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Calculating some of Biochemistry indicators for patients of β-Thalassemia major in Misan

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By

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Dedication

I dedicate this work

To my parents ***My father*** *who accompanied me throughout my journey step by step* ***My mother*** *who gave me confidence , encouraged and supported me to achieve my dream*

To ***My close friends*** *who supported me and wished my success*

To ***My Supervisors*** *who Gave me their Time and Knowledge*

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*First , I thank **Allah** for everything , without his mercy , I wouldn't do what I have done .*

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List of Abbreviations

Abbreviations	Key
Hb, HGB	Hemoglobin
HbF	Fetal hemoglobin
HbA	Hemoglobin A
HbA2	Hemoglobin A2
TI	Thalassemia Intermediate
TM	Thalassemia Major
TDT	Transfusion Dependent Thalassemia
NTDT	Non Transfusion Dependent Thalassemia
MRI	magnetic resonance imaging
DFO	deferoxamine drug
BMT	Bone marrow transplantation
CBC	complete blood count
LDH	Lactate dehydrogenase
sTfR	Soluble transferrin receptors
Zn	Zinc
Cu	Copper
TP	Total protein
Cl^-	Chloride
RBC	Red blood cell

Abbreviations	Key
HCT	Hematocrit
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
RDW-SD	Red blood cell distribution width – Standard deviation
RDW-CV	Red blood cell distribution width – Coefficient of variation
WBC	White blood cell
LYM	Lymphocytes

Abstract

This study was conducted during the period from November 2020 to June 2022, 150 samples were taken consisting of (100 patients and 50 healthy people), the total patients were 50 females and 50 males with thalassemia major and their ages ranged from 10-25 years , and from the auditors of the Misan Center for Mediterranean Anemia, while the control group They matched in age and gender.

The two groups of patients and healthy subjects were divided into two groups according to age, the first group aged 10-17 and the second group aged 18-25 years.

This study aimed to know the chemical parameters and the differences in serum concentrations (LDH, sTfR, Cu, Zn, TP, Cl^{-},and Ferritin) in the blood, and they were studied in terms of sex, total, as well as age. The results showed a significant ($p < 0.05$) increase in the level of (LDH, sTfR, Cu, TP, Cl^{-},and Ferritin) in patients with thalassemia major compared to the healthy ones, as well as the increase in the level in males compared to females, in addition to the effect of age, where the older the individuals with thalassemia, a clear rise in the level was observed compared to ($P < 0.05$), in younger individuals. The results showed significantly low levels of (Zn) in patients compared to healthy controls, as well as statistically significant low levels in females compared to male patients. As people with thalassemia get older, the concentration of (Zn) in the blood serum decreases.

As for CBC, these factors have been studied (RBC, HCT, HGB, MCV, MCH, MCHC, RDW-SD, RDWCV, WBC,LYM%, and LYM#) and a statistically significant decrease ($P < 0.05$) was found in each of (RBC, HCT, HGB, MCH and MCV) compared to healthy subjects, in contrast, an increase in (MCHC, RDW-SD, RDWCV, WBC, LYM%, and LYM#) in patients compared to

healthy subjects. As for the gender of patients, it was found that (RBC, HCT, HGB, MCH, MCHC, RDW-SD, RDWCV, WBC,LYM%, and LYM#) in female patients with thalassemia was high compared to male patients, in contrast to MCV, which was found low in female patients. According to the age, both (RBC, HCT, HGB, RDWCV, WBC, LYM%, and LYM#) increased with age, in contrast to (MCV and MCH), it was noted that they decreased significantly with age. As for (MCHC and RDW-SD), it was observed that it decreased in male patients with age, in contrast to females of the same type.

CHAPTER ONE

INTRODUCTION

&

LITERATURE REVIEW

1.1 Thalassemia syndromes:-

Thalassemia is a collection of inherited hemoglobin abnormalities defined by decreased synthesis of one or more globin chains, resulting in imbalanced globin synthesis, which is the most important determinant in defining the severity of the disease in thalassemia syndromes[1]. Thalassemia are most common in Southeast and South Asia, the Middle East, Mediterranean countries, and North and Central Africa; however, due to continued migration, thalassemia are now becoming more common in North America and North Europe as well[2,3].

Thalassemia is divided into two types based on the afflicted chain type. It could be either thalassemia alpha or beta. It is divided into subtypes based on where the defect is located. As a result, thalassemia is the most frequent beta type[4].

The severity of the condition is determined by the balance of alpha and beta chain production as a loss of this balance causes an accumulation of alpha and beta chains within the red blood cell so that the hemoglobin becomes insoluble[5].

In fact, there are many types of thalassemia diseases alpha and beta and also it has subtypes as mentioned before but this study focused one type which is beta type because it is spread in Misan city where this study was hold.

1.1.1 Historical Review:

The disorder was first recognised in 1925 by Thomas Cooley, described a series of infants who became profoundly anaemic and developed clinical symptoms of severe anemia, enlarged liver and spleen, and discoloration of skine . They all had a leukocytosis which was not of the leukaemic type but predominantly normoblasts and in Two patients many reticulated cells. There was normal or increased resistance to red blood cells from these to haemolysis by hypotonic solutions. In addition all these children had a classical mongoloid facies[6,7] .

In 1936, the pathological changes were described by Whipple and Bradford, who observed that many of their patients came from the Mediterranean region and coined the word thalassemia from the Greek “thalassa”, meaning ‘the sea’. More recently it has become clear that the Mediterranean and parts of north and West Africa through the Middle East and Indian subcontinent to Southeast Asia (figure 2.1)[8].

These children usually presented within the first year of life and rarely survived beyond the first few years of life. Profound anemia, failure to thrive, recurrent infections, and progressive abdominal distension due to hepatosplenomegaly were uniformly described. Those with the severest anemia would die within the first few months of life from cardiac failure and or infection and those with less severe anemia would start to develop other manifestations of the anemia such as poor growth and venous ulceration. Thrombocytopenia and neutropenia secondary to hypersplenism along with associated infective and bleeding complications was also seen. X ray images of the bones would show cortical thinning, and a moth eaten or lace like appearance, of the intramedullary space in the small bones.

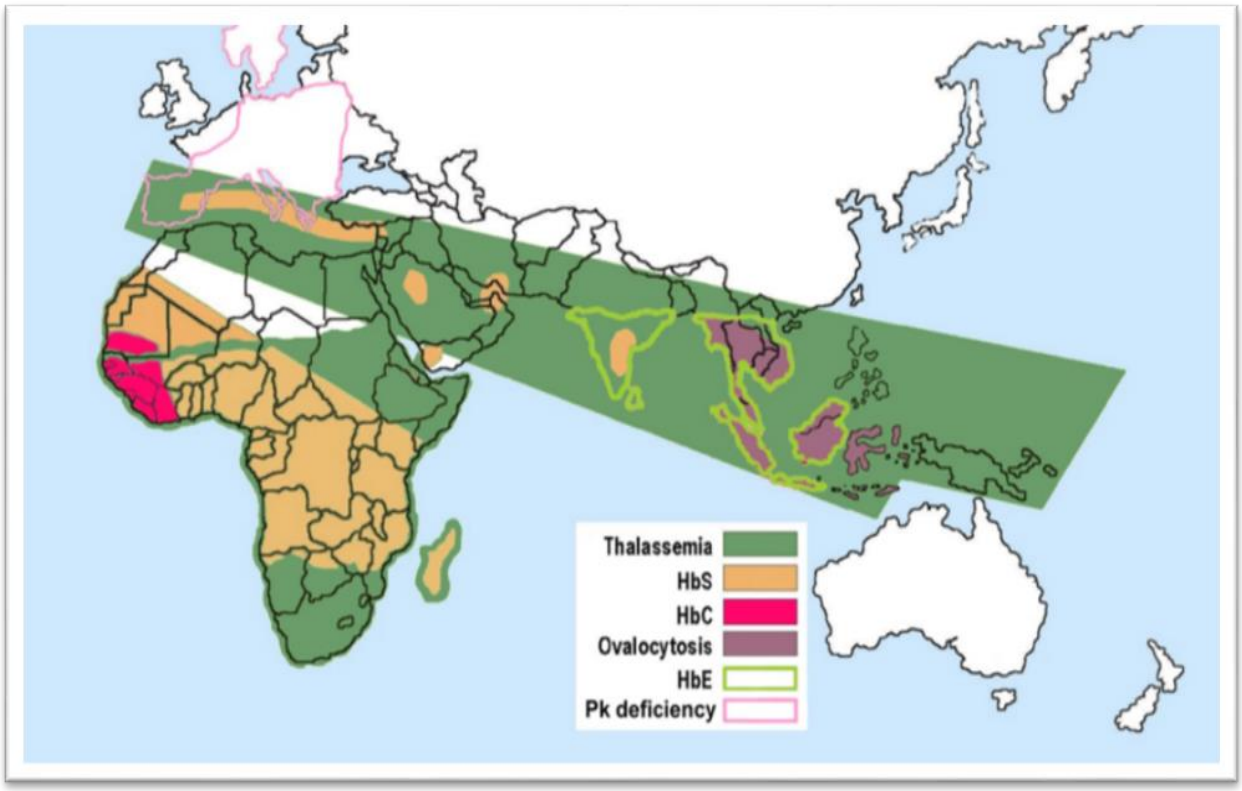


Fig. (1-1): Global distribution of different Hemoglobin disorder

The skull X rays were the most dramatic with symbol 'hair on end' appearance due to expansion of the diploic space. Pathological fractures were frequent occurrences. Extramedullary haematopoiesis could be seen on chest X rays and some children would go onto develop spinal cord paraparesis due to cord compression. As children developed beyond the age of 7 years there was evidence of delayed growth and sexual development that was thought to be secondary to severe anemia and possibly the siderosis from increased gastrointestinal iron absorption[9].

The term thalassemia intermedia was used to describe disorders that were milder than the major form but more severe than the traits. But, until the 1950s, there was no understanding of the underlying cause[10]. Later there was rapid progress toward an understanding of the structure and function of human hemoglobin the genetic basis of the thalassemia diseases was proposed[10], linking them to unbalanced globin chain synthesis, when David Weatherall and associates labeled reticulocytes of thalassemic patients with radioactive amino acids in vitro and were able to demonstrate that in patients with alpha and beta-thalassemia, alpha- or beta-chain production was defective because of unbalanced globin chain synthesis [11].

1.1.2 Pathophysiology

Hemoglobin is a tetramer of two alpha globin chains combined with two non-alpha globin chains. The chains are designated by Greek letters, which are used to describe the particular hemoglobin. Fetal hemoglobin (HbF) is the primary hemoglobin until six months of age and consists of two alpha chains and two gamma chains. Adult hemoglobin is primarily hemoglobin A (HbA), consisting of two alpha chains and two beta chains. A smaller component of adult hemoglobin is hemoglobin A2 (HbA2), consisting of two alpha chains and two delta chains. There is no substitute for alpha globin in the formation of any of the normal hemoglobin (Hb) following birth (eg, HbA, HbA2, and HbF) [12].

The pathogenesis of beta-thalassemia is two-fold. First, there is decreased hemoglobin synthesis causing anemia and an increase in HbF and HbA2 as there are decreased beta chains for HbA formation. Second, and of most pathologic significance in beta-thalassemia major and intermedia, α-chain synthesis proceeds

at a normal rate, resulting in an excess of α-chains. Despite absent or reduced β-chain production, These α-chains are unstable and precipitate in the red cell precursors causing cell destruction in the bone marrow as well as cell maturation and release into the circulation followed by premature cell destruction in the spleen. The resulting anemia is therefore a product of both ineffective erythropoiesis and shortened red-cell survival. Increases in plasma volume as a result of shunting through expanded marrow and progressive splenomegaly exacerbate the anemia[13] . The bone marrow expansion leads to bony deformities, characteristically of the facial bones which cause frontal bossing and maxillary protrusion. Biochemical signaling from marrow expansion involving the bone morphogenetic protein (BMP) pathway inhibits hepcidin production causing iron hyper absorption[10].

Patients who have received insufficient treatment or who rely on blood transfusions are at risk for end-organ damage from iron overload. Thrombocytopenia and hepatic dysfunction are also caused by hepatosplenomegaly caused by extra medullary hematopoiesis and continuing hemolysis. As a result of diminished HbA production, beta-thalassemia minor produces microcytosis and, at most, moderate anemia. Because people with beta-thalassemia minor have one unaffected beta-globin gene, they can still manufacture enough hemoglobin to meet the body's needs without causing erythroid hyperplasia. Furthermore, an increase in alternative hemoglobin types, most typically HbA2, compensates for the hemoglobin shortage. The clinical syndromes associated with thalassemia are heterogeneous due to the many possible mutations affecting the human globin chain loci[14] .

Beta-thalassemia can also coexist with other hemoglobinopathies and cause variably clinically significant anemia’s in the heterozygous beta-thalassemia

carrier. Delta-beta-thalassemia is clinically similar to beta-thalassemia, and it occurs when there is a deletion of the neighboring delta and beta genes. The pathophysiology of delta-beta-thalassemia parallels that of beta-thalassemia, except there is not an increased HbA2 since the delta chain is also affected.

Variable severity of fundamental biosynthetic abnormalities and coinherited modifying variables, such as increased fetal globin subunit synthesis or reduced or increased synthesis of -globin subunits, contribute to this variability[15].

1.1.3 Classification

Thalassemia syndromes can be subdivided according to their genetic causation or according to their phenotypic presentation. Normal globin production results from balanced synthesis of alpha globin from duplicated genes on chromosome 16 and singe β genes on chromosome 11. Thalassemia is classified according to genetic basis into α, β, γ, δβ, δ, and εγδβ [16,17,18,19] .

Alpha-thalassemia is divided into four types due to the absence or deficiency of one or more of four α-globin genes that result: silent carrier (one α gene inactive) asymptomatic without any hematologic abnormalities, α-thalassemia trait (two α gene inactive) familial microcytic anemia; commonly mistaken as iron deficiency but normal iron indices, Hemoglobin H disease (three α genes inactive) mild to moderate microcytic hemolytic anemia at birth ;evidence of chronic hemolysis, and fetal hydropes (four α genes inactive) severe intra uterine anemia , anemic heart failure and resulting in intrauterine fetal death ,still birth or early neonatal death[20,21].

The β-thalassemia is classified into three classes according to the clinical severity of the anemia into the (Thalassemia trait) Carriers (single β mutation) of the defect with asymptomatic anemia, (Thalassemia Intermedia; TI) Intermediate severity of anemia that may or may not require transfusion support :genetically heterogeneous and (Thalassemia Major; TM) transfusion dependent anemia : usually homozygote or compound heterozygote for p mutations Severe[22]. Classification of β-thalassemia according to genotype β-thalassemia mutation globin chain that is produced. With some mutations no globin is synthesized and is termed β° thalassemia. In others the globin chain is synthesized but in reduced amounts and is known as β^{+}-thalassemia[23,24].

In recent years, thalassemia has been classified according to the clinical pattern into thalassemia dependent on Transfusion Dependent Thalassemia (TDT) and Non Transfusion Dependent Thalassemia (NTDT)[25,26].

1.1.4 Incidence and Frequency of Thalassemia

The frequency of the alpha and beta globin defects have not been analysed in all countries worldwide, primarily due to lack of resources and greater clinical priorities. However a number of researchers have undertaken these studies in individual countries. Worldwide figures from the WHO suggest that there are 270 million carriers for globin gene defects and that 300,000 to 400,000 children are born annually with a severe hemoglobin disorder[27]. Thalassemia is spread in the Mediterranean countries and parts of Africa, throughout the Middle East, the Indian subcontinent, Iran, southern China and countries along the northern coast of Africa, South America, Thailand and Cambodia[17,28,29,24,30,31].

Population migration and intermarriage between different ethnic groups has introduced thalassemia in almost every country of the world, including Northern Europe where thalassemia was previously absent. It has been estimated that about 1.5% of the global population. (80 to 90 million people) are carriers of β-thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world. The total annual incidence of symptomatic individuals is estimated at 4.4/10,000 throughout the world and 1 in 10,000 people in the European Union. According to Thalassemia International Federation, only about 200,000 patients with thalassemia major are alive and registered as receiving regular treatment around the world, In the developing world, the majority of patients die before the age of 20[32].

