassemia

Results of the correlation between hemoglobin levels in the blood of the two tested groups the (patient-group), and the (control-group) showed that levels of hemoglobin in the (patient- group) significantly reduced concerning the (control-group), the patient-group found 7.88% while in the control group which found 13.42 % (Table 4.6 and Figure 4.6).

These findings are in line with the outcomes of (Panda and Sharma 2018) that studied a total of 66 β-thalassemia patients and found the mean of Hb was recorded at 8.7 %, the ferritin level was 666.69 ± 325 ng /mL.

The patients of β-thalassemia have genetic mutations that lead to the inhibition of hemoglobin production and this cause to reduce of hemoglobin levels in the(blood) of b-thalassemia patients and emerging of blood anemia (Fucharoen and Weatherall 2012, Shafique *et al.* 2021).

Also, the results indicated that there is a great relation between hemoglobin and β-thalassemia disease which appears in the P value that recorded <0.001**, the standard deviation of the patient group recorded 1.36 while in the control group recorded 7.88 respectively. The finding of the current results is in agreements with (Hendy *et al.* 2010).

Table 4.6 Statistics group of hemoglobin parameter

	Study group	**Mean**	**S. Deviation**	**P value**
Hb%	Thalassemia	7.885	1.3636	<0.001**
	Control	13.425	1.0242	
	Total	10.655	3.0305	

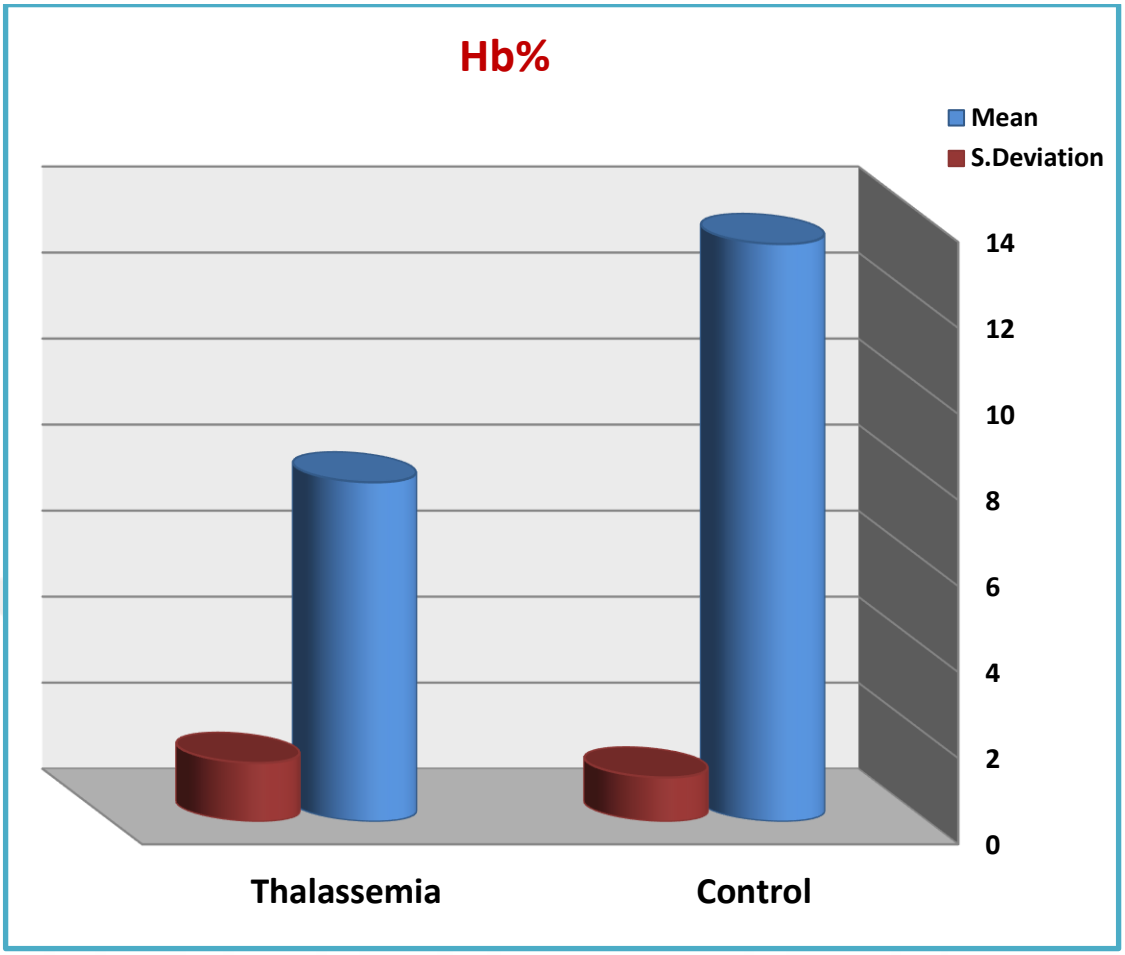

Figure 4.6 Statistics group of hemoglobin parameter

4.7 The Packed Cell Volume (PCV) and β-Thalassemia

The results of the correlation between PCV and β-thalassemia illustrated in Table and Figure 4.7. Revealed that the patient-group was significantly reduced in PCV value as compared to the (control group). The (patient group) recorded in PCV mean of 23.24 while the control group recorded 40.18 (Table 4.7).

These decrements in PCV values were correlated with hemoglobin levels in the blood of the patient group so this situation led to decreased values of PCV in the patients, with B-thalassemia. The findings indicated acorrelation between PCV value, and β-thalassemia in a positive great correlation (0.001**), the standard deviation of the patient group recorded at 3.98 while the control group recorded 4.05 (Table 4.7).

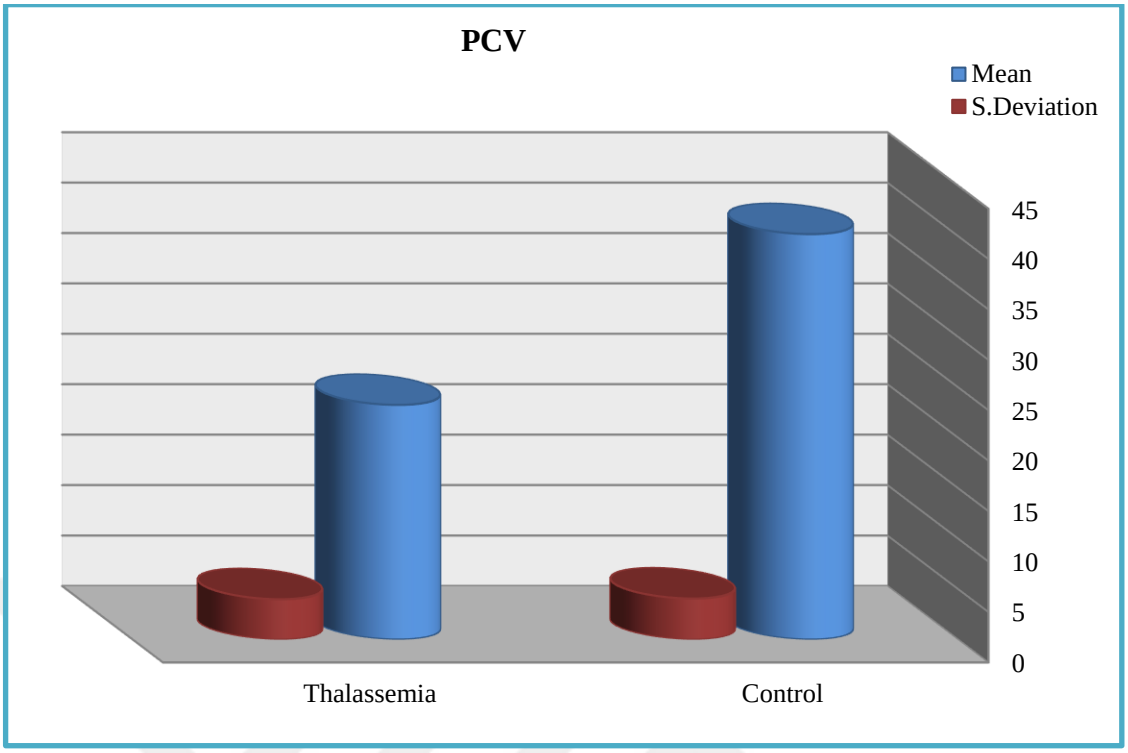

Figure 4.7 The packed cell volume (PCV) and β-thalassemia

Table 4.7 Statistics group of PCV parameter

	Study group	Mean	S. Deviation	P value
PCV	β-Thalassemia	23.242	3.9861	
	Control	40.187	4.0577	<0.001**
	Total	31.715	9.4061	

