Republic of Iraq Ministry of Higher Education and Scientific Research University of Babylon/College of Medicine Department of Biochemistry

Assessment of NGAL, B2-microglobulin, and Cystatin-C for analysis renal insufficiency in Iraqi patients with Beta-Thalassemia major

A Thesis

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بسم الله الرحمن الرحيم

﴿ قَالُوا سبْحَانكَ لاَ عِلْم لَنَا الاَ مَا عَلمْتَنَا أنكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العظيم

سورة البقرة الآية (32)

Dedication

To my parents for their endless supportand they were always a source of strength during moments of despair.

If the dedication is part of the fulfilment

So the dedication to….

my dear father ….. Icon supreme fatherhood

my dear mother ……My first love

To my dear brothers and sisters

To everyone supports me

Estabraq

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تقيم NGAL
وبيتا 2 مايكروكلوبيولين مع تحليل السيستاتين للقصور الكلوي في مرضى الثلاسيميا في العراق

رسالة
مقدمة إلى عمادة كلية الطب في جامعة بابل
وهي جزء من متطلبات نيل درجة الماجستير
في الكيمياء الحياتية السريرية
من قبل

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Summary

Beta-thalassemia is most prevalent genetic hemoglobinopathy in the world. It is caused by a reduction or absence of beta globin chain production, which is typically a portion of adult hemoglobin (HbA, which is α2β2). A compensatory inefficient erythropoiesis, severe anemia, and accelerated erythrocyte turnover will result from this genetic abnormality. Thalassemia syndrome is frequently accompanied with a wide range of problems resulting from both the disease and the treatments used to treat it. The life of patients with β-thalassemia major depends on blood transfusion. Regular blood transfusion leads to hemosiderosis in their main organs. The aim of the present study is to identify diagnostic, prognostic and predictive parameters, which can be used to diagnose deterioration of kidney function in thalassemia patients in early renal impairment with high sensitivity and specificity, improve prognostication or predict and monitor treatment effectiveness and tolerability for the individual patient through determination of new biomarkers

This study was designed as a case-control study and was constructed to study the renal complications in beta-thalassemia major patients with repeated blood transfusion. To achieve this aim, 45 patients with beta-thalassemia major, and with 45 apparently healthy individuals as control group.

All samples were collected from Al Karama center of hereditary blood disorder from November 2021 to January 2022 and this study was performed at the laboratory of Baghdad International Research Center.

The serum samples were used to measure biochemical parameters, Serum creatinine, blood urea nitrogen, ferritin, CBC, GOT, GPT, neutrophil gelatinase-associated lipocalin, and Cystatin-C were measured from blood samples. Furthermore, urinary B2-microglobulin. Results S. NGAL, and Cystatin-C

parameters in the Patient group were significantly higher than those in the control group ($p < 0.001$), ($p < 0.003$). Urinary B2-microglobulin increased in Patient groups compared to the control group ($p < 0.001$). Ferritin is in the Patient group were significantly higher than those in the control group ($p < 0.001$), CBC are in the Patient group were lower than those in the control group and GOT, GPT are within normal range. The findings of this study Glomerular and Tubular dysfunction among patients with β-thalassemia has been related to iron overload, chronic anemia and indicate that after taking deferasirox and deferoxamine (DFO), there was renal damage and an increase in inflammatory factors. Also, minor renal impairment was observed after deferoxamine administration. Therefore, it seems that patients who are taking these two drugs should be monitored carefully. In this study significant, positive correlation between ferritin and NGAL, β2MG. There is a statistically significant positive correlation between the ferritin level and B.Urea. positive significant correlation between NGAL, β2-MG,CYS-C and S.Cr. In conclusion, renal hemosiderosis and asymptomatic renal dysfunction are prevalent among β-thalassemia major patients with repeated blood transfusion, which are not found in routine renal investigations, which required regular screening with early parameters of glomerular and tubular dysfunction.

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List of Abbreviations

Abbreviation	The Meaning
A	
AUC	Area under curve
B	
BTM	Beta thalassemia major
B2M	Beta2-microglobulin
C	
CYS-C	Cystatin-C
CKD	Chronic renal disease
CBC	Complete blood count
D	
DFO	Desferrioxamine
DFP	Deferiprone
DFX	Deferasirox
E	
ELISA	The enzyme-linked immunosorbent assay
F	
fl	Femtoliter
G	
GFR	Glomerular filtration rate
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
H	
HbA	Adult hemoglobin
HbF	hemoglobin Fetal
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HbA2	Minor Adult hemoglobin
HCV	Hepatitis C virus
HLA	Human leukocyte antigen
HPLC	High pressure liquid chromatography
HRP	Horseradish Peroxidase

HSCT	Hematopoietic stem cell transplantation
HB	Hemoglobin
I	
IE	ineffective erythropoiesis
IL- 18	Interleukin-18
K	
KDa	Kilo Dalton
M	
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
mRNA	Messenger Ribonucleic acid
N	
NGAL	Neutrophil gelatinase–associated lipocalin
NTDT	Non-transferrin dependent thalassemia
O	
OD	Optical density
P	
pg	Pico gram
PCV	Pack cell volum
R	
RDW	red cell distribution width
ROC	Receiver operator characteristic
S	
SD	Standard deviation
SPR	Solid Phase Receptacle
STP	Strip
T	
TDT	Transfusion-dependent thalassemia
V	
VIDAS	Vitek Immuno Diagnostic Assay System
W	
WHO	World Health Organization
WBC	White blood cell
α	Alpha

β	Beta
γ	Gama
δ	Sigma
ζ	Zeta
ε	Epsilon

1-Introduction:-

Thalassemia is a genetic disorder characterized by the complete absence or reduced synthesis of the alpha- or beta-globin chain of hemoglobin. Although thalassemia is usually asymptomatic or associated with only mild anemia, patients with severe disease require lifelong blood transfusions for survival[1].

The word of thalassemia is derived from two Greek words: which means "the sea" and "Haima" which means "blood". According to polypeptide chain defective of hemoglobin in red blood cells, there are two types of thalassemia, alpha thalassemia that is caused by a defect in the rate of synthesis of α chains, and beta thalassemia that is caused by a defect in the rate of synthesis of β chains[2]. Approximately, 1.5% of the global population are heterozygotes (carriers) of the β- thalassemia gene [3]. It occurs in a high frequency in a broad belt and Iran is located on thalassemia belt. In Iran, the thalassemia gene prevalence rate is 4% to 10% in different parts [4], but fortunately the thalassemia prevention program in this country was formulated in 1995 and started to be implemented across Iran in 1997. Hereafter, the prevalence of the thalassemia has beenreduced dramatically [5]

The survival of patients has significantly improved in recent decades; however, complications of this disease in different organs can affect the quality of life among sufferers [6] Consequently, β-thalassemia leads to reduced haemoglobin production and accumulation of α-globins which form insoluble hemi chromes [7].

The clinical and haematological spectrum of β-thalassemia disease ranges from mild to clinically overt conditions including transfusion dependent (TDT) β-thalassemia major (TM) and non-transfusion dependent (NTDT)β-thalassemia intermedia (TI) or thalassemia minor (TMin)[8].

Hypoxia and chronic anemia lead to oxidative stress and lipid peroxidation, resulting in tubular cell function impairment [9]. Moreover, iron overload has an important role in the pathogenesis of kidney injury in thalassemic patients [10]. In addition, iron chelator toxicity can result in glomerular dysfunction. Hepatitis B or C infections may lead to a decrease in the glomerular filtration (GFR); moreover, hepatic and cardiac dysfunction caused by iron overload may result in renal impairment [11].

The deposition of hemosiderin in proximal and distal tubules can lead to interstitial fibrosis, tubular necrosis, and cortical atrophy. Injured tubules release cytotoxines and growth factors that result in tubulo-interstitial fibrosis and glomerular sclerosis [10]

1.1- Hemoglobin:-

Hemoglobin (Hb) is a hemoprotein of the red blood cells that mainly function to carry oxygen from the lungs to the tissues. Other functions include the transport of carbon dioxide (CO2) and a buffering action. The molecular weight of hemoglobin is 64–64.5 kDa. Heme is required for oxygen transport, while globin protects heme from oxidation, makes it soluble, and allows for variation in oxygen affinity[12].

Hb is a protein that allows Red Blood Cells (RBCs) to carry oxygen. The deficiency of Hb lowers the survival rate of RBCs resulting in hemolytic anemia leading to a limited supply of oxygen in the body which can be life-threatening. Two protein chains, α, and β, are required to synthesize Hb. RBCs will not be able to carry oxygen efficiently if either of the aforementioned protein chains is insufficient[13].this causes anemia that begins in early childhood and lasts throughout life, Moderate to severe thalassemia (Hb less than 5 to 6g/dl) [14].

1.1.1- Structure of the hemoglobin:-

Hemoglobin comprises four subunits, each having one polypeptide chain and one heme group. All hemoglobins carry the same prosthetic heme group iron protoporphyrin IX associated with a polypeptide chain of 141 (alpha) and 146 (beta) amino acid residues. The ferrous ion of the heme is linked to the N of a histidine. The porphyrin ring is wedged into its pocket by a phenylalanine of its polypeptide chain. The polypeptide chains of adult hemoglobin themselves are of two kinds, known as alpha and beta chains, similar in length but differing in amino acid sequence shown in Figure1.1 [15]

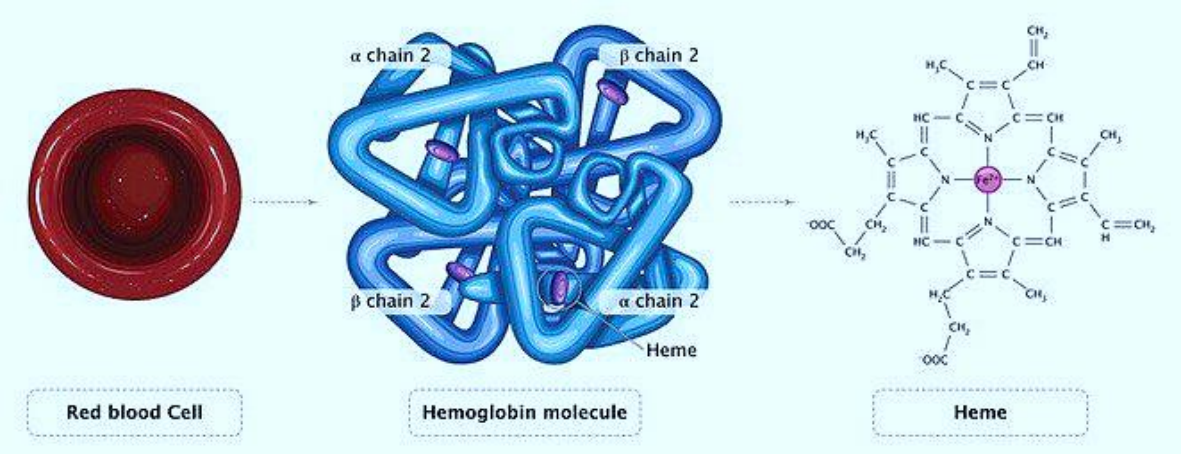


Figure 1.1 Structure of the hemoglobin tetramer. [16]

1.1.2- Hemoglobin Types:-

A single hemoglobin molecule has two types of globin chains, each with its own heme protein. One globin chain is alpha and the other is beta. Two hemoglobin molecules combine to produce a functional hemoglobin tetramer[17]. The following four major types of haemoglobin:

1- “Embryonic” haemoglobins, which are detectable from the 3rd to the 10th week of gestation and represent ζ2ε2 (Hb Gower 1), α2ε2 (Hb Gower 2), ζ2γ2 (Hb Portland 1); and ζ2β2 tetramers (Hb Portland 2)

2- "Foetal" haemoglobin (HbF), which constitutes the predominant oxygen carrier during pregnancy and is a α2γ2 molecule;

3- Adult" haemoglobin (HbA α2β2), which replaces HbF shortly after birth.

4-A minor adult component, HbA_2 (α2δ2)[18].

The process of different haemoglobin species being produced and stop at certain period of human development is known as "haemoglobin switching". Under normal conditions, the red cells of the adult human contain approximately 97-98% of HbA, 2-3% of HbA_2 and traces of HbF. as shown in Figure1. 2.

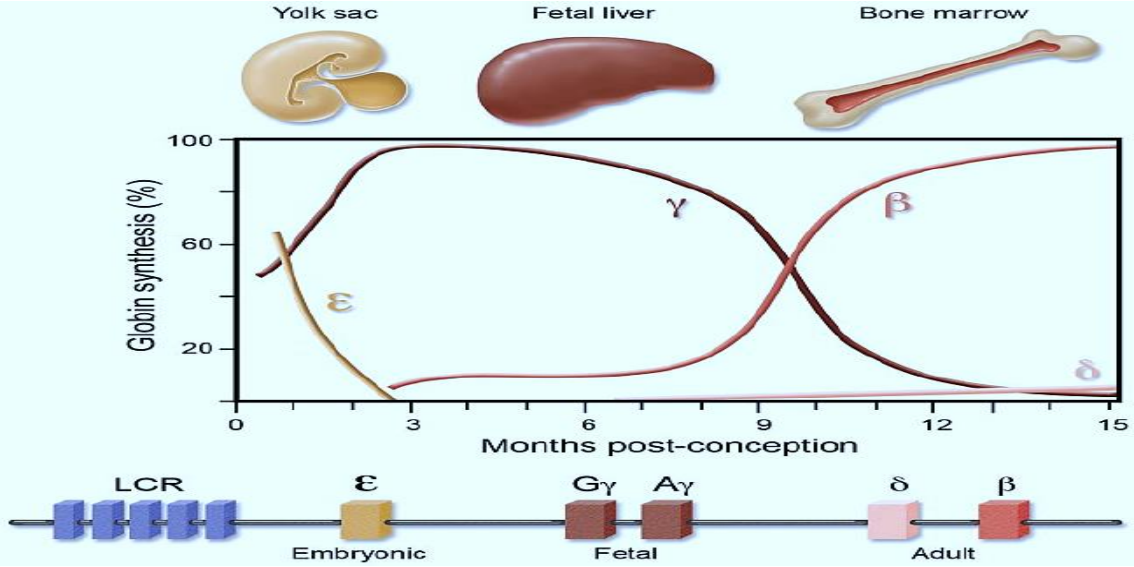


Figure 1.2.Globin synthesis at various stages of embryonic, foetal and adult erythroid development.[19]

1.1.3. General Classification of hemoglobin disorders:-

Hemoglobin disorders can be broadly classified into two general categories:

1. Those in which there is a quantitative defect in the production of one of the globin subunits, either total absence or marked reduction. These are called the thalassemia syndromes.

2. Those in which there is a structural defect in one of the globin subunits[20].

1.2- Thalassemia:-

Thalassemia syndromes are inherited hemoglobinopathies characterized by impaired or absent production of one of the globin chains of adult hemoglobin with subsequent accumulation of the unpaired chains. The most common form is β-thalassemia related to a defective production of the b-globin chains causing an unbalanced ratio of α-globin to β-globin shown in Figure 1.3 [21].

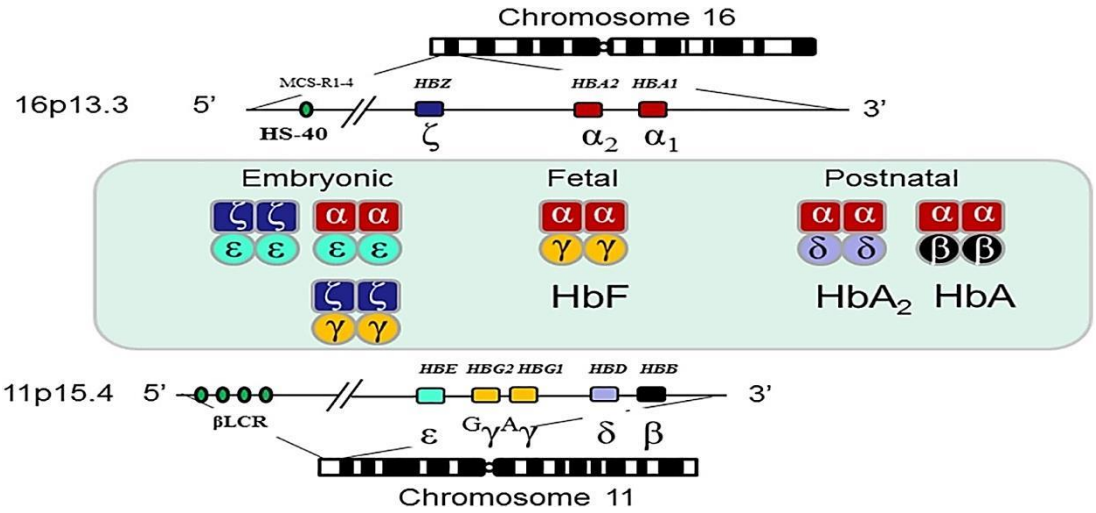
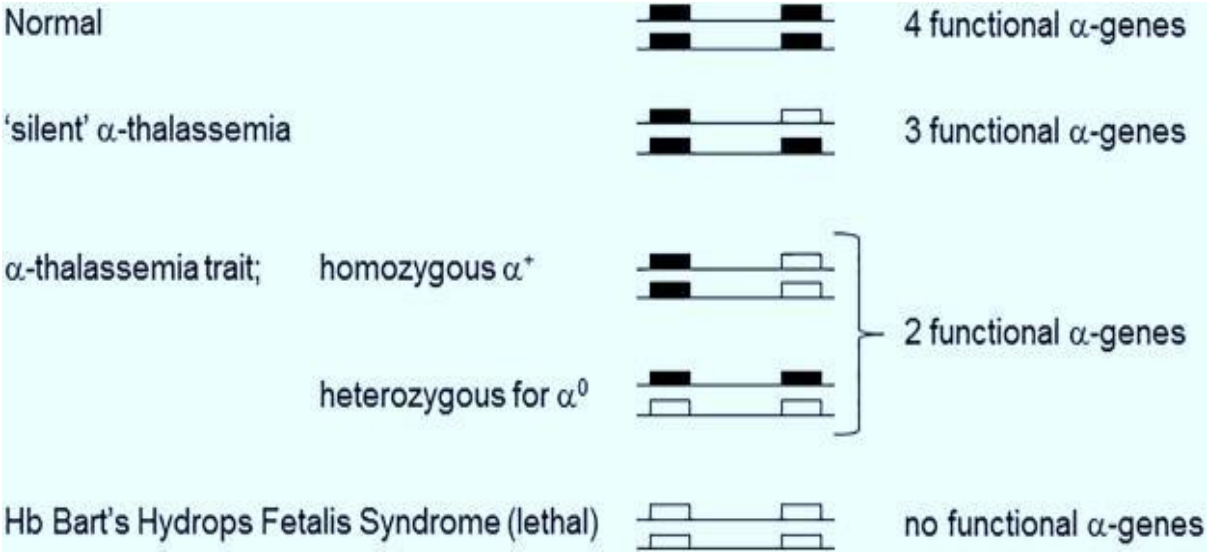


Figure1. 3. Schematic presentation of the chromosomal location of the α- and β-globin gene clusters on 16p and 11p respectively. [22]

1.2.1-Alfa-Thalassemia:-

Alpha-thalassemia is one of the most common hemoglobin genetic abnormalities and caused most frequently by deletions involving one or both alpha globin genes and less commonly by non deletional defects[23]. Normal

adult hemoglobin consists of pairs of α and β chains ($\alpha_2\beta_2$), and fetal hemoglobin has two α chains and two γ chains ($\alpha_2\gamma_2$). defective production of α chains is reflected by the presence of excess γ chains, which form γ_4 tetramers, called hemoglobin Bart's; in adults, excess β chains form β_4 tetramers, called hemoglobin H(HbH). Because of their very high oxygen affinity, both tetramers cannot transport oxygen, and, in the case of HbH, its instability leads to the production of inclusion bodies in the red cells and a variable degree of hemolytic anemia.[24] Classification of α-thalassemia defects is shown in Figure 1.4.



Figer1. 4 Classification of α-thalassemia defects and phenotypic expression[22]

1.2.2-Beta Thalassemia:-

β-Thalassemia are highly prevalent in the Mediterranean, Middle East, and the Indian subcontinent; however, due to recent migrations, they are becoming more common worldwide, making their management and care an increasing concern for health care systems [25]

β-thalassemia is caused by β-globin gene (*HBB*) mutations that either reduce (β^+) or abrogate (β^0) production of functional β-globin. In β-thalassemia,

an excess of unpaired α-globin impedes red-cell development and survival, leading to ineffective erythropoiesis, hemolysis, chronic anemia, and compromised quality of life. Patients with severe anemia receive lifelong red-cell transfusions and regular iron chelation to prevent iron overload [26]. Untreated, β-thalassemia leads to hepatosplenomegaly, bone deformities due to bone marrow expansion, and heart failure due to severe anemia [27].

Patients who are homozygous or compound heterozygous for β-thalassemia mutations can have β-thalassemia major or intermedia. Patients with β-thalassemia major generally present early in life, with severe anemia and symptoms, whereas patients with β-thalassemia intermedia tend to present later in life, with mild-to-moderate anemia and symptoms [28].

1.2.2.1 Epidemiology:-

Thalassemia, the most common form of hereditary anemia, is caused by the impaired synthesis of one of the two globin chains in hemoglobin. This disorder has been found to be highly prevalent in tropical and sub-tropical regions of the world (e.g., Southeast Asia, the Mediterranean area, the Indian subcontinent, and Africa), where the estimated prevalence rates are 12–50% in the case of alpha thalassemia and 1–20% in that of beta thalassemia. [29]

In the world, Maldives has the highest incidence of thalassemia with an 18% carrier rate of the population. The estimated frequency of β-thalassemia in Cyprus is up to 16%, Thailand 1%, Iran 5%–10%, and China 3%–8%.[30] According to the World Health Organization (WHO)in 2018, at least 5.2 percent of people worldwide have thalassemia, and around 1.1 percent of couples are at risk of producing children with a hemoglobin abnormality[31].

1.2.2.2-Etiology:-

Thalassemia is autosomal recessive, which means both the parents must be affected with or carriers for the disease to transfer it to the next generation[32]. They are caused by mutations that nearly all affect the β globin locus and are extremely heterogeneous. Almost every possible defect affecting gene expression at transcription or post-transcriptional level, including translation, have been identified in β thalassemia. These genetic defects lead to a variable reduction in β globin output ranging from a minimal deficit (mild β^+ thalassemia alleles) to complete absence (β° thalassemia).[33]

1.2.2.3. Pathophysiology:-

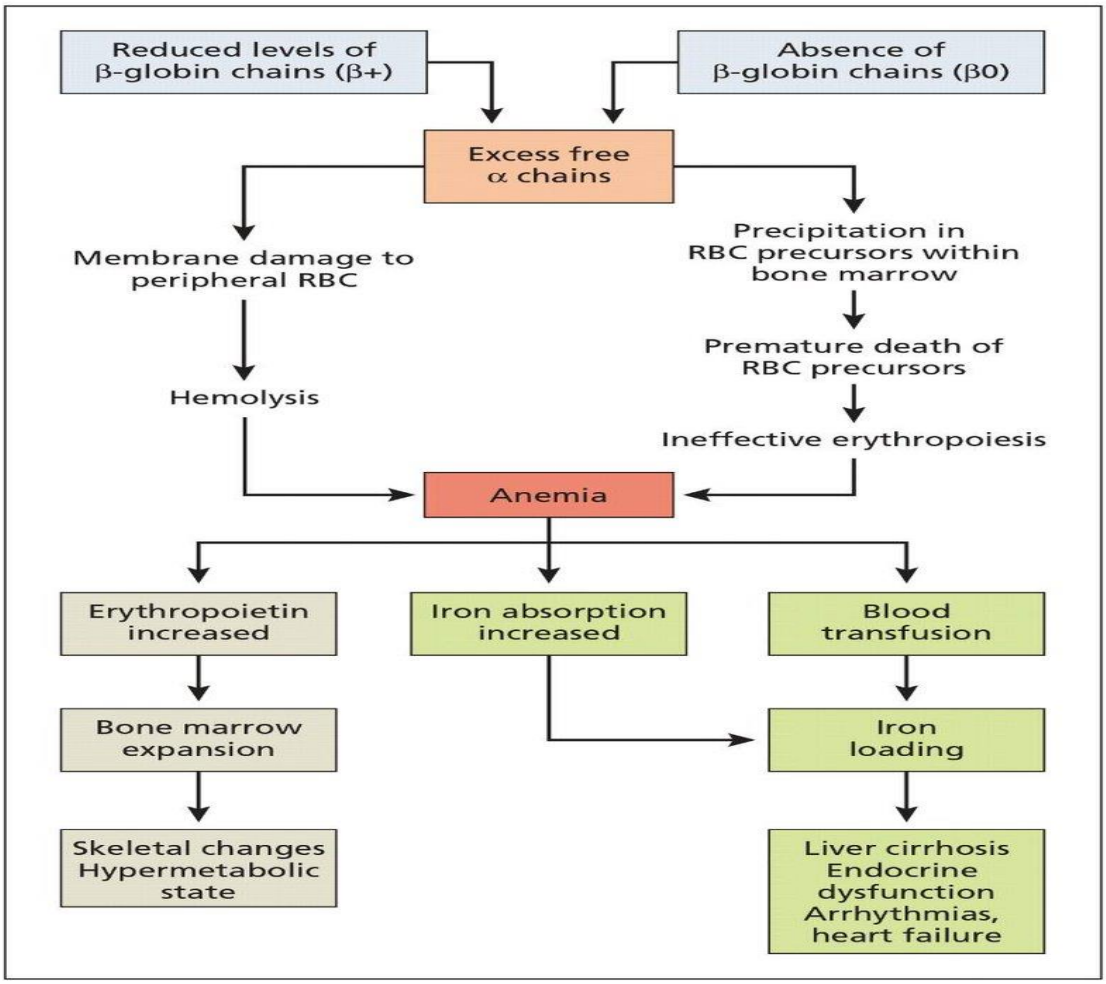
The main characteristic of the pathophysiology of β-thalassemia is reduced β-globin chain production. The inevitable imbalance in the α/β-globin ratio and α-globin accumulation lead to oxidative stress in the erythroid lineage, apoptosis, and ineffective erythropoiesis. The result is compensatory hematopoietic expansion and iron overloud in liver leads impaired hepcidin production from it that causes increased intestinal iron absorption and progressive iron overload[34] .

