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Department of Pharmacology and Toxicology

The Impact of ABCC2 Gene Polymorphism on Deferasirox Induced Hepatic Toxicity in Thalassemia Patients in Kerbala

A thesis

Submitted to the Council of the College of Pharmacy, University of Kerbala, as a Partial Fulfillment of the Requirements for the Master Degree of Science in Pharmacy and Toxicology

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بسم الله الرحمن الرحيم

﴿وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّن الْعِلْمِ إِلاَّ قَلِيلاً﴾

صدق الله العلي العظيم

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Supervisor Certification

We certify that this M.Sc. thesis titled: -

The effect of ABCC2 gene polymorphism on the occurrence of hepatic toxicity in thalassemia patients treated with deferasirox in Iraq

was prepared by (**Zahraa Hussain Abd Ayoub**) under our supervision at the University of Kerbala College of Pharmacy, Department of Pharmacology and Toxicology as a partial fulfillment of the requirements for the Master Degree of Science in Pharmacology and Toxicology

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Dedication

I dedicate this work with special thanks to my best friend and husband Ali Kamil, my daughter Zainab and my parents for being there for me throughout the entire Master program. All of them have been my best cheerleaders.

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Abbreviations	**Full-Text**
AAR	Ast:Alt Ratio
ABCC2	Atp Binding Cassette Subfamily C Member2
ADME	The Absorption, Distribution, Metabolism, And Elimination
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ARMS- PCR	Amplification Refractory Mutation System
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BCRP	Breast Cancer Resistance Protein
BMI	The Body Mass Index
C3	Complement Component 3 Is A Protein Of Immune System
DDH2O	Deionized Water
DFO	Deferoxamine
DFP	Deferiprone
DFX	Deferasirox
EDTA	Ethylene Diamine Tetra Acetic
EMA	European Medicines Agency
FDA	Food And Drug Administration
GH-IGF-1 axis	The growth hormone (GH)–insulin-like growth factor (IGF)-I axis is a key endocrine mechanism regulating linear growth in children.
GIT	Gastrointestinal tract
GSH	Glutathione
GSH	Glutathione
Hb pattern	Hb Pattern (By Cellulose Acetate Electrophoresis Or Hig Performance Liquid Chromatography [Hplc])
HbA2	HbA2, composing of two α chains and two δ chains, is a minor component of the hemoglobin.
HbE	Hemoglobin E (HbE) is an extremely common structural hemoglobin variant
HbF	HbF (α2 γ2), the main haemoglobin component in the foetus
HbH disease	Haemoglobin H Disease is a form of alpha thalassemia

HLA	Human Leukocyte Antigen
HPLC	High Performance Liquid Chromatography
HSCT	Hematopoietique Stem Cell Transplant
IgG	Immunoglobin G
IMM	The Inner Mitochondrial Membrane
MCH	Mean Corpuscular Hb
MCV	Mean Corpuscular Volume
MDR1	is one of the most extensively studied transporters in the ATP binding cassette (ABC) super family of transport proteins.
MRP2	Multidrug Resistance Protein Multidrug Resistance Protein 2
NCBI	National Center for Biotechnology Information
NS	Nonsignificant.
PASA	Pcr Amplification of Specific Alleles
PBS	Phosphate Buffered Saline
Pcr	Polymerase Chain Reaction
RC	Respiratory Chain
S	Significant
SD	Standard Deviation
SNPs	Single Nucleotide Polymorphisms
TBE	Tris-Borate-Edta Buffer
TBIL	Total Serum Bilirubin
TDT	Transfusion-Dependent Thalassemia
TE buffer	Tris Edta Buffer
TFR	Transferrin Receptor
TI	Thalassemia intermedia
TM	Thalassemia major
Tmax	The Time To Reach Maximum Plasma Concentration
UGT	Uridine Diphosphate Glucuronosyltransferase
Vd	Volume of Distribution
βTM	Beta thalassemia major

Abstract

Background: β thalassemia is a hereditary disorder in which genetic mutations influencing the β-globin gene lead to reduced synthesis of functional β-globin protein, which results in an imbalance between α- and β-globin chains and ineffective erythropoiesis. severe anemia requiring life-long blood transfusions. In transfusion-dependent patients, regular transfusion leads to iron overload. the accumulation of iron results in progressive dysfunction of the heart, liver, and endocrine glands. Iron chelation in the transfusion-dependent thalassemia management is essential to prevent organ damage and to improve survival. Deferasirox is a once-daily oral iron chelator for therapy of blood transfusion-related iron overload in patients with thalassemia. Deferasirox is mainly metabolized in the liver by (glucuronidation) and eliminated through hepatobiliary excretion in feces. Deferasirox and metabolites are mostly excreted in bile through multidrug resistance protein 2 (MRP2, also known as ABCC2). Multidrug resistance protein 2 is a unidirectional efflux transporter. multidrug resistance protein 2 localizes to the apical membrane domain of polarized cells such as hepatocytes, renal proximal tubule cells, and intestinal epithelia, where it mediates unidirectional transport of substrates to the luminal side of the organ, therefore acting as an ATP-dependent efflux pump. Multidrug resistance protein 2 appears to have a role in the deferasirox anion's elimination from the liver into the bile. As a result, people who have a genetic variation in the ABCC2 gene may be more likely to have hepatotoxicity. Multidrug resistance protein 2 may reduce the biliary elimination of deferasirox, according to ABCC2 polymorphisms.

Aim of study: the study aims to investigate the effect of ABCC2 gene polymorphism on hepatotoxicity by deferasirox in thalassemia patients.

Patients and methods: Cross sectional study with a total of 200 participants, both male and female, ranging in age from 14 to 61 years old.100 thalassemia patients and 100 healthy control to compare the parameters receiving deferasiox as monotherapy for at least three months were involved in the study. Five ml of blood was drawn from the vein of all subjects by using a disposable syringe and then divided into two parts: The first part (3ml) was placed in a gel tube which was used for the determination of biomarker levels. The remaining blood was saved in an EDTA tube and stored freezing at -40˚C until used for DNA extraction and molecular analysis.The allele specific polymerase chain reaction technique (pcr) was used to detect the (G>A, rs 8187710) and (C>T, rs 717620 single nucleotide polymorphism (SNP).

Results: The genotypes for ABCC2 (G>A, rs 8187710), the percentage of wild genotype (GG) in 100 thalassemia patients was 58%, the heterozygous type (GA) presented with percentage of 32%, and finally the homozygous type (AA) appeared with percentage of 10%. And forABCC2 (C>T, rs 717620), the percentage of wild genotype (CC) in 100 thalassemia patients was 56%, the heterozygous type (CT) presented with percentage of 31%, and finally the homozygous type (TT) appeared with percentage of 13%.The results were indicted that there was highly significant difference found between the measured biomarkers and ABCC2 (G> A, rs 8187710) genotype, (p =<0.001). The results were indicted that there was highly significant difference found between the measured biomarkers and ABCC2 (C>T, rs717620) genotype, (p =<0.001).

Conclusion: According to the results of the currents study, the genetic variation of ABCC2 gene is associated with hepatotoxicity in patients taking deferasirox and the wild group of patients are at risk of hepatotoxicity.

Chapter One

Introduction

1. Introduction:

1.1 Thalassemia

Thalassemia is an inherited disease, meaning that at least one of the parents must be a carrier for the disease. It is caused by either a genetic mutation or a deletion of certain key gene fragments (***Thein, 2018***). It is a heterogeneous group of blood diseases affecting the hemogloin genes, resulting in ineffective erythropoiesis (***Bouva et al., 2006***). Thalassemia can be categorized into:

1.1.1 Alpha thalassemia is caused by alpha-globin gene deletion, which results in decreased or absent production of alpha-globin chains. Alpha globin gene has 4 alleles, and disease severity ranges from mild to severe depending on the number of deletions of the alleles. Four allele deletion is the most severe form in which no alpha globins are generated, and the excess gamma chains (present during the fetal period) form tetramers. It is incompatible with life and results in hydrops fetus. One allele deletion is the mildest form and is mostly clinically silent (***Coelho et al., 2010***).

1.1.2 Beta thalassemia results from point mutations in the beta-globin gene. It is classified into three groups based on the zygosity of the beta-gene mutation. A heterozygous mutation (beta-plus thalassemia) results in beta-thalassemia minor in which beta chains are non productive. It is mild and usually asymptomatic. Beta thalassemia major is caused by a homozygous beta-globin gene mutation (beta-zero thalassemia), resulting in the total absence of beta chains. It manifests clinically as jaundice, growth retardation, hepatosplenomegaly, endocrine abnormalities, and severe anemia requiring life-long blood transfusions. The condition between these two types is called beta-thalassemia intermedia with mild to moderate clinical symptoms (***Makis et al., 2021***).

1.2 Epidemiology of β Thalassemia

β-Thalassemia is widespread in Mediterranean countries, the Middle East, the Indian subcontinent, and South and Southeast Asia; however, emigration has led to a worldwide distribution (***Betts et al., 2020***).

The World Health Organization report suggests that about 60 000 infants are born with major thalassemia, including homozygous beta-thalassemia, E/beta-thalassemia, homozygous alpha 0 thalassemia, and HbH disease, every year (***Modell and Darlison, 2008***) (***Rafi et al., 2021).*** Worldwide, approximately 1.5% of people are β-thalassemia carriers (***Coelho et al., 2010***). Even within small geographic regions, there are considerable variances even though the total number of patients with the disease and those who are carriers is known in the majority of countries (***De Sanctis et al., 2017***).

The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia (***Weatherall et al., 2010***).

According to an Iranian study, the impacted birth rate decreased from 2.53 per 1,000 live births in 1995 to 0.82 per 1,000 live births in 2004 (***Karimi et al., 2007***). Thalassemia major occurred in Oman between 2005 and 2007, with a carrier rate of 2.6% (***Alkindi et al., 2010***). In 1995, the frequency was reported to be 0.4 per 1000 births, which was roughly 10 years earlier (***Rajab et al., 2000***). Thalassemia incidence in Iraq declined from 72.4 per 100 000 live births in 2010 to 34.6 per 100 000 live births in 2015 (***Kadhim et al., 2017***). In Iraq, the prevalence of beta-thalassemia was 36/100,000 In a single population-based analysis (***Jabbar et al.,2023).***

A comprehensive national hemoglobinopathy control program was put into place by law and got to effect on October 24, 2002, in 33 provinces of Turkey. By 2008,

the number of babies with the condition has decreased by 90% in Turkey (***Canatan, 2011***).

Thalassemia major cases have only been documented in a small number of European nations. In Belgium, 1 in 25 000 newborns are affected (***Gulbis et sl., 2010***). According to a French registry study, between 2005 and 2008, the frequency was 1 in 112 881 births (***Thuret et al., 2010***).

The Greek National Registry for Hemoglobinopathies reported a significantly lower incidence of β-thalassemia than expected based on the prevalence of carriers, thereby demonstrating the efficacy of thalassemia prevention programs (***Voskaridou et al., 2012***).

Only roughly 200,000 thalassemia major patients are known to be alive and listed as receiving regular therapy worldwide, according to Thalassemia International Federation. The most widespread kind of HbE/beta thalassemia, where the carrier frequency is around 50%, is beta-thalassemia with unusual Hb or structural Hb variants with thalassemic characteristics (***Cao and Galanello, 2010***).

Thalassemia has a high frequency in the Middle East due to a high carrier rate and a tendency for consanguineous marriages in the region's culture (***Joulaei et al., 2014***).

However, the introduction of preventative initiatives in many different countries in this region has decreased prevalence over the last decades.

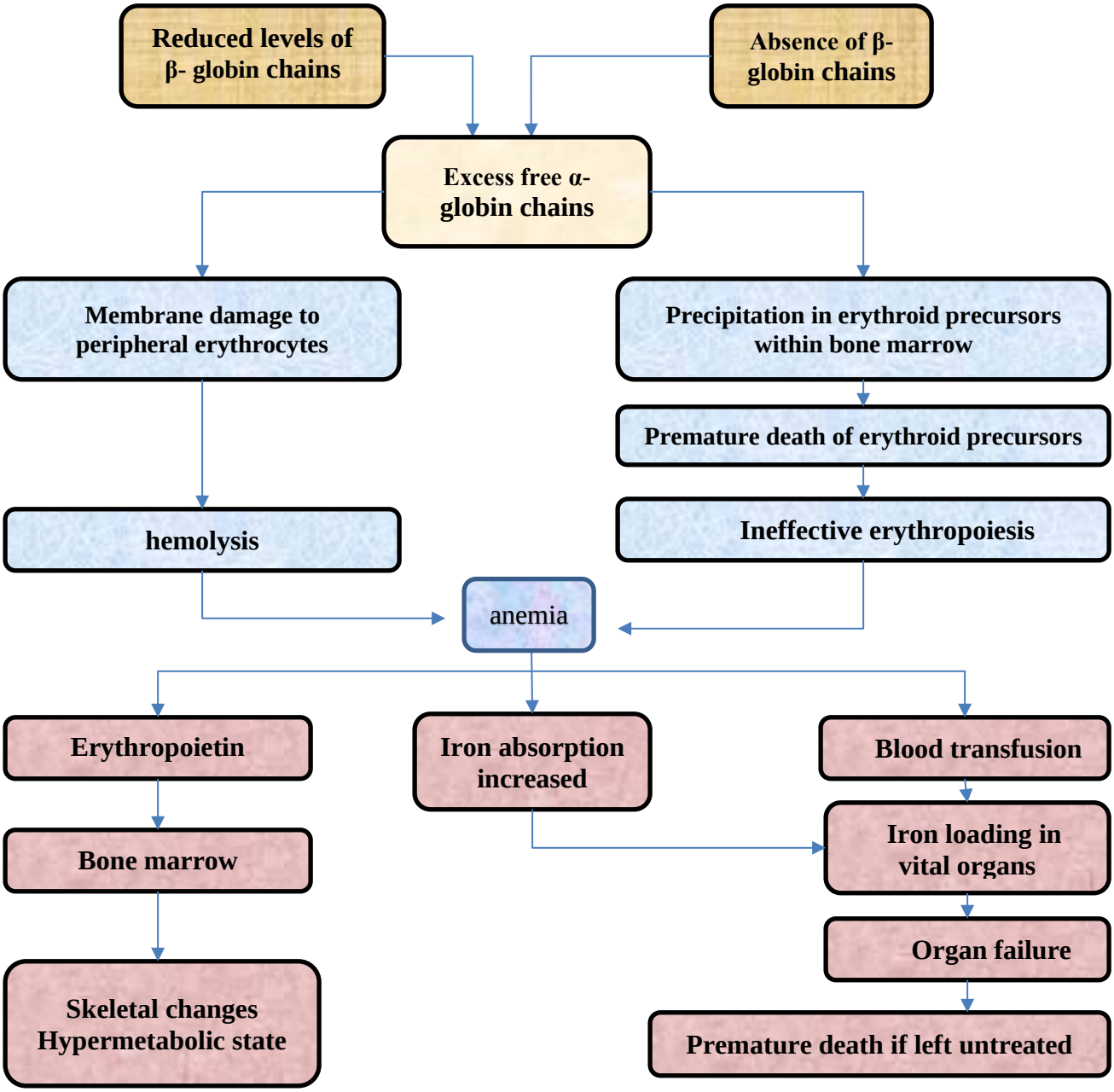
1.3 Pathophysiology of β Thalassemia

The main problem in β thalassemia is a decreased or absent production of β globin chains with a relative excess of α chains, which accumulate and precipitate in the erythroid precursors forming inclusion bodies that bound to the membrane skeleton, causing oxidative membrane damage and extensive premature destruction by apoptosis of the red blood cell precursors in the bone marrow (ineffective erythropoiesis) (***Sankaran and Nathan, 2010***). An increase in the production of erythropoietin is the body's initial response to anemia and ineffective erythropoiesis. This increased production of erythropoietin, in turn, may result in hyperplasia of erythroid marrow in medullary and extramedullary sites with characteristic deformities of the skull and face, cortical thinning and pathological fractures of long bones, extramedullary erythropoietic tissue masses and splenomegaly. Particularly in patients who have undergone splenectomies, the lipid membrane composition of unnatural red blood cells may cause thrombotic problems (***Weiss and dos Santos, 2009***).

