

KARADENIZ TECHNICAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

BIOTECHNOLOGY DEPARTMENT

**EFFECT OF DEFEROXAMINE AND DEFEROSIROX ON SOME
IMMUNOLOGICAL RESPONSES, BIOCHEMICAL, HORMONAL, AND
HEMATOLOGICAL CHARACTERISTICS IN BETA THALASSEMIA MAJOR
PATIENTS**

MASTER THESIS

HAMZAH MAHMOOD NAJM NAJM

FEBRUARY 2025
TRABZON



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**This thesis accepted to give the degree of
"MASTER OF SCIENCE"
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PREFACE

This study, named “Effect of Deferoxamine and Deferosirox on Some Immunological Responses, Biochemical, Hormonal, and Hematological Characteristics in Beta Thalassemia Major Patients” was prepared as a “Master thesis” at Karadeniz Technical University, Institute of Natural and Applied Sciences, Department of Biotechnology.

First, I give thanks to God for granting me the endurance and direction needed to complete this endeavor.

I would like to express my deep and sincere gratitude to my first supervisor, Prof. Dr. Remziye NALÇACIOĞLU, for her invaluable guidance, unwavering support, and encouragement throughout this journey. Her cooperation and enthusiasm have been instrumental in fostering my passion for this field.

I am profoundly grateful to my second supervisor, Prof. Dr. Özlen BALTA, for her assistance throughout the research period.

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Last but not least, I am deeply thankful to my parents for their unwavering support and to my mother for her heartfelt prayers. I am deeply grateful to all of you.

HAMZAH MAHMOOD NAJM NAJM

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THESIS ETHICS DECLARATION

I declare that I have completed this study titled as “Effect of Deferoxamine and Deferosirox on Some Immunological Responses, Biochemical, Hormonal, and Hematological Characteristics in Beta Thalassemia Major Patients” and presented as a master's thesis is written under my advisor's supervision. I hereby declare that I have completed this study under the responsibility of Prof. Dr. Remziye NALÇACIOĞLU and Prof. Dr. Özlen BALTA, that I have collected the data/samples myself, that I have performed the experiments/analyses in the relevant laboratories, that I have fully cited the information I have obtained from other sources in the text and bibliography, that I have acted in accordance with scientific research and ethical rules during the study process and that I accept all legal consequences in case of any contrary results. 24/02/2025

HAMZAH MAHMOOD NAJM NAJM

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ÖZET

**BETA TALASEMİ MAJÖR HASTALARINDA DEFEROKSAMİN VE
DEFEROSİROKS BAZI İMMÜNOLOJİK YANITLAR, BİYOKİMYASAL,
HORMONAL VE HEMATOLOJİK ÖZELLİKLER ÜZERİNE ETKİSİ**

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Talasemi, genetik bir kan hastalığı olup, düşük hemoglobin oranıyla karakterizedir ve her iki cinsiyette de ciddi anemiye neden olur. Bu hastalık, alfa ve beta talasemi olmak üzere iki ana kategoriye ayrılır. Hayatta kalabilmek için düzenli kan transfüzyonlarına ihtiyaç duyulur, ancak bu süreç zamanla vücutta aşırı demir birikimine yol açabilmektedir. Deferoksamin ve deferasiroks gibi şelasyon tedavileri, BTM hastalarında demir yükünü kontrol altına almak amacıyla uygulanmaktadır.

Bu çalışma, Azadi Eğitim Hastanesi Talasemi Merkezi'nde yürütülmüş ve 18 yaş üzeri 200 β -talasemi majör hastasını kapsamaktadır. Hastalar, demir şelatlayıcı olarak deferasiroks veya deferoksamin reçete edilen transfüzyona bağımlı hastalar olmak üzere iki gruba ayrılmıştır. Ayrıca, talasemi hastalığı bulunmayan 100 sağlıklı bireyden oluşan bir kontrol grubu da çalışmaya dahil edilmiştir.

İstatistiksel analizler (ANOVA, Kruskal-Wallis, Pearson correlation coefficient), deferoksaminin deferasiroks'tan daha etkili olduğunu ve HCT, RBC, MCH, Hb, GH, WBC, lenfositler, kreatinin, TSH, prolaktin (hem erkeklerde hem kadınlarda) ve TLR3 seviyelerini daha fazla iyileştirdiğini göstermiştir. Buna karşılık, deferasiroks'un trombosit sayısı, ferritin, demir, kalsiyum, AST, ALT, ALP, GGT, LH, FSH (hem erkeklerde hemde kadınlarda), IL6 ve IL8 seviyelerini düşürmede daha etkili olduğu belirlenmiştir.

Ayrıca, deferasiroks, tiroid bezindeki demir yükünü azaltmada daha başarılı olup, vücutta genel demir seviyeleri üzerinde daha geniş kapsamlı bir etkiye sahiptir. Uzun yarı ömrü sayesinde daha güvenli bir kullanım sunmaktadır. Sonuçları genel olarak, Sonuçları deferasiroksun, deferoksamine kıyasla daha etkili ve güvenli olduğunu söyleyebiliriz.

Anahtar Kelimeler: Beta-talasemi majör, Deferoksamin, Deferasiroks, Ferritin ve demir, endokrin sistem, GRP78, CHOP, interlökin, karaciğer enzimleri.



Master Thesis

SUMMARY

EFFECT OF DEFEROXAMINE AND DEFEROSIROX ON SOME IMMUNOLOGICAL RESPONSES, BIOCHEMICAL, HORMONAL, AND HEMATOLOGICAL CHARACTERISTICS IN BETA THALASSEMIA MAJOR PATIENTS

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2025, 93 Pages

Thalassemia is a genetic blood disease known by an abnormally low hemoglobin ratio, leading to severe anemia in both genders. It is classified into alpha and beta thalassemia. Frequent blood transfusions are essential for survival but can lead to excess iron. Chelating therapies, such as deferasirox, and deferoxamine are used to manage iron overload in patients with BTM.

This study was conducted at Azadi Teaching Hospital's Thalassemia Centre, involving 200 patients with beta thalassemia major over 18 years old. The patients were divided into two categories: transfusion-dependent patients who were prescribed deferasirox or deferoxamine as iron chelators. The control group included 100 healthy individuals (patients without thalassemia disease) was also included.

Statistical analysis (ANOVA, Kruskal-Wallis, Pearson correlation coefficient) revealed that deferoxamine was more beneficial than deferasirox in improving levels of HCT, RBC, MCH, Hb, GH, EBC, lymphocytes, creatinine, TSH, prolactin (in both males and females), and TLR3. In contrast, deferasirox was more effective in reducing platelet count, ferritin, iron, calcium, AST, ALT, ALP, GGT, LH, FSH (in both males and females), and IL6 and IL8.

Furthermore, Deferasirox was more effective in reducing iron overload in the thyroid gland and had a more comprehensive impact on iron levels throughout the body, which made

it safer for use due to its extended half-life. When we evaluate the results in general, we can say that deferasirox is more effective and safer than deferoxamine.

Key Words: Beta-thalassemia major, Deferoxamine, Deferasirox, Ferritin and iron, endocrine system, GRP78, CHOP, interleukin, liver enzymes.



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ABBREVIATIONS

ALP	: Alkaline phosphatase
ALT	: Alanine aminotransferase
AST	: Aspartate aminotransferase
CBC	: Complete blood count
CHOP	: C/homologous protein
DFO	: Deferoxamine
DFP	: Deferiprone
DFX	: Deferasirox
ELISA	: Enzyme linked immunosorbent assay
ER	: Endoplasmic reticulum
FDA	: Food and drug administration
FSH	: Follicle stimulating hormone
FUS	: Fused in sarcoma
GGT	: Gamma glutamyl transferase
GH	: Growth hormone
GRP	: Glucose regulated protein
Hb	: Hemoglobin
HbA	: Adult hemoglobin
HbF	: Fetal hemoglobin
HbH	: Hemoglobin H
HCT	: Hematocrit
HIV	: Human immunodeficiency virus
HPLC	: High performance liquid chromatography
HRP	: Horseradish peroxidase
IL	: Interleukin
LH	: Luteinizing hormone
MCH	: Mean corpuscular hemoglobin
MCV	: Mean corpuscular volume
mL	: Milliliter
ng	: Nanogram
nm	: Nanometer

NTBI	: Non-transferrin bound iron
OD	: Optical density
PCR	: Polymerase chain reaction
Pg	: Picogram
P-value	: Probability value
RBC	: Red blood cell
ROS	: Reactive oxygen species
T. protein	: Total protein
T3	: Triiodothyronine hormone
T4	: Thyroxine hormone
TDT	: Transfusion dependent thalassemia
TIBC	: Total iron binding capacity
TLR	: Toll like receptor
TM	: Thalassemia major
TNF- α	: Tumor necrosis factor alpha
TSH	: Thyroid stimulating hormone
Vit. D	: Vitamin D
WBC	: White blood cell
β	: Beta
β T	: Beta thalassemia
β TM	: Beta thalassemia major
α	: Alpha
γ	: Gamma
δ	: Delta

1. INTRODUCTION

1.1. Overview of Thalassemia

Thalassemia is a hereditary blood disorder characterized by an abnormal low hemoglobin ratio (Al-Allawi et al., 2010). Thalassemia is derived from Greek words "Thalassa," which means "sea", and "emia", which means blood. Thalassemia disrupts the hemoglobin production and leads to severe anemia (Shirzadfar & Mokhtari, 2018). The clinical recognition of thalassemia dates back to 1925 when Thomas Cooley documented a condition characterized by anemia, splenomegaly, and bone deformities in individuals of Italian descent (Nigam et al., 2017). This disease is sometimes referred as Mediterranean anemia due to its prevalence in the Mediterranean regions. It has an impact on individuals of both genders (Muncie & Campbell, 2009).

Thalassemia disease is prevalent throughout large regions that include the Mediterranean basin, Africa, the Eastern Mediterranean, the Indian Peninsula, and Southeast Asia (Sanctis et al., 2017) as illustrated in Figure 1. The incidence rate on the Eastern Mediterranean coast, despite the availability of numerous prevention programs, is high at 31.4 per 100,000 newborns, and the number of new cases at birth could increase over the next 30 years (Weatherall, 2018). On the Southern Mediterranean coast, where the incidence rate is even higher at 36.8 per 100,000 newborns, the region continues to suffer from inadequate healthcare infrastructure, which could lead to a significant rise in new cases by 2050 (Bellis et al., 2021; Vichinsky, 2005).

There are two main types of thalassemia: alpha and beta. Beta thalassemia (β T) is found in various regions at a prevalence of between 1% to 20%, sometimes higher than this range. In Africa south of the Sahara, the prevalence ranges from 10 to 20%, but in certain Middle Eastern and Indian regions, it might be 40% or more (Tari et al., 2018). Around 270 million people worldwide have defective hemoglobin and thalassemia, with 80 million carriers of beta thalassemia. Annually, worldwide, 300,000-400,000 children are born with severe Hb disease, 23,000 with thalassemia major (TM) (Williams & Weatherall, 2012).

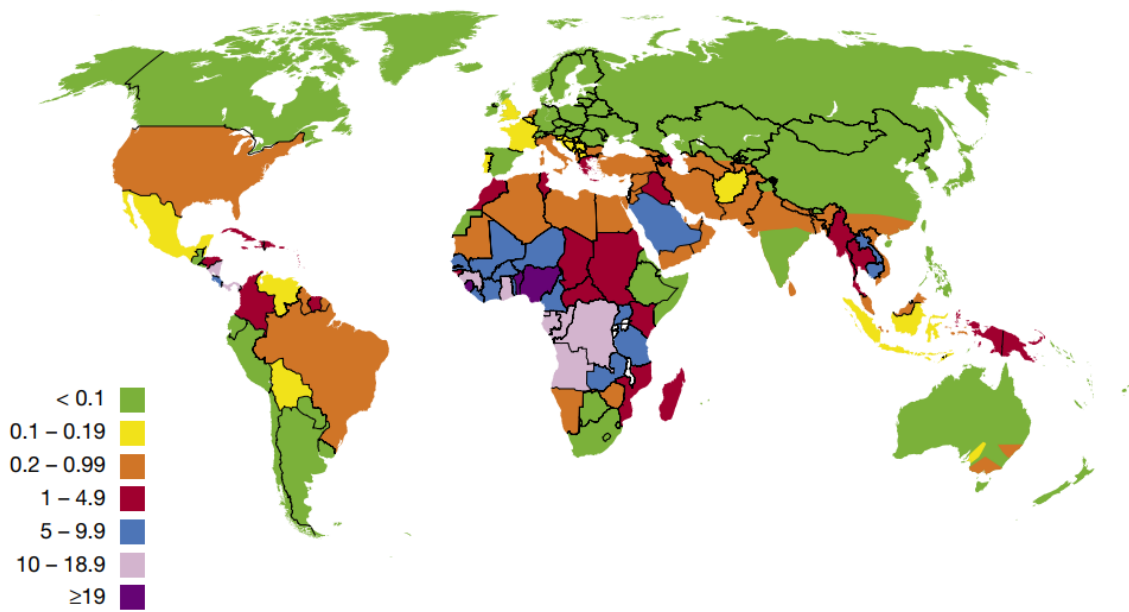


Figure 1. The global distribution of pathological hemoglobin disorders per 1,000 live births (WHO, 1996).

The estimated annual incidence of symptomatic individuals worldwide is 1/100,000, whereas in the European Union it is 1/10,000 individuals. However, specific information regarding carrier rates in numerous populations is lacking, especially in regions of the world that are anticipated or known to be significantly impacted due to this disease (Taher et al., 2014).

In Iraq, hemoglobinopathies accounted for 75% of all cases, with β -thalassemia major comprising 67% of all types. The incidence in Iraq increased from 33.5 per 100,000 population in 2010 to 37.1 per 100,000 population in 2015. Nevertheless, within the same duration, the rate of occurrence has declined from 72.4 cases per 100,000 live births to 34.6 cases per 100,000 live births. In 2015, there were 335 new cases of symptomatic thalassemia recorded, resulting in an incidence rate of 34.6 per 100,000 live births (Kadhim et al., 2017). Figure 2. shows the incidence of thalassemia per 100,000 individuals in each governorate in 2015. Thalassemia was most prevalent in Basra governorate with a rate of 54.5 per 100,000 people, followed by Dohuk governorate with a rate of 54.1 per 100,000 populations. The lowest incidence was in Muthanna with a rate of 19.7 per 100,000 populations.

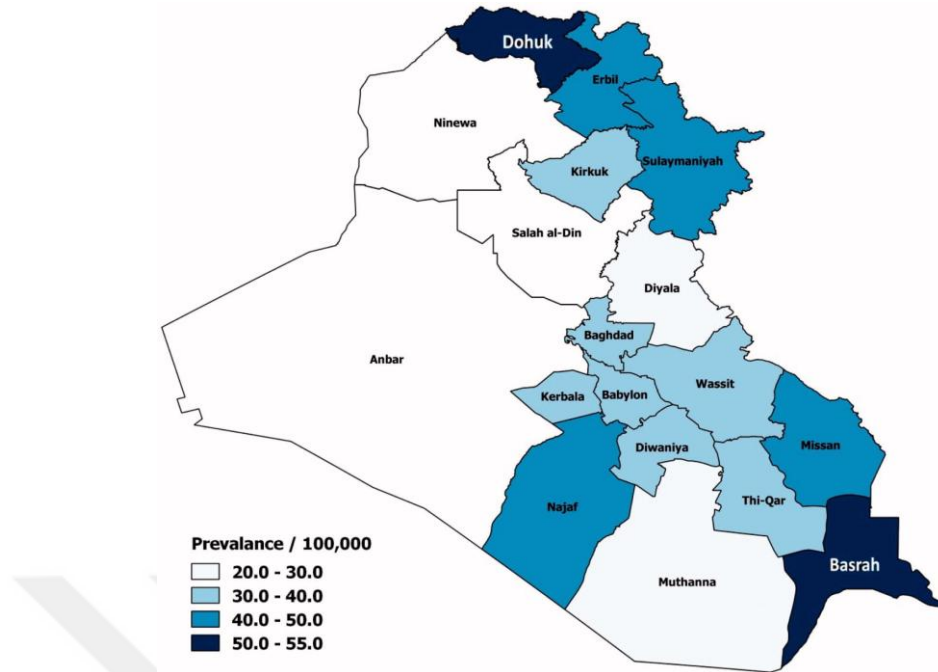


Figure 2. The estimated prevalence of thalassemia per 100,000 people in Iraq according to governorate (Kadhim et al., 2017).

1.2. Hemoglobin Structure and Types in Thalassemia

Hemoglobin is an approximately 64,456 Daltons complex globular protein. Its primary role is to carry oxygen (O_2) from the lungs to the body's tissues and transport carbon dioxide (CO_2) from the tissues back to the lungs. The hemoglobin contains four polypeptide chains interconnected by non-covalent interactions. Each one contains a heme group and a single oxygen-binding site (Mok, 2011). There are four types of globin chains present named alpha (α) beta (β) gamma (γ) and delta (δ). Two distinct globin chains combine to form hemoglobin, includes two alpha (α) and two non-alpha chains like (β , γ , and δ) (Figure 3). 95% of hemoglobin in healthy humans is hemoglobin A (Hb A), which is made up of $\alpha_2\beta_2$, with a minor proportion (3.5%) of hemoglobin A2 (Hb A2) which is made up of $\alpha_2\delta_2$, and hemoglobin F (Hb F) consisting of $\alpha_2\gamma_2$ (Viana et al. 2017). After birth, the production of gamma chains decreases, while the production of beta chains increases, leading to the formation of Hb A (Mcconnell, 2011).

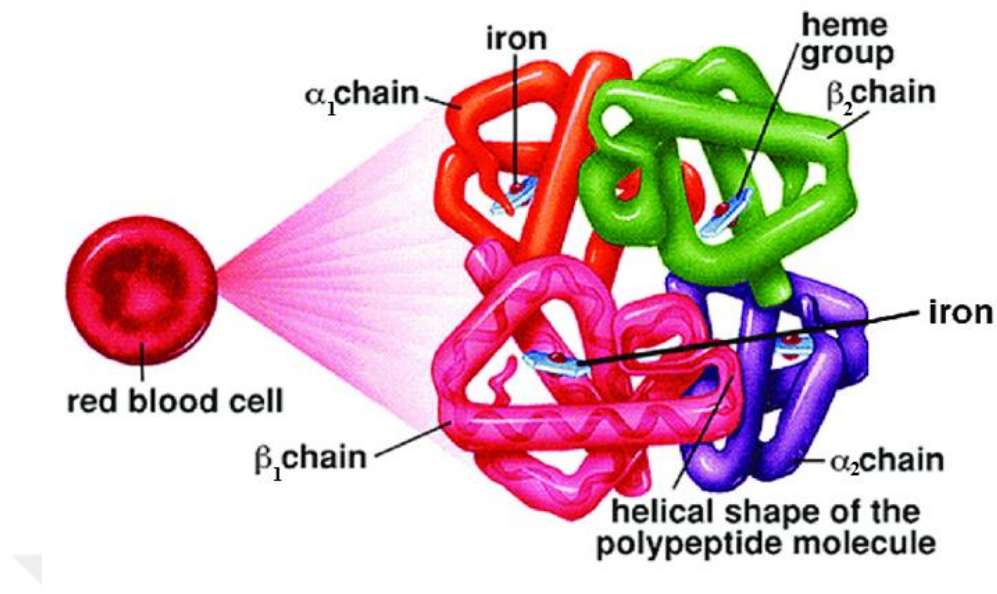


Figure 3. 4D structure of hemoglobin (Storz, 2018).

Thalassemia patients have hemoglobin (HbA) ($\alpha_2\beta_2$) deficiency due to the inability of their bone marrow cells to produce the alpha or beta polypeptide chains of human hemoglobin (Clarke & Higgins, 2000). Hemoglobin is produced concurrently within the red blood cell synthesis process in the bone marrow (Masuda et al., 2016) as in Figure 4.

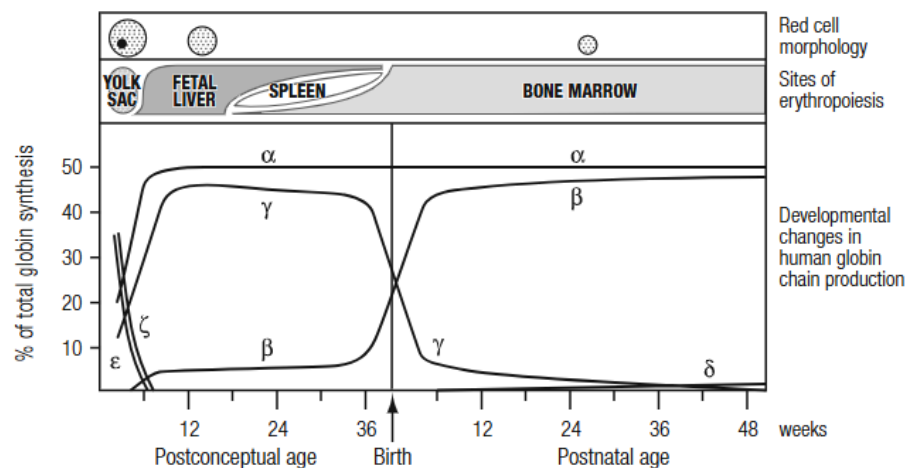


Figure 4. The stages of human hemoglobin development (Marengo-Rowe, 2007). It shows the location of hemoglobin formation along with changes in globin chains production during both embryonic and postnatal development.

1.3. Pathophysiology of the Thalassemia Disease

Thalassemic individuals suffer health issues due to insufficient hemoglobin, which is essential for appropriate oxygenation in the body. These patients also lack healthy hemoglobin. Therefore, the patient's body tries to produce additional red blood cells. That is why a genetic abnormality in the production of hemoglobin leads to the overproduction of harmful hemoglobin, causing thalassemia disease. Individuals with thalassemia receive treatment based on the severity of the disease, as certain minor forms of thalassemia may not be detected. It can result in mild anemia and iron shortages in patients, but more severe cases can be fatal (Bernaudin et al., 2008). Iron overload from frequent blood transfusions affects metabolism and damages some tissues and organs. Several studies indicate that iron chelation treatment improves patients' chances of survival with transfusion-dependent thalassemia (TDT) (Tari et al., 2018). Whereas the accumulation of excess iron in essential organs such as the heart, endocrine glands, liver, and spleen results from non-using iron chelation treatment (Dorgaleleh et al., 2020). Iron overload is the main reason for the majority of thalassemia-related deaths (Wood, 2015). Chronic anemia in thalassemia patients is linked to several complications, including renal failure, cirrhosis, cardiomyopathy, arrhythmias, hypothyroidism, hyperthyroidism, hypogonadism, and endocrine dysfunction (Malik et al., 2009).