Subsequent clinical manifestations include splenomegaly, marrow expansion, extramedullary hemopoiesis, and bone marrow. Besides, excess iron from blood transfusion and ineffective erythropoiesis deposits in major organs, causing cardiac dysfunction, liver fibrosis, diabetes, and neurological complications[35].

The underlying hypothesis is that the globin chains carrying heme, hemichromes and iron could generate reactive oxygen species (ROS) that would, in turn, damage cellular compartments. For both a- and b-thalassemic RBCs the evidence,[36]. The excess globin chain is deposited on the RBC

membrane skeleton, where oxidant injury produces changes in red cell material properties, hydration, and the organization of the phospholipid bilayer. These changes are detected by macrophages and the affected RBCs are rapidly removed.[37]

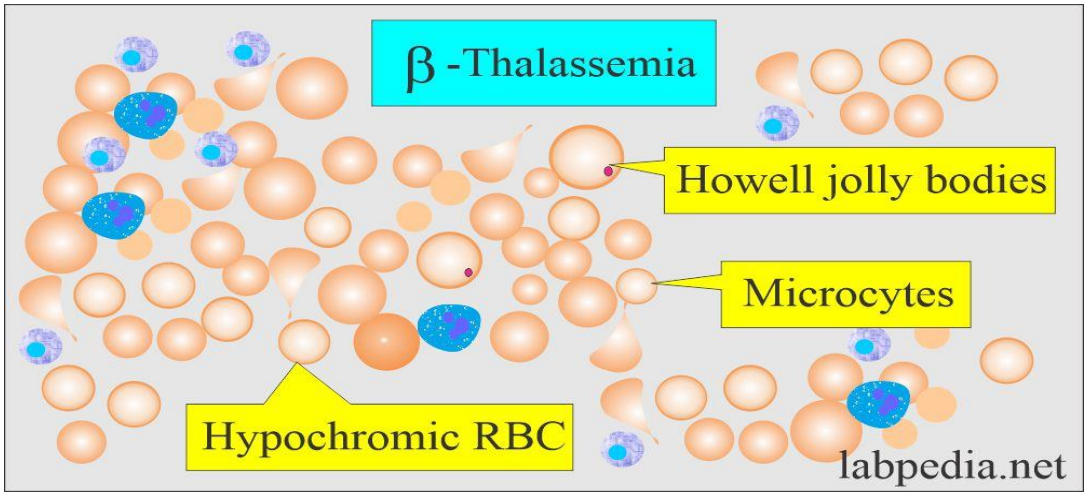
Erythroid marrow hypertrophy in medullary and extramedullary locations causes cranial and facial malformations, as well as extramedullary erythropoietic tissue masses, cortical thinning, and pathological fractures of long bones. The abnormal RBCs' lipid membrane composition might cause thrombotic complications, especially in splenectomized patients.The complation are shown in Figure1.5[38].



Figer1.5.Mechanism of iron overload development due to ineffective erythropoiesis in β-TI.[39]

1.2.2.4. Clinical presentation and Diagnosis of β-thalassemia major:-

Usually occurs between 6 and 24 months of life, with severe microcytic/normocytic anemia, mild jaundice, and hepatosplenomegaly. The hematological diagnosis is based on reduced hemoglobin level (<7 g/dL) and very low MCH (<20 pg). The peripheral blood smear shows severe erythrocyte morphologic changes with marked poikilocytosis (speculated tear-drop cells), target cells, and numerous erythroblasts this shown in Figure1.6. The number of erythroblasts is related to the degree of ineffective erythropoiesis and is markedly increased after splenectomy [40].



Figer1. 6- Beta-thalassemia-smear[30].

In the classical form of β-thalassemia major (homozygotes β°), at hemoglobin analysis, HbA is absent and HbF represents the 92–95% of the total hemoglobin. In thalassemia major forms due to double heterozygosity of β°/β+, the HbA levels can be variable between 10 and 30% and HbF between 70 and 90%. In such condition, both parents usually have typical hematological parameters of β-carriers.[41]

Beta-thalassemia intermedia should be suspected in subjects who present at a later age with similar but milder clinical findings than thalassemia major. The clinical spectrum of thalassemia intermedia is very wide as well as the hematological phenotype. Patients with milder forms may have moderate-to-mild anemia, and the levels of HbA and HbF are very much dependent of the underlying molecular defects and the degree of ineffective erythropoiesis.[42]

Clinically, they present in later childhood or even in early adulthood with a mild to moderate anemia, with a hemoglobin (Hb) level ranging between 7 and 10 g/dL, that only requires occasional or short-course of regular transfusions in certain clinical settings (e.g., in the course of infections or pregnancy). [43] More severely affected patients present younger, between 2 and 6 years of age, and require transfusions for normal and sustained growth.[44]

1.2.2.5. Complications of β-thalassemia major:-

A- Chronic anemia:

Chronic anemia and hypoxia can lead to oxidative stress and lipid peroxidation that are correlated to tubular cells dysfunction. Increased metabolic demand in association with chronic hypoxia in tubular cells may lead to apoptosis and then development of tubulo -interestitial injury and consequent glomerulosclerosis and kidney fibrosis. [45] Moreover, in some studies, hyperfiltration was discovered in β -thalassemia. At first, this hyperdynamic circulation leads to increased plasma flow and glomerular filtration rate, but eventually stretching of glomerular capillary wall with subsequent endothelial and epithelial injury may result in glomerular dysfunction and a progressive decline in GFR[46].

B-Iron overload:

There is no physiological mechanism for the body to eliminate excess iron. When iron consumption is increased over time, either as a consequence of red blood cell transfusions or as a result of increased iron absorption through the gastrointestinal (GI) tract, iron overload (haemosiderosis) develops. Both of these occur in patients with β-thalassemia major[47].

Another mechanism that accounts for iron overload is dyserythropoiesis. It's been proven that it's caused by the liver producing less hepcidin, an iron-regulating hormone. Hepcidin deficiency causes the cellular iron exporter ferroportin to become activated; this causes an increase in the amount of iron that enters the plasma[48] Excess free iron is known to be a catalyst of lipid peroxidation which damages cells[49].

Toxicity from iron overload most commonly damages the liver, heart, and endocrine system and can adversely affect survival. For example, in thalassemia without iron chelation, death usually occurs from cardiac failure or arrhythmia at an early age, long-term efficacy and survival data of chelation therapy is lacking This can occur in autoimmune disorders, inflammatory states, malignancy, chronic renal insufficiency, metabolic syndromes, and hepatopathies [50].

C- Cardiac complications:-

Iron overload is the most important a potential cause of heart failure in patient with βTM. When the heart is exposed to a high quantity of circulating non-transferrin bound iron over an extended period, this is known as cardiac iron loading.[51] Despite advancements in therapeutic care of thalassemia major and the resultant significant increase in patient survival, heart disease has

always been and continues to be the primary cause of death and illness[52], [53].

Cardiac compensation is a frequent mechanism observed in chronic anemia caused by TDT to ensure sufficient oxygenation to peripheral tissues and organs. Chronic anemia in TDT patients is known to impair the cardiac sympathetic and parasympathetic signals, creating a spectrum of heart rate and rhythm abnormalities [54]

D- Enlargement of the Liver and Liver Disease:-

Transfusion-acquired hepatitis C virus (HCV) remains an important problem among patients with thalassemia, In thalassemia patients with HCV infection, liver iron does not play a major role in influencing the chronicity rate, whereas it is significantly associated with the fibrosis.[55]

Accumulating in the hepatocytes, iron plays a direct role in cancer development .[56] In addition to the risk of hepatocellular carcinoma(HCC) development in beta-thalassemia is linked to several factors: the high risk of infections transmitted by blood transfusions, responsible of chronic liver diseases as HCV and HBV. [57]

E- Enlargement of the Spleen:-

Increased red blood cell breakdown by the reticuloendothelial system, particularly in the spleen, resulting in spleen enlargement (splenomegaly). Modern transfusion regimens, on the other hand, have significantly decreased the incidence of splenomegaly and splenectomy in BTM patients by establishing more appropriate pretransfusional haemoglobin levels and transfusion intervals [58],[59].

F- Endocrine Complications:-

The most common main consequences of beta -thalassemia are endocrine problems, including hypogonadism, hypoparathyroidism, hypothyroidism, and pancreatic and adrenal insufficiency [60].

Failure of pubertal growth, delay or lack of sexual progress, amenorrhea, sexual dysfunction and infertility due to hypogonadism are well-known disorder of the hypothalamic - pituitary - gonadal axis in β-thal patients[61].

Primary hypothyroidism that may affect thalassemia patients is major lead to gland infiltration by iron overload. Central hypothyroidism caused by low secretion of Thyrotropin Stimulating Hormone (TSH) from the anterior pituitary gland, or by decreased secretion of Thyrotropin-Releasing Hormone (TRH) from the hypothalamus is less common[62].

G- Bone Abnormalities and Osteoporosis:-

Bone disorders in patients with thalassemia major (TM) and intermedia (TI) constitute complex conditions that result from various factors affecting the growing skeleton[63]

Iron overload, a state with excessive iron storage in the body, is a common complication in thalassemia patients which leads to multiple organ dysfunctions including the bone. Iron overload-induced bone disease is one of the most common and severe complications of thalassemia including osteoporosis. Currently, osteoporosis is still frequently found in thalassemia even with widely available iron chelation therapy[64].

H- Renal complication:-

The emerging renal complications in beta-thalassemia patients have raised the global exchange of views. Despite better survival due to blood transfusion and iron chelation therapy, the previously unrecognized renal complication remain a burden of disease affecting this population [65].

Less is known about the effects of thalassaemia on the kidney. Abnormalities of renal function, such as increased renal plasma flow, decreased urine concentrating ability, and renal tubular acidosis, Anaemia and iron-mediated toxicity are the speculated causes of these abnormalities.Chelation therapy may also affect renal function in thalassaemia patients [66].

1.2.2.6. Management of β-thalassemia major:-

A-Transfusions

The goals of transfusion therapy are correction of anemia, suppression of erythropoiesis, and inhibition of gastrointestinal iron absorption, which occurs in transfused patients as a consequence of increased, although ineffective, erythropoiesis [67]

Before starting transfusions, diagnosis of thalassemia should be confirmed; the molecular defect, the severity of anemia on repeated measurements, the level of ineffective erythropoiesis, and clinical criteria such as failure to thrive or bone changes with facial deformities should be taken into account. [68]

The decision to start transfusion in patients with confirmed diagnosis of thalassemia should be based on the presence of severe anemia (Hb < 7 g/dl for more than two weeks, excluding other contributory causes such as infections)[69].

B- Iron Chelating Therapy:-

A unit of transfused packed RBC contains approximately 200–250 mg of iron and as humans essentially do not have a mechanism to excrete iron from the body, transfusional iron gets accumulated. Additionally, increased intestinal absorption of iron due to suppression of hepcidin secretion from the liver . [70].

The use of iron chelators for the treatment of systemic diseases such as thalassemia major and hemochromatosis is already a proven therapeutic approach , chelator treatment in iron overload patients induces substantial iron excretion and a negative iron balance [71] Iron chelation therapy is also needed in many of these patients even if they are not transfused [72].

There are now three iron chelators approved for clinical use, each with unique iron binding characteristics, absorption, elimination, and metabolism pathways. These is summarized in Table 1.1

Table1.1. Comparison of currently available iron chelators[73].

Compound	Desferrioxamine (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Advantages	extensive experience, low cost, better availability	better compliance	chelates cardiac iron more efficiently
Disadvantages	poor compliance	limited safety data	not recommended as monotherapy

Dose (mg/kg/day)	30-60	20-40 (TDT); 5-20 (NTDT)	75-100
Frequency	8-12hr infusion, 5-7 days /week	once daily	three times daily
Main adverse effects	ocular and auditory symptoms, retardation of bone growth, local reactions, allergy	GI symptoms, increased creatinine, increased hepatic enzymes	GI symptoms, arthralgia, agranulocytosis/ neutropenia
Metabolism	Intrahepatic to metabolite B which binds iron	>90% of it is excreted in the feces, and 60% of it is unmetabolized. Iron is bound by the majority of metabolites	Glucuronide formed in liver does not bind iron
Route	subcutaneous or intravenous	Oral	Oral
Route of iron excretion	urinary and faecal	Faecal	Urinary

TDT: transfusion-dependent thalassaemia, NTDT: non-transfusion dependent thalassaemia, GI: gastrointestinal

C. Splenectomy:-

In thalassemia major and thalassemia intermedia, overactivity of the spleen occurs as a consequence of severe hemolysis. Splenectomy protects the patients against poor health and growth retardation by decreasing the transfusion requirement, improving the level of Hb as well as decline the accumulation of iron [74].

Removal of the spleen is suggested when a requirement of transfusion is greater than 200 to 220 ml RBCs/kg with 70% hematocrit as well as packed RBCs 250–275 ml/kg with 60% hematocrit per year[75].

D. Transplantation of hematopoietic stem cells:-

This scheme is used in the management of various ailments such as thalassemia. In this therapy, hematopoietic stem cells from the marrow squash of healthy individuals are sequestered and transmitted to patients of thalassemia. Nearly 80% of transplant recipients were successful by this treatment[76].

Graft versus host disease (GVHD) is the most significant and hazardous problem in the transplantation of bone marrow which might lead to the death of transfer recipients[77].

E. Gene Therapy:-

The curative therapies of hematopoietic stem cell transplant (HSCT) and gene therapy or editing are increasingly used to treat patients with hemoglobinopathies, including thalassemia and sickle cell disease [78].

Recent progress in gene therapy has been made by targeting discrete groups of patients with certain immunodeficiencies and hemophilia.β-thalassemia is one of the first examples in which gene therapy could be applied

to a large population of patients who reside mostly in developing countries.[79] Complications and menegmentes are shown in Figure1.7.

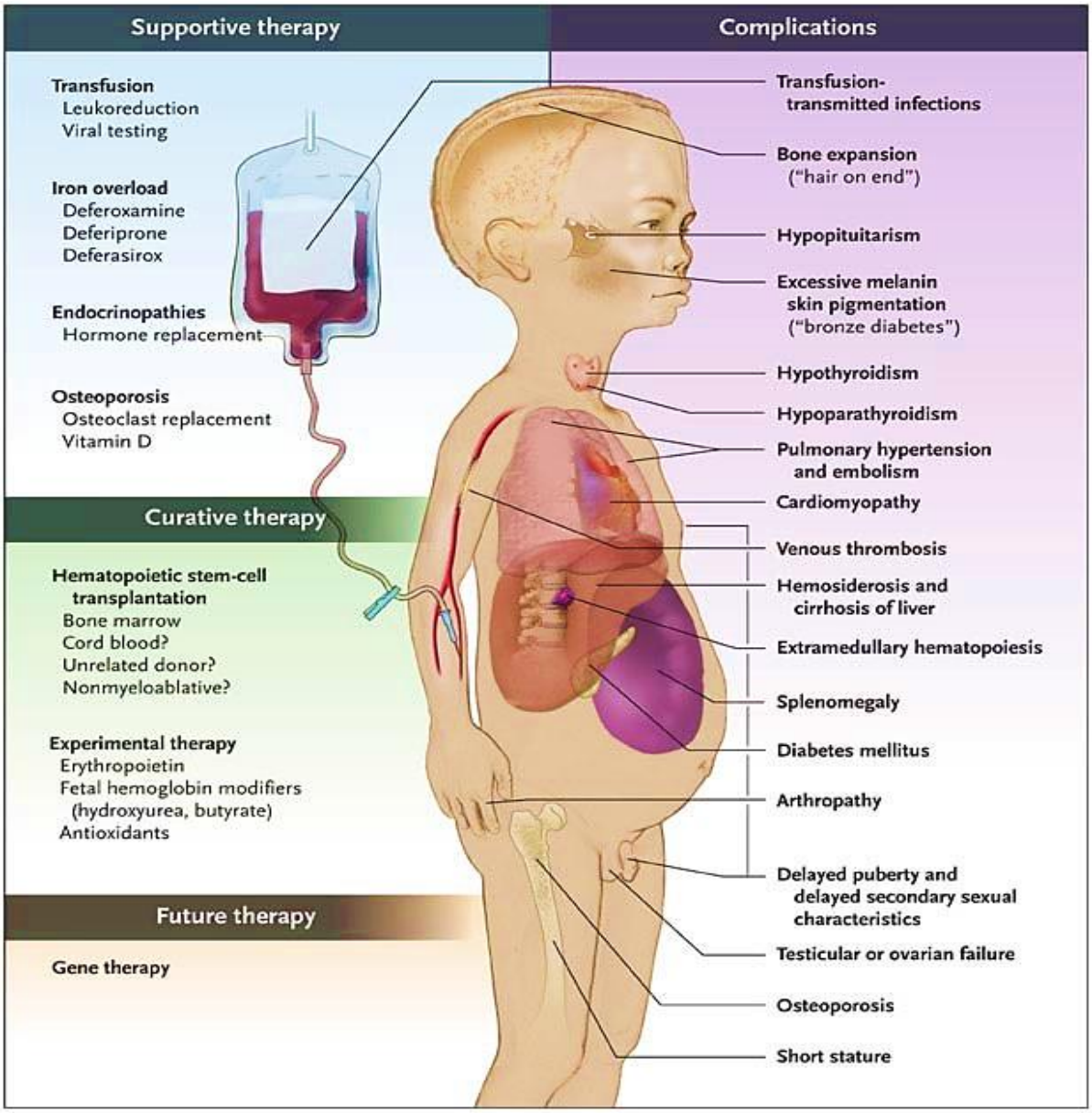


Figure1.7. Management of Thalassemia and Treatment-Related Complications.[80]

1.3. Renal dysfunction in β-TM:-

Advances in the management of patients of beta-thalassemia major (β-TM) and the advent of effective chelators have led to the discovery of many renal complications. Mechanisms of renal impairment in β-TM are still not fully investigated[81].

Chronic anemia and hypoxia may result in oxidative stress and lipid peroxidation and finally impairment in tubular cells function. In addition, iron overload due to repeated blood transfusions is a critical factor in the pathogenesis of kidney injury in thalassemic patients[82].

1.3.1. Mechanisms of renal disease in β-TM:-

Several major factors are responsible for functional abnormalities found in β-TM which include shortened red cell life span, rapid iron turnover, and tissue deposition of excess iron. Moreover, the uses of specific iron chelators are not without harm to the kidney[83].

1- The potential effects of thalassaemia-related chronic hypoxia and anaemia:-

Observed that the most striking effects of anemia on renal morphology were damages in the proximal tubules and a volume increase of the peri-tubular spaces[84]. A naemia can lead to activation of the oxidative stress cascade once again with the end result of lipid peroxidation and cell damage and eventual functional change of the tubules [85].

The anaemia may also lead to changes in the morphology of cells in terms of size and vascular supply. Therefore, clinical caution is warranted with recommended monitoring for renal tubular and glomerular function [86].

2-The potential effects of iron loading on the kidney:-

Post-mortem studies demonstrate haemosiderin deposition in visceral and parietal glomerular epithelial cells in both proximal and distal convoluted tubules leading to the possibility of tubular dysfunction. Also from studies in iron overloaded rats, it is possible that high levels of iron are sufficient to cause cellular damage via stimulation of reactive oxidative elements[87].

The mechanism of iron overload-associated renal damage has not been fully elucidated. Non-transferrin iron can lead to organelle membrane dysfunction and subsequent cell injury/death. Iron-catalyzed generation of reactive oxygen species (ROS) is responsible for initiating the peroxidative reaction. The possible association of oxidative stress and its impact on nitric oxide (NO) metabolism in iron overload associated renal injury was investigated through a randomized study on rats[88].

Iron overload may also lead to Damage and loss of peritubular capillaries, epithelial-mesenchymal transdifferentiation of tubular cells to myofibroblasts, tubulointerstitial injury, glomerulosclerosis [80].

3- The potential effects of iron Chelation therapy on the kidney:-

Consumption of all three available iron chelators (deferoxamine, deferiprone, and deferasirox) may result in glomerular dysfunction. This glomerulopathy ranges from mild increase in serum creatinine up to acute kidney injury. Deferasirox and deferoxamine can cause renal injury in thalassemia patients more than deferiprone, especially when appropriate dosage monitoring is absent Iron depletion due to higher doses of iron chelators can play a critical role in pathogenesis of kidney injury in thalassemia.[89]

The most probable mechanism of GFR reduction in iron deprived nephrons is mitochondrial dysfunction and consequent production of adenosine and adenosine triphosphate. This phenomenon may lead to activation of the tubulo-glomerular feedback and vasoconstriction of the afferent preglomerular arterioles[10].

1.3.2. Types of renal injury:-

Kidney injury in thalassemia increases with age and duration of blood transfusions [90]. These patients can also manifest both tubular and glomerular dysfunction [91].

A- Tubular dysfunction:-

Tubular dysfunction among patients with β-thalassemia has been related to iron overload, chronic anaemia, as well as deferoxamine (DFO) toxicity [92]. Protein are reabsorbed and catabolized within the proximal tubule after being freely filtered at the glomerulus. As a result, the presence of significant amounts of these proteins in the urine indicates that tubular reabsorptive processes have failed[93].

Common signs of tubulopathy, such as proteinuria, hypercalciuria, phosphaturia, hyperuricosuria, magnesiumuria, and increased excretion of β2-microglobin (β2M), Cystatin C was demonstrated in a TDT Iranian population[94].

Early recognition of renal dysfunction is of great importance both for intervening in a timely manner and for improving prognosis. Therefore, in recent years several products produced and released by proximal tubular cells as measurable proteins in a variety of renal diseases have been tested, as possible valid biomarkers of renal injury. [95] Such proteins include N-Acetyl-beta-D-glycosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), Liver-type Fatty Acid-Binding Protein (L-FABP) and Interleukin-8 (IL-8), all of which were studied mainly in children and younger patients with TM. Further studies are needed to evaluate the significance of these biomarkers as predictors of renal disease in thalassemia patients[96].

B-Glomerular dysfunction:-

Changes in glomerular function are also evident in multitransfused patients with beta-thalassemia. Chronic anemia is thought to reduce systemic vascular resistance leading to hyperdynamic circulation and subsequent increased renal plasma flow and glomerular filtration rate (GFR)[97]. Although reduction in GFR rarely occurs in pediatric patients with β thalassemia major , gradual decrease in GFR may happen with increasing age and progressive kidney damage[98].

The current study results showed decreased estimated GFR in eleven percent of thalassemic patients. Glomerular capillary wall stretching, and subsequent endothelial and epithelial injury, can induce the transudation of macromolecules into the mesangium and glomerular dysfunction, which may cause a progressive decline in GFR[99].