Furthermore, patients with thalassemia major who are not or are not adequately treated may have growth retardation as a result of anemia and the increased metabolic burden caused by erythroid enlargement. Anemia can result in severe heart failure and cardiac hypertrophy. The body has a good mechanism for retaining iron but not eliminating It (***Kowdley, 2016***). The mechanism by which iron is absorbed in the gut is tightly controlled. Iron absorption is up and downregulated by a hormone made in the liver called hepcidin. Hepcidin is a small circulating peptide secreted from the liver in response to circulating iron signals (***Wojciechowska, 2021***). Hepcidin, a 25-amino acid peptide generated by hepatocytes that plays a key role in the regulation of iron homeostasis, is downregulated by erythropoiesis, anemia, and hypoxia in non-transfused patients, leading to increased intestinal iron

absorption and iron overload (***Porter et al., 2023***)Receptors on hepatocytes since the amount of iron in circulation and accordingly up or downregulate hepcidin production. Hepcidin binds to the basolateral transporter (ferroportin), following which ferroportin is internalized and then degraded by lysosomes ***(Nemeth and Ganz, 2021).***

Consequently, the iron export out of the gut epithelium is reduced, leading to a secondary reduction in iron import at the level of the gut on the absorptive side. Thus, hepcidin has a negative feedback loop. The liver injury could potentially lead to a reduction in the production of hepcidin from hepatocytes or a reduction in the sensing of iron by the liver. Therefore, less hepcidin is made, and more iron is absorbed. This explains how increased iron buildup could be caused in the liver secondary to liver disease. However, iron can also accumulate in the liver because of liver injury (***Kowdley, 2016***). Once liver cells undergo necrosis, they are scavenged by macrophages in the liver. Thus, part of what might lead to excess iron in the liver could be the absorption or scavenging of dying hepatocytes by Kupffer cells. Once this process occurs and these macrophages become iron-loaded, a secondary process of liver injury and more damage may be precipitated. That excess iron in the liver can occur independent of liver damage or due to liver damage, and that iron buildup in the liver can also be secondary to more advanced liver disease (***Poordad et al., 2016***). The pathophysiology of β thalassemia is summarized in Figure (1.1).



(Figure 1-1): Pathophysiology of β-thalassemia due to the imbalance in chain synthesis (***Porter & Taher, 2023***).

1.4 Clinical Features β Thalassemia

β-Thalassemia is caused by reduced (β+) or absent (β0) synthesis of the β-globin chains of hemoglobin.

β-thalassemia includes three main forms:

(A) β-thalassemia major (TM), also referred to as "Cooley's anemia" and "Mediterranean anemia".

(B) β-thalassemia intermedia (TI).

(C) β-thalassemia minor, called "β-thalassemia carrier," "β-thalassemia trait," or "heterozygous β-thalassemia.

1.4.1 β-Thalassemia Major

Individuals with TM are usually brought to medical attention between ages 6 and 24 months; they subsequently require regular red blood cell (RBC) transfusions to survive. Affected infants fail to thrive and become progressively pale. Feeding problems, diarrhea, irritability, recurrent bouts of fever, and progressive enlargement of the abdomen caused by splenomegaly may occur. The most relevant features of untreated or poorly transfused individuals are growth retardation, pallor, jaundice, brown pigmentation of the skin, poor musculature, genuvalgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes that result from expansion of the bone marrow. These skeletal changes include deformities of the long bones of the legs, typical craniofacial changes, and osteoporosis. Individuals who have not undergone regular transfusions usually die from high-output heart failure. If a regular transfusion program that maintains a minimum hemoglobin (Hb) concentration of 9.0 to 10.5 g/dl is initiated, then ineffective erythropoiesis is inhibited and growth and development tend to be normal up to 10 to 12 years (***Galanello and Origa, 2010***).

1.4.2 β-Thalassemia Intermedia

Individuals with TI present later than TM, have milder anemia, and by definition do not require or only occasionally require transfusions. Sometimes, they are completely asymptomatic until adult life. Clinical features are pallor, mild to moderate jaundice, cholelithiasis, liver and spleen enlargement, moderate to severe bone modifications, leg ulcers, extramedullary masses of hyperplastic erythroid marrow (***Weatherall and Clegg, 2001***) (***Eldor and Rachmilewitz, 2002***) (***Langer and Esrick, 2021***).

1.4.3 β-Thalassemia Trait

Carriers of thalassemia are usually clinically asymptomatic but sometimes have mild anemia (***Origa., 2017***).

1.5 Hematological Features

1.5.1 Thalassemia Major

Patients with thalassemia major have severe microcytic and hypochromic anemia, associated with increased red blood cells and low mean corpuscular volume (MCV) and mean corpuscular Hb (MCH). Anisocytosis, poikilocytosis (cells with spiculated teardrop and elongated shapes), and nucleated red blood cells (i.e., erythroblasts) can also be seen in the peripheral blood smear in addition to microcytosis and hypochromic. The percentage of erythroblasts increases significantly after splenectomy and is correlated with the severity of anaemia. Depending on the kind of beta-thalassemia, the Hb pattern (as determined by cellulose acetate electrophoresis or high-performance liquid chromatography [HPLC]) differs. HbA is nonexistent, HbF is 95-98%, and HbA2 is 2-5% in beta-thalassemia, which is characterized by the absence of beta globin chain production. HbA levels range from 10 to 30%, HbF levels range from 70 to 90%, and HbA2 levels range from 2% to 5% in beta-thalassemia homozygotes with a residual variable beta-globin production or beta0/beta complex

heterozygotes. Typically, a bone marrow test is not required to diagnose those who are impacted. The myeloid/erythroid ratio in the bone marrow has been reversed from the normal 3 or 4 to 0.1 or less, which is mostly attributable to severe erythroid hyperplasia. Beta-thalassemia is characterized by a complete lack of globin beta chains and a notable excess of alpha globin chains relative to gamma globin chains; the alpha/gamma ratio is 2.0. This information was obtained by in vitro synthesis of radioactively tagged globin chains in affected individuals. The clinical manifestations of beta-thalassemia range from severe (thalassemia major) to mild (thalassemia intermedia) depending on the degree of beta-globin chain decrease. The imbalance in the alpha/beta gamma ratio is quite similar to a beta 0-thalassemia major. (***Cao and Galanello , 2010***).

1.5.2 Thalassemia Intermedia

Patients with thalassemia intermedia have moderate anemia and have a clearly heterogeneous hematological picture, with severity ranging from beta-thalassemia carrier condition to thalassemia major.

1.5.3 Beta-Thalassemia Carrier State

Beta-thalassemia carriers are clinically asymptomatic. The characteristic hematological features are microcytosis (reduced red blood cell volume), hypochromic (reduced red blood cell Hb content), increased HbA2 level (the minor component of the adult Hb, which is made up of two alpha and two delta chains [alpha2delta2]), and slightly imbalanced alpha/beta-gamma globin chain synthesis ratio (1.5–2.4), by in vitro synthesis of radioactive-labeled globin chains. The Hb pattern of beta-thalassemia heterozygotes is characterized by 92–95% HbA, 3.8 HbA2, and a variable amount of HbF (0.5– 4%). Examination of the blood smear reveals microcytosis, hypochromia, and marked variations in the size and shape of the red blood cells (***Cao and Galanello , 2010***).

1.6. The Complications of β Thalassemia

Iron overload: In healthy humans, regulating absorption helps maintain iron homeostasis. Only one milligram (mg) is lost per day, mostly as a result of epithelial cells from the intestine, urinary tract, skin, and other mucosal organs. Receiving a unit of packed red cells normally causes the deposition of 200 mg of iron in the tissues during red cell senescence as each milliliter of transfused blood contains around 1 mg of iron. Thalassemia patients who receive blood transfusions will invariably experience notable iron overload due to their lack of excretory mechanisms (***Ganz and Nemeth, 2012***).

Iron overload, sometimes referred to as hemochromatosis, is defined by inappropriate iron accumulation in the functional components of an organ, which leads to organ failure and damage (***Aronow, 2018***).

The most significant β thalassemia consequence is tissue iron excess. After about a year of transfusions, iron starts to accumulate in parenchymal tissues (***Ricchi., 2024***) which may be significantly more dangerous than reticuloendothelial cells. As iron loading increases, serum transferrin, the primary iron transport protein, may be unable to bind and detoxify all of the iron, and a portion of the plasma iron that is not bound to transferrin may encourage the production of free hydroxyl radicals, which are the principal cause of oxygen-related cellular damage. (***Ginzburg and Rivella, 2011***).

These and other oxygen-derived species produced by iron have toxic characteristics that may cause extensive tissue damage, which have been demonstrated by developments in free-radical chemistry.

Superoxide dismutase, catalase, and glutathione peroxidase act as part of the body's antioxidant defences against free radical damage, but in patients with high iron loads, these defences might not be enough to stop oxidative damage. Lack of chelating

therapy causes the heart, liver, and endocrine glands to gradually become dysfunctional as a result of iron build up. Changes related to chronic anemia in the heart are typically seen in patients not receiving transfusions and are exacerbated by iron deposition (***Kim et al., 2011***).

Extensive iron deposits are associated with cardiac hypertrophy and dilatation, degeneration of myocardial fibers, and in rare cases fibrosis. In patients who are receiving transfusions but not chelating therapy, symptomatic cardiac disease has been reported within 10 years after the start of transfusions and may be aggravated by myocarditis and pulmonary hypertension. Iron-induced liver disease is a common cause of death in older patients and is often aggravated by infection with hepatitis C virus. Within two years after the start of transfusions, collagen formation and portal fibrosis have been reported; in the absence of chelating therapy, cirrhosis may develop in the first decade of life. Iron loading within the anterior pituitary is the primary cause of disturbed sexual maturation, early secondary amenorrhea occurs in approximately one quarter of female patients over the age of 15 years. diabetes mellitus is observed. As the iron burden increases and iron-related liver dysfunction progresses, hyperinsulinemia occurs as a result of reduced extraction of insulin by the liver, leading to exhaustion of beta cells and reduced circulating insulin concentrations. Reduced serum concentrations of trypsin and lipase suggest that the exocrine pancreas is also damaged by iron loading. Over the long term, iron deposition also damages the thyroid, parathyroid, and adrenal glands. bone density is markedly reduced in patients with b-thalassemia, particularly those with hypogonadism. Osteopenia may be related to marrow expansion, even in patients who receive transfusions, or to iron-induced osteoblast dysfunction, diabetes, hypoparathyroidism, or hypogonadism (***Yousaf et al., 2023***).

1.7 Therapeutic Options for Thalassemia

People who have thalassemia might be deliberate genetic counseling because mutant genes can pass to their offspring (***Mutar et al., 2019***).

Patients with severe beta-thalassemia need to receive treatment for all of their lives in order to prevent and treat the signs and complications of the condition (***Persons, 2010***) .Current treatment options for thalassemia relies on blood transfusions TDT includes Blood Transfusion, Iron Chelation, and Splenectomy, as presented in Figure (1.2). On the other hand, hematopoietic stem cell transplant (HSCT) is an option for a subgroup of individuals (***Kababi et al., 2020***).

1.7.1 Iron Chelation Therapy

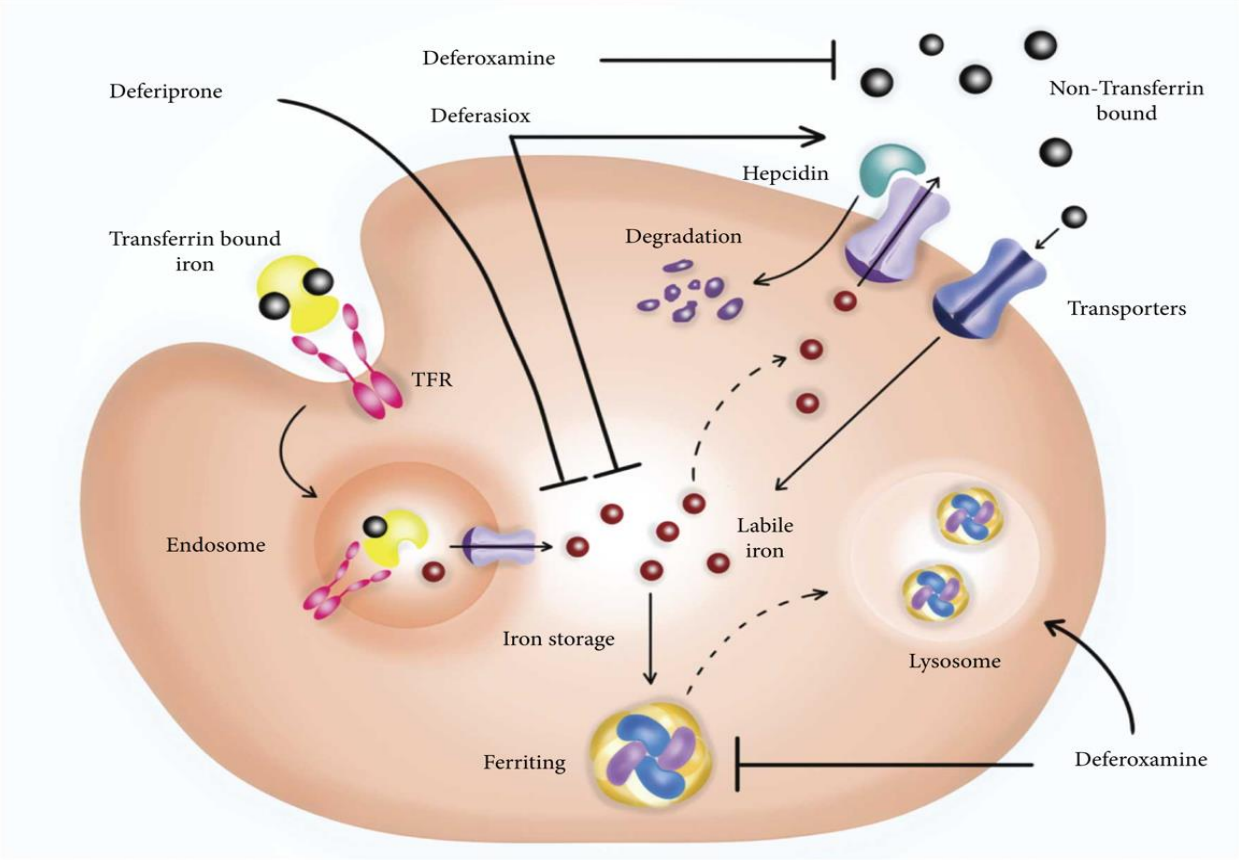
Since the early 1980s, iron-chelation treatment has been used successfully to treat iron overload in thalassemia and other related disorders (***Kontoghiorghe and Kontoghiorghes, 2016***).

The primary goals of chelation therapy are to treat and prevent iron excess. Chelation can reduce the excess iron burden and maintain normal iron levels. Iron chelators can be used to treat patients to reduce the harmful effects of iron overload. Iron chelators enter cells, bind free iron, and eliminate it from the body. Iron chelation therapy is widely accepted in young individuals with severe chronic anemia, but is dubious for older patients with the same condition brought on by myelodysplastic syndromes. Iron chelator action can also help with certain aspects of haematological illnesses, such as increasing platelet formation, reducing leukemic cell proliferation, and inducing leukemic cell differentiation (***Fibach and Rachmilewitz, 2017***).

For the treatment of iron overload, a variety of iron chelators can be used. Important iron chelators including the DFP deferiprone, DFX deferasirox, and DFO deferoxamine each have advantages and adverse effects (***Mobarra et al., 2016***).

1.7.2 Mechanism of Action

Different mechanisms of action in managing iron overload are reported for deferoxamine, deferiprone, and deferasirox (as shown in Figure 1.3). Deferoxamine forms a compound with ferritin or non-transferrin-bound iron that is later eliminated by the kidneys. Degradation of ferritin in lysosomes is another effect of deferoxamine. Cryosolic labile iron is chelated by deferiprone and deferasirox. Deferasirox may also raise hepcidin levels, which results in the degradation of ferroportin. TFR, transferrin receptor (***Poggiali et al., 2012***).



(Figure 1-2): Mechanism of action in the management of iron overload by the Deferasirox family (*Silva, 2013*).