The prevalence of thalassemia varies in the Arab countries, where alpha thalassemia is prevalent in most Arab countries, with a frequency ranging from 1 to 58 %,while β-thalassemia carrier rates of 1-11 %[17,33] .

In Iraq, there is little data on the epidemiology and burden of thalassemia .increased from 33.5/100,000 in 2010 to 37.1/100,000 in 2015, while the incidence rate had decreased from 72.4/100,000 live births to 34.6/100,000 live births between 2010 and 2015. β-Thalassemia major (β-TM) represented 73.9% of all types of thalassemia[34].

1.1.5 Management of β-thalassemia major

1.1.5.1 Transfusion

Goals of transfusion therapy are the primary means of treatment for patients with severe β-thalassemia. To avoid anemia, inhibition of gastrointestinal iron absorption and fill the deficiency caused by the increased ineffective erythropoiesis. 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). Post-transfusion Hb level of 9 to 10 g/dl - 13 to 14 g/dl prevents growth impairment, organ damage and bone deformities, allowing normal activity and quality of life[35,36].

The frequency of transfusion is usually every two to four weeks. The amount of blood to be transfused to the patient depends on several factors including Hb, target increase in Hb level and hematocrit of blood unit. In general, the amount of transfused RBC should not exceed 15 to 20 ml/kg/day, infused at a maximum rate of 5 ml/kg/hour, to avoid a fast increase in blood volume. Over time, due to complications of frequent blood transfusions and iron overload, iron chelators (like Dessferrioxamine B treatment) are used to help remove the excess iron[37,38,31].

These transfusion regimens will provide a marked improvement in survival, growth and sexual development, prevent disfiguring bony abnormalities, decrease cardiac effort, and limit the development of hepatosplenomegaly. The red blood cell transfusions are lifesavers for patients with thalassemia. They are responsible for a series of inevitable complications and expose the patients to a variety of risks of long term transfusion therapy is iron overload[39].

1.1.5.2 Complications

Complications in β-thalassemia major are linked to overstimulation of the bone marrow, dysfunctional erythropoiesis, Iron overload of tissue is fatal with or without transfusion if not prevented or adequately treated[40,41].

but the most serious disadvantage of life saving transfusions is the inexorable accumulation of iron within tissues and blood-borne infections. After approximately one year of transfusions, iron begins to be deposited in parenchymal tissues. where it may cause substantial toxicity as compared with that within reticuloendothelial cells[38,39].

most patients die of heart dysfunction of iron deposition[32]. and a human have no physiologic mechanism for active elimination of excess iron, and loses only 1–2 mg iron per day[39,42].

Iron accumulation in the liver causes fibrosis and cirrhosis The harmful effects of excessive iron deposition are primarily observed in the heart, liver, skin, Endocrine abnormalities include diabetes mellitus and impaired glucose tolerance, adrenal insufficiency, hypothyroidism, osteoporosis, hypoparathyroidism and hypogonadism[43].

In the presence of excess metal iron can generate reactive oxygen specie ,scause oxidative damage to lipids, protein, and DNA. A well-studied physiologic biochemical iron reacts with O2 species to form cytotoxic hydroxyl radicals by the Fenton and Haber-Weiss reactions. The hydroxyl radical can non-selectively attack proteins, nucleic acids, polysaccharides and lipids. Indeed, the production of hydroxyl radicals has been demonstrated in rats exhibiting iron overload[44,45].

1.1.5.3 Assessment of iron overload

Patients maintained on a regular transfusion regimen progressively develop clinical manifestations of iron overload: hypogonadism (35-55% of the patients), hypothyroidism (9-11%), hypoparathyroidism (4%), diabetes (6-10%), liver fibrosis, and heart dysfunction (33%). the clinical condition of iron must be carefully evaluated to determine the need for treatment and the timing and monitoring of chelation therapy[46,47].

this done by many ways including serum ferritin, which is related to iron stores in the body, however, it cannot be relied upon as a single value, as it is affected by factors such as liver disease, inflammatory disorders and malignant tumors. Nevertheless, the method remains reliable and easier to assess iron overload, Determination of liver iron concentration in a liver biopsy specimen shows a high correlation with total body iron accumulation and is considered the gold standard for the evaluation of iron overload[48,49].

As well as the presence of hepatic fibrosis and HCV infection, as well as, nuclear magnetic resonance imaging (MRI) techniques for assessing iron loading in the liver and in the heart have been introduced, As the body has no effective means for removing iron, the only way to remove excess iron is to use iron binders (chelators), which allow iron excretion through the urine and/or stool. As a general rule, patients should start iron chelation treatment once they have had 10-20 transfusions or when ferritin levels rise above 1000 ng/ml[50,51].

Chelation of iron with desferoxamine is effective in reducing iron load and extending life expectancy. However, to be effective, this drug must be given by subcutaneous continuous infusion each day and is not without side effects including hearing and visual loss, Several oral agents for chelation are in various

stages of testing, It improves the quality of life for patients, In addition to vitamin and folic acid supplements[18,52].

The first drug available for treatment of iron overload was deferoxamine (DFO), an exadentate iron chelator that is not orally absorbed and usually as a subcutaneous 8- to 12-hour nightly infusion, 5-7 nights a week. Average dosage is 20-40 mg/kg body weight for children and 30-50 mg/kg body weight for adults[53].

In high risk cases, continuous administration of DFO via an implanted delivery system (Port-acath) or subcutaneously, at doses between 50 and 60 mg/ kg per day, were the only options to intensify the chelation treatment before the advent of the combined therapy with DFO and deferiprone[54]. Implanted delivery systems are associated with risk of thrombosis and infection. With DFO, iron is excreted both in faeces (about 40%) and in urine. The most frequent adverse effects of DFO are local reactions at the site of infusion, such as pain, swelling, induration, erythema, burning, pruritus, wheals and rash, occasionally accompanied by fever, chills and malaise[55,56].

1.1.5.4 Iron overload-related complications

Iron overload of tissue with or without transfusion is fatal, which is the most important complication of β-thalassemia if not prevented or adequately treated. Regular transfusions double rate of iron accumulation, After approximately one year of transfusions, iron begins to be deposited in parenchymal tissues, where it may cause substantial toxicity as compared with that within retic-uloendothelial cells[57,58].

a non-transferrin bound fraction of plasma iron may promote the generation of free hydroxyl radicals, that leading to oxygen-related damage. The advances in free-radical chemistry have clarified the toxic properties of free hydroxyl radicals, which may cause widespread tissue damage. Extensive iron deposits are associated with cardiac hypertrophy and dilatation, degeneration of myocardial fibers, and in rare cases fibrosis[59,60].

In addition to multiple blood transfusions and hemolysis of red blood cells, the increased gastrointestinal iron absorption due to paradoxical hepcidin suppression from dyserythropoiesis lead to iron overload in β-thalassemia major patients[61]. Iron loading within the anterior pituitary is the primary cause of disturbed sexual maturation, for both sexes, and also cause growth deficiency[62].

As the iron burden increases and iron-related liver dysfunction progresses, hyperinsulinemia occurs as a result of reduced extraction of insulin by the liver, leading to exhaustion of beta cells and reduced circulating insulin concentrations (cause diabetes and impaired glucose tolerance)[63]. Studies reporting suggest that the exocrine pancreas is also damaged by iron loading. Over the long term, iron deposition also damages the thyroid (hypothyroidism)and adrenal glands, and may provoke pulmonary hypertension, right ventricular dilatation, and restrictive lung disease[64]. The degree and the severity of iron toxicity depends upon on need for transfusions , Each milliliter of transfused blood contains about one mg of iron, thus receipt of a unit of packed red cells typically results in the deposition of 200 mg of iron ultimately in the tissues following red cell senescence[65].

1.1.5.5 Splenectomy

If the annual red cell requirement exceeds 180-200 ml/Kg of RBC ,splenectomy should be considered, provided that other reasons for increased consumption, such as hemolytic reactions, have been excluded. Other indications for splenectomy are symptoms of splenic enlargement, leukopenia and or thrombocytopenia and increasing iron overload despite good chelation, Splenectomy also prevents extra medullary hematopoiesis[66,67] .

1.1.5.6 Bone marrow and cord blood transplantation

Bone marrow transplantation and stem cell transplantation are currently considered the curative treatment for Thalassemia patients. Successful allogeneic bone marrow transplant for severe β-thalassemia was first reported in 1982, Later numerous transplants have been performed successful, Bone marrow transplantation (BMT) from HLA (Human leukocyte antigen) identical donors has been successfully performed worldwide, Bone marrow transplantation depends on the clinical situation specifically the presence of hepatomegaly, extent of liver fibrosis, history of regular chelation and hence severity of iron accumulation. In patients without the above risk factors, stem cell transplantation from an HLA identical sibling has a disease free survival rate over 90%[68,69].

The main problem of BMT the lack of an HLA identical sibling donor for the majority of affected patients. BMT from unrelated donors has been carried out on a limited number of individuals with β-thalassemia. Provided that selection of the donor is based on stringent criteria of HLA compatibility and that individuals have

limited iron overload, results are comparable to those obtained when the donor is a compatible sib, but it is not available for all patients[70].

If marrow transplantation is successful, iron overload and iron-induced cardiac impairment may be reduced or the progression of these processes may be prevented[71].Cord-blood transplantation, the use of unrelated phenotypically matched donors, and in utero transplantation. offers a good probability of a successful cure and is associated with a low risk of Graft versus host disease[72] .

Usually, the diagnosis of thalassemia depends on the measurement of some characteristic variables in the blood cells counts and related indicators, like the complete blood count test (CBC) using a special fully automated blood analyzer or counter. However, the CBC test cannot be relied upon alone in diagnosing thalassemia due to its similarity with characteristics that can be seen in other pathological disorders, which leads to a poor diagnosis. Therefore, other additional tests must be performed.

As it is mentioned before, many studies were showed the importance of studying some biofactors for thalassemia patients therefore this study was took about 100 patients' samples men and women, and 50 healthy samples. Furthermore, specific biofactors was measured for these 150 persons that are; LDH, sTfR, Cu, Zn, TP, Cl^{-}, Ferritin, RBC, HCT, HGB, MCV, MCH, MCHC, RDW-SD, RDWCV, WBC, LYM%, and LYM#.

1.2 Lactate dehydrogenase (LDH) in patients with thalassemia major

Lactate dehydrogenase is an enzyme involved in metabolism and energy production in cells[73]. Basically, the enzyme is involved in the anaerobic metabolism of glucose when oxygen is absent or in limited supply[74]. It is found in all tissues of the body and in high concentrations in heart muscle cells, skeletal muscles, red blood cells, liver, kidneys, lungs and brain[75]. LDH is one of the specific cardiac enzymes used to assess heart injury in patients when there is inflammation or injury, and to assess the percentage of liver and lung tissue damage and disease progression. It is known to be high in intravascular hemolysis. The LDH level also rises when metabolic processes are accelerated in cases of lymphoma cancers. In cerebrospinal fluid, LDH increases in bacterial meningitis, while it is observed to be normal in viral meningitis. The ratio of fluid LDH compared to the upper limit of normal serum LDH indicates an inflammatory process[76]. Typically, the normal range of LDH is between 140 to 280 U/L. The LDH activity also increases during strenuous exercise to generate lactic acid under normal physiological conditions[77]. A study conducted in 2020 in Kurdistan, northern Iraq, on 100 Thalassemia major patients under the age of 17 reported that the lactate dehydrogenase enzyme ratio appeared to be significantly elevated[78].

Another study showed elevated levels of LDH enzyme in the blood of thirty Thalassemia patients[79].

1.3 Soluble transferrin receptors (sTfR) in patients with thalassemia major

Transferrin is a glycoprotein that binds to iron in the blood plasma[80]. The formation of erythrocytes in the bone marrow depends on the uptake of transferrin bound iron via the transferrin receptor[81]. The level of soluble transferrin receptors (sTfR) in the plasma has been shown to be closely related to the number of red cell precursors in the bone marrow and to provide a reliable quantitative assessment of the rate of erythropoiesis. The levels of sTfR reflect the receptor density on cells (tissue iron status) and the number of cells with receptors (erythropoietic activity), the sTfR is used as an alternative to the ferritin test due to the proportion of ferritin affected by acute inflammation in the body [82,83].

In a Greece study that conducted on 40 subjects with beta-thalassemia (21 patients dependent on blood transfusion and 19 non-transfused or intermittently transfused) compared with 30 healthy subjects, it was found that the sTfR level in affected subjects was significantly higher compared to healthy individuals and also in non-accredited patients. On blood transfusion or transfusion intermittently compared to patients dependent on blood transfusion[84] . Another study evaluated sTfR levels on 57 adults with beta thalassemia divided into groups according to the genotype, where there was a significant difference between healthy and sick patients, as well as a positive correlation between all types of thalassemia[85].

In 2019, significantly higher sTfR levels were found in 94 transfusion-dependent thalassemia patients compared to 101 non-transfusion-dependent patients[86].

1.4 Zinc (Zn) in patients with thalassemia major

Zinc is an essential element for the human body to perform several functions. It contributes to building proteins, regulating gene expression, supporting and interacting enzymes, and is important in strengthening the immune system[87]. The body needs it in small quantities, not exceeding 15 mg per day, and not exceeding to 40 mg. Excessive consumption of it causes ataxia and lethargy[88]. Its deficiency also causes delayed growth[89]. It has been found through studies that the level of zinc in the blood is low in thalassemia patients and has been linked to many reasons, including low zinc in the blood serum with growth retardation as well as low bone mineral density and others[90].

In a study conducted on 56 patients with thalassemia to clarify how iron chelation treatment affects zinc levels in patients, it was found that iron chelation treatment leads to zinc deficiency[91]. Several studies have reported that Zn deficiency has been associated with regular use of iron chelation therapy[92,93]. While some reported a deficiency regardless of the use of iron chelates[94,95]. Another study was conducted in Thailand for thalassemia patients between the ages of 2-20 years, their zinc level was measured and found a significant decrease in its percentage in the blood[96]. Also, another research in 2020 aimed to know the difference in the concentration of Zn in patients thalassemia and healthy people and found a decrease in its concentration[97].

1.5 Copper (Cu) in patients with thalassemia major

One of the minerals that the body needs and has several properties that act as an antioxidant and antibacterial[98]. It is a cofactor for more than 300 enzymes and is related to albumin and serotonin, which protect cells from free radical damage. Copper deficiency leads to anemia, neutropenia and growth disturbance[99]. On the other hand, the accumulation of Cu in the body leads to copper toxicity and Wilson's disease, where human liver excretes copper in the bile. Sometimes copper accumulates in the liver, causing damage. After sufficient damage, the liver releases Cu into the bloodstream [100,101].

Copper has a key role in the growth of healthy nerves and bones, collagen production, the pigment melanin in the skin. In normal cases, Cu is absorbed from the food we eat and gets rid of it through the bile[102].

Cu levels in several studies were higher in thalassemia patients than in healthy controls[103,104,105,106,107]. But in other studies, the results of copper showed a decrease in its level in the blood[108].

Another study was conducted on children under 12 years of age comparing Cu and Zn levels, and it showed a deficiency of Zn, but Cu levels were normal without an increasing or decreasing[109].

1.6 Total protein (TP) in patients with thalassemia major

Calculating of total protein means measuring two types of proteins (albumin and globulin)[110]. Total protein is the most abundant protein in the blood serum (more than 50%), Albumin is made in the liver[111]. Globulin is a group of proteins, some of them are made in the liver and the others are produced by the immune system[112] .

Many medical conditions affect the percentage or level of total protein in the blood, including leading to its rise, such as multiple myeloma[113], bone marrow disorders[114], and infections[115]. Kidney disease, malnutrition, malabsorption in the intestine[116]. Measuring total protein is important for thalassemia patients, as it is important to assess the health status of the individual by monitoring kidney disease or evaluating liver functions and nutritional problems, or acute liver disease also leads to its rise.