Thalassemia is classified into two main types based on the changes that occur in the peptide chains that make up hemoglobin: the first type is α -thalassemia, which results from a mutation in the alpha chains, and the second type is β -thalassemia, which results from a mutation in the beta chains (Giardine et al., 2007).

1.3.1. Alpha Thalassemia

Alpha thalassemia is result of a mutation in the α -globin genes (*HBA1 and HBA2*) on chromosome 16's short arm, which regulates the production of alpha globin chains in hemoglobin. An individual inherits two alpha globin chain genes from each parent, as four genes are responsible for producing the alpha globin chain. So far, over 80 genetic mutations have been discovered. Alpha thalassemia is a rare disorder caused by mutations in a number of alpha hemoglobin genes. The disorder becomes more severe as more genes are affected (Musallam et al., 2013). Alpha thalassemia has four types based on the number of affected α globin genes.

Alpha thalassemia carrier results from a mutation or deletion in one of the four genes involved in alpha globin chain production. Carriers typically remain asymptomatic (Langlois et al., 2008).

Alpha thalassemia minor occurs when two of the four genes which are responsible for alpha-globin chain formation are mutated or deleted, resulting in mild anemia (Luo et al., 2017).

Alpha thalassemia intermedia (Hemoglobin H disease) is caused by absence in three of four alpha-globin genes. Hemoglobin H disease, severe anemia that requires blood transfusion, occurs when beta-globin chains accumulate in RBCs (Vichinsky, 2009).

Alpha thalassemia major results from the complete absence of all four alpha-globin genes, preventing alpha-chain formation and causing gamma-globin chains to predominate. This shift leads to the unstable deposition of γ_4 tetramers (Vichinsky, 2009).

The condition is associated with fetal hydrops, a severe disorder characterized by the accumulation of fluid in various parts of the fetus's body (Prathyusha et al., 2019). The hemoglobin produced in this case is known as hemoglobin Bart and affected infants either die in utero or shortly after birth due to insufficient oxygenation (Darvishi et al., 2016).

1.3.2. Beta Thalassemia

Beta thalassemia is caused by changes occurring in the *HBB* gene located on chromosome 11 (Polat, 2021). The β -genes on chromosome 11 are mutated to produce defects in the production of β -globin chains. Beta thalassemia, produced by over 200-point mutations, may cause mild anemia or asymptomatic (Rivella, 2015). Various genotypes can be differentiated. Homozygosity for the genetic trait itself is the most severe form of thalassemia is referred to as major thalassemia or Cooley's anemia (Salvatory, 2008). Heterozygosity is characterized by the presence of both wild allele and one carrying the mutated β -globin gene. Heterozygous for two distinct mutations of the β -globin gene is associated with the different severity of thalassemia based on the specific point mutation present (Salvatory, 2008).

Beta thalassemia is classified into four different types based on the hemoglobin type and associated symptoms, as indicated in Table 1.

Table 1. Types of beta thalassemia and their characteristics (Susanto, 2021).

Phenotypes	Hemoglobin type	Anemia	Genotype
Beta thalassemia silent carrier	Normal	Normal	$\beta\beta^+$
Beta thalassemia minor (Trait)	HbA2	High	$\beta\beta^\circ$
Beta thalassemia intermedia	a small amount of HbA and HbF, HbA2	Moderate or light	$\beta^+\beta^+$
Beta thalassemia major	HbA absent, only HbA2 amount High and HbF present	Severe	$\beta^\circ\beta^\circ$ or $\beta^+\beta^\circ$ $\beta^+\beta^+$

Beta thalassemia silent is a heterozygous state of β thalassemia, difficult to detect due to normal hematological parameters, and Hb A2 and Hb F levels remain normal, with minor beta chain imbalance (Salvatory, 2008). Beta thalassemia minor refers to β thalassemia carriers who do not show clinical symptoms, although sometimes they get mild anemia (Galanello & Origa, 2010). This condition involves a decrease in RBC count and Hb content, with levels of hemoglobin reaching 10 g/100 ml (Romanello et al., 2018). If both parents have thalassemia, there is a 25% probability of their children being born with homozygous thalassemia in each pregnancy (Galanello & Origa, 2010). β thalassemia carriers are generally heterozygous, carrying one standard hemoglobin β -globin and one thalassemia allele, indicated as β^+ or β° (Quek & Thein, 2007). Beta Thalassemia intermedia experience moderate anemia that may not always require blood transfusions, while some may exhibit signs of severe anemia similar to beta thalassemia major (Galanello et al., 2001). β -thalassemia major, the most severe kind of beta thalassemia, is described by chronic anemia with clinical symptoms that usually occur between the ages of 6 and 24 months (Tari et al., 2018).

1.4. Signs and Symptoms of Thalassemia

Patients with thalassemia intermedia may need blood transfusions from the age of 2 to 6 years old. Some can live without regular transfusions and remain without symptoms until adulthood, experiencing only mild anemia and erythrocytosis and possibly extramedullary erythropoiesis. This is a compensatory process by the bone marrow to chronic anemia, which can lead to facial bone abnormalities, osteopenia, fractures of long bones, and the production of erythropoietic in numerous organs, especially affecting the spleen due to its function in removing damaged red blood cells (Vogiatzi et al., 2009; Taher et al., 2021).

Extramedullary erythropoiesis can lead to neurological complications, including paraplegia, due to spinal cord compression (Galanello et al., 2001; Sidhu et al., 2021).

Beta thalassemia major causes malnutrition, pallor, feeding difficulties, diarrhea, irritability, frequent febrile episodes, abdominal enlargement due to splenic enlargement, and liver enlargement in infants. The patient presents with deformities of the long bones of the leg, craniofacial changes (alteration in the bridge of the nose, propensity towards a Mongoloid eye shape, including craniofacial enlargement, and enlarged jaws that expose the upper teeth), muscle weakness, pallor, and ulcers of the legs are structural changes that arise from bone marrow expansion (Tari et al., 2018). Individuals in this population run the risk of developing iron overload complications, which manifest in children as growth delays, sexual immature development, or failure, and maintain into adulthood as endocrine disorders (diabetes, hypogonadism, thyroid gland disorder, pituitary insufficiency, and adrenal insufficiency, but less frequently). Additional complications consist of splenomegaly, viral infections (including hepatitis B and C virus), HIV infection from blood transfusion, thrombosis, and osteoporosis (Lei, 2019).

The risk of infection increases in thalassemia patients due to repeated blood transfusions and splenectomy (Unissa et al., 2018). Thalassemia can lead to enlargement to the bone marrow which in turn causes bone widening and abnormal bone structure, particularly in the face and skull. The increased bone marrow growth can also result in osteoporosis, making the bones thinner and softer, and increasing susceptibility to fractures (Vogiatzi et al., 2009). The spleen plays a crucial role in impaired red blood cells. In thalassemia removing these cells by spleen results in splenomegaly, which can exacerbate anemia and decrease the lifespan of transfused red blood cells. Severe splenomegaly can require surgical removal (Unissa et al., 2018). Thalassemia patients suffer cardiac problems

such as congestive cardiac failure and arrhythmias, which are frequently observed in chronic forms of thalassemia (Soliman et al., 2014).

1.5. Clinical Complication of Beta Thalassemia

1.5.1. Anemia

Deposition of excess unbound globin protein (α -globin) leads to cell membrane damage. This can happen to immature erythroid progenitors and make erythropoiesis (intramedullary) less effective. It can also happen to mature erythropoiesis and make hemolysis (extramedullary), both of which cause anemia (Kato & Gladwin, 2009) (Figure 5). To compensate the severe anemia, red blood cells (RBC) formation increases, but this is about 95% ineffective, and this characterizes beta thalassemia. In individuals with this disease, bone marrow contains five times more levels of RBC when compared with healthy individuals. This is significantly detected throughout the maturation pathway of the RBC. The cell was identified to have died in the marrow at the beginning of the cell cycle. Furthermore, deaths that occur in the medulla of the late RBC are the main reason for inefficient RBC formation (Testa, 2001).

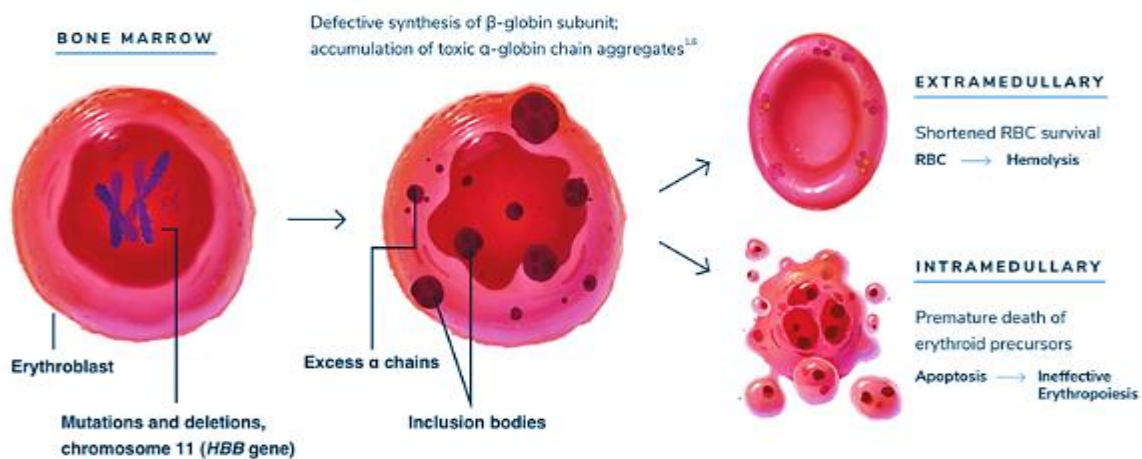


Figure 5. Ineffective erythropoiesis and hemolysis mechanisms in beta thalassemia (Eliezer A. et al., 2018).

1.5.2. Iron Overload

Iron Metabolism and Homeostasis: Iron is essential for many different important activities. It plays an essential role in the synthesis of proteins necessary for oxygen transport, DNA replication, cell signaling, and other essential functions. However, because excess iron is harmful for the body, there are a lot of risks involved. Under standard physiological circumstances, the body's iron levels are strictly regulated (Gordeuk & Brannon, 2017). Additionally, iron is effectively retained and recycled by the body, so the amount of iron lost must be made up by diet in the range of (1-2) mg each day (Fleming & Ponka, 2012).

Increased Iron Concentration in Thalassemia Patients: The ability of the human body to excrete excess iron from the body is limited. Iron overload occurs when repeated transfusions of excess iron exceed the body's adequate storage capacity due to the absence of a physiological pathway for iron excretion (Wardhani, 2020). Approximately 200 mg of iron is contained in one unit of packaged red blood cells utilized in a transfusion system. For example, a 6-year-old thalassemia patient who received 60–75 units of packed RBCs accumulated 12–15 grams of excess iron through regular blood transfusions, in contrast to the 3–4 grams detected in a healthy adult who did not receive transfusions (Benjamin, 2019). Iron first builds up in reticuloendothelial macrophages before being deposited in parenchymal cells. This causes fibrosis, tissue damage, and organ damage finally (D' Antiga, 2019).

Iron Toxicity Mechanism: Through continuous blood transfusions, patients acquire iron far faster than it can bind to transferrin, leading to an increase in blood levels of free iron. This free iron, also known as non-transferrin-bound iron, is directly harmful to the heart and other tissues (Vichinisky, et al., 2012). Unless treated with chelation therapy, the presence of excess free iron can cause significant harm to many tissues in the body and can be fatal. Iron has a high reaction rate, rapidly transiting between two valence states (Fe III and Fe II), resulting in the transfer of electrons and the production of detrimental free radicals (atoms or molecules with unpaired electrons). In the Fenton reaction, this happens (Figure 6).

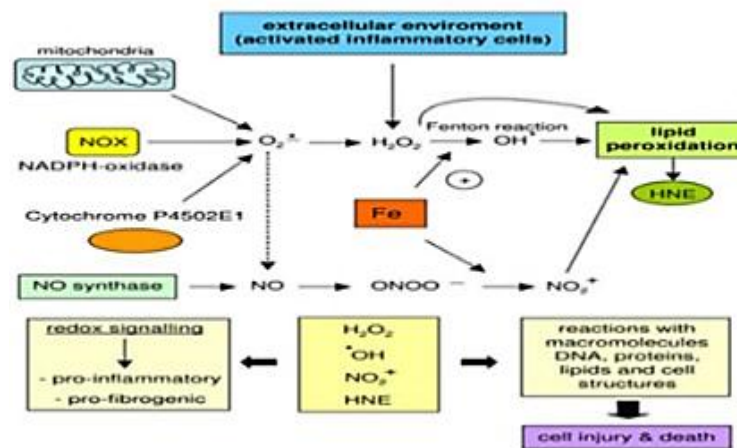


Figure 6. Fenton reaction; a process where hydrogen peroxide (H_2O_2) reacts with iron ion (Fe^{2+}) to produce hydroxyl radicals ($\cdot OH$) (Gutteridge, 1986).

Iron free radicals have the ability to damage organelles, lipid membranes, and DNA, leading to cell death and fibrosis (Capellini, et al., 2008). Recent research has demonstrated that oxidation caused by free radicals is an important factor in the tissue damage observed during ischemia, the hydroxyl radicals are produced through the Fenton reaction.

Hydroxyl radicals exhibit a high degree of reaction, leading to their attack with proteins, lipids, and DNA (Prabhu. et al., 2009). Organ dysfunction often arises over a prolonged period of (3-10) years of chronic exposure to elevated iron levels. This allows for the beginning of a treatment strategy designed for reducing iron loading. Nevertheless, the duration required to significantly decrease iron levels might vary depending on the specific organ. Therefore, it is advisable to take prompt action and try to prevent severe iron accumulation at an early stage (Vichinsky, et al., 2012).

1.6. Diagnosis of Thalassemia

Diagnosis of thalassemia is made by measuring hemoglobin levels, examining erythrocyte morphology, performing quantitative and qualitative evaluations of hemoglobin, and conducting molecular genetic tests. In thalassemia, hemoglobin (Hb) levels are typically below 7 g/dL, and there are alterations in the morphology of red blood cells, such as the presence of target cells (Badens et al., 2011).

Individuals with thalassemia exhibit alternations in the shape and structure of red blood cells, including microcytosis, hypochromic, anisocytosis and poikilocytosis (elongated and teardrop cells), target cell and nucleated RBC (erythroblasts). The degree of anemia is associated with the count of erythroblasts, which increases significantly after splenectomy. However, at healthy people erythroblasts are not visible (Munkongdee et al., 2020).

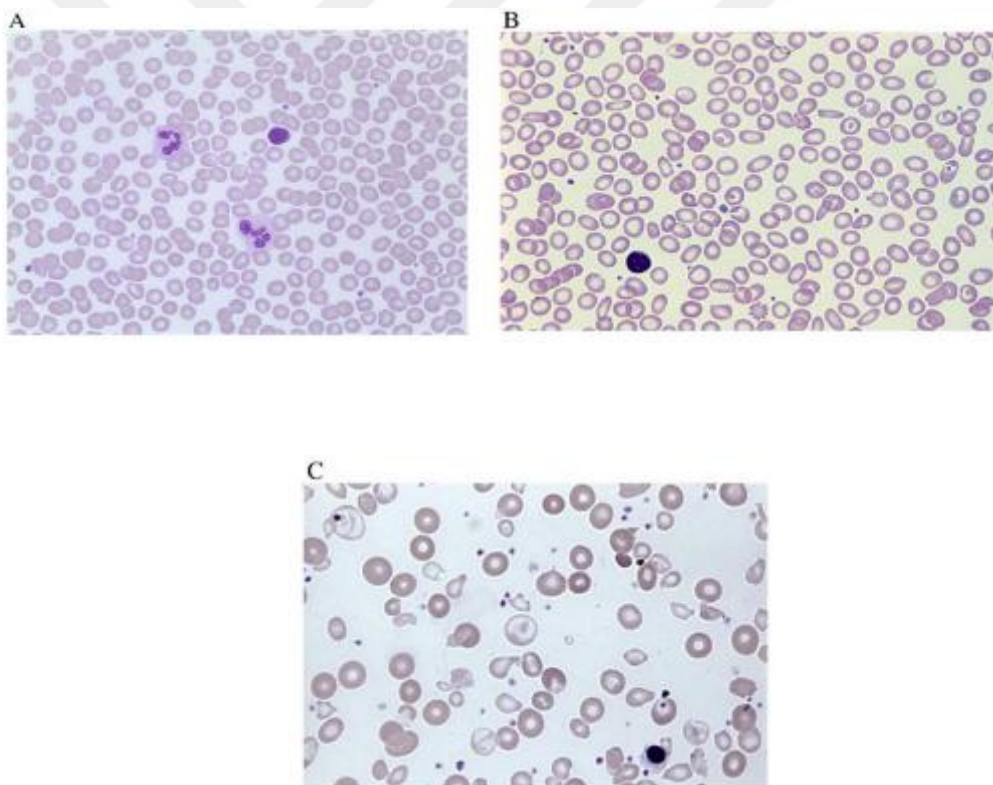


Figure 7. Standard blood smears for β -thalassemia major, β -thalassemia trait and normal (Thein, 2018).

- A) Normal, B) thalassemia trait characterized by red blood cells with hypochromic microcytic features, and C) thalassemia major exhibiting red blood cells with irregular nucleation and extensive aniso-poikilocytosis.

Quantitative and qualitative evaluations of hemoglobin are done by measuring the level and type of Hb present via DE-52 micro chromatography, or high-performance liquid chromatography (HPLC), cellulose acetate electrophoresis (Langlois, et al., 2008). Hb patterns vary depending on the kind of β T. Beta 0 is characterized by the absence of homozygotes HbA, with HbF comprising 92–95% of the total Hb. In Beta+ / Beta 0 genetic compounds and Beta+ thalassemia homozygotes, the levels of Hb F between 70-90% and HbA are between 10-30%. In beta-thalassemia homozygotes, the levels of HbA2 are variable, in beta-thalassemia minor, they are enhanced (Munkongdee et al., 2020).

The prevalence of a limited number of mutations in each population has greatly simplified molecular genetic analyses, especially since beta-globin gene mutations are often identified by PCR, one of the most widely used techniques, that amplifies a set of primers that are complementary to the mutations (Monni et al., 2018).

Iron overload early detection to prevent possible iron-related problems, there are numerous ways to identify excess iron in blood, including transferrin, iron, ferritin, non-transferrin-bound iron (NTBI) (High ferritin and transferrin saturation), and total iron-binding capacity (TIBC) (Cross et al., 2015).

1.7. Treatment of Thalassemia

1.7.1. Prevention

Preventive measures such as premarital exams and medical guidance are used to ensure that the parents' thalassemia status is determined prior to conception. If the embryo is found to be affected, it is recommended to terminate the pregnancy (Prathyusha et al., 2019).

1.7.2. Treatment

Blood Transfusions: Regular transfusions are necessary to treat chronic anemia in patients with thalassemia major and most other severe types once to twice a month, but those with the less severe types may only need it occasionally (Dev & Babitt, 2017).

Splenectomy: Thalassemia patients undergo splenectomy in order to reduce their blood consumption and transfusion needs. Splenectomy is typically avoided in children under the age of five (Taher & Cappellini, 2018). Problems linked to splenectomy include infection, increased susceptibility to blood clots (Kumfu et al., 2017).

Bone Marrow Transplantation: Transplantation of bone marrow during infancy represents the sole curative treatment for beta-thalassemia major. Hematopoietic stem cell transplantation usually produces positive results in low-risk individuals who meet the following criteria: absence of hepatomegaly, absence of portal cirrhosis on liver history, and consistent chelation treatment. These individuals should have no more than two of these abnormalities (Muncie Jr et al., 2009).

Iron Chelation Treatment: Chelating agents are the mainstay in the treatment of iron overload, as these agents work by forming groups with iron that do not bind to transferrin (NTBI) present in the plasma and inside cells to get rid of excess iron. There are currently three iron chelating agents: Deferoxamine, Deferasirox, and Deferiprone, and they work better on the pools of iron present in the plasma because this source is more accessible than stored iron in the form of ferritin or hemosiderin. The liver secretes iron chelate through the biliary system into the stool, and the duration of elimination in the stool or urine depends on the type of chelate used (Ghazanfari et al., 2019).

The first iron chelator implemented was deferoxamine (DFO) (Chiani et al., 2010). The binding of a single iron molecule to a single molecule of deferoxamine results in weak oral absorption, requiring subcutaneous or intravenous injection, it is not given intramuscularly and is primarily eliminated via urine. Deferoxamine is absorbed by liver cells with high efficiency, while iron is eliminated into bile through hepatocytes (Marsella & Borgna-Pignatti, 2014). The primary adverse effects of deferoxamine therapy include injection site reactions in the skin, including redness and itching. Additionally, the medication has the potential to induce sensorineural hearing loss, tinnitus, bone deformities, growth retardation, and night blindness. In addition, treatment at large doses induce pulmonary reactions (Ware & Kwiatkowski, 2013). The Food and Drug Administration of the United States (FDA) has approved deferoxamine as a chelating agent to treat chronic iron excess caused by transfusion-dependent anemia, and DFO is an adjunct to prolonged survival in thalassemia patients who start treatment (Hoffbrand et al., 2012).

Deferiprone (DFP) is an oral iron chelator. In 2011, the FDA approved DFP to get rid of excess iron (Stewart, 2021). When taken orally, it is quickly absorbed. Its levels increased in blood after 45 minutes of consumption. It is generally eliminated through the urine, similar to deferoxamine. In patients with thalassemia major, deferiprone has higher binding rates

than deferoxamine. Three deferiprone molecules attach to one iron atom, which chelates excess iron from mitochondria and lysosomes (Brittenham, 2011).

Some of the adverse effects caused by deferiprone include agranulocytosis, myelotoxicity, stomach and liver disorders, zinc deficiency, arthropathy, increased liver enzyme levels, abdominal discomfort, nausea, vomiting, diarrhea, and dyspepsia (Elalfy et al., 2014).

Deferasirox (DFX), a new oral chelator for triple iron chelation, exhibited superior efficacy and comparatively reduced toxicity in comparison to deferiprone. Intestinal distress is among the adverse effects associated with deferasirox. Among the adverse effects are abdominal pain, nausea, vomiting, diarrhea, a rash on the skin, and elevated levels of liver enzymes. It is processed by the liver and then removed through the stools (Chang et al., 2015). Among the three chelators, DFX has the longest half-life. One iron atom is coupled to two DFX molecules (Chen et al., 2021). The FDA has approved DFX for the treatment of iron excess caused by chronic blood transfusions in patients two years of age and older, in the presence of nausea and vomiting (Garcia et al., 2021).