1.7.3. Deferasirox

Deferasirox is a potent and specific oral N-substituted bis-hydroxyphenyl-triazole tridentate iron (***Barani et al., 2021***) chelator. The FDA approved the medication in 2005, and the European Medicines Agency (EMA) approved it in 2006 as the first-line treatment for iron excess brought on by blood transfusions. In March 2002, the EMA classified deferasirox as an "orphan medicine."

The European Medicines Agency states that deferasirox is recommended for patients the age of ≥2 years who have persistent iron overload brought on by thalassemia major, other forms of thalassemia, or anemia. It was used alone or in combination therapy with deferiprone or deferoxamine, particularly in cases of iron-related cardiac disease. According to the EMA, treatment should only begin if there is evidence of chronic iron overload, which can occur after a packed red blood cell transfusion of more than 100 ml/kg (for example, 20 units for a person weighing 40 kg) or when serum ferritin levels are greater than 1,000 mcg/l (***Pepe et al., 2017***). Deferasirox (DFX) is known to be a successful treatment to reduce the body's iron level and stop future tissue damage. DFX is marked by non-negligible problems and toxicities, despite the medication's tolerability, which may necessitate the temporary cessation of medication administration or additional supportive therapy (***Cappellini et al., 2011***).

1.7.4. Dose of Deferasirox

Most patients can achieve negative iron balance with a daily deferasirox dose of 20 mg/kg (or roughly 1,500 mg for an individual weighing 80 kg) (***Chirnomas et al., 2008***). The FDA and EMA have approved a daily maximum dose of 40 mg/kg. With

a similar safety profile of around 30 mg/kg per day, such large doses are said to be effective (***Díaz-García et al., 2014***).

1.7.5. Side effects of Deferasirox

Phase II and III clinical trials have investigated the deferasirox's safety. Deferasirox was usually well accepted by adults, (***Piga et al., 2006)*** children, and adolescents in the phase II studies (***Galanello et al., 2006***). With β-thalassemia major over a treatment period of 48 weeks. Compared to patients receiving deferoxamine, transient, mild-to-moderate nausea, vomiting, and stomach pain were more common, but they resolved on their own without a dosage termination (***Piga et al., 2006***).

The most common side effects of deferasirox in children and adolescents between the ages of 2 and 17 were mild nausea and a moderate skin rash (***Galanello et al., 2006***). The most frequent side effects associated with deferasirox therapy in the pivotal phase III trial, which involved 296 patients with b-thalassemia major receiving the medication for a year, were gastrointestinal issues (15.2% of patients) and rash (10.8% of patients), neither of which necessitated dose adjustment or treatment discontinuation (***Cappellini, 2008***). The stomach discomfort remained for around 8 days. Serum creatinine levels also showed mild, non-progressive increases in 38% of patients; these increases were typically within the normal range and infrequently went above twice the upper limit of normal. Some patients' liver enzyme levels became higher, and this was correlated with higher serum ferritin levels.

Patients who received low dosages of deferasirox frequently experienced transaminase increases. 2 out of the 296 patients had elevations that were above twice the upper limit of normal (***Porter et al., 2008***).

Regular monitoring to determine iron overload as precisely as possible is necessary in order to develop an efficient iron chelation regimen with deferasirox for each patient and summarized in Table (1.1)

(Table 1-1): Monitoring requirements during iron chelation therapy with Deferasirox (*Cappellini, 2008*).

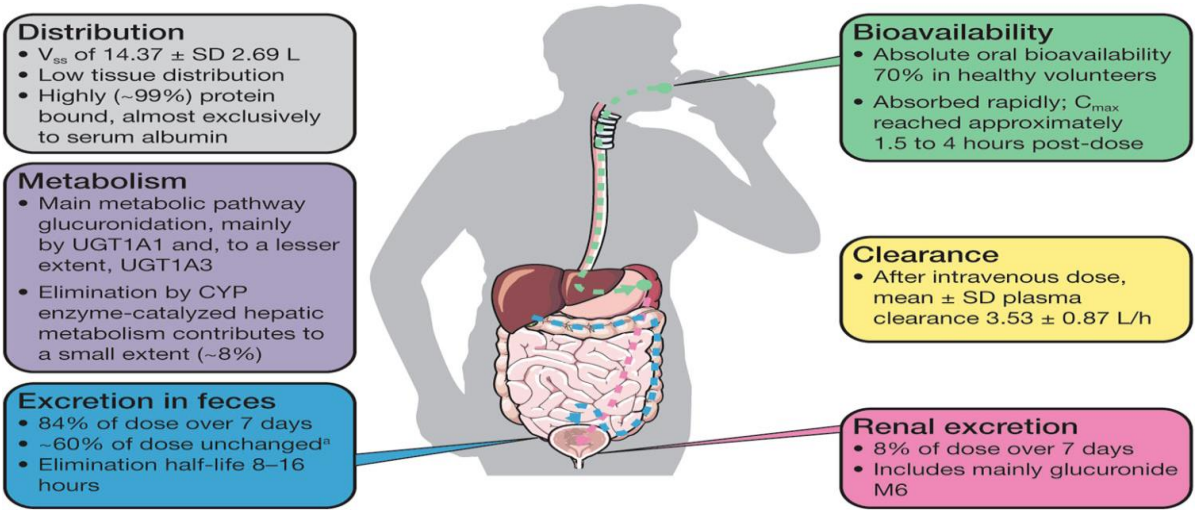
Material to monitor	**Frequency**
Blood intake	Ongoing
Serum ferritin	Monthly, with dose adjustment based on 3 6-month trends
Serum creatinine	In duplicate before initiating Deferasirox treatment; monthly during treatment.
Proteinuria	Monthly
Liver function tests	Monthly
Auditory and ophthalmic testing	Before the start of treatment; annually during treatment
Proteinuria	Monthly
Liver function tests	Monthly
Auditory and ophthalmic testing	Before the start of treatment; annually during treatment

1.7.6 Deferasirox pharmacokinetic:

Deferasirox (DFX), the first once-daily oral iron chelator, is a tridentate ligand with high affinity and specificity for iron. The lipophilic active form is highly bound to protein, above all albumin. The main pathway of DFX metabolism is via glucuronidation by uridine diphosphate glucuronosyltransferase (UGT) to metabolites M3 (acyl glucuronide) and M6 (2-O-glucuronide); 6% of the prodrug is metabolized by cytochrome P450 1A1 and 2D6 enzymes to M1 (5-hydroxy DFX) and M4 (5'-hydroxy DFX), respectively (***Waldmeier et al., 2010***). Only 8% of DFX and its metabolites are eliminated in the urine, while 84% is excreted by bile through multidrug resistance protein, multidrug resistance protein 2 (MRP2, also known as ABCC2), and breast cancer resistance protein (***Bruin et al., 2008***).

With a volume of distribution (Vd) of 14.37 -2.69 l in humans, DFX is quickly absorbed and diffused throughout the body; the time to attain maximum plasma concentration (Tmax) is 1-4 h after administration (***Novartis, 2006***).

Deferasirox's ADME (absorption, distribution, metabolism, and elimination) profile is summarized in Figure (1-3) (***Tanaka, 2014***).



(Figure1-3): Overview of absorption, distribution, metabolism, and elimination of Deferasirox (***Tanaka, 2014***)**.**

1.8: Mechanism of liver Injury in Thalassemia Patients Treated with Deferasirox

Human organ dysfunction is often caused by the toxicity of therapeutic medications (***Hartung, 2009***).The heart, kidney, and liver are examples of aerobic organs where mitochondria are commonly targets of damage (***Vuda et al., 2016***).

It is often tacitly assumed that toxicity occurs due to the inhibition of respiratory chain (RC) function and induction of oxidative stress; however, there may be other ways by which drugs can harm mitochondria (***Lee and Thévenod, 2006***).

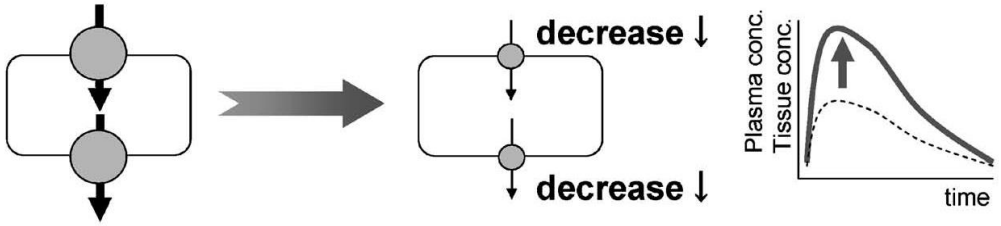
The mechanism of injury accounting for serum enzyme elevations during deferasirox therapy is unknown. The injury may be due to direct, intrinsic toxicity and have a more severe outcome in patients with pre-existing liver disease due to iron overload or concurrent hepatitis B or C. Deferasirox is metabolized primarily by glucuronidation and biliary excretion in the liver (***Bruin et al., 2008***). Polymorphisms of the hepatic genes known to be involved in deferasirox excretion were linked to at least one serious liver injury caused by the drug (***Lee et al., 2013***). The pattern of liver injury was typically hepatocellular or mixed with prominent elevations in serum aminotransferase levels. Immunologic and autoimmune features were absent (***Enna et al., 2008***).

1.9 Effect of Genetic Polymorphisms of ABCC2 gene

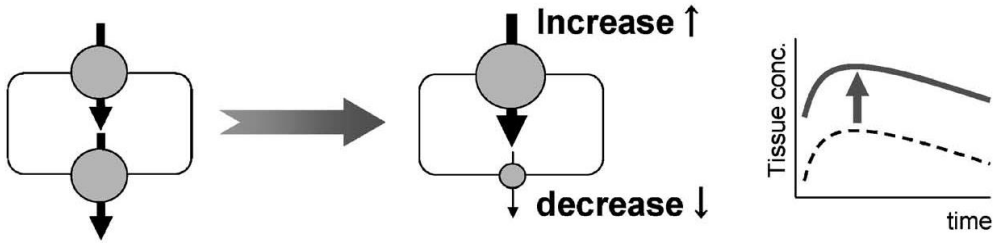
It is well-recognized that different patients respond differently to the same medication. Although many factors influence drug response, including age, organ function, concomitant therapy, drug-drug interactions, and the nature of the disease, it is estimated that genetics can account for 20–95 percent of the variability in drug disposition and effects (***Kalow et al., 1999) (Liu, 2009***). Once a drug is administered, it is absorbed and distributed to its site of action, where it interacts with targets (such as receptors and enzymes), undergoes metabolism, and is excreted. Each process influences the drug response and potentially involves clinically significant genetic variation. The field of pharmacogenetics began with a focus on drug-metabolizing enzymes (***Meyer, 2004***), but it has been extended to membrane transporters that influence drug absorption, distribution, and excretion (***Fischer et al., 2005***).

In many organs, several kinds of drug transporters are expressed on both sides of the plasma membrane, and coordination of the function of uptake and efflux transporters determines the efficient transcellular transport of drugs. For example, the transport of organic anions in human biliary excretion from hepatocytes is supported by several ABC transporters (MDR1, MRP2, BCRP, etc.), which results in the efficient vectorial transport of drugs from the blood circulation to the bile (***Giacomini and Sugiyama, 2005***). The accumulated evidence has revealed that drug transporters dominate the pharmacokinetics of substrate drugs; several examples demonstrate that functional changes in some transporters sometimes affect the pharmacological and toxicological effects and pharmacokinetics. Generally speaking, the cases in which change in the transport activity alters the pharmacological effect are divided into two categories as follows (Figure. 1-4);

① Decreased transport activity of transporters in clearance organs (liver, kidney) → the increase in the plasma and tissue concentration



② Increased uptake activity or decreased efflux activity of transporters in pharmacological target organs (brain etc.)
→ the increase in the target concentration



(Figure 1-4): The effect of a functional change in the transporter activity on the pharmacological and toxicological actions of drugs (*Maeda and Sugiyama, 2008*).

1. When the transport activity of uptake and efflux transporters in major clearance organs (liver, kidney) is decreased, total clearance of drugs is also decreased, and their plasma concentration becomes higher, leading to increased drug exposure to whole organs.
2. When the increase in the transport activity of uptake transporters or the decrease in that of efflux transporters in the pharmacological (toxicological) target organs occurs, target tissue concentration is increased. At the several barriers which protect important organs (brain, testis, etc.) from severe toxic xenobiotics, the reduced activity of efflux transporters increases the exposure of drugs to important organs. It should be noted that a change in the local drug exposure at the target tissues whose distribution volumes are relatively small does not always lead to a change in the plasma concentration of drugs. One

important factor that can alter the transport function of transporters is the genetic polymorphism of drug transporters, and their clinical significance has been gradually disclosed by multiple clinical and in vitro studies (***Maeda and Sugiyama, 2008***).

1.10 MRP2 (Multidrug Resistance-Associated Protein 2)

ABCC2/MRP2 is a unidirectional efflux transporter that transports organic anions, including drug conjugates and conjugated bilirubin. However, expressed in numerous human tissues, particularly in the liver, kidney, gastrointestinal tract (GIT), and placenta. MRP2 localizes to the apical membrane domain of polarized cells such as hepatocytes, renal proximal tubule cells, and intestinal epithelia, where it mediates unidirectional transport of substrates to the luminal side of the organ, therefore acting as an ATP-dependent efflux pump (***Nies and Keppler, 2007***).

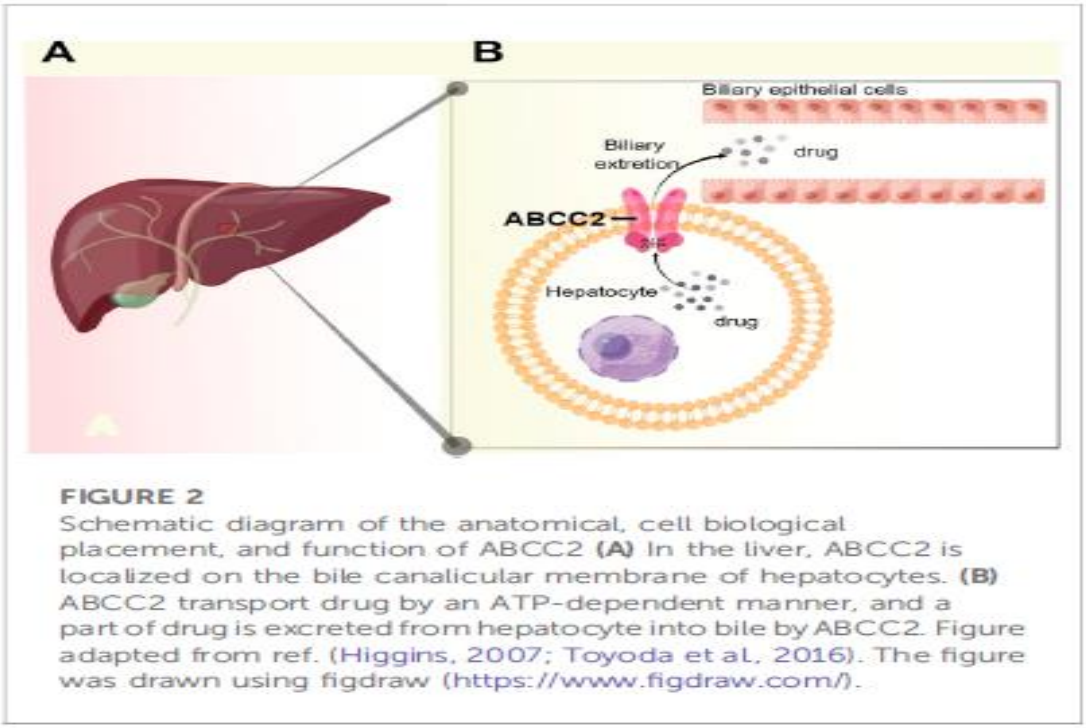


FIGURE 2
Schematic diagram of the anatomical, cell biological placement, and function of ABCC2 **(A)** In the liver, ABCC2 is localized on the bile canalicular membrane of hepatocytes. **(B)** ABCC2 transport drug by an ATP-dependent manner, and a part of drug is excreted from hepatocyte into bile by ABCC2. Figure adapted from ref. (Higgins, 2007; Toyoda et al, 2016). The figure was drawn using figdraw (https://www.figdraw.com/).

(Figure 1-5): ABCC2 location and function (*Chen et al., 2022*).