4.8 The Alanine Aminotransferase (ALT) and β-thalassemia

The link between ALT levels and β-thalassemia revealed that the patient group had considerably more ALT levels compared to the (control group). The patient-group found 41.17 U/l, while it was found in the control group 14.50 U/l (Table and Figure 4.8), the correlation between ALT levels and β-thalassemia is a positive correlation (0.007*) and the standard deviation of the patient group recorded 5.90 while the control group recorded 3.57 respectively. Increased liver enzymes (such as ALT) are symptomatic of a liver infection in patients with -thalassemia major who are transport-reliant (Ibrahim *et al*. 2011).

The results here agree with the findings of (Salama *et al*. 2015), who discovered that the main cause of increased liver enzymes is an (Fe) overload in b-thalassemia patients, and they discovered that the levels of ALT and AST in b-thalassemia patients, were elevated and had considerably more serum ferritin levels when compared to the (control group). (Ibrahim *et al*. 2011) demonstrated that SGPT and SGOT levels were increased, and they discovered a much substantial relation between ALT and AST as well as a strong association between ferritin level and HCV status.

Table 4.8 Statistics group of ALT parameter

	Study group	Mean	S. Deviation	P value
ALT	Thalassemia	41.1725	5.90261	<0.001**
	Control	14.5091	3.57912	
	Total	27.8408	14.24684	

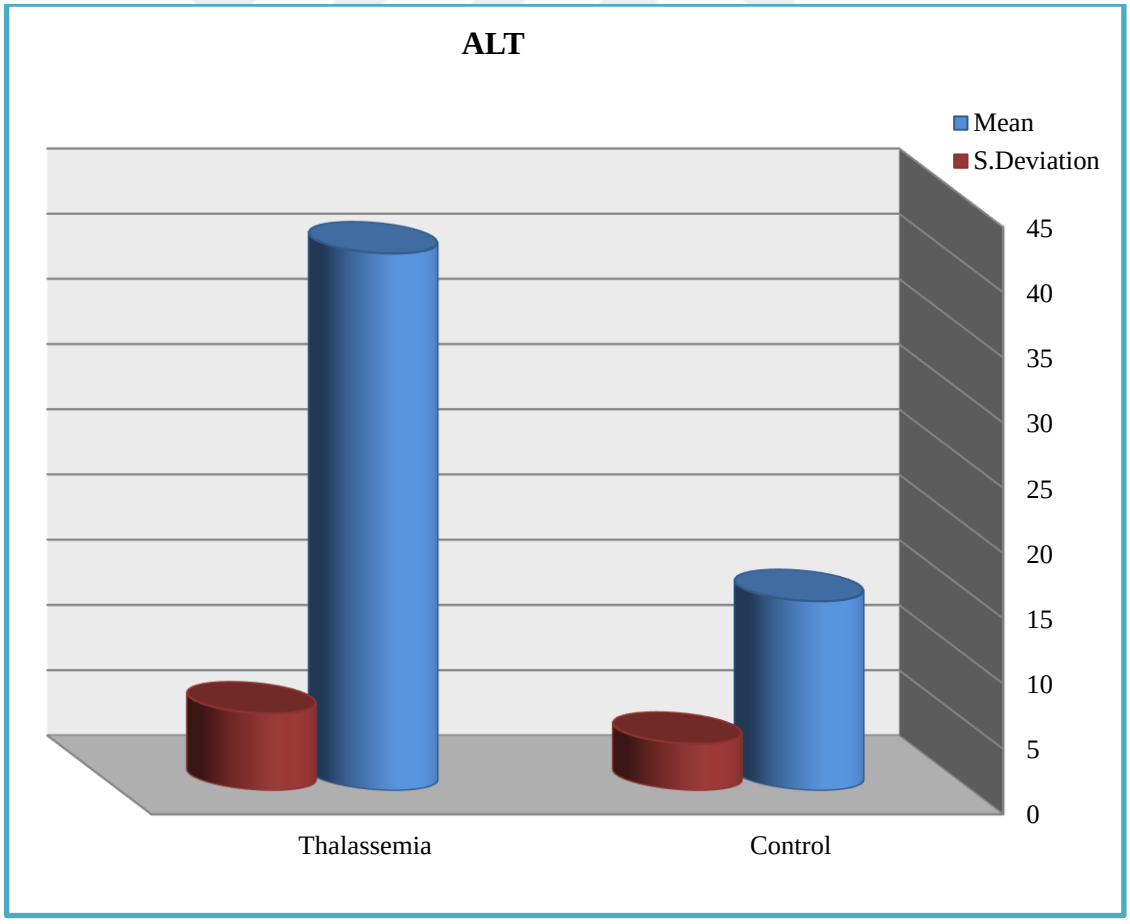

Figure 4.8 Statistics group of ALT

4.9 The Aspartate Aminotransferase (AST) and Thalassemia

The patient group recorded a level of AST that was 44.14 U/L, but the control group only recorded a level of 23.67 U/L, as shown in Table 4.9 and Figure 4.9. This indicates that the patient group had greater levels of AST, than the (control group). In addition, the standard deviation of the (group of patients) was 7.70, while the standard deviation of the group of controls was 6.33 (Table 4.9).

The presence of an infection or inflammation in the liver is indicated by a high AST level; the liver is a prominent target for damage in individuals with beta-thalassemia since it is one of the organs most vulnerable to damage (Nienhuis and Nathan 2012).

In egypt, beta-thalassemia is the most prevalent (chronic hemolytic anemia) (85,1%), with a carrier incidence of 9-10.2%. The serum concentrations of ALT, and AST gradually rise after liver damage, whether it is acute or chronic (AST) (Salama *et al.* 2015). The results here agree with the findings of (Israa Imad Jabbar 2021).

Table 4.9 Statistics group of AST parameter

	Study group	Mean	S. Deviation	P value
AST	β-Thalassemia	44.14535	7.707559	<0.001**
	Control	23.67273	6.333493	
	Total	33.90904	12.451771	