1.8. Aim of Study

Thalassemia leads to severe anemia. Blood transfusions are crucial for survival, but frequent transfusions can lead to complications due to iron excess. Chelating treatments, deferoxamine and deferasirox are often used to reduce iron overload of iron in patients with β -thalassemia major because they are less harmful (side effect) than deferiprone, which was not included in this study because it has a high disadvantage (side effect). The hypothesis in our study suggests that deferoxamine and deferasirox may have significant effects on the management of iron accumulation, potentially helping to reduce iron deposits in organs and improve the effectiveness of immune and inflammatory responses. The aim of this study is to assess the effectiveness of these chelators for improving immunological, hormonal, biochemical, and hematological levels by regulating iron balance by using statistical analysis of the results.

2. MATERIALS AND METHODS

2.1. Equipments, Instruments and Kits

The equipment, disposable materials, and kits used in the study are presented in Tables 2, 3, and 4.

Table 2. The equipment and instruments used in the study.

No.	Equipments & Instruments	Company	Country
1	Complete blood count Bc-30s	Mindray	China
2	Centrifuge	HITACHI	Japan
3	Enzyme-linked immunosorbent assay system (ELISA)	BIOTEK	USA
4	Incubator	Memmert	Germany
5	Deep freezer	Arcelik	Korean
6	Cobas e 411	Roche	USA
7	Cobas c 311	Roche	USA
8	Micropipettes	Winlab	Germany
9	Rack for tubes	Afco	Jordan
10	Rack for Eppendorf	Afco	Jordan
11	Cool box	Blow kings	Germany

Table 3. Disposable materials used in the study and their sources.

No.	Disposable Material	Company	Country
1	Blue tip	Jual	Lebanon
2	Yellow tip	Jual	Lebanon
3	Eppendorf (1.5 ml)	Afco	Jordan
4	Vacuum tube with gel and clot activator	Afco	Jordan
5	Ethylene diamine tetra acetic acid (EDTA) tube	Afco	Jordan
6	Disposable sterile syringe (5 ml)	Medjecte	UAE

Table 4. The kits used in the study.

No.	Kit	Corporation	Country
1	Interleukin-6 (IL-6) ELISA Kit	Sunlong Biotechnology	China
2	Interleukin-8 (IL-8) ELISA Kit	Sunlong Biotechnology	China
3	Toll like receptor-3 (TLR-3) ELISA Kit	Bioassay Technology	China
4	Toll like receptor-4 (TLR-4) ELISA Kit	Sunlong Biotechnology	China
5	Glucose regulates protein-78 (GRP78) ELISA Kit	Sunlong Biotechnology	China
6	Human CHOP (FUS) ELISA Kit	ELK Biotechnology	USA
7	Creatinine Kit	Cobas	USA
8	Ferritin Kit	Cobas	USA
9	Iron Kit	Cobas	USA
10	Alanine transaminase (ALT) Kit	Cobas	USA
11	Aspartate transferase (AST) Kit	Cobas	USA
12	Gamma glutamyl transferase (GGT) Kit	Cobas	USA
13	Bilirubin Kit	Cobas	USA
14	Alkaline phosphate Kit	Cobas	USA
15	Calcium Kit	Cobas	USA
16	Vitamin D Kit	Cobas	USA
17	Total protein Kit	Cobas	USA
18	Albumin Kit	Cobas	USA
19	Globulin Kit	Cobas	USA
20	Testosterone Kit	Cobas	USA
21	Prolactin Kit	Cobas	USA
22	Growth hormone Kit	Cobas	USA
23	Follicle-stimulating hormone Kit	Cobas	USA
24	Luteinizing hormone Kit	Cobas	USA
25	Thyroid-stimulating hormone Kit	Cobas	USA
26	Thyroxine Kit	Cobas	USA
27	Triiodothyronine Kit	Cobas	USA

2.2. Thalassemia Patients and Collection of Blood Samples

The hospital management allowed patient sample collection (No: 41163). Institute approval was also granted for the intended research (EYK No: 2039). The research was carried out at the Thalassemia Centre of Azadi Teaching Hospital from June 2023 to September 2023. The study included 200 patients with β -thalassemia major who were all over 18 years old and diagnosed by the center's clinicians through Hb electrophoresis and complete blood counts. The personal information and details (gender, age, blood type, thalassemia type, date of infection, duration of treatment and type of chelator) of each individual patient are present in the questionnaire form (appendix1). Signed written consent was taken from each individual participating in the study. The first group consisting of 100 (50 males and 50 females). The group comprised transfusion-dependent patients who were all prescribed oral deferiasirox (Exjade®), an iron chelator, at a daily dose of 20 mg/kg. The second group, include 100 (50 males and 50 females) transfusion-dependent thalassemia patients, was administered deferoxamine (Desferal®), an iron chelating agent, at a daily dose ranging from 20 to 40 mg/kg. The patients were evaluated at the Thalassemia Centre, a leading facility for the treatment of thalassemia patients, at a dosage of 40 mg/kg per day, several times per week. This study does not include patients who are taking deferiprone, as the number of individuals using it is small due to its higher side effects compared to other chelator agents (DFO, DFX), particularly its toxic effects on the liver and kidney. The control group consisted of 100 healthy individuals (50 male, 50 female), all over 18 years old, with no family history of hereditary blood diseases. Patients under 18 years old, those with non-beta-thalassemia major, individuals not using deferoxamine or deferiasirox chelators, and those with kidney or liver diseases were excluded from the study. The study stages are shown in Figure 8.

2.2.1. Preservation and Collection of Blood Sample

From the left cephalic vein of each participant and healthy control group, 5 ml of peripheral venous blood was collected. From each blood, 2 ml was placed in containers containing EDTA anticoagulant and the remaining 3 ml was transferred directly into clot activator and gel serum separation tubes for hematological study, including complete blood count (CBC). The tubes were then permitted to rest at room temperature (18-22°C). The serum was transferred into Eppendorf tubes and stored at -20°C after undergoing 15 minutes

of separation by centrifugation at 3000 rpm. The serum was then used for immunological, biochemical, hormonal analysis.

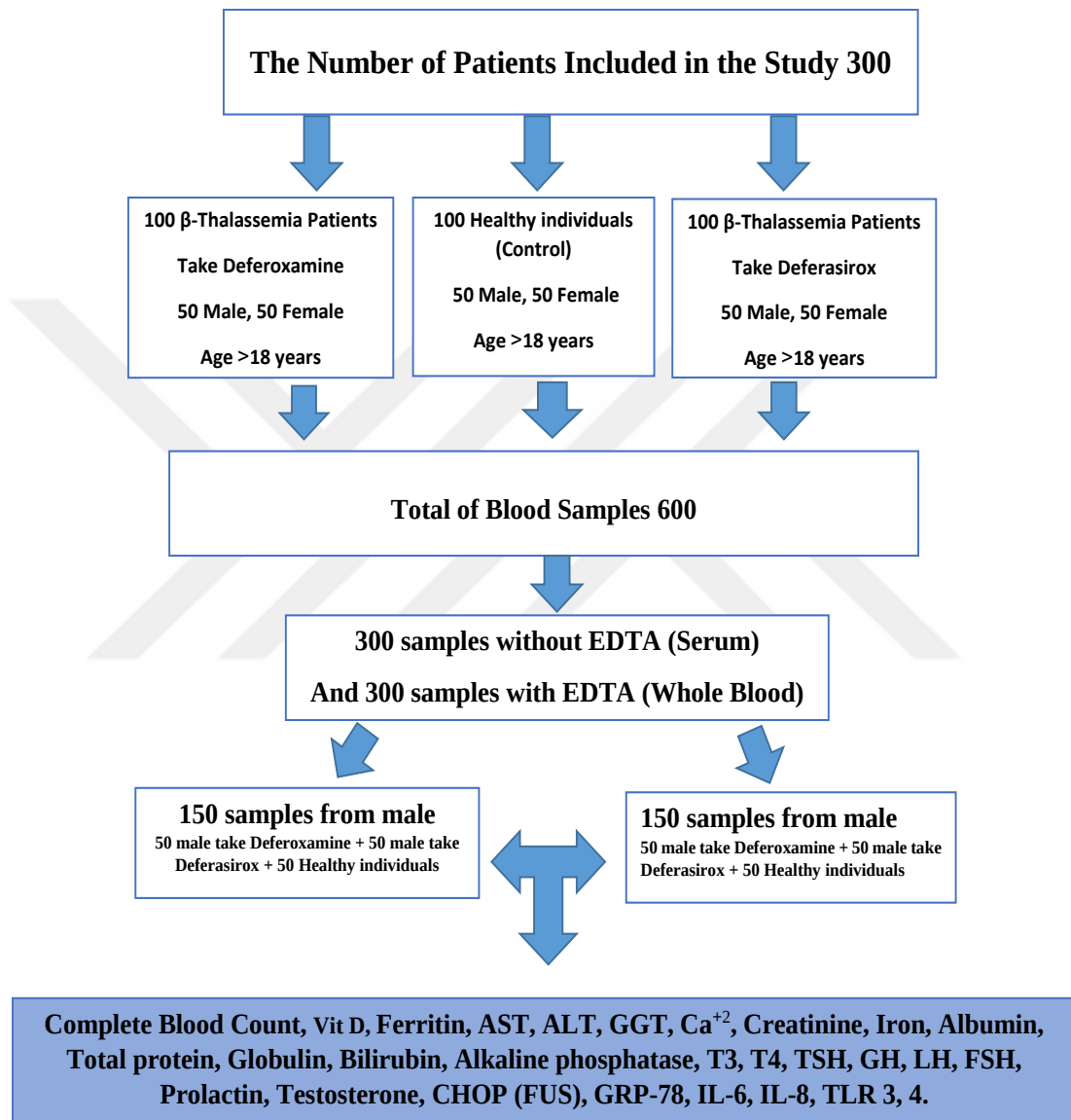


Figure 8. The Study Stages.

2.3. Hematological Tests

2.3.1. Complete Blood Count

The Mindray BC-30s model device was used to measure parameters in patients' blood, including WBCs, LYM, RBCs, Hb, HCT, MCV, MCH, and PLT.

After the machine's startup procedure, calibration is performed to ensure a minimal error rate in the final result. The blood samples, in anticoagulant-containing tubes, were subsequently placed on a specialized holder and started to analysis via 10 microliters of blood through a capillary tube.

2.4. Biochemical Tests

A device made in the United States, Cobas model (c 311), which is a self-analyzing instrument capable of measuring multiple variables at the same time with fast and accuracy, was utilized. In this instrument ferritin, iron, creatinine, alanine transaminase, aspartate transferase, gamma glutamyl transferase, bilirubin, alkaline phosphate, calcium, vitamin D, total protein, and albumin levels are measured. The self-analyzing device receives samples and is accompanied by a specialised computer. Each blood sample is encoded with a unique barcode that is entered by the individual using a calculator while inserting the samples into the device and entering the code number into the computer. The computer screen displays the results for each patient.

2.5. Hormonal Tests

The only difference between the instrument and the device used for biochemical analysis is its model Cobas model (e 411). The usage of this device is similar to the one used in biochemical tests. We measured prolactin, growth hormone, follicle stimulating hormone, luteinizing, thyroid stimulating hormone, thyroxine and triiodothyronine levels in this device.

2.6. Immunological Tests

Specific immunological parameters were determined in patients' blood using a specialized instrument called the "ELISA Biotek" model TS-800. These parameters comprised Interleukin-6 (IL-6), Interleukin-8 (IL-8), Toll like receptors-3 (TLR3), Toll like receptors-4 (TLR4), Glucose regulates protein 78 (GRP78), CHOP (FUS).

2.6.1. Estimation of the Human Interleukin-6 Concentration in Serum

Human Interleukin 6 (IL-6) ELISA Kit (Sunlong Biotech, SL1001Hu_1) was used for the measurement of IL-6.

The sandwich-ELISA technique is used in this kit. Standard or samples were added into the wells of microelisa stripplate, which were pre-coated with an antibody specific to IL-6. These samples were then combined with the stripplate that was included. Then, a Horseradish Peroxidase (HRP) conjugated antibody that is specific for IL-6 is added into one microelisa and then the plate is incubated. Following incubation, a washing stage was implemented to remove any unbound components. Subsequently, a tetramethylbenzidine (TMB) chromogen substrate solution was added to each well. After adding the stop solution, the color changed from blue to yellow. Spectrophotometric calculations were performed at a wavelength of 450 nanometers.

To calculate the results a linear calibration curve was created by putting the IL-6 standard concentration values on the horizontal axis against the average absorbance values on the vertical axis. By comparing the resulting curves, the IL-6 concentration of the samples under study was calculated. Assay range 3-200 pg/ml.

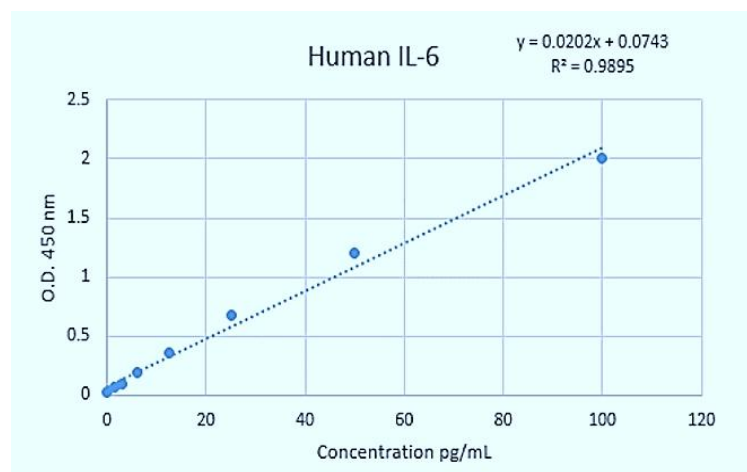


Figure 9. Standard curve for Interleukin-6. It displays the relationship between IL-6 concentration (pg/mL) on the X-axis and O.D. at 450 nm on the Y-axis. The data points represent absorbance values for standard concentration of IL-6, and the trendline is used to estimate IL-6 levels in study samples.

2.6.2. The Estimation of the Concentration of Human Interleukin-8 in Serum

Human Interleukin 8 (IL-8) ELISA Kit (Sunlong Biotech, SL1004Hu) was used for the measurement of IL-8. This ELISA kit uses Sandwich-ELISA strategy. Standards or samples were placed into the specified wells of the microelisa stripplate, which has been pre-coated with an antibody that targets only IL-8. Then, samples were mixed with the attached microelisa stripplate. Next, we added HRP to the wells and incubated the plate. Unbound components were removed by washing. The TMB solution is added to all well. The blue color that appears, followed by yellowing upon the addition of the stop solution, occurs only in wells containing IL-8 and HRP-conjugated IL-8 antibodies. At a wavelength of 450 nanometers, the optical density (OD) can be calculated via spectrophotometric techniques.

To calculate the results a linear calibration curve was generated by plotting the concentration of the IL-8 standard values on the x-axis and the average absorbance values on the y-axis. The IL-8 concentration in the samples was determined by comparing their values to the generated curve. Assay range 6-200 pg/ml.

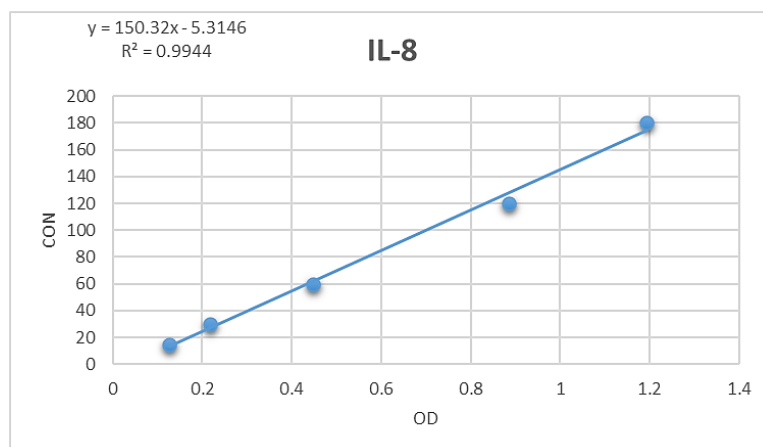


Figure 10. Standard curve for Interleukin-8. It displays the relationship between IL-8 concentration (pg/mL) on the Y-axis and O.D. at 450 nm on the X-axis. The data points represent absorbance values for standard concentration of IL-8, and the trendline is used to estimate IL-8 levels in study samples.

2.6.3. The Estimation of the Concentration of Human Toll Like Receptor 3 (TLR3) in Serum

Human Toll-Like Receptor 3 (TLR3) ELISA Kit (Bioassay Technology Laboratory, E0365Hu) was used for the measurement of toll-like receptor 3 (TLR3).

The plate has been pre-coated with an antibody specific to Human TLR3. The TLR3 present in the sample is added and creates a binding interaction with the antibodies that are fixed on the wells, using a sandwich approach in this particular test. Then, the biotinylated Human TLR3 Antibody is put in and forms a binding interaction with TLR3 contained within sample. Next, add streptavidin-HRP, which forms a bond with the biotinylated TLR3 antibody. Following the incubation period, all unbound HRP is removed using a washing procedure. When the substrate solution is added, the color intensity increases in direct correlation with the concentration of TLR3. After adding an acidic stop solution, the reaction is stopped, and the amount of absorption is measured at 450 nm.

To calculate the results, a standard curve was created by drawing the optical density (OD) for each standard on the Y-axis and the concentration on the X-axis. Then, design a curve that best fits the points on the graph. The best method for making these calculations is to use computer-based curve-fitting software, which employs regression analysis to obtain the optimal fit line. Assay range 50 – 10000 ng/ml.

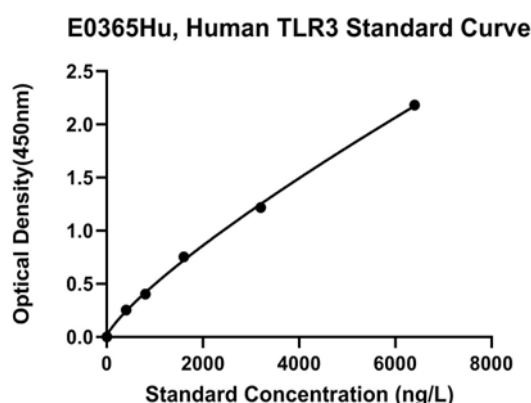


Figure 11. Standard curve for Toll-Like Receptor 3. It displays the relationship between TLR3 concentration (ng/L) on the X-axis and O.D. at 450 nm on the Y-axis. The data points represent absorbance values for standard concentration of TLR3, and the trendline is used to estimate TLR3 levels in study samples.

2.6.4. The Estimation of the Concentration of Human Toll Like Receptor 4 (TLR4) in Serum

Human Toll-Like Receptor 4 (TLR4) ELISA Kit (Sunlong Biotech, SL2269Hu) was used for the measurement of toll-like receptor 4 (TLR4).

This kit applies to the Sandwich-ELISA technique. A plate pre-coated with an antibody that specifically targets TLR4, has samples or standards placed into designated wells. Then combine the supplied sample with the microelisa stripplate that is attached. After that, add a horseradish peroxidase (HRP)-conjugated antibody that specifically targets TLR4 to one microelisa stripplate well and let it incubate. Washing out all parts that are not linked. Each well gets an addition of tetramethylbenzidine (TMB) chromogen substrate solution. Blue color, followed by yellowing upon the addition of the stop solution, will appear only in wells that contain TLR4 and HRP-conjugated TLR4 antibodies. Spectrophotometric methods allow one to determine O.D at a wavelength of 450 nanometers.

To calculate the results a linear calibration curve was produced by plotting the average absorbance values on the y-axis and the standard concentration of TLR4 are plotted on x-axis. Through curve comparison, the TLR4 concentration of the analysed samples was determined. Assay range 85-4000 pg/ml.

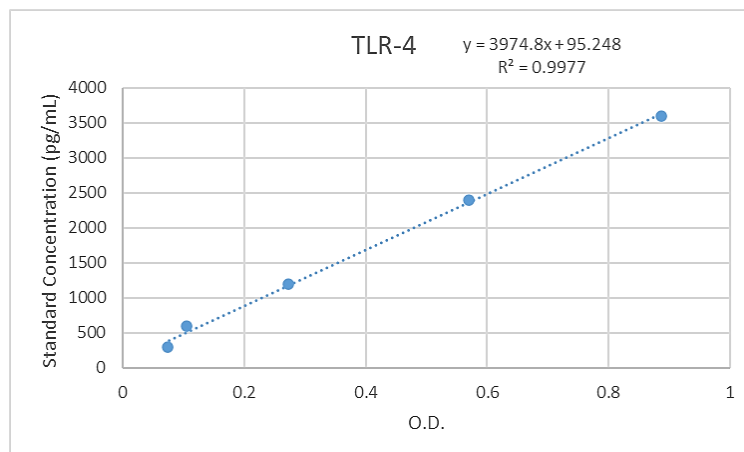


Figure 12. Standard curve for Toll Like Receptors 4. It displays the relationship between IL-8 concentration (pg/mL) on the Y-axis and O.D. at 450 nm on the X-axis. The data points represent absorbance values for standard concentration of IL-8, and the trendline is used to estimate IL-8 levels in study samples.

2.6.5. The Estimation of the Concentration of Human Glucose Regulated Protein 78 in Serum

Human Glucose Regulated Protein 78 (GRP78) ELISA Kit (Sunlong Biotech, SL2048Hu) was used for the measurement of GRP78.

The method used in this ELISA kit is Sandwich-ELISA. The microelisa stripplate that comes with this kit has already been covered with an antibody that is specific to GRP78. Standards or samples are then put on the appropriate microelisa stripplate wells and mixed with the antibody. Then, an antibody that is linked to Horseradish Peroxidase (HRP) with a specific for GRP78 is added to each microelisa strip plate well and left to sit for some time. The free parts are washed away. This is where tetramethylbenzidine (TMB) chromogen substrate solution goes into each well. Only the wells containing GRP78, and an antibody attached to HRP will be blue. When the stop solution is added, the colour will change to yellow. Spectrophotometry is used to measure the optical density (OD) at a wavelength of 450 nm. The OD number is related to how much GRP78 is present. If you compare the OD of the samples to the standard graph, you can find out the amount of GRP78 is in the samples.

To calculation the results a simple calibration curve was made by putting the average absorbance values on the y-axis and the GRP78 standard concentration values on the x-axis. The GRP78 concentration of the samples that were examined was found by comparing the curves. Assay range 0.08-4 ng/ml.

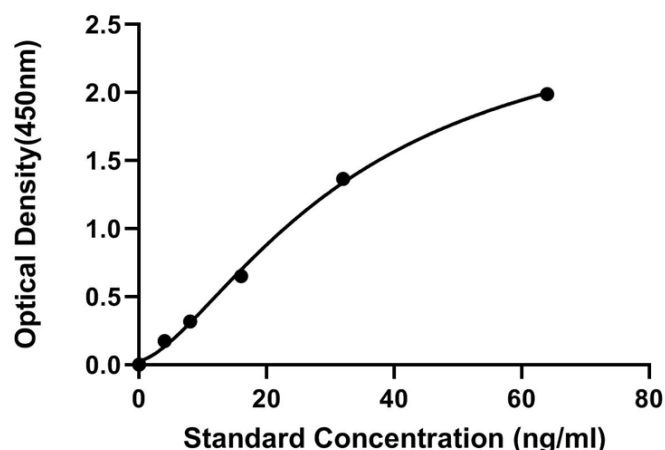


Figure 13. Standard curve for Glucose Regulated Protein 78. It displays the relationship between GRP78 concentration (ng/L) on the X-axis and O.D. at 450 nm on the Y-axis. The data points represent absorbance values for standard concentration of GRP78, and the trendline is used to estimate GRP78 levels in study samples.