Many studies conducted on thalassemia patients, there was no significant change between patients and healthy controls, or a marked increasing than normal level in the blood serum[117,118,119].

Moreover, section of studies showed an increase in the level of total protein, including a study conducted in 2019 in Al-Hilla / Iraq on Thalassemia patients, where a noticeable difference was found[120]. Another study conducted in Iraq on 150 children from the age of (4-18) who had thalassemia, found a decrease in total protein[121].A study also dealt with 13 children with thalassemia. It was also noted a decrease in the level of total protein and an increase in the rate of erythrocyte sedimentation compared to healthy children[122].

1.7 Chloride (Cl^-) in patients with thalassemia major

Chloride as KCl a type of electrolyte, an electrically charged mineral, Works to help control the amount of fluid and acid balance in the body[123]. If there are symptoms of acid or fluid imbalance, that including persistent vomiting, diarrhea, fatigue, weakness, dehydration, and breathing problems, this needs a chloride test[123]. High levels of Cl^- indicate dehydration and kidney disease, as well as when the patient has a lot of acid in the blood and if the blood contains an amount of alkali[124]. On the other hand, low levels indicate heart failure and lung disease[125]. There are many factors that affect the level of Cl^- in the blood, including fluid intake or loss due to vomiting or diarrhea, as well as some antacid medications that lead to abnormal results[126].

A section of the research reported on the change in the electrolyte level in the blood of patients with thalassemia major[127,128]. Studies have revealed that changes in electrolyte levels occur in other types of anemia, such as sickle cell anemia[129,127,130].

In a study conducted on Thalassemia patients at ages of (12-28 years), it found that chloride levels did not show differences between the control group and the patients[131].

1.8 Ferritin in patients with thalassemia major

It is a protein responsible for storing iron inside cells. Thus, a low ferritin deficiency indicates a lack of iron stores, and a high iron reserve indicates many health problems caused by the accumulation of iron in the body. Including liver disease and hemochromatosis. In general, infections are considered one of the most common causes of high levels of ferritin in the blood, as well as endocrine glands such as hyperthyroidism, liver disease and frequent blood transfusions[132,133].

In a study hold on 32 Thalassemia patients who had chronic inflammation of the liver, it was found that the level of ferritin was higher than the groups without hepatitis[134]. In several studies, the level of ferritin in the blood of children with thalassemia patients was twenty times higher than the normal range[135,136].

Many studies and research is have dealt with a significant and noticeable increase in the level of iron stores in the blood thalassemia patients compared to healthy subjects[137,138,139].

A section of researchers highlighted the association of endocrine complications with the level of ferritin, which was positive[140,141,142].

1.9 Complete Blood Count (CBC) in patients with thalassemia major

The CBC blood monitoring test is one of the common and simple tests by which thalassemia is diagnosed. Thalassemia is classified on the basis of the affected chain of the hemoglobin molecule beta thalassemia or alpha thalassemia[143]. A blood test that measures many components of blood, giving details about red and white blood cells in addition to the physical characteristics of these cells, such as size, shape, and components. CBC measures many components such as : (RBC, HGB, HCT, MCH, MCHC, MCV, RDW-SD, RDW-CV, LYM%, LYM# and WBC), some of this bioindicators were calculation and them details are:

The number of red blood cells or the number of red blood cells in hemoglobin, which is the key to differentiating between anemia and normal people. It works to transport oxygen to every parts of the body are called (RBC).

Hemoglobin This test measures the level of hemoglobin, a protein found inside red cells are called (HGB or Hb).

Which expresses the percentage of red blood cells of the total blood volume are called (HCT).

MCH: mean corpuscular hemoglobin, MCH is the average hemoglobin in the average red blood cells.

Mean corpuscular hemoglobin concentration (MCHC). MCHC checks the average amount of hemoglobin in a group of red blood cells.

The mean corpuscular volume(MCV) is the average size of your red blood cells.

White blood cells that are also one of the body's main types of immune cells are called Lymphocytes, They are made in the bone marrow and found in the blood and lymph tissue. These cells work together to defend the body against foreign substances, such as bacteria, viruses, and cancer cells that can threaten its functioning[144]. Lymphocyte count is one part of a complete blood count (CBC). In β-thalassemia Besides the high risk of blood-borne infections associated with multiple transfusions, the increased susceptibility of these patients to infectious diseases has been attributed to a coexistent immune deficiency[145].

Low RBC is evidence of acute or chronic bleeding, to that indicates a deficiency in nutrients such as iron, vitamin B12 and folic acid, as well as bone marrow disorders, chronic infections and kidney disease. The function of the RBC is not limited in transporting oxygen and nutrients, but it considers an important communication systems where it is involved in nitric oxide metabolism and redox regulation[146]. One of these studies aimed at evaluating the characteristics of blood and oxidative stress through their dependence on taking RBC concentrations before and after using several experimental treatments as a means to relieve some side effects in thalassemia patients[147]. The aim of another study was to extend the survival period of RBC by taking some vitamins, leading increase in level of Hb, but no improvement in the level of Hb was observed in patients[148].

Rising in MCH above the normal rate may hide behind major anemia and diseases of the liver and thyroid gland, While its low indicates that the red blood cells are smaller than normal, as occurs in thalassemia major.

If values of RBC, HCT and HGB less than normal, this is an indication of anemia may refer to iron deficiency, bleeding or hemolysis and other problems, but if the opposite occurs, it indicates heart disease and red blood cells that result from

smoking. Another research dealt with the ratio of HCT and Hb concentrations and found it to be significantly lower in thalassemia patients[149]. Another study confirmed that thalassemia major patients had lower HCT levels compared to thalassemia trait carriers as well as healthy subjects[150]. According to another research conducted on thalassemia patients, it was found that the percentage of hemoglobin, MCV and MCHC is lower in patients compared to normal people[151].

White blood cells rise as a result of bacterial or viral infections, infections, leukemia and myelomas, as well as in allergic conditions such as asthma and conditions that cause tissue death such as exposure to burns or heart attacks[152]. WBC is of several types. In this study was limited to lymphocytes (LYM) which rises in infections such as infections of viral origin, colitis and some blood cancers, and decrease in autoimmune diseases, AIDS, viral hepatitis, Corona virus, or by the action of cortisone medications[153].

Elshami and Alhalees in 2012 presented an investigation for thalassemia existence by using data mining classifiers depending on CBC. The data set consisted of 46,920 samples and in the end the authors concluded that MCV was the main feature to indicate the presence of thalassemia[154].

A study was conducted in Thailand for a group of adult samples that were examined for the detection of Thalassemia, the aim of the study was to differentiate between people with abnormal hemoglobin based on complete blood count and hemoglobin data. The results indicated that HB was a frequent feature of this study[155]. Masala et al. used characteristics of haemochromocytometric data to distinguish normal patients from α-thalassemia carriers and β-thalassemia carriers as attribute for the classification process including RBC count, Hb, hematocrit (HCT), HbA2 and MCV, The dataset consisted of 304 clinical records[156].

Evaluation of 153 cases diagnosed and distinguished from iron deficiency anemia characterized by low RBC, Hb, MCV, serum iron, transferring saturation and serum ferritin , beta thalassemia cases were confirmed with low Hb, MCV, and normal serum iron[157].

Several studies have reported that RDW is a good indicator for distinguishing between thalassemia and anemia[158,159,160]. Contrary to some studies, it was demonstrated that RDW alone is not specific or sensitive enough to distinguish between anemia and thalassemia (α _ β) as they had a high level in all cases[161,162,].

The Aims of study

Thalassemia is one of the most globally common chronic hematological disorders. The average life expectancy of it evolved from 17 years in 1970, to 27 years in 1980 and to 37 years in 1990[(163)]. This high ratio led this study to search about the reason behind this fatal disease because it may be not genetically it may be anything else such specific microbes. The results were so good; the location of this disease, changeable ions concentrations (such as Zn and Cu), thalassemia status with malaria patients …etc. However, these results are not enough for solving this argumentative matter so additional biofactors were calculated; LDH, sTfR, Cu, Zn, TP, Cl^{-}, Ferritin, RBC, HCT, HGB, MCV, MCH, MCHC, RDW-SD, RDWCV, WBC,LYM%, and LYM#.

For finding more additional biofactors supporting this study previous results and also may find a suitable assay for detecting thalassemia disease before it get worse. It is obvious that when these show right reason for thalassemia this should lead to find successful treatment for this fatal disease and this study gave satisfied results for this aim.

CHAPTER Two

Materials & Methods

2.1 Materials

2.1.1 Instruments and Apparatus

the apparatus and equipment's utilized used in this study as shown in table (2-1)

Table (2-1) The apparatus and equipment used in the study

No.	Apparatus	Manufacturer
1.	Centrifuge	Nuve NF-400(Turkey) Hettich Zentifugen(Germany)
2.	Deep freezer	Vestel (Turkey)
3.	EDTA tubes	Medical Technology (China)
4.	Elisa	Biotek (USA)
5.	Hot Plate	Stuart (UK)
6.	Jel Tubes	Alibaba (China)
7.	Oven	Binder (USA)
8.	Mini Vidas	BioMérieux (France)
9.	UV/VIS Spectrophotometer , UV-9200	Biotech (UK)
10.	Micropipette	(China)
11.	CBC	Sysmex ,XP-300 (Germany)
12.	Water path	Daiki KBLEE 2010
13.	Gloves	Broche(Malaysia)
14.	Blue Tips	(China)
15.	Yellow Tips	(China)
16.	Plastic Cuvette	Alibaba (China)

2.1.2 Kits

The kits used in this study are listed in table (2-2)

Table (2-2) The kits used in the study

No.	Kits and Chemicals	Manufacturer
1.	Ferritin Kit	BioMérieux (France)
2.	Total protein Kit	Spinreact (Spain)
3.	Chloride Kit	Biolabo (France)
4.	Copper Kit	LTA (Italy)
5.	Zinc Kit	LTA (Italy)
6.	LDH Kit	Spinreact (Spain)
7.	Human soluble transferrin receptor (sTfR) ELISA Kit	Sunlong Biotech(China)

2.2 Study Subjects

This study was conducted over an eight month period, from November 2020 to the end of July 2021, Blood samples were collected from 150 subjects (100 β-thalassemia major patients and 50 healthy controls). A 100 (50 male and 50 female) patients with β- thalassemia major patients, and the ages range from 10 years to 25 years. Diagnosis of beta thalassemia patients was based on their medical history and examination, in addition to their data from complete blood count and hemoglobin electrophoresis. In addition, all patients undergo regular blood transfusions as part of their treatment.

All blood collections were undertaken when the patient had received no desferrioxamine drug for 48 hrs, and did not receive a blood transfusion during that time. While the healthy group included 50 people who were selected from the general population according to age and gender match with the group of patients. Then the patients and healthy people were divided into two subgroups on the basis of age from 10-17 years and 18-25 years to measure the chemical functions required in this study. A study project was designed as scheme, as shown in fig. (2-1).

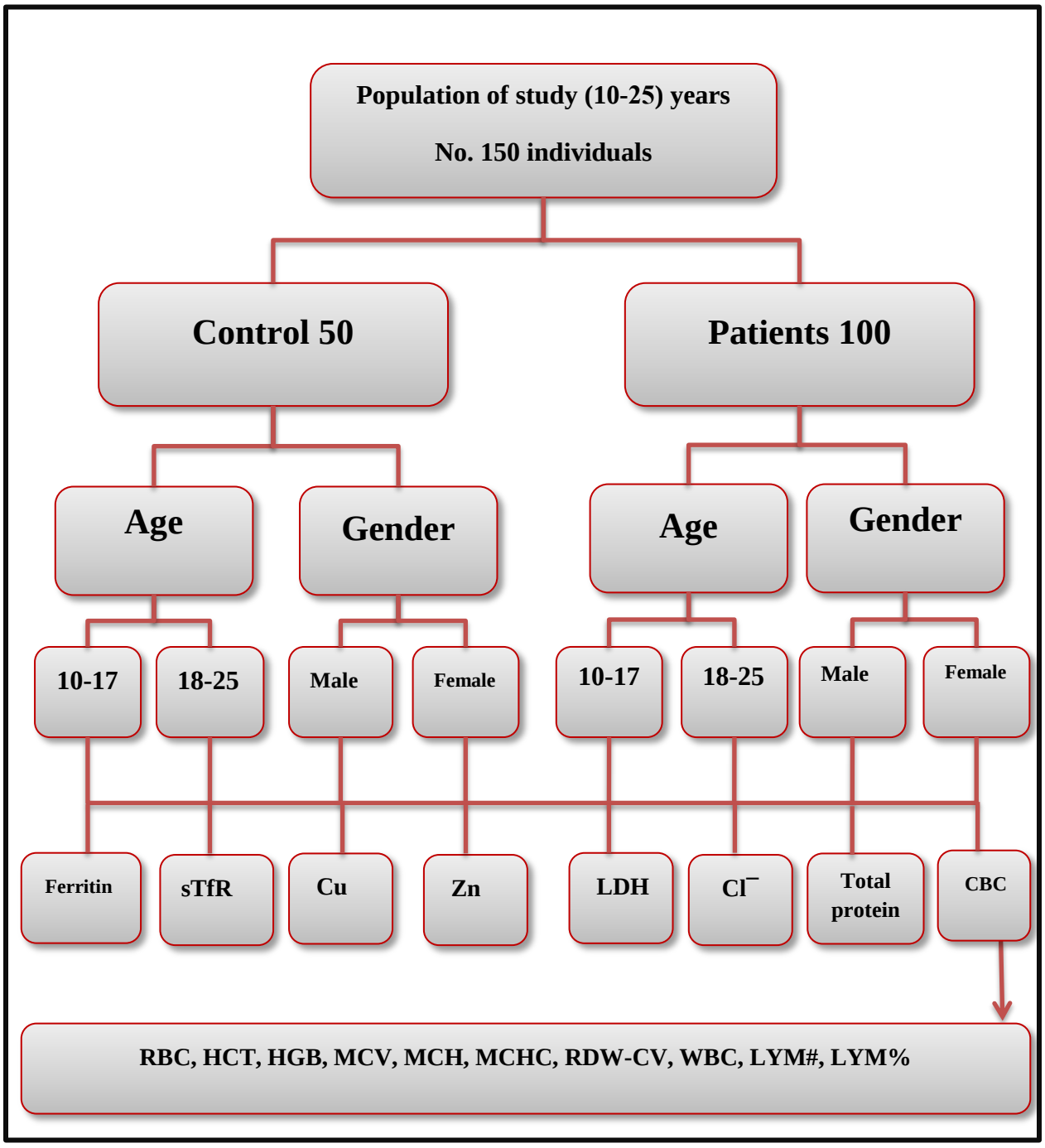


Fig. (2-1): Design of the current study scheme

2.3 Methods:

2.3.1 Collection of blood and samples preparation

Blood samples were collected from 150 subjects (100 β-thalassemia major patients from the thalassemia Center in Missan and 50 healthy controls),10ml of blood was taken from each one and collected in two tubes,8 ml in Gel tube and 2 ml in ethylene di amine tetra acetic acid (EDTA) tube . The serum preparation After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10 - 20 min. Remove the clot by centrifuging at 2.000 - 3.000 rpm. for 20 minutes. If precipitates appear during reservation, the sample should be centrifuged again. The serum were separated and stored at (- 20 °C) until time of use.

2.3.2 Determination of serum ferritin value

2.3.2.1 Principle

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection Enzyme Linked Fluorescent Assay (ELFA)[(164)]. The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. The sample has taken and transferred into the well containing alkaline phosphatase labeled anti-ferritin (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a “sandwich”. Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the

hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of ferritin present in the sample.

Table (2-3) Content of the ferritin (FER) kit

60 FER Reagent Strips	STR	Ready to use.
60 FER SPRs (2 x 30)	SPR	Ready-to-use. Interior of SPRs coated with monoclonal anti- ferritin Immunoglobulins (mouse).
FER control 1 x 3 ml (liquid)	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 Range").
FER Calibrator (liquid) (1 x 2 ml)	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 (1st IRP 80/578) ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range").
FER Dilution Buffer (liquid) (1 x 25 ml)	R1	Ready to use. TRIS buffer (0.1 mol/l, pH 7.4) and protein and chemical stabilizers.
Specifications for the factory master data required to calibrate the test:		

• MLE data (Master Lot Entry) provided in the kit, or • MLE bar codes printed on the box label.
1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib.