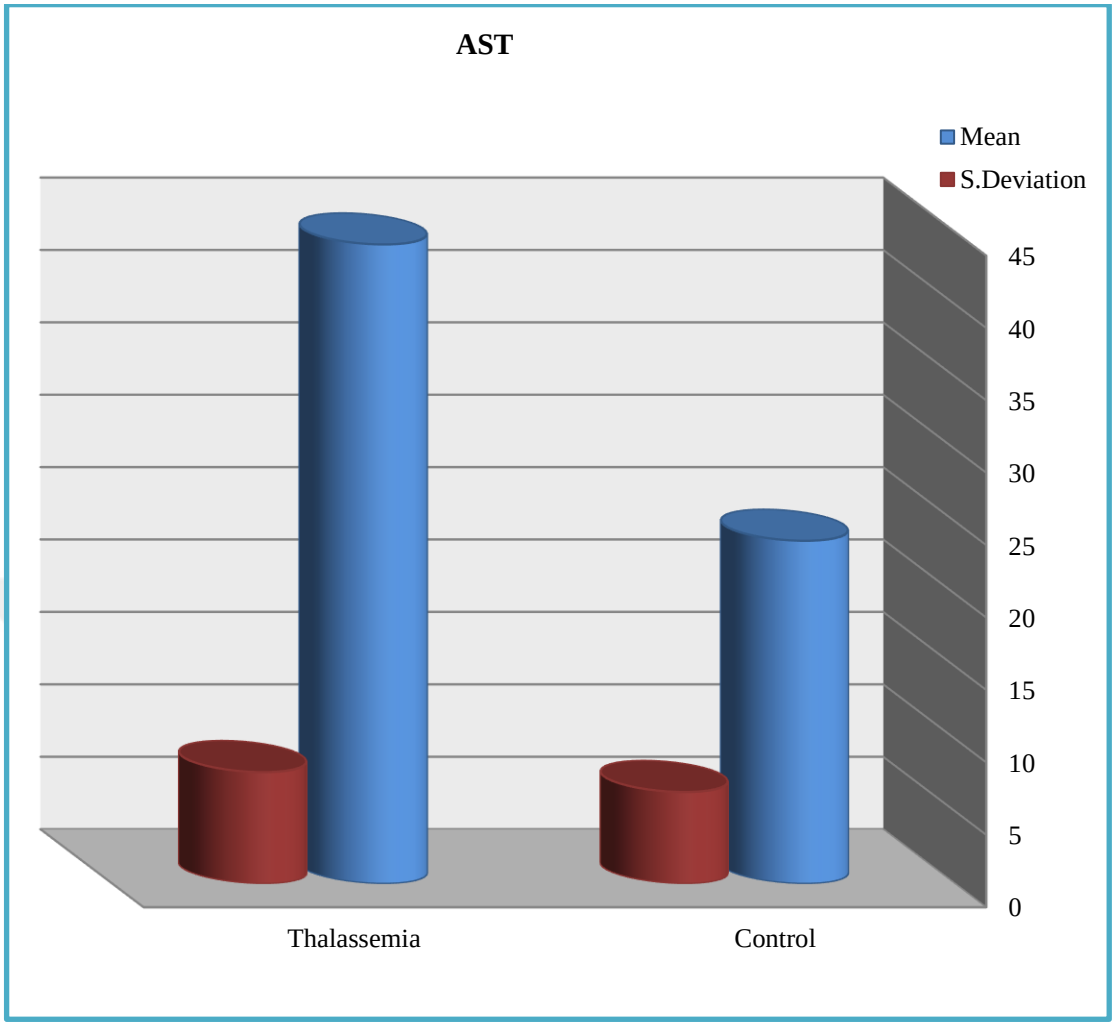

Figure 4.9 The Aspartate aminotransferase (AST) and thalassemia

4.10 The Albumin Level and Thalassemia

The link between albumin level and thalassemia illness revealed that the two groups of our study (control and patient) had different means for the investigated parameter (albumin), with the patient group having significantly lower albumin levels than the (control-group). The patient-group registered 39.98g/dL, whereas the control group registered 45.49g/dL (Table 4.10 and Figure 4.10). The results were consistent with those of (Alhillawi *et al.* 2021), the levels of albumin in the patient group with thalassemia were considerably lower in this study's control group compared to the patient group with thalassemia.

Table 4.10 Statistics group of albumin parameter

	Study group	Mean	S. Deviation	P value
Albumin	Thalassemia	39.98	9.148	<0.001**
	Control	45.49	3.179	
	Total	42.74	7.357	

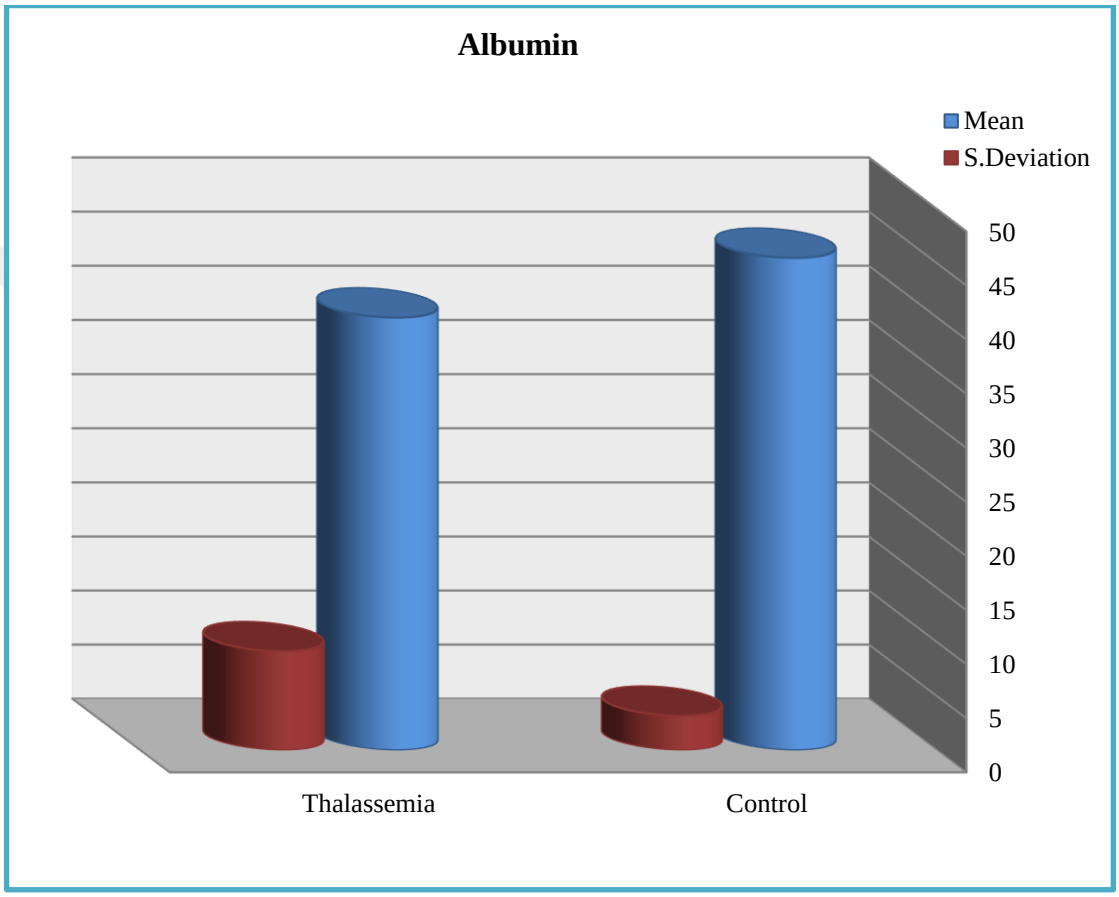

Figure 4.10 The albumin level and thalassemia

4.11 The Copper Level and Thalassemia

The results of serum copper levels for the two tested groups revealed that the patient group was a lower copper concentration than the control group, with a score of 12.7125 mol/L compared to 18.0845 mol/L for the control group. These outcomes are in line with those of (Al-Omari and Takruri 2022).

People with - thalassemia are known to have insufficient hepcidin, which increases the bodies ability to absorb (Fe) from the diet, and release iron from storage. This causes an increase in oxidative stress, necessitating the enzyme superoxide dismutase (SOD).

Iron absorption competes with the absorption of zinc and copper, the two essential SOD components, resulting in Zn and Cu deficiency. Dietary intakes of Zn and Cu were similarly inadequate in -thalassemia patients (Table 4.11 and Figure 4.11).

In addition, the findings showed that the standard deviation of the patient population was 1.26568mol/l, whereas the standard deviation of the comparison group was 3.04684mol/l, with a P value of 0.001** indicating that there was a highly strong correlation here between copper concentration in the serum blood of the patient group and with there concentration in the control group.

This was demonstrated by comparing the standard deviations of the sick group and the control group (Table 4.11).

These findings indicate that copper concentration considers not an indicator in the prediction of β-thalassemia disease. The published papers on serum copper levels in β-thalassemia patients revealed some incongruent findings with some data displaying decreased or increased copper levels or may be no significant changes (Mahyar *et al.* 2010, Zekavat *et al.* 2018).