1.3.3. Biomarker of renal dysfunction in TBM:-

1.3.3.1. Blood urea:-

Nitrogen metabolism is necessary for normal health. Nitrogen is an essential element present in all amino acids; it is derived from dietary protein intake, is necessary for protein synthesis and maintenance of muscle mass, and is excreted by the kidneys. Renal nitrogen excretion consists almost completely of urea and ammonia[100].

Renal excretion accounts for more than 90% of urea elimination from the body. Urea is filtered across the glomerulus and enters the proximal tubule. The concentration of urea in the ultrafiltrate is similar to plasma [101] , (40 % -70 %) of urea passes passively out of the tubule and into the renal interstitial space, eventually returning to plasma. Urea back-diffusion is also affected by urine

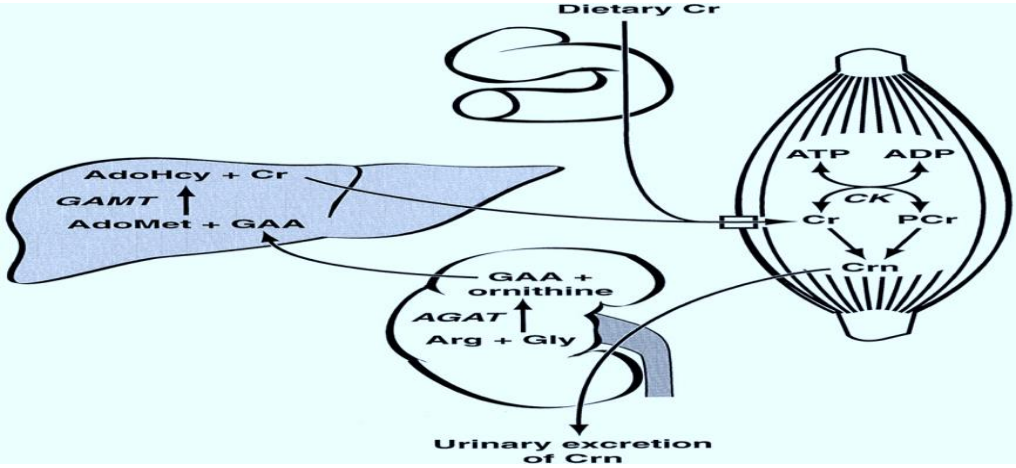
flow rate, during high flow; the entry of urea into the interstitium is reduced and more in low-flow situations. For many years, blood and serum urea measurements have been used to assess kidney function[102].

1.3.3.2. Serum Creatinine:-

creatinine are nitrogenous end products of metabolism. Creatinine is the product of muscle creatine catabolism. Thus, creatinine production essentially reflects lean body mass. Because this mass changes little from day to day, the production rate is fairly constant[103].

In normal subjects, creatinine is excreted primarily by the kidneys. There is minimal extrarenal disposal or demonstrable metabolism, As a small molecule (molecular weight of 113 daltons)[104].

Creatinine formation begins with the transamidination from arginine to glycine to form glycocyamine or guanidoacetic acid (GAA). This reaction occurs primarily in the kidneys, but also in the mucosa of the small intestine and the pancreas. The GAA is transported to the liver where it is methylated by S-adenosyl methionine (SAM) to form creatine. Creatine enters the circulation, and 90% of it is taken up and stored by muscle tissue. In a reaction catalyzed by creatine phosphokinase (CPK), most of this muscle creatine is phosphorylated to creatine phosphate. Each day, about 2% of these stores is converted nonenzymatically and irreversibly to creatinine show as figure1.8[105].



Figer1.8.Metabolism and excretion of Creatnine [106].

1.3.3.3. Beta2-microglobulin (β2M):-

Beta2-microglobulin is a single-chain polypeptide, with low molecular weight (11.8 kDa) released by all nucleated human cells [107]. The production of beta 2-microglobulin in normal subjects is quite constant, about 0.13 mg/h kg. The catabolism almost exclusively through renal elimination [108].

The protein readily passes the glomerular membrane; subsequently more than 99.9% of the filtered beta 2-microglobulin is reabsorbed and degraded in the proximal tubules, only about 5 micrograms/h of the protein appearing in the final urine. Proximal tubular dysfunction leads to an increased urinary concentration this shown in Figure1.9[109].

The amount of this parameter is very low in healthy individuals, but its level increases in conditions such as neoplastic, inflammatory, and immunologic conditions [110].

Impaired uptake as a result of tubular injury results in increased β2M urinary excretion, and thus β2M is considered a direct marker of tubular dysfunction. However, increased β2M production or isolated glomerular disease may increase urinary excretion as well [111].

Furthermore, clinical studies have investigated β2-MG as a useful biomarker for predicting poor outcomes in patients with kidney.[112] In CKD the serum β2-microglobulin had the best diagnostic value. Periodic renal assessment of renal patients is mandatory as they may be affected by hidden renal dysfunction[113].

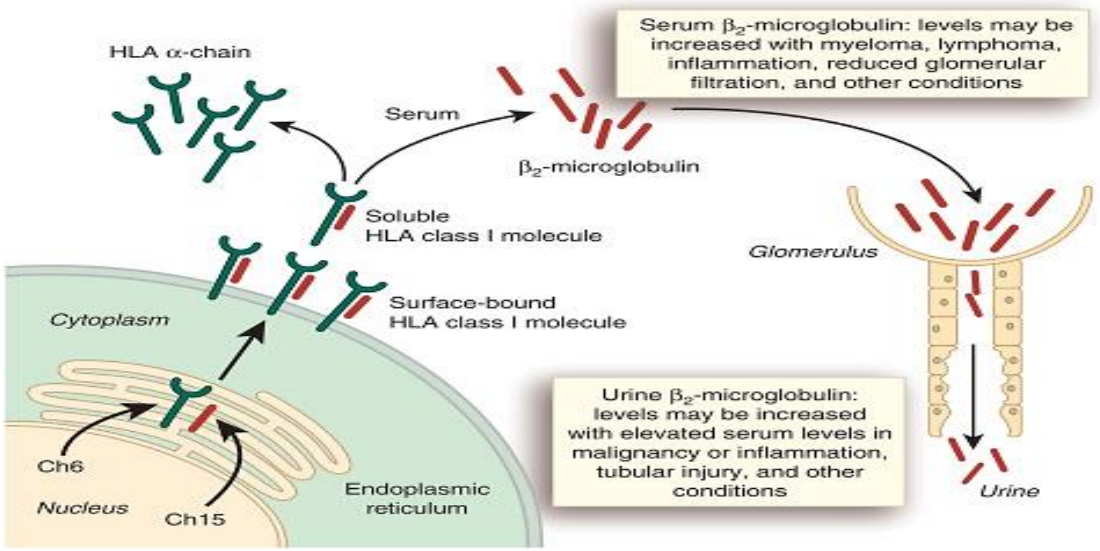


Figure1.9. production, circulation, catabolism and excretion of B2 macroglobulin in health and disease[114].

1.3.3.4. Neutrophil gelatinase associated lipocalin (NGAL):-

Neutrophil gelatinase-associated lipocalin (NGAL), a small 25 kDa stress-protein released from injured tubular cells after various damaging stimuli, is already known by nephrologists as one of the most promising biomarkers of incoming Acute Kidney Injury[115].

Plasma NGAL were filtered in glomerulus, and many undergone reabsorption by proximal tubular; therefore, NGAL excretion in urine only occurs when there is proximal tubular damage which disrupts NGAL reabsorption or increase NGAL synthesis [116].

The main function of NGAL is related to its capacity to bind iron - siderophore complexes, leading to a bacteriostatic property by preventing iron uptake with bacteria. NGAL is detected at very low level in various cell types. The primary site of NGAL production in kidneys was the ascending loop of Henle and collecting duct cells [117].

Renal tubular and glomerular dysfunctions are not uncommon in patients with β-TM. Iron over load, anemia, and iron chelation therapy are likely to be the main factors responsible for these tubular and glomerular abnormalities. Iron over load leads to iron deposition in the glomeruli, proximal renal tubules, and renal interstitium. Anemia causes glomerular hyperperfusion, and hyperfiltration can lead to stretching of the glomerular capillary wall, resulting in endothelial and epithelial injury and hypoxia, which in turn leads to tubuleinterstitial injury [118].The rise of NGAL in serum is regarded a sensitive and reliable measure of proximal tubular toxicity[119].

Kidney injury may result in NGAL secretion from the epithelial cells of kidney. NGAL is filtered by glomeruli and then reabsorbed by proximal tubule. After acute kidney injury, the reabsorption of NGAL in tubular cells decreases and therefore serum and urinary NGAL concentration increases show in Figure 1.10 [120].

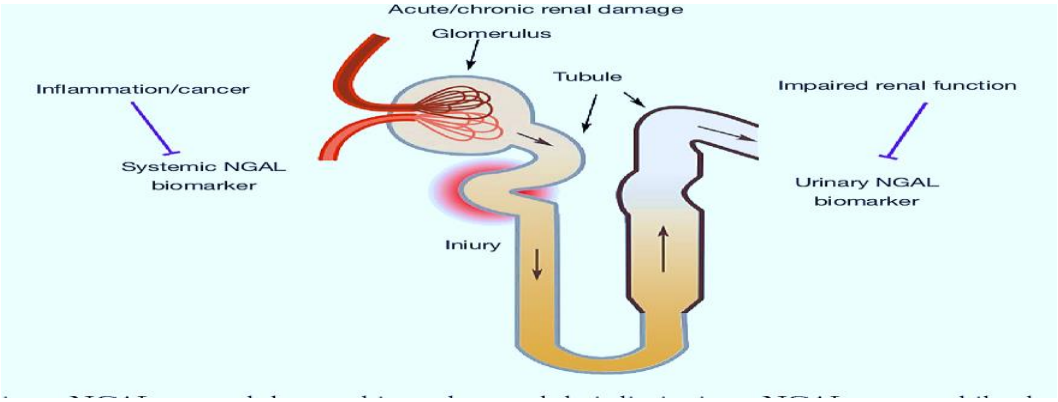


Figure 1.10 Systemic and urinary NGAL as renal damage biomarkers and their limitations [121].

Neutrophil gelatinase-associated lipocalin (NGAL) is highly modulated in a wide variety of pathological situations, making it a useful biomarker of various disease states. It is one of the best markers of acute kidney injury, as it is rapidly released after tubular damage. However, a growing body of evidence highlights an important role for NGAL beyond that of a biomarker of renal dysfunction[122]. Moreover, after acute kidney injury (AKI), hepatic production of NGAL increases. Thus, both urine and plasma NGAL can be used to predict the onset and course of kidney injury [123].

NGAL levels clearly correlate with severity of renal impairment, probably expressing the degree of active damage underlying the chronic condition. For all these reasons, NGAL may become one of the most promising next-generation biomarkers in clinical nephrology and beyond[124].

1.3.3.5. Cystatin-C:-

Cystatin-C is low-molecular-weight non-glycosylated protein that can inhibit cysteine protease, synthesized and secreted by all human nucleated cells[125]. The mature, active form of human cystatin-C is a single non-glycosylated polypeptide chain consisting of 120 amino acid residues, with a molecular mass of 13,343–13,359 kDa, and containing four characteristic disulfide-paired cysteine residues[126].

Cystatin-C is a sensitive biomarker for glomerular filtration rate (GFR) and not secreted by the renal tubules or reabsorbed back into the serum. It is better than creatinine clearance in the diagnosis of renal function impairment, as it is not affected by height, sex, diet, and muscle mass[127]. Additionally, β Thalassemia patients had a high frequency of glomerular dysfunction , and cystatin-C was a promising marker for monitoring of glomerular dysfunction in these patients [128].

Estimated GFR based on cystatin-C could be used as a confirmatory test for an adverse prognosis in patients with chronic kidney disease. The validity and accessibility of cystatin-C testing have been greatly improved by the release of a certified reference material for calibrating laboratory assays[129].

Report the development and validation of two new equations for estimating GFR — one using standardized cystatin-C alone and the other using cystatin-C combined with standardized creatinine [130]. The early and accurate detection of renal impairment is crucial to prevent its progression, and thereby, to potentially improve its outcome. In clinical practice, the detection of renal impairment, which is characterized by a rapid decline of the glomerular filtration rate (GFR), is based on an increase of serum creatinine. However, there are major limitations to the use of creatinine for estimating glomerular filtration rate (GFR)[131]. Thus, minor changes of creatinine, as typically seen early in acute renal failure, may already reflect substantial declines in GFR. Furthermore, serum creatinine inaccurately estimates GFR due to tubular secretion and reabsorption of creatinine, To overcome these obstacles, there is an extensive search for improved laboratory markers of impaired renal function[132]. Cross-sectional studies in chronic renal insufficiency identified serum cystatin C as a promising, easily measurable marker to estimate GFR with higher diagnostic value than serum creatinine [133].

Limitations to its routine use in clinical practice has been primarily cost considerations where, for example, it costs up to ten times that of a serum Creatinine assay, and it can be affected by thyroid disease, adiposity, and underlying inflammation shown in Figure1.11[134].

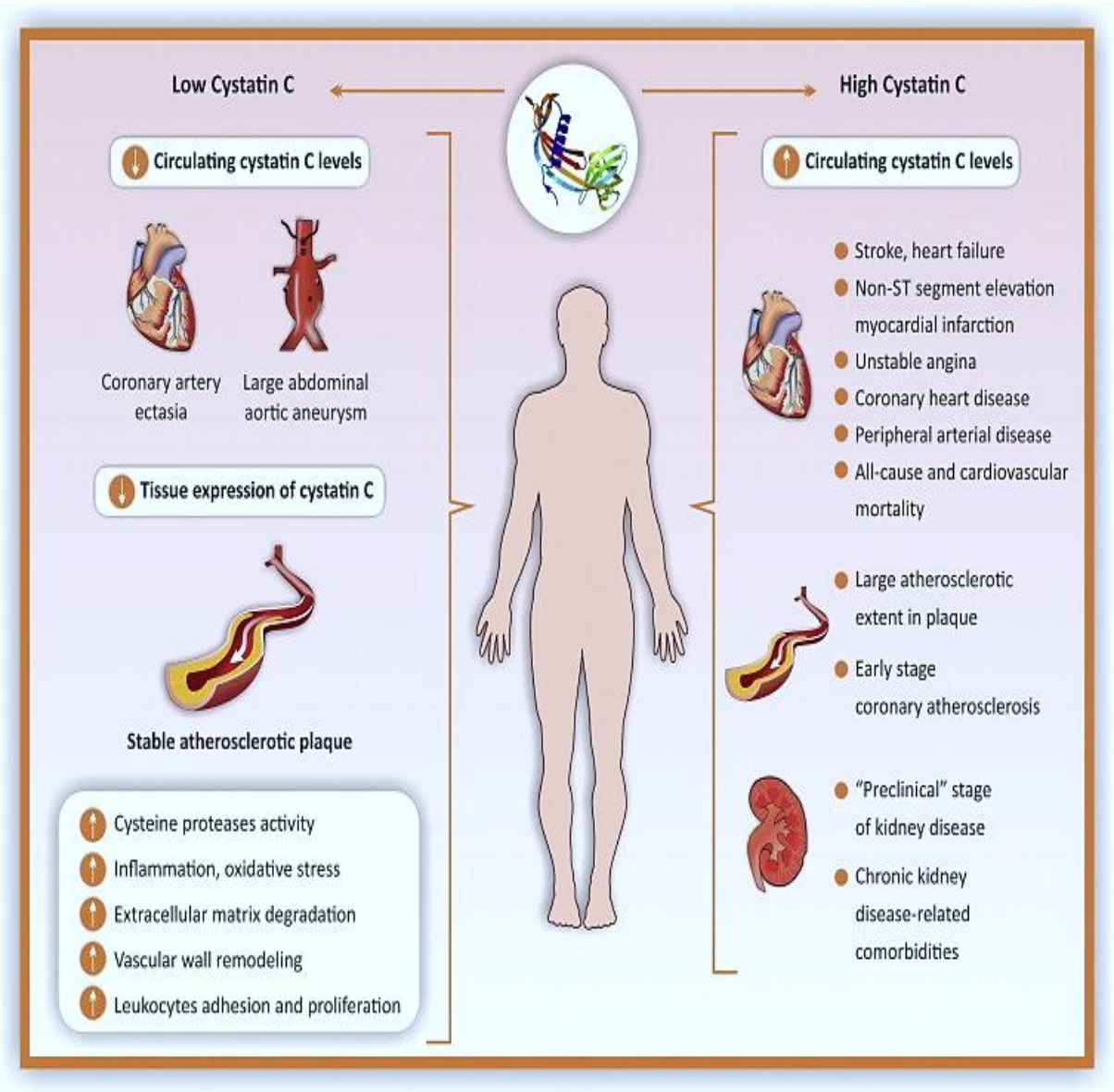


Figure1.11. Controversial findings regarding the relationship between Cystatin-C and Kidney diseases.[135]

Aim of the study:-

The objective of the present study is to identify diagnostic, prognostic and predictive parameters, which can be used to diagnosis deterioration of kidney function in β-thalassemia major patients in early renal impairment with high sensitivity and specificity, improve prognostication or predict and monitor treatment effectiveness and tolerability for the individual patient through determination of new parameters.

To achieve this aim it should apply the following points:-

1. Assessment the changes in β2-microglobulin in BTM patient for prediction renal impairment and correlation with duration of disease.

2. Evalution the changes in the NGAL as parameter in BTM.

3. Evaluation the changes in The Cystatin-C level among BTM.

2. Material and methods:-

2.1. Chemicals.

All the kits used in this study were shown in table (2.1).

Table: (2.1)-Kits used in this study

NO	Kit	Name of the Company	Manufacture Country
1	Creatinine (Colorimetric) Kit	Biolabo	France
2	Ferritin Kit	BioMrieux Mini Vidas	France
3	GOT Kit	Biolabo	France
4	GPT Kit	Biolabo	France
5	Human Beta2-microglobulin ELISA Kit	Mybiosource	USA
6	HumanCystatin(CYS-C)ELISA Kit	Mybiosource	USA
7	HumanNeutrophil Gelatinase Associated Lipocalin(NGAL) ELISA Kit	Mybiosource	USA
8	Urea (Colorimetric) Kit	Biolabo	France

2.2. Instruments and equipment.

The instruments and equipment used in this study were shown in table (2.2).

Table: (2.2) - Instruments and equipment

NO.	Instruments and equipment	Origin
1	Centrifuge	Hettich, Germany
2	Deep freeze	GFL, Germany
3	Disposable syringe (5 ml)	Jordan
4	Disposable test tube (10 ml)	Meheco, China
5	Distilletor	GFL, Germany
6	Elisa reader and washer	Biotek, USA
7	Eppendorf tube (1.5 µl)	China
8	Hematology Analyzer (CBC)	China
9	Incubator	Memmert,Germany
10	Micropipettes (5-50 µl), (2-20 µl), (20-200 µl), (100-1000 µl)	Slamed, Germany
11	Multichannel micropipette reservoir	Mybiosource USA
12	pipette tips 0.2 ml	China
13	pipette tips 1 ml	China
14	Test tube with separation gel	AFCO, Jordan

15	Vidas	BioMrieux,France
16	Water bath	Memmert,Germany

2.3. Subjects

In this study, there are two groups: the first includes patients with beta thalassemia major, and the second includes those who appear to be healthy. The sample size was determined according to the Daniel formula for sample size.

This formula is:

$$\mathbf{N = Z^2\ P\ (1\text{-}P) / d^2}$$

Where n= sample size

Z= Z statistic for the level of confidence interval 95% which = 1.96.

P= Prevalence of β-thalassemia which is 3.0 % in the Iraq [136]. d= precision (in proportion of one; if 5%, d = 0.05).

$n= (1.96)^2 *0.03*0.97 / 0, 0025$

n= 45

2.3.1. The Place and Date of Study

The samples were collected from Karama Hospital-Genetic Hematology center/Baghdad during the period from 1st of September until 1st of January 2023. Questionnaires were created to collect data from the control and patients group. The questionnaire is shown in appendix.

2.3.2. Study Designs

The study was designed as a case-control study.

2.3.3. Patients group

The patients group that consisted of 45 patients with β-thalassemia major with repeated blood transfusion.

2.3.4. Control Group

The control group consists of 45 individuals. They were collected from apparently healthy individuals Age (18-35) according to the plan study.

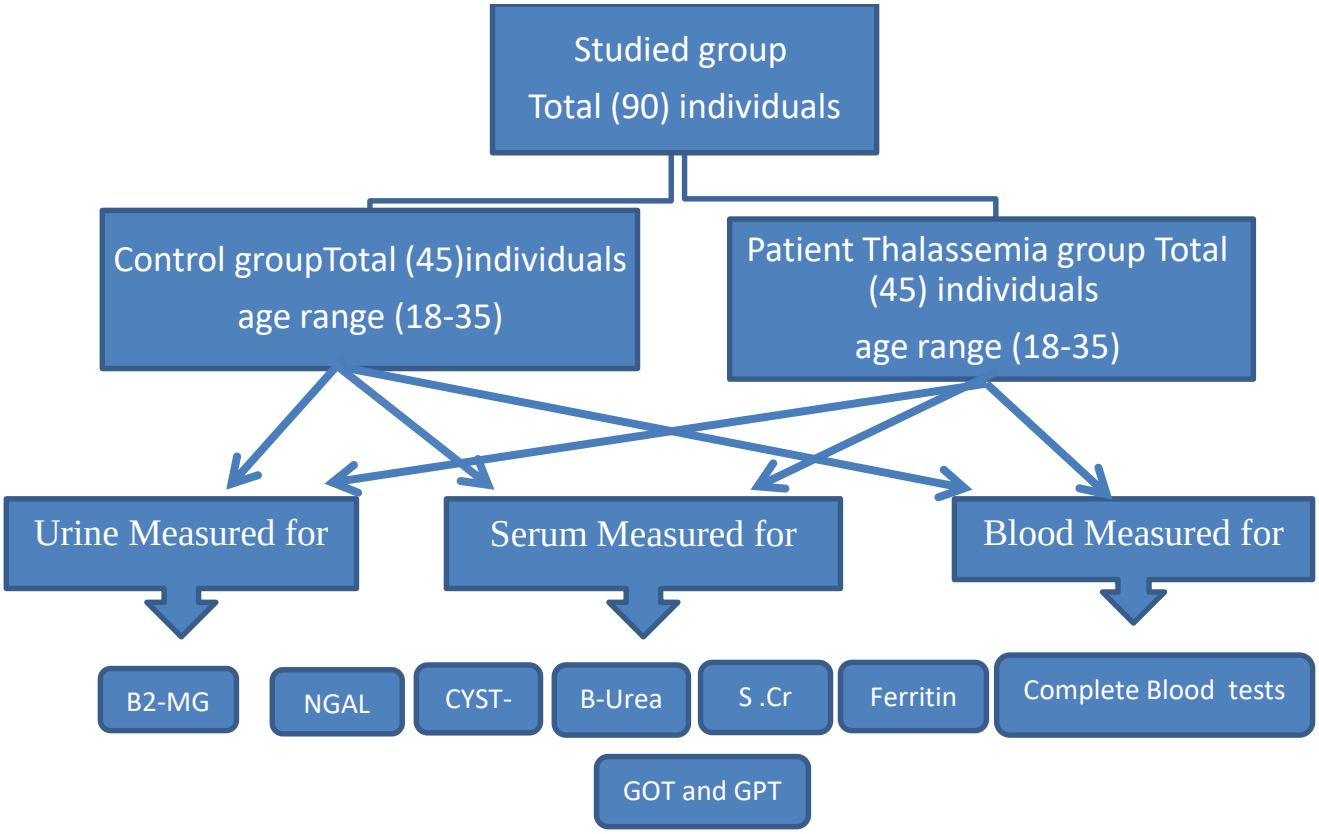


Figure (3-1) Studied groups

2.3.5. Criteria for Exclusion

1. Any patient with diabetes mellitus.

2. Patients with other hemoglobinopathies.

3. Pregnant.

4. Any patient with chronic liver disease.

2.3.6. Inclusion Criteria

The patients group that consisted of 45 patients with β-thalassemia major with repeated blood transfusion, in addition to apparently healthy individuals as control group that consisted of 45 individuals.

2.3.7. Ethical Approval and Consent

All participants in this study were informed before to collecting samples, and verbal agreement was obtained from each of them.

2.4. Methods

2.4.1. Collection of Samples

Using a disposable syringe (5 ml), venous blood samples were obtained from control and patients. Subjects were asked to come in for a blood sample. Five milliliters of blood were extracted through vein puncture and progressively pumped into disposable tubes containing separating gel. The blood in the gel-containing tubes was allowed to clot for 10 minutes at room temperature before being centrifuged for 10 minutes at 2000 xg, then separated into small volumes and kept in a deep freezer (-20 C) to carry out the assay. The blood samples obtained from the groups were used to estimate serum Ferritin, serum Creatinine, Blood Urea, B2-MG, Cyst-C and NGAL.