The localization of MRP2 (figure 1-5) supports a function in the terminal excretion and detoxification of endogenous and xenobiotic organic anions, particularly conjugates of glutathione (GSH), glucuronate or sulfate, as well as a contribution to resistance towards anticancer drugs targeting solid malignant tumors (***Jemnitz et al., 2010***).

MRP2 transports conjugated endogenous and xenobiotic substances, including hormones, toxins, and carcinogens, into the bile, urine, and intestinal lumen (***Herédi-Szabó et al., 2009***). The hepatic MRP2 pump contributes to the driving forces of bile flow and is the major transporter responsible for the biliary excretion of bilirubin glucuronides (***Järvinen et al., 2020***).

Genetic polymorphism is the variation in the sequence of DNA among populations or individuals. These may involve single nucleotide polymorphisms (SNPs), insertion, deletion, sequence repeats. A single-nucleotide polymorphism (SNP) is a variation in the sequence of DNA when a single nucleotide (adenine (A), guanine (G), thymine (T) or cytosine (C)) differs between members of the same species. In human population genetics, it has been noted that the prevalence of certain SNPs can differ substantially between different ethnicities. SNPs can be inherited or occur de novo. SNPs are the most frequent form of genetic variations among individuals, and present at a specific nucleotide site (***Ismail and Essawi, 2012***). an SNP may occur in intronic, non-coding regions or exonic, coding regions, where the change may be synonymous (no difference in amino acid sequence) or non-synonymous (alters the amino acid sequence) (***Hunt et al., 2009***). A non-synonymous SNP may lead to protein truncation (nonsense mutation) or affect folding or biophysical properties (missense mutations) and therefore may have important functional consequences (***Cregg et al.,2013).***

Several SNPs have also been reported for ABCC2, some affecting transporter expression and function. Among those SNPs, namely -24C>T (rs717620), and

4544G>A (rs8187710), located in 5'-UTR, and exon 32, respectively, have been studied extensively mainly because of their high allele frequency in humans (***Haenisch et al., 2007***)

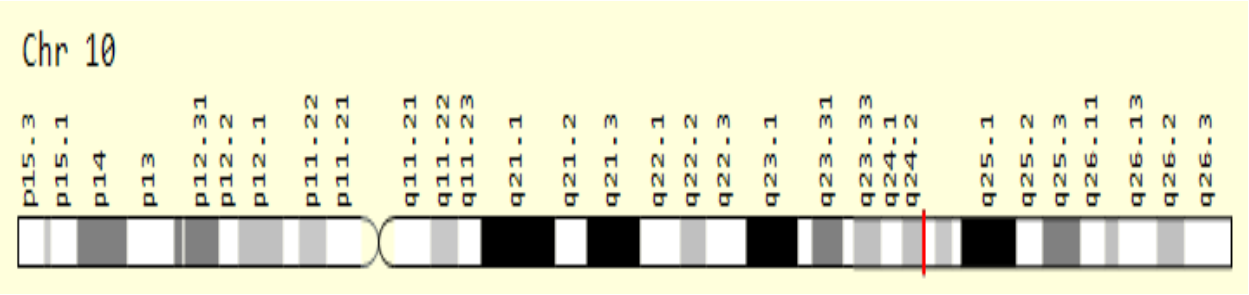


Figure 1. 6: ABCC2 gene located in chromosome 10 which encodes MRP2(*Hulot et al., 2005*).

MRP2 appears to have a role in the deferasirox anion's elimination from the liver into the bile. As a result, people who have a genetic variation in the ABCC2 gene may be more likely to have hepatotoxicity. MRP2 may reduce deferasirox the of biliary elimination, according to ABCC2 polymorphisms (***Hartung, 2009***).

1.11 Aim of the study: The aim of this study is to investigate the effect of ABCC2 gene polymorphism (G>A, rs 8187710) and (C>T, rs 717620) on hepatotoxicity by deferasirox in thalassemia patients.

Chapter Two

Patients, Materials and Methods

2. Patients, Materials, and Methods

2.1 Patients

The present work includes a cross-sectional study of 100 patients suffering from iron overload and thalassemia out of 650 patients in the thalassemia center and 100 control participants used to compare the parameters of liver function tests of thalassemia patients with control group. Thalassemia patients took deferasirox as monotherapy at least three months, medical history, physical examinations, and complete investigations were recorded on a standardized proform by respective ward physicians' patients during their visit to Kerbala Teaching Hospital of Children (Thalassemia Center). This study was performed in the period from October 2022 to June 2023. All patients were diagnosed by a consultant pediatrician. The patients age ranged from 14 to 56 years. Available clinical data were gender, age, weight and height,Ferritin,ALP,TBIL,ALT,AST.

2.1.1 Patients Criteria

2.1.1.1 Inclusion Criteria

- ❖ 100 patients suffered iron overload and thalassemia.
- ❖ All were treated with deferasirox as monotherapy for at least three months.
- ❖ The patient's age ranged from 14 to 56 years.
- ❖ Th serum ferritin level not more than 1500 ng/ml.

2.1.1.2 Exclusion Criteria

- Patient with liver disease (hepatitis, liver cirrhosis, SLE related hepatitis, liver cancer, fatty liver and hepatitis A, B, C)
- Age more than 56yr and less than 14yr.
- Duration of treatment less than three months.

2.1.2 Ethical and Scientific Approval

The Scientific and Ethical Committee of Pharmacy College/Kerbala University approved the study's protocol, and each participant signed a consent form after being informed of the nature and purpose of the study.

2.1.3 Blood Sampling

Five ml of blood was drawn from the vein of all subjects by using a disposable syringe and then divided into two parts: The first part (3ml) was placed in a gel tube and left at room temperature for about (30 min) for clotting, samples were put in the centrifuge at 4000 x g to get serum which was used for the determination of biomarker levels. The remaining blood was saved in an EDTA tube and stored freezing at -40˚C until used for DNA extraction and molecular analysis.

2.1.4 Determination of Body Mass Index

- The body mass index (BMI) was estimated by the following equation:

BMI (kg/m^2) = Wight in kg / (Height in meter)^2

For both patients and control groups, weight was classified according to their BMI as shown below **(** ***World Health Organization, 2016)*****.**

a. Underweight < 18.5

b. Normal weight 18.5-24.9

c. Overweight 25.0 – 29.9

d. Obese ≥ 30.0

2.1.5 Determination of Dose for Deferasirox:

The Dose of Deferasirox depends on the level of serum Ferritin

- If S. Ferritin = 3000 ⟶ Dose = 40mg/kg
- If S. Ferritin = 1500 - 3000 ⟶ Dose = 30mg/kg
- If S. Ferritin = 500 - 1500 ⟶ Dose = 20mg/kg

If S. Ferritin is less than 500 ⟶ The drug will be stopped for three months and we repeat the Liver function tests S. Ferritin (***Porter et al., 2023***).

2.2 Materials

2.2.1 Instruments and Equipment and Their Suppliers

All instruments used in this study are listed in Table (2-1) accompanied by their manufacturing company.

(Table 2-1): Instruments and the manufacturing companies.

Equipment	Company	Country
Anticoagulant tube (EDTA tube)	AFMA-Dispo	Japan
Centrifuge	SIGMA	Germany
Centrifuge	Hettich	Germany
Cobas e 411	Roche	Germany
Cobas integra 400 plus	Roche	Germany
Digital camera	Digital camera	Digital camera
Distillation	Distillation	Distillation
DNA extraction tubes 100 µl.	Eppendorf	Germany
Gel documentation system	Techin me	England
Hood	LabTech	Korea
Incubator	Binder	Germany
Micropipettes	SLAMMED	Japan
PCR machine	TECHINE	UK
PCR tubes 50µl.	Hirschmann	Germany
Refrigerator	Concord	Lebanon
Sensitive balance	AND	Taiwan
Syringes	Abo-Dhabi Med. Devices	UAE
Tips	Slammed	Germany
UV- Transilluminator	Syngene	England
Vortex mixer	HumanTwist	Germany
Water bath	LabTech	Korea

2.2.2 Chemicals, Kits, Drug, and Their Suppliers

All chemicals and kits used in this study are listed in table (2-2) accompanied by the producing company.

(Table 2-2): Chemicals and kits and their producing companies.

N0.	Chemicals	Company/country
1.	**Alcohol (Ethanol) 70% and 95%.**	Fluka chemika/ Switzerland
2.	**Ethidium bromide, Agarose, 100 bp DNA, ladder, Loading dye (bromophenol blue)**	Promega/USA
3.	**Tris-Borate-EDTA (TBE) buffer, TE buffer**	Bio Basic/Canada
4.	**Nuclease free water**	Intron/ Korea

Kit	Company/country
DNA extraction kit	Add Prep Genomic DNA Extraction Kit Korea
Pcr master mix 2X Kit	Promega/ USA
Biochemical kit	Roche Germany
primers	Promega/ USA
Ferritin	Roche Germany

DNA extraction kit

1	Proteinase K
2	2ml collection tube

3	Wash Buffer
4	Elution Buffer
5	Lysozyme

Pcr master mix Kit

1	DNA polymerase enzyme (Taq).
2	dNTPs
3	MgCl2
4	Reaction buffer

2.3 Methods

2.3.1. Method of Determining liver Enzymes

Principle:

AST and ALT activity is measured by measuring the change in absorbance as aspartate and alanine transfer their amino group to the appropriate respective α-keto acids. Pyridoxal-5-phosphate is the coenzyme for these reactions.

$$Aspartate + \alpha - Ketoglutarate \leftrightarrow Oxaloacetate + Glutamate$$

$$Oxaloacetate + NADH + H^{+} \leftrightarrow Malate + NAD^{+}$$

$$Alanine + \alpha - \text{Ketoglutarate} \leftrightarrow \text{Pyruvate} + \text{Glutamate}$$

$$Pyruvate + NADH + \text{H}^{+} \leftrightarrow Lactate + \text{NAD}^{+}$$

Change in absorbance is directly proportional to the concentration of ALT and AST (***Bishop et al., 2020)***.

ALP activity is measured by a method devised by Bowers and McComb8 that involves the calculation of activity based on the molar absorptivity of p-nitrophenol. Para-nitrophenylphosphatase, a colorless compound, is hydrolyzed to a yellow-colored p-nitrophenol, and an increase in absorbance can be measured in the following reaction

$$p-Nitrophenylphosphate \leftrightarrow p-Nitrophenol + Phosphate\ ion$$

The increase in absorbance of p-nitrophenol is directly proportional to ALP activity. Samples collected for ALP analysis must be free of hemolysis and must be analyzed soon after collection. Ingestion by the patient of a high-fat meal before specimen collection may also cause falsely increased values due to the intestinal fraction (***Bishop, 2020)***. However, fasting specimens are not recommended because the interference is negligible.

2.3.2 Molecular Analysis

2.3.2.1Extraction of Genomic DNA from Blood Sample

DNA was extracted from blood using a DNA isolation kit (DNA kit (AddPrep Genomic DNA Extraction kit). Genotyping was carried out by allele-specific PCR for two types of SNPs (rs 717620 CT rs 8187710 GA) Gene ABCC2. Primers and a green master mix kit (Promega/ USA) were used, and PCR products were separated on a 1.5% agarose gel.

Principle

This kit of DNA extraction involves using the first step "proteinase K" enzyme and other reagents to lyse cells and degrade protein, promoting the binding of DNA to the silica gel fiber matrix of the spin column. The next step involves removing Contaminants using a Wash Buffer and ultimately purified genomic DNA is eluted

with an elution buffer. The purified DNA is suitable for use in PCR or other enzymatic reactions ***(Dairawan, and Shetty, 2020***).

Procedure:

1. Sample Preparation

Transfer 200 µl of blood to a 1.5 ml microcentrifuge tube with PBS, add 20 µl of Proteinase K then mix by vortex for 10 seconds, incubate at 60ºCfor at least 10 minutes, invert the tube every 3 minutes, and proceed with step 2 Lysis.

2. Lysis

Add 200 µl of GSB Buffer to the sample and mix by vortex for 10 seconds, Incubate at 70ºC for at least 10 minutes to ensure the sample lysate is clear, invert the tube every 3 minutes, and at this time, pre-heat the required Elution Buffer (200 µl per sample) to 70ºC (for step 5 DNA Elution).

3. DNA Binding

Add 200 µl of absolute ethanol to the sample lysate and mix immediately by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Place a GS Column in a 2 ml Collection Tube. Transfer mixture (including any insoluble precipitate) to the GS Column then centrifuge at 14-16,000 x g for 2 minutes. If the mixture did not flow through the GS column membrane after centrifugation, extend the centrifuge time until it does. Discard the 2 ml Collection Tube containing the flow-through then place the GS Column in a new 2 ml Collection Tube.

4. Wash

Add 400 µl of W1 Buffer to the GS Column. Centrifuged at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml

Collection Tube. Add 600 µl of Wash Buffer (make sure ethanol was added) to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube, and centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

5.Elution

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA elution step to increase DNA recovery and the total elution volume to approximately 200µl.In a clean 1.5 ml microcentrifuge tube, transfer the dried GS column. 100 µl of pre-heated elution buffer should be added to the column matrix's center. Allow at least 3 minutes for elution buffer to be completely absorbed. To elute the purified DNA, centrifuge at 14-16,000 x g for 30 seconds.
The DNA was stored at 2-8$_{o}$C.

2.3.2.2Amplification of DNA.

The allele-specific PCR, which is also known as an ARMS- PCR (amplification refractory mutation system) or PASA (PCR amplification of specific alleles), or AS-PCR, was used to detect the SNPs (***Darawi et al., 2013***). Allele-specific PCR reaction protocol was used for SNP detection ABCC2 gene of (rs717620 CT Rs8187710 GA). The ALLEL SPECIFIC -PCR reactions were performed in 25 µl volumes in PCR tubes under sterile conditions, all the volume of the reaction mixture was completed to 2 µl using DDH2O and the master mix which contained optimum concentrations of reaction requirements (MgCl2 , Tag polymerase , dNTP) has been used, table (2.3).

(Table 2-3): PCR mix reaction for detection of ABCC2 gene.

No.	Material	Volume(µl)
1	Master Mix	10
2	Forward	2
3	Reverse	2
4	Template DNA	4
5	DDH2O	7
Total		25µl

2.3.2.3 Primers for PCR

A primer is a short single strand of DNA fragments consisting of bases known as oligonucleotides that have a sequence that is complementary to the target DNA region. Without the use of primers, the amplification process cannot begin on a single DNA molecule. Thus, it should first be annealed to the single strands that result from the denaturation of the double-stranded DNA (***Chaitanya et al., 2013***).

The polymerase chain reaction was performed using specific primer pairs designed for the ABCC2 gene. Based on the national center for biotechnology information (NCBI) database, all gene information and SNPs detail were collected using Genius software designed.

2.3.2.3.1 Preparation of the primers in the following steps: -

Polymerase Chain Reaction (PCR) was performed by using specific primers to amplify ABCC2 gene rs 8187710 and rs 717620. The primers of this study were designed by Asst. Prof. Dr. Hayder Ali Muhammed .using primer-blast software and purchased from Promega/ USA, as a lyophilized product of different picomols concentrations. Lyophilized forward and revers primers were dissolved in specific volumes of nuclease-free water to get a concentration of 100 pmol/ µl (stock solution). To prepare 10pmol/ µl of working solution of each primer, 10µl of each primer (stock solution) was diluted with 90 µl of nuclease-free water. The primers were kept at -20 C until further use. Table (2-4) illustrates the primers used to amplify the gene alleles.

2.3.2.3.2Materials: Lyophilized primers, sterile dH_2O

1. The tube was spun down before opening the cap.
2. Prepare Master Stock, pmoles/µl, the desired amount of sterile dH_2O was added according to the manufacturer to obtain 100 pmoles/µl (Master Stock).
3. The tube was mixed properly to re-suspend the primers equally.
4. The prepared Working Stock, 1.5 pmoles/µl, 1.5 microliters of the master stock were transferred to a 0.5 ml Eppendorf tube that contains 98.5µl of sterile dH_20 to obtain a 1.5 pmoles/µl (Working Stock).
5. The master stock was stored at -40 C^o.

The sequence of primers used for PCR amplification of the ABCC2 gene (rs717620 CT rs 8187710 GA) was illustrated in Table (2-4).

(Table 2-4): Specific primers of ABCC2 gene (rs 717620) and (rs 8187710).