Table 4.11 Statistics group of copper parameter

	Study group	**Mean**	**S. Deviation**	**P value**
Copper	Thalassemia	162.213	43.2963	<0.001**
	Control	88.571	24.5009	
	Total	125.392	50.9343	

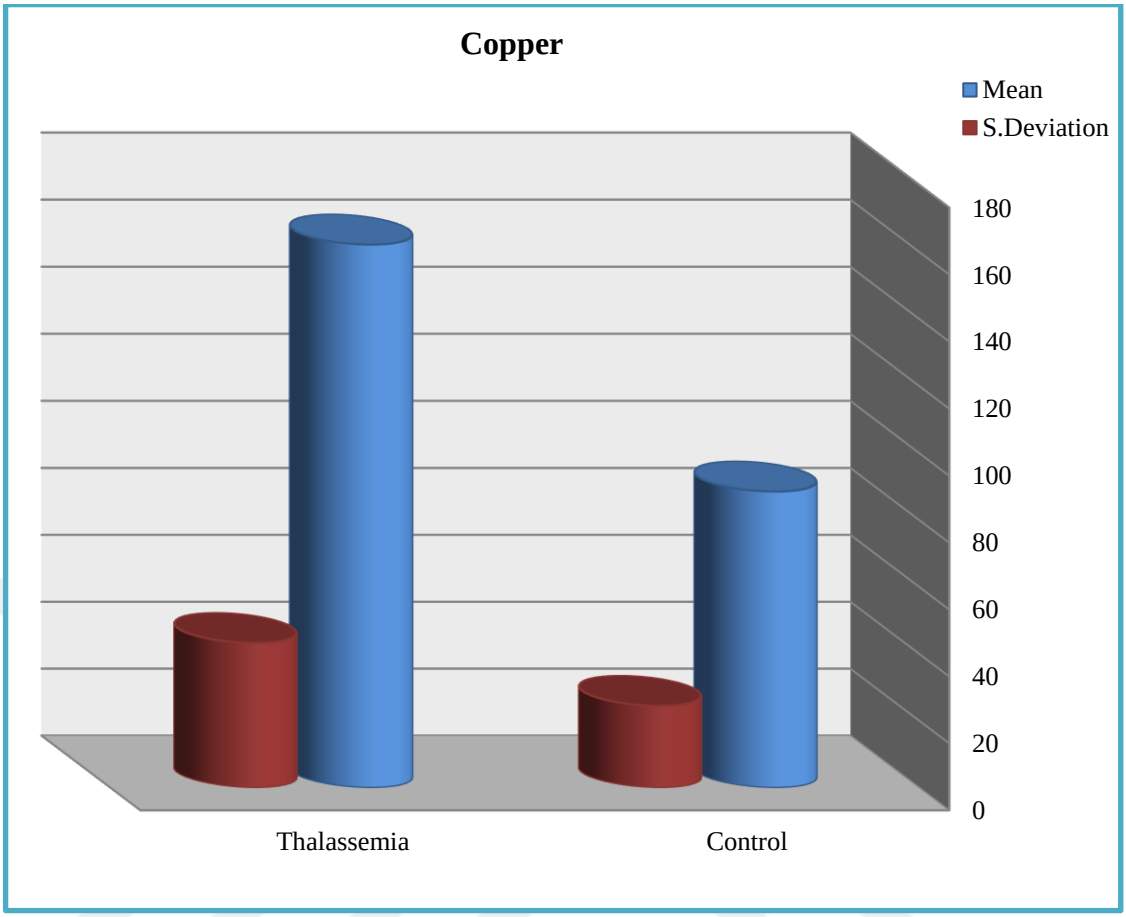

Figure 4.11 The Copper level and β-thalassemia

4.12 Correlations between Hepcidin and Another Parameter

4.12.1 Correlations between hepcidin and ferritin parameter

Pearson correlation test (Table 4.12 and Figure 4.12) showed that hepcidin levels had no significance with ferritin levels (P = <0.001**, r = -0.899), which is consistent with the previous study of (Salah Al-Zuhairi *et al.* 2021), which showed no significant association between serum (ferritin and hepcidin) in patients with (BTM).

This indicates that a deficiency of hepcidin levels contributes to an increase in ferritin levels in (patients with β-thalassemia), and these results are consistent with those of aprevious study (Tantiworawit *et al.* 2021).

Abd El-Salam *et al.* (2018), Elizabeth Nemeth, and Tomas Ganz (2006) showed that the level of hepcidin in (patients with beta-thalassemia) was significantly lower than in healthy individuals.

Table 4.12 Correlations between Hepcidin and ferritin parameter

No	Parameters	N	Correlation	P value
Pair 1	Hepcidin and Ferritin	110	-0.899-**	<0.001**

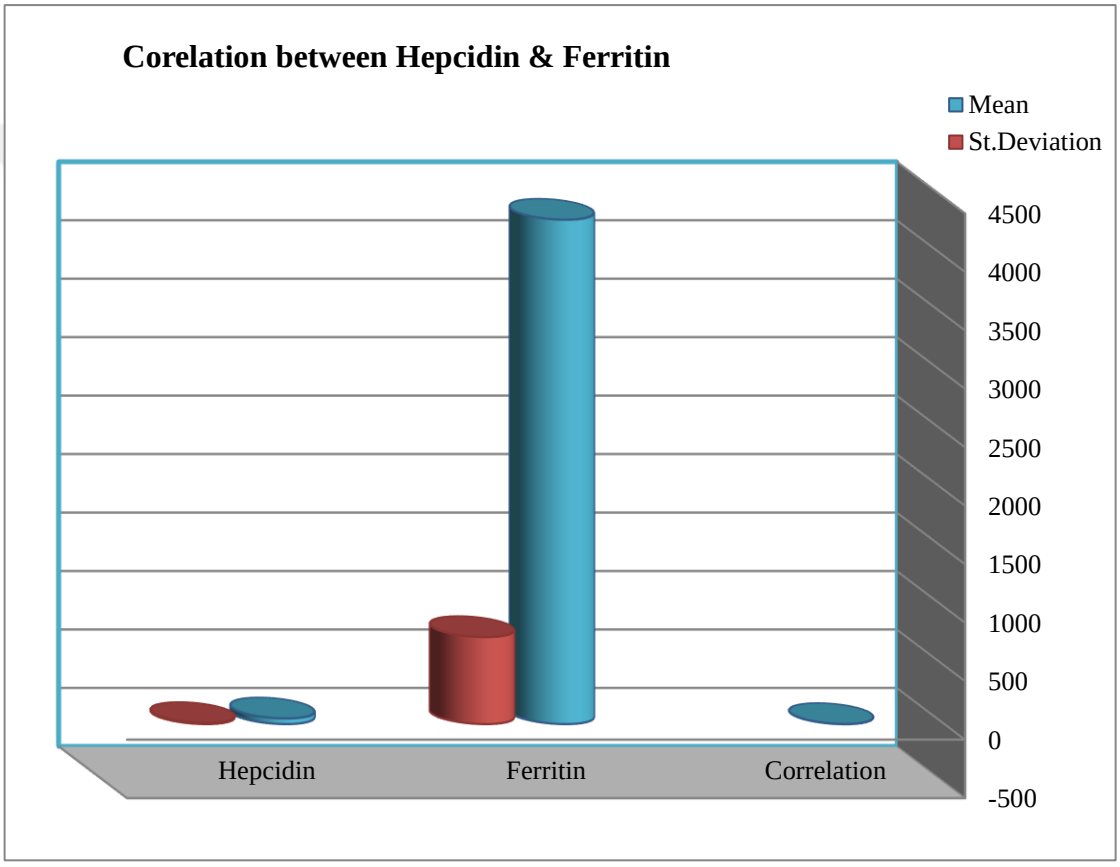

Figure 4.12 Correlations between hepcidin and ferritin parameter

4.12.2 Correlations between hepcidin and Iron parameter

The Pearson correlation test (Table 4.13 and Figure 4.13) showed that the levels of hepcidin did not correlate with iron levels (P = <0.001**, r = -0.823-).

The finding of the current results is in agreement with (Tantiworawit *et al.* 2021). Documented research demonstrated a negative relationship between hepcidin, (Fe) and ferritin (Hasoon *et al.* 2020).