2.6.6. The Estimation of the Concentration of Human CHOP (FUS) in Serum

Human CHOP (FUS) ELISA Kit (ELK Biotechnology, ELK4043) was used for the measurement of GRP78.

The test principle used in this kit sandwich enzyme immunoassay. An antibody specific to CHOP (FUS) has already been pre-coated on the microtiter plate included in this kit. After adding standards or samples to the right microtiter plate wells, a biotin-conjugated antibody that is particular to CHOP (FUS) is applied. Subsequently, populate each microplate well with HRP-conjugated avidin and allow the mixture to incubate. Only the wells containing CHOP (FUS), biotin-conjugated antibody, and enzyme-conjugated avidin will exhibit a change in color after the addition of tetramethylbenzidine (TMB) chromogen substrate solution. A solution of sulfuric acid is introduces to stop the enzyme-substrate reaction, and then the color change is measured spectrophotometrically at $450 \text{ nm} \pm 10 \text{ nm}$. The concentration of CHOP (FUS) in the samples is determined by comparing their OD to the standard curve.

To calculate a result, take the average of the two readings for each control, standard, and sample. Next, take away the average zero reading for standard value. Set a standard curve by placing the absorbance on the x-axis and human FUS content on the y-axis. Then, join the points on the graph to make a best fit curve. If samples have been diluted, you need to multiply the percentage read from the standard curve by the dilution factor. Using some tools to plot, like curve expert. Assay range 0.16-10 ng/ml.

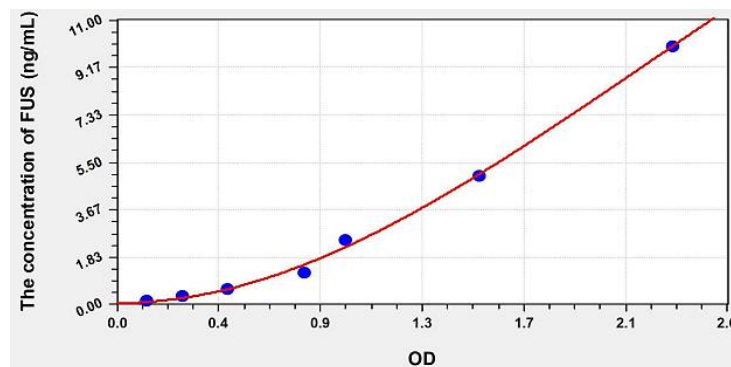


Figure 14. Standard curve for CHOP (FUS). It displays the relationship between CHOP (FUS) concentration (ng/mL) on the X-axis and O.D. at 450 nm on the Y-axis. The data points represent absorbance values for standard concentration of FUS, and the trendline is used to estimate FUS levels in study samples.

2.7. Statistical Analysis

2.7.1. ANOVA and Kruskal-Wallis Test

Statistical analysis was performed using SPSS V26 software in this study. To determine the presence or absence of significant differences in the measurements among groups. At first, the Shapiro-Wilk test was used to evaluate the proof of significant variations between groups and to check the data's normality. In the case that Shapiro-Wilk test indicated a non-normal distribution ($P < 0.01$), the Kruskal-Wallis test used to determine the differences between the groups. A one-way ANOVA was conducted in cases where the data followed a normal distribution ($P > 0.01$) in the Shapiro-Wilk test. the homogeneity of variances was evaluated using Levene's test. If the results indicated equal variances ($P > 0.01$), a standard one-way ANOVA was used. In cases of non-normal data, transformation procedures such as the Arc-sine transformation were taken into consideration. In order to compare the differences between groups, post-hoc analysis was done using Tukey test following ANOVA results and two independent tests after the Kruskal-Wallis test.

The standard deviation was determined for each group. Each test was subjected to three independent replicates to guarantee the accuracy and reliability of the results.

2.7.2. Correlation Analysis

Correlation analysis was used to explain the impact of iron overload problems and their treatments on various organ functions (liver, kidney, pituitary gland, thyroid gland, ovaries, and tests) and immune responses which might assist to understanding and explanation of this physiological issue that has been associated with the disease and its treatments. To evaluate the intensity and direction of the relationship between these variables, a non-parametric (Bivariate) Spearman test (Two-Tail) has been used. The statistical significance criterion was decided on at $P \leq 0.01$ for all analysis.



3. RESULTS

3.1. Hematological Tests

The measurement of the hematological parameters for all groups are presented in Table 5.

Table 5. Results of hematological parameters in the study groups

parameters	Normal Value	DFX (mean $\pm$ SD)	DFO (mean $\pm$ SD)	Control (mean $\pm$ SD)	P value
RBC $\times 10^{12}/L$	4-6	2.97 $\pm$ 0.57	3.40 $\pm$ 0.59	5.21 $\pm$ 0.68	≤ 0.01
WBC $\times 10^9/L$	4.0-12.0	8.48 $\pm$ 1.79	7.94 $\pm$ 0.95	5.42 $\pm$ 1.29	≤ 0.01
Lymphocyte $\times 10^9/L$	1.0-5.0	5.01 $\pm$ 0.78	4.96 $\pm$ 0.81	3.49 $\pm$ 0.56	≤ 0.01
HCT %	35.0-55.0	22.08 $\pm$ 3.32	24.79 $\pm$ 3.13	37.37 $\pm$ 1.91	≤ 0.01
Hb g/dL	11.0-17.0	7.14 $\pm$ 1.17	8.22 $\pm$ 1.13	13.98 $\pm$ 12.15	≤ 0.01
MCV fl	80 - 100	72.19 $\pm$ 6.39	72.48 $\pm$ 3.50	86.14 $\pm$ 2.48	≤ 0.01
MCH pg	25 - 35	24.56 $\pm$ 3.32	26.35 $\pm$ 2.23	31.16 $\pm$ 4.73	≤ 0.01
Platelet $\times 10^9/L$	150-400	394.89 $\pm$ 65.83	447.04 $\pm$ 60.18	272.76 $\pm$ 33.46	≤ 0.01

3.1.1. Red Blood Cell Counts

As shown in Figure 15, the RBC counts of the DFO, DFX, and control groups exhibited a normal distribution, by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One way ANOVA test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$), and the F-value is 366.9 with df (2, 297). The Post Hoc test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and DFX ($P \leq 0.01$). Additionally, there is a significant difference was observed between DFX and DFO ($P \leq 0.01$).

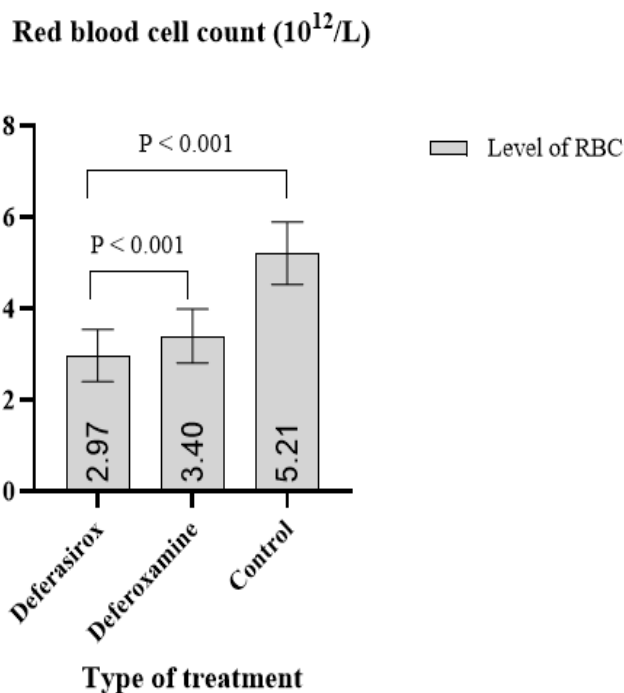


Figure 15. Mean value of red blood cell counts.

3.1.2. White Blood Cell and Lymphocytes Count

As shown in Figure 16, the WBC and lymphocyte counts of the DFO, DFX, and control groups exhibited a normal distribution, by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One way ANOVA test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$), the F-value of WBC is 134.54 with df (2, 297), and the F-value of lymphocyte is 142.66 with df (2, 297). The Post Hoc test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and DFX ($P \leq 0.01$). Additionally, there is a significant difference was observed between DFX and DFO ($P \leq 0.01$). While the lymphocyte showed no difference between DFX and DFO ($P > 0.01$)

White blood cells and lymphocyte Counts ($10^9/L$)

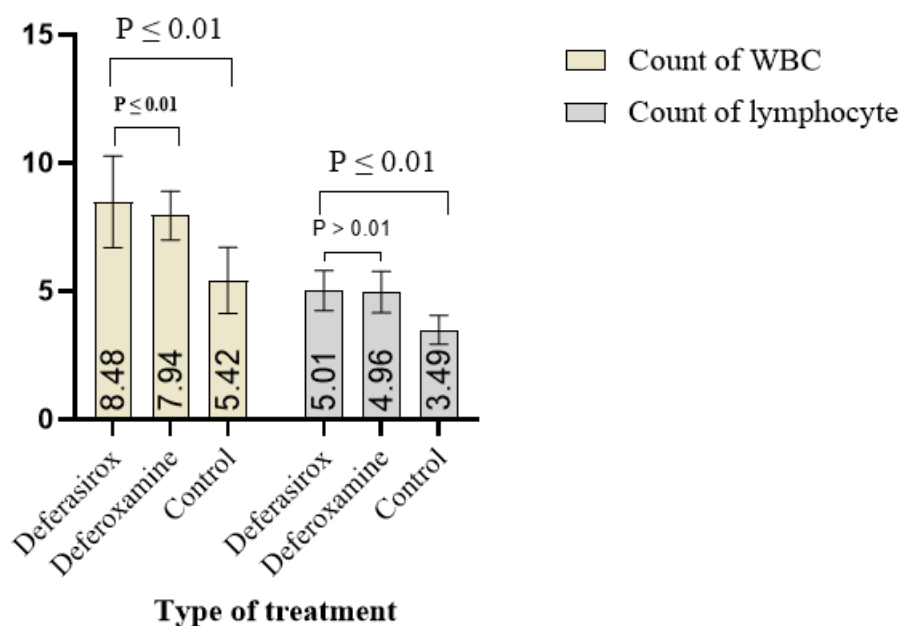


Figure 16. Mean value of white blood cell and lymphocyte counts.

3.1.3. Level of Hemoglobin and Hematocrit

As shown in Figure 17, the Hb levels of the DFO, DFX, and control groups exhibited a normal distribution by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One-way ANOVA test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$), and the F-value is 26.94 with df (2, 297). The Tukey test further confirmed that there are significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

However, Figure 17. shows the HCT rates of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the

control group ($P \leq 0.01$). Additionally, there is a significant difference was observed between DFX and DFO ($P \leq 0.01$).

Percentage of hematocrit (%) and hemoglobin concentration rate (g/dl)

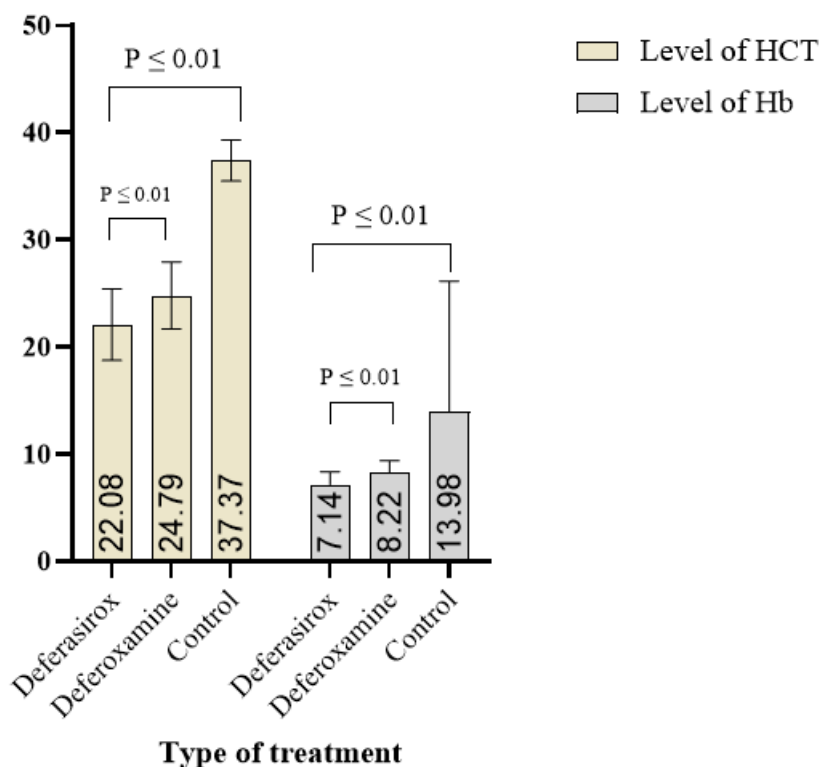


Figure 17. Mean value of the hematocrit and hemoglobin concentration rates.

3.1.4. Percentage Values of Mean Corpuscular Volume (MCV) and Corpuscular Hemoglobin (MCH)

As shown in Figure 18, the MCV levels of the DFO, DFX, and control groups exhibited a normal distribution by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One-way ANOVA test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$), and the F-value is 321.8 with df (2, 297). The Tukey test further confirmed that there are significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO ($P > 0.01$).

However, Figure 18. shows the MCH levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference was observed between DFX and DFO ($P \leq 0.01$).

Percentage of mean corpuscular volume (MCV) (fl) and mean corpuscular hemoglobin (MCH) (pg)

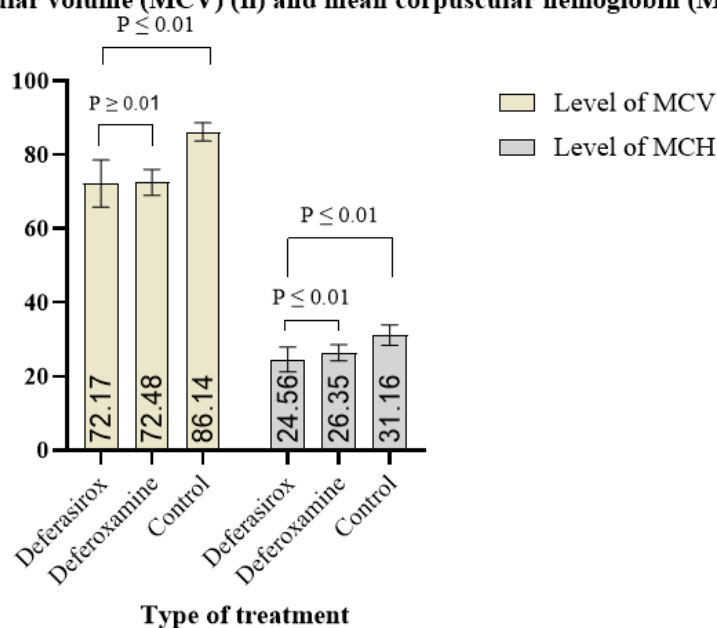


Figure 18. Mean value of the corpuscular volume and corpuscular hemoglobin levels.

3.1.5. Platelet Counts

As shown in Figure 19, the platelet counts of the DFO, DFX, and control groups exhibited a normal distribution by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One-way ANOVA test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$), and the F-value is 264.48 with df (2, 297). The Tukey test further confirmed that there are significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

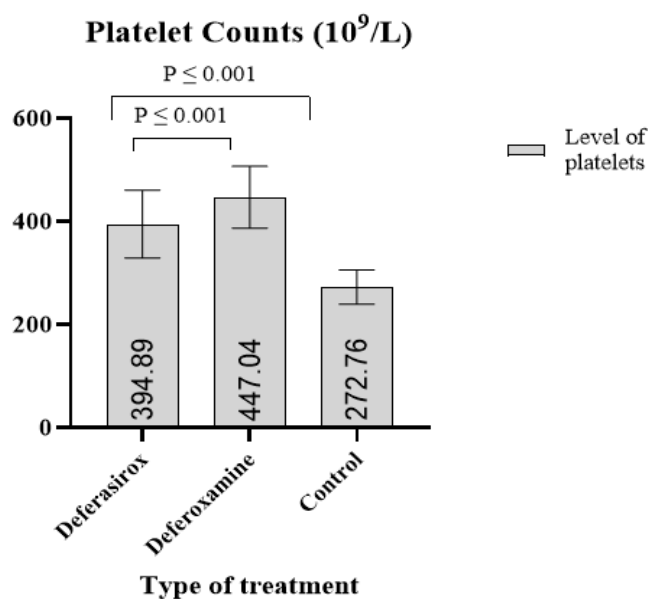


Figure 19. Mean value of platelet counts.

3.2. Biochemical Tests

The analysis of the biochemical tests for all groups are presented in Table 6.

Table 6. Results of biochemical parameters in the study groups

parameters	Normal Value	DFX (mean $\pm$ SD)	DFO (mean $\pm$ SD)	Control (mean $\pm$ SD)	P value
Ferritin (ng/L)	20-250	1687.27 $\pm$ 312.71	2018.42 $\pm$ 272.09	31.20 $\pm$ 7.55	≤ 0.01
Iron (μ mol/L)	8.1-28.3	37.88 $\pm$ 4.59	51.07 $\pm$ 7.96	17.17 $\pm$ 4.50	≤ 0.01
AST (U/L)	≤ 35	33.45 $\pm$ 8.28	71.99 $\pm$ 8.79	16.12 $\pm$ 3.56	≤ 0.01
ALT (U/L)	≤ 40	30.32 $\pm$ 8.11	62.44 $\pm$ 7.41	17.76 $\pm$ 4.19	≤ 0.01
ALP (U/L)	35-105	74.24 $\pm$ 36.18	111.43 $\pm$ 35.12	72.64 $\pm$ 17.17	≤ 0.01
GGT (U/L)	3-40	22.15 $\pm$ 12.12	34.28 $\pm$ 19.19	18.03 $\pm$ 11.39	≤ 0.01
Bilirubin (mg/dL)	0.1-1.2	2.59 $\pm$ 1.79	3.00 $\pm$ 2.72	0.74 $\pm$ 0.33	≤ 0.01

Table 6 continued

parameters	Normal Value	DFX (mean $\pm$ SD)	DFO (mean $\pm$ SD)	Control (mean $\pm$ SD)	P value
Albumin (g/L)	38-54	43.06 $\pm$ 25.30	42.16 $\pm$ 3.80	46.40 $\pm$ 1.97	≤ 0.01
Total Protein (g/L)	60-80	65.31 $\pm$ 6.48	65.17 $\pm$ 5.93	74.41 $\pm$ 2.76	≤ 0.01
Globulin (g/L)	20-38	23.77 $\pm$ 4.12	22.90 $\pm$ 4.50	27.29 $\pm$ 2.42	≤ 0.01
Creatinine (mg/dL)	0.6-1.2	0.74 $\pm$ 0.12	0.59 $\pm$ 0.12	0.33 $\pm$ 0.039	≤ 0.01
Ca (mg/dL)	> 10.5	8.03 $\pm$ 0.68	7.82 $\pm$ 0.60	12.55 $\pm$ 1.26	≤ 0.01

3.2.1. Levels of Ferritin and Iron

As shown in Figure 20, the ferritin and iron levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

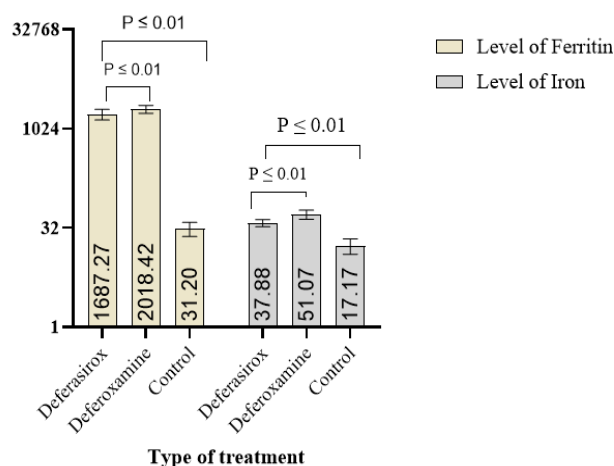
Levels of Ferritin (ng/L) and Iron ($\mu\text{mol/L}$)

Figure 20. Mean value of ferritin and iron levels.

3.2.2. Liver Enzyme Levels (Aspartate Aminotransferase, Alanine Amino Transaminase and Alkaline Phosphatase) in the Serum

As shown in Figure 21, the AST, ALT, and ALP levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

Levels of Aspartate aminotransferase, Alanine aminotransaminase and Alkaline phosphatase (U/L)

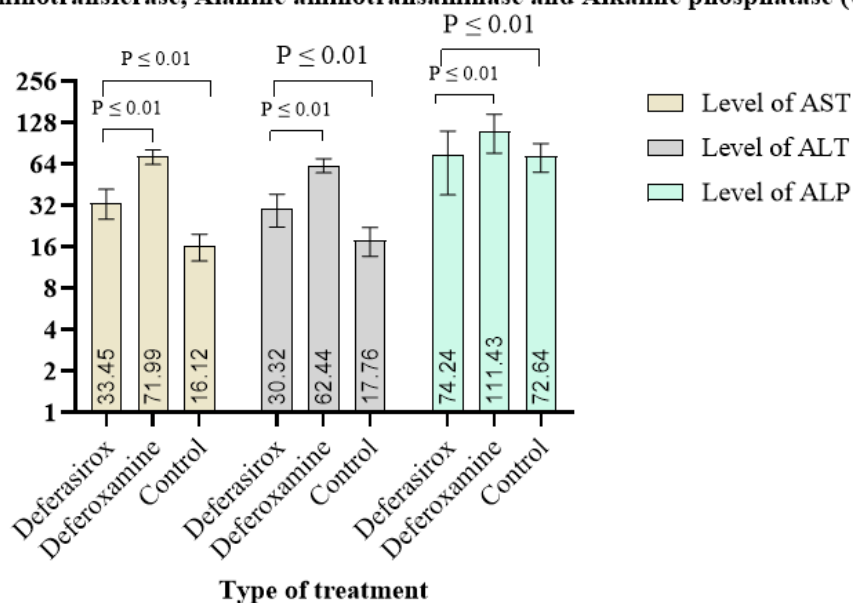


Figure 21. Mean value of aspartate aminotransferase, alanine transaminase and alkaline phosphatase levels.