Rs 717620	**C>T polymorphism**	C allele T allele	385 bp
Reverse 1	5-ATTCCTGGACTGCGTCTGGAACG-3		
reverse 2	5-ATTCCTGGACTGCGTCTGGAACA-3		
Forward	5-CCCTCTACTGATGCTGCCCTTTGTG -3		
rs 8187710	**G>A polymorphism**	G allele A allele	550 bp
Forward 1	5-CCTAGACAACGGGAAGATTATAGAGTG-3		
Forward 2	5-CCTAGACAACGGGAAGATTATAGAGTA-3		
reverse	5-GCATCACCATGGATGAATCTCAGATA-3		

2.3.2.4 Polymerase Chain Reaction

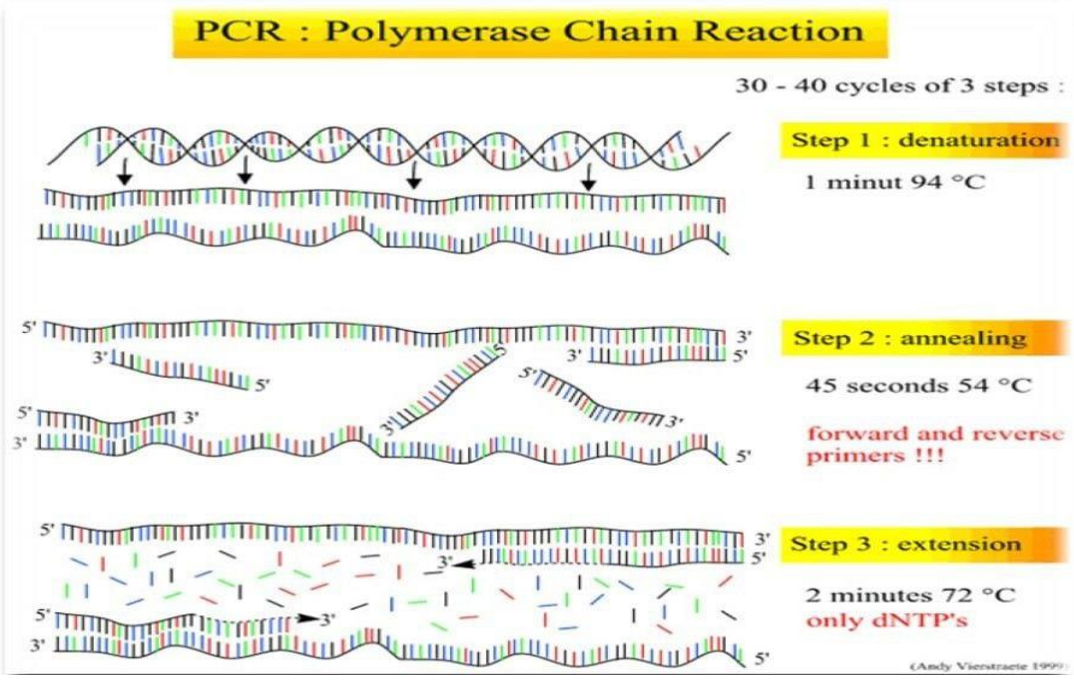
The polymerase chain reaction, also known as PCR, has rapidly emerged as one of the most essential methods in modern biological and medical research (***Ehtisham et al., 2016)***. It amplifies a specific region of a DNA strand to generate thousands to millions of copies of a particular DNA sequence ***(Church, 2006).***

A PCR requires the following:

1. DNA template containing the target DNA region.
2. Two primers to initiate DNA synthesis.
3. A thermostable DNA polymerase to catalyze DNA synthesis.
4. Deoxynucleotide triphosphates (dNTPs, the building blocks of new DNA strands).
5. Buffer including bivalent cations, usually Mg2+.

There are three steps of a PCR that are cycled about 25-35 times these steps including the following:

1. **Denaturation:** This step includes the separation of the double DNA strands into two single strands accomplished by heating for about 94-95°C.
2. **Annealing:** At lower temperature (55-65°C), DNA primers (which are short single-strand DNA fragments) are attached to the ends of each strand of the DNA and initiates the reaction.
3. **Extension:** This step occurs at 72-74°C, where each primer binding to the DNA template will be extended complementary to the template DNA. This process is carried out in the presence of the Tag DNA polymerase, because of its ability to operate efficiently at high temperatures **(Shahzad *et al.,2020).***



(Figure 2-1): Steps of PCR Cycling

2.3.2.4.1 Thermos Cycler Program for DNA Amplification

The PCR reaction program procedures for both SNPs of the ABCC2 gene was presented in table (2-5).

(Table 2-5): Polymerase Chain Reaction (PCR) Program

Type of Cycle	Temperature ^{o}C	Time	No. of Cycle
Initial denaturation	95	5 min	1 cycle
Denaturation	95	45 sec	35 cycle
Annealing	60	55 sec	35 cycle
Extension	72	1 min	35 cycle
Final Extension	72	5 min	1 cycle

2.3.2.5 Agarose Gel Electrophoresis:

Procedure

100 ml of 1.5% agarose solution was prepared according to the following steps**.**

A. Preparation of Solution

1X TBE buffer (tris borate EDTA) was prepared by diluting 10X TBE buffer with deionized water (10 ml of 10X TBE buffer with 90 ml of deionized water: 1:10 dilution).

B. Preparation of Agarose Gel

1. In order to make agarose gel, 1.5 g of agarose powder was dissolved in 100 ml of 1x TBE (Tries Borate EDTA) buffer (pH 8), and after that, the mixture was heated to boiling with a heater until all of the gel particles were dissolved.

2. The solution was stirred on a hot plate until the agarose is dissolved and the solution was clear and then the solution was left to cool.

3. 3 µl of ethidium bromide was added to the solution.

4. Gel chamber ends were closed with a rubber gasket.

5. Combs were pushed in the gel chamber about 1 inch from one end of the tray.

6. A gel solution was poured into the chamber and permitted to be hardened for approximately 30 minutes at room temperature.

7. The combs were removed, and then samples and DNA ladder were loaded (5 µl) on each well with extreme caution to avoid damage to the wells and cross-contamination of neighboring wells.

8. The chamber is placed in a horizontal electrophoresis system and covered with the same TBE buffer that is used to prepare the gel.

9. The cathode (black) was connected to the wells side of the unit and the anode (red) to the other side.

10. Electrophoresis was attached to a direct current power source until dye markers migrated to a suitable distance, according to the size of the DNA fragment that is recognized.

2.3.2.5.1 DNA Electrophoresis

About 5µl of the PCR product was loaded into each well with great precaution to prevent damage to the wells and cross-contamination of neighboring wells. An electric field (50V for 35 min) was established in the system causing the negatively charged nucleic acids to travel across the gel to the positive electrode (anode).

(Figure 2-2): Agarose Gel Electrophoresis

2.3.2.5. 2 DNA Ladder

In the current study, 5µL of DNA ladder (1500 bp, Intron) was used as standard, and the band size ladder was 100- 1500 bp.

2.3.2.5.3 Gel - Band Visualization

To visualize the DNA bands, the agarose gel was placed in the UV trans illuminator device and exposed to UV light and the photos were captured by a digital camera linked to a PC.

2.4 Statistical analysis:

The data of the present study was entered and analyzed through the Statistical Package for the Social Sciences (SPSS version 24). The data were presented as frequencies and percentages or mean and standard deviation in appropriate tables and graphs. Chi square test, ANOVA test, T- test, Hardy–Weinberg Equilibrium and Multiple linear regression were used where is appropriate to find out the possible association between the related variables of the current study. Statistical association was considered significant when p value equal or less than 0.05 (P value ≤ 0.05).

Chapter Three

Results

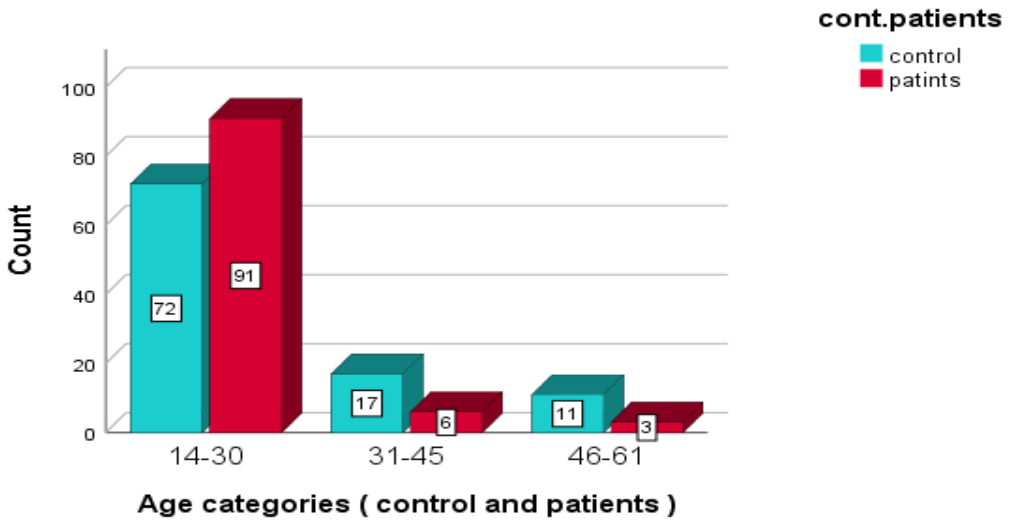
3. Results

3.1. Demographic Characteristics

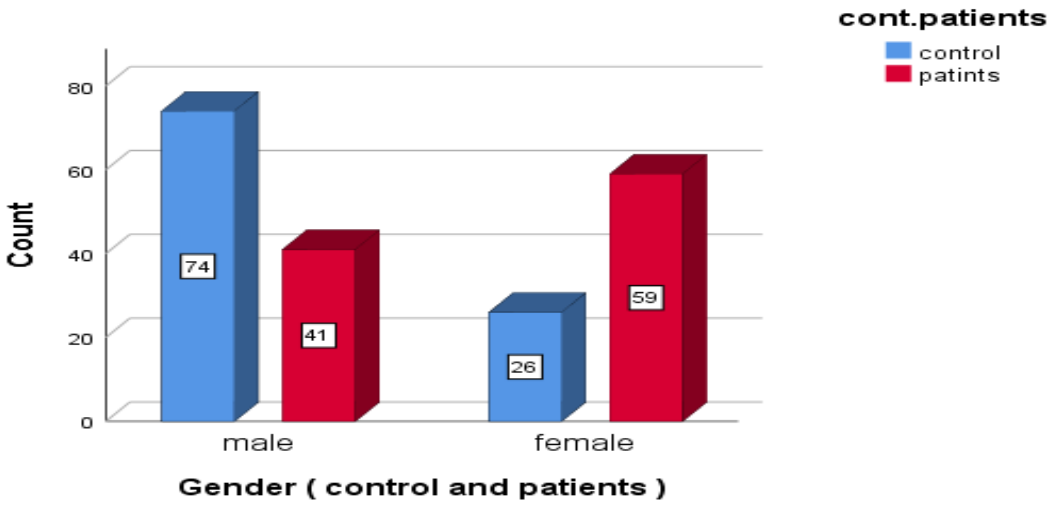
A total of 200 participated were included in this study, which were divided into subgroups based on gender, age, BMI, and duration of deferasirox treatment. The demographic characteristics are summarized in Table (3.1). The gender distribution of the participants were (74) male in control group and (41) male in patients group while (26) female in control group (59) female in patients group. The range of participant´s age was within (14-61) years old, (91) of the patients and (72) of control were within (14- 30) year, while (6) of the patients and(17) of control were within age range (31-45), and (3) of the patients and (11) of control were in age range (46-61) year. The study groups were divided in to subgroups based on the BMI, (33) of the patients and only (2) of control were classified as under weight, (65) of the patients and (43) of control were within normal weight and (2) of patients and (48) of control were over weight. Additionally, the analysis of data was illustrated that mostly patients (79) were having duration of treatment within (111-144) months, (8) of the patients group were having duration of treatment within (74-110) months , (7) of the patients group were having duration of treatment within (37-73) months and (6) of the patients group were having duration of treatment within (2-36) months.

(Table 3-1) :- Descriptive Statistics for Socio-Demographic Data (N 200) .

Variable		Control N=100	Patients N=100
Sex	**Male**	74	41
	Female	26	59
Age (Year)	**14-30**	72	91
	31-45	17	6
	46-61	11	3
BMI Body Mass Index	**Underweight**	2	33
	Normal	43	65
	Overweight	48	2
	Obese	7	0
Duration of Treatment (months)	**2-36**	-	6
	37-73	-	7
	74-110	-	8
	111-144	-	79



(Figure 3-1): Descriptive statistics of socio demographic for participants for the age (n= 200)



(Figure 3-2): Descriptive statistics of socio demographic for participants for the gender (n= 200)

3.2. Difference between continuous variables in the patient and control groups

Table (3.2) shown the analysis of basic thalassemia characteristics. There was a significant difference in the mean levels of Age, Weight, Height, BMI, AST, ALT, ALP and TBIL.

(Table 3-2):- Results of the analysis of basic thalassemia characteristics

Parameter	Control	Patients	P value
	Mean±SD	Mean±SD	
Age	28.360± 11.246	21.460±7.621	0.001
Weight	74.260±10.922	49.200±8.076	0.001
Height	171.120±8.870	157.020±6.619	0.001
BMI	25.399±3.711	19.901±2.624	0.001
ALT	25.850±8.543	35.561±31.966	0.004
AST	23.050±7.441	37.509±30.122	0.001
ALP	80.950±21.579	182.478±104.810	0.001
TBIL	0.338±.204	2.3776±1.41436	0.001

T-test was *: significant at $p \leq 0.05$
aminotransferase (AST), Total serum bilirubin (TBIL) Body Mass Index (BMI) , alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), Total serum bilirubin(TBIL), Standard deviation(SD)

3.3. Association Between Age and Duration of Treatment

(Table 3-3) clarifies that the mean difference of age in relation to the duration of treatment with deferasirox was statistically significant ($p<0.05$).

The age group (14-30) years was highly significant with the duration of treatment.

(Table 3-3):- Association between Age and duration of treatment

		Duration of treatment(months)				
variable		**2-36**	**37-73**	**74-110**	**111-144**	**P value**
Age (years)	14-30*	4	6	7	74	**0.043**
	31-45	2	0	1	3	
	46-61	0	1	0	2	

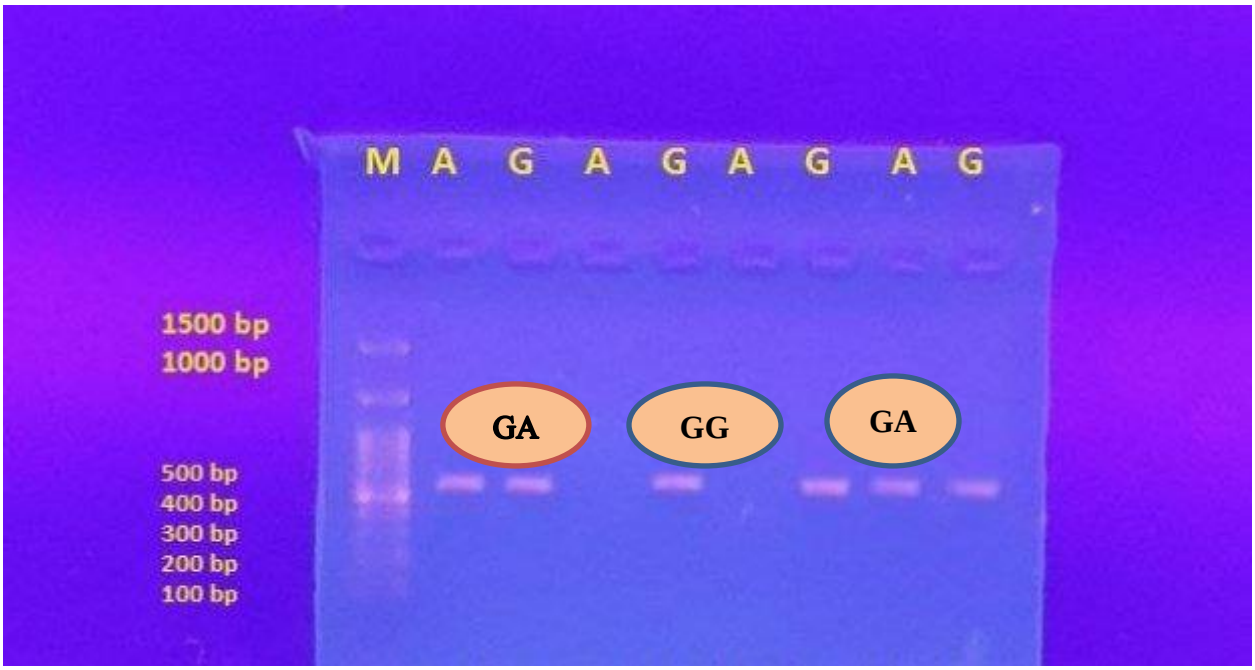
*Chi-square test was used with a significant p value ≤ 0.05.