A decrease in hepcidin levels will improve (Fe) absorption from the food and (Fe) mobilization from macrophages and hepatocytes, hence increasing the amount of iron available for RBC formation, hepcidin has been demonstrated to be suppressed by hypoxia and anemia (Nemeth and Ganz 2006).

Lack of hepcidin has been associated with b-thalassemia, which increases (Fe) absorption from the diet, and iron release from the bodies iron stores (Al-Omari and Takruri 2022).

Table 4.13 Correlations between hepcidin and iron parameter

No	Parameters	N	Correlation	P value
Pair 2	Hepcidin and Iron	110	-0.823-**	<0.001**

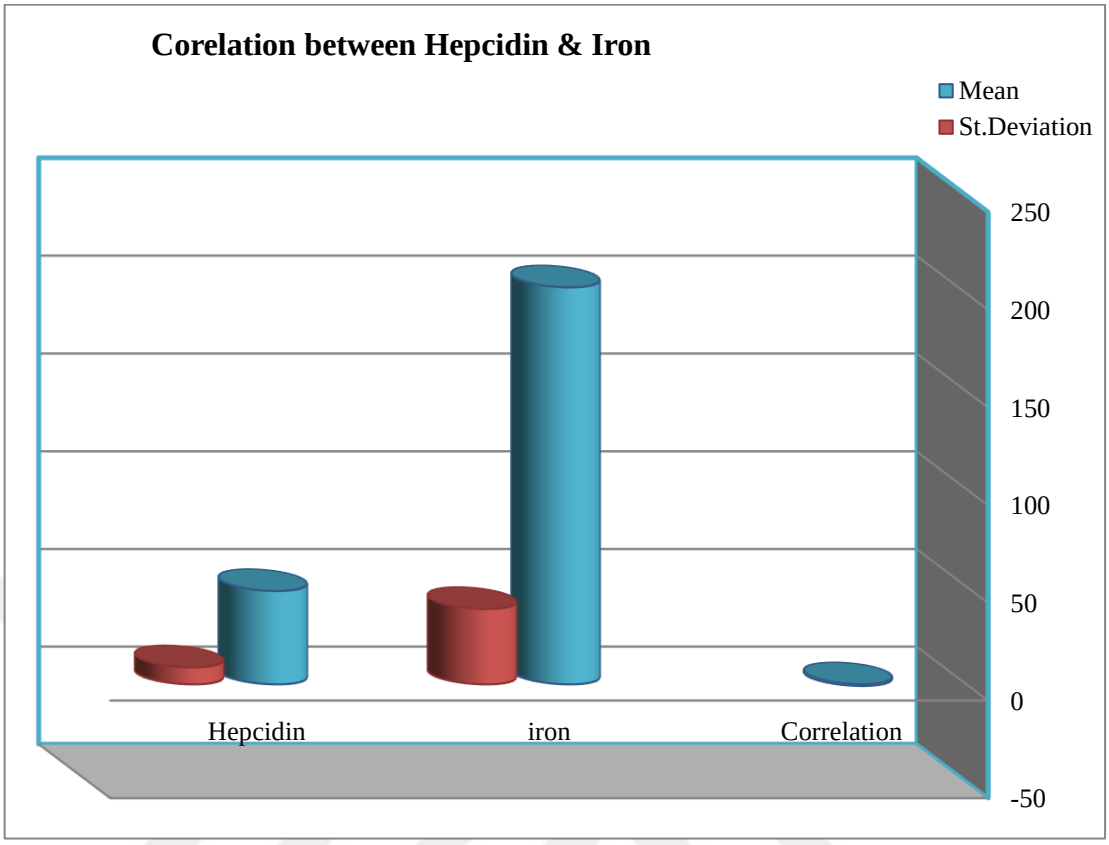

Figure 4.13 Correlations between hepcidin and iron parameter

4.12.3 Correlations between hepcidin and hemoglobin parameter

The association between hepcidin and hemoglobin levels in serum blood indicated a strong correlation (P <0.001**, r = 0.843), which indicates that hemoglobin has a strong positive link with hepcidin. These results were in line with (saadoon, M. Q. and Ali, B. H. 2018).

The link between serum and liver hepcidin mRNA expression and hemoglobin concentration was positively correlated in thalassemic patients compared to controls.

Al-Shemery and Al-Dujili (2019) discovered a significant drop in hemoglobin, hepcidin, and body mass index in - thalassemia patients compared to healthy persons, whereas serum iron and ferritin levels increased significantly **(Table 4.14 and Figure 4.14).**

Table 4.14 Correlations between hepcidin & hemoglobin parameter

No	Parameter	N	Correlations	P value
Pair 3	Hepcidin & Hb	110	-0.823-**	<0.001**

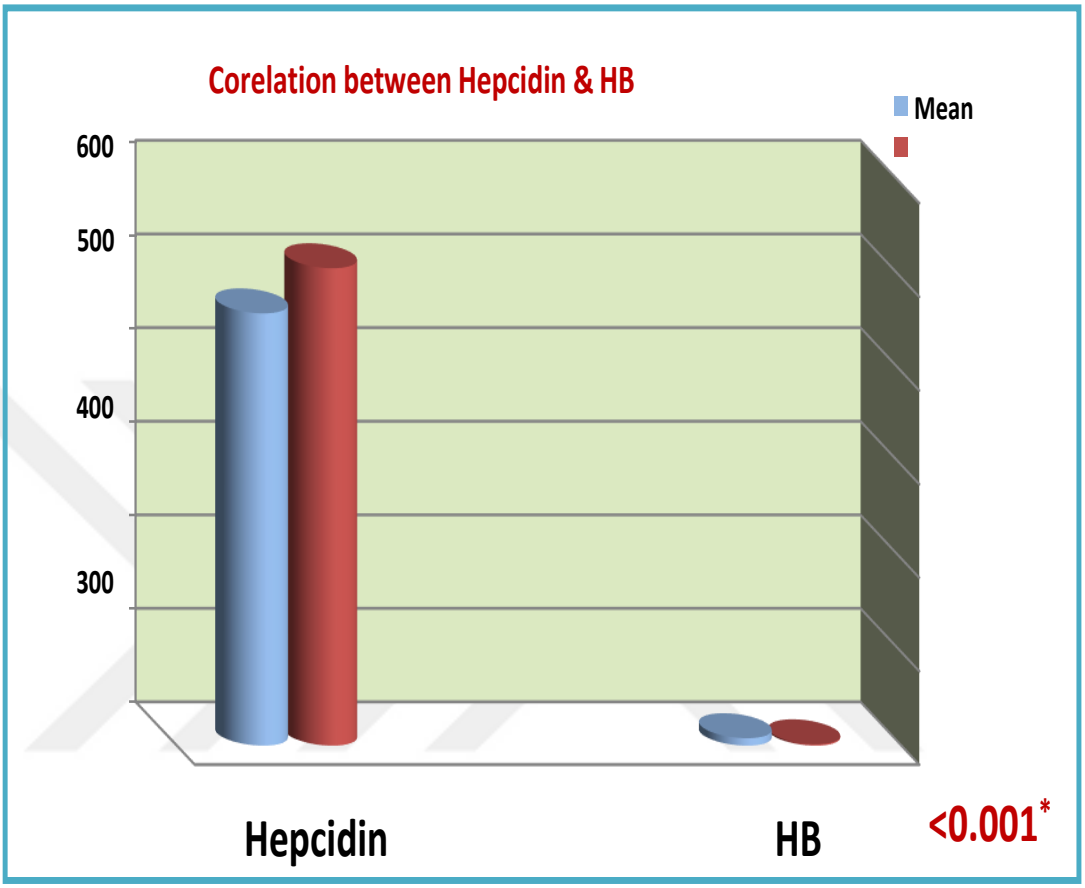

Figure 4.14 Correlations between hepcidin and hemoglobin parameter

4.12.4 Correlations between Hepcidin and PCV parameter

The results of the correlation between hepcidin level and PCV level in serum blood revealed there is a (strong correlation) between them (P <0.001**, r = 0.821), which indicates that PCV has a strong positive link with hepcidin (Table 4.15 and Figure 4.15).