Early morning urine sample collected from patients and control, and putted into disposable container, than centrifuged for 20 minutes at 1000xg, then the supernatant was collected and separated into small volumes and kept in a deep freezer (-20 C) to carry out the assay.

2.4.2. Determination of Human Neutrophil Gelatinase Associated Lipocalin (NGAL)

Human Neutrophil Gelatinase Associated Lipocalin (NGAL) level was measured by enzyme linked immunosorbent assay kit.

Principle:-

The sandwich-ELISA technique is used in this ELISA kit. This kit includes a micro ELISA strip plate that has been pre-coated with anti-human NGAL monoclonal antibody. In the micro ELISA plate wells, standards or samples are mixed with the specific antibody. After that, a Biotinylated detection antibody specific for human NGAL and an Avidin-Horseradish Peroxidase (HRP) conjugate are incubated in each microplate well. The components that are not needed washed away. Each well is filled with substrate solution. Only those wells that contain human NGAL, the color of Biotinylated detection antibody and the Avidin- HRP conjugate will be blue. The enzyme-substrate reaction is stopped when stop solution is added, and the color turns to yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The optical density value is proportional to the amount of human NGAL in the sample. By comparing the absorbance of the samples to the standard curve, the concentration of NGAL in the samples was determined.

Reagent Preparation

1- All materials were placed at room temperature (18~25℃) before use.

2- A volume of 30 mL of concentrated wash buffer was added to 720 mL of deionized or distilled water to prepare 750 mL of wash buffer.

3- A standard solution of 50 pg/mL was produced by addition the original 120µl (100 ng/mL) with 120µl of standard diluent. The working solution was let stand for 10 min. Then, as needed, the serial dilutions were produced, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/mL.

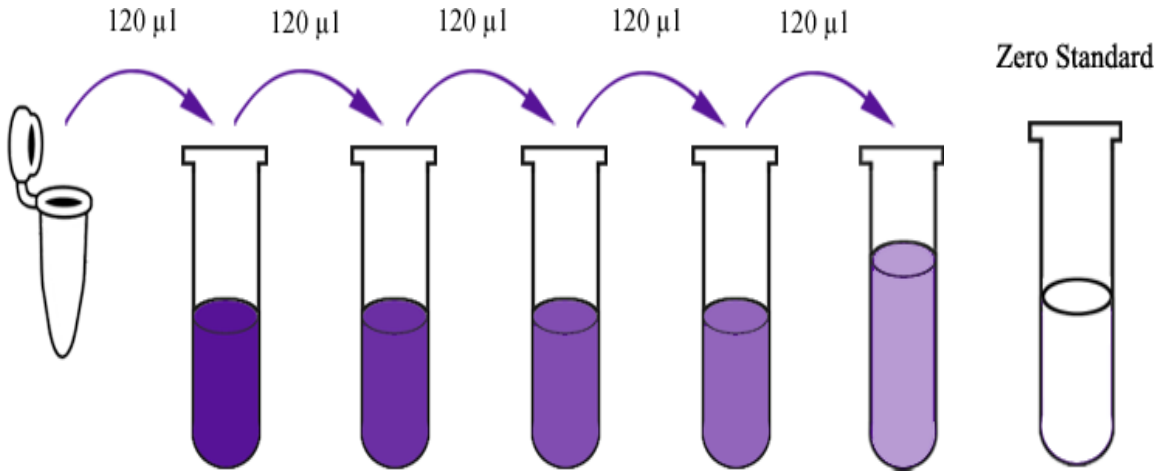


Figure (2.1): Concentration of standards of NGAL

4- The required amount of Biotinylated Detection Ab working solution was calculated before use (100µL/well). The stock tube was utilized by centrifuge before use; the Concentrated Biotinylated Detection Ab was diluted as 100x with Biotinylated Detection Ab Diluent.

5- The required amount of HRP Conjugate working solution was calculated before use (100µL/well). The Concentrated of HRP Conjugate was diluted as 100x with Concentrated HRP Conjugate Diluent.

Assay procedure

1- A volume of 100µl of standard was added to well standard, antibody was not added to a standard well because the standard solution contains Biotinylated antibody.

2- A volume of 100µl from sample was added to each sample wells. The wells were mixed. The plate was covered with sealer. Incubate for 90 min, at 37 °c.

3- Without washing, the liquid was decanted from each well. Each well was filled immediately with 100µl of Biotinylated Detection Ab working solution. The surface of plate was protected by plate sealer. The wells were mixed and incubated for 1 hour at 37°C.

4- The solution was decanted from each well after incubation and added 350 µl of wash buffer to each well. After 1-2 minutes of soaking, the solution was decanted from each well and patted it dry with clean absorbent paper. This wash phase was repeated three times. The ELISA reader was used to complete this phase.

5- Each well was received 100 µl of HRP Conjugate working solution. The surface of plate was protected by plate sealer. The plate was incubated at 37°C, for 30 minutes.

6- The solution was decanted from each well, and then the wash procedure was performed in five times as in step 4.

7- Each well was received 90 µl of Substrate Reagent. The new plate sealer were covered the wells. At 37°C, The plate was incubated for around 15 minutes. The plate was protected from light.

8- The reaction was stopped by adding 50µl of stop solution to each well, and the color change from the blue to yellow immediately.

9- The optical density (OD value) of each well identified directly after applying the stop solution by utilize a microplate is measured spectrophotometrically at 450 nm within 10 min.

Calculation of Results

Known concentration of Human NGAL standard and its corresponding reading absorbance was plotted on the scale (Y-axis) and the scale (X-axis) respectively. The level of Human NGAL in sample is determined by plotting the sample's absorbance on the X-axis as shown in figure (2-3). The dilution factor must be multiplied by the concentration determined from the standard curve for each sample diluted.

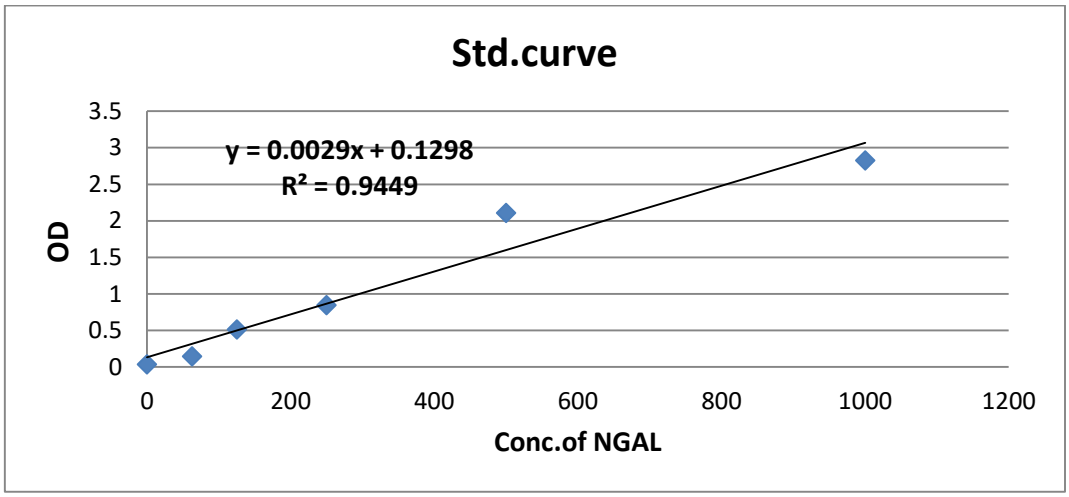


Figure (2-2) Standard curve of NGAL

2.4.3. Determination of Beta2-microglobulin (B2M).

Beta2-microglobulin (B2M) was measured by enzyme linked immunosorbent assay kit.

Principle:-

The sandwich-ELISA technique is used in this ELISA kit. This kit includes a micro ELISA strip plate that has been pre-coated with anti-human

B2M monoclonal antibody. In the micro ELISA plate wells, standards or samples are mixed with the specific antibody. Antibodies of the patient, if present in the specimen, binds to the antigen. In the following step, the unbound fraction is washed off. Anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are then incubated and the samples in the microplates react with the antigen-antibody complex. In the following stage, the unbound conjugate is washed off. The addition of TMB-substrates causes a colorimetric (blue) enzymatic reaction that is stopped by diluted acid (color changes to yellow). The strength of the chromogenic color formation depends on the amount of conjugate attached to the antigen-antibody complex and is proportional to the initial concentration of the respective antibodies in the sample of the patient.The optical density (OD) is measured by spectrophotometrically at a wavelength of 450 nm.

Kit composition of Reagents

Table: (2.3) - Components of B2M Kit

Reagents	Quantity
Adhesive Strip (For 96 well)	4
Antibody diluent	1x 10 ml
Antibody(100 x concentrate)	1x 60 µl
Assay plate (12 x 8 coated microwells)	1(96well)
HRP-conjugate diluent	1x 20 ml
HRP-conjugate(100 x concentrate)	1x 120 µl
Instruction manual	1

Sample diluent	2x 20 ml
standard	2x 250 µl
Stop Solution	1x 10 ml
TMB Substrate	1x 10ml
Wash buffer(25 x concentrate)	1x 20 ml

Procedure

1. A volume of 100µl was pipette from each patient and healthy control diluted serum into the designated microwells.

2. A volume of 100 µl was pipette from calibrators in the suitable wells.

3. Incubate for 30 minutes at 25°C.

4. Wash 3 times using 300 µlof washing buffer.

5. Pipette 100 µl conjugate into each well.

6. Incubate for 30 minutes at 25°C.

7. Wash 3 times using 300 µl of washing buffer.

8. Pipette 100µl TMB substrate into each well.

9. Incubate for 30 minutes at 25°C° and should be protected from intense light.

10. Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.

11. Incubate 5 minutes minimum.

12. Agitate plate carefully for 5 sec.

13. Read absorbance at 450 nm within 30 minutes.

Calculation of Result

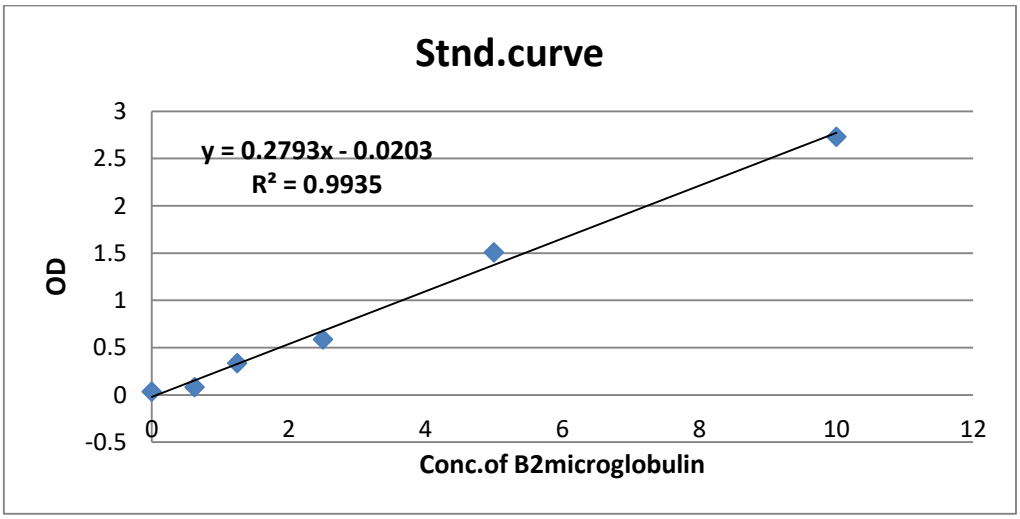


Figure (2.3) Standard curve of B2-microglobulin test

2.4.4. Determination of Human Cystatin-C (CYS-C)

Principle

ELISA kit uses the Sandwich-ELISA principle. The plate has been pre-coated with a human Cystatin C antibody. Cystatin C antibody present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human Cystatin C Antibody is added and binds Cystatin C antibody in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated Cystatin C antibody. After incubation unbound Streptavidin-HRP has washed away during a washing step. The substrate solution is then added and color develops in proportion to the amount of human Cystatin-C antibody. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm.

Kit composition of Cystatin-C:- As shown in the Table (2.4).

Table: (2.4) - Components of Cystatin-C Kit

Reagents	Quantity
20x Wash Solution	25ml x 1 bottle
Chromogen Solution A	6.0mlx 1 bottle
Chromogen Solution B	6.0mlx 1 bottle
Closure plate membrane	2x pieces
HRP-Conjugate Reagent	10.0 ml x 1 bottle
Manual	1x paper
Microelisa Stripplate	96 well plate
Sample diluent	6.0 ml x 1 bottle
Standards	0.5 ml x 6 vials
Stop Solution	6.0ml x 1 bottle

Procedure

1- All reagents were Prepare, standard solutions, and samples as instructed. All reagents were Bring to room temperature before use. The assay is performed at room temperature.

2- I added 50µl standard was added to a standard well (S1,S2,S3,S4,S5,S6).

3- I added 50µl sample was Add to sample wells and then add 10µl anti-Cystatin C antibody to sample wells, 50µl streptavidin-HRP was added to sample wells and standard wells. We Mix and Cover the plate with a sealer. and we Incubate 60 minutes at 37°C.

4- I remove the sealer and wash the plate 5 times with wash buffer at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.

5-I added 50µl substrate solution A was to each well and then we add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

6- I added 50µl Stop Solution to each well, the blue color will change into yellow immediately.

7- The optical density (OD value) determined each well immediately by using a microplate is measured spectrophotometrically at 450 nm within 10 minutes after adding the stop solution.

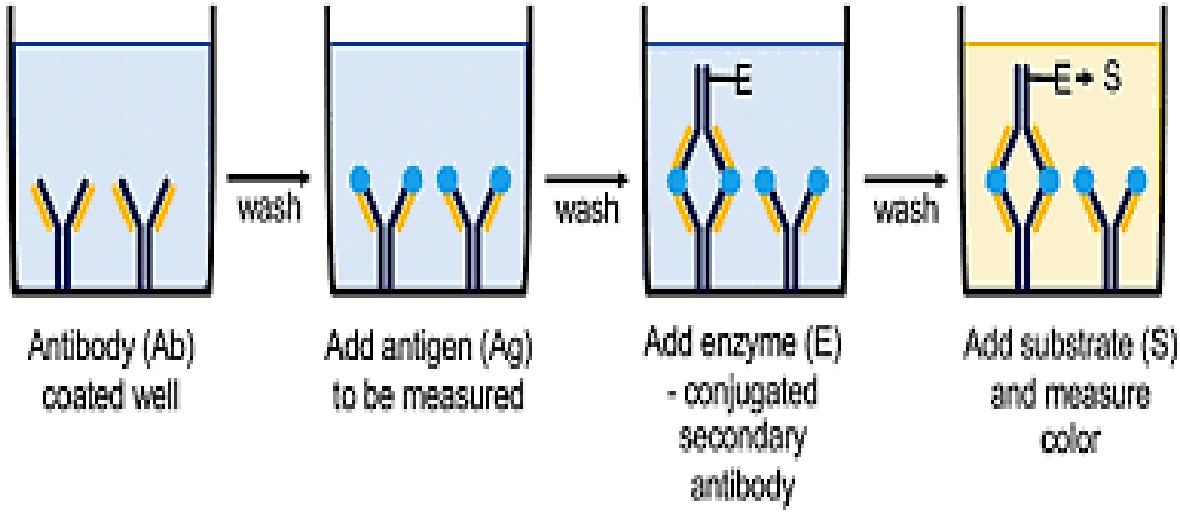


Figure (2-4) principle of sandwich ELISA[137].

Calculation of Result

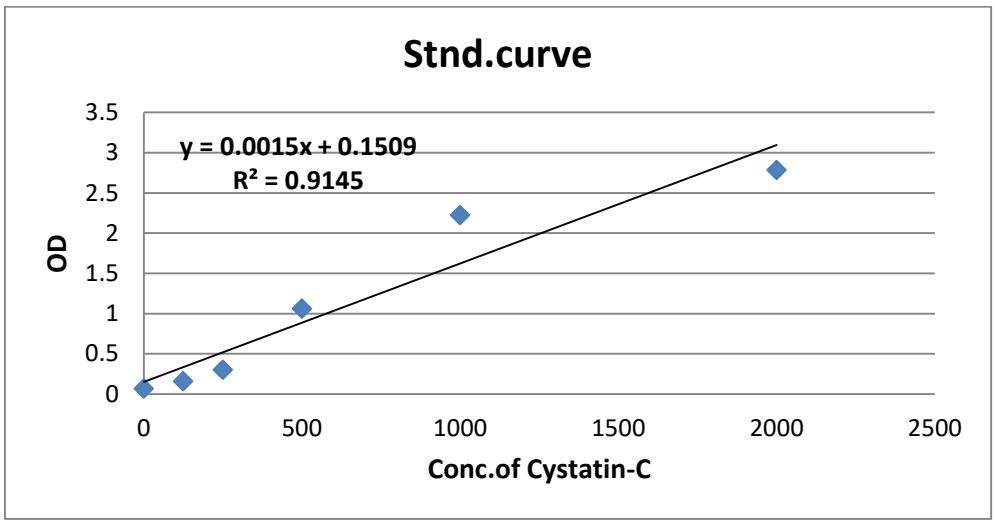


Figure (2.5) standard curve of Cystatine-C test

2.4.5. Determination of Serum Creatinine.

Principle this procedure is based upon a modification of the original picrate reaction (Jaffe). Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid $\xrightarrow{\text{PH>12}}$ Red complex

Reagents Composition

Table: (2.5) - Reagents composition of Creatinine Kit

Reagent 1	**R1**	**Disodium Phosphate 6.4 mmol/L Sodium hydroxide 150 mmol/L**
Reagent 2	**R2**	**Sodium dodecyl sulfate 0.75 mmol/L Picric acid 4.0 mmol/L**

Standard	**R3**	**Standard 177 µmol/L (2 mg/dL)**

Procedure

All reagents and specimens were placed at room temperature before using

Table: (2.6) - Procedure of serum creatinine.

Working Reagent (R1+R2)	1000 Ml
Serum	1000 Ml

The contents were mixed and the kinetic test were performed at 37 o C. After 30 seconds, the absorbance A1 was read and 120 seconds later, the absorbance A2 was read against distilled water is measured spectrophotometrically at 490 nm (490-510).

Calculations

The results were calculated as follows:

$$\text{Result} = \frac{\text{Abs Assay (A2 − A1) – Abs Blank (A2 − A1)}}{\text{Standard(A2−A1)}} \text{ x (2 mg/dl)}$$

Reference Intervals[138].

Male = 0.9 -1.3 mg/dl

Female = 0.6 - 1.1 mg/dl

2.4.6. Determination of Blood Urea.

Principle

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated forms with chloride and salicylate a blue – green complex. The intensity of the color formed is proportional to the concentration of urea in the sample.

Reagents Composition

Table: (2.7) - Reagents composition of Urea Kit

Reagent 1	R1	Salicylate 31 mmol/L Nitroprussiate 1.67 mmol/L
Reagent 2	R2	Urease ≥ 15 UI/L
Reagent 3	R3	Sodium hypochloride 7 mmol/L Sodium hypooxide 62 mmol/L
Standard	R4	Standard urea 40 mg/dL

Procedure

The reagents and specimens were placed at room temperature before using.

Table: (2.8) - Procedure of blood urea.

Pipette into test tubes	Blank	Standard	Sample
Working Reagent (R1+R2)	1ml	1ml	1ml

Distilled water	10 µl		
Standard		10 µl	
Serum			10 µl
The tubes were mixed and incubated for 2 min at 37oC			
Reagent 3 (R3)	1ml	1ml	1ml

The contents were mixed and let stand for 8 min at room temperature or 5 min at 37o C. the absorbance is measured spectrophotometrically at 600 nm against blank.

Calculations

The results were calculated as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (standard)}} \text{ x Standard concentration (40 mg/dl).}$$

Abs (standard)

2.4.7. Determination of Serum Ferritin

Serum Ferritin level was measured by VIDAS technique.

Principle

The VIDAS Ferritin assay is enzyme-linked fluorescent immunoassay (ELFA). Performed in an automated instrument all assay steps and assay temperature are controlled by the instrument apipette tip-like disposable device, The Solid Phase Receptacle (SPR) functions as both the solid phase and the

assay's pipetting mechanism. The SPR is coated at time of manufacture with mouse monoclonal anti-ferritin antibodies The test reagents are predispensed in sealed reagent strips and are ready to use. The instrument performs all of the assay processes automatically.

The ferritin in the sample binds to the specific monoclonal antibody conjugate with alkaline phosphatase on the interior of the SPR to retained ferritin during the washing procedure, any unbound conjugate is removed.

A fluorescent substrate, 4-Methylumbelliferyl phosphate is cycled in and out of the SPR during the final detection stage. The conjugate enzyme catalyzes the hydrolysis of this substrate to produce a fluorescent product (4-Methylumbelliferone) with 450 nm fluorescence. The fluorescence intensity is proportional to the amount of ferritin present in the sample.

Kit composition

Table: (2.9) - Components of Ferritin Kit

60 FER SPR (2X30)	SPR	Ready to use .SPRs coated with mouse monoclonal anti-Ferritin antibodies
FER Control (liquid) (1x2ml)	C1	Ready to use .TRIS buffer(0.1 mol/l,PH 7.4) with human spleen ferritin and protein and chemical stabilizers ,MLE data indicate the confidence interval in ng/ml (control C1 dose value renge)
FER strips (60 STP)	STR	Ready to use.

FER Dilution Buffer (liquid) (1x25ml)	R1	Ready to use. TRIS buffer(0.1 mol/l,PH 7.4) and protein and chemical stabilizers
FER Calibrator (liquid) (1x2ml)	S1	Ready to use. TRIS buffer(0.1 mol/l,PH 7.4) with human spleen ferritin and protein and chemical stabilizers ,MLE data indicate the Calibrator concentration in ng/ml(1^{st} IRP 80/578)(calibrator (S1) dose value) and the confidence interval in Relative fluorescence value (calibrator(S1) RFV Range)

Procedure

The Ferritin strips and Ferritin SPR were inserted into the instrument then 100 µl were added from each sample to wells no.1 of strips. The instrument performs all the assay steps automatically. Calibration was performed each 14 days and its curve is stored in memory while quality.

Reference Range

Normal serum ferritin: Man 70-435 ng /ml

Women 25-280 ng/ml

2.4.8. Determination of CBC Testes

A CBC gives your provider a picture of your overall health. Using a small amount of blood, a CBC can help detect hundreds of conditions, disorders and infections. It allows your provider to monitor your health, screen for disease and plan and adjust treatment.

Performed by an automated hematology analyzer, which counts cells and collects information on their size and structure. The concentration of hemoglobin is measured, and the red blood cell indices are calculated from measurements of red blood cells and hemoglobin. This test(s) was done by automated system SYSMEX (CE approved)

Normal ranges for a complete blood count:-

Hemoglobin normal range:

- Male (ages 15+): 13.0 - 17.0 g/dL
- Female (ages 15+): 11.5 - 15.5 g/dL

Hematocrit normal range:

- Male: 40 - 55%
- Female: 36 - 48%

Red blood cell normal range:

- adult : 4.35- 5.65 $*10^{12}$ / L

Platelet Count normal range:

- Adult: 150,000 - 400,000/mL

White blood cell (WBC) normal range:

- Adult: 5,000-10,000/mL

2.4.8. Determination of GOT and GPT Testes

Serum GOT, GPT level was measured by Cobas E411 analyzer technique

The Cobas E411 analyzer is a fully automated analyzer that uses a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis. It is designed for both quantitative and qualitative in vitro assay determinations for a broad range of applications (including anemia; bone, cardiac and tumor

markers; critical care; fertility/hormones; and infectious diseases). The analyzer is available as a rack sample handling system**.**

The Clinical Biochemistry Analyzer is an instrument that uses the pale yellow supernatant portion (serum) of centrifuged blood sample, and induces reactions using reagents to estimate the values of serum transaminases (GOT, GPT)

Normal ranges:-

GOT = 0 – 40 U/L

GPT = 0 – 41 U/L

2.5. Statistical Analysis.

Statistical analysis was carried out using SPSS version 26. Continuous variables were given as (Mean± SD) while categorical variables were provided as frequencies and percentages. Student t-test was used to compare means between two groups and the correlation test (Pearson test) was performed to find the association between variables. P-value less than 0.05 was considered as significant. ROC survival test was used for evaluating the ability of study parameters to discriminate disease from non-disease subjects[139].