3.4. Genotyping of ABCC2 (rs 8187710)

The results of genotype rs **8187710** were clear band with a molecular size 400 bps (Figure 3-3) The size of amplicon was determined by making comparsion with DNA ladder 100 - 1000 bp

Genotype of rs 8187710 which was classified into three genotypes:

1. The major genotype group (GG) for the allele G.
2. The homozygous genotype group (AA) for the allele A.
3. Heterozygous (GA).



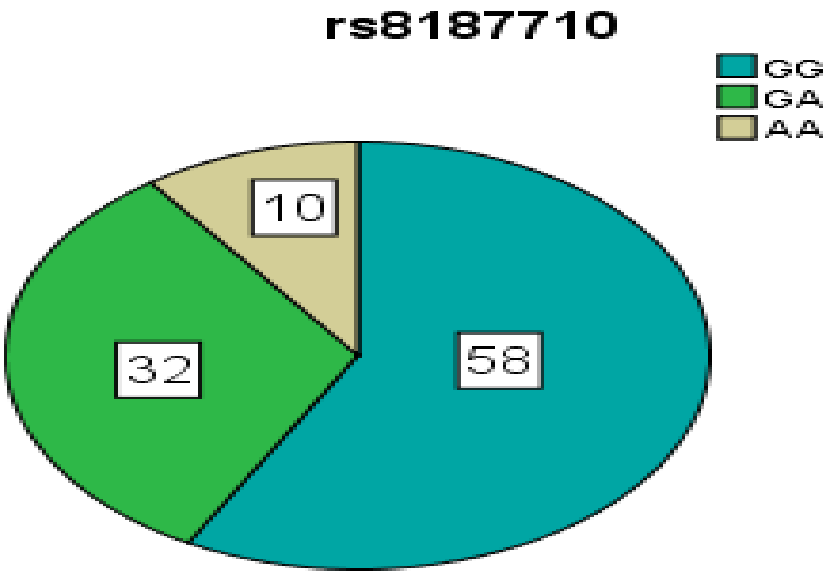
(Figure 3-3): Detection of Gene ABCC2 (G> A, rs 8187710) genetic polymorphism by ARMS.PCR products with three possible genotype (GG, AA, and GA).

Table (3-4) and figure (3-4) summarizes the distribution of genotyping groups of rs 8187710 in patients with thalassemia.

(Table 3-4): Distribution of gene polymorphism for (G> A, rs 8187710) genotype Patients (N.100)

Variable		Frequency	Percent
Genotype (G> A, rs 8187710)	GG wild	58	58%
	GA hetero	32	32%
	AA homo	10	10%

Data Presented by numbers and percentage



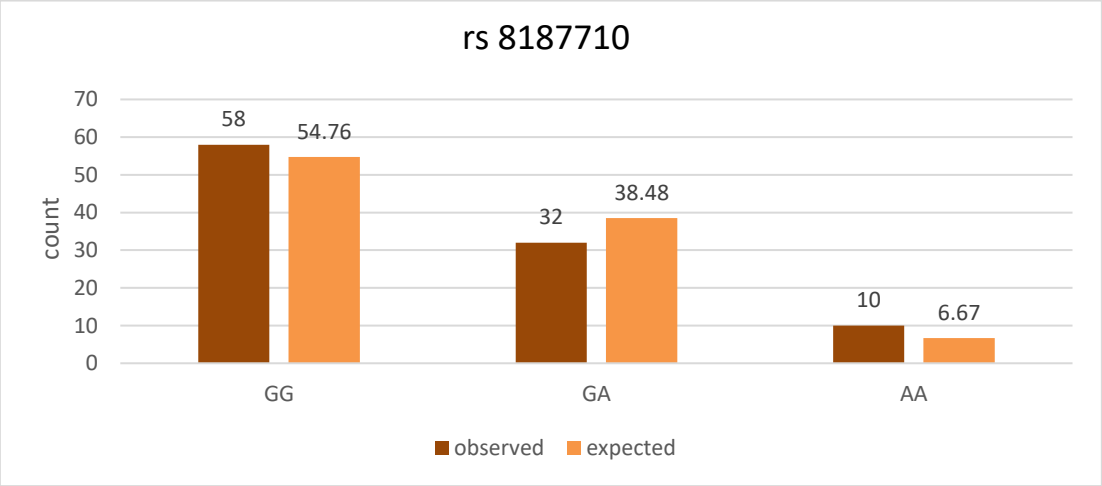
(Figure 3-4): Detection of Gene ABCC2 (G> A, rs 8187710) genotype in Thalassemia patients.

3.5.Hardy–Weinberg Equilibrium for G>A, rs 8187710) in Patients.

The results of comparison between the observed and anticipated values for ABCC2 (G>A, rs 8187710) tested population were shown in figure (3.4). Table (3.5) illustrated the distribution and percentage of individuals having rs 8187710 differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: GG (58, 54.76); AA (10, 6.67); GA (32, 38.48) (goodness-of-fit $\chi 2$ for rs 8187710; 2.836, P<0.0922 (NS)} and therefore it was statistically nonsignificant. The result states that the amount of genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors.

(Table 3-5) : Hardy–Weinberg equilibrium for G>A, rs 8187710) in patients.

Variable			Frequency	Percent	Alleles		Hardy–Weinberg equilibrium X^2 test
rs 8187710	GG wild	Observed expected	58 54.76	58 (%) 54.76 (%)	G	A	
	GA hetero	Observed expected	32 38.48	32 (%) 38.48 (%)	48 (74%)	52 (26%)	P<0.0922 (NS)
	AA homo	Observed expected	10 6.67	10 (%) 6.67 (%)			



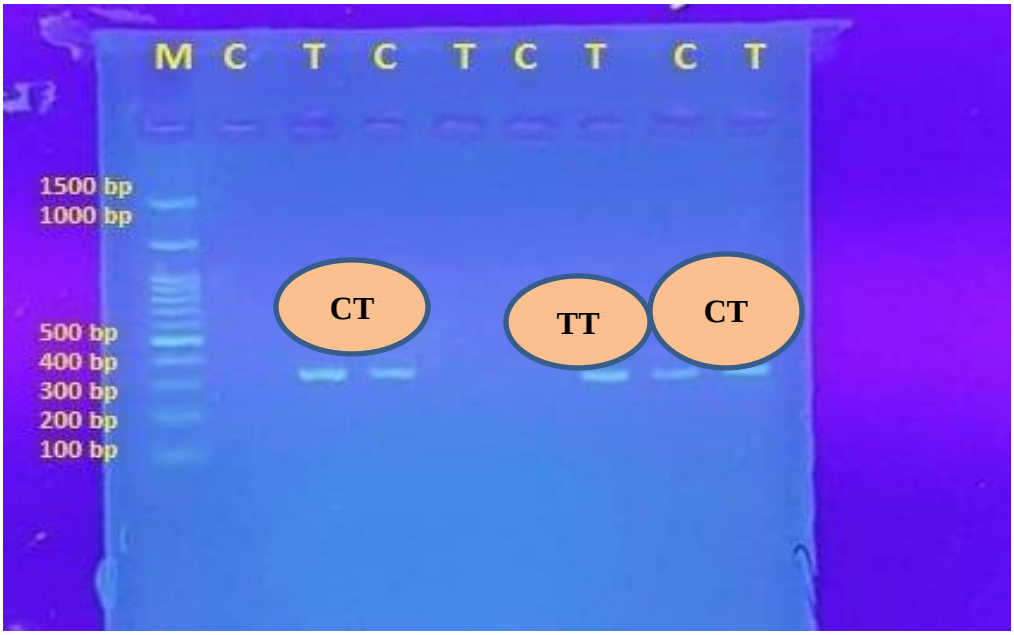
(Figure 3-5): Observed (Obs.) vs expected (Exp.) genotype frequencies % of ABCC2 (G>A, rs 8187710) among individuals' sample

3.6. Genotyping of ABCC2 (C>T, rs 717620).

The results of genotype rs **717620** were a clear band with a molecular size 350 bps (Figure 3.6) The size of amplicon was determined by making comparsion with DNA ladder 100 - 1000 bp

Genotype of rs **717620** which was classified into three genotypes:

1. The major genotype group (CC) for the allele C.
2. The homozygous genotype group (TT) for the allele T.
3. The heterozygous (CT).



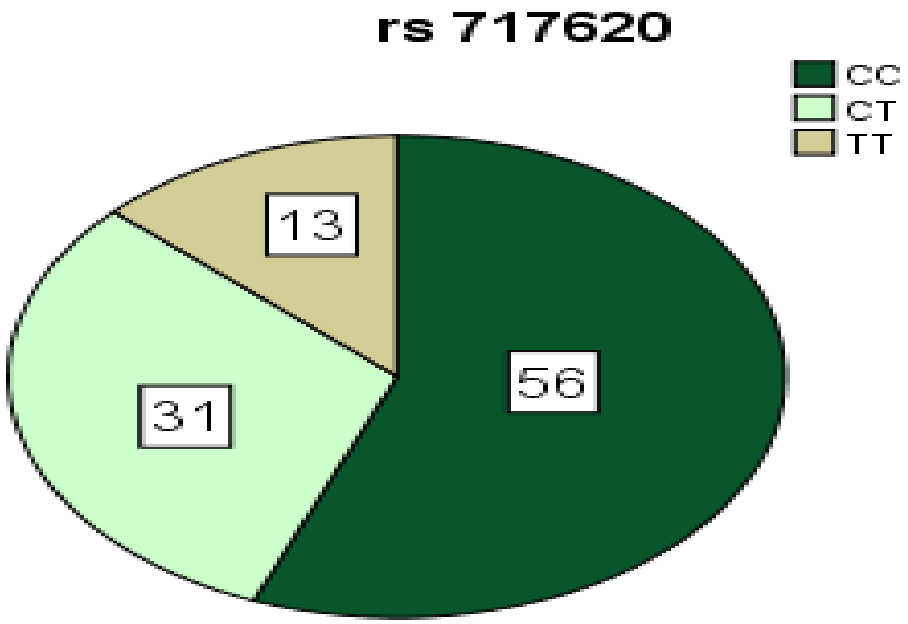
(Figure 3-6): Detection of ABCC2 (C>T, rs 717620) genetic polymorphism by ARMS.PCR products with three possible genotypes (CC, TT, CT).

Table (3.6) and figure (3.7) summarizes the distribution of genotyping groups of rs 717620 in patients with thalassemia.

(Table3-6) : Distribution of ABCC2 (C>T, rs 717620) genotype in thalassemia patients.

Variable		Frequency	Percent
Genotype (C>T, rs 717620)	CC wild	56	56
	CT hetero	31	31
	TT homo	13	13
	Total	100	100.0

Data Presented by numbers and percentage



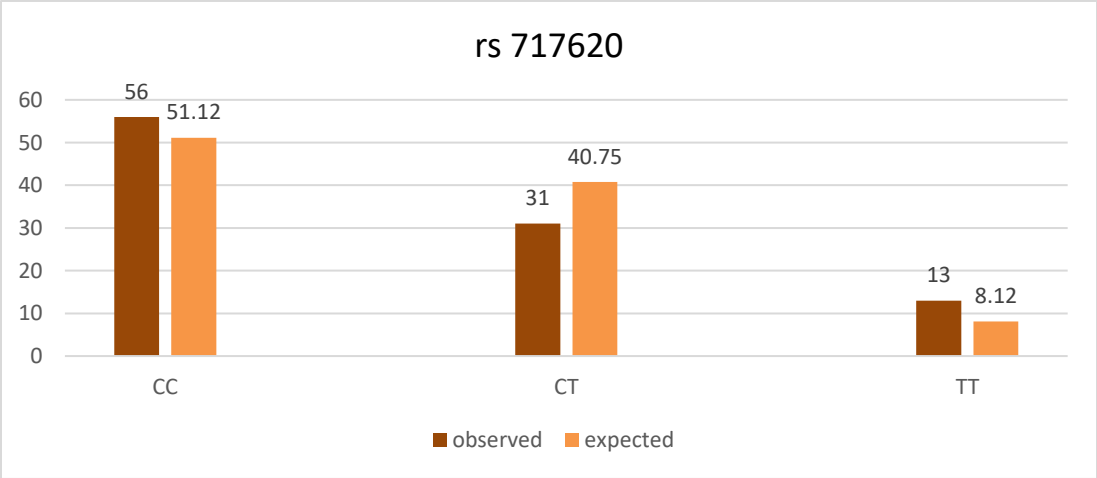
(Figure 3-7): Detection of Gene ABCC2 (C>T, rs 717620) genotype in patients with thalassemia.

3.7. Hardy–Weinberg Equilibrium for (C>T, rs 717620) in Patients.

The results of comparison between the observed and anticipated values for ABCC2 (G>A, rs 717620) the tested population were shown in figure (3.7). Table (3.7) demonstrated the distribution and percentage of individuals having rs **717620** differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: CC (56, 51.12); TT (13, 8.12); CT (31, 40.75) (goodness-of-fit $\chi 2$ for rs 717620 = 6.551, P<0.0167 (S)} and therefore it was statistically significant. The result states that the amount of genetic variation in a population will change from one generation to the next

(Table 3-7) : Hardy–Weinberg equilibrium for (C>T, rs 717620) in patients.

Variable			Frequency	Percent	Alleles		Hardy–Weinberg equilibrium test X^2
rs 717620	**CC wild**	Observed expected	56 51.12	56 (%) 51.12 (%)	C	T	
	CT hetero	Observed expected	31 40.75	31 (%) 40.75 (%)	43 (71.5 %)	57 (28.56%)	**P<0.0167 (S)**
	TT homo	Observed expected	13 8.12	13(%) 8.12 (%)			



(Figure 3-8): Observed (Obs.) vs expected (Exp.) genotype frequencies % of ABCC2 (C>T, rs 717620) among individuals' sample

3.8. Effect of Deferasirox Treatment on Laboratory Biomarkers in Thalassemia Patients having ABCC2 (G> A, rs 8187710) genotype.

Table (3.8) shows the mean levels of biomarkers AST, ALT, ALP, and TBIL based on the genotypes of rs 8187710 SNP groups. Data were analyzed by combining the control, wild, heterozygous and homozygous variant groups. Results are indicted that there was highly significant difference found between the measured biomarkers and ABCC2 (G> A, rs 8187710) genotype, (p =<0.001).

(Table 3-8):- Difference between alleles of (G> A, rs 8187710) genotype mean levels of biomarkers in thalassemia Patients

Parameters	Groups				Pairwise comparison	P value
	control	GG	GA	AA		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
BMI	**25.39 ± 3.71**	**19.89 ± 2.59**	**19.7 ± 2.88**	**20.71 ± 1.83**	Control vs GG	0.001
					Control vs GA	0.001
					Control vs AA	0.001
ALT	**25.85 ± 8.54**	**40.97 ± 37.4**	**30.5 ± 22.38**	**20.47 ± 12.02**	GG vs control	0.021
					GG vs AA	0.011
AST	**23.05 ± 35.41**	**43.11 ± 35.4**	**31.9 ± 19.32**	**22.88 ± 14.11**	GG vs control	0.001
					GG vs AA	0.021
ALP	**80.95 ± 21.57**	**181 ± 99.4**	**197 ± 122.5**	**143.6 ± 64.14**	GG vs control	0.001
					GA vs control	0.001
TBIL	**0.33 ± 0.2**	**2.42 ± 1.65**	**2.41 ± 1.05**	**2 ± 0.71**	GG vs control	0.001
					GA vs control	0.001
					AA vs control	0.001

One way ANOVA test was used with a significant p value ≤ 0.05.. Results are presented as mean ± SD,[S]= Significant, [NS]= Non significant

3.9. Effect of Deferasirox Treatment on Laboratory Biomarkers in Thalassemia Patients having ABCC2 (C> T, rs717620) genotype.