2.3.2.2 Procedure

1. Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.
2. Use one "FER" strip and one "FER" SPR from the kit for each sample, control or calibrator to be tested. Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.
3. The test is identified by the "FER" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the control is to be tested, it should be identified by "C1".
4. If necessary, clarify samples by centrifugation.
5. Mix the calibrator, control and samples using a vortex- type mixer (for serum or plasma separated from the pellet).
6. Insert the "FER" Reagent Strips and 2SPRs into appropriate position on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
7. Initiate the assay as directed in the operator's manual. All the assay steps are performed automatically by the instrument.
8. Reclose the vials and return them to 2-8°C after pipetting.

9. The assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
10. Dispose of the used SPRs and strips into an appropriate recipient.

2.3.3 Quantitative determination of total protein in serum

2.3.3.1 Principle

Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant.

The intensity of the color formed is proportional to the total protein concentration in the sample[165].

2.3.3.2 Reagents

Table (2-4) Content of The total protein Kit - Reconstitution of Reagents

R Biuret	Sodium potassium tartrate Sodium iodide Potassium iodide Copper (II) sulphate Sodium hydroxide	15 mmol/L 100 mmol/L 5 mmol/L 19 mmol/L 1000 mmol/L
T protein cal	Bovine albumin primary standard	7 g/dL

2.3.3.3 Procedure

1. Adjust the instrument to zero with distilled water.
2. Pipette into a cuvette :

	Blank	Standard	Sample
R	1.0 mL	1.0 mL	1.0 mL
Standard	-	25	-
Sample	-	-	25 µl

3. Mix and incubate 5 minutes at 37°C or 10 minutes at room temperature, and wavelength:540(530-550)nm
4. Reed the absorbance (A) of the samples and Standard, against the Blank. The color is stable for at least 30 minutes.

2.3.3.4 Calculations

$$\frac{(\mathrm{A})\ \mathrm{Sample} - (\mathrm{A})\mathrm{Blank}}{(\mathrm{A})\mathrm{Standard} - (\mathrm{A})\mathrm{Blank}} \times 7(\mathrm{Standard\ concentration}) = \mathrm{g/dL\ of\ total\ protein\ in\ the\ sample}\ .$$

2.3.4 Quantitative determination of chloride in serum

2.3.4.1 Principle

$$Hg(SCN)_2 + 2Cl^- \longrightarrow HgCl_2 + 2SCN^-$$

$$3(SCN) + Fe^{3+} \longrightarrow Fe(SCN)_3$$

Chloride ions react with undissociated mercuric thiocyanate to form undissociated mercuric chloride and free thiocyanate ions. Thiocyanate ions react with ferric ions to form a highly colored reddish complex of ferric thiocyanate which absorbance, proportional to the amount of chloride in the specimen, is measured at 500 nm (450-500).

2.3.4.2 Reagents

Table (2-5) Content of The chloride Kit - Reconstitution of Reagents

Vial R1 Thiocyanate reagent	Ferric nitrate	22.2 mmol/L
	Chloride mercuric	0.55 mmol/L
	Mercuric Thiocyanate	1.33 mmol/L
	Nitric acid	30 mmol/L
	Surfactant	1 mmol/L
Vial R2 Standard	Chloride	100 mmol/L

2.3.4.3 Procedure

Let stand reagent and specimen at room temperature.

Pipette into well identified test tubes:	Blank	Standard	Sample
Reagent	1 mL	1 mL	1 mL
Demineralised water	10 µL	-	-
Standard	-	10 µL	-
Specimen	-	-	10 µL

Mix well, Let stand for 5 minutes at room temperature. Record absorbances at 500 nm (450-500) against reagent blank. Colour is stable for 30 minutes away from light.

2.3.4.4 Calculations

$$Result = \frac{\text{Abs(Assay)}}{\text{Abs(Standard)}} \times 100(\text{Standard concentration})$$

2.3.5 Quantitative colorimetric determination of Copper in serum

2.3.5.1 Principle

The chromogen 3,5-Di-Br-PAESA react with cupric ions and forming a blue-violet compound, which intensity is proportional to the copper concentration in the sample.

The method does not require de-proteinization of the serum nor the blank sample.

2.3.5.2 Reagents

Table (2-5) Content of The copper Kit - Reconstitution of Reagents

Reagent A	Acetate buffer 0.1M pH 4.9; reducing agents and preservatives.
Reagent B	3,5-Di-Br-PAESA.
Standard	Ion copper 200µg/dl; preservatives.

Prepare the work reagent mixing in the equal quantity the reagent A with reagent B. Reagent are stored at 2-8ºC and is stable until.

2.3.5.3 Procedure of this kit

Reagents	Blank	Standard	Sample
Work Reagent	1 mL	1 mL	1 mL
Distilled water	66 µL	-	-
Standard	-	66 µL	-
Sample	-	-	66 µL

Mix and wait for 10 minutes then read the absorbance's against the blank at 580 nm.

The colour is stable for 30 minutes.

2.3.5.4 Calculations

$$Copper\ \mu g/dl = \frac{\text{A(sample)}}{\text{A(Standard)}} \times 200(\text{Standard concentration})$$

2.3.6 Colorimetric determination of Zinc in serum

2.3.6.1 Principle

Zinc reacts with the chromogen present in the reagent forming a coloured compound which colour intensity is proportional to the zinc concentration present in the sample.

2.3.6.2 Reagents

Table (2-6) Content of the Zinc Kit - Reconstitution of Reagents

Reagent A	Borate buffer 0.37 M pH 8.2; Saliciladoxime.
Reagent B	Nitro-PAPS; 0.4 mM, preservatives.
Standard	Zinc ion 200 µg/dl (30.6µmole/l); stabilizers and preservatives.

Add 2 ml of reagent B to a vial of reagent A. Reagents are stable until expiration date on label, stored at 2-8ºC.

2.3.6.3 Procedure of this kit

Reagents	Blank	Standard	Sample
Work Reagent	1 mL	1 mL	1 mL
Distilled water	55 µL	-	-
Standard	-	55 µL	-
Sample	-	-	55 µL

Mix and read the absorbance against blank at 578 nm. Colour is stable for 30 minutes.

2.3.6.4 Calculations

$$Zinc\ \mu g/dl = \frac{\text{A(sample)}}{\text{A(Standard)}} \times 200(\text{Standard concentration})$$

2.3.7 Quantitative determination of lactate dehydrogenase (LDH) in serum

2.3.7.1 Principle

Lactate dehydrogenase (LDH) catalysis the reaction of pyruvate by NADH, according the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-lactate} + NAD^+$$

The rate of decrease in concentration of NADPH, measured photo metrically, is proportional to the catalytic concentration of LDH present in the sample.

2.3.7.2 Reagents

Table (2-7) Content of the LDH Kit - Reconstitution of Reagents

R 1 Buffer	Imidazole Pyruvate	65 mmol/L 0.6 mmol/L
R 2 Substrate	NADH	0.18 mmol/L

Work reagent (WR):

Dissolve 1 tablet of R2 in one vial of R1. Cap and mix gently to dissolve contents.

Stability: 2 days at 2-8ºC or 12 hours at room temperature (15-25ºC).

2.3.7.3 Procedure of this kit

1. Assay conditions:

   Wavelength: 340 nm
2. Adjust the instrument to zero with distilled water or air.
3. Pipette in to a cuvette:

	25ºC – 30 ºC	37ºC
Work Reagent	3 mL	3 ml
Sample	100 µL	50 µL

4. Mix, incubate for 1 minute.
5. Read initial absorbance at 1 minute intervals thereafter for 3 minute.
6. Calculate the difference between absorbance's and the average absorbance differences per minute (ΔA/minute).

2.3.7.4 Calculations

25ºC – 30 ºC ΔA/minute × 4925 = U/L LDH

2.3.8 Determination of soluble transferrin receptor (sTfr) in serum

2.3.8.1 Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to sTfR. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for sTfR is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain sTfR and HRP conjugated sTfR antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of sTfR. You can calculate the concentration of sTfR in the samples by comparing the OD of the samples to the standard curve.

Table (2-8) Materials provided with the (sTfR) Kit

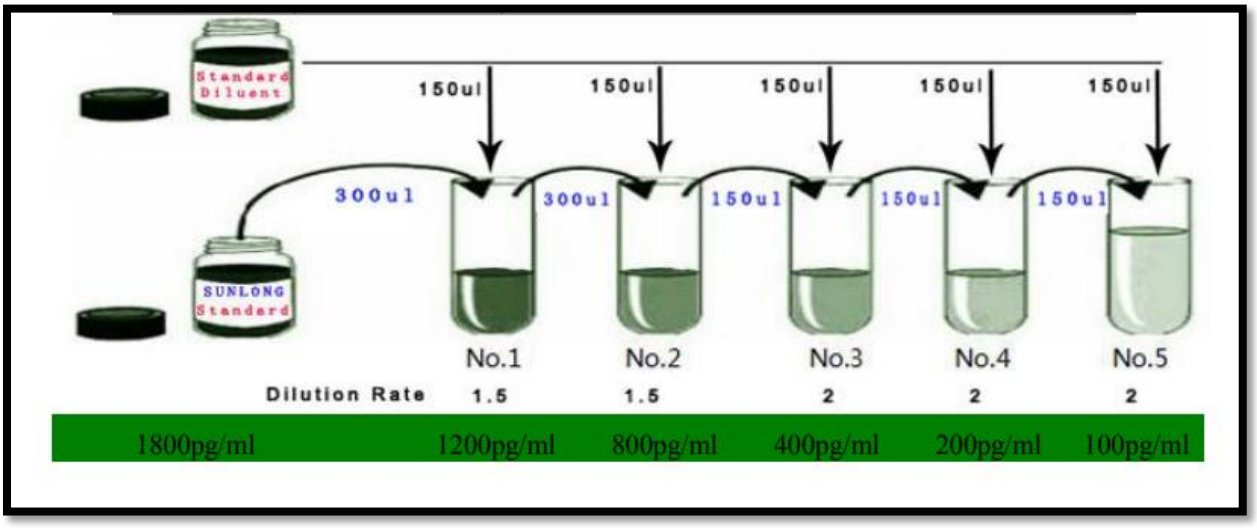
	Materials provided with the Kit	96 determination	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microelisa stripplate	1	2-8ºC
5	Standard: 1800 ng/ml	0.5ml×1 bottle	2-8ºC

6	Standard diluent	1.5ml×1 bottle	2-8ºC
7	HRP-Conjugate reagent	6ml×1 bottle	2-8ºC
8	Sample diluent	6ml×1 bottle	2-8ºC
9	Chromogen solution A	6ml×1 bottle	2-8ºC
10	Chromogen solution B	6ml×1 bottle	2-8ºC
11	Stop solution	6ml×1 bottle	2-8ºC
12	Wash solution	20ml (30X)×1bottle	2-8ºC

2.3.8.2 Procedure of this kit

1. Dilution of Standards Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube use two wells, total ten wells.

1200pg/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
800pg/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
400pg/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
200pg/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
100pg/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent

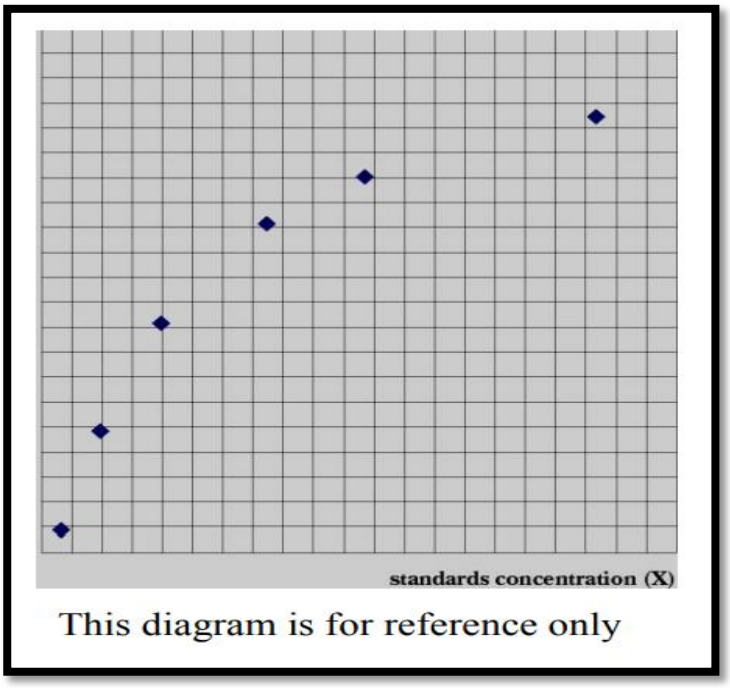


2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 µl HRP-Conjugate reagent to each well except the blank control.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.

9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

2.3.8.3 Calculation of Results

Known concentrations of Human sTfR Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human sTfR in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.



2.4 Statistical Analysis of Data

Statistical analysis was performed by a one-way ANOVA (Analyses Variation) followed by LSD test. Data were expressed as Mean±SD. Statistical significance was set at $P<0.005$ (SPSS,2001).

CHAPTER Three

Results

&

Discussion

3. Results and discussion

3.1 Lactate dehydrogenase (LDH):

Results of this study revealed a signification ($p < 0.05$) increase in serum LDH level (228.56± 91.28 U/L) in female and male (283.26±109.73 U/L) β-thalassemia major patients comparison to female and male in control groups (103.90± 31.96U/L and 112.87± 30.79 U/L) respectively, the LDH levels were significantly higher in patients group (225.91±107.73 U/L) than in Controls group (108.39±31.39 U/L), The results are shown that in table (3-1), and fig. (3-1).