3. Results and Discussion.

3.1. Demographic and Clinical Characteristics of the Study Group

3.1.1Gender Distribution in patients and control

The gender distribution of the studied groups was 45 patients with β-thalassemia major, 18 (40.00%) male and 27 (60.00%) female, matching with controls and the results represented in figure (3-1).

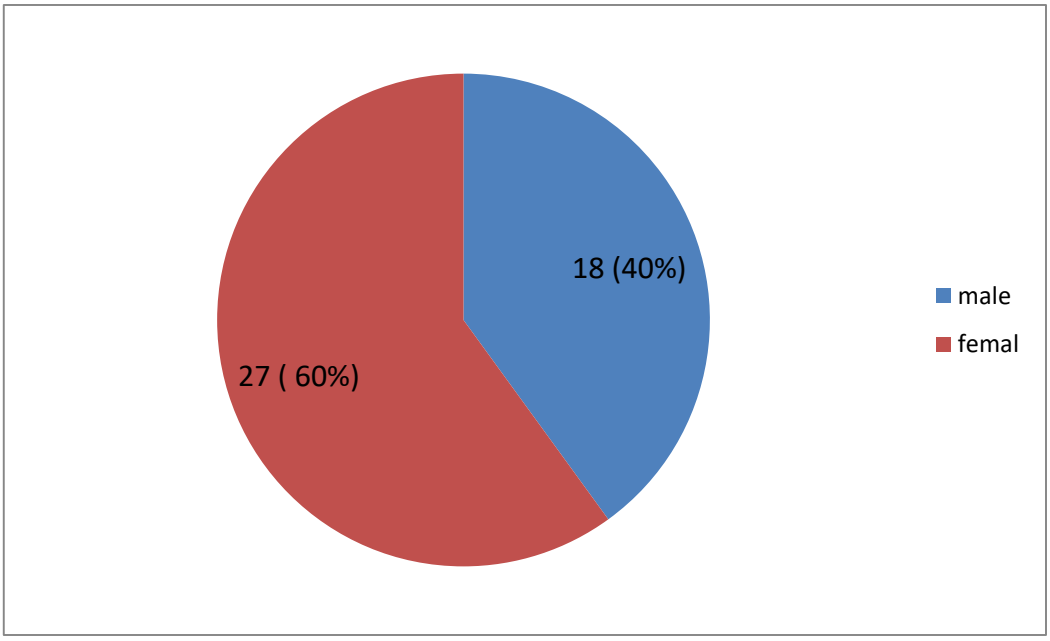


Figure (3-1) Gender distribution of patients & controls

3.1.2 Age Distribution in patients and control

The distribution of patients with β- thalassemia major according to age is shown in Figure (3.1). A total of patients with thalassemia included in this study whose ages ranged (18-35) years. Control group apparently healthy subjects with an age range (18-35) years

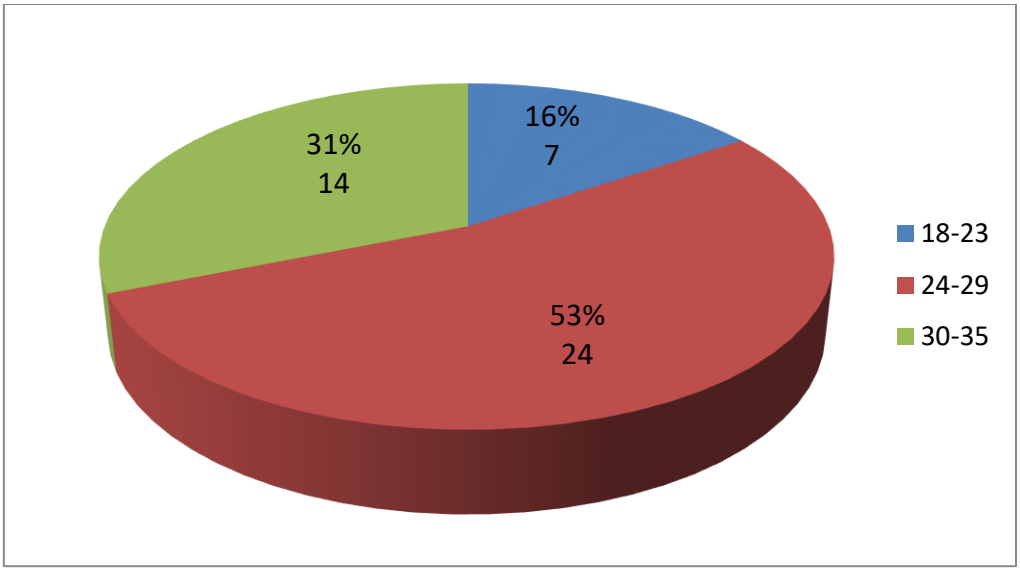


Figure (3-2) Age groups of patients with BTM

3.2. Biochemical Parameters.

3.2.1. Neutrophil Gelatinase Associated Lipocalin (NGAL).

Assessment of NGAL in Thalassemia patient is showed that the Mean ± SD for patient was (711.0 ± 198.6) pg/ml and for healthy control was (187.4 ± 41.82) pg/ml respectively. The findings demonstrated a significant difference in NGAL levels between patients and their control group (P< 0.01), as shown in the Table (3.1) and Figure (3.1).

Table: 3.2- Comparison of NGAL level in patients and control groups.

	Group	N	Mean ± SD	p-value
NGAL pg/ml	Patients	45	711.0 ± 198.6	0.001
	Control	45	187.4 ± 41.82	

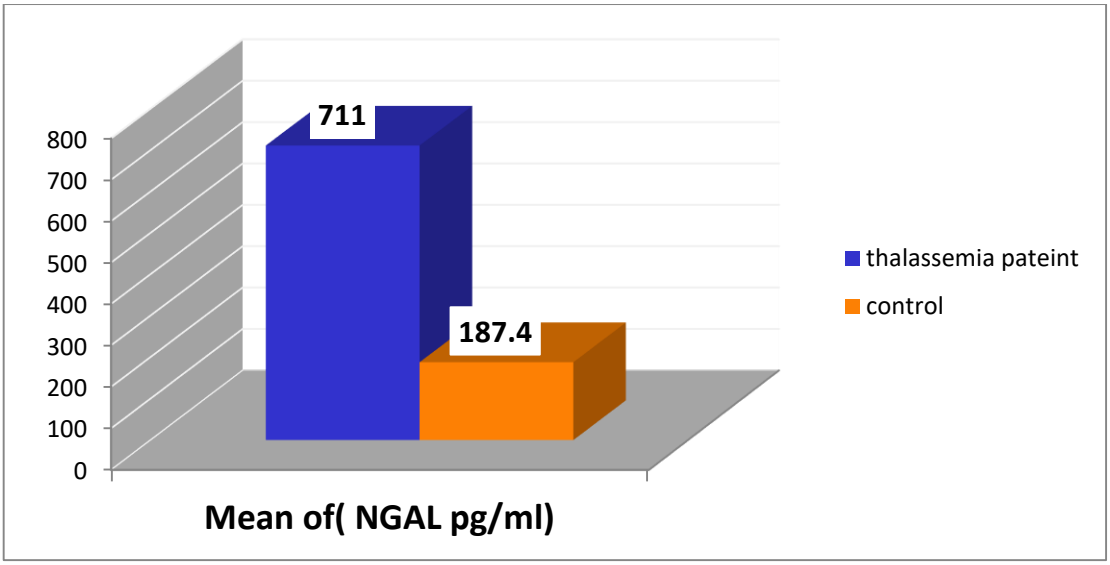


Figure (3-3) Comparison of NGAL level in patients and control groups.

Increased NGAL level in the serum indicate the presence of an injury to the renal tubes. Elevated serum NGAL in individuals with βthalassemia major related to renal dysfunction [140] .

According to the results of our study, renal tubular dysfunction is prevalent in patients with βTM. Also, we found that increased plasma NGAL level can be considered as the beginning of renal tubular injury in patients with βTM. Laboratory measurements of the renal function in these patients at certain time points can prevent further complications. Regarding the prevalence of renal tubular dysfunction in patients with TM and unawareness of the patients about it, a periodic evaluation of renal function can prevent progression of renal dysfunction by identifying them early this agreed with other study (Sadeghi, Mohsen Vakili, et al. 2021)[92]

The results of the current study are agreed with others studies reported that β-thalassemia major patients had higher levels of serum NGAL compared to control (Zainab Fathi, et al. 2021; Maria Domenica Cappellini MD,et al. 2017). The principal finding of the current study is that patients with β-thalassemia major had considerably elevated levels of serum NGAL compared

to control independent of age and sex which is in agreement with another study by (Karaman K, Şahin S, Geylan H, et al. 2019) alrevealed that BTM patients receiving deferasirox and regular blood transfusions had considerably greater levels of NGAL[119].

3.2.2. Beta2-microglobulin (β2M)

Assessment of β2-microglobulin in Thalassemia patient is showed that the Mean ± SD for patient was (7.1 ±1.6) mg/ml and for healthy control was (1.4 ± 0.95) mg/ml respectively. The results revealed that there was a significant difference in the level of β2M between patients and their control group (P<0.05), the means, standard deviation, and statistical parameters are listed in the Table (3.2) and in Figure (3-2).

Table: 3.3- Comparison of β2M level in patients and control groups.

	group	N	Mean ± SD	P-value
β2-microglobulin mg/ml	patients	45	7.1 ±1.6	0.001
	control	45	1.4 ± 0.95	

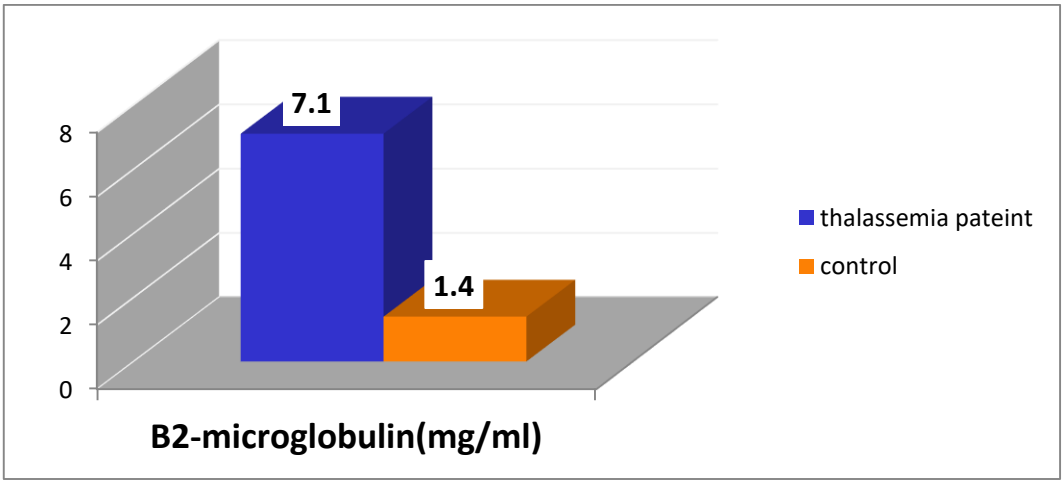


Figure (3-4) Comparison of B2-microglobulin level in patients and control groups

Low-molecular-weight protein known as urinary β2 microglobulin is freely filtered by glomeruli, reabsorbed by renal tubules, and subsequently excreted. Is considered to be a more accurate endogenous indicator of early glomerular filtration rate (GFR). A sensitive early biomarker for glomerular and tubular failure in BTM is β2MG[141].

According to our study and other studies, the damage's exact cause is unknown, although it may be related to an increase in oxidative stress brought on by the buildup of iron in the tissues[142]. In the present study thalassemic patients showed higher levels of β2-microglobulin than control groups. The underlying mechanisms for tubulopathy in patients with β-TM include long-standing anemia, chronic hypoxia, and iron overload and DFO, this is in agreement with Hamed and ElMelegy,(2010) [143].

The current study revealed that there was significant difference in urine β2M between patient groups and the healthy group the results are agreed with others studies according to Kacar et al.[144] β2M as well as serum ferritin and liver iron deposition were found to be significantly positively correlated, this results agreement with study by Uzun et al[145].

3.2.3. Cystatin-C (CYT-C):-

Assessment of CYT-C in β-Thalassemia major patient is showed that the Mean ± SD for patient was (1091.8 ± 485.79) ng/mL and for healthy control was (360.7 ± 53.88) ng/mL respectively. The findings demonstrated a significant difference in Cystatin-C levels between patients and their control group ($P< 0.05$), as shown in the Table (3.3) and in Figure (3-3).

Table: 3.4- Comparison of CYT-C level in patients and control groups.

Group	group	N	Mean ± SD	p-value
Cystatin-C ng/ml	patients	45	1091.8 ±485.79	0.003
	control	45	360.7 ± 53.88	

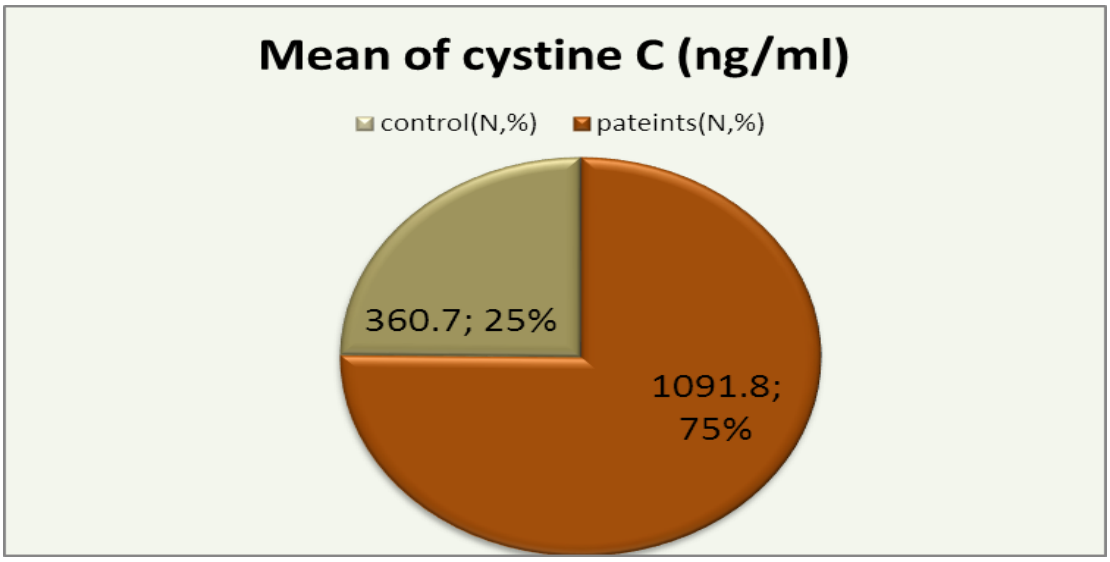


Figure (3-5) Comparison of Cystatin-C level in patients and control groups.

The result show in Table (3.3) is comparison of the level of CYT-C in serum between the patient group and the healthy control group that revealed a high significant increase in the patient group ($p < 0.05$). Increased CYT-C level in the serum indicate the presence of an injury to the renal tubes[127].

The current study revealed that little variations in cystatin C levels after deferasirox therapy did reflect kidney damage which is in agreement with another study by Papassotiriou et al. (2010) [146]. As well, elevated Cys-C levels were found to be related with take DFX doses in our study, Besides among patients with hyperfiltration. That's why elevated Cys-C levels should be thought as an early sign of renal injury in βTM patients, On the other hand, it

is known that most commonly used oral chelator, DFX, has nephrotoxic side effects causes increase Cystatin-C parameter this agree with study by Gokce, MKup, HTugcu, et al[147].

Several findings in our study are Increased CYT-C level in β-thalassemia major patient comparison with healthy group also agree in line with our study regarding β-thalassemia major (Behairy et al. 2017b; Mohammed Abdulrazzaq 2020). Our study Compared to serum creatinine, cystatin C is expected to be a more potent and reliable endogenous biomarker for GFR. Interestingly, a study by Badeli et al. (2019) [146].

3.2.4. Serum Creatinine and Blood Urea.

The results revealed that the level of serum Creatinine and Urea is within the normal limit, the Mean± SD for B.Urea mg/dl in β-TM and control groups are(30.4 ± 8.97) (19.2 ± 7.47) respectively. As well the Mean± SD for Creatinine mg/dl in β-TM and control groups are (0.45 ± 0.10) (0.60 ± 0.23) respectively are listed in Figure (3.4).

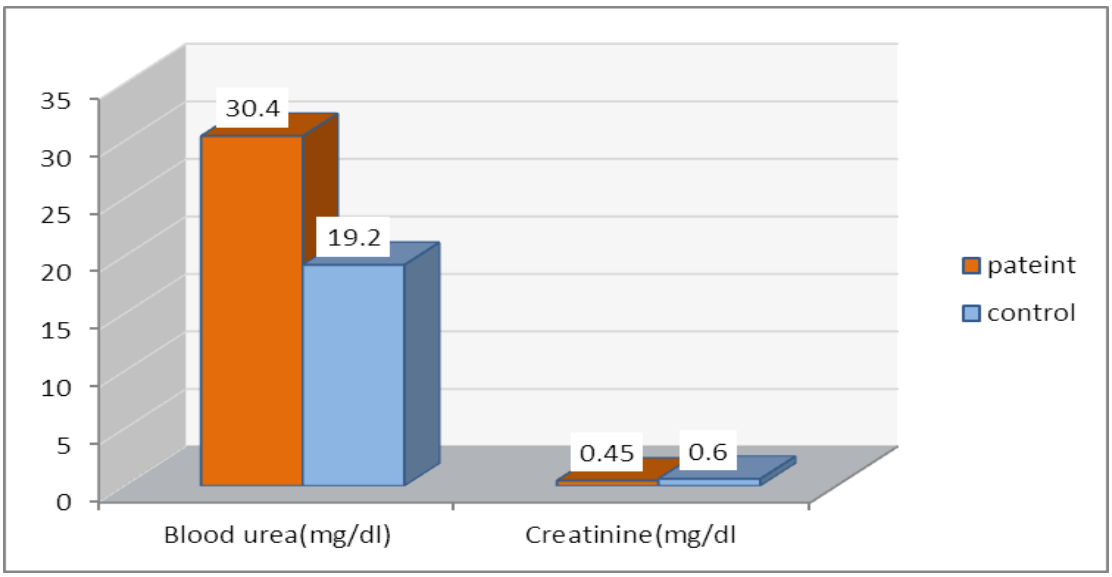


Figure: 3.6- Comparison of S-Cr, Urea level in patients and control groups.

Although the level of urea and creatinine is within the normal limit, there is difference between the patients and the control group, and this is similar to the study conducted by Dr.Zeina A.Althanoon. et al .2020, Reem K Dwaik BSc. et al. 2022.[148].

The current study is agree with recent studies are shown that to control renal function in deferasirox users, the levels of serum urea, creatinine are measured. However, Serum creatinine and serum urea levels do not accurately determine the primary renal injury stages[149] . The fact that serum creatinine and urea are affected by several factors such as ; muscle mass, protein intake, inflammatory disease and liver disorder this agree with study by A. Nickavar, A. Qmarsi, S. Ansari, and E. Zarei,2017 [150].

Our study shown that serum Creatinine and serum urea were non-significantly and their levels are within the normal range in the β-TM group and the control group. Consistent with study by Karaman, Kamuran Şahin, Serdar Geylan, et al.[151].

3.2.5. Serum Ferritin

Assessment of Ferritin in Thalassemia patient is showed that the Mean ± SD for patient was (3701±1974) Ng/ml and for healthy control was (135 ± 88) Ng/ml respectively. The findings demonstrated a significant difference in Ferritin levels between patients and their control group ($P< 0.01$), as shown in the Table (3.5) and Figure (3-5).

Table: 3.6- Comparison of Ferritin level in patients and control groups.

	Group	N	Mean ± SD	p-value
Ferritin Ng/ml	Patients	45	3701 ±1974	0.001
	Control	45	135 ± 88	

S.Ferritin levels provide another option for prognostic factors to predict a range of clinical outcomes in patients with β-thalassemia[152]. Evaluated frequency, pattern, and associations of renal iron accumulation in β-thalassemia major. β-thalassemia major patients had a significantly high frequency of renal Iron overload[153].

The current study revealed that because of frequent blood transfusion, iron deposition occurs in visceral organs such as the heart, liver, endocrine glands, kidney, etc., causing tissue damage and ultimately, organ dysfunction or failure. Although chelating therapy considerably improves the survival in patients with chronic transfusion, multi-organ failure secondary to hemosiderosis is still common[154].

On the other hand, serum ferritin measurement is still a repeatable and low-cost method to roughly assess both iron overload and the effectiveness of iron chelation therapy[155].

Serum ferritin levels may be used as a prognostic marker for predicting renal recovery in renal impairment patients[156]. This result similar to study was done by (Musallam et al. 2014).(Mohamed R. El-Shanshory. et al. 2021)[157].

3.2.6. Complete Blood Study

Assessment of HB, PCV, WBC, Platelet in β-thalassemia major patient is showed that the Mean ± SD for patient and for healthy control as shown in the Table (3.6).The findings demonstrated a significant difference in HB, PCV, WBC, levels between patients and their control group (P ≤0.05) and The findings demonstrated no significant difference in Platelet between βTM patients and their control group that the Mean ± SD for patient and for healthy control (359.8 ± 131.4) (289.8 ± 71.7) 10/UL respectively.

Table: 3.7- Comparison of HB, PCV and WBC level in patients with βTM and control groups.

	Group	N	Mean ± SD	p. value
HB g/dL	Patients	45	8.7 ± 1.1	0.001
	Control	45	12.6 ± 0.96	
PCV %	Patients	45	27.7 ± 4.2	0.001
	Control	45	39.4 ± 3.0	
WBC 10/UL	Patients	45	12.6 ± 5.3	0.03
	Control	45	7.57 ± 2.1	

Complete blood count (CBC) is a quick, inexpensive, and easily accessible test which is used as the primary test for the diagnosis of anemia. To date, numerous red blood cell (RBC) indices have been investigated and various parameters have been proposed for each index.[158]

All parameters (HB, PCV, WBC) we analyzed were lower in the βTM group compared to the control group, in agreement with the results of Batebi et

al. The result will be completely different when the comparison is done between βTM group and control group [159]**.**

3.2.7. GOT and GPT Testes

The results revealed that the level of serum GOT and GPT is within the normal limit, the Mean± SD for GOT U/L in β-TM and control groups are(36.2 ± 11.2) (14.178 ± 9.0) respectively. As well the Mean± SD for GPT U/L in β-TM and control groups are (33.7 ± 24.8) (18.6 ± 8.32) respectively.

Table: 3.8- Comparison of GOT, GPT level in patients and control groups.

	group	**N**	**Mean ± SD**
GOT U/L	Patients	45	36.2 ± 11.2
	Control	45	14.178 ± 9.0
GPT U/L	Patients	45	33.7 ± 24.8
	Control	45	18.6 ± 8.32

The patients with β-thalassemia major need periodic blood transfusions that can result in accumulation of body iron, so treatment with iron chelating agent is required. Complications of this iron overload affecting many vital organs, including the liver[160].

Hepatic iron overload leads to different degrees of liver fibrosis, the severity of which is closely correlated with the severity of liver iron overload.The pattern of iron deposition seen in the initial stages of thalassemia major is preferentially sinusoidal with a more or less diffuse distribution within the acinus. Hepatic dysfunction was mild in patients with BTM who were seronegative for hepatitis[161].

Our results show that the results are within normal, and there is a possibility that the liver will be affected after a while and it needs follow-up in thalassemic patients. So the proper use of chelating agents in thalassemic patients seems to be of great importance in delaying progression of the liver disease.[162] Iron chelation therapy (with each of the three available chelators) seems to restore iron balance and reduce the risk of mortality. Several studies are needed to better understand all the possible consequences of iron overload (e.g., in maintaining chronic infections or in accelerating tumorigenesis) and how chelators can reverse tissue damage[163].