Table (3-9) shows the mean levels of biomarkers AST, ALT, ALP, and TBIL based on the genotypes of **rs717620**SNP groups. Data were analyzed by combining the control, wild, heterozygous and homozygous variant groups. Results were indicted that there was highly significant difference found between the measured biomarkers and ABCC2 **(C> T, rs717620)** genotype., (p =<0.001).

Table (3-9):- Difference between alleles of (C> T, rs717620) genotype with mean levels of biomarkers in thalassemia Patients

Parameters	Groups				Pairwise comparison	P value
	control	CC	CT	TT		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
BMI	25.39 ± 3.71	19.95 ± 2.81	20.23 ± 2.03	18.84 ± 2.92	Control vs CC	0.001
					Control vs CT	0.001
					Control vs TT	0.001
ALT	25.85 ± 8.54	38.12 ± 34.56	29.59 ± 24.56	38.74 ± 36.22	NS comparison	0.065
AST	23.05 ± 7.44	33.81 ± 23.97	42.61 ± 38.27	41.24 ± 32.28	CC vs control	0.01
					CT vs control	0.047
ALP	80.95 ± 21.57	187.07 ± 96.5	175.14 ± 125.7	180.13 ± 90.53	CC vs control	0.001
					CT vs control	0.001
					TT vs control	0.011
TBIL	0.33 ± 0.2	2.19 ± 1.28	2.55 ± 1.58	2.74 ± 1.51	CC vs control	0.001
					CT vs control	0.001
					TT vs control	0.001

One way ANOVA test was used with a significant p value ≤ 0.05. Results are presented as mean ± SD, [S]= Significant, [NS]= Non significant .

3.10. Effect of the Genetic Variation on the Gender

3.10.1. Effect of (G> A, rs 8187710) on the Gender

Table (3-10) shows the number and percent of GG,GA,AA in male and female. The results indicated that there was no difference between male and female with genetic variation. p value (0.080 NS).

Table (3-10) :- Association between gender and genetic variation. (G> A, rs 8187710)

Group		rs 8187710			Total	P value
		GG	GA	AA		
Gender	Male	24	16	1	41	0.080
		58.5%	39.0%	2.4%	100.0%	
	Female	34	16	9	59	
		57.6%	27.1%	15.3%	100.0%	

*Chi-square test was used with a significant p value ≤ 0.05.

3.10.2. Effect of (C> T, rs717620) on the gender

Table (3-11) shows the number and percent of CC, CT, TT in male and female. The results indicated that there was no difference between male and female with genetic variation. p¬ value (0.063 NS).

Table (3-11):- Association between gender and genetic variation of gene(C> T, rs717620) genotype

Group		rs717620			Total	P value
		CC	CT	TT		
Gender	Male	25	8	8	41	0.063
		61.0%	19.5%	19.5%	100.0%	
	Female	31	23	5	59	
		52.5%	31.0%	13.0%	100.0%	

*Chi-square test was used with a significant p value ≤ 0.05.

Table (3-12):-Multiple linear regression for the effect of genetic polymorphism of rs8187710 and rs717620 on the parameters under study.

Dependent variable	Pearson correlation rs8187710	P value	Pearson correlation rs717620	P value	R squared	F value	F significance	Beta value
ALT	-0.218	0.015	-0.045	0.328	0.049	2.482	0.089	rs8187710 (-0.216) rs717620 (-0.034)
AST	-0.235	0.009	0.120	0.117	0.72	3.786	0.026	rs8187710 (-0.241) rs717620 (0.132)
ALP	-.044	0.333	-0.039	0.351	0.003	0.158	0.854	rs8187710 (-0.042) rs717620 (-0.037)
TBIL	-0.067	0.255	0.150	0.068	0.028	1.395	0.253	rs8187710 (-0.074) rs717620 (0.154)

In order to find out the relationship between parameters under the study and genetic variations of rs8187710 and rs717620, a multiple linear regression model was used in which rs8187710 and rs717620 were considered as explanatory variables and the parameters under the study as dependent variables. Pearson correlation is used to brief the relationship between the genetic variation and the parameters. For ALT, Pearson correlation of rs8187710 is -0.218 means the correlation is inversely between ALT and rs8187710 (as the genetic variation decrease (GG). The ALT level will increase). P value is 0.015 significant while Pearson correlation rs717620 is -0.045 weak correlation. P value is 0.328 nonsignificant.

R squared means the effect of both rs8187710 and rs717620 on the parameters. R squared for ALT is 0.049 is weak, for AST, Pearson correlation of rs8187710 is -0.235 which means the correlation is inversely between AST and rs8187710 (as the genetic variation decrease (GG), the AST level will increase). P value is 0.009 significant while Pearson correlation rs717620 is 0.120 means the correlation is directly proportional between AST and rs717620 (as the genetic variation increase, the AST level will increase). P value is 0.117 nonsignificant. R squared for AST is 0.72. F significance is 0.026 significant. for ALP, Pearson correlation of rs8187710 is

-.044 which means the correlation is inversely between ALP and rs8187710 (as the genetic variation decrease (GG), the ALP level will increase). P value is 0.333 nonsignificant while Pearson correlation rs717620 is --0.039weak correlation. P value is 0.351 nonsignificant. R squared for ALP is 0.003is very weak. For TBIL, Pearson correlation of rs8187710 is -0.067 which means the correlation is inversely between TBIL and rs8187710 (as the genetic variation decrease (GG), the TBIL level will increase). P value is 0.255 nonsignificant while Pearson correlation rs717620 is 0.150 means the correlation is directly proportional between TBIL and rs717620 (as the genetic variation increase, the ALP level will increase). R squared for TBIL is 0.028is weak.

Chapter Four

Discussion

4. Discussion

Transfusion therapy for β-thalassemia treatment often leads to hemosiderosis development, as the body is unable to excrete iron excess (The transferrin–transferrin receptor system allows this iron to enter into the cell and to be stored in ferritin or physiologically utilized. The normal transport of iron throughout the circulation is mediated by protein transferrin, which limits its toxic effect (***Akiki et al., 2023)*** .When the transferrin binding capacity is saturated, labile iron is bound to citrate and albumin, and it may load cells through alternative pathways like calcium channels (***Oudit et al., 2006***), with significant tissue damage); chelation treatment occurs when toxic iron levels accumulate. The accumulation of iron in different organs leads to additional clinical complications. Reactive oxygen species produced by the metabolism of non-transferrin-bound iron contribute to cellular

dysfunction, apoptosis, and necrosis (***Bruzzese et al., 2023)***. Chronic hypoxia and uncontrolled iron overload possess serious clinical consequences of morbidity and mortality. Almost every system of the body is damaged in these conditions, most commonly the liver, heart and endocrine glands (***Tanin et al.,2020***). Chelators remove iron by binding its labile form and then by urine and feces elimination.

Deferasirox, a potent and specific iron chelator, has been widely used in clinical practice (***Chirnomas et al., 2009***).

It has much higher patient compliance compared with early chelators such as deferiprone and deferoxamine due to its long half-life and once-daily administration regimen (***Cianciulli et al., 2009***) (***Osborne, 2019***).

Only 8% of DFX and its metabolites are removed in the urine, while the remaining 84% is excreted in the bile via multidrug resistance protein, multidrug

resistance protein 2 (also known as ABCC2), and breast cancer resistance protein ***(Bruin et al., 2008).***

Multidrug resistance protein 2 (MRP2) is a member of the ATP-binding cassette (ABC) transporter family of membrane proteins encoded by the Abcc2 gene and expressed in the hepatocyte's canalicular part. MRP2 acts upon the biliary transport of several endogenous compounds and many drugs, including the iron chelator deferasirox (***Choi et al., 2007***).

The genetic polymorphisms of drug transporters could contribute to the high interindividual variability of deferasirox. Therefore, it is important to understand the critical role of ABCC2gene polymorphisms on the occurrence of hepatic toxicity in thalassemia patients treated with Deferasirox.

The present study is the first that focused on the genetic polymorphism of the ABCC2 gene in Iraqi patients with thalassemia treated with deferasirox.

4.1 Demographic data

The ages of the 100 patients who participated in the study ranged from 14 to 56 years with a mean of 21.46±7.62years. (33) of the patients and only (2) of the control were classified as underweight, (65) of the patients and (43) of the control were within normal weight and (2) of patients and (48) of the control were overweight. Pairwise comparison for BMI (Control>GG,GA,AA) in the first snp (G> A, rs 8187710) and (Control > CC,CT,TT) in the second snp(C>T,, rs717620) respectively .

According to the findings of present study, most thalassemia patients with low BMI. BMI is one of the most preferred methods to assess underweight and obesity. Additionally, as both underweight and obesity are associated with developing many

health problems. Individuals diagnosed with thalassemia major may be susceptible to various growth anomalies due to the illness itself or the adverse effects of their chelating medication (***Hammod et al., 2018)***.

There are numerous possible explanations for that finding, but the existence of multiple endocrinopathies may be the most significant (***Yang, et al.,2020***).

One study showed that around two-thirds (60%) of thalassemia patients are underweight while the remaining individuals are healthy and overweight (***Salih and Al-Mosawy, 2013***)

Similar results were found in another study, which found that patients with thalassemia major had lower BMI and a slower rate of growth. This was linked to poor hemoglobin, elevated ferritin levels, and inadequate iron chelation, as was previously reported (***Sexna., 2003***).

In an Indian study, Kumari V et al. found that 68.9% of children with thalassemic disorders were underweight (***Kumari et al., 2012***). Tanphaichitr et al. observed that 74.5% of thalassemics were underweight (***Sheikh et al., 2017***).

According to Tienboon et al.'s research, 64% of males and 78% of females are underweight (***Yost, 2009.***).

One prominent characteristic of thalassemia major is problems with development. Its pathogenesis is multifactorial. Thalassemia patients have short red blood cell life spans, which leads to ineffective erythropoiesis and accelerated red cell turnover. This means that the body needs more energy and nutrients to maintain normal erythropoiesis, while their average daily energy intake is below recommended levels. Additionally, many individuals have numerous vitamin and

mineral deficiencies, including calcium and zinc, as well as deficits in vitamin A, vitamin E, folate, and vitamin B12 (***Sheikh et al., 2017***).

In this case, it is possible that zinc and copper will be chelated in addition to or instead of iron because of the chemical similarities between the two minerals. Furthermore, there is a negative correlation between liver iron concentration and zinc and vitamin C levels. Hepatic metallothionein concentrations rise with iron overload and bind zinc, causing sequestration. Folate requirements are particularly higher in patients with thalassemia who have hyperactive erythropoiesis, and deficits are frequently described (***Ozdem et al., 2008***) (***Goldberg et al., 2018***).

Growth problems may result from endocrinopathies (hypogonadism, delayed puberty, hypothyroidism), emotional variables, intense use of chelating drugs, and dysregulation of the GH-IGF-1 axis (***Skordis and Kyriakou, 2011***).

The mean difference of the age in relation to duration of treatment with deferasirox was statistically significant ($p<0.05$). The age group (14-30) years was highly significant with the duration of treatment. The number of the patients over 30 years old is small, their life of span is short. There are no chelating agents or thalassemia centers present when they are born. Thalassemia centers first appeared in 1997. In addition, the chelating agent desferral was available in small amount and through international committee of the red cross. Deferasirox arrived the Iraq in 2010yr. For these reasons, the elderly patients suffered from iron overload and organ dysfunction mainly cardiac dysfunction.

4.2 Biochemical Finding

In this study, 100 patients had their AST, ALT, ALP, and TBIL levels tested. Liver enzymes are raised and these indicate that liver injury was noticed in transfusion-dependent β-thalassemia major patients (***Suman et al., 2016***).

In thalassemia, the abnormal liver function appears to be related to the high ferritin levels and the age when transfusions were initiated (***Vogel et al., 2011***).

In thalassemia, the liver is the earliest organ affected by iron, and serum ALT and AST are raised due to peroxidative injury and the direct toxic effect of iron on liver cells (***Suman et al., 2016***).

The results were consistent with others, which indicate that age did not adversely affect liver functions. Soliman et al. (2014) (***Soliman et al., 2014***), reported that the variations in ALT and AST activities in βTM patients under regular treatment with iron chelating agents were not correlated with the age of the patients.

Liver enzyme functions can be influenced by many personal and environmental factors, including age, gender, body mass index (BMI), malnutrition, and extrahepatic diseases such as cardiac, musculoskeletal, or endocrine diseases.

ALT is an enzyme that is found predominantly in hepatocytes and normally in low serum levels. However, during hepatocyte injury, levels of ALT increase substantially and are a sensitive and reliable marker of hepatic inflammation (***Kim et al., 2008***). With advancing age, ALT levels progressively declined while AST levels remained stable, leading to a higher. The AST: ALT ratio. Although higher AAR is often used as a surrogate measure of advanced fibrosis, advancing age can also contribute to increased AAR. (***Goh et al., 2015***). ALT levels were not constant but decreased with increasing age for both men and women, independent of

components of the metabolic syndrome, surrogate markers of adiposity, and other markers of hepatic function (***Dong et al., 2010***). ALT is highly specific for hepatocellular damage, and its elevation could be due to liver damage secondary to iron overload. Our result is consistent with what has been found in different studies, where thalassemic patients showed increased liver enzymes (***Prati et al., 2004***). ALP test measures alkaline phosphatase, which can be elevated in many liver diseases. Forms of this enzyme (isoenzymes) are produced by bone, intestine, and placenta, so other sources of high levels should be considered. Alkaline phosphatase levels are generally high during adolescence due to bone growth and development (***Prati et al., 2004***) (***Lowe et al., 2022***). Serum ALP was measured in a patient aged 15 years and older. It was demonstrated that the concentrations of ALP were very high in early adolescence and decreased to a low point after the completion of bone growth (***Strauch et al., 2023***). Elevated serum ALP activity may be due to a splenomegaly disorder that thalassemic patients have already been diagnosed with by physicians. This study agrees with that of Bayraker et al., who observed an increase in serum ALP activity in patients with splenomegaly only (***Ali et al., 2020***).

4.3. The effect of genetic polymorphism in the ABCC2 gene encodes MRP2 protein on thalassemia patients treated with Deferasirox.

The genetic polymorphisms related to the Abcc2 gene, which encodes the MRP2 protein, may influence individual susceptibility to hepatotoxicity related to Deferasirox. The previous studies show the effect of genetic polymorphism in the ABCC2 gene, which encodes MRP2protein on patients treated with deferasirox, including:

A study in Korea that included 98 patients with hematologic diseases showed that the patients without wildtype alleles carrying two MRP2 haplotypes containing 21774 deals and 224T were at increased risk of hepatotoxicity compared to patients with the wild-type allele (***Lee et al., 2013***).

A study in Brazil in15 patients with sickle cell disease and hemochromatosis receiving deferasirox (DFX) observed that four patients developed hepatotoxicity: One had the rs717620 (-24C>T) polymorphism in heterozygosity; three, however, had no polymorphism in any of their alleles and had hepatotoxicity. Moreover, considering the four patients who presented hepatotoxicity, only one had the homozygous-17774 del G polymorphism, and three of these patients, despite having no polymorphism, still experienced hepatotoxicity (***Braga et al., 2017***).

In another study, thirty-eight healthy Chinese subjects were administered a single dose of 20 mg/ kg. In this study for the ABCC2 c.-24 C>T rs717620; (25) wild, (12) hetero, (1) variant. ABCC2 c.-24 C>T was associated with the pharmacokinetic variability of deferasirox in Chinese subjects, ABCC2 c.-24 C>T (rs717620) was significantly associated with the pharmacokinetics of deferasirox in the human body. Individuals carrying the c.-24 T allele had a 65% higher clearance than non-carriers (***Cao et al., 2020***).