3.2.3. Concentration of Blood Proteins (Albumin, Total Protein and Globulin)

As shown in Figure 22, the albumin, total protein, and globulin levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further

confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO in all parameters ($P > 0.01$).

Concentration of blood proteins (Albumin, Total protein and Globulin) (g/L)

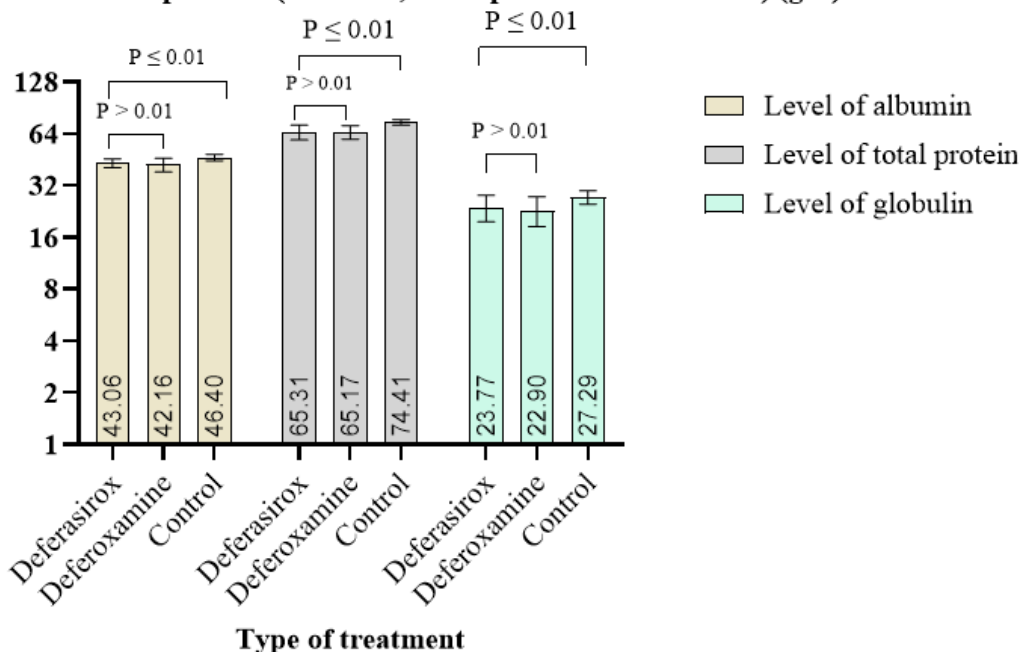


Figure 22. Mean value of albumin, total protein and globulin levels.

3.2.4. Level of Creatinine

As shown in Figure 23, the creatinine levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

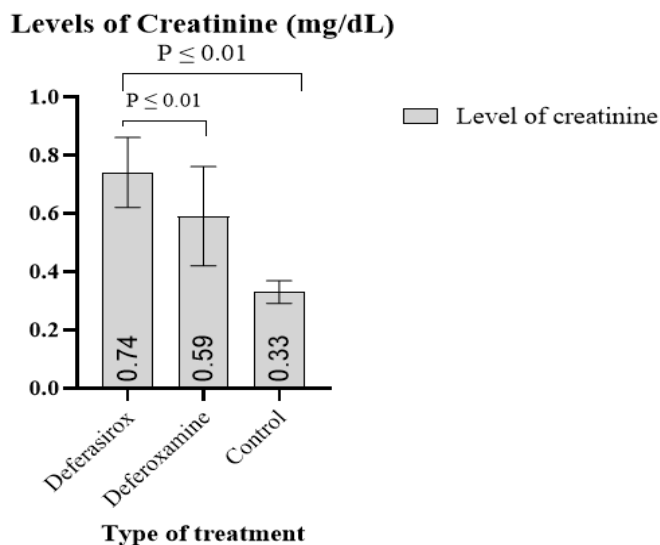


Figure 23. Mean value of creatinine levels.

3.2.5. Level of Calcium

As shown in Figure 24, the calcium levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO ($P > 0.01$).

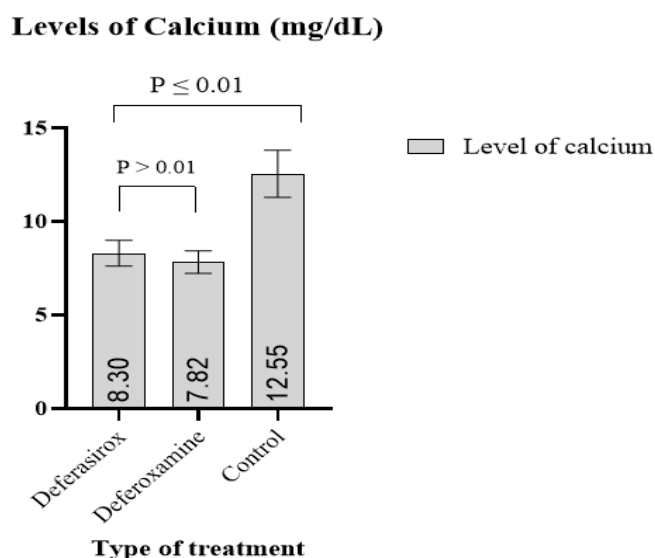


Figure 24. Mean value of calcium levels.

3.2.6. Level of Bilirubin

As shown in Figure 25, the bilirubin levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO ($P > 0.01$).

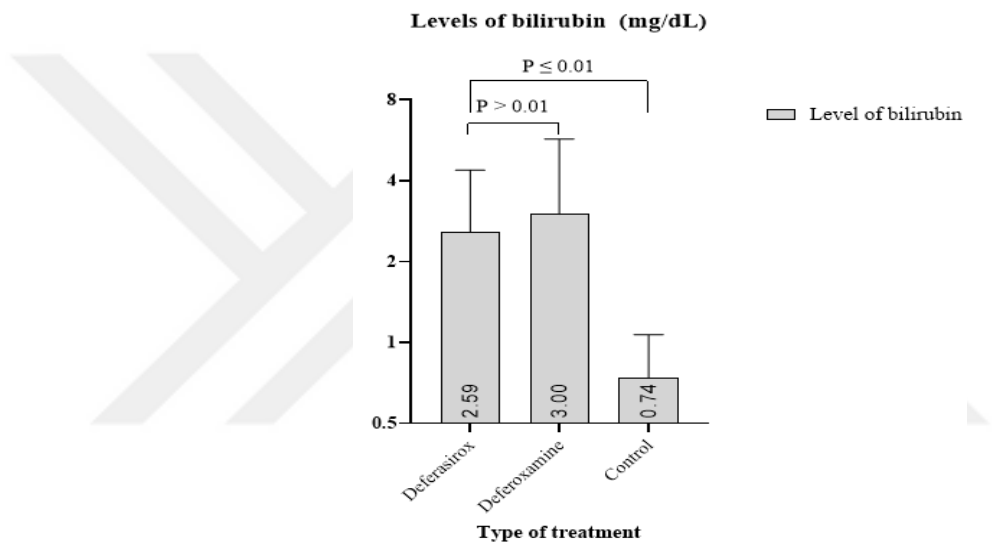


Figure 25. Mean value of bilirubin levels.

3.2.7. Level of Gamma Glutamyl Transferase

As shown in Figure 26, the GGT levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

Levels of Gamma Glutamyl transferase (U/L)

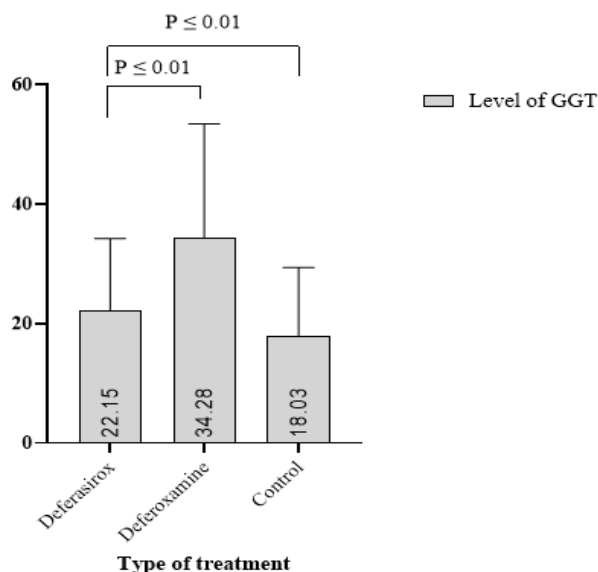


Figure 26. Mean value of gamma glutamyl transferase levels.

3.3. Hormonal Tests

Hormonal tests results for all groups are presented in Table 7.

Table 7. Results of hormonal parameters in the study groups

parameters	Normal Value	DFX (mean $\pm$ SD)	DFO (mean $\pm$ SD)	Control (mean $\pm$ SD)	P value
T3 (nmol/L)	1.2-3.5	2.32 $\pm$ 0.66	2.41 $\pm$ 0.67	2.28 $\pm$ 0.64	> 0.01
T4 (nmol/L)	66-181	127.35 $\pm$ 23.21	129.30 $\pm$ 22.56	125.0 $\pm$ 22.93	> 0.01
TSH (nmol/L)	0.27-4.3	3.96 $\pm$ 2.19	2.58 $\pm$ 1.23	2.44 $\pm$ 1.00	$\leq$ 0.01
GH (ng/mL)	Up to 10	1.15 $\pm$ 0.82	1.72 $\pm$ 0.91	2.04 $\pm$ 0.86	$\leq$ 0.01
Testosterone in male (ng/mL)	2.49-8.36	3.86 $\pm$ 2.48	3.93 $\pm$ 2.55	5.23 $\pm$ 1.74	$\leq$ 0.01
Testosterone in female (ng/mL)	0.084-0.481	0.27 $\pm$ 0.11	0.28 $\pm$ 0.12	0.30 $\pm$ 0.11	> 0.01
Prolactin in male (μ U/mL)	86-324	157.83 $\pm$ 41.56	138.09 $\pm$ 34.39	137.92 $\pm$ 43.75	$\leq$ 0.01

Table 7 continued

Parameters	Normal Value	DFX (mean $\pm$ SD)	DFO (mean $\pm$ SD)	Control (mean $\pm$ SD)	P value
Prolactin in female (μ U/mL)	102-496	289.19 $\pm$ 119.2	268.41 $\pm$ 162.57	216.15 $\pm$ 90.93	≤ 0.01
LH in male (IU/mL)	1.7-8.6	4.15 $\pm$ 2.56	2.29 $\pm$ 2.54	8.94 $\pm$ 1.87	≤ 0.01
LH in female (IU/mL)	7.7-58.5	7.37 $\pm$ 3.87	5.76 $\pm$ 2.37	30.58 $\pm$ 15.10	≤ 0.01
FSH in male (IU/mL)	1.5-12.4	3.51 $\pm$ 2.62	2.40 $\pm$ 2.11	8.47 $\pm$ 2.91	≤ 0.01
FSH in female (IU/mL)	25.8-134.8	23.77 $\pm$ 4.14	22.15 $\pm$ 3.83	53.42 $\pm$ 24.63	≤ 0.01
Vit. D (ng/mL)	25 - 30	21.26 $\pm$ 5.40	22.62 $\pm$ 3.59	36.25 $\pm$ 5.13	≤ 0.01

3.3.1. Levels of Triiodothyronine Hormone (T3)

As shown in Figure 27, the T3 levels of the DFO, DFX, and control groups exhibited a normal distribution by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One-way ANOVA test revealed no significant differences between the chelating agents (DFO, DFX) and the control group ($P > 0.01$), and the F-value is 1.011 with df (2, 297). The Tukey test further confirmed that there are no significant differences between DFX and the control group ($P > 0.01$), as well as between DFO and the control group ($P > 0.01$). Additionally, there is no significant difference between DFX and DFO ($P > 0.01$).

Levels of Triiodothyronine hormone (nmol/L)

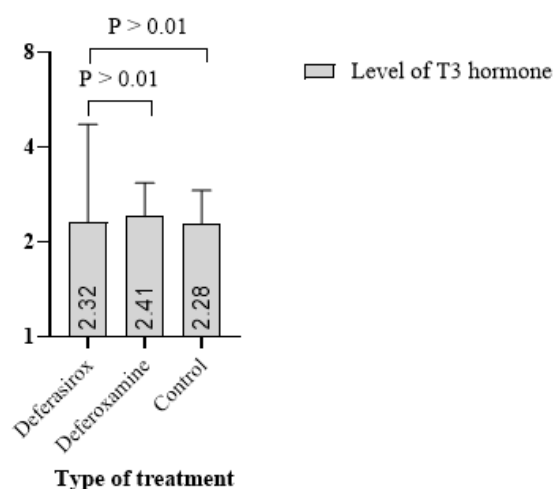


Figure 27. Mean value of triiodothyronine hormone levels.

3.3.2. Levels of Thyroxine Hormone (T4)

As shown in Figure 28, the T4 levels of the DFO, DFX, and control groups exhibited a normal distribution by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One-way ANOVA test revealed no significant differences between the chelating agents (DFO, DFX) and the control group ($P > 0.01$), and the F-value is 0.809 with df (2, 297). The Tukey test further confirmed that there are no significant differences between DFX and the control group ($P > 0.01$), as well as between DFO and the control group ($P > 0.01$). Additionally, there is no significant difference between DFX and DFO ($P > 0.01$).

Levels of Thyroxine hormone (nmol/L)

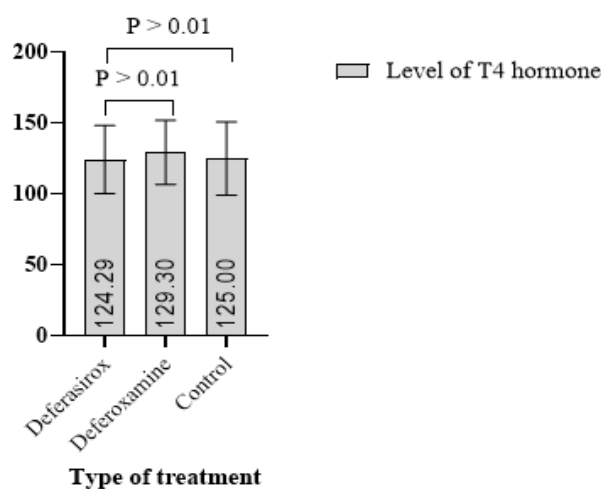


Figure 28. Mean value of thyroxine hormone levels.

3.3.3. Level of Thyroid Stimulating Hormone (TSH)

As shown in Figure 29, the TSH levels of the DFO, DFX, and control groups exhibited a normal distribution, by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One way ANOVA test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$), and the F-value is 28.88 with df (2, 297) The Post Hoc test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and DFX ($P \leq 0.01$).

Levels of Thyroid Stimulating hormone (nmol/L)

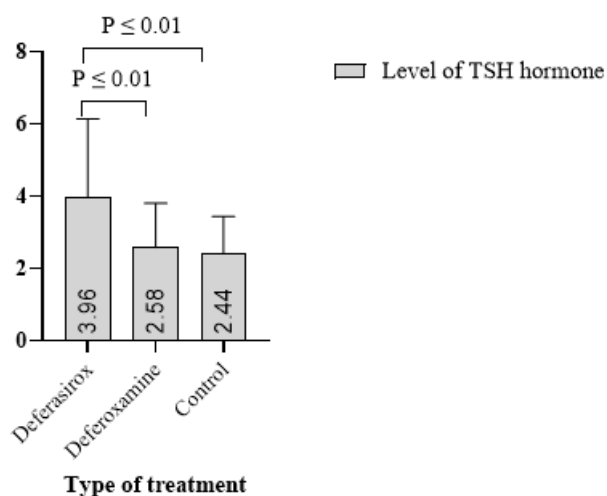


Figure 29. Mean of thyroid stimulating hormone levels.

3.3.4. Level of Growth Hormone (GH)

As shown in Figure 30, the GH levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

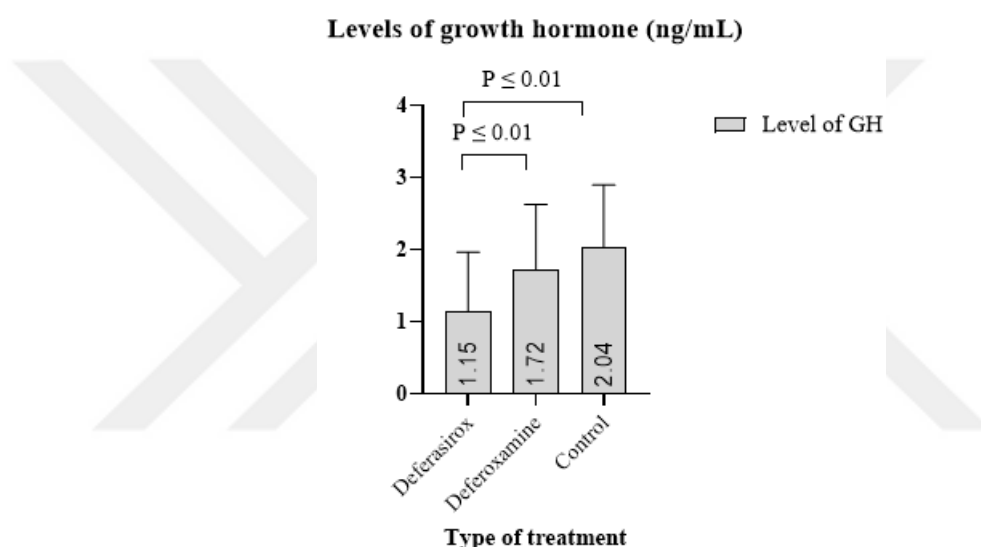


Figure 30. Mean value of growth hormone levels.

3.3.5. Level of Testosterone Hormone

As shown in Figure 31, the testosterone hormone levels in males of the DFO, DFX, and control groups exhibited a normal distribution, by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One way ANOVA test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$), and the F-value is 5.68 with df (2, 297). The Post Hoc test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO ($P > 0.01$).

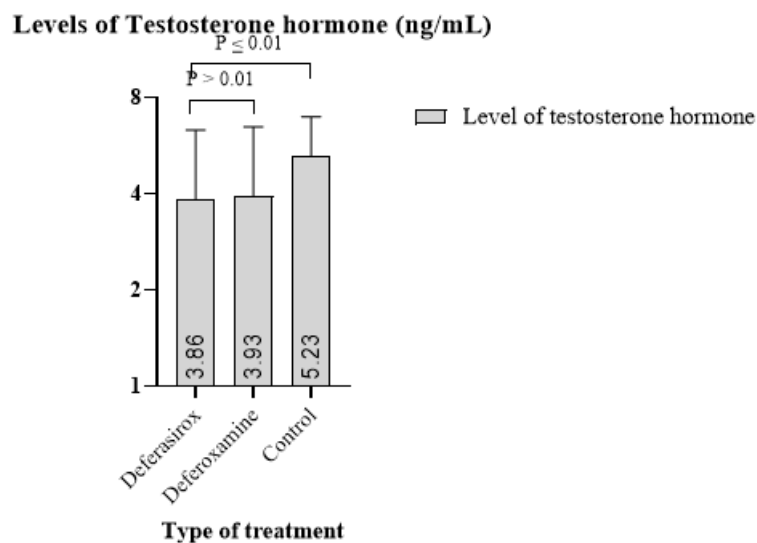


Figure 31. Mean value of testosterone hormone levels in males.

As shown in Figure 32, the testosterone hormone levels in females of the DFO, DFX, and control groups exhibited a normal distribution by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One-way ANOVA test revealed no significant differences between the chelating agents (DFO, DFX) and the control group ($P > 0.01$), and the F-value is 0.751 with df (2, 297). The Tukey test further confirmed that there are no significant differences between DFX and the control group ($P > 0.01$), as well as between DFO and the control group ($P > 0.01$). Additionally, there is no significant difference between DFX and DFO ($P > 0.01$).

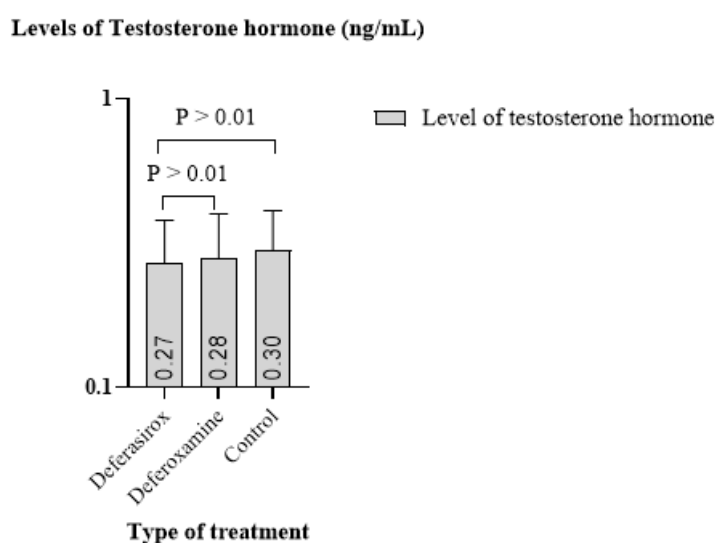


Figure 32. Mean value of testosterone hormone levels in females.

3.3.6. Level of Prolactin Hormone

As shown in Figure 33, the prolactin hormone levels in females of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), but not significant differences between DFO and the control group ($P > 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

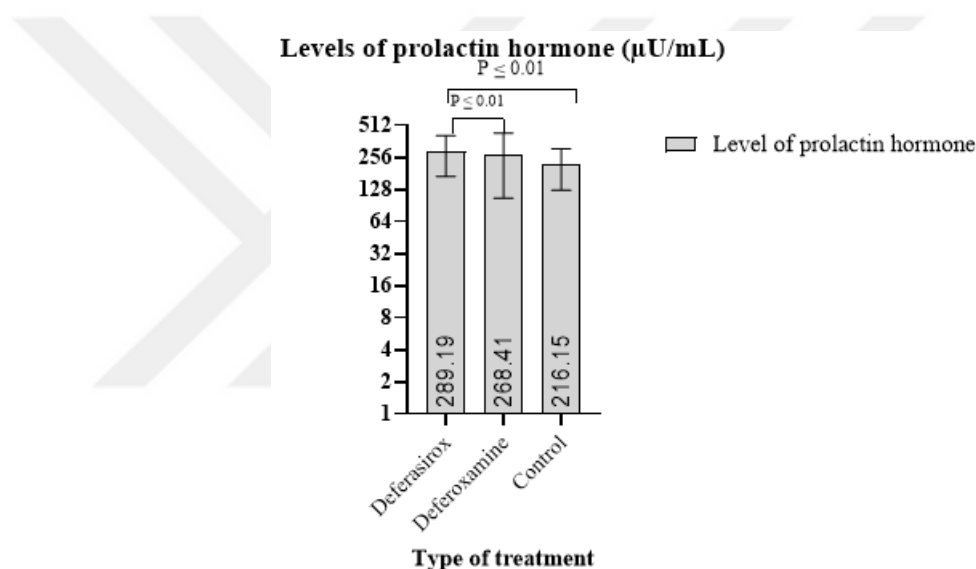


Figure 33. Mean value of prolactin hormone levels in females.