Table (3-1): Serum LDH value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Controls group	Patients group
LDH (U/L)	Female	103.90± 31.96[a]	228.56± 91.28[b]
	Male	112.87± 30.79[a]	283.26±109.73[b]
	Total	108.39±31.39[a]	225.91±107.73[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

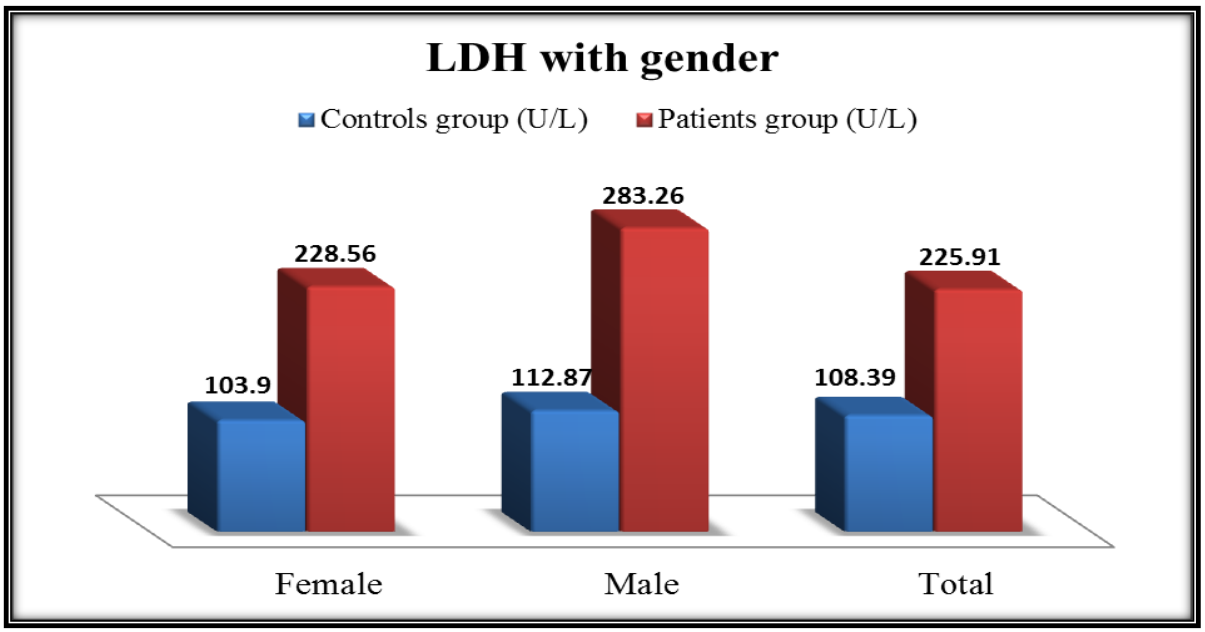


Fig. (3-1): LDH in healthy and β-thalassemia major patients according to gender and group

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both ages have a significant (P <0.05) increase in serum LDH level (10 – 17 years =186.02±59.64 U/L, and 18 – 25 years = 271.11±98.41 U/L) in comparison to female control groups (99.52±28.66 U/L, and 108.90±35.85 U/L respectively), and also male patients in both age subgroups have a statistically significant (P <0.05) increase in serum LDH (10 – 17 years = 241.19±86.08 U/L, and 18 – 25 years=325.33±129.04 U/L) in comparison to male control groups (103.22±24.71 U/L, and 123.33±30.79 U/L) respectively, The results are shown that in table (3-2),and fig. (3-2)

Table (3-2): Serum LDH value in healthy and β-thalassemia major patients according to age and gender

Variable	Gender	Age	Controls group	Patients group
LDH (U/L)	Female	10 - 17	99.52±28.66[a]	186.02±59.64[b]
		18 - 25	108.90±35.85[a]	271.11±98.41[b]
	Male	10 – 17	103.22±24.71[a]	241.19±86.08[b]
		18 - 25	123.33±30.79[a]	325.33±129.04[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

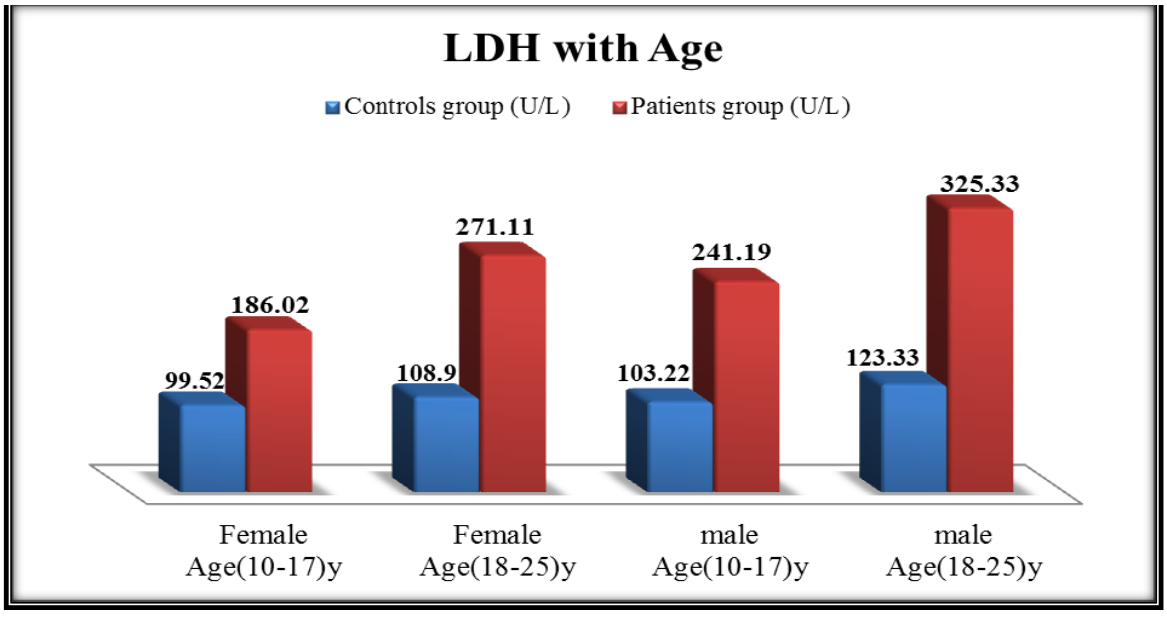


Fig. (3-2): LDH in healthy and β-thalassemia major patients according to age and gender

Lactate dehydrogenase (LDH) used as indicator for hemolysis in patients with thalassemia disease. The concentration of LDH increases due to vascular disease and dysfunction[166]. It is believed that the increased concentration as a result of microvascular disease is related to hemolysis and its levels[(167)].

Also, low oxygen saturation leads to high LDH as a result of low hemoglobin. LDH is considered as a prognostic indicator of the mechanism of intravascular hemolysis[(168)]. The infections that thalassemia patients are exposed to as a result of infection and weak immunity cause an increase in LDH[(169)]. The level of LDH decreases with a decrease in the rate of hemolysis[(170)]. Most thalassemia patients suffer from a deficiency of vitamin B_{12} and folic acid due to the supply of ineffective red blood cells and their early death, which leads to an increase in LDH[(171)].

Moreover proved a correlation between the level of ferritin and LDH in thalassemia patients[(172)]. The degree of bone damage and Hb levels correlate with increased LDH and may indicate hemolysis within the bone[(173)].

3.2 Soluble transferrin receptors (sTfR)

Results of this study revealed a signification ($p < 0.05$) increase in serum soluble transferrin receptors level (2421.26±1349.10 pg/ml) in female and for male (2690.12±2114.40 pg/ml) β-thalassemia major patients comparison to female and male in control groups (904.765±463.53 pg/ml and 551.70±313.67 pg/ml) respectively, the sTfR levels were significantly higher in patients group (2554.33±1765.79 pg/ml) than in Controls group (735.29±433.07 pg/ml), as it is clarify in table (3-3), and fig. (3-3).

Table (3-3): Serum Soluble transferrin receptors value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Controls group	Patients group
sTfR (pg/ml)	Female	904.765±463.53[a]	2421.26±1349.10[b]
	Male	551.70±313.67[a]	2690.12±2114.40[b]
	Total	735.29±433.07[a]	2554.33±1765.79[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

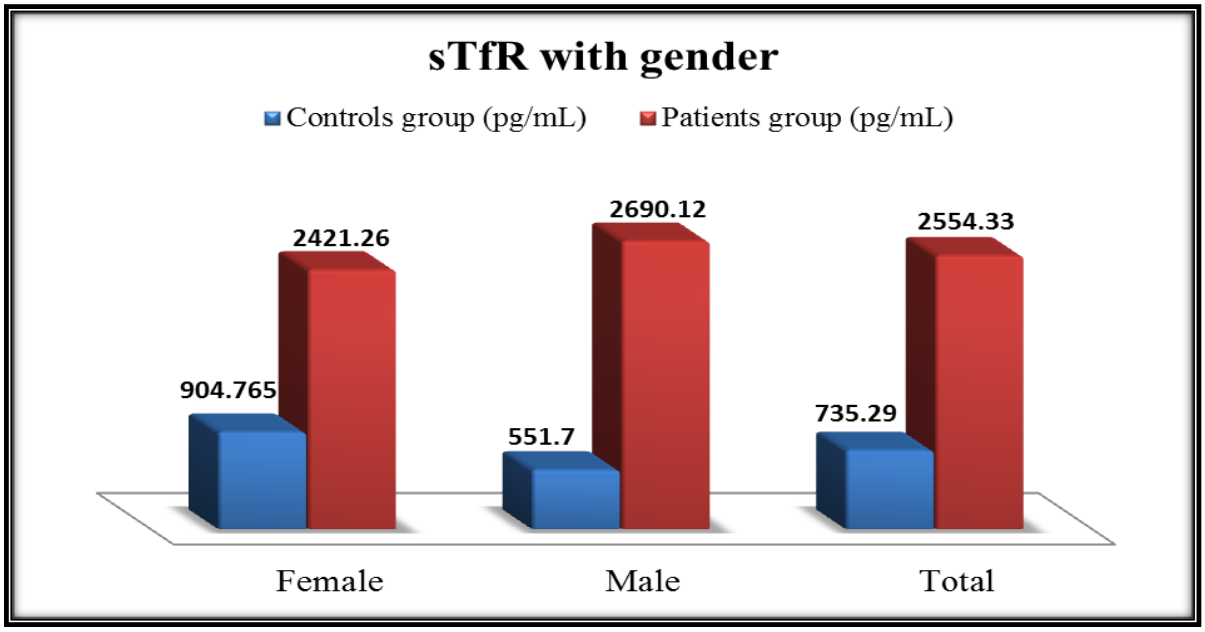


Fig. (3-3): Soluble transferrin receptors in healthy and β-thalassemia major patients according to gender and group

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) increase in serum sTfR level (10 – 17 years =2121±1190.80 pg/ml, and 18 – 25 years = 2370.29±1994.54 pg/ ml) in comparison to female control groups (976.68±383.86 pg/ml, and 488.97±312.85 pg/ml) respectively, and also male patients in both age subgroups have a statistically significant (P <0.05) increase in serum sTfR (10 – 17 years = 2721.05±1452.84 pg/ml, and 18 – 25 years=2997.15±2220.00 pg/ml) in comparison to male control groups (832.84±537.66pg/ml, and 614.43±315.05 pg/ml) respectively, as it is identified in table (3-4),and fig. (3-4).

Table (3-4): Serum Soluble transferrin receptors value in healthy and β-thalassemia major patients according to age and gender

Variable	Gender	Age	Controls group	Patients group
sTfR (pg/ml)	Female	10 - 17	976.68±383.86[a]	2121±1190.80[b]
		18 - 25	488.97±312.85[a]	2370.29±1994.54[b]
	Male	10 – 17	832.84±537.66[a]	2721.05±1452.84[b]
		18 - 25	614.43±315.05[a]	2997.15±2220.00[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

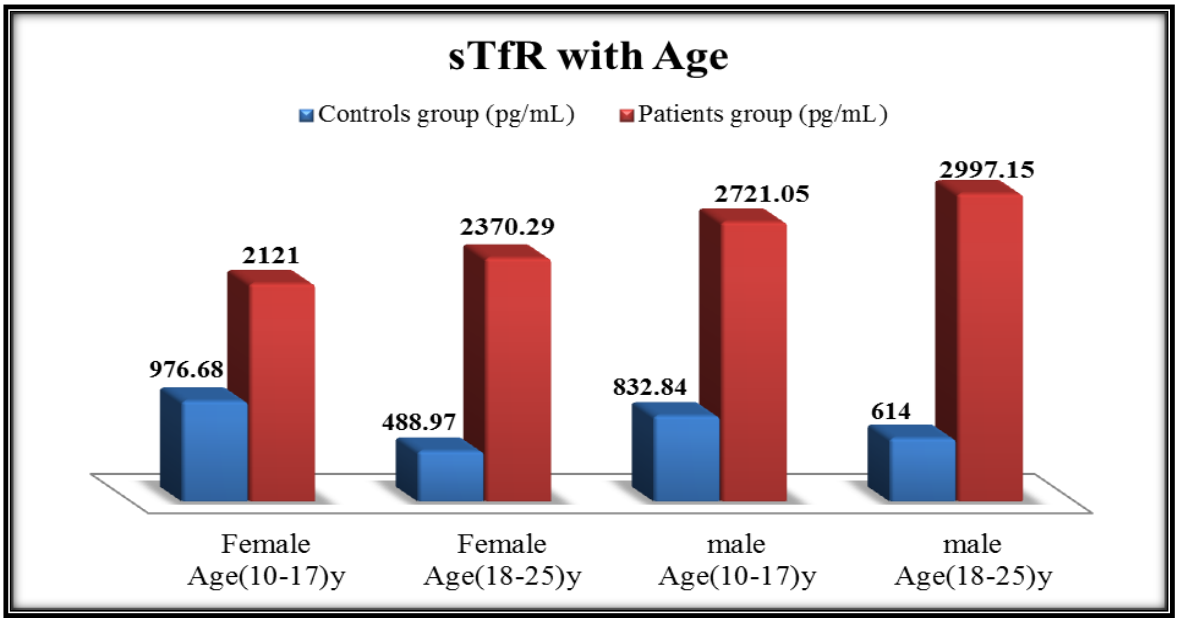


Fig. (3-4): Soluble transferrin receptors in healthy and β-thalassemia major patients according to age and gender

This study showed high levels of Soluble transferrin receptors (sTfR) compared with the control, which indicates an increased activity of erythrocytes in the bone marrow[174]. Transferrin receptor measurements used to detect and assess iron deficiency and to diagnose iron deficiency anemia. High levels of sTfR indicate the inactivity of erythrocytes, as high levels of sTfR indicate in case Hemolysis an increased demand for iron. The sTfR demonstrated astonishing diagnostic accuracy in predicting the risk of extramedullary hematopoiesis and clinical severity[175,176].

The levels of sTfr and erythropoiesis are associated with globin chain imbalance in thalassemia, and the increase in iron stores, transferrin saturation and inflammation leads to the formation of inactive blood cells and the possibility of shortening the survival period of red cells.

The significant difference in the height of ferritin compared with sTfr in thalassemia patients confirms that ferritin is affected by inflammation unlike sTfr, as it is shown in the table (3-13) specific to ferritin and a table (3-4) specific to transferrin.

Osteitis or bone marrow inflammation causes protein deposition in red blood cells with a decrease in oxygen concentration as a result of a lack of hemoglobin as previously described in this study, which is the first clinical sign that appears on a child with thalassemia and continues until the period of receiving treatment to alleviate these symptoms, and this raises doubts that this infection appeared from the beginning of the production of red blood cells.

3.3 Zinc (Zn)

Results of this study revealed a signification ($p < 0.05$) decreased in serum Zn level (61.96±24.63 µg/dl) in female and male (75.10±29.92 µg/dl) β-thalassemia major patients comparison to female and male in control groups (120.80±67.40 µg/dl, 121.38±35.49 µg/dl) respectively, Zinc levels were significantly lower in patients group (68.544±28.06 µg/dl) comparing to Controls group (121.09±53.33 µg/dl), The results are shown that in table (3-5),and fig (3-5).

Table (3-5): Serum Zn value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Controls group	Patients group
Zn (µg/dl)	Female	120.80±67.40[a]	61.96±24.63[b]
	Male	121.38±35.49 [a]	75.10±29.92 [b]
	Total	121.09±53.33 [a]	68.544±28.06 [b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

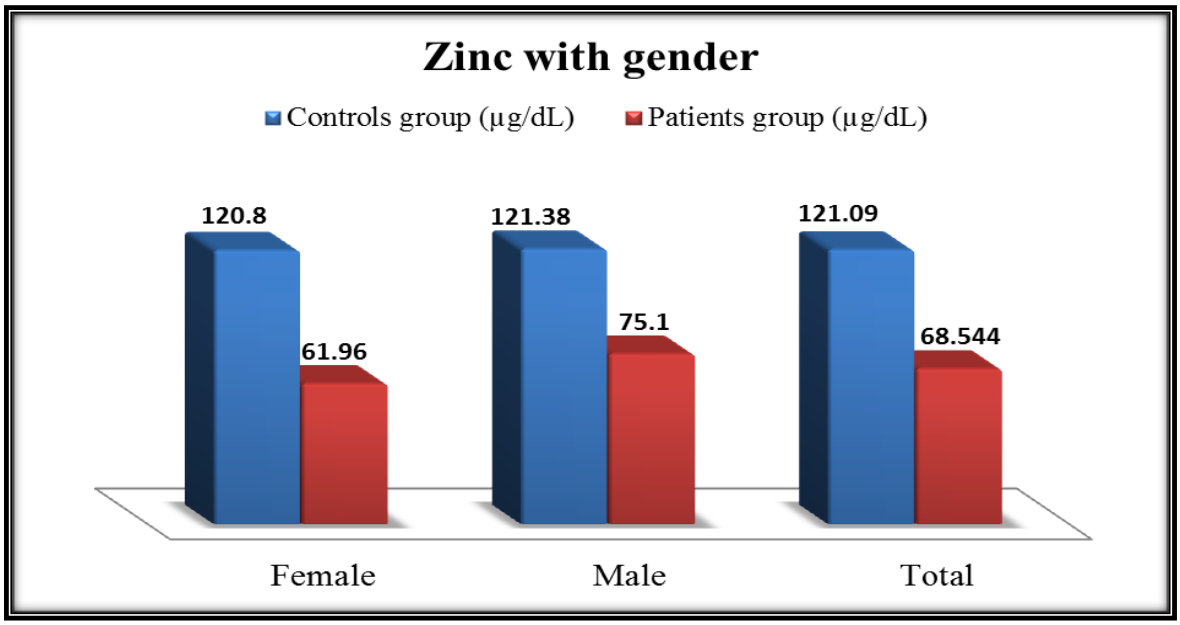


Fig. (3-3): Zinc in healthy and β-thalassemia major patients according to gender and group

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) decreased in serum Zn level (10 – 17 years =73.81±22.51 µg/dl, and 18 – 25 years = 50.11±20.97 µg/dl) in comparison to female control groups (104.63±14.35 µg/dl, and 136.98±93.23 µg/dl) respectively, and also male patients in both age subgroups have a statistically significant (P <0.05) decreased in serum Zn (10 – 17 years = 88.74±34.77 µg/dl, and 18 – 25 years=61.49±15.25 µg/dl) in comparison to male control groups (108.40±22.71 µg/dl, and 134.37±41.75 µg/dl) respectively, The results are shown that in table (3-6),fig(3-6).