3.3. Correlation between parameter

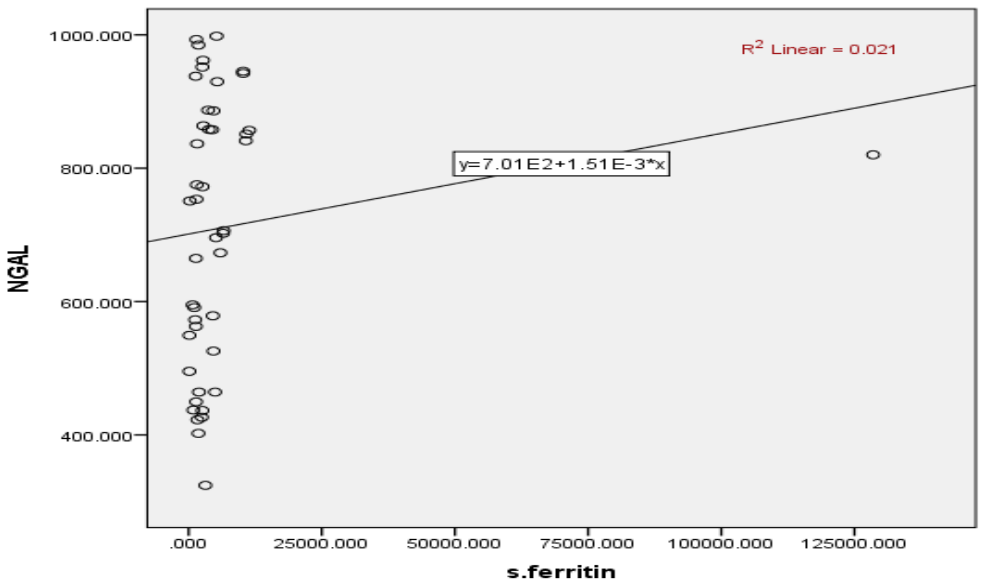
The correlation coefficient is a statistical measure of the strength of a linear relationship between two variables. Its values can range from -1 to 1. A correlation coefficient of -1 describes a perfect negative, or inverse, correlation, with values in one series rising as those in the other decline, and vice versa. Coefficient of 1 show a perfect positive correlation, or a direct relationship. A correlation coefficient of 0 means there is no linear relationship[164].

Table 3.9-Correlation between, CYS-C, NGAL, B2M and ferritin in BTM and control

Correlations							
		S.ferritin	B.Urea	Createnine	NGAL	Cystatin-C	B2-microglobulin
S.ferritin	Pearson Correlation	1	.220*	-0.101	.269*	0.274	0.25*
	Sig. (2-tailed)		0.037	0.344	0.010	0.069	0.054
	N	90	90	90	90	90	90
B.Urea	Pearson Correlation	.220*	1	.218*	.476**	0.132	.274**
	Sig. (2-tailed)	0.037		0.039	0.000	0.215	0.009
	N	90	90	90	90	90	90

Createnine	Pearson Correlation	-0.101	.218*	1	-.358	-0.156	-0.122
	Sig. (2-tailed)	0.344	0.039		0.001	0.142	0.252
	N	90	90	90	90	90	90
NGAL	Pearson Correlation	.269*	.476**	-.358	1	.425**	.341**
	Sig. (2-tailed)	0.010	0.000	0.001		0.000	0.001
	N	90	90	90	90	90	90
Cystatin-C	Pearson Correlation	-0.010	0.132	-0.156	.425**	1	.271**
	Sig. (2-tailed)	0.928	0.215	0.142	0.000		0.010
	N	90	90	90	90	90	90
B2-microglobulin	Pearson Correlation	0.25	.274**	-0.122	.341**	.271**	1
	Sig. (2-tailed)	0.054	0.009	0.252	0.001	0.010	
	N	90	90	90	90	90	90

3.3.1. Correlation between Ferritin and other parameter



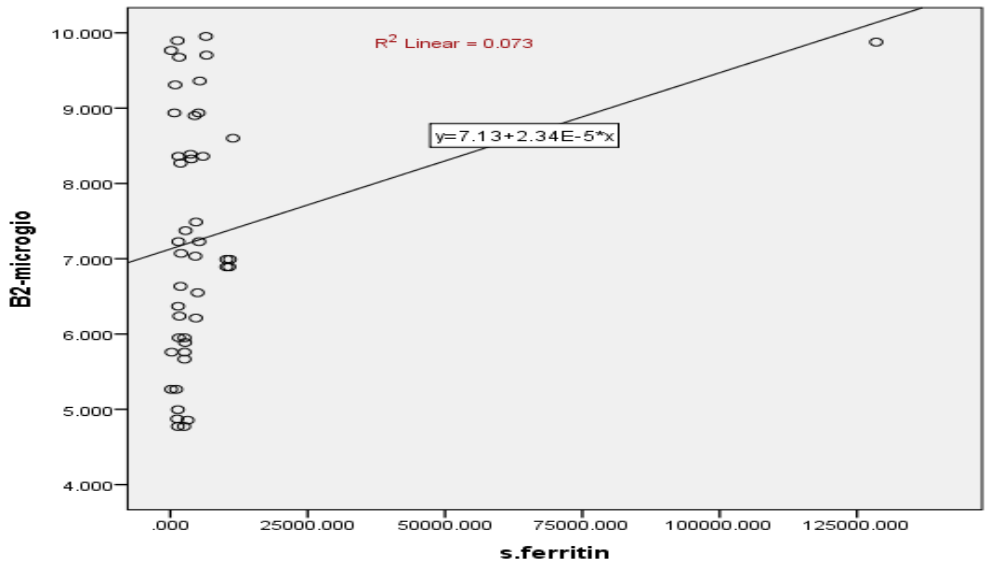
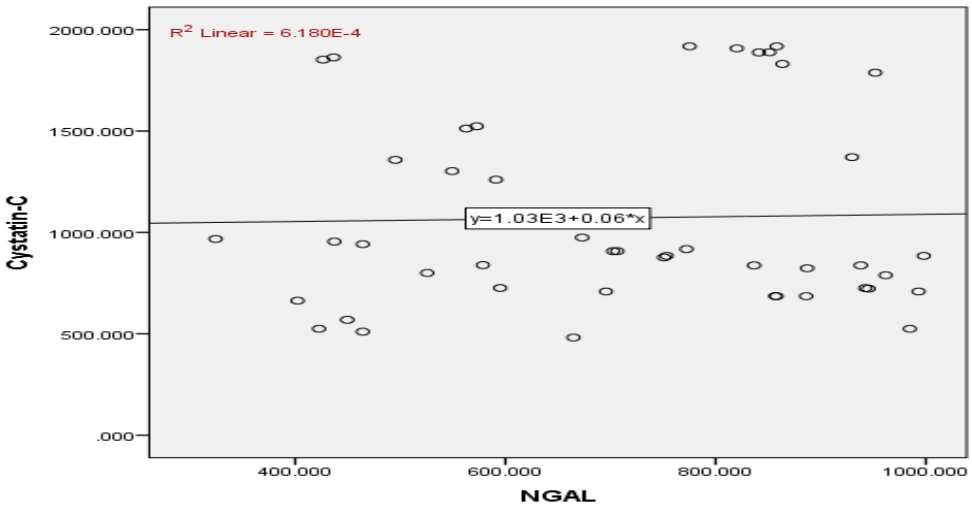


Figure 3.7Correlation between Ferritin and other parameter

The present study showed no significant correlation between ferritin and (Cr, Cystatin-C) in BTM patients (P>0.05) .otherwise in our study, there was a significant positive correlation between ferritin and NGAL (P≤0.01) (r=0.26), and between ferritin and B.Urea where (P≤0.05) (r=0.22), ferritin and B2-microglobulin (P≤0.05) (r = 0.25). The information exposed in Table (3-9) and Figure (3-7). This confirms that there is a strong correlation between Ferritin and B.Urea and NGAL this may be related to renal dysfunction in β-thalassemia major patients. These results confirm that there is risk of glomerular and tubular dysfunction may increase with iron buildup in the body. The finding of current study is decided with a previos study of Pradana Zaky Romadhon, Ami Ashariati, et al.(2022)[65].

3.3.2. Correlation between NGAL and other parameter



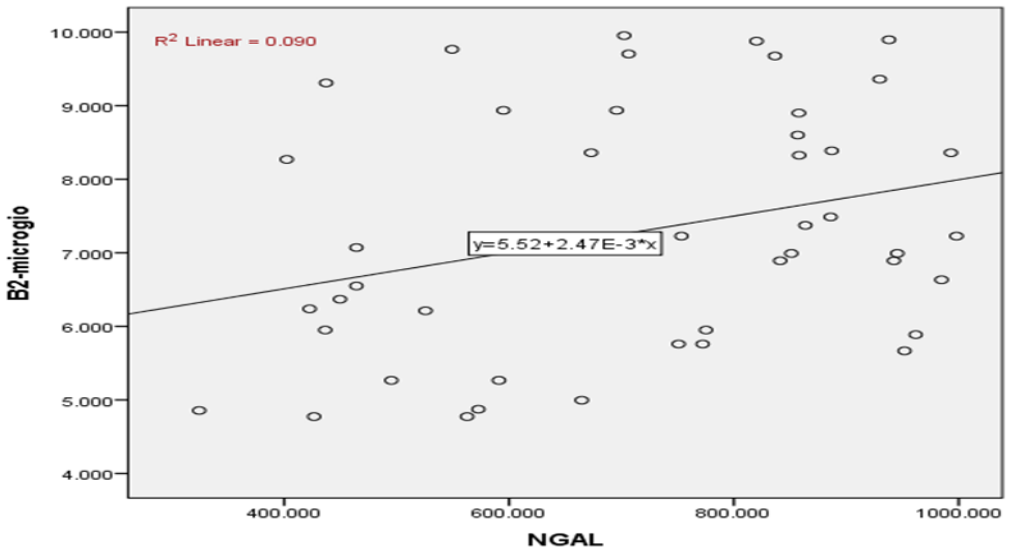
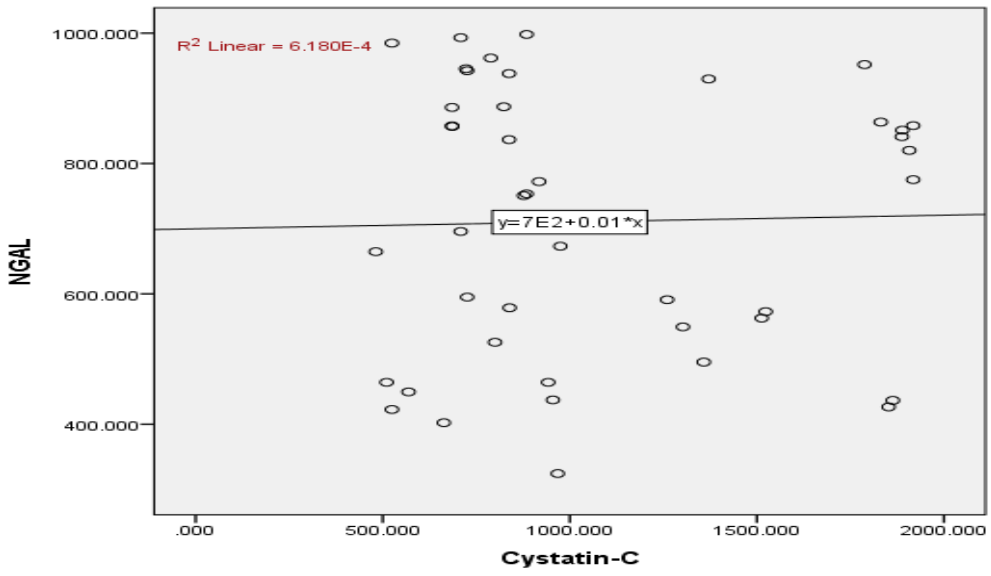


Figure 3.8 Correlation between NGAL and other parameter

The result of the current study showed significant correlation between NGAL and traditional parameters ferritin and B.Urea ($p<0.05$)($r=0.26$),($p<0.01$) ($r=0.47$) respectively for diagnosis renal impairment in βTM and NGAL with new parameters Cystatin-C, B2-microglobulin ($p<0.01$)($r=0.42$)($r=0.34$) respectively showed in a Table (3-9) and Figure (3-8). This confirms that there is a strong correlation between NGAL and all other parameters this is related to renal dysfunction in β-thalassemia major patients. It is possible that the increase in the renal dysfunction markers with increasing NGAL with other parameters. After ischemic or nephrotoxic injury, intra renal NGAL is upregulated and increase the finding of current is agree with of Hashemieh, Mozhgan,(2020)[3].

3.3.3. Correlation between Cystatin-C and other parameter



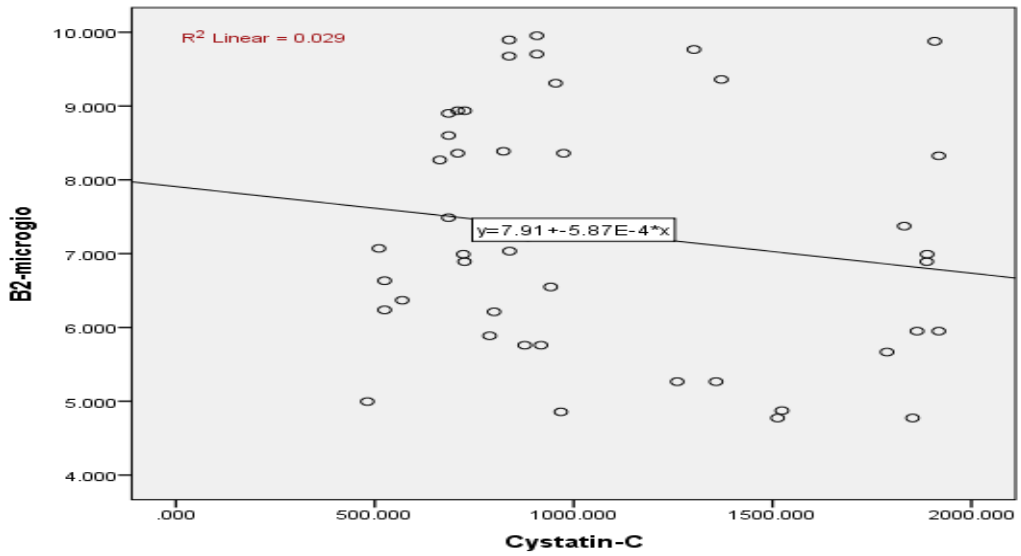
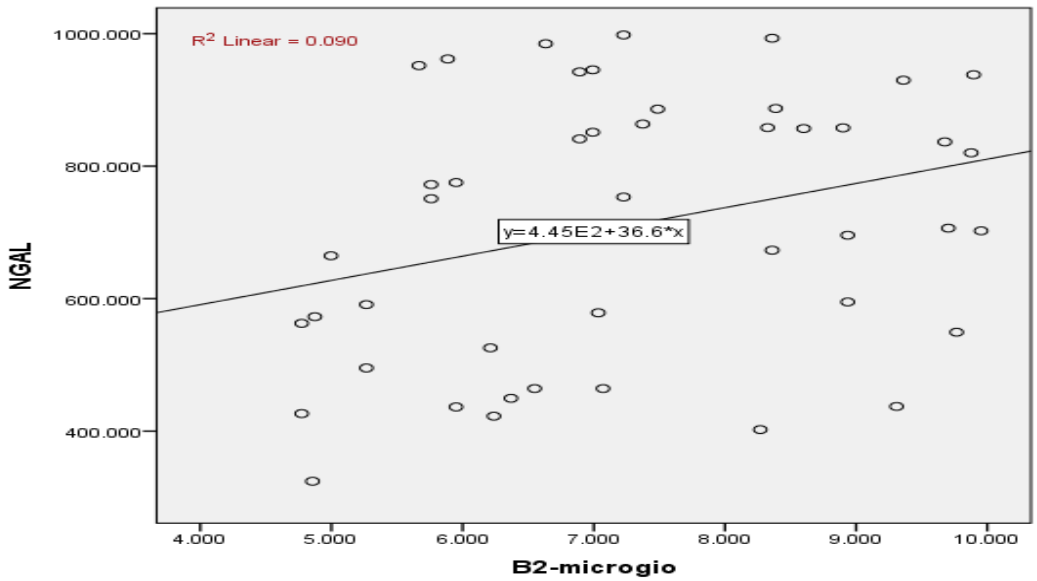


Figure 3.9 Correlation between Cystatin-C and other parameter

The recent study showed significant negative correlation between Cystatin-C and Ferritin, B.Urea and Cr ($p>0.001$) within βTM patients, as shown in table (3-9). Moreover, in our study, there was a significant positive correlation between Cystatin-C and (NGAL and β2-microglobulin) ($p\leq0.001$) ($r=0.42$) ($r=0.27$) respectively as publicized in Figure (3-9). From this we conclude that a strong and direct relationship between Cystatin-C and NGAL and β2MG in Renal impairment in β-thalassemia major patients. An increase in Cystatin-C, NGAL and β2MG is evidence of tubular dysfunction that may be a consequence of chronic anemia, hypoxia or nephrotoxicity[165].

3.3.4. Correlation between B2-microglobulin and other parameter



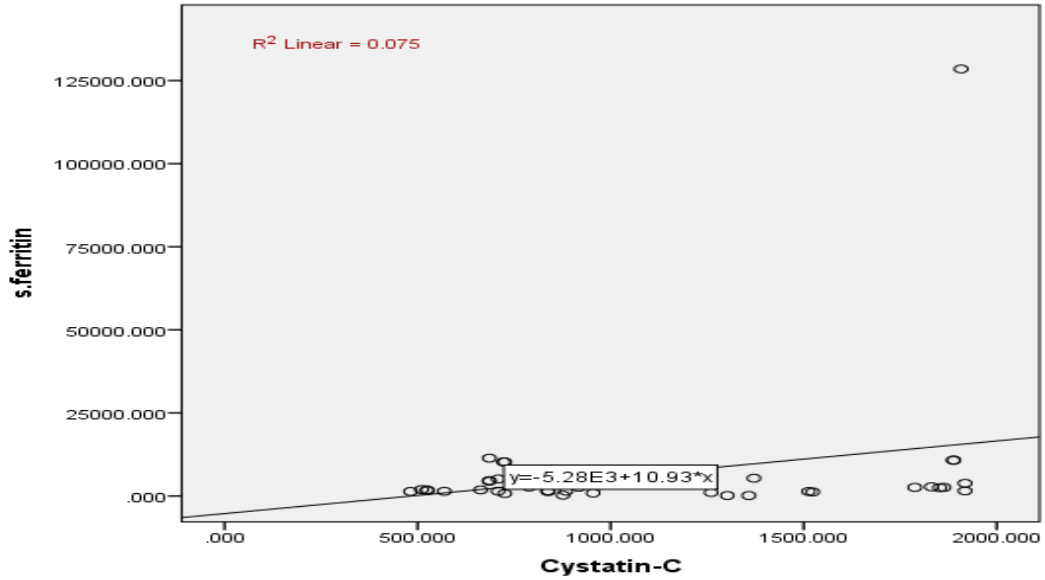


Figure 3.10 Correlation between B2-microglobulin and other parameter

The current study showed no significant negative correlation between B2-microglobulin with B.Urea and Cr ($p>0.001$) within βTM patients. Otherwise in our study showed a significant positive correlation between β2-microglobulin and other biomarker (NGAL, ferritin, and Cystatin-C) ($p<0.001$) Details are exposed in Table (3-9) and Figure (3-10). This confirms that there is a strong correlation between β2-microglobulin and (NGAL, Cystatin-C and ferritin) in renal dysfunction in β-thalassemia major patients. From these result we can explain the relationship between β2-microglobulin with (NGAL, Cystatin-C and ferritin) associated with renal impairment in β-thalassemia major patients. Our findings are similar to findings of Jalaly et al. are demonstrated that there is a positive relation between renal dysfunction in thalassemia major patients with increasing of B2-MG with other parameter lead to glomerular and tubular dysfunction[166].

3.4. ROC curve of biochemical parameters

The receiver operating characteristic curve (ROC curve) was used to evaluate the diagnostic values of parameters in discrimination between beta thalassemia major patients and controls.

3.4.1. ROC Curve of Neutrophil Gelatinase Associated Lipocalin (NGAL)

ROC curve for the sensitivity and specificity of NGAL (Pg/mL) for diagnosis of renal dysfunction in beta thalassemia major, (Cut-off point was ≥ 296 (Pg/ml)) , AUC=0.99, P ≤ 0.006, 95% CI (651.8-770.2), the sensitivity and the specificity was 97.1 %, 89.9 % respectively, as shown in Figure (3.11)

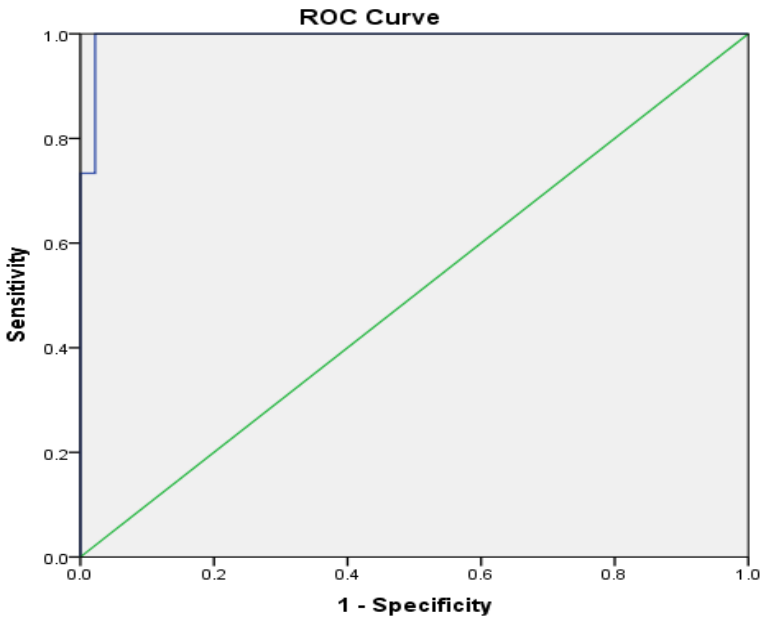


Figure (3.11) ROC Curve of (NGAL)

3.4.2. ROC Curve of Beta2-microglobulin (B2M).

ROC curve for the sensitivity and specificity of B2M (mg/L) for diagnosis of renal dysfunction in beta thalassemia major , (Cut-off point was ≥ 3.8 (mg/l)) , AUC=0.96, P ≤ 0.001, 95% CI (6.62-7.58), the sensitivity and the specificity was 95.6 %, 91.2 % respectively, as shown in Figure (3-12) .

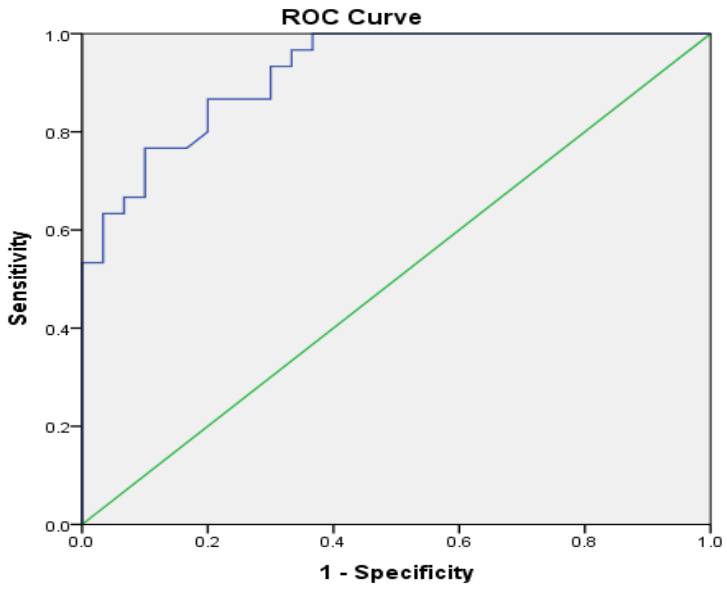


Figure (3.12) ROC Curve of Beta2-microglobulin (B2M)

3.4.3. ROC Curve of Cystatin-C

ROC curve for the sensitivity and specificity of Cystatin-C (ng/mL) for diagnosis of renal dysfunction in beta thalassemia major, (Cut-off point was ≥ 506.2 (mg/l)), AUC=0.91, P ≤ 0.002, 95% CI (946.2-1235.08), the sensitivity and the specificity was 95.6 %, 84.4 % respectively, as shown in Figure (3-13)

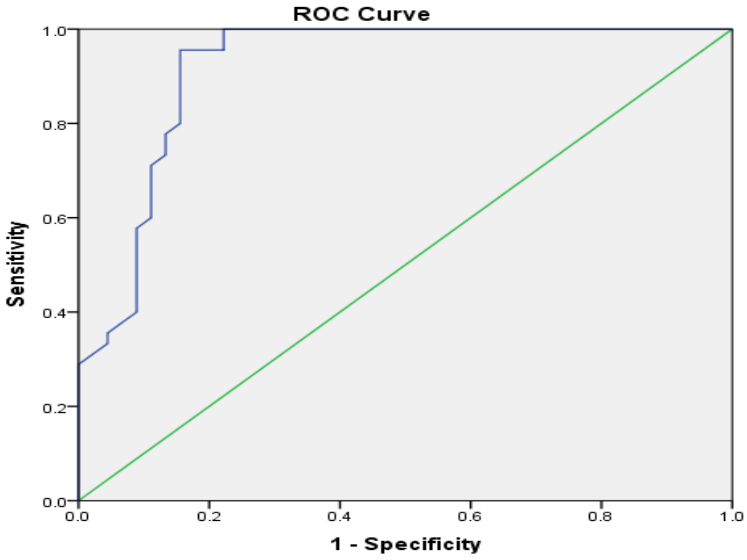


Figure (3.13) ROC Curve of Cystatin-C

Conclusion

From result of this study it could conclude the following points:-

1-All parameters included in this study are significantly higher in β-thalassemia major patients than in healthy subjects. And this indicate renal impairment.