As shown in Figure 34, the prolactin hormone levels in males of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed no significant differences between the chelating agents (DFO, DFX) and the control group ($P > 0.01$). The Mann-Whitney U test further confirmed no significant differences between DFX and the control group ($P > 0.01$), as well as between DFO and the control group ($P > 0.01$). Additionally, there is no significant difference between DFX and DFO ($P > 0.01$).

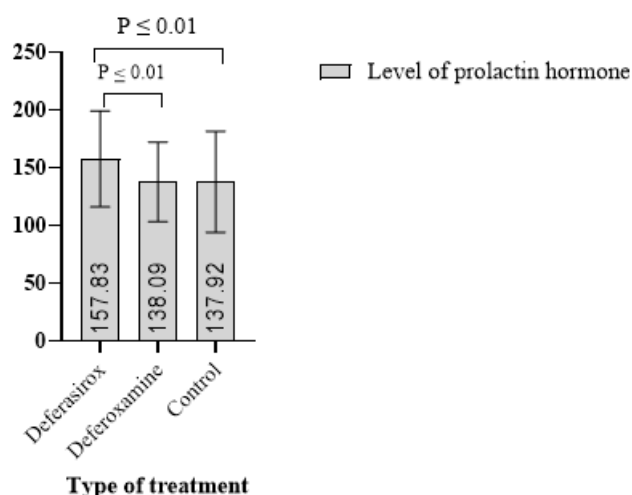
Levels of prolactin hormone ($\mu\text{U/mL}$)

Figure 34. Mean of prolactin hormone levels in males.

3.3.7. Level of Luteinizing Hormone (LH)

As shown in Figure 35, the LH levels in females of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

Levels of luteinizing hormone (IU/L)

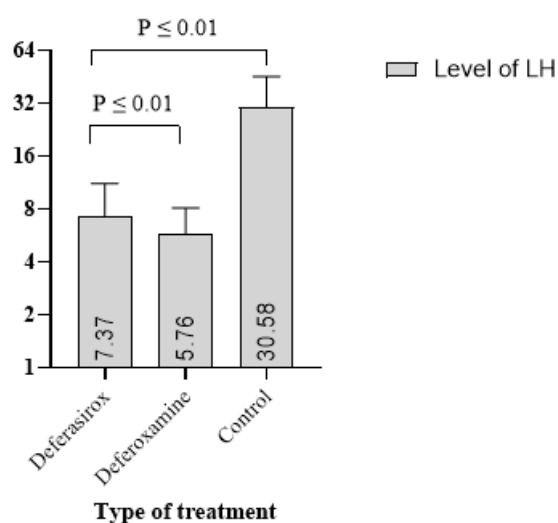


Figure 35. Mean value of luteinizing hormone levels in females.

As shown in Figure 36, the LH levels in males of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

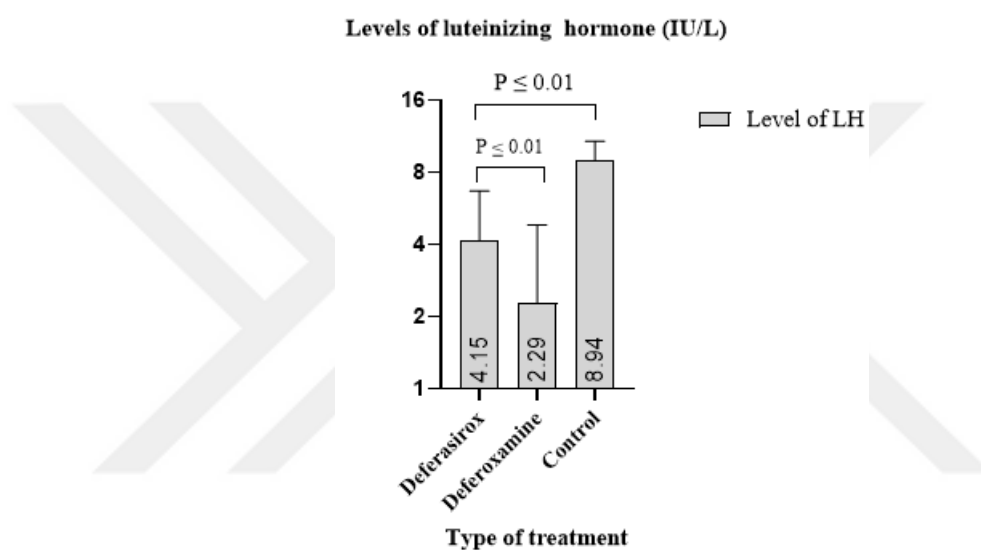


Figure 36. Mean of luteinizing hormone levels in males.

3.3.8. Level of Follicle Stimulating Hormone (FSH)

As shown in Figure 37, the FSH hormone levels in females of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

Levels of Follicle stimulating hormone (IU/L)

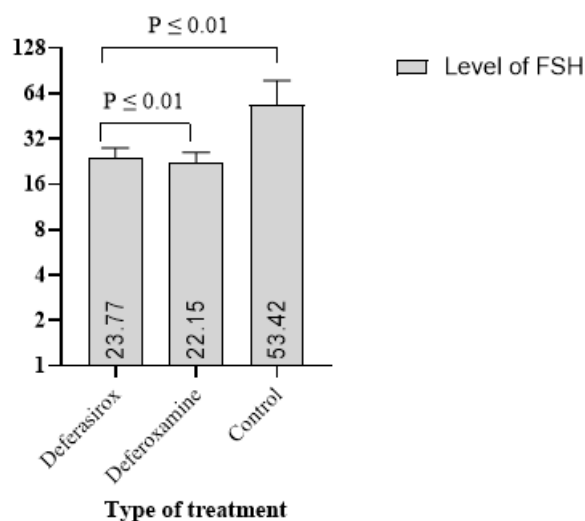


Figure 37. Mean value of follicle stimulating hormone levels in females.

As shown in Figure 38, the FSH hormone levels in females of the DFO, DFX, and control groups exhibited a normal distribution by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One-way ANOVA test revealed no significant differences between the chelating agents (DFO, DFX) and the control group ($P > 0.01$), and the F-value is 1.35 with df (2, 297). The Tukey test further confirmed that there are no significant differences between DFX and the control group ($P > 0.01$), as well as between DFO and the control group ($P > 0.01$). Additionally, there is no significant difference between DFX and DFO ($P > 0.01$).

Levels of Follicle stimulating hormone (IU/L)

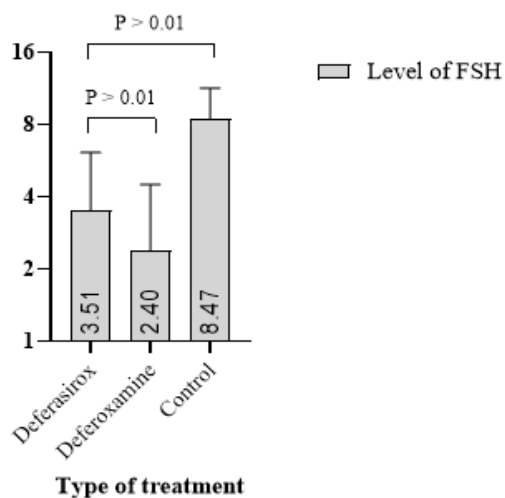


Figure 38. Mean of follicle stimulating hormone levels in males.

3.3.9. Level of Vitamin D (Vit. D)

As shown in Figure 39, the Vit. D levels of the DFO, DFX, and control groups exhibited a normal distribution by the Shapiro-Wilk test. After performing the Levene test, which was non-significant ($P > 0.05$) the one-way ANOVA and Post Hoc tests (Tukey test) were used to assess the differences among these three groups. One-way ANOVA test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$), and the F-value is 300.55 with df (2, 297). The Tukey test further confirmed that there are significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFO and DFX ($P > 0.01$).

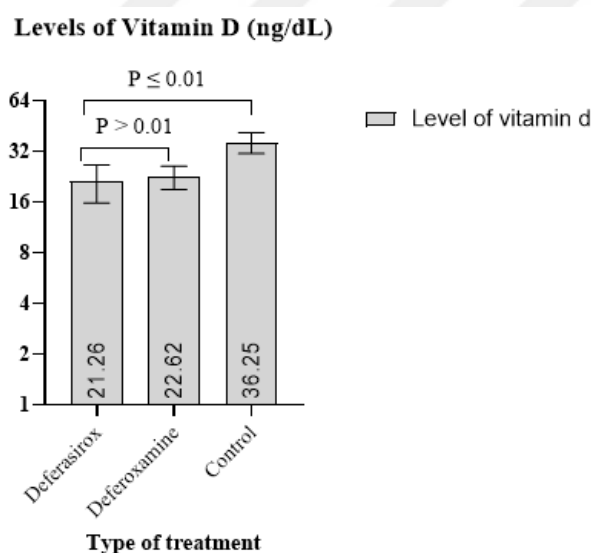


Figure 39. Mean value of vitamin D levels.

3.4. Immunological Tests

Immunological tests results for all groups are presented in Table 8.

Table 8. Results of immunological parameters in the study groups.

parameters	DFX (mean $\pm$ SD)	DFO (mean $\pm$ SD)	Control (mean $\pm$ SD)	P value
IL6 (pg/mL)	3.60 $\pm$ 0.98	6.10 $\pm$ 1.21	0.90 $\pm$ 0.38	≤ 0.01
IL8 (pg/mL)	202.15 $\pm$ 10.0	205.75 $\pm$ 1.83	93.63 $\pm$ 12.54	≤ 0.01
TLR3 (ng/L)	19.55 $\pm$ 13.58	2876.73 $\pm$ 2245.64	3980.72 $\pm$ 2742.68	≤ 0.01
TLR4 (ng/L)	241.15 $\pm$ 258.8	201.82 $\pm$ 218.47	1575.62 $\pm$ 1158.88	≤ 0.01
CHOP (ng/mL)	5.11 $\pm$ 1.68	5.62 $\pm$ 0.58	3.52 $\pm$ 1.55	≤ 0.01
GRP78 (ng/mL)	0.67 $\pm$ 1.29	0.30 $\pm$ 0.94	2.06 $\pm$ 0.93	≤ 0.01

3.4.1. Level of Interleukin 6 (IL6)

As shown in Figure 40, the IL6 levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

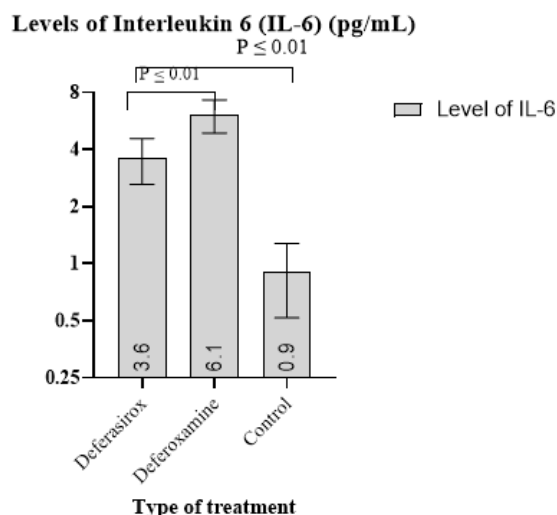


Figure 40. Mean value of interleukin 6 levels.

3.4.2. Level of Interleukin 8 (IL-8)

As shown in Figure 41, the IL8 levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO ($P > 0.01$).

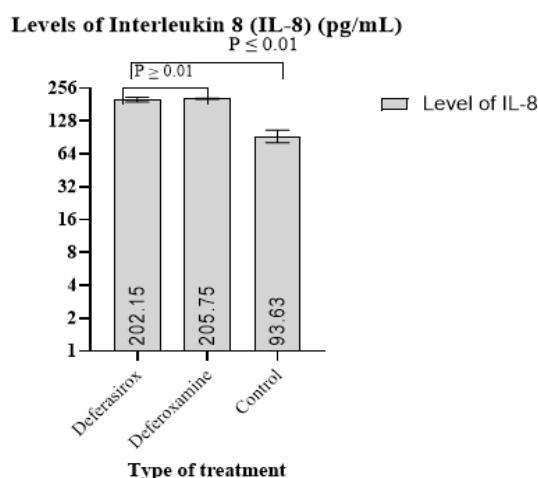


Figure 41. Mean value of interleukin 8 levels.

3.4.3. Level of Toll-Like Receptor 3 (TLR3)

As shown in Figure 42, the TLR3 levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). Additionally, there is a significant difference between DFX and DFO ($P \leq 0.01$).

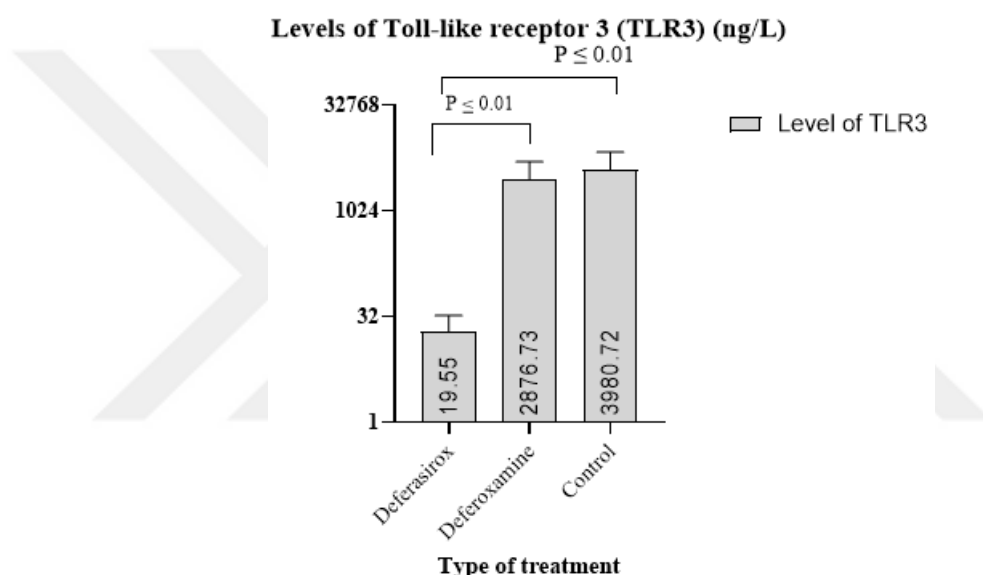


Figure 42. Mean value of toll-like receptor 3 levels.

3.4.4. Level of Toll-Like Receptor 4 (TLR4)

As shown in Figure 43, the TLR4 levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO ($P > 0.01$).

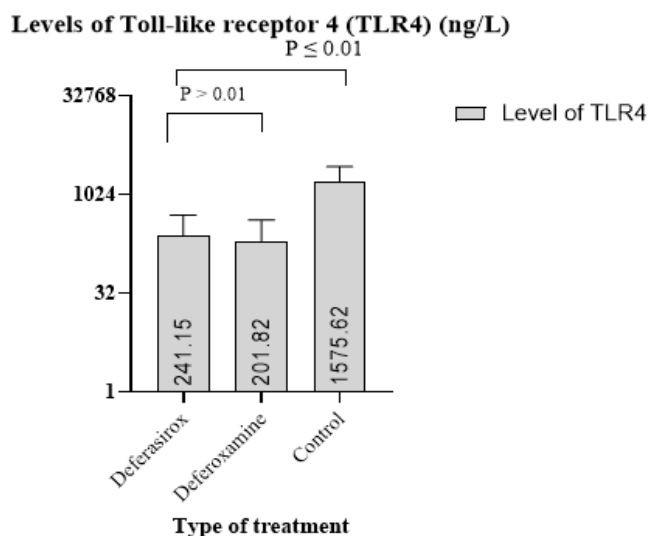


Figure 43. Mean value of toll-like receptor 4 levels.

3.4.5. Level of CHOP (FUS)

As shown in Figure 44, the CHOP (FUS) levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO ($P > 0.01$).

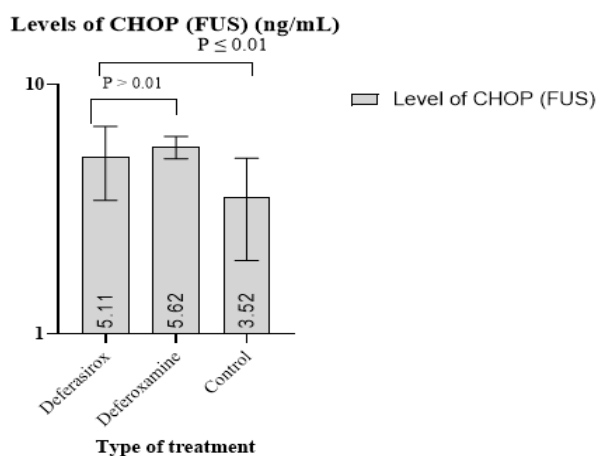


Figure 44. Mean value of CHOP (FUS) levels.

3.4.6. Level of Glucose-Regulated Protein 78 (GRP78)

As shown in Figure 45, the GRP78 levels of the DFO, DFX, and control groups exhibited an abnormal distribution, by the Shapiro-Wilk test. The Kruskal-Wallis and Mann-Whitney U tests were used to assess the differences among these three groups. Kruskal-Wallis test revealed significant differences between the chelating agents (DFO, DFX) and the control group ($P \leq 0.01$). The Mann-Whitney U test further confirmed significant differences between DFX and the control group ($P \leq 0.01$), as well as between DFO and the control group ($P \leq 0.01$). However, there is no significant difference between DFX and DFO ($P > 0.01$).

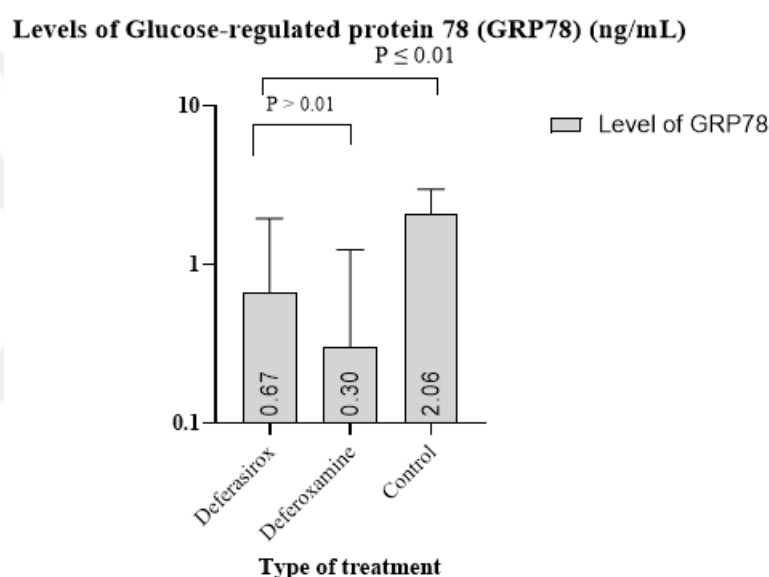


Figure 45. Mean value of glucose-regulated protein 78 levels.

3.5. Correlation Analysis Results

The results of correlation analysis after using a nonparametric Spearman test to determine relationship between tests.

Table 9. Correlation coefficients (r) between hematological tests related to levels (RBC, Hb, WBC, lymphocyte) with biochemical, hormonal, and immunological parameters.

Test	RBC	Hb	WBC	Lymphocyte
WBC	-0.539*	-0.578*	NC	0.492*
lymphocyte	-0.569*	-0.503*	0.492*	NC
RBC	NC	0.784*	-0.539*	-0.539*
Hb	0.784*	NC	-0.578*	-0.578*
HCT	0.786*	0.937*	-0.574*	-0.574*

Table 9 continued

Test	RBC	Hb	WBC	Lymphocyte
MCV	0.567*	0.621*	-0.549*	-0.549*
MCH	0.665*	0.684*	-0.487*	-0.487*
Platelet	-0.582*	-0.509*	0.497*	0.497*
Ferritin	-0.662*	-0.728*	0.609*	0.609*
AST	-0.562*	-0.589*	0.541*	0.541*
ALT	-0.527*	-0.525*	0.519*	0.519*
creatinine	-0.610*	-0.597*	0.554*	0.429*
Iron	-0.533*	-0.558*	0.532*	0.593*
Albumin	NC	0.418*	-0.412*	NC
Total protein	0.536*	0.563*	-0.455*	-0.439*
Bilirubin	-0.455*	-0.465*	NC	0.416*
Calcium	0.645*	0.648*	-0.557*	-0.598*
Vit. D	0.608*	0.660*	-0.544*	-0.584*
TSH	-0.415*	NC	NC	NC
LH	0.536*	0.583*	-0.625*	-0.514*
FSH	0.624*	0.628*	-0.584*	-0.528*
GRP78	0.460*	0.437*	-0.465*	-0.423*
CHOP	NC	NC	NC	0.452*
TLR 3	0.455*	0.525*	NC	-0.408*
TLR 4	0.538*	0.567*	-0.446*	-0.496*
IL 6	-0.677*	-0.694*	0.550*	0.591*
IL 8	-0.672*	-0.701*	0.557*	0.597*

NC = No Correlation (Coefficient near to zero, and p -value > 0.05).

* High Significant = p -value < 0.01 .

Positive value = Direct correlation, Negative value = Inverse correlation.

In Table 9. of the current study showed a high significant positive correlation ($P \leq 0.01$) between RBC and (HB, HCT, MCV, MCH, total protein, calcium, Vit. D, LH, FSH, GRP78, TLR 3, TLR 4).

The results also showed a high significant negative correlation ($P \leq 0.01$) between RBC and (WBC, lymphocytes, platelet, ferritin, AST, ALT, creatinine, iron, bilirubin, TSH, IL 6, IL 8).

And study showed a high significant positive correlation ($P \leq 0.01$) between Hb and (RBC, HCT, MCV, MCH, albumin, total protein, calcium, Vit. D, LH, FSH, GRP78, TLR 3, TLR 4).

The results also showed a high significant negative correlation ($P \leq 0.01$) between Hb and (WBC, lymphocytes, platelets, ferritin, iron, AST, ALT, creatinine, bilirubin, IL 6, IL 8).

Also, the current study showed a high significant positive correlation ($P \leq 0.01$) between WBC and (lymphocytes, platelets, ferritin, iron, AST, ALT, creatinine, IL 6, IL 8).

The results also showed a high significant negative correlation ($P \leq 0.01$) between WBC and (RBC, Hb, HCT, MCV, MCH, albumin, total protein, calcium, Vit. D, LH, FSH, GRP78, TLR 3, TLR 4).