Table (3-6): Serum Zn value in healthy and β-thalassemia major patients according to age and gender

Variable	Gender	Age	Controls group	Patients group
Zn (µg/dl)	Female	10 - 17	104.63±14.35 [a]	73.81±22.51 [b]
		18 - 25	136.98±93.23 [a]	50.11±20.97 [b]
	Male	10 – 17	108.40±22.71 [a]	88.74±34.77 [b]
		18 - 25	134.37±41.75 [a]	61.49±15.25 [b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

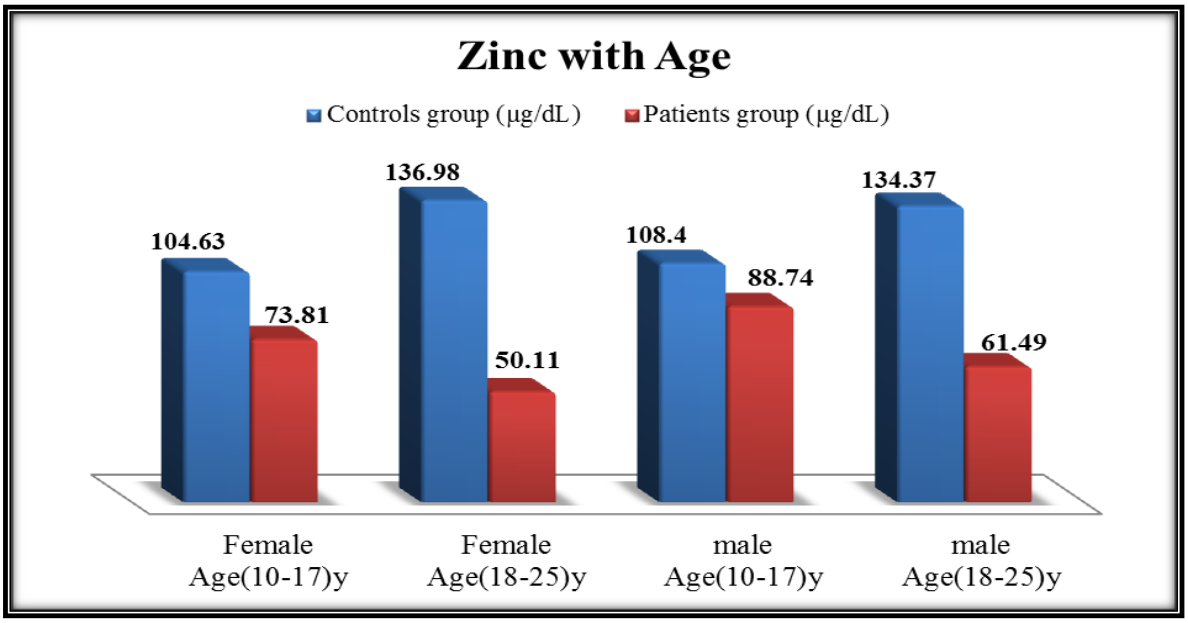


Fig. (3-6): Zinc in healthy and β-thalassemia major patients according to age and gender

Zinc is one of the factors that participate in cellular metabolism and has several functions. It is found in vital organs also it considered an anti-inflammatory. The growth of the body is affected by Zn levels[177]. Zn affects oxidative stress and thus prevents many diseases such as cancers as well as mutations[178]. Zn deficiency in thalassemia patients leads to many diseases; including failure to reach puberty, as well as delayed growth of the body, as a study showed the linear effect of Zn supplementation on children's growth[90]. Some attributed the reason for the lack of Zn and the lack of blood weight in thalassemia patients to the excessive excretion of Zn in urine due to the decomposition of blood cells[105].

There are more than 1000 enzymes that depend on the element Zn[179]. Zinc deficiency affects the thymus glands and weakens the immune response against fungi as well as the secretion of antibodies to cells[180]. Zinc is of great importance in microorganisms, including fungi (Candida) that grow and deplete zinc and other minerals from the body, so when it takes Zn from the body, you will be able to stay alive more to end the weakening of the immune system as the work of other microbes[181].

A study has shown that age is related to Zn deficiency in Thalassemia patients, as the higher the age, the more noticeable the Zn deficiency is, The results are shown that in fig. (3-6).

3.4 Copper (Cu)

Results of this study found a signification ($p < 0.05$) increase in serum Copper level (101.66±33.57 µg/dl) in female and male (120.89±45.98 µg/dl) β-thalassemia major patients comparison to female and male in control groups (81.27±23.78 µg/dl, 86.45±27.74 µg/dl) respectively, the Cupper levels were significantly higher in patients group (111.28±41.20 µg/dl) than in Controls group (83.81±25.67 µg/dl), The results are shown that in table (3-7),and fig. (3-7)

Table (3-7): Serum Cu value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Controls group	Patients group
Cu (µg/dl)	Female	81.27±23.78[a]	101.66±33.57[b]
	Male	86.45±27.74[a]	120.89±45.98[b]
	Total	83.81±25.67[a]	111.28±41.20[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

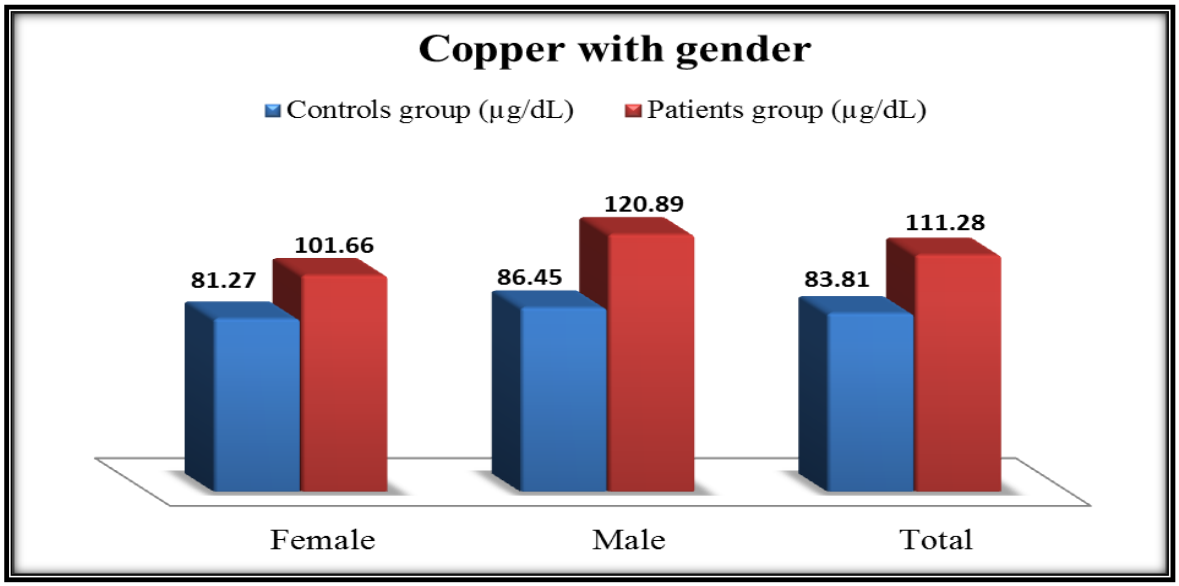


Fig. (3-7): Copper in healthy and β-thalassemia major patients according to gender and group

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) increase in serum Cupper's level (10 – 17 years =94.13±32.20 µg/dl, and 18 – 25 years =108.89±33.91 µg/dl) in comparison to female control groups (74.50±20.17 µg/dl, and 88.05±25.91 µg/dl) respectively, while male patients in both age subgroups have a statistically significant (P <0.05) increase in serum Cu (10 – 17 years = 102.83±38.49 µg/dl, and 18 – 25 years=138.24±46.57 µg/dl) in comparison to male control groups (77.55±32.14 µg/dl, and 96.09±18.89 µg/dl) respectively, The results are shown that in table (3-8),and fig.(3-8)

Table (3-8): Serum Cu value in healthy and β-thalassemia major patients according to gender and age

Variable	Gender	Age	Controls group	Patients group
Cu (µg/dl)	Female	10 - 17	74.50±20.17[a]	94.13±32.20[b]
		18 - 25	88.05±25.91[a]	108.89±33.91[b]
	Male	10 – 17	77.55±32.14[a]	102.83±38.49[b]
		18 - 25	96.09±18.89[a]	138.24±46.57[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

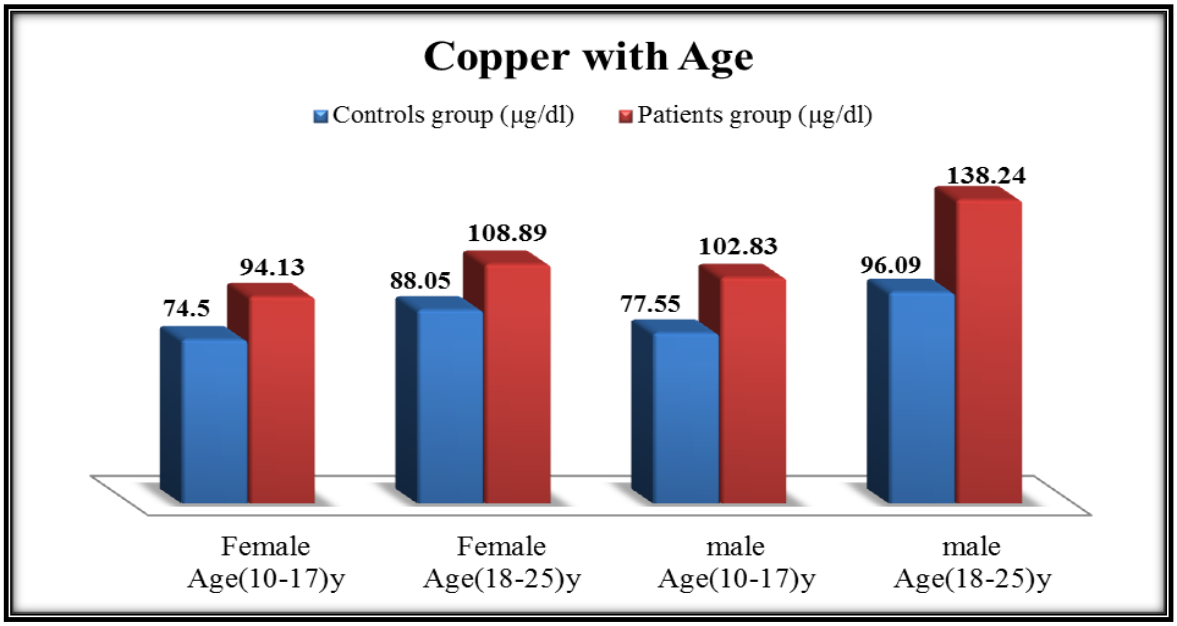


Fig. (3-6): Copper in healthy and β-thalassemia major patients according to age and gender

Cupper is an important and essential component in the construction of many proteins, it participates as a cofactor in many enzymes and plays a role in maintaining cellular homeostasis[182]. An increase in the concentration of Cu in the blood has serious consequences because it becomes toxic to the nervous system and liver, as in Wilson's disease[183]. The liver is the regulator responsible for the metabolism of nutrients, including Cu, as it regulates its absorption and transport, because too much or too little Cu is associated with oxidative cell damage, organ dysfunction, and immune deficiency[184]. Therefore, an excess of Cu in the blood is associated with an injury or defect in the liver due to its damage to a viral infection[185]. Usually continuous blood transfusion for thalassemia patients as a treatment method for the disease leads to transmission of viral infections such as hepatitis virus HCV. High level of Cu in the blood has been observed in these patients[183,186]. Research indicates that the target of copper toxicity is the mitochondria because excess copper leads to oxidation, formation of reactive oxygen species (ROS) and mitochondrial damage[187].

The high level of ceruloplasmin enzyme in patients with thalassemia major may be the reason for the high level of Cu in the blood[188]. It is noteworthy that ceruloplasmin and Cu are elevated in inflammatory patients[189]. Zn and Cu maintain a homeostasis in the blood, as changes tend to be inversely related to each other, thus making them have an antagonistic effect on each other[190].

3.5 Total protein (TP)

Results of this study observed a signification ($p < 0.05$) increase in serum total protein level (7.68±0.70 g/dl) in female and male (7.51±0.75 g/dl) β-thalassemia major patients comparison to female and male in control groups (6.25±0.83 g/dl, 6.03±0.94 g/dl) respectively, the TP levels were significantly higher in patients group (7.59±0.73 g/dl) than in Controls group (6.03±0.94 g/dl), The results are shown that in table (3-9),and fig.(3-9).