2-Renal hemosiderosis and asymptomatic renal dysfunction are prevalent among β-thalassemia major patients with repeated blood transfusion, which renal dysfunction testes are not found in routine renal investigations.

3-The NGAL is the most sensitive, specific, and highly predictive early indicators for acute renal injury in individuals with βTM when subclinical kidney damage or dysfunction is expected before serum creatinine increases and It is followed in importance for acute renal injury in individuals with βTM by the Cystatin-C parameter.

Recommendations

- A future study is to be done on larger sample size to give results that are more accurate.

- Other early markers of renal dysfunction such as GFR, N-acetyl-beta-Glucosaminidase (NAG) and Kidney injury molecule-1(KIM1) are needed to be measured.

- Assessment of oxidative stress such as nitric oxide and carbon tetrachloride caused by iron deposition.

- Evaluation of NGAL in other types of Hemoglobinopathies.

References

References:-

[1] U Yu, L Chen, X Wang, X Zhang, Y Li, F Wen, S Liu, "Evaluation of the vitamin D and biomedical statuses of young children with β-thalassemia major at a single center in southern China," *BMC Pediatr.*, vol. 19, no. 1, pp. 4–11, 2019.

[2] F. R. Aszhari, Z. Rustam, F. Subroto, and A. S. Semendawai, "Classification of thalassemia data using random forest algorithm," in *Journal of Physics: Conference Series*, 2020, vol. 1490, no. 1, p. 12050.

[3] M. Hashemieh, "Early Detection of renal dysfunction in β thalassemia with focus on novel biomarkers," *Iran. J. Pediatr. Hematol. Oncol.*, vol. 10, no. 1, pp. 57–68, 2020.

[4] F. H. Nezhad, K. H. Nezhad, P. M. Choghakabodi, and B. Keikhaei, "Prevalence and genetic analysis of α-and β-thalassemia and sickle cell anemia in Southwest Iran," *J. Epidemiol. Glob. Health*, vol. 8, no. 3–4, p. 189, 2018.

[5] M. Hashemieh, H. T. Naghadeh, M. T. Namini, H. Neamatzadeh, and M. H. Dehshal, "The Iran thalassemia prevention program: success or failure?," *Iran. J. Pediatr. Hematol. Oncol.*, vol. 5, no. 3, p. 161, 2015.

[6] F. Koohi, T. Kazemi, and E. Miri-Moghaddam, "Cardiac complications and iron overload in beta thalassemia major patients—a systematic review and meta-analysis," *Ann. Hematol.*, vol. 98, no. 6, pp. 1323–1331, 2019.

[7] S. Rivella, "β-thalassemias: paradigmatic diseases for scientific discoveries and development of innovative therapies," *Haematologica*, vol. 100, no. 4, p. 418, 2015.

References

[8] A Lal, T Wong, S Keel, M Pagano, J Chung, A Kamdar, L Rao, A Ikeda, G Puthenveetil, "The transfusion management of beta thalassemia in the United States," *Transfusion*, vol. 61, no. 10, p. 3027, 2021.

[9] A. Bakr, Y. Al-Tonbary, G. Osman, and R. El-Ashry, "Renal complications of beta-thalassemia major in children," *Am. J. Blood Res.*, vol. 4, no. 1, p. 1, 2014.

[10] J. Sleiman, A. Tarhini, and A. T. Taher, "Renal complications in thalassemia," *Thalass. Reports*, vol. 8, no. 1, p. 7481, 2018.

[11] K. M. Musallam and A. T. Taher, "Mechanisms of renal disease in β-thalassemia," *J. Am. Soc. Nephrol.*, vol. 23, no. 8, pp. 1299–1302, 2012.

[12] M. H. Ahmed, M. S. Ghatge, and M. K. Safo, "Hemoglobin: structure, function and allostery," *Vertebr. Invertebr. Respir. proteins, lipoproteins other body fluid proteins*, pp. 345–382, 2020.

[13] F. Rustam, Furqan Ashraf, Imran Jabbar, Shehbaz Tutusaus, Kilian Mazas, Cristina Barrera, Alina Eugenia Pascual de la Torre Diez, Isabel, "Prediction of β-Thalassemia carriers using complete blood count features," *Sci. Rep.*, vol. 12, no. 1, p. 19999, 2022.

[14] S. Sadiq, MU Khalid, S Ullah, W Aslam, A Mehmood, GS Choi, BW On, "Classification of β-Thalassemia Carriers From Red Blood Cell Indices Using Ensemble Classifier," *IEEE access*, vol. 9, pp. 45528–45538, 2021.

[15] A. J. Marengo-Rowe, "Structure-function relations of human hemoglobins," in *Baylor University Medical Center Proceedings*, 2006, vol. 19, no. 3, pp. 239–245.

[16] A. Cao, "Galanello R. b-thalassemia," *Genet Med*, vol. 12, no. 2, pp. 61–76, 2010.

References

[17] J. Powrel and N. P. Adhikari, “Elastic property of sickle and normal hemoglobin protein: Molecular dynamics,” *AIP Adv.*, vol. 12, no. 4, p. 45308, 2022.

[18] V. Viprakasit, R. Origa, and S. Fucharoen, “Genetic basis, pathophysiology and diagnosis,” in *Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT)[Internet]. 3rd edition*, Thalassaemia International Federation, 2014.

[19] I. Cantú and S. Philipsen, “Flicking the switch: adult hemoglobin expression in erythroid cells derived from cord blood and human induced pluripotent stem cells,” *Haematologica*, vol. 99, no. 11, p. 1647, 2014.

[20] B. G. Forget and H. F. Bunn, “Classification of the disorders of hemoglobin,” *Cold Spring Harb. Perspect. Med.*, vol. 3, no. 2, p. a011684, 2013.

[21] M. D. Cappellini and A. T. Taher, “The use of luspatercept for thalassemia in adults,” *Blood Adv.*, vol. 5, no. 1, pp. 326–333, 2021.

[22] S. Farashi and C. L. Harteveld, “Molecular basis of α-thalassemia,” *Blood Cells, Mol. Dis.*, vol. 70, pp. 43–53, 2018.

[23] R. Galanello and A. Cao, “Alpha-thalassemia,” *Genet. Med.*, vol. 13, no. 2, pp. 83–88, 2011.

[24] F. B. Piel and D. J. Weatherall, “The α-thalassemias,” *N. Engl. J. Med.*, vol. 371, no. 20, pp. 1908–1916, 2014.

[25] A. T. Taher, D. J. Weatherall, and M. D. Cappellini, “Thalassaemia,” *Lancet*, vol. 391, no. 10116, pp. 155–167, 2018.

[26] F Locatelli, AA Thompson, JL Kwiatkowski, JB Porter, AJ Thrasher, S

References

Hongeng, MG Sauer., “Betibeglogene autotemcel gene therapy for non–β0/β0 genotype β-thalassemia,” *N. Engl. J. Med.*, vol. 386, no. 5, pp. 415–427, 2022.

[27] F. T. Shah, F. Sayani, S. Trompeter, E. Drasar, and A. Piga, “Challenges of blood transfusions in β-thalassemia,” *Blood Rev.*, vol. 37, p. 100588, 2019.

[28] A. T. Taher, K. M. Musallam, and M. D. Cappellini, “β-Thalassemias,” *N. Engl. J. Med.*, vol. 384, no. 8, pp. 727–743, 2021.

[29] JS Lee, TM Rhee, K Jeon, Y Cho, SW Lee, KD Han, MW Seong, SS Park, YK Lee., “Epidemiologic Trends of Thalassemia, 2006–2018: A Nationwide Population-Based Study,” *J. Clin. Med.*, vol. 11, no. 9, p. 2289, 2022.

[30] S. Ali, S Mumtaz, HA Shakir, M Khan, HM Tahir, S Mumtaz, TA Mughal, A Hassan, “Current status of beta-thalassemia and its treatment strategies,” *Mol. Genet. Genomic Med.*, vol. 9, no. 12, p. e1788, 2021.

[31] T. Apidechkul, F. Yeemard, C. Chomchoei, P. Upala, and R. Tamornpark, “Epidemiology of thalassemia among the hill tribe population in Thailand,” *PLoS One*, vol. 16, no. 2, p. e0246736, 2021.

[32] K. M Musallam, A Vitrano, A Meloni., “Risk of mortality from anemia and iron overload in nontransfusion-dependent β-thalassemia,” *Am. J. Hematol.*, vol. 97, no. 2, pp. E78–E80, 2022.

[33] H. Frangoul, D Altshuler, MD Cappellini., “CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia,” *N. Engl. J. Med.*, vol. 384, no. 3, pp. 252–260, 2021.

[34] A. Makis, E. Voskaridou, I. Papassotiriou, and E. Hatzimichael, “Novel

References

therapeutic advances in β-thalassemia," *Biology (Basel).*, vol. 10, no. 6, p. 546, 2021.

[35] N Li, P An, J Wang, T Zhang, X Qing, B Wu, L Sun, X Ding, L Niu, Z Xie, M Zhang, X Guo, "Plasma proteome profiling combined with clinical and genetic features reveals the pathophysiological characteristics of β-thalassemia," *Iscience*, vol. 25, no. 4, p. 104091, 2022.

[36] G Pizzino, N Irrera, M Cucinotta, G Pallio, F Mannino, V Arcoraci, F Squadrito, D Altavilla, "Oxidative stress: harms and benefits for human health," *Oxid. Med. Cell. Longev.*, vol. 2017, 2017.

[37] K. Gwozdzinski, A. Pieniazek, and L. Gwozdzinski, "Reactive oxygen species and their involvement in red blood cell damage in chronic kidney disease," *Oxid. Med. Cell. Longev.*, vol. 2021, 2021.

[38] E Ferru, A Pantaleo, F Carta, F Mannu, A Khadjavi, V Gallo, L Ronzoni, G Graziadei, "Thalassemic erythrocytes release microparticles loaded with hemichromes by redox activation of p72Syk kinase," *Haematologica*, vol. 99, no. 3, p. 570, 2014.

[39] N. Ben Salah, R. Bou-Fakhredin, F. Mellouli, and A. T. Taher, "Revisiting beta thalassemia intermedia: past, present, and future prospects," *Hematology*, vol. 22, no. 10, pp. 607–616, 2017.

[40] A. Tamaddoni, L. Gharehdaghly, and M. Bahadoram, "Mutation in thalassemia syndrome and clinical manifestation," *Immunopathol. Persa*, vol. 6, no. 2, pp. e29–e29, 2020.

[41] V. Brancaleoni, E. Di Pierro, I. Motta, and M. D. Cappellini, "Laboratory diagnosis of thalassemia," *Int. J. Lab. Hematol.*, vol. 38, pp. 32–40, 2016.

[42] R. Origa, A. Baldan, M. Marsella, and C. Borgna-Pignatti, "A complicated

References

disease: what can be done to manage thalassemia major more effectively?," *Expert Rev. Hematol.*, vol. 8, no. 6, pp. 851–862, 2015.

[43] M. A. Yassin, A. T. Soliman, V. De Sanctis, K. S. Yassin, and M. A. J. Abdulla, "Final height and endocrine complications in patients with β-thalassemia intermedia: our experience in non-transfused versus infrequently transfused patients and correlations with liver iron content," *Mediterr. J. Hematol. Infect. Dis.*, vol. 11, no. 1, 2019.

[44] A. Inati, M. A. Noureldine, A. Mansour, and H. A. Abbas, "Endocrine and bone complications in β-thalassemia intermedia: current understanding and treatment," *Biomed Res. Int.*, vol. 2015, 2015.

[45] A. Podkowińska and D. Formanowicz, "Chronic kidney disease as oxidative stress-and inflammatory-mediated cardiovascular disease," *Antioxidants*, vol. 9, no. 8, p. 752, 2020.

[46] L Téllez, L Ibáñez-Samaniego, CP Del Villar, R Yotti, "Non-selective beta-blockers impair global circulatory homeostasis and renal function in cirrhotic patients with refractory ascites," *J. Hepatol.*, vol. 73, no. 6, pp. 1404–1414, 2020.

[47] A. V. Hoffbrand, D. R. Higgs, D. M. Keeling, and A. B. Mehta, *Postgraduate haematology*. John Wiley & Sons, 2016.

[48] T. Ganz and E. Nemeth, "Hepcidin and iron homeostasis," *Biochim. Biophys. Acta (BBA)-Molecular Cell Res.*, vol. 1823, no. 9, pp. 1434–1443, 2012.

[49] A. Ahmadzadeh, A. Jalali, S. Assar, H. Khalilian, K. Zandian, and M. Pedram, "Renal tubular dysfunction in pediatric patients with beta-thalassemia major," *Saudi J. Kidney Dis. Transplant.*, vol. 22, no. 3, p.

References

497, 2011.

[50] A. W. Chalmers and J. M. Shammo, “Evaluation of a new tablet formulation of deferasirox to reduce chronic iron overload after long-term blood transfusions,” *Ther. Clin. Risk Manag.*, vol. 12, p. 201, 2016.

[51] S. Kumfu, S. Fucharoen, S. C. Chattipakorn, and N. Chattipakorn, “Cardiac complications in beta-thalassemia: From mice to men,” *Exp. Biol. Med.*, vol. 242, no. 11, pp. 1126–1135, 2017.

[52] DT Kremastinos, D Farmakis, A Aessopos, G Hahalis, E Hamodraka, D Tsiapras, A Keren, “β-thalassemia cardiomyopathy: history, present considerations, and future perspectives,” *Circ. Hear. Fail.*, vol. 3, no. 3, pp. 451–458, 2010.

[53] T Cogliandro, G Derchi, L Mancuso, MC Mayer, B Pannone, A Pepe, M Pili, P Bina, “Guideline recommendations for heart complications in thalassemia major,” *J. Cardiovasc. Med.*, vol. 9, no. 5, pp. 515–525, 2008.

[54] PI Fianza, AA Pramono, M Ghozali, TA Sihite, D Setiabudi, MRAA Syamsunarno, “Diagnostic Modalities in Detecting Cardiovascular Complications of Thalassemia,” *Rev. Cardiovasc. Med.*, vol. 23, no. 8, p. 267, 2022.

[55] ME Lai, R Origa, F Danjou, GB Leoni, S Vacquer, F Anni, C Corrias, P Farci, G Congiu, “Natural history of hepatitis C in thalassemia major: a long-term prospective study,” *Eur. J. Haematol.*, vol. 90, no. 6, pp. 501–507, 2013.

[56] H Gao, Z Jin, G Bandyopadhyay, G Wang, D Zhang, KC e Rocha, X Liu, H Zhao, T Kisseleva, “Aberrant iron distribution via hepatocyte-stellate cell axis drives liver lipogenesis and fibrosis,” *Cell Metab.*, vol. 34, no. 8,

References

pp. 1201–1213, 2022.

[57] A Mangia, D Bellini, U Cillo, A Laghi, G Pelle, VM Valori, E Caturelli, "Hepatocellular carcinoma in adult thalassemia patients: an expert opinion based on current evidence," *BMC Gastroenterol.*, vol. 20, no. 1, pp. 1–14, 2020.

[58] A Piga, M Serra, F Longo, G Forni, G Quarta, MD Cappellini, R Galanello, "Changing patterns of splenectomy in transfusion-dependent thalassemia patients," *Am. J. Hematol.*, vol. 86, no. 9, pp. 808–810, 2011.

[59] L. Okar, M. Ali, J. Parengal, and M. A. Yassin, "COVID-19 and thalassemia beta major in splenectomized patient: Clinical case progression and literature review," *Clin. Case Reports*, vol. 8, no. 12, pp. 2917–2921, 2020.

[60] K. M. Belhoul, M. L. Bakir, M.-S. Saned, A. Kadhim, K. M. Musallam, and A. T. Taher, "Serum ferritin levels and endocrinopathy in medically treated patients with β thalassemia major," *Ann. Hematol.*, vol. 91, no. 7, pp. 1107–1114, 2012.

[61] A. A. Mahmud, A. M. Al-Gharawi, and F. M. Mustafa, "Evaluation of delayed puberty of patients with beta thalassemia major in–diyala governorate," *Diyala J. Med.*, vol. 15, no. 2, pp. 76–79, 2018.

[62] C. E. Seow, A. S. Goh, and S. L. Lim, "High prevalence of central hypothyroidism among patients with transfusion dependent thalassemia in Hospital Pulau Pinang: A cross sectional study," *Med J Malaysia*, vol. 76, no. 6, pp. 799–803, 2021.

[63] V De Sanctis, AT Soliman, H Elsefdy, N Soliman, "Bone disease in β thalassemia patients: past, present and future perspectives," *Metabolism*,

vol. 80, pp. 66–79, 2018.

[64] P. Piriyakhuntorn, A. Tantiworawit, M. Phimphilai, K. Shinlapawittayatorn, S. C. Chattipakorn, and N. Chattipakorn, “Impact of iron overload on bone remodeling in thalassemia,” *Arch. Osteoporos.*, vol. 15, no. 1, pp. 1–30, 2020.

[65] PZ Romadhon, A Ashariati, SUY Bintoro, M Thaha, SD Suryantoro, C Windradi, BA Mahdi, “Markers of Renal Complications in Beta Thalassemia Patients with Iron Overload Receiving Chelation Agent Therapy: A Systematic Review,” *J. Blood Med.*, pp. 725–738, 2022.

[66] CT Quinn, VL Johnson, HY Kim, F Trachtenberg, MG Vogiatzi, JL Kwiatkowski, EJ Neufeld., “Renal dysfunction in patients with thalassaemia,” *Br. J. Haematol.*, vol. 153, no. 1, pp. 111–117, 2011.

[67] R. Origa, “β-Thalassemia,” *Genet. Med.*, vol. 19, no. 6, pp. 609–619, 2017.

[68] M. Cappellini, A. Cohen, A. Eleftheriou, A. Piga, J. Porter, and A. Taher, “Guidelines for the Clinical Management of Thalassaemia [Internet],” 2014.

[69] K. Mishra, A. Shah, K. Patel, K. Ghosh, and S. Bharadva, “Seroprevalence of HBV, HCV and HIV-1 and correlation with molecular markers among multi-transfused thalassemia patients in Western India,” *Mediterr. J. Hematol. Infect. Dis.*, vol. 12, no. 1, 2020.

[70] R. Bou-Fakhredin, A.-H. Bazarbachi, B. Chaya, J. Sleiman, M. D. Cappellini, and A. T. Taher, “Iron overload and chelation therapy in non-transfusion dependent thalassemia,” *Int. J. Mol. Sci.*, vol. 18, no. 12, p. 2778, 2017.

References

[71] M. T. Nuñez and P. Chana-Cuevas, “New perspectives in iron chelation therapy for the treatment of neurodegenerative diseases,” *Pharmaceuticals*, vol. 11, no. 4, p. 109, 2018.

[72] M. Karimi, N. Cohan, V. De Sanctis, N. S. Mallat, and A. Taher, “Guidelines for diagnosis and management of Beta-thalassemia intermedia,” *Pediatr. Hematol. Oncol.*, vol. 31, no. 7, pp. 583–596, 2014.

[73] S. Sheth, A. T. Taher, T. D. Coates, A. Kattamis, and M. D. Cappellini, “Management of luspatercept therapy in patients with transfusion-dependent β-thalassaemia,” *Br. J. Haematol.*, 2023.

[74] AG Olabi, HM Maghrabie, OHK Adhari, “Battery thermal management systems: recent progress and challenges,” *Int. J. Thermofluids*, p. 100171, 2022.

[75] V De Sanctis, D Canatan, JLV Corrons, “Preliminary data on COVID-19 in patients with hemoglobinopathies: a multicentre ICET-a study,” *Mediterr. J. Hematol. Infect. Dis.*, vol. 12, no. 1, 2020.

[76] ME Bernardo, E Piras, A Vacca, G Giorgiani, M Zecca, A Bertaina, D Pagliara, B Contoli, “Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan,” *Blood, J. Am. Soc. Hematol.*, vol. 120, no. 2, pp. 473–476, 2012.

[77] H. Cario, “Hemoglobinopathies—genetically diverse, clinically complex, and globally relevant,” *memo-Magazine Eur. Med. Oncol.*, vol. 11, no. 3, pp. 235–240, 2018.

[78] A. Srivastava and R. V Shaji, “Cure for thalassemia major–from allogeneic hematopoietic stem cell transplantation to gene therapy,”

References

Haematologica, vol. 102, no. 2, p. 214, 2017.

[79] A. Biffi, "Gene therapy as a curative option for β-thalassemia," *New England Journal of Medicine*, vol. 378, no. 16. Mass Medical Soc, pp. 1551–1552, 2018.

[80] C Demosthenous, E Vlachaki, C Apostolou, P Eleftheriou, A Kotsiafti, E Vetsiou, E Mandala, "Beta-thalassemia: renal complications and mechanisms: a narrative review," *Hematology*, vol. 24, no. 1, pp. 426–438, 2019.

[81] M. Y. K. Zeid, H. M. A. Hassab, D. N. Younan, and O. Arab, "Study of the effect of different iron-chelating agents on early renal glomerular and tubular function markers in children with beta-thalassemia," *Alexandria J. Pediatr.*, vol. 32, no. 3, p. 116, 2019.

[82] M. Hamdy, I. Shaheen, Z. M. El-Gammal, and Y. M. Ramadan, "Detection of Renal Insufficiency in a Cohort of Patients With Beta-thalassemia Major Using Cystatin-C," *J. Pediatr. Hematol. Oncol.*, vol. 43, no. 8, pp. e1082–e1087, 2021.

[83] F Nalesso, M Rigato, I Cirella, MP Protti, R Zanella, B Rossi, MC Putti, FK Martino, "The Assessment of Renal Functional Reserve in β-Thalassemia Major Patients by an Innovative Ultrasound and Doppler Technique: A Pilot Study," *J. Clin. Med.*, vol. 11, no. 22, p. 6752, 2022.

[84] S. Raz, A. Koren, and C. Levin, "Attention, response inhibition and brain event-related potential alterations in adults with beta-thalassaemia major," *Br. J. Haematol.*, vol. 186, no. 4, pp. 580–591, 2019.

[85] E. Fibach and M. Dana, "Oxidative stress in β-thalassemia," *Mol. Diagn. Ther.*, vol. 23, no. 2, pp. 245–261, 2019.

References

[86] B. Kaissling, S. Spiess, B. Rinne, and M. Le Hir, “Effects of anemia on morphology of rat renal cortex,” *Am. J. Physiol. Physiol.*, vol. 264, no. 4, pp. F608–F617, 1993.

[87] S. Bhandari and R. Galanello, “Renal aspects of thalassaemia a changing paradigm,” *Eur. J. Haematol.*, vol. 89, no. 3, pp. 187–197, 2012.

[88] M El-Shanshory, NM Hablas, MS Aboonq, “Nigella sativa improves anemia, enhances immunity and relieves iron overload-induced oxidative stress as a novel promising treatment in children having beta-thalassemia major,” *J. Herb. Med.*, vol. 16, p. 100245, 2019.

[89] M. Hashemieh, “Early detection of renal dysfunction in β thalassemia with focus on novel biomarkers,” *Iran. J. Pediatr. Hematol. Oncol.*, vol. 10, no. 1, pp. 57–68, 2020.

[90] A Jalali, H Khalilian, A Ahmadzadeh, S Sarvestani, F Rahim, K Zandian, S Asar, “Renal function in transfusion-dependent pediatric beta-thalassemia major patients,” *Hematology*, vol. 16, no. 4, pp. 249–254, 2011.

[91] N. S. Mallat, S. G. Mallat, K. M. Musallam, and A. T. Taher, “Potential mechanisms for renal damage in beta-thalassemia.,” *J. Nephrol.*, vol. 26, no. 5, pp. 821–828, 2013.