And showed a high significant positive correlation ($P \leq 0.01$) between lymphocytes and (WBC, platelets, ferritin, iron, AST, ALT, bilirubin, creatinine, IL 6, IL 8, CHOP).

The results also showed a high significant negative correlation ($P \leq 0.01$) between lymphocytes and (RBC, Hb, HCT, MCV, MCH, total protein, calcium, Vit. D, LH, FSH, GRP78, TLR 3, TLR 4).

Table 10. Correlation coefficients (r) biochemical tests (AST, ALT, ferritin, iron) with hematological, biochemical, hormonal, and immunological parameters.

Test	AST	ALT	Ferritin	Iron
WBC	0.541*	0.519*	0.609*	0.532*
lymphocyte	0.546*	0.532*	0.618*	0.593*
RBC	-0.562*	-0.527*	-0.662*	-0.533*
Hb	-0.589*	-0.525*	-0.728*	-0.558*
HCT	-0.600*	-0.551*	-0.700*	-0.558*
MCV	-0.644*	-0.611*	-0.684*	-0.697*
MCH	-0.469*	-0.428*	-0.621*	-0.545*
Platelet	0.751*	0.685*	0.509*	0.743*
Ferritin	0.540*	0.486*	NC	0.564*
AST	NC	0.874*	0.540*	0.867*
ALT	0.874*	NC	0.486*	0.826*
Alkaline phosphatase	0.400*	NC	NC	NC
creatinine	0.422*	0.430*	0.625*	0.433*
Iron	0.867*	0.826*	0.564*	NC

Table 10 continued

Test	AST	ALT	Ferritin	Iron
Albumin	-0.508*	-0.474*	-0.434*	-0.496*
Total protein	-0.589*	-0.537*	-0.590*	-0.621*
Globulin	-0.430*	NC	-0.430*	-0.494*
Bilirubin	0.442*	0.410*	0.439*	0.454*
Calcium	-0.714*	-0.636*	-0.649*	-0.689*
Vit. D	-0.552*	-0.554*	-0.722*	-0.584*
LH	-0.584*	-0.547*	-0.597*	-0.587*
FSH	-0.688*	-0.641*	-0.623*	-0.695*
GRP78	-0.607*	-0.571*	-0.436*	-0.590*
CHOP	0.548*	0.532*	NC	0.518*
TLR 3	NC	NC	-0.598*	NC
TLR 4	-0.543*	-0.511*	-0.599*	-0.604
IL 6	0.645*	0.581*	0.745*	0.631*
IL 8	0.640*	0.586*	0.740*	0.625*

NC = No Correlation (Coefficient near to zero, and p -value > 0.05).

* High Significant = p -value < 0.01 .

Positive value = Direct correlation, Negative value = Inverse correlation.

In Table 10. of the current study showed a high significant positive correlation ($P \leq 0.01$) between AST and (WBC, lymphocytes, platelets, ferritin, ALT, ALP, bilirubin, creatinine, iron, IL 6, IL 8, CHOP).

The results also showed a high significant negative correlation ($P \leq 0.01$) between AST and (RBC, Hb, MCV, MCH, HCT, albumin, total protein, globulin, calcium, Vit. D, LH, FSH, GRP78, CHOP, TLR 4).

And showed a high significant positive correlation ($P \leq 0.01$) between ALT and (WBC, lymphocytes, platelets, ferritin, AST, bilirubin, creatinine, iron, IL 6, IL 8, CHOP).

The results also showed a high significant negative correlation ($P \leq 0.01$) between ALT and (RBC, Hb, MCV, MCH, HCT, albumin, total protein, calcium, Vit. D, LH, FSH, GRP78, CHOP, TLR 4).

Also showed a high significant positive correlation ($P \leq 0.01$) between ferritin and (WBC, lymphocytes, platelets, AST, ALT, creatinine, iron, bilirubin, IL 6, IL 8).

The results also showed a high significant negative correlation ($P \leq 0.01$) between ferritin and (RBC, Hb, MCV, MCH, HCT, albumin, total protein, globulin, calcium, Vit. D, LH, FSH, GRP78, TLR 3, TLR 4).

And showed a high significant positive correlation ($P \leq 0.01$) between iron and (WBC, lymphocytes, platelets, ferritin, AST, ALT, creatinine, bilirubin, CHOP, IL 6, IL 8).

The results also showed a high significant negative correlation ($P \leq 0.01$) between iron and (RBC, Hb, MCV, MCH, HCT, albumin, total protein, globulin, calcium, Vit. D, LH, FSH, GRP78, TLR 4).

Table 11. Correlation coefficients (r) between immunological tests related to inflammations (TLR3, TLR4, IL6, IL8, GRP78, and CHOP levels) with biochemical, hormonal, and immunological parameters.

Test	TLR3	TLR4	IL6	IL8	GRP78	CHOP
WBC	NC	-0.446*	0.546*	0.557*	-0.465*	NC
lymphocyte	-0.408*	-0.496*	0.532*	0.597*	-0.423*	0.452*
RBC	0.455*	0.538*	-0.642*	-0.672*	0.460*	NC
Hb	0.525*	0.567*	-0.685*	-0.701*	0.437*	NC
HCT	0.536*	0.560*	-0.681*	-0.693*	0.413*	NC
MCV	NC	0.590*	-0.676*	-0.683*	0.522*	-0.469*
MCH	0.420*	0.495*	-0.562*	-0.570*	0.401*	NC
Platelet	NC	-0.479*	0.520*	0.533*	-0.555*	0.406*
Ferritin	-0.598*	-0.599*	0.734*	0.740*	-0.436*	NC
AST	NC	-0.543*	0.632*	0.640*	-0.607*	0.548*
ALT	NC	-0.511*	0.567*	0.586*	-0.571*	0.532*
creatinine	-0.525*	-0.404*	0.561*	0.575*	NC	NC
Iron	NC	-0.604*	0.612*	0.625*	-0.590*	0.518*
Albumin	NC	0.475*	-0.531*	-0.547*	0.414*	NC
Total protein	NC	0.495*	-0.573*	-0.598*	0.476*	-0.422*
Globulin	NC	0.409*	-0.440*	-0.457*	0.450*	NC
Bilirubin	NC	-0.416*	0.472*	0.481*	-0.403*	NC
Calcium	NC	0.586*	-0.652*	-0.649*	0.511*	-0.402*
Vit. D	NC	0.547*	-0.672*	-0.688*	0.425*	-0.429*
GH	0.480*	NC	NC	NC	NC	NC
GRP78	NC	0.492*	-0.510*	-0.516*	NC	NC

Table 11 continued

Test	TLR3	TLR4	IL6	IL8	GRP78	CHOP
TLR 3	NC	NC	NC	-0.403*	0.492*	NC
TLR 4	NC	NC	-0.621*	-0.632*	-0.516*	NC
IL 6	NC	-0.612*	NC	0.567*	-0.510*	NC
IL8	-0.403*	-0.632*	0.514*	NC	-0.516*	NC

NC = No Correlation (Coefficient near to zero, and p -value > 0.05).

* High Significant = p -value < 0.01 .

Positive value = Direct correlation, Negative value = Inverse correlation.

In Table 11. of the current study showed a high significant positive correlation ($P \leq 0.01$) between TLR3 and (RBC, Hb, HCT, MCH, GH).

The results also showed a high significant negative correlation ($P \leq 0.01$) between TLR3 with (lymphocytes, ferritin, creatinine, IL8).

And showed a high significant positive correlation ($P \leq 0.01$) between TLR4 and (RBC, Hb, HCT, MCV, MCH, albumin, total protein, calcium, Vit. D, GRP78).

The results also showed a high significant negative correlation ($P \leq 0.01$) between TLR4 with WBC, lymphocytes, platelets, AST, ALT, ferritin, creatinine, iron, bilirubin, IL6, IL8.

Also showed a high significant positive correlation ($P \leq 0.01$) between IL6 and (WBC, lymphocytes, platelet, ferritin, AST, ALT, creatinine, iron, bilirubin, IL8).

The results also showed a high significant negative correlation ($P \leq 0.01$) between IL6 with (RBC, Hb, HCT, MCV, MCH, albumin, total protein, globulin, calcium, Vit. D, GRP78, TLR4).

Finally, the current study showed a high significant positive correlation ($P \leq 0.01$) between IL8 and (WBC, lymphocytes, platelet, ferritin, AST, ALT, creatinine, iron, bilirubin, IL6).

The results also showed a high significant negative correlation ($P \leq 0.01$) between IL8 with (RBC, Hb, HCT, MCV, MCH, albumin, total protein, globulin, calcium, Vit. D, GRP78, TLR3, TLR4).

Also showed a high significant positive correlation ($P \leq 0.01$) between GRP78 and (RBC, HB, HCT, MCV, MCH, albumin, total protein, globulin, calcium, Vit. D, TLR3).

The results also showed a high significant negative correlation ($P \leq 0.01$) between GRP78 with (WBC, lymphocytes, platelets, ferritin, AST, ALT, iron, bilirubin, TLR 4, IL6, IL8).

And the current study showed a high significant positive correlation ($P \leq 0.01$) between CHOP and (lymphocytes, platelet, AST, ALT, iron).

The results also showed a high significant negative correlation ($P \leq 0.01$) between CHOP with (MCV, total protein, calcium, Vit. D).



4. DISCUSSION

Patients with beta thalassemia major require frequent blood transfusions to maintain their health as a result of their low hemoglobin levels. These processes lead to the accumulation of free iron in important organs and tissues, including the heart, endocrine glands, liver, and reproductive system. Additionally, they cause stress in the endoplasmic reticulum, which disrupts the protein folding process, thereby leading to functional disorders in these organs. Deferoxamine and deferasirox are iron chelation medications that are administered to these patients to get rid of the accumulated free iron in their bodies. In regard to the widespread prevalence of this disease in numerous countries and the widespread use of these drugs, this study evaluated the impact of deferoxamine and deferasirox on specific hematological, biochemical, hormonal, and immunological parameters to determine their relative efficacy and benefits in modulating these parameters.

4.1. Hematological Parameters Tests

We have found a significant decrease in the RBC count between the thalassemia groups in comparison with the control group. Patients with thalassemia show a significant decrease in the total number of RBCs. We have obtained results that agree with a study performed by Khawaji et al. (2020), which also showed there is a significant reduction in the RBC count among the thalassemia patients compared to the control group. Genetic mutations in the genes responsible for the synthesis of the protein chains that comprise hemoglobin cause the disruption in the biosynthesis of globin chains and an imbalance in the synthesis of hemoglobin. This impacts the number, shape, and size of red blood cells during the formation process in the bone marrow, which produces small red blood cells and, as a result, lacks the same volume as normal red blood cells (Scianò et al., 2021). The increased breakdown of red blood cells in the spleen in patients with beta thalassemia can be the cause of the decreased red blood cell count (Hashim et al., 2020). The deficiency in red blood cells is caused by a two-process defect characterized by a higher rate of destruction than production (Agapidou et al., 2018). Spleen and Kupffer cells in the liver engulf red blood cells that are abnormal or aged entering the circulation (Abdulla et al., 2021). In addition the absence of beta globin chains in beta thalassemia results in the accumulation of alpha chains inside red blood cells and produces oxidative stress. Membrane damage,

breakdown, and dissolve because of this stress causes the number of red blood cells decreases (Chen et al., 2018).

We have found a significant increase in the WBCs count and lymphocytes between the thalassemia groups in comparison with the control group. Patients with thalassemia show a significant increase in lymphocytes, and in total number of WBCs, the results of our research are agreement with a study done by Sonmez et al. (2017), which also showed there is a significant increase in WBC count and lymphocytes between the thalassemia patients compared to the control group.

The abnormal increase in white blood cells may be linked to the presence of pathogenic causes, such as thalassemia, which forms cells in the bone marrow, liver, spleen, and lymphatic tissues. Thalassemia can result in bone marrow injury and immune system diseases (Kadhem., 2014). Hemolysis, both internal and external to the bone marrow, may be responsible for the numerical increase in total WBC and LYM in thalassemia patients. This process induces the kidneys to produce erythropoietin, which in change stimulates bone marrow to increase production of a variety of blood cells, including WBC. Additionally, infection in thalassemia patients results in an increase in lymphocytes and monocytes. Because white blood cells, including lymphocytes, are essential for the innate immune response, they are impacted by stress, infection, and inflammation (Fadlyana et al., 2017). We have proven statistically that there is a direct (positive) correlation between increased WBC and lymphocyte levels and the disorder that occurs in the liver enzymes, increased iron levels, increased immunological markers that indicate inflammation, but proven that there is an inverse (negative) correlation with Hb decrease level.

In our study, mean Hb and Plt. levels were significantly higher in the DFO group than in the DFX group, our results are consistent with a study conducted by Sirdah et al. (2018) and Henter et al. (2007), which also reported a significant increase in Hb and Plt. levels in the DFO group relative to the DFX group. This may be due to the protective effects of DFO on hematopoiesis or preventing apoptosis in the bone marrow. Wu at all. Demonstrated that, both DFO and DFX promote the recovery of hematopoiesis in iron-overload. DFO may exhibit higher efficacy in improving white blood cell (WBC) and platelet (PLT) counts compared to DFX. DFO has counteract the suppressive effects of iron overload on erythroid burst-forming unit (BFU-E) colony formation and lineage differentiation by reducing intracellular reactive oxygen species (ROS) levels. In contrast, DFX acts by inhibiting NF- κ B-mediated gene expression and reducing the levels of activated T-cell-derived tumor

necrosis factor (TNF), which is detrimental to normal hematopoiesis. However, it has also been reported that DFX exposure may induce ROS production, which can impair the growth and viability of hematopoietic progenitor cells (HPCs) from myelodysplastic syndromes (MDS), leading to a reduction in CD34 expression in normal HPCs. In addition, DFO and DFX effect the expression of apoptotic regulatory proteins. Specifically, DFO was more effective in reducing the expression of pro-apoptotic proteins (Bax, caspase-3, and PARP), whereas DFX was more effective in increasing the expression of the anti-apoptotic protein BCL-2 and inhibiting caspase-9 expression. The superior protective effect of DFO on hematopoiesis may be attributed to its more pronounced role in safeguarding the bone marrow from apoptosis (Wu et al.,2017).

4.2. Biochemical Parameters Tests

Hossaini and Haeri (2019) explained that the serum ferritin level in thalassemia patients has shown a significant increase. This increase results from the production of ineffective RBCs. Additionally, the body is unable to excrete more than 1 mg of iron per day, which results in an increase in iron levels due to repetitive blood transfusions. Each unit of transfused RBC contains approximately 250 mg of iron. Besides repeated blood transfusions, the excess production of a protein known as 15 growth differentiation factors (15-GDF) may contribute to the elevated iron concentration in thalassemia patients. This protein inhibits the production of the liver protein hepcidin, which is responsible for increasing the absorption of iron from food in the intestines. Many studies have indicated that this increase in iron concentration in the blood is a risk factor (Bonfils, 2015).

The study performed by Goruntla et al. (2020), agrees with our study that deferasirox is more effective than deferoxamine in reducing iron accumulation and storage in the form of ferritin. However the potential adverse effects of deferasirox are significantly lower than those of deferoxamine. This is due to the drug's half-life, as previously indicated, in contrast to deferoxamine, which is restricted to the time of administration (just for 8 hours) while deferasirox provides 24-hour protection against effects of iron overload, moreover, a study showed that deferasirox was more effective than deferoxamine in reducing excess iron in the liver and heart (Al-Kuraishy & Al-Gareeb, 2017).

Choi et al. (2020), discovered that the levels of alanine transaminase (ALT), aspartate aminotransferase (AST), and Abdalla et al. (2011), Allegra et al. (2016) they found the levels

of ALP and GGT in individuals with β TM who were treated with the iron chelation drugs deferoxamine and deferasirox was significantly increased in compared to a control group. Figure 21. and 26. shows that the results of our current study, which displayed a notable rise in the levels of ALT, AST, GGT and ALP between the target groups are in conformity with these results. The GGT, AST, ALP, and AST levels were significantly higher in the deferoxamine-taking group, than in the deferasirox-taking group, according to the results.

Liver enzymes (AST) and (ALT) are two enzymes that are members of the transaminase family. Due to liver diseases, these enzymes are elevated. Upon the damage of specific liver cell types, these enzymes are released into the circulation, which results in elevated liver enzymes, and they can be quantified as indicators of liver cell damage caused by excess iron (Ali et al., 2020). Ferritin accumulation leads to chronic inflammation of the liver (Goruntla et al., 2020). We have proven statistically that there is a direct (positive) correlation between increased ferritin levels and the disorder that occurs in the liver enzymes.

In our study, both ferritin and liver tests were reduced in the DFX arm more than in the DFO arm. It can be explained by the fact that lower body iron causes less liver damage. This is because the iron chelator DFX has a longer half-life, which means that its effects last for a longer period after taking doses compared with deferoxamine (Fung, 2010).

Salih and Al-Mosawy (2016) found that the concentration of bilirubin in patients with β -thalassemia major who were treated with the iron chelation drugs deferoxamine and deferasirox was significantly increase in compared to a control group. According to Figure 25. the data from our current study displayed a significant increase in bilirubin concentration between the target groups that are in conformity with these results. The results indicated increase in bilirubin levels in the group that took deferoxamine compared to the group that took deferasirox but not significant. Despite the use of chelation medications, Salih and Al-Mosawy (2016) reported that iron overload and high ferritin levels impact liver function and in turn affect bilirubin levels. A relative increase in the concentration of ALT significantly impacts the secretion of bile, thereby affecting liver functions. The enzyme level is also higher when there is hemolysis and more red blood cell destruction, as well as when the liver is inflamed or cirrhosis, the bile ducts are blocked, gallstones, and when use medications and antibiotics (Waseem et al.,2011). We have proven statistically that there is a direct (positive) correlation between increased bilirubin levels and the disorder that occurs in the liver

enzymes, increased levels of ferritin, and increased markers that indicate inflammation, but proven that there is an inverse (negative) correlation with Hb decrease level.

In a study conducted by Adly et al. (2017) and Abd and Zainal (2020), it was found that albumin levels, globulin, and t.protein in patients with β -thalassemia major who were treated with the iron chelation drugs deferoxamine and deferasirox were significant lower than compared to a control group. Figure 22. shows that the data of this study, which displayed a significant reduction in the levels of globulin, albumin, & total protein between the target groups, are in conformity with these results. The results indicated not a significant decrease in globulin, albumin and total protein levels between deferoxamine and deferasirox.

Proteins are essential for the generation of enzymes, hormones, and antibodies, as well as regulating osmotic pressure, making their evaluation diagnostically important for evaluating nutritional status, liver, and kidney illnesses. Total protein levels indicate the equilibrium between synthesis and degradation, mainly consisting of albumin and globulin, which severe as important biomarkers for many diseases (Abbass et al., 2011; Hosen et al., 2015).

In thalassemia patients, repeated blood transfusions induce iron overload, causing hepatic and renal damage, and then albuminuria (Abdulla et al., 2021).

Chronic anemia causes kidney injury by elevating renal plasma flow and induced glomerular hyperfiltration. Globulin levels, mostly synthesized by the liver, decrease as a result of hepatic and renal damage induced by oxidative stress, iron accumulation, and the harmful impact of iron chelators (Malik et al., 2010). We have proven statistically that there is an inverse (negative) correlation between decreased albumin, globulin and total protein levels and disorder that occurs in the liver enzymes, increased levels of ferritin, and increased markers that indicate inflammation, but proven that there is a direct (positive) correlation is with Hb level decrease.

Annayev et al. (2007) found that the level of creatinine in patients with β -thalassemia major who were treated with the iron chelation drugs deferoxamine and deferasirox was significantly increase in compared to a control group. Figure 23. shows that the outcomes of our current data demonstrated a notable elevation in creatinine level between the target groups, are in conformity with these results. The results indicated a significant increase in creatinine levels in the group that took deferasirox compared to the group that took deferoxamine. Creatinine is an accurate marker of normal kidney function, and an increase in its concentration in the serum is indicative of kidney dysfunction. This can be affected by

many kinds of factors, including liver disease, gastrointestinal bleeding, excessive protein intake, inflammatory markers increase, and oxidative stress from chronic inflammation lead to increases in the production of inflammatory cytokines (IL-6, IL-8, TNF- α), which impairs blood flow to the kidney and leads to accumulation and increase of creatinine levels (Taher et al., 2018). We have proven statistically that there is a direct (positive) correlation between increased creatinine levels and the disorder that occurs in the liver enzymes, increased levels of ferritin, and increased markers that indicate inflammation (WBC, lymphocyte, IL-6, IL8), but proven that there is an inverse (negative) correlation with Hb, and RBC decrease level.

The kidneys may be impacted by iron chelation medications, as deferoxamine does not affect kidney function but deferasirox chelate increases creatinine levels (Huang et al., 2015). Recent clinical studies indicate that DFX causes partial necrosis of renal tubules and increases creatinine and urination excretion (papadopoulos et al., 2010).

Cappellini et al. (2018) noticed that deferasirox has direct toxicity to tubular cells and causes apoptosis and mitochondrial dysfunction. It was found in a study published by Al-Kuraishy et al. (2017) that the renal tubules were damaged as a result of a decrease in the glomerular filtration rate after use of deferasirox.

Lertsuwan et al. (2018) found that the concentration of calcium in individuals with β -thalassemia major who were treated with the iron chelation drugs deferoxamine and deferasirox was significantly decrease in compared to a control group. Figure 24. shows that the results of our current study, which displayed a significant decrease in the concentration of calcium between the target groups, are in conformity with these results. The results indicated a significant increase in calcium concentration in the group that took deferasirox compared to the group that took deferoxamine.

The clinical condition osteomalacia, which is known to be associated with thalassemia major, and hypoparathyroidism, which reduces calcium absorption from the gastrointestinal tract and results in reduced calcium levels, are believed to be associated with hypocalcemia (Shetty et al., 2018). We have proven statistically that there is a direct (positive) correlation between decreased calcium levels and decreased levels of Vit. D (which is essential for the absorption of Ca^{+2} in the intestine), but proven there are an inverse (negative) correlation with the disorder that occurs in level of liver enzymes, elevated levels of TSH, increased ferritin levels, and increased markers that indicate inflammation (WBC, lymphocyte, IL-6, IL8) which these markers lead to promotes Ca^{+2} loss through the kidneys.

Multiple blood transfusions can also cause hypocalcemia because citrate (an anticoagulant used in maintained blood prepared for transfusion) binds to calcium in the patient's blood. Platelets have higher concentrations of citrate than red blood cells, so when platelet units are transfused, more of it may enter the bloodstream. This can raise the risk of low calcium levels (Kamal, 2014).