Table (3-9): Serum total protein value in healthy and β-thalassemia major patients according to age and gender

Variable	Gender	Controls group	Patients group
Total protein (g/dl)	Female	6.25 ± 0.83^{a}	7.68 ± 0.70^{b}
	Male	6.03 ± 0.94^{a}	7.51 ± 0.75^{b}
	Total	6.03 ± 0.94^{a}	7.59 ± 0.73^{b}

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

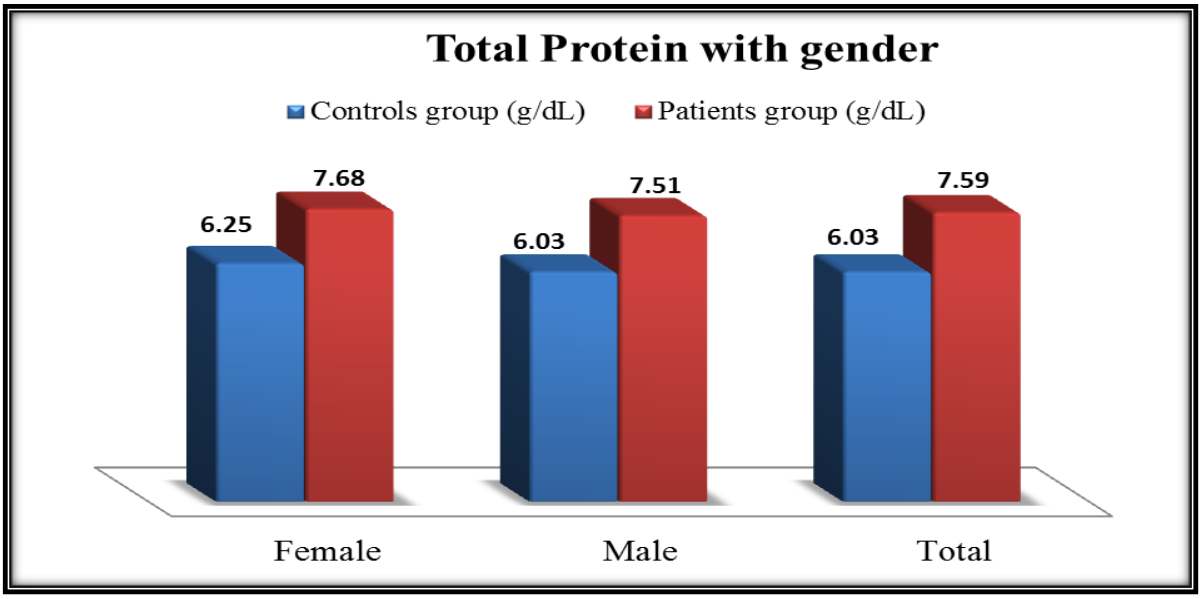


Fig. (3-9): Total Protein in healthy and β-thalassemia major patients according to gender and group

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) increase in serum TP level (10 – 17 years =7.42±0.62 g/dl, and 18 – 25 years =7.93±0.69 g/dl) in comparison to female control groups (6.16±0.91 g/dl, and 6.35±0.70 g/dl) respectively, as same male patients in both age subgroups have a statistically significant (P <0.05) increase in serum TP (10 – 17 years = 7.18±0.80 g/dl, and 18 – 25 years=7.83±0.54 g/dl) in comparison to male control groups (6.02±0.98 g/dl, and 6.03±0.94 g/dl) respectively, The results are shown that in table (3-10),and fig.(3-10)

Table (3-10): Serum TP value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Age	Controls group	Patients group
Total protein (g/dl)	Female	10 - 17	6.16 ± 0.91^{a}	7.42 ± 0.62^{b}
		18 - 25	6.35 ± 0.70^{a}	7.93 ± 0.69^{b}
	Male	10 – 17	6.02 ± 0.98^{a}	7.18 ± 0.80^{b}
		18 - 25	6.03 ± 0.94^{a}	7.83 ± 0.54^{b}

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

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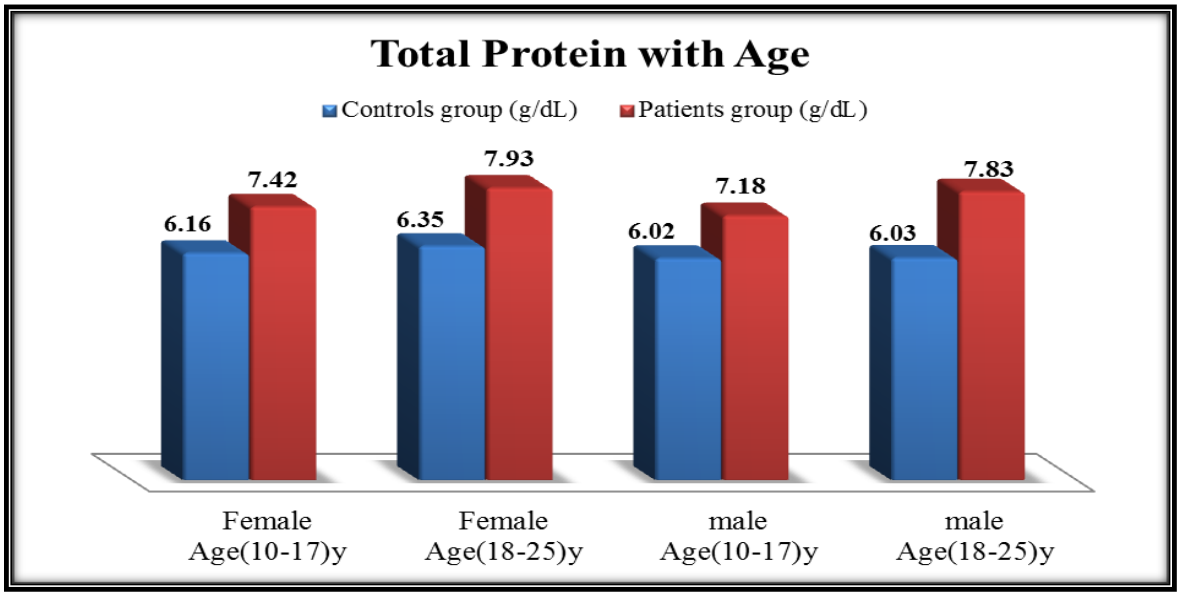

Fig. (3-10): Total Protein in healthy and β-thalassemia major patients according to age and gender

The level of protein indicates the balance between the two protein structures[110]. Globulin which makes up about 40% of the proteins in the blood, while albumin makes up 60%[191]. TP helps regulate blood osmotic pressure[192], transport nutrients, and remove waste products. The normal level of TP in the blood serum is (6.6 - 8.3 g/dL)[193].

Abnormal indications indicate abnormal liver function[194]. Elevated levels of TP indicate an increase in protein synthesis in the liver[195]. In Thalassemia patients, the patient is exposed to frequent blood transfusions, which leads to an increase in the load on the liver and thus damages it[196].

In this study TP levels were significantly ($P < 0.05$) higher than the healthy ones, but they were not higher than the necessary or normal levels in the blood. Also it is noted that the older patient, higher level of TP in the blood. This is what is noticed in studies conducted on different age groups, research that included children, the TP level was low to normal, while studies conducted on older ages[121,122], high TP was noted[119]. This is because the liver is damaged in the later stages as a result of repeated infections and iron overload, in addition to not adhering to a customized and balanced diet for each patient.

3.6 Chloride (Cl^-)

Results of this study found a signification ($p < 0.05$) increase in serum Cl^- level (109.44±18.86 mmol/L) in female and male (114.21±24.06 mmol/L) β-thalassemia major patients comparison to female and male in control groups (95.46±12.40 mmol/L, 102.69±5.96 mmol/L) respectively, the Cl^- levels were significantly higher in patients group (111.83±21.64 mmol/L) than in Controls group (99.08±10.30 mmol/L), The results are shown that in table (3-11), and fig. (3-11).

Table (3-11): Serum Cl^- value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Controls group	Patients group
Cl^- (mmol/L)	Female	95.46±12.40[a]	109.44±18.86[b]
	Male	102.69±5.96[a]	114.21±24.06[b]
	Total	99.08±10.30[a]	111.83±21.64[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

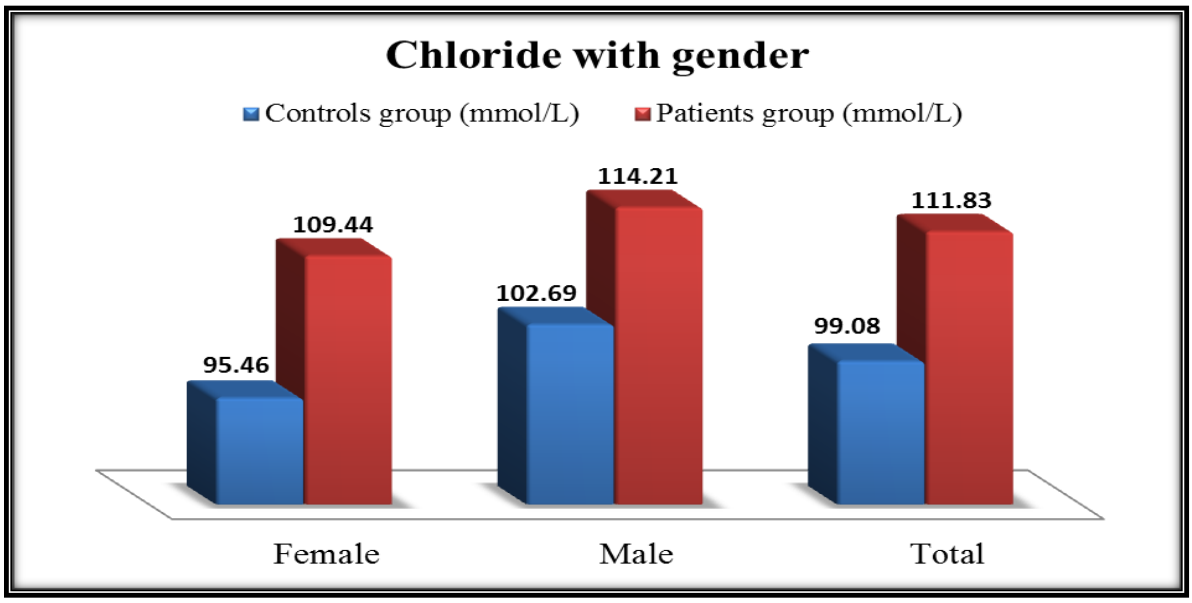

Fig. (3-11): Chloride in healthy and β-thalassemia major patients according to gender and group

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant ($P < 0.05$) increase in serum Cl^- level (10 – 17 years =107.16±18.26 mmol/L, and 18 – 25 years = 111.73±19.55 mmol/L) in comparison to female control groups (91.45±14.15 mmol/L, and 99.47±9.24 mmol/L) respectively beside, male patients in both age subgroups have a statistically significant ($P < 0.05$) increase in serum Cl^- (10 – 17 years = 106.68±9.35 mmol/L, and 18 – 25 years=121.75±31.25 mmol/L) in comparison to male control groups (104.33±5.50 mmol/L, and 101.06±6.16 mmol/L) respectively, The results are shown that in table (3-12),and fig. (3-12).

Table (3-12): Serum TP value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Age	Controls group	Patients group
Cl (mmol/L)	Female	10 - 17	91.45±14.15[a]	107.16±18.26[b]
		18 - 25	99.47±9.24[a]	111.73±19.55[b]
	Male	10 – 17	104.33±5.50[a]	106.68±9.35[b]
		18 - 25	101.06±6.16[a]	121.75±31.25[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

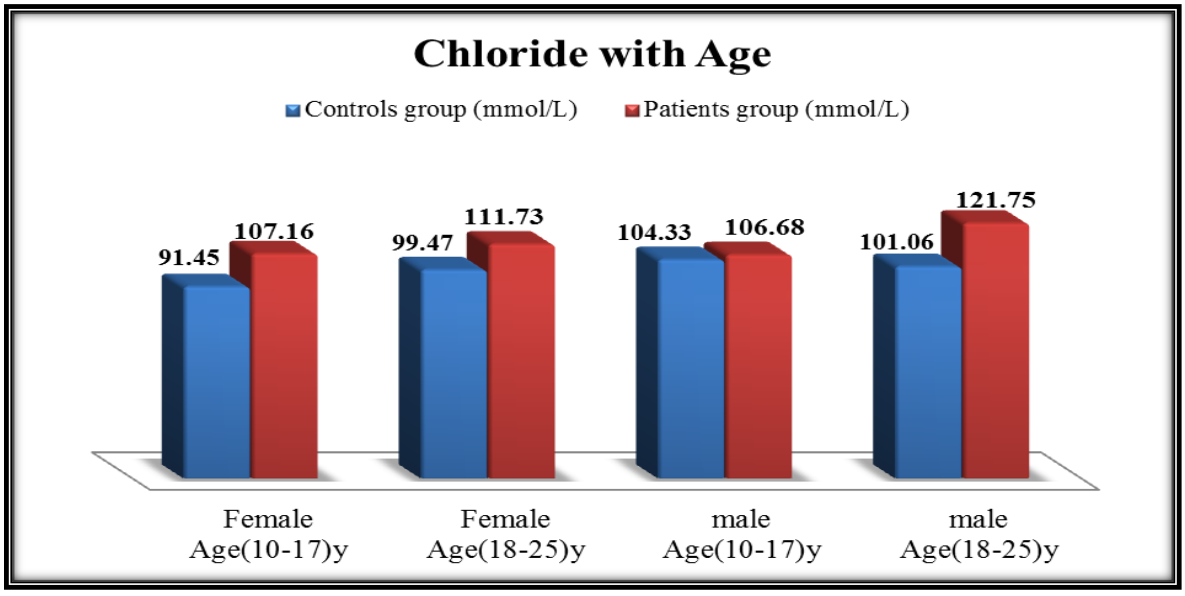

Fig. (3-12): Total Protein in healthy and β-thalassemia major patients according to age and gender

Electrolytes, including Cl^-, make up about 1% of the blood plasma. They are necessary for the metabolism process where they play a role in fluid level and balance. Electrolyte balance is necessary for cell function as well as maintaining the shape of red blood cells and the exchange of oxygen and carbon dioxide between tissues and red blood cells[197]. Abnormal activation of the chloride transport system is the reason for the accumulation of Cl^- and dehydration[198], which is one of the clinical symptoms appearing in Thalassemia patients, where patients suffer from dryness in the body and skin and change in skin color. In addition, Cl^- is active in red blood cells that contain positively charged Hb, such as hemoglobin (Hbs)[199].

Some researchers believe that the oxidative damage caused by free globulin chains causes membrane abnormalities[126]. It was also mentioned that the sodium and potassium pump increases in alpha and beta thalassemia cells, and therefore, chloride transport increases as a result of oxidative damage[200,201].

While others suggested that the increased level may be due to kidney damage resulting from the excess of iron[128].

3.7 Ferritin

Results of this study revealed a signification ($p < 0.05$) increase in serum Ferritin level (4142.67±2682 ng/ml) in female and male (4199.02±2671.88 ng/ml) β-thalassemia major patients comparison to female and male in control groups (48.00±28.01 ng/ml, 74.20±66.57 ng/ml) respectively, the Ferritin levels were significantly higher in patients group (6470.85±2663.57 ng/ml) than in Controls group (61.10±52.27 ng/ml), The results are shown that in table (3-13), and fig. (3-13).

Table (3-13): Serum Ferritin value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Controls group	Patients group
Ferritin (ng/ml)	Female	48.00±28.01[a]	4142.67±2682[b]
	Male	74.20±66.57[a]	4199.02±2671.88[b]
	Total	61.10±52.27[a]	6470.85±2663.57[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

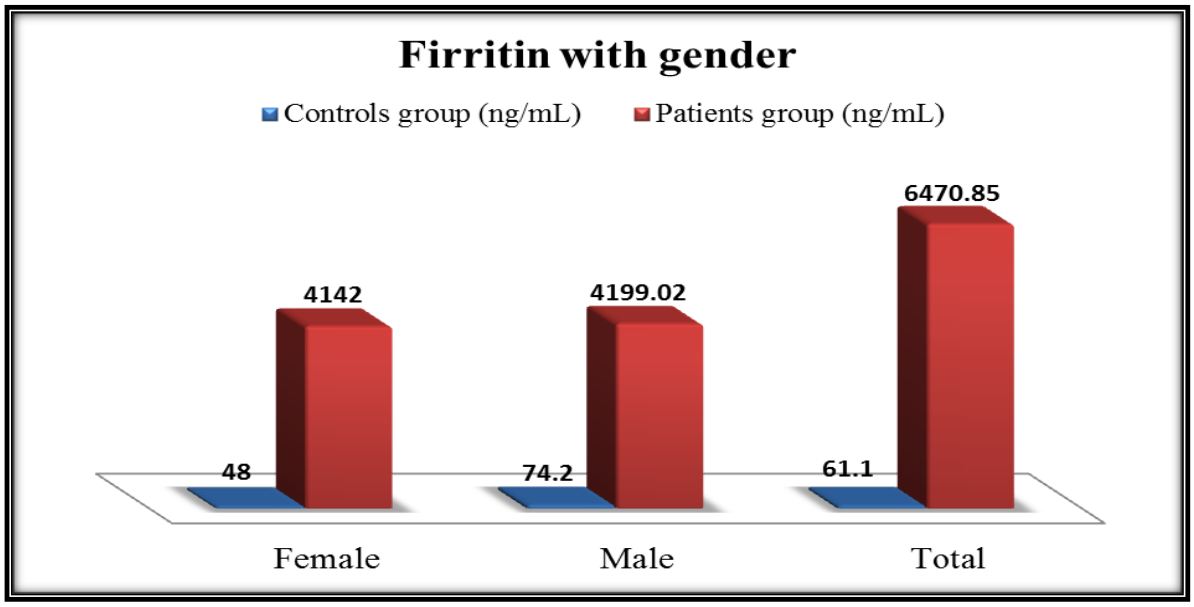

Fig. (3-13): Ferritin in healthy and β-thalassemia major patients according to gender and group

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) increase in serum Ferritin level (10 – 17 years =3911.94±2614 ng/ml, and 18 – 25 years = 4373.41±2782.45 ng/ml) in comparison to female control groups (40.45±24.34 ng/ml, and 55.55±30.31 ng/ml) respectively in addition, male patients in both age subgroups have a statistically significant (P <0.05) increase in serum Ferritin (10 – 17 years = 4014.76±2353.91 ng/ml, and 18 – 25 years=4383.28±2993.95 ng/ml) in comparison to male control groups (64.68±39.60 ng/ml, and 83.73±86.43 ng/ml) respectively, The results are shown that in table (3-14),and fig. (3-14).