The finding of the current results is in agreement with (Saadoon, M. Q. and Ali, B. H. 2018). The iron concentration showed a strong influence through the level of hepcidin-25, so hepcidin -25 used to be a surprisingly important exchange in the thalassemia-

affected person after which the blood progressed with much of the behavior of iron overload. We, therefore, inferred the low level of (Hb) before and after transfusion due to the lack or absence of globin formation due to gene perturbation and inferred the association of hepcidin with Hb and PCV.

Table 4.15 The correlation between hepcidin level and PCV level

No	Parameters	N	Correlation	P value
Pair 4	Hepcidin & PCV	110	0.821**	<0.001**

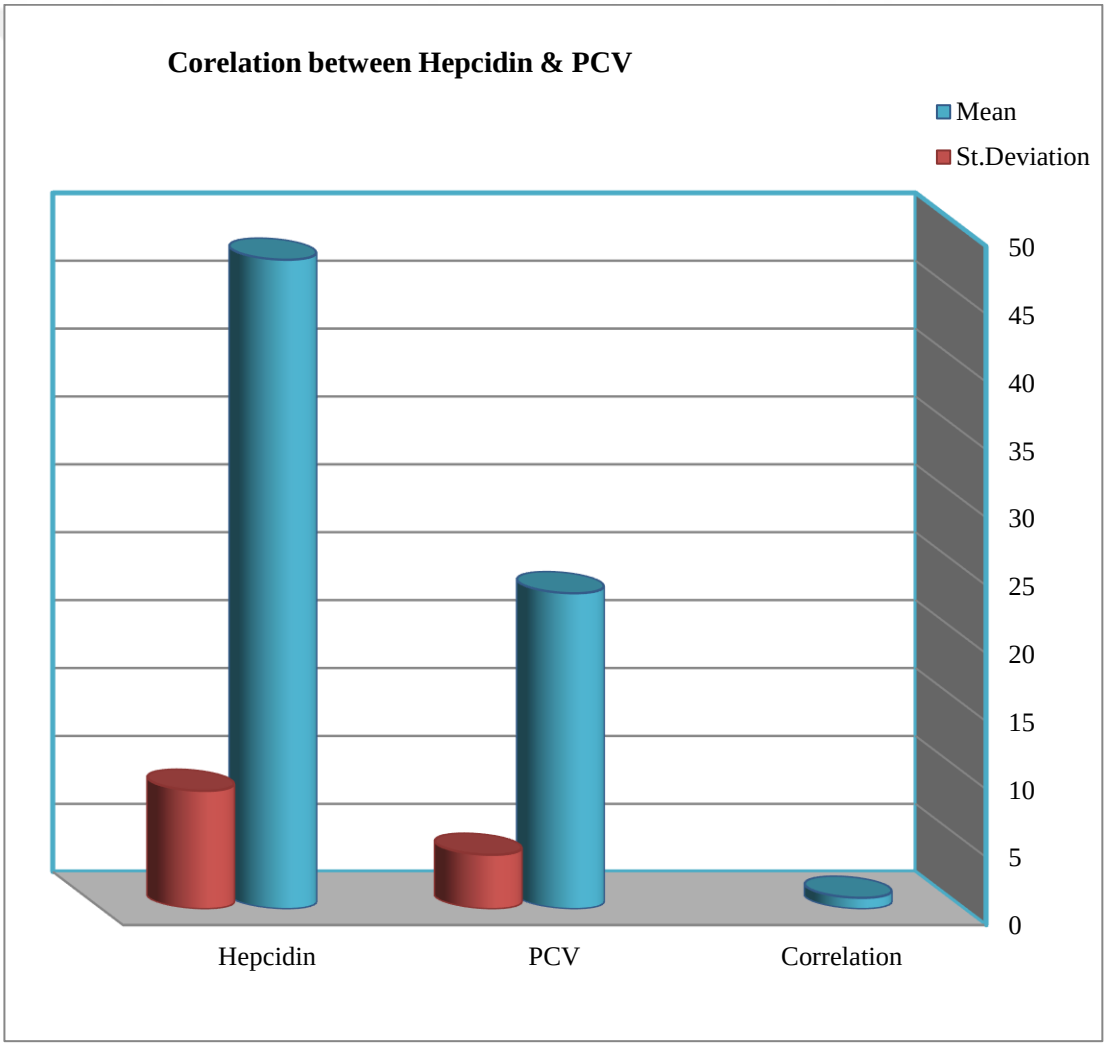

Figure 4.15 The correlations between hepcidin and PCV

4.12.5 Correlations between Hepcidin and ALT parameter

Correlation results between hepcidin level and blood ALT level showed no correlation between them (P<0.001**, r = -0.862**). Since hepatocytes predominantly produce hepcidin, pathological abnormalities in the liver may impair its synthesis (Table 4.16 and Figure 4.16).

Symptoms of ALT activity include liver damage. In all patients with thalassemia,an injured liver is the obvious primary for excessive iron deposition (Hasoon *et al.* 2020). The current findings do not match those of (An *et al.* 2015), because the results of hepcidin were low in the current study, while in the study (An *et al.* 2015) the result of hepcidin were high, and this is why the relationship became negative.

Table 4.16 The results of correlation between hepcidin level and ALT

No	Parameters	N	Correlation	P value
Pair 5	Hepcidin & ALT	110	-0.862-**	<0.001**

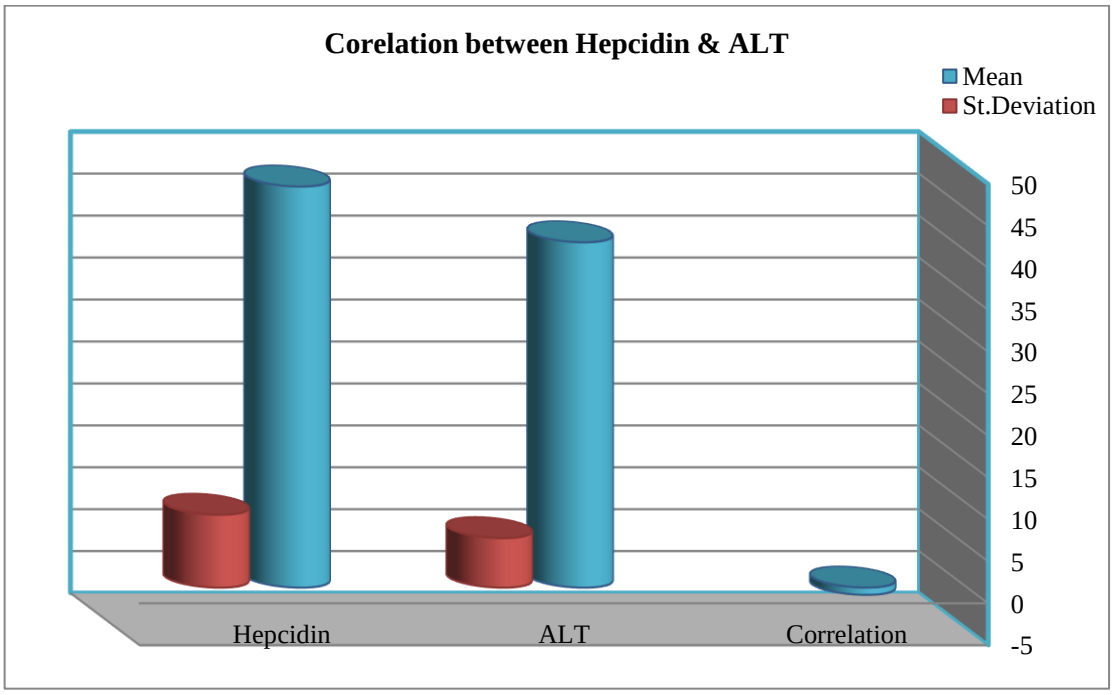

Figure 4.16 Correlations between hepcidin and ALT parameter

4.12.6 Correlations between Hepcidin and AST parameter

The results of the association between hepcidin level and AST level in the blood showed a no correlation between them (P 0.001**, r = -0.752).