4.3. Hormonal Parameters Tests

Endocrine disorders are among the most common complications associated with thalassemia. However, how often they occur is difficult to determine due to the continuous increase in life span of well-chelated individuals and the variations in the age of initial chelation therapy exposure. Patients with thalassemia major are more likely to develop endocrine diseases as a result of frequent blood transfusions. They appear to be the second most common cause of death in this population, following heart disease (Hussein, S. Z. 2022). The primary endocrine complications noticed in thalassemia patients include abnormal bone mineralization, diabetes mellitus, thyroid gland disorder, hypoadrenalism, and disorders of sexual development, growth, and fertility. The endocrine system's involvement continues to burden the lives of these patients, despite improvements in treatment. In fact, numerous studies have indicated that thyroid gland disorder may result in sexual dysfunction, pubertal failure, and infertility in as many as 50% to 65% of patients (Yenzeel, 2017).

According to reports, thalassemia patients experience chronic thyroid dysfunction at higher rates when their ferritin levels exceed 1800 mg/L or when they receive late-chelation therapy (Faiq et al., 2022). The most prevalent causes of thyroid dysfunction are chronic inflammation, chronic tissue hypoxia, organ siderosis, free radical leakage, and gland damage (Hussein, S. Z. 2022). We have proven statistically that there is a direct (positive) correlation between increased TSH levels and the disorder that occurs in the liver enzymes, increased levels of ferritin, and increased markers that indicate inflammation (WBC, lymphocyte, IL-6, IL8), but proven that there is an inverse (negative) correlation with Hb, and RBC decrease level.

In a study conducted by Hussein, S. Z. (2022) and Abdull et al. (2019) it was found that the levels of triiodothyronine (T3), Thyroxine (T4) and Thyroid stimulating hormone (TSH) in patients with β -thalassemia major was significantly increased in compared to a

control group. Respectively figures 3.13., 3.14, show that the results of our current study, which displayed increase in the levels of T3 and T4 between the target groups are in conformity with these results but not significant. The results indicated increase in T3, T4 levels in the group that took deferoxamine compared to the group that took deferasirox but not significant. Figure 29., show that the results of our current study, which displayed a significant increase in the levels of TSH hormone between the target groups are in conformity with these results. The results indicated a significant increase in TSH levels in the group that took deferasirox compared to the group that took deferoxamine.

Compared to health levels, beta-thalassemia patients showed a modest increase in thyroid hormones and a high level of TSH, as found in the present study. The hypothalamus produces an abundance of thyrotrophic-releasing hormone (TRH) because of an increase in iron synthesis. It produces an increase in the release of the hormone TSH by the pituitary gland, which promotes the reduction of thyroid hormones or their maintenance at normal levels. The accumulation of iron in the thyroid gland as a result of excessive blood transfusion and hemolysis can lead to gland malfunction (Hussein, S. Z. 2022).

The inability of the reproductive system to produce testosterone due to a main disorder (female reproductive organs or male reproductive organs) or another disorder (pituitary or hypothalamus) is known as hypogonadism (Dohle et al., 2012). Correlation between hypogonadism and pituitary iron excess is established (Kuşkonmaz., 2014). Iron deposition in the hypothalamus, pituitary, and gonads of patients with thalassemia leads to hypogonadism and delayed puberty (Khan, 2017). Researchers found that elevated iron deposition in the anterior portion of the pituitary gland damages gonadotrophs and reduces the production of gonadotrophins (LH and FSH) (Srisukh et al., 2016).

The iron depositions in the endocrine glands, which have been believed to be the primary causes of male infertility in β -thalassemia, are the result of the "free iron" that acts as a catalyst to produce oxygen species that peroxide the membrane and damage cells and lipids, resulting in cell destruction. Additionally, there are other potential causes of hypogonadism in β -thalassemia, such as chronic hypoxia and liver disorders (Yenzeel .2017).

In a study conducted by Chahkandi et al. (2017), Daraghme (2016), and Majeed (2017) it was found that the levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone hormone in patients with β -thalassemia major was significantly decreased (in males and females) when compared to a control group (healthy individuals).

Respectively, Figures 3.17, 3.18, 3.21, 3.22, 3.23, and 3.24. demonstrate the outcomes obtained from our analysis, which indicated a significant drop in LH levels, FSH, and testosterone hormones between the target groups are in conformity with these results. The results indicated a significant increase in LH and FSH values in the groups that took deferasirox compared to the group that took deferoxamine but indicated increase in testosterone levels in the group that took deferoxamine compared to the group that took deferasirox but not significant.

According to a study conducted in Egypt by Hegazi et al. (2013), a significant increase in the mean iron and ferritin levels was the main cause of the change in the activity of gonadotrophs in the pituitary gland anterior portion (which produce LH and FSH). This alteration occurs early in life and has a significant impact on the gonads' function during adulthood. The immediate impact associated with iron, particularly NTBI, on the ovaries and testes is currently unknown. Most female thalassemia patients, including those with amenorrhea, maintain their ovarian reserve. When testicular tissues from male autopsies, they showed testicular interstitial fibrosis (Srisukh et al., 2016). 40.2% of the individuals in the 2017 study, which had 97 female and male transfusion-dependent patients with thalassemia between the ages of 15 and 32, showed lower testosterone levels (Khan, 2017). About 71% of patients had disrupted puberty, according to a different study by Chahkandi et al. (2017) that was published in Iran. Additionally, there is mounting evidence that iron overload from excessive blood transfusions may be the primary cause of hypogonadism. This highlights the importance of using iron chelators early and regularly in order to ensure normal sexual maturation and avoid permanent damage to the testis and ovaries. Furthermore, because females with thalassemia are more prone to hypogonadism and may need hormonal replacement therapy to become pregnant, it emphasizes the importance of routine gonadal hormone monitoring, including the evaluation of adulthood (Wu et al., 2017). We have proven statistically that there is an inverse (negative) correlation between the glands disorders with the disorder that occurs in the liver enzymes, increased levels of ferritin, and increased markers that indicate inflammation (WBC).

GH levels were significantly lower in those with BTM than in a control group, according to a study published by Faiq et al. (2022). Figure 30. shows that the results of our current study, which displayed a significant decrease in the levels of GH between the target groups, are in conformity with these results. The results indicated a significant increase in GH levels in the group that took deferoxamine compared to the group that took deferasirox.

Growth impairment is one of the most common complications of β -thalassemia. This condition is characterized by growth retardation (GR), alterations in appearance, bone deformity, and the failure of pubertal development in thalassemia patients (Chhabra et al., 2016; Badfar et al., 2017).

Previous research indicated that patients with elevated accumulation of iron in their liver exhibited reduced stature. Furthermore, they exhibited reduced levels of insulin-like growth factor-I (IGF-I) compared to TM patients with less hepatic iron deposition (Yassin et al., 2018). A common issue in BT adults is abnormal backbone development, due to platyspondyly. Vertebral abnormalities may result from multiple reasons, including iron precipitation in RBCs and deficiency of essential elements. Furthermore, these patients are predisposed to growth hormone deficiency, gonadal insufficiency, or thyroid dysfunction. (Arab-Zozani et al., 2021).

We have proven statistically that there is a direct (positive) correlation between decreased GH levels and the disorder that occurs in the Hb and Ca^{+2} but proven that there is an inverse (negative) correlation with liver enzymes, increased levels of ferritin, increased levels of TSH and increased markers that indicate inflammation (WBC, lymphocyte, IL-6, IL8).

In a study conducted by Majeed (2017) it was found that the levels of prolactin hormone in patients with β -thalassemia major was significantly increased (in males and females) when compared to a control group (healthy individuals). Figures 3.19., and 3.20., show that the results of our current study, which displayed a significant increase in the levels of prolactin hormones between the target groups are in conformity with these results. The results indicated a significant increase in prolactin levels in the group that took deferiasirox compared to the group that took deferoxamine.

Most tissues, particularly the liver, spleen, heart, endocrine glands, and other critical organs, accumulate excessive iron. In particular, the anterior pituitary gland is highly susceptible to free radicals. It is seen in magnetic resonance imaging that the anterior pituitary's function can be disrupted by deposits of iron (Yenzeel, 2017).

In a study conducted by Jumaa et al. (2021) it was found that the level of Vit. D in patients with β -thalassemia major was significantly decreased compared to a control group (healthy individuals). Figure 39. shows the results of our current study, which displayed a significant decrease in the level of Vit. D between the target groups are in conformity with these results. The results indicated increase in Vit. D levels in the group that took

deferoxamine compared to the group that took deferiasirox but not significant. As proven by research, the defective 25-hydroxylation of vit. D in the liver is due to vitamin D deficiency in thalassemia patients. This defect is caused by an excess of iron. The liver primarily stores vitamin D. Excess iron in the liver can affect vitamin D metabolism, potentially leading to vitamin D deficiency because of reduced 25-hydroxylase activity. The increased risk of vitamin D deficiency is due to inherited and cultural ethnic factors that may result in low serum vit. D levels and limited exposure to sunlight (Abdelmotaleb et al., 2021). Increased iron levels and insufficient nutritional support affect vitamin D levels, necessitating frequent monitoring (Chatterjee & Bajoria, 2009). We have proven statistically that there is a direct (positive) correlation between decreased Vit. D levels and decreased levels of Ca^{+2} , but proven there are a inverse (negative) correlation with the disorder that occurs in the liver enzymes, increased levels of ferritin, and increased markers that indicate inflammation (WBC, lymphocyte, IL-6, IL8).

4.4. Immunological Parameters Tests

In a study conducted by Noori et al. (2017) and Sarhat et al. (2020) it was found that the level of interleukin 6 (IL-6) in patients with β -thalassemia major was significantly increased in compared to a control group. Figure 40. shows that the results of our current study, which displayed a significant increase in IL-6 level between the target groups, are in conformity with these results. The results indicated a significant increase in IL6 levels in the group that took deferoxamine compared to the group that took deferiasirox.

IL-6 is a cytokine that is involved in the systemic inflammatory response as well as local tissue inflammation. It promotes the differentiation of helper T cells into Th17 cells, which are composed of macrophages and T lymphocytes and are essential to the immune response (Deokar et al., 2018; Sarhat et al., 2020). Patients with thalassemia suffer elevated levels of inflammation and oxidative stress due to an excess of iron (Kohgo et al., 2008). The serum levels of IL-6 in thalassemia patients were found to be higher than those in healthy controls in the current study. Thalassemia patients experience chronic inflammatory condition. The secretion of IL-6 in thalassemia patients may be significantly affected by iron overload. It is probable that the abnormality of iron metabolism is a result of the overstimulation of macrophages, which is responsible for the increased production of IL-6 (Mohammed et al., 2020). The rise in non-transferrin bound iron (NTBI) has been linked to

an increase in reactive oxygen species (ROS), leading to the activation of ROS and the production of pro-inflammatory cytokines, including IL-6. The stimulation of mononuclear cells and the production of cytokines, including IL-6, are facilitated by the direct injury that IL-6 causes to compromised cells. Iron is an important factor to cell damage and death, as it stimulates the formation of free radicals (Nur et al., 2011).

Deferasirox chelator agent in thalassemia major shows a significant decrease in IL-6 compared to deferoxamine. The inability of deferoxamine to continuously regulate the levels of the labile pool of intracellular iron (LPI) and non-transferrin-bound iron (NTBI) may be linked to its shorter half-life in plasma (Jialal et al., 2002). Deferasirox has a longer half-life, and its effect persists for a significantly longer period after dosing. Deferasirox is an iron chelator with a longer half-life. This may help reduce inflammation by better controlling LPI and NTBI levels after it is given. This is because the continuous presence of the iron chelator in the plasma may prevent excess iron accumulation, thereby preventing iron-related diseases and death (Ragab et al., 2015; Jialal et al., 2002; Neufeld, 2006).

Nevertheless, the primary cause of elevated levels of these cytokines is poor compliance in patients treated with deferoxamine. This is because patients who are well-compliant with deferoxamine don't have a significant difference in serum IL-6 levels compared to individuals who are not treated with deferoxamine. Therefore, the advantages of excellent compliance with deferoxamine over low compliance were not limited to the reduction of serum ferritin but also to the decrease of an inflammatory marker that was elevated in patients who had low compliance. This indicates iron chelators are efficient in reducing these markers when patients with thalassemia are well-compliant with their treatment (Hershko et al., 2005).

In a study conducted by Jya Yilmaz et al. (2001) and Hanoon et al. (2018) it was found that the level of interleukin 8 (IL-8) in patients with β -thalassemia major was significantly increased in compared to a control group. Figure 41. shows that the results of our current study, which displayed a significant increase in IL-8 level between the target groups, are in conformity with these results. The results indicated a significant increase in IL8 levels in the group that took deferoxamine compared to the group that took deferiasirox.

Rougier et al. (1998) demonstrated that bone marrow stromal cells may serve as a significant source of cytokines and regulate the local production of IL-8 in human bone marrow. The bone marrow is under compensation stress because of ineffective erythropoiesis, which allows it to enhance its capacity by a factor of several. The increased

activity of bone marrow and stromal cells may affect IL-8 plasma levels. These elevated levels of cytokines can enhance the phagocytic and chemotactic functions of macrophages and neutrophils. The ineffective erythropoiesis results in the accumulation of iron in several parenchymal organs, particularly the liver, heart, and endocrine glands, which leads to iron-mediated damage. This iron is also delivered from transfused RBCs and, to a lesser extent, from increased intestinal absorption of iron and found a substantial correlation between the degree of hepatic siderosis and IL-8 serum levels (Uguccioni et al., 1993). The increased production of IL-8 may be attributed to continuous antigen stimulation and iron overload caused by transfusions, which activate macrophages. This hypothesis is supported by the correlations detected between the total number of transfusions and the ferritin concentrations of the patients and IL-8 levels (Cazzola et al., 1996). We have proven statistically that there is a direct (positive) correlation between increased IL6, IL8 levels and the disorder that occurs in the liver enzymes, increased levels of ferritin, and increased markers that indicate inflammation (WBC, lymphocyte), but proven that there is an inverse (negative) correlation with Hb, and RBC decrease level.

According to a study performed by Khalaf et al. (2022), it was found that the level of toll-like receptor 3 (TLR3) in patients with β -thalassemia major was significantly increased compared to a control group. Figure 42. shows that the results of our current study, which displayed a significant decrease in TLR3 level between the target groups, are in no conformity with these results because Khalaf depended on children for his research. The results indicated a significant decrease in TLR3 levels in the group that took deferasirox compared to the group that took deferoxamine.

The levels of TLR3 can be affected by various factors, such as the duration of the disease and age. In adults the immune system may have been subjected to protracted stress over the years, leading to a decrease in TLR receptor activity. Previous studies have shown that adults with β -thalassemia appear persistent immune responses that differ from those of children (Huang & Wang, 2021). One of the key factors affecting TLR3 response is oxidative stress, which is the result of the accumulation of free iron in the body as a result of frequent blood transfusions. The accumulation of free iron results in the production of free radicals, which cause harm to immune cells, including those that express TLR3. Research has shown that innate immune responses, such as the activation of TLR3, can be reduced by oxidative stress (Smith & Johnson, 2020).

Chronic inflammation and immune suppression appear frequently in patients who require blood transfusions as a result of iron excess and immune system dysfunction. The expression of TLR receptors, such as TLR3, may be affected as an effect of these chronic inflammatory conditions, leading to a decrease in their activity (Kumar & Singh, 2019). Different immune system responses may result from iron chelation therapies, including deferoxamine and deferasirox (Chen & Zhang, 2018).

In the research carried out by Buttari et al. (2020) it was found that the level TLR4 in patients with β TM was significantly decreased compared to a control group. Figure 43. shows that the results of our current study, which displayed a significant decrease in the TLR4 level between the target groups, are in conformity with these results. The results indicated a decrease in TLR4 levels in the group that took deferoxamine relative to the group that took deferasirox but not significant.

In β -thalassemia patients, immune abnormalities, particularly those involving neutrophils, are a significant concern. These cells are essential for the body's response to infections. The bone marrow releases immature neutrophils that function differently from mature ones during severe and persistent inflammation. In patients with more severe disease forms, key immune receptor abnormalities include altered expression of CD45, TLR4, and CD32, in addition to diminished oxidative burst production and increased membrane lipid peroxidation. Hypoxia, inflammation, and iron accumulation contribute to impaired neutrophil function, resulting in an activated neutrophil phenotype. TLR signaling may be influenced by chronic inflammation, which is frequently induced by persistent oxidative stress. The immune response may be regulated by reducing TLR4 in response to increased ROS levels, which reduces the possibility of tissue damage as a result of protracted inflammation (Buttari et al., 2020). We have proven statistically that TLR3 and TLR4 inverse (negatively) affected by increased ferritin levels and markers of inflammation.

In a study published by Lithanatudom et al. (2010), it was found that the levels of CHOP in patients with β -thalassemia major were significantly increased compared to a control group, but GRP78 was significantly decreased compared to a control group. Figure 44. shows that the results of our current study, which displayed a significant increase in CHOP levels between the target groups, are in conformity with these results. The results indicated an increase in CHOP levels in the group that took deferoxamine compared to the group that took deferasirox but not significant. While Figure 45. shows that the results of our current study, which displayed a significant decrease in GRP78 levels between the target

groups, are in conformity with these results. The results indicated a decrease in GRP78 levels in the deferoxamine-treated group comparison to the group that took deferasirox but the difference was not significant.

The important organelle known as ER is responsible for the formation, folding, and transportation of new produced proteins into the ER lumen, to acquire their correct structure. It also acts as dynamic calcium storage that coordinates downstream reactions in response to growth factors, hormones, and other stimuli. ER stress arises when unfolded or misfolded proteins accumulate due to elevated protein folding needs. This stress may be caused by metabolic circumstances, including increased cytokine levels, leading to release of Ca^{+2} from the ER and generation of ROS (Zhang et al., 2008).

Unfolded protein response (UPR) occurs to reduce ER stress. This essential biological process reduces protein synthesis, increases protein breakdown, and regulates molecular chaperones. Nonetheless, if normal ER function is not returned, the UPR finally leads to apoptosis to destroy stressed cells. In BTM, the accumulation of unpaired α chains causes significant ER stress. GRP78 (Glucose-Regulated Protein 78), a key molecular chaperone that maintains ER homeostasis and activates the UPR, is upregulated during normal ER stress to restore cellular balance. However, in β -thalassemia major, prolonged ER stress can exhaust the capacity to upregulate GRP78, leading to reduced adaptive UPR efficiency and an inability to counteract protein misfolding. This dysregulation results in the activation of the PERK pathway, which upregulates CHOP (C/EBP Homologous Protein), a transcription factor that activates promoting apoptotic factors, inhibits anti-apoptotic protein such Bcl-2 to increase apoptosis. Consequently, erythroid precursor cells undergo apoptosis, leading to ineffective erythropoiesis a hallmark of β -thalassemia major (Noulsri et al., 2023).

Moreover, the chronic iron overload associated with transfusion-dependent β -thalassemia exacerbates ER stress by generating ROS, which intensifies CHOP activation and further diminishes GRP78-mediated stress relief (We have proven statistically that there is an inverse (negative) correlation between increased CHOP levels and decreased levels of GRP78). Studies have shown that in thalassemic erythroblasts, unpaired α -globin chains activate UPR signaling by dissociating BiP (a form of GRP78) from ER stress sensors, ultimately leading to apoptosis through caspase-3 activation and GATA-1 cleavage. Modulating intracellular calcium levels in thalassemic erythrocytes may restore UPR activity and reduce apoptosis, highlighting the interconnection between ER stress and calcium signaling (Zhang et al., 2008; Noulsri et al., 2023).

Additionally, ER stress and inflammation are tightly linked. Accumulated unfolded proteins and ER stress can trigger inflammation through ROS production and calcium release, IL-6, TNF- α , and IL-1 β are examples of inflammatory cytokines that could increase ER stress and activate the UPR. The reduction in GRP78 expression in serum-derived erythroblasts from β -thalassemia patients underscores the failure of UPR adaptation, while elevated CHOP levels indicate a transition toward apoptosis (Zhang et al., 2008; Noulis et al., 2023). We have proven statistically that there is a direct (positive) correlation between increased CHOP levels with increased levels of IL6, and IL8 that indicate inflammation, but proven that there is an inverse (negative) correlation between increased IL6 and IL8 with GRP78.

These findings suggest that low GRP78 and high CHOP levels are critical biomarkers of disease severity in β -thalassemia major and may represent promising therapeutic targets. Strategies aimed at enhancing GRP78 expression or inhibiting CHOP activation could mitigate ER stress-induced apoptosis and improve erythropoiesis, offering potential novel therapies for managing this disorder.

5. CONCLUSION

Statistical analysis results indicated that deferiasirox was more effective than deferoxamine in reducing excess iron levels in patients. Additionally, it had a significant impact on reducing inflammation by lowering inflammatory markers such as IL6 and IL8. Deferiasirox also demonstrated a beneficial effect in improving liver enzyme levels (AST, ALT, GGT, and ALP) as well as hormones such as FSH, and LH.

In contrast, deferoxamine was found to negatively impact kidney function and some pituitary hormones, as evidenced by significant changes in creatinine levels and alterations in TSH, prolactin, and growth hormone levels.

Correlation analysis further revealed that elevated liver enzymes and inflammatory markers were associated with increased iron levels in the body, reinforcing the link between iron overload and its detrimental effects on organ function. However, there were no significant variations in vitamin D levels, T3, T4, and testosterone hormone, or markers of endoplasmic reticulum stress (such as GRP78 and CHOP).

Moreover, the findings suggested that the accumulation of unpaired alpha chains within the ER contributes to cellular stress, leading to apoptosis through increased CHOP levels.

Overall, these results indicate that deferiasirox is a safer and more effective treatment for iron overload compared to deferoxamine, offering additional benefits in reducing inflammation and improving organ function.

6. RECOMMENDATION

1. We suggest that genetic studies be conducted by researchers to facilitate the early detection of β -thalassemia.
2. We suggest that researchers conduct more studies on the immunological parameters of patients with thalassemia.
3. Reducing cellular stress resulting from ER dysfunction could contribute to improved red blood cell production and provide new therapeutic strategies for this disease.
4. It is recommended that a study be conducted that evaluates and tracks glandular function in patients who are using the iron chelation medications deferoxamine and deferasirox, regardless of their age.
5. Examine the role of other interleukin and cytokine, including IL11 and IL3, in the development of β -thalassemia.
6. Conduct genetic-level research to investigate the effects of deferasirox and deferoxamine.
7. Perform a study to evaluate the levels of cortisol, estradiol, insulin, and adrenocorticotrophic hormone in individuals who get treated with the iron chelation medications deferoxamine and deferasirox.

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8. APPENDIX

Appendix 1: Form of information for patients with thalassemia

- Sample Number Day Date
- Gender :
 - Age :
 - Blood Type :
 - Thalassemia Type :
 - Date of infection :
 - Date or duration of treatment
 - Type of treatment :
 - 1- Deferosirox. ()
 - 2- Deferoxamin. ()
 - Family history :
 - Occupation or Economic Status :
 - Do you have other diseases:
 - Other notes :

CURRICULUM VITAE

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