Another study in Italy analysed the case of a 3-year-old girl affected by major thalassemia. She was treated with deferasirox, an oral iron chelator. The girl was admitted to the hospital with elevated liver and renal function tests. The genetic analysis indicates the Functional effect in MRP2 ABCC2 c.-24C>T rs 717620 Decreased activity and c.4544G>A rs 8187710 Impaired ATPase activity. Deferasirox is eliminated into the bile through multidrug resistance protein 2 (MRP2) and breast cancer resistance protein (BCRP) (***Bruin et al., 2008***). Since MRP2 transports the drug, impaired transporter activity leads to intracellular drug accumulation and toxicity (***M. Marano1 et al., 2015***).

In contrast to the present study, the genotype testing determined the frequencies and percentages of ABCC2gene polymorphisms within thalassemia patients of this study as existing in tables (3-4) and (3-6).

In the present study, for ABCC2 (G>A, rs 8187710), the percentage of wild genotype (GG) in 100 thalassemia patients was 58%, the heterozygous type (GA) presented with percentage of 32%, and finally the homozygous type (AA) appeared with percentage of 10%. And forABCC2 (C>T, rs 717620), the percentage of wild genotype (CC) in 100 thalassemia patients was 56%, the heterozygous type (CT) presented with percentage of 31%, and finally the homozygous type (TT) appeared with percentage of 13%.Table (3.8) show the mean levels of biomarkers AST, ALT, ALP, BTIL, s. based on the genotypes of rs 8187710 SNP groups. The results were indicted that there was highly significant difference found between the measured biomarkers and ABCC2 (G> A, rs 8187710) genotype, ($p =<0.001$). The observed results for rs 8187710 and liver biomarker levels showed that the patients who were carrying the (GG) genotype have more effect on ALT and AST. ALP levels are high in the patients who were carrying the (GG) and (GA) genotypes. TBIL levels were affected in patients with (GG), (GA) and (AA)genotypes. Table (3.9) show the mean

levels of biomarkers AST, ALT, ALP, BTIL, s. based on the genotypes of rs 717620 SNP groups. The results were indicted that there was highly significant difference found between the measured biomarkers and ABCC2 (C>T, rs717620) genotype, (p =<0.001). the patients who were carrying the (CC) and (CT) genotypes have more effect on AST than those with (TT) genotype, ALP and TBIL levels were affected in patients with (CC), (CT) and (TT)genotypes. ALT not be affected by genetic variation in this snp. Table (6) brief the effect of both ABCC2 (G> A, rs 8187710) and (C>T, rs717620) genotypes on the parameters. ALT, from Pearson correlation and R squared we observed (as the genetic variation decrease, the ALT level will increase). For AST, ABCC2 (G> A, rs 8187710) affect inversely and (C>T, rs717620) genotype affect proportionally (as the genetic variation increase, the AST level will increase). Both ABCC2 (G> A, rs 8187710) and (C>T, rs717620) genotypes have little effect on ALP and TBIL levels .The parameters are high in the patients who were carrying wild (GG)and(CC) this means those patients at risk of hepatotoxicity .The explanation of this results that show all biomarkers are highly statically significant difference among different groups, which means that the genetic variation can affect on the hepatotoxicity. The genetic polymorphism (C>T, rs717620 is located in 5´-UTR in noncoding region of the gene in chromosome 10, this type of snp can affect on the gene expression, therefore the presence of MRP2can be affected (***Lu et al., 2015***). G> A, rs 8187710 is located in the exon 32 of chromosome 10, this type of genetic polymorphism can change the sequence of amino acid of protein which leads to transporter dysfunction)(***AL-Haggar et al., 2012***) in ABCC2gene that encodes MRP transporters that by which deferasirox is eliminated, polymorphism in this gene decreases MRP activity; as a result, deferasirox will be accumulated in the organ. Toxicity from therapeutic drugs is a major cause of human organ dysfunction (***Hartung, 2009***).

Mitochondria frequently target toxicity in aerobic organs such as the heart, kidney, and liver (***Vuda and Kamath, 2016***).

It is often tacitly assumed that toxicity occurs due to the inhibition of respiratory chain (RC) function and induction of oxidative stress; however, there may be other ways by which drugs can harm mitochondria. At the same time, toxicity can occur in the liver, which has a very high density of mitochondria and depends on aerobic metabolism to generate ATP (***Díaz-García et al., 2014***).

Iron is essential for various aspects of mitochondrial function; for example, iron-Sulphur clusters are co-factors in components of the RC, the citric acid cycle, and anti-oxidant defenses (***Xu et al., 2013***).

Thus, the depletion of mitochondrial iron by DFX might lead to adverse changes in RC activity or redox state, which could explain the observed toxicity (***Díaz-García et al., 2014***).

Moreover, iron chelators can also have beneficial effects on mitochondria; for example, by inhibiting cell death due to ferroptosis (***Dixon et al., 2012***).

Therefore, the reason why DFX alone causes toxicity in organs like the liver has remained a conundrum. that DFX has a direct but subtle effect on the permeability of the IMM, which results in an influx of water into the matrix and partial uncoupling of the RC, but without causing depolarization. As a highly lipophilic drug, DFX most likely partially inserts into the hydrophobic part of the inner mitochondrial membrane without causing sufficient membrane disorder to induce a large generalized increase in permeability to small molecules. Since it contains polar groups (OH, COOH) and is negatively charged at physiological pH, it may favor the enrichment of water, protons, and H3O+ in the vicinity, thus specifically enhancing the movement of protons and water across the inner mitochondrial membrane. Water movement across

the IMM is critical in regulating mitochondrial morphology within living cells and the genesis of pathological swelling (***Gottwald et al., 2020).***

A previous study used the ferritin level as an independent variable for predicting the risk of hepatotoxicity, but there was no association between the ferritin level and the development of hepatotoxicity (***Uchino et al., 2016) (Kowdley et al.,2012)***. Furthermore, all patients with hepatotoxicity showed a decreased AST/ALT and bilirubin after discontinuing deferasirox. These findings suggested that the hepatotoxicity observed in our study was mainly associated with the administration of deferasirox (***Lee et al., 2013***).

4.4 limitations of the Study

The sample size is one of the limitations of this study, but it may be considered a relatively large number compared with other studies dealing with deferasirox's pharmacogenetics. Additional parameters should be measured in the study but these parameters not available in hospital and are expensive in external laboratories.

4.5 Conclusions

Depending on the results, there are some conclusions:

- ABCC2 gene was highly polymorphic and detected with different genotypes and variable frequencies in Iraqi thalassemia patients.
- The observed results for rs 8187710 and liver biomarker levels showed that the patients who were carrying the (GG) genotype had more effect on ALT, AST, and TBIL levels than those with (GA) and (AA) genotypes. The patients carrying the (GA) genotype had more effect on ALP level than those with (GG) and (AA) genotypes.
- The patients carrying the (CT) genotype had more effect on AST than those with (CC) and (TT) genotypes. The patients carrying the (TT) genotype had more effect on ALT and TBIL than those with (CC) and (CT) genotypes. The patients carrying the (CC) genotype had more effect on ALP than those with (CT) and (TT) genotypes.
- The genetic variation in ABCC2gene encodes MRP transporters that by which Deferasirox is eliminated; the polymorphism in this gene decreases MRP activity. As a result, Deferasirox will be accumulated in liver and causes toxicity.

4.6 Recommendations

some recommendations can be given:

- It is recommended to study many SNPs for the ABCC2 gene along with many thalassemia patients.
- It is recommended to study the genetic variations in enzymes involved in the metabolism of Deferasirox might contribute to individual variations in drug response.
- It is recommended that prevention of thalassemia by prenuptial screening through a medical examination and by avoiding the marriage of people in the same family who carry genetic diseases.

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Appendix

وزارة الصحة
دائرة صحة كربلاء
مركز التدريب والتنمية البشرية
لجنة البحوث

استمارة رقم ٢٠٢١/٠٣

رقم القرار ٢٠٠

تاريخ القرار ٢٠٢٢/١١/٢١

قرار لجنة البحوث

درست لجنة البحوث في دائرة صحة كربلاء مشروع البحث ذي الرقم (٢٠٢٢٠٢٠١/كربلاء) المعنون:

(تاثير تعدد الاشكال الجينية على ظهور تسمم الكبد في مرضى الثلاسيميا الذين يستخدمون علاج الديفيرازيروكس)

والمقدم من الباحثين :- **(زهراء حسين عبد ايوب)**

الى شعبة ادارة المعرفة / وحدة ادارة البحوث في مركز التدريب والتنمية البشرية في دائرة صحة كربلاء
بتاريخ ٢٠٢٢/١١/٢٠ وقررت:

قبول مشروع البحث اعلاه كونه مستوفيا للمعايير المعتمدة في وزارة الصحة والخاصة بتنفيذ البحوث ولا مانع من تنفيذه في مؤسسات الدائرة.

الدكتورة تقوى خضر عبد الكريم طبيبة اختصاص

مقرر لجنة البحوث

21/11/2022

وزارة الصحة دائرة صحة كربلاء لجنة البحوث

المرفقات:

Choose an item.

ملاحظات:

- تم تخويل عضو لجنة البحوث (د.تقوى خضر عبد الكريم) او مقرر اللجنة (د.نعيم عبيد طلال) للتوقيع على هذا القرار استنادا الى النظام الداخلي للجنة البحوث.
- الموافقة تعني ان مشروع البحث قد استوفى المعايير الاخلاقية والعلمية لإجراء البحث والمعتمدة في وزارة الصحة. اما التنفيذ فيعتمد على التزام الباحث بتعليمات المؤسسة الصحية التي سينفذ فيها البحث.

University of Kerbala

Consent to be in Research

Study Title: The effect of ABCC2 gene polymorphism on the occurrence of hepatic toxicity in thalassemia patients treated with deferasirox in Iraq

The Researcher Name: Zahraa Hussain

This is a medical research study, and you do not have to take part. The researcher and doctor, Mohammen naji atiyah Consultant pediatrician, will explain this study to you. If you have any questions, you may ask me and/or the doctor.You are being asked to participate in this study because you had been taking daily oral doses of deferasirox as monotherapy for iron overload in thalassemia patients. In this study, the researcher is collecting blood samples from you to learn more about the association of genetic polymorphism of ABCC2gene with hepatotoxicity in thalassemia patients taking deferasirox .If you agree to be in this study, you will go to the laboratory and give a blood sample for one time only. The blood will be drawn by putting a needle into a vein in your arm. One small tube of blood will be taken. This will take about five minutes.

Will my medical information be kept confidential?

We will do our best to protect the information we collect from you and your medical record. Information that identifies you will be kept secure and restricted. If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used. Information that identifies you will be destroyed when this research is complete. You have been given copies of this consent form to keep.

The Consent:

If you wish to be in this study, please sign below.

Name of participant:

Date Participant's Signature for Consent

Date Person Obtaining Consent (Researcher)

Questionnaire for Patients taking Deferasirox therapy Demographic characterization

الاسم: **رقم الهاتف:**

الوزن: **الطول:**

Parameters	variable	Notes
Age		
Gender		
Marital status	Yes No	
BMI		
Education	Primary Secondary Collage	
Family history of liver disease	Yes No	
Other disease	Yes No	
Dose of deferasirox		
Duration of treatment		
Side effects		
Other drugs used		

الخلاصة

المقدمة الثلاسيميا بيتا هو اضطراب وراثي تؤدي فيه الطفرات الجينية التي تؤثر على جين بيتا جلوبين إلى انخفاض تخليق بروتين بيتا جلوبين الوظيفي، مما يؤدي إلى خلل بين سلاسل ألفا وبيتا جلوبين وتكون الكريات الحمر غير فعالة.فقر الدم الحاد يحتاج الى نقل دم مدى الحياه في المرضى المعتمدون على نقل الدم ,نقل الدم المنتظم يؤدي الى زياده الحديد في الجسم . يؤدي تراكم الحديد إلى خلل تدريجي في القلب والكبد والغدد الصماء. تعد عملية إزالة معدن ثقيل من الحديد في إدارة الثلاسيميا المعتمدة على نقل الدم ضرورية لمنع تلف الأعضاء وتحسين البقاء على قيد الحياة. ديفيراسيروكس هو مخلب الحديد يعطى عن طريق الفم مرة واحدة يوميا لعلاج الحديد الزائد المرتبط بنقل الدم لدى المرضى الذين يعانون من مرض الثلاسيميا. يتم استقلاب ديفيراسيروكس بشكل أساسي في الكبد بواسطة (الجلوكورونيدات) ويطرح من خلال إفراز الكبد في البراز . يُفرز ديفيراسيروكس ومستقلباته في الغالب في الصفراء من خلال بروتين المقاومة للأدوية المتعددة 2 (MRP2، المعروف أيضًا باسم ABCC2). لبروتين المقاوم للأدوية المتعددة 2 هو ناقل تدفق أحادي الاتجاه. يتمركز بروتين المقاومة للأدوية المتعددة 2 في مجال الغشاء القمي للخلايا المستقطبة مثل خلايا الكبد وخلايا الأنابيب الكلوية القريبة والظهارة المعوية، حيث يتوسط النقل أحادي الاتجاه للركائز إلى الجانب اللمعي للعضو، وبالتالي يعمل كمضخة تدفق تعتمد على الطاقه . يبدو أن بروتين المقاومة متعدد الأدوية 2 له دور في القضاء على أنيون ديفيراسيروكس من الكبد إلى الصفراء. نتيجة لذلك ، قد يكون الأشخاص الذين لديهم تباين وراثي في جين ABCC2 أكثر عرضة للإصابة بالسمية الكبدية. بروتين المقاومة متعدد الأدوية 2 قد يقلل من التخلص الصفراوي من ديفيراسيروكس ، وفقا لأشكال تعدد الأشكال ABCC2.

الهدف من الدراسة: تهدف الدراسة إلى دراسة تأثير تعدد أشكال الجين ABCC2 على التسمم الكبدي بعقار الديفيراسيروكس في مرضى الثلاسيميا.

المرضى والأساليب: تضمن العمل الحالي دراسة حالة ، 100 مريض يعانون من فرط الحديد والثلاسيميا من أصل 650 مريضاً في مستشفى كربلاء التعليمي للأطفال (مركز الثلاسيميا) و 100 مريض أصحاء. تم علاج مرضى الثلاسيميا باستخدام ديفيراسيروكس كعلاج أحادي لمدة ثلاثة أشهر على الأقل. تراوحت أعمار المرضى من 14 إلى 57 عامًا. تم سحب خمسة مل من الدم من وريد جميع الأشخاص باستخدام حقنة يمكن التخلص منها ومن ثم تقسيمها إلى قسمين: الجزء الأول (3 مل) تم وضعه في أنبوب هلامي يستخدم لتحديد مستويات العلامات الحيوية. تم حفظ الدم المتبقي في أنبوب EDTA وتخزينه متجمدًا عند -40 درجة مئوية حتى يتم استخدامه لاستخراج الحمض النووي والتحليل الجزيئي.تم استخدام تقنية تفاعل البلمرة المتسلسل النوعي للألليل للكشف عن تعدد الأشكال (G> A، rs 8187710) و (C> T، rs 717620 تعدد أشكال النوكليوتيدات المفردة (SNP).

النتائج : الأنماط الجينية لـ ABCC2 (G>A, rs 8187710)، كانت النسبة المئوية للنمط الوراثي البري (GG) في 100 مريض بالثلاسيميا 58%، والنوع المتخالف (GA) بنسبة 32%، وأخيراً النوع المتماثل (AA). ظهرت بنسبة 10%. وبالنسبة لـ(ABCC2 (rs 717620، كانت نسبة النمط الجيني البري (CC) في 100 مريض بالثلاسيميا 56%، والنوع المتغاير (CT) بنسبة 31%، وأخيراً ظهر النوع المتماثل (TT) مع نسبة 13٪. أشارت النتائج إلى وجود فرق كبير للغاية بين التحاليل المقاسة والتغاير الجيني.

الاستنتاجات: وفقًا لنتائج الدراسه الحاليه، يرتبط الاختلاف الوراثي لجين ABCC2 مع تسمم الكبد في المرضى الذين يتناولون العلاج والمجموعة البرية من المرضى معرضة لخطر السمية الكبدية.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة
فرع الأدوية والسموم

تأثير تعدد الأشكال الجيني ABCC2 على حدوث التسمم الكبدي في مرضى الثلاسيميا المعالجين بديفيراسيروكس في العراق

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كجزء من متطلبات نيل درجة الماجستير في علم الادوية والسموم

بواسطة
زهراء حسين عبدايوب
بكالوريوس صيدلة

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