Since hepatocytes are responsible for the majority of hepcidin synthesis, pathological changes in the liver may have an effect on hepcidin synthesis (Table 4.16 and Figure 4.16).

AST and ALT values are greater in β-thalassemia patients, which means they are more likely to develop heart and liver problems. While treating patients with beta thalassemia, clinicians should monitor the heart and liver function of their patients (Hosseini and Bouzari 2016).

The effects of thalassemia are known as cirrhosis and cirrhosis of the liver. Transaminases multiplied over and beyond the usual range, indicating that iron overload may contribute to cirrhosis.

The current findings do not match those of (An *et al.* 2015), because the results of hepcidin were low in the current study, while in the study (An *et al.* 2015) the result of hepcidin were high, and this is why the relationship became negative.

Table 4.17 The results of correlation between hepcidin level and AST

No	Parameters	N	Correlation	P value
Pair 6	Hepcidin & AST	110	-0.752-**	<0.001**

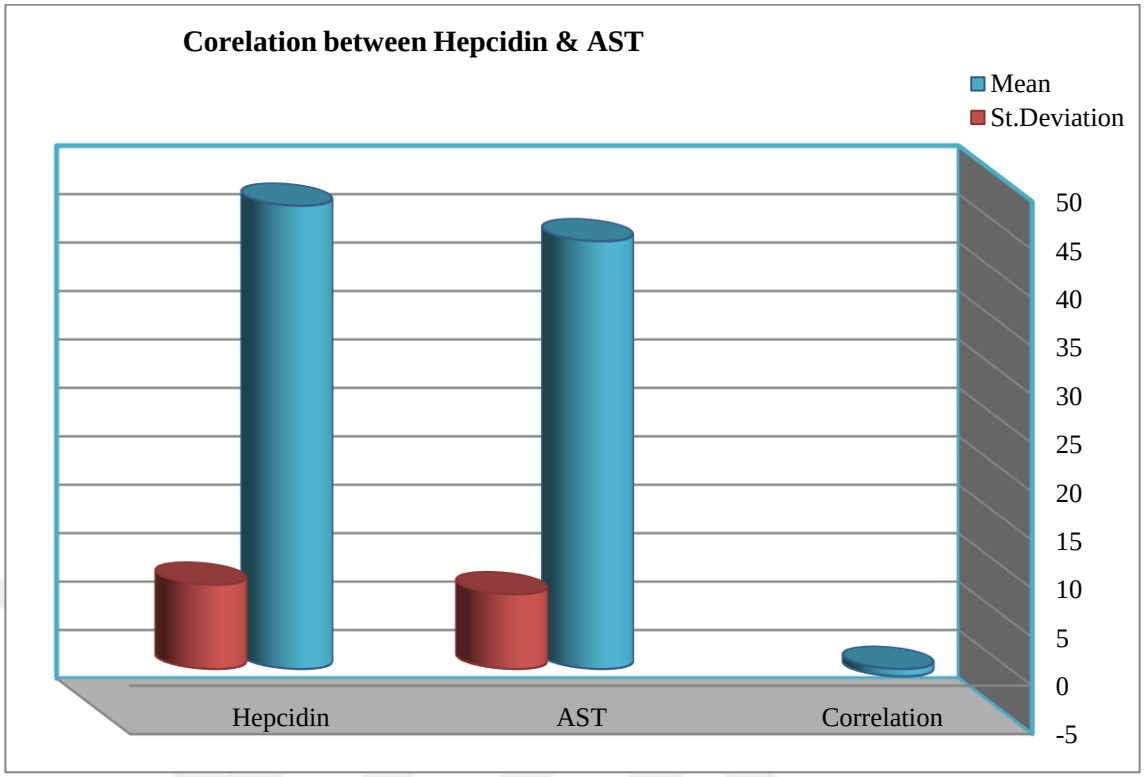

Figure 4.17 Correlations between hepcidin and AST

4.12.7 Correlations between Hepcidin and albumin parameter

The results of the correlation between hepcidin level and albumin level in serum blood (Table 4.18 and Figure 4.18) indicated there is a significant relation between the hepcidin level and albumin level and it eas a significant correlation between them (P< 0.001**, r = 0.356**). Hepcidin is substantially linked with lipids, albumin, creatinine, urea, and remaining renal function in hemodialyzed individuals (Malyszko *et al.* 2006). Thus, this leads to a decrease in albumin in the blood due to cirrhosis. The finding of the current results is in agreements with (Malyszko *et al.* 2006).

Table 4.18 The results of correlation between hepcidin level and albumin level

No	Parameters	N	Correlation	P value
Pair 7	Hepcidin and Albumin	110	0.356^{**}	$<0.001^{**}$

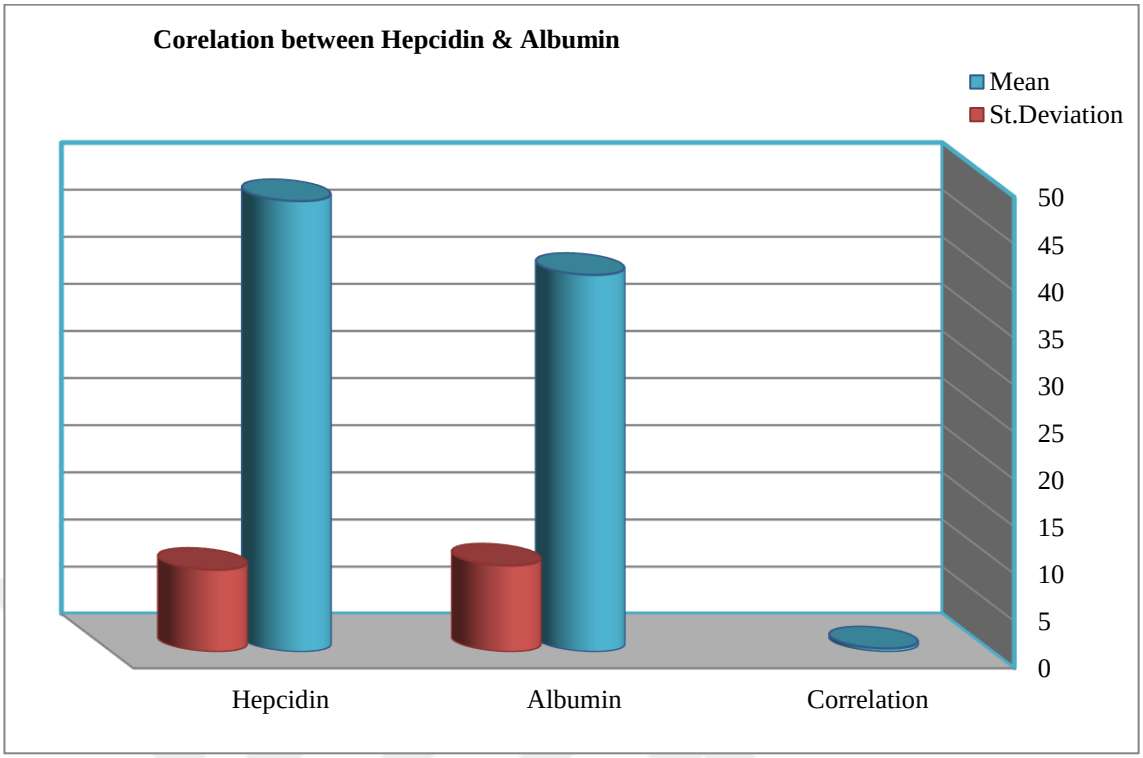

Figure 4.18 Correlations between hepcidin and albumin

4.12.8 Correlations between Hepcidin and Copper parameter

The results of the correlation between hepcidin level and copper level in serum blood (Table 4.19 and Figure 4.19) indicated there is a relation between the hepcidin level and copper level and it was a significant correlation between them (P< 0.001**, r= 0.693). The finding of the current results is in agreement with (Al-Omari and Takruri 2022).