[92] M. V. Sadeghi, M. Mirghorbani, and R. Akbari, “β-Thalassemia minor & renal tubular dysfunction: is there any association?,” *BMC Nephrol.*, vol. 22, no. 1, pp. 1–7, 2021.

[93] N. Rifai, *Tietz textbook of clinical chemistry and molecular diagnostics*. Elsevier Health Sciences, 2017.

[94] P. U. Cetinkaya, F. M. Azik, V. Karakus, B. Huddam, and N. Yilmaz,

References

"β2-Microglobulin, Neutrophil Gelatinase-Associated Lipocalin, and Endocan Values in Evaluating Renal Functions in Patients with β-Thalassemia Major," *Hemoglobin*, vol. 44, no. 3, pp. 147–152, 2020.

[95] M. B. Hosen, M. S. Hasan, M. F. Azim, R. Sarder, and M. Uddin, "Evaluation of Renal Function in Beta-Thalassemia Patients in Bangladesh," *BM J.*, vol. 6, no. 1, pp. 11–14, 2015.

[96] C. Demosthenous, P. Eleftheriou, C. Apostolou, P. Sarafidis, V. Perifanis, and E. Vlachaki, "β-Thalassemia and renal complications. A narrative review of pathophysiologic mechanisms," *Population (Paris).*, vol. 11, p. 12, 2018.

[97] MS ElAlfy, NHK Elsherif, FSE Ebeid, EAR Ismail, "Renal iron deposition by magnetic resonance imaging in pediatric β-thalassemia major patients: Relation to renal biomarkers, total body iron and chelation therapy," *Eur. J. Radiol.*, vol. 103, pp. 65–70, 2018.

[98] D Stefanopoulos, N Nasiri-Ansari, I Dontas, A Vryonidou, A Galanos, L Psaridi, IG Fatouros, "Fibroblast growth factor 23 (FGF23) and klotho protein in beta-Thalassemia," *Horm. Metab. Res.*, vol. 52, no. 03, pp. 194–201, 2020.

[99] AA Mahmoud, DM Elian, NMS Abd El Hady, HM Abdallah, S Abdelsattar, FO Khalil, "Assessment of subclinical renal glomerular and tubular dysfunction in children with beta thalassemia major," *Children*, vol. 8, no. 2, p. 100, 2021.

[100] C. Higgins, "Urea and the clinical value of measuring blood urea concentration," *Acutecaretesting. Org*, pp. 1–6, 2016.

[101] J. D. Klein, M. A. Blount, and J. M. Sands, "Urea transport in the kidney,"

References

Compr. Physiol., vol. 1, no. 2, pp. 699–729, 2011.

[102] L. Bankir and B. Yang, “New insights into urea and glucose handling by the kidney, and the urine concentrating mechanism,” *Kidney Int.*, vol. 81, no. 12, pp. 1179–1198, 2012.

[103] I Popovych, A Gozhenko, I Kuchma, W Zukow, V Bilas, G Koval'chuk, A Ivasivka, “Immunotropic effects of so-called slag metabolites (creatinine, urea, uric acid and bilirubin) at rats,” *J. Educ. Heal. Sport*, vol. 10, no. 11, pp. 320–336, 2020.

[104] A Louvado, F Coelho, M Palma, LC Tavares, ROA Ozorio, L Magnoni, I Viegas, “Effect of glycerol feed-supplementation on seabass metabolism and gut microbiota,” *Appl. Microbiol. Biotechnol.*, vol. 104, no. 19, pp. 8439–8453, 2020.

[105] X. He, J. Sotelo-Orozco, C. Rudolph, B. Lönnerdal, and C. M. Slupsky, “The role of protein and free amino acids on intake, metabolism, and gut microbiome: a comparison between breast-fed and formula-fed rhesus monkey infants,” *Front. Pediatr.*, vol. 7, p. 563, 2020.

[106] M. Wyss and R. Kaddurah-Daouk, “Creatine and creatinine metabolism,” *Physiol. Rev.*, 2000.

[107] S Qun, F Hu, G Wang, J Wu, Q Tang, J Zhang, Z Chen, X Wang, Q Liu, W Ge, “Serum beta2-microglobulin levels are highly associated with the risk of acute ischemic stroke,” *Sci. Rep.*, vol. 9, no. 1, pp. 1–8, 2019.

[108] H. Wang, B. Liu, and J. Wei, “Beta2-microglobulin (B2M) in cancer immunotherapies: biological function, resistance and remedy,” *Cancer Lett.*, vol. 517, pp. 96–104, 2021.

[109] NH Dung, NT Kien, NTT Hai, PT Cuong, NTT Huong, DBQ Quyen, NM

References

Tuan, DM Ha, “Measuring serum beta2-microglobulin to predict long-term mortality in hemodialysis patients using low-flux dialyzer reuse,” *Ther. Clin. Risk Manag.*, pp. 839–846, 2019.

[110] A. G. Kacar, I. Silfeler, A. Kacar, F. Pekun, E. Turkkan, and E. Adal, “Levels of beta-2 microglobulin and cystatin C in beta thalassemia major patients,” *J. Clin. Anal. Med.*, vol. 6, no. 3, pp. 269–273, 2015.

[111] MH Rosner, T Reis, F Husain-Syed, “Classification of uremic toxins and their role in kidney failure,” *Clin. J. Am. Soc. Nephrol.*, vol. 16, no. 12, pp. 1918–1928, 2021.

[112] R. Wang, H. Hu, S. Hu, H. He, and H. Shui, “β2-microglobulin is an independent indicator of acute kidney injury and outcomes in patients with intracerebral hemorrhage,” *Medicine (Baltimore).*, vol. 99, no. 8, 2020.

[113] A. O. Eguvbe, M. U. Nwagu, E. S. Idogun, and A. A. Akande, “The role of urine albumin creatinine ratio and serum β2 microglobulin as biomarkers of chronic kidney disease,” *Universa Med.*, vol. 38, no. 3, pp. 172–178, 2019.

[114] P. A. Keown, “Predicting long-term outcome in renal transplantation,” *Kidney Int.*, vol. 84, no. 4, pp. 650–652, 2013.

[115] V. Marakala, “Neutrophil Gelatinase-Associated Lipocalin (NGAL) in kidney injury-A systematic review,” *Clin. Chim. Acta*, 2022.

[116] M. Mohammed, J. Mohammad, Z. Fathi, M. Al-Hamdany, and N. Alkazzaz, “Comparative evaluation of cystatin C and neutrophil gelatinase-associated lipocalin in patients with thalassemia major versus thalassemia intermedia,” *Pharmacia*, vol. 68, no. 4, pp. 741–746, 2021.

[117] G Coppolino, N Comi, D Bolignano, G Patella, A Comi, M Provenzano, L

References

Rivoli, M Andreucci, “Urinary neutrophil gelatinase-associated lipocalin (NGAL) predicts renal function decline in patients with glomerular diseases,” *Front. cell Dev. Biol.*, vol. 8, p. 336, 2020.

[118] M. G. Shaalan, M. K. Hassan, H. J. Al-Shanoof, and L. M. Al Naama, “Renal Dysfunction in Pediatric Patients in Iraq With β-Thalassemia Major and Intermedia,” *Cureus*, vol. 14, no. 9, 2022.

[119] P. D. Sanchez-Gonzalez, F. J. Lopez-Hernandez, A. I. Morales, J. F. Macias-Nunez, and J. M. Lopez-Novoa, “Effects of deferasirox on renal function and renal epithelial cell death,” *Toxicol. Lett.*, vol. 203, no. 2, pp. 154–161, 2011.

[120] E. V Schrezenmeier, J. Barasch, K. Budde, T. Westhoff, and K. M. Schmidt-Ott, “Biomarkers in acute kidney injury–pathophysiological basis and clinical performance,” *Acta Physiol.*, vol. 219, no. 3, pp. 556–574, 2017.

[121] M. Ning, X. Mao, Y. Niu, B. Tang, and H. Shen, “Usefulness and limitations of neutrophil gelatinase-associated lipocalin in the assessment of kidney diseases,” *J. Lab. Precis. Med. Vol 3, No 1 (January 2018) J. Lab. Precis. Med.*, 2018, [Online]. Available: https://jlpm.amegroups.com/article/view/3964

[122] M. Buonafine, E. Martinez-Martinez, and F. Jaisser, “More than a simple biomarker: the role of NGAL in cardiovascular and renal diseases,” *Clin. Sci.*, vol. 132, no. 9, pp. 909–923, 2018.

[123] J. L. Alge and J. M. Arthur, “Biomarkers of AKI: a review of mechanistic relevance and potential therapeutic implications,” *Clin. J. Am. Soc. Nephrol.*, vol. 10, no. 1, pp. 147–155, 2015.

References

[124] A. Yamamoto, S. Nakayama, Y. Wakabayashi, Y. Yoshino, and T. Kitazawa, “Urine neutrophil gelatinase-associated lipocalin as a biomarker of adult pyelonephritis,” *J. Infect. Chemother.*, 2023.

[125] Q. Yang and Y. Luo, “Progress on the Relationship between Serum Cystatin C Level and Chronic Heart Failure after Coronary Heart Disease,” 2019.

[126] M. SaleamKhalaf, “Evaluation of Serum Cystatin C Level in Patients with Diabetic Nephropathy,” *Ann. Rom. Soc. Cell Biol.*, pp. 11704–11707, 2021.

[127] OG Behairy, ER Abd Almonaem, NT Abed, OM Abdel Haiea, RM Zakaria, RI AbdEllaty, “Role of serum cystatin-C and beta-2 microglobulin as early markers of renal dysfunction in children with beta thalassemia major,” *Int. J. Nephrol. Renovasc. Dis.*, vol. 10, p. 261, 2017.

[128] B. A. Ali and A. M. Mahmoud, “Frequency of glomerular dysfunction in children with beta thalassaemia major,” *Sultan Qaboos Univ. Med. J.*, vol. 14, no. 1, p. e88, 2014.

[129] YL Lin, IC Chang, HH Liou, CH Wang, YH Lai, CH Kuo, “Serum indices based on creatinine and cystatin C predict mortality in patients with non-dialysis chronic kidney disease,” *Sci. Rep.*, vol. 11, no. 1, p. 16863, 2021.

[130] LA Inker, CH Schmid, H Tighiouart, JH Eckfeldt, HI Feldman, T Greene, JW Kusek, J Manzi, “Estimating glomerular filtration rate from serum creatinine and cystatin C,” *N. Engl. J. Med.*, vol. 367, no. 1, pp. 20–29, 2012.

[131] M. A. Khusainova, “CYSTATIN C IS AN EARLY MARKER OF

References

DECREASED KIDNEY FUNCTION," *Orient. Renaiss. Innov. Educ. Nat. Soc. Sci.*, vol. 3, no. 1, pp. 485–490, 2023.

[132] MG Shlipak, K Matsushita, J Ärnlöv, LA Inker, R Katz, KR Polkinghorne,, "Cystatin C versus creatinine in determining risk based on kidney function," *N. Engl. J. Med.*, vol. 369, no. 10, pp. 932–943, 2013.

[133] K Soto, S Coelho, B Rodrigues, H Martins, F Frade, S Lopes, L Cunha, AL Papoila, "Cystatin C as a marker of acute kidney injury in the emergency department," *Clin. J. Am. Soc. Nephrol.*, vol. 5, no. 10, pp. 1745–1754, 2010.

[134] S. Fernando and K. R. Polkinghorne, "Cystatin C: not just a marker of kidney function," *Brazilian J. Nephrol.*, vol. 42, pp. 6–7, 2020.

[135] J. V. Salgado, F. L. Souza, and B. J. Salgado, "How to understand the association between cystatin C levels and cardiovascular disease: Imbalance, counterbalance, or consequence?," *J. Cardiol.*, vol. 62, no. 6, pp. 331–335, 2013.

[136] A. Kattamis, G. L. Forni, Y. Aydinok, and V. Viprakasit, "Changing patterns in the epidemiology of β-thalassemia," *Eur. J. Haematol.*, vol. 105, no. 6, pp. 692–703, 2020.

[137] M. S. Tabatabaei and M. Ahmed, "Enzyme-linked immunosorbent assay (ELISA)," in *Cancer Cell Biology: Methods and Protocols*, Springer, 2022, pp. 115–134.

[138] N. W. Tietz, "Clinical Guide to Laboratory Tests. 4 [sup] th ed." Philadelphia: WB Saunders Company, 2006.

[139] M. S. Shafi, T. Faisal, S. Naseem, and S. Javed, "Postgraduate Medical Trainees' Understanding of Biostatistics: A Pre-and Post-Research

References

Methodology Workshop Experience," *J Coll Physicians Surg Pak*, vol. 28, no. 3, pp. 196–200, 2018.

[140] E. Crescenzi, A. Leonardi, and F. Pacifico, "NGAL as a potential target in tumor microenvironment," *Int. J. Mol. Sci.*, vol. 22, no. 22, p. 12333, 2021.

[141] B. Stephenson, G. Chertow, Y. Yang, and D. Rosenbaum, "PUK2 Hospitalization in Patients Receiving Maintenance Dialysis with Hyperphosphatemia Randomized to Tenapanor or Sevelamer," *Value Heal.*, vol. 24, p. S234, 2021.

[142] M Althubiti, M Elzubier, GS Alotaibi, MA Althubaiti, "Beta 2 microglobulin correlates with oxidative stress in elderly," *Exp. Gerontol.*, vol. 150, p. 111359, 2021.

[143] E. A. Hamed and N. T. ElMelegy, "Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study," *Ital. J. Pediatr.*, vol. 36, pp. 1–10, 2010.

[144] A. G. Kaçar, İ. Şilfeler, A. Kacar, F. Pekun, E. Türkkan, and E. Adal, "Levels of beta-2 microglobulin and cystatin C in beta thalassemia major patients," 2015.

[145] E. Uzun, Y. I. Balcı, S. Yüksel, Y. Z. Aral, H. Aybek, and B. Akdağ, "Glomerular and tubular functions in children with different forms of beta thalassemia," *Ren. Fail.*, vol. 37, no. 9, pp. 1414–1418, 2015.

[146] M. Mohammed, J. Mohammad, Z. Fathi, M. Al-Hamdany, and N. Alkazzaz, "Comparative evaluation of cystatin C and neutrophil gelatinase-associated lipocalin in patients with thalassemia major versus thalassemia intermedia," *Pharmacia*, vol. 68, p. 741, 2021.

References

[147] M Gokce, H Kup, D Tugcu, Z Salcioglu, G Aydogan, A Akcay, F Akici, "Insidious renal damage in patients with thalassemia major: Is it more serious than appreciated," *J Hematol Thrombo Dis*, vol. 2, no. 6, pp. 1–4, 2014.

[148] Z. A. Althanoon and N. A. Alkazzaz, "Comparison of The Effects Of Deferasirox And Deferoxamine On Uric Acid And Renal Function In Patients with Beta Thalassemia," *Syst. Rev. Pharm.*, vol. 11, no. 11, pp. 214–222, 2020.

[149] I. Z. Fouad, M. S. ElNahid, M. F. Youssef, and Y. M. Amroussy, "Urinary neutrophil gelatinase-associated lipocalin as a marker of kidney injury in Egyptian patients with thalassemia," *Egypt. J. Intern. Med.*, vol. 31, no. 3, pp. 343–352, 2019.

[150] A. Nickavar, A. Qmarsi, S. Ansari, and E. Zarei, "Kidney function in patients with different variants of beta-thalassemia," *Iran. J. Kidney Dis.*, vol. 11, no. 2, p. 132, 2017.

[151] K Karaman, S Şahin, H Geylan, AŞ Yaşar, M Çetin, AU Kömüroğlu, AF Öner, "Evaluation of Renal Function Disorder With Urinary Neutrophil Gelatinase–associated Lipocalin Level in Patients With β-Thalassemia Major," *J. Pediatr. Hematol. Oncol.*, vol. 41, no. 7, pp. 507–510, 2019.

[152] F Shah, K Huey, S Deshpande, M Turner, M Chitnis, E Schiller, A Yucel, L Moro Bueno, "Relationship between Serum Ferritin and Outcomes in β-Thalassemia: A Systematic Literature Review," *J. Clin. Med.*, vol. 11, no. 15, p. 4448, 2022.

[153] A Meloni, L Barbuto, L Pistoia, V Positano, S Renne, "Frequency, pattern, and associations of renal iron accumulation in sickle/β-thalassemia patients," *Ann. Hematol.*, vol. 101, no. 9, pp. 1941–1950,

References

2022.

[154] S. Afshan, M. H. Parisa, A. Shahram, A. Azita, and M. Hashemieh, "Renal iron load estimation in thalassemia patients using T2* magnetic resonance imaging," *Int. J. Med. Res. Heal. Sci*, vol. 8, pp. 182–189, 2019.

[155] A Spasiano, A Meloni, S Costantini, E Quaia, F Cademartiri, P Cinque, A Pepe, "Setting for 'Normal' Serum Ferritin Levels in Patients with Transfusion-Dependent Thalassemia: Our Current Strategy," *J. Clin. Med.*, vol. 10, no. 24, p. 5985, 2021.

[156] Z. M. Dimitrijevic, S. S. Salinger-Martinovic, R. J. Jankovic, and B. P. Mitic, "Elevated serum ferritin levels are predictive of renal function recovery among patients with acute kidney injury," *Tohoku J. Exp. Med.*, vol. 248, no. 2, pp. 63–71, 2019.

[157] MR El-Shanshory, LM Sherief, HM Hassab, "Prevalence of iron deficiency anemia and beta thalassemia carriers among relatives of beta thalassemia patients in Nile Delta region, Egypt: a multicenter study," *J. Egypt. Public Health Assoc.*, vol. 96, pp. 1–8, 2021.

[158] H. Ayyıldız and S. A. Tuncer, "Determination of the effect of red blood cell parameters in the discrimination of iron deficiency anemia and beta thalassemia via Neighborhood Component Analysis Feature Selection-Based machine learning," *Chemom. Intell. Lab. Syst.*, vol. 196, p. 103886, 2020.

[159] E. Bordbar, M. Taghipour, and B. E. Zucconi, "Reliability of different RBC indices and formulas in discriminating between β-thalassemia minor and other microcytic hypochromic cases," *Mediterr. J. Hematol. Infect. Dis.*, vol. 7, no. 1, 2015.

References

[160] A. Y. D. Bashi and F. H. Fathi, “Evaluation of Hepatic Enzymes in major β-thalassemic Patients using Deferasirox,” *Iraqi J. Pharm. Sci. (P-ISSN 1683-3597 E-ISSN 2521-3512)*, vol. 31, no. 2, pp. 237–243, 2022.

[161] R. A. Risdon, M. Barry, and D. M. Flynn, “Transfusional iron overload: the relationship between tissue iron concentration and hepatic fibrosis in thalassaemia.,” *J. Pathol.*, vol. 116, no. 2, pp. 83–95, 1975.

[162] X Lai, L Liu, Z Zhang, L Shi, G Yang, M Wu, “Hepatic veno-occlusive disease/sinusoidal obstruction syndrome after hematopoietic stem cell transplantation for thalassemia major: incidence, management, and outcome,” *Bone Marrow Transplant.*, vol. 56, no. 7, pp. 1635–1641, 2021.

[163] A Bruzzese, EA Martino, F Mendicino, “Iron chelation therapy,” *Eur. J. Haematol.*, vol. 110, no. 5, pp. 490–497, 2023.

[164] D. Edelmann, T. F. Móri, and G. J. Székely, “On relationships between the Pearson and the distance correlation coefficients,” *Stat. Probab. Lett.*, vol. 169, p. 108960, 2021.

[165] C Demosthenous, E Vlachaki, C Apostolou, P Eleftheriou, A Kotsiafti, E Vetsiou, E Mandala, “Beta-thalassemia: renal complications and mechanisms: a narrative review,” *Hematol. (United Kingdom)*, vol. 24, no. 1, pp. 426–438, 2019.

[166] M. Zafari and A. Aghamohammady, “Comparison of Beta-2 Microglobulin Level and Some Variables Between Thalassemia Major Patients Who Treated by Desferal and Control Group,” *Zahedan J. Res. Med. Sci.*, vol. 19, no. 10, 2017.

الملخص:-

ثلاسيميا بيتا هي أكثر أنواع اعتلال الهيموغلوبين الوراثي انتشارًا في العالم. يحدث بسبب قلة أو عدم إنتاج (HbA2, α2β2)وهو إنتاج سلسلة بيتا غلوبين ، والتي عادة ما تكون جزءًا من الهيموغلوبين البالغ ينتج عن هذا الشذوذ الجيني عملية تكوين الكريات الحمر التعويضية غير فعاله ، سلسلة بيتا غلوبين وهو وفقر الدم الحاد، وتسارع تحلل وانتاج كريات الدم الحمراء ، وغالبًا ما تترافق متلازمة الثلاسيميا مع مجموعة واسعة من المشاكل الناتجة من المرض والعلاجات المستخدمة لعلاجه. تعتمد حياة مرضى بيتا ثلاسيميا الكبرى على نقل الدم. يؤدي نقل الدم المنتظم إلى داء هيموسيديريات في أعضائهم الرئيسية. الهدف من هذه الدراسة هو تحديد المؤشرات الحيوية التشخيصية والإنذارية والتنبؤية ، والتي يمكن استخدامها لتشخيص تدهور وظائف الكلى لدى مرضى الثلاسيميا في وقت مبكر من مسار المرض بحساسية ونوعية عالية ، وتحسين التنبؤ ومراقبة فعالية العلاج والتحمل المريض من خلال تحديد المؤشرات الحيوية الجديدة

تم تصميم هذه الدراسة لدراسة المضاعفات الكلوية في مرضى الثلاسيميا بيتا الرئيسيين الذين يعانون من عمليات نقل الدم المتكررة. لتحقيق هذا الهدف تم اجراء اختبار على 90 شخص قسموا على مجموعتين،ضمت المجموعه الأولى على45 مريضا مصابين بالثلاسيميا بيتا الكبرى ، والمجموعه الثانية 45 من الافراد الاصحاء ضاهريا كمجموعة السيطرة.

جمعت جميع العينات من مركز الكرامة لاضطرابات الدم الوراثية من تشرين الثاني 2021 الى كانون الثاني 2022 وأجريت هذه الدراسة في مختبر مركز بغداد الدولي للبحوث في بغداد.

تم استخدام عينات المصل لقياس التحاليل الكيميائية الحيوية ، تم قياس الكرياتينين في الدم ، اليوريا في الدم،الفرتين,CBC,GPT,GOT, الليبوكالين المرتبط بالجيلاتيناز (NGAL) ،والسيستاتين-سي من عينات الدم. علاوة على ذلك ،بيتا2- ميكروغلوبولين من عينات الادرار. النتائج كانت المؤشرات الحيوية S. NGALو Cystatin-C في مجموعة المرضى أعلى بكثير من تلك الموجودة في المجموعة الضابطة (P <0.001) ، (P <0. 003) كذالك B2-microglobulin في مجموعات المرضى مقارنة بمجموعة التحكم اعلى . تحليل الفرتين في مجموعة المرضى أعلى بكثير من تلك الموجودة في المجموعة الضابطة (P <0.001) ، وكان CBC في مجموعة المرضى أقل من تلك الموجودة في المجموعة الضابطة و GOT و GPT ضمن النطاق الطبيعي (P <0.001). نتائج هذه الدراسة بينت

الخلل الوظيفي الكبيبي والأنبوبي لدى مرضى الثلاسيميا بيتا مرتبطة بالحمل الزائد للحديد وفقر الدم المزمن وتشير إلى أنه بعد تناول ديفيراسيروكس وديفيروكسامين (DFO) ، كان هناك تلف كلوي وزيادة في عوامل الالتهاب. أيضا ، لوحظ ضعف كلوي طفيف بعد إعطاء ديفيروكسامين. لذلك ، يبدو أنه يجب مراقبة المرضى الذين يتناولون هذين العقارين بعناية. في هذه الدراسة علاقة ارتباط موجبة معنوية بين الفيريتين وNGAL ,وبيتا2-مايكروكلوبيولين توجد علاقة ارتباطية موجبة ذات دلالة إحصائية بين مستوى الفيريتين واليوريا. علاقة ارتباط موجبة بين NGAL و B2-MG و CYS-C و S.Cr .

الاستنتاج هو ان داء ترسب الحديد في الكلية واختلال وظيفي كلوي بدون أعراض منتشر بين مرضى الثلاسيميا العضمى الذين يعانون من عمليات نقل الدم المتكررة ، والتي لا توجد في الفحوصات الروتينية للكلى ، والتي تتطلب فحصًا منتظمًا بعلامات مبكرة للخلل الكبيبي والأنبوبي.