A deficiency of hepcidin has been associated with beta-thalassemia, which increases (Fe) absorption from the diet, and iron release from the body's iron stores. As a result, oxidative stress increases, necessitating the superoxide dismutase enzyme (SOD). Increased iron absorption competes with the two crucial zinc and copper components of the SOD, causing deficits in both. Dietary intakes of zinc and copper were shown to be deficient in β-thalassemia patients. Hepcidin and copper are positively correlated, and this has been proven.

Table 4.19 The results of correlation between hepcidin level and copper level

No	Parameters	N	Correlation	P value
Pair 8	Hepcidin & Copper	110	-.690-**	<0.001**

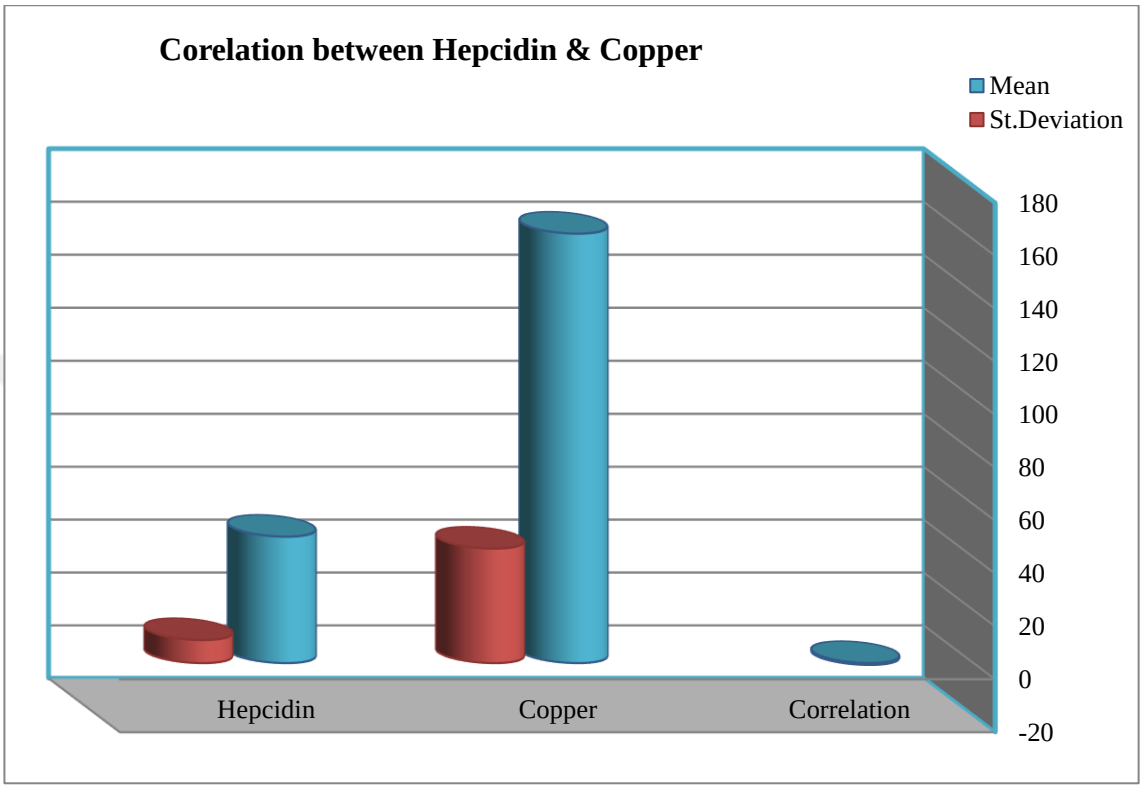

Figure 4.19 Correlations between hepcidin and copper parameter

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The level of hepcidin in the (patient group) was, significantly lower compared to the control-group, and this indicates a clear association between the level of hepcidin and beta-thalassemia.

The level of iron in the blood in the (patient-group) was more than that of the (control-group), and this indicates that the statistics showed that iron levels in the serum of the (diseased-group) were much higher than those in the control- group.

The results showed that hepcidin levels have no significance with ferritin levels, and this indicates that deficiency of hepcidin levels may be contributing to increased ferritin in patients and also ferritin can be used as a tool for examining (Fe) burden on patients.

There is no significant relationship between (serum hepcidin and serum (Fe) in patients.

The results showed that hepcidin levels have no significance with ALT and AST in patients.

Hepcidin levels have a strong correlation with hemoglobin and PCV in patients.

Hepcidin levels with albumin and copper had a significant positive relationship in patients.

5.2 Recommendations

Ferritin and hepcidin concentrations in the serum blood of -thalassemia individuals can be utilized as a prediction for -thalassemia patients or in a person or healthy person who does not display indications of the illness, as these data indicated.

The hepcidin hormone has a significant role in early identification and subsequent treatment selection for -thalassemias.

Monitoring the abruptness of changes in biochemical markers of -thalassemia daily may protect patients from the dangers of -thalassemia. A complete blood count and hemoglobin test are the most useful diagnostic tools for investigating –thalassemias.

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APPENDICES

APPENDIX 1.The clinical criteria of the study

APPENDIX 2. Abbott i 1000 sr

APPENDIX 3. Abbott C4000

APPENDIX 4. Human Reader HS

APPENDIX 5. Human Combiwash

APPENDIX 1.The clinical criteria of the study

Name: **Namber of pation**

Age(5-35): **Control group:A**

Gender: **Pateint group:B**

Biochemical parameters

1-Ferritin

2-Iron

3-Hb

4-PCV

5-ALT

6-AST

7-Albumin

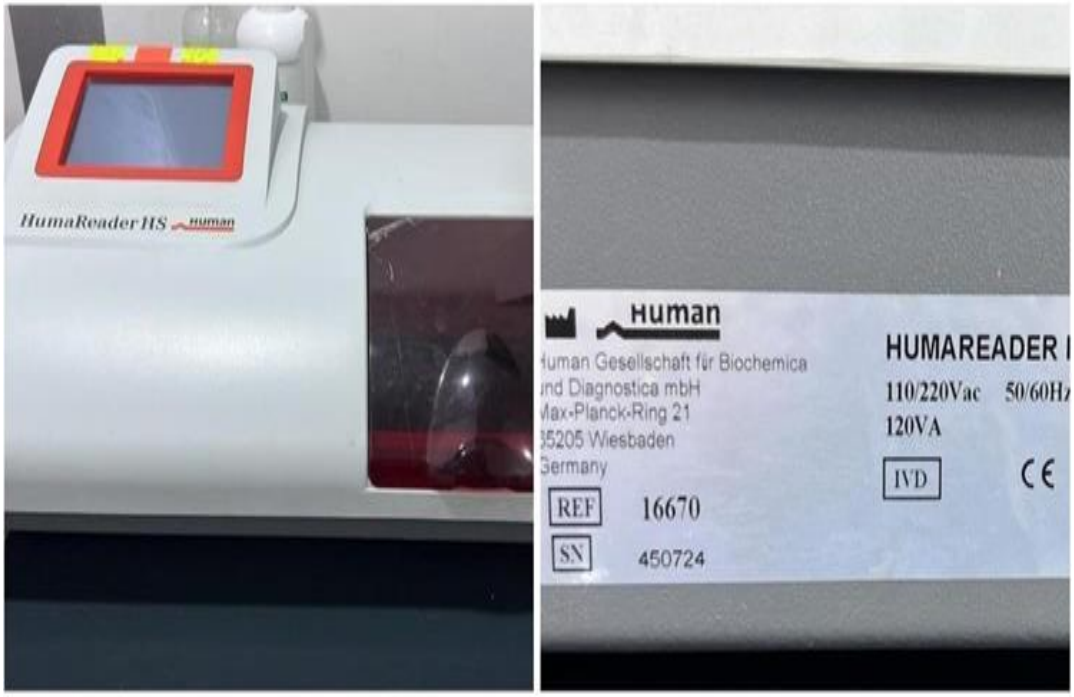
8- Copper

9-Hepcidin

APPENDIX 2. Abbott i 1000 sr

APPENDIX 3. Abbott C4000

APPENDIX 4. Human Reader HS



APPENDIX 5. Human Combiwash