Table (3-14): Serum Ferritin value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Age	Controls group	Patients group
Ferritin (ng/ml)	Female	10 - 17	40.45±24.34[a]	3911.94±2614[b]
		18 - 25	55.55±30.31[a]	4373.41±2782.45[b]
	Male	10 – 17	64.68±39.60[a]	4014.76±2353.91[b]
		18 - 25	83.73±86.43[a]	4383.28±2993.95[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

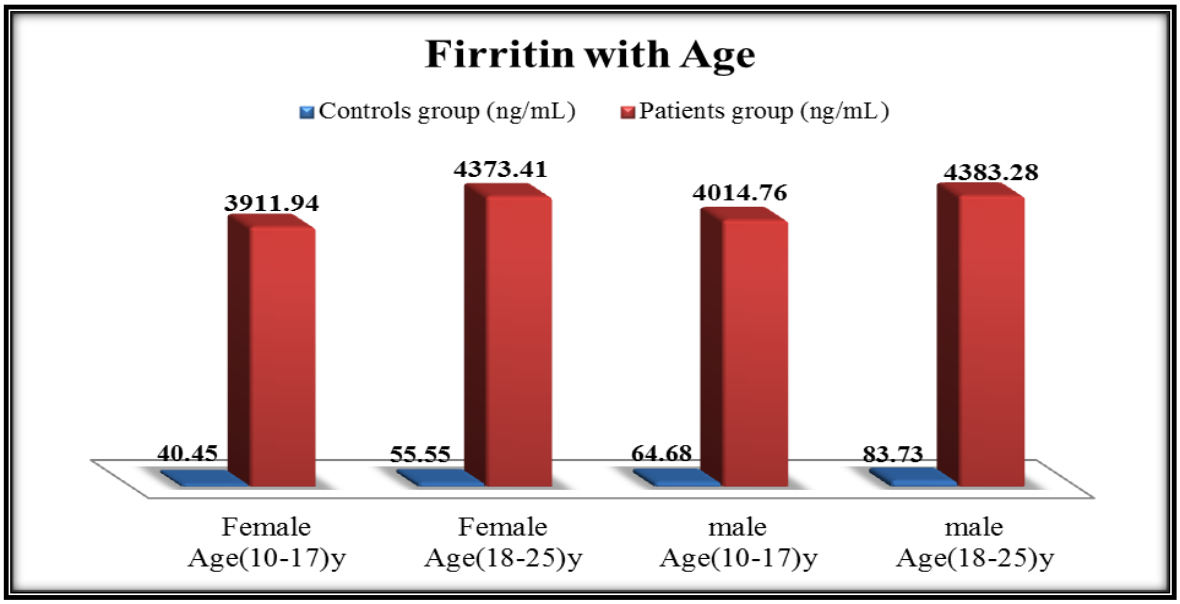

Fig. (3-14): Ferritin in healthy and β-thalassemia major patients according to age and gender

Iron overload is an inevitable disorder in thalassemia patients. Serum ferritin measurement is an inexpensive and easy way to determine iron burden. Chelation therapy is used to lower the iron level, reduce the level of ferritin, and reduce the incidence of various complications[202]. Several researchers also addressed the relationship between the use of iron chelation therapy with the level of ferritin[203,202].

The results showed that all of our patients had increased ferritin compared to the same gender in the control group. however the ferritin level is not statistically affected by the gender of thalassemia patients, The results are shown that in table (3-13).

We also note in this study that the older the patients, the higher the ferritin ratio fig.(3-14), and this is what is observed in a study that revealed a positive correlation between the level of ferritin in the blood with age[204].

Iron overload due to regular and recurrent blood transfusions for patients with beta thalassemia major is one of the main causes as well as the result of hemolysis of red blood cells[205,206]and also liver damage because to high ferritin levels[207].

3.8 Complete Blood Count (CBC)

3.8.1 Red blood cell (RBC)

Results of this study find a signification ($p < 0.05$) decreased in serum RBC level ($3.36\pm0.41 \times10^6$/µL) in female and male ($3.15\pm0.53 \times10^6$/µL) β-thalassemia major patients comparison to female and male in control groups ($4.88\pm0.69 \times10^6$/µL, $5.80\pm0.62 \times10^6$/µL) respectively, the RBC levels were significantly lower in patients group ($3.26\pm0.49 \times10^6$/µL) than in Controls group ($5.34\pm0.80 \times10^6$/µL), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant ($P < 0.05$) decreased in serum RBC level (10 – 17 years = $3.33\pm0.32\times10^6$/µL, and 18 – 25 years = $3.39\pm0.50\times10^6$/µL) in comparison to female control groups ($5.14\pm0.73\times10^6$/µL, and $4.62\pm0.57\times10^6$/µL) respectively, and so male patients in both age subgroups have a statistically significant ($P < 0.05$) decreased in serum RBC (10 – 17 years = $3.06\pm0.47\times10^6$/µL, and 18 – 25 years= $3.24\pm0.59\times10^6$/µL) in comparison to male control groups ($5.77\pm0.80\times10^6$/µL, and $5.82\pm0.39\times10^6$/µL) respectively, The results are shown that in table (3-16).

3.8.2 Hematocrit (HCT)

Results of this study showed a signification ($p < 0.05$) decreased in serum HCT level (25.98±3.54 %) in female and male (23.66±4.10%) β-thalassemia major patients comparison to female and male in control groups (38.66±4.75 %, 47.70±3.55 %) respectively, the HCT levels were significantly lower in patients

group (24.83±3.98 %) than in Controls group (43.18±6.17 %), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) decreased in serum HCT level (10 – 17 years = 25.87±2.90%, and 18 – 25 years = 26.09±4.15%) in comparison to female control groups (37.94±4.42%, and 39.37±5.14%) respectively, and so male patients in both age subgroups have a statistically significant (P <0.05) decreased in serum HCT (10 – 17 years = 22.92±3.18%, and 18 – 25 years= 24.38±4.79%) in comparison to male control groups (45.97±3.44%, and 47.70±3.55%) respectively, The results are shown that in table (3-16).

3.8.3 Hemoglobin (HGB)

Results of this study showed a signification (p <0.05) decreased in serum HGB level (8.64±1.11 g/dL) in female and male (7.76±1.21 g/dL) β-thalassemia major patients comparison to female and male in control groups (12.79±1.31 g/dL, 15.31±1.43 g/dL) respectively, the HGB levels were significantly lower in patients group (8.20±1.23 g/dL) than in Controls group (14.05±1.86 g/dL), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) decreased in serum HGB level (10 – 17 years = 8.58±0.97 g/dL, and 18 – 25 years = 8.70±1.25 g/dL) in comparison to female control groups (13.100±1.01 g/dL, and 12.49±1.54 g/dL) respectively, and so male patients in both age subgroups have a statistically significant (P <0.05) decreased in serum HGB (10 – 17 years =

7.62±1.07 g/dL, and 18 – 25 years= 7.90±1.34 g/dL) in comparison to male control groups (14.58±1.30 g/dL, and 16.05±1.19 g/dL) respectively, The results are shown that in table (3-16).

3.8.4 Mean corpuscular volume (MCV)

Results of this study found a signification ($p < 0.05$) decreased in serum MCV level (75.55±9.21 fL) in female and male (75.93±4.74 fL) β-thalassemia major patients comparison to female and male in control groups (84.76±5.70 fL, 83.11±6.85 fL) respectively, the MCV levels were significantly lower in patients group (75.74±7.29 fL) than in Controls group (83.94±6.29 fL), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant ($P < 0.05$) decreased in serum MCV level (10 – 17 years = 77.01±3.91 fL, and 18 – 25 years = 74.09±12.38 fL) in comparison to female control groups (84.24±3.64 fL, and 85.27±7.34 fL) respectively, and so male patients in both age subgroups have a statistically significant ($P < 0.05$) decreased in serum MCV (10 – 17 years = 75.14±4.33 fL, and 18 – 25 years= 76.72±5.07 fL) in comparison to male control groups (81.30±8.97 fL, and 84.93±3.19 fL) respectively, The results are shown that in table (3-16).

3.8.5 Mean corpuscular hemoglobin (MCH)

Results of this study observed a signification ($p < 0.05$) decreased in serum MCH level (26.47±2.64 pg) in female and male (25.48±2.86 pg) β-thalassemia

major patients comparison to female and male in control groups (27.82±2.61 pg, 26.15±2.76 pg) respectively, the MCH levels were significantly lower in patients group (25.98±2.79 pg) than in Controls group (26.98±2.79 pg), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) decreased in serum MCH level (10 – 17 years = 26.59±3.04 pg, and 18 – 25 years = 26.35±2.23 pg) in comparison to female control groups (28.05±2.43 pg, and 27.59±2.86 pg) respectively, and so male patients in both age subgroups have a statistically significant (P <0.05) decreased in serum MCH (10 – 17 years = 25.42±2.81 pg, and 18 – 25 years= 25.54±2.98 pg) in comparison to male control groups (25.30±3.61 pg, and 27.00±1.11 pg) respectively, The results are shown that in table (3-16).

3.8.6 Mean corpuscular hemoglobin concentration (MCHC)

Results of this study showed a signification (p <0.05) increase in serum MCHC level (34.25±1.99 g/dl) in female and male (33.37±2.62 g/dl) β-thalassemia major patients comparison to female and male in control groups (332.15±2.20 g/dl, 31.76±0.96 g/dl) respectively, the MCHC levels were significantly higher in patients group (33.81±2.36 g/dl) than in Controls group (31.96±1.69 g/dl), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) increase in serum MCHC level (10 – 17 years = 34.02±2.13 g/dl, and 18 – 25 years = 34.47±1.86 g/dl) in comparison to female control groups (32.89±2.29 g/dl, and

31.41±1.90 g/dl) respectively, and so male patients in both age subgroups have a statistically significant (P <0.05) increase in serum MCHC (10 – 17 years = 33.48±2.51 g/dl, and 18 – 25 years= 33.26±2.78 g/dl) in comparison to male control groups (31.77±1.23 g/dl, and 31.76±0.65 g/dl) respectively, The results are shown that in table (3-16).

3.8.7 Red blood cell distribution width – Standard deviation (RDW-SD)

Results of this study found a signification (p <0.05) increase in serum RDW-SD level (50.68±13.19 fL) in female and male (49.71±11.01 fL) β-thalassemia major patients comparison to female and male in control groups (42.07±2.07 fL, 42.98±4.64 fL) respectively, the RDW-SD levels were significantly higher in patients group (50.19±12.09 fL) than in Controls group (42.53±3.59 fL), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) increase in serum RDW-SD level (10 – 17 years = 47.23±13.17 g/dl, and 18 – 25 years = 54.12±12.52 fL) in comparison to female control groups (41.97±1.35 fL, and 42.17±2.67 fL) respectively, and so male patients in both age subgroups have a statistically significant (P <0.05) increase in serum RDW-SD (10 – 17 years = 50.25±11.06 fL, and 18 – 25 years= 49.16±11.15 fL) in comparison to male control groups (40.96±2.98 fL, and 45.00±5.21 fL) respectively, The results are shown that in table (3-16).

3.8.9 Red blood cell distribution width – Coefficient of variation (RDW-CV)

Results of this study showed a signification ($p < 0.05$) increase in serum RDW-CV% level (19.61±6.18 %) in female and male (19.09±5.14 %) β-thalassemia major patients comparison to female and male in control groups (13.24±1.01 %, 14.27±1.73 %) respectively, the RDW-CV% levels were significantly higher in patients group (19.35±5.66 %) than in Controls group (13.76±1.50 %), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant ($P < 0.05$) increase in serum RDW-CV% level (10 – 17 years = 17.81±5.94 g/dl, and 18 – 25 years = 21.42±5.99 g/dl) in comparison to female control groups (13.20±0.92 g/dl, and 13.29±1.13 g/dl) respectively, and so male patients in both age subgroups have a statistically significant ($P < 0.05$) increase in serum RDW-CV% (10 – 17 years = 19.44±5.54 g/dl, and 18 – 25 years= 18.75±4.81g/dl) in comparison to male control groups (14.26±1.89 g/dl, and 14.28±1.64 g/dl) respectively, The results are shown that in table (3-16).

3.8.10 White blood cell (WBC)

Results of this study observed a signification ($p < 0.05$) increase in serum WBC level (15.40±15.54 $\times 10^3/\mu L$) in female and male (12.16±8.31 $\times 10^3/\mu L$) β-thalassemia major patients comparison to female and male in control groups (7.06±1.59 $\times 10^3/\mu L$, 7.39±2.09 $\times 10^3/\mu L$) respectively, the WBC levels were significantly higher in patients group (13.79±12.51 $\times 10^3/\mu L$) than in Controls group (7.22±1.84 $\times 10^3/\mu L$), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) increase in serum WBC level (10 – 17 years = 13.95±16.39 $\times10^3$/µL, and 18 – 25 years = 16.86±14.84 $\times10^3$/µL) in comparison to female control groups (6.70±1.21$\times10^3$/µL, and 7.41±1.87$\times10^3$/µL) respectively, and so male patients in both age subgroups have a statistically significant (P <0.05) increase in serum WBC (10 – 17 years = 11.57±9.19 $\times10^3$/µL, and 18 – 25 years= 12.74±7.49$\times10^3$/µL) in comparison to male control groups (7.97±2.48$\times10^3$/µL, and 6.81±1.49$\times10^3$/µL) respectively, The results are shown that in table (3-16).

3.8.11 Lymphocytes (LYM)

Results of this study showed a signification (p <0.05) increase in serum LYM% level (44.07±16.57 %) in female and male (42.20±12.76 %) β-thalassemia major patients comparison to female and male in control groups (33.16±11.52 %, 36.68±6.38 %) respectively, the LYM% levels were significantly higher in patients group (43.14±14.74 %) than in Controls group (34.92±9.36 %), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant (P <0.05) increase in serum LYM% level (10 – 17 years = 41.69±15.94 %, and 18 – 25 years = 46.44±17.16 %) in comparison to female control groups (33.62±7.44 %, and 32.70±14.86 %, respectively), and so male patients in both age subgroups have a statistically significant (P <0.05) increase in serum LYM% (10 – 17 years = 40.85±11.47 %, and 18 – 25 years= 43.54±14.04 %) in comparison to male control

groups (37.10±6.45 %, and 36.26±6.38 %) respectively, The results are shown that in table (3-16).

3.8.12 Lymphocytes (LYM#)

Results of this study observed a signification ($p < 0.05$) increase in serum LYM# level (7.09±11.05 $\times 10^3/\mu L$) in female and male (3.05±1.62 $\times 10^3/\mu L$) β-thalassemia major patients comparison to female and male in control groups (2.31±0.73 $\times 10^3/\mu L$, 2.64±0.88 $\times 10^3/\mu L$) respectively, the LYM# levels were significantly higher in patients group (5.07±8.11 $\times 10^3/\mu L$) than in Controls group (2.47±0.82 $\times 10^3/\mu L$), The results are shown that in table (3-15),and fig (3-15).

According to the age subgroups in control and β- thalassemia major individuals groups, female patients in both age subgroups have a significant ($P < 0.05$) increase in serum LYM# level (10 – 17 years = 5.76±10.77$\times 10^3/\mu l$, and 18 – 25 years = 8.42±11.38$\times 10^3/\mu l$) in comparison to female control groups (2.42±0.61$\times 10^3/\mu l$, and 2.21±0.86$\times 10^3/\mu l$, respectively), and so male patients in both age subgroups have a statistically significant ($P < 0.05$) increase in serum LYM# (10 – 17 years = 3.02±1.27$\times 10^3/\mu l$, and 18 – 25 years= 3.08±1.93$\times 10^3/\mu l$) in comparison to male control groups (2.94±1.10$\times 10^3/\mu l$, and 2.36±0.53$\times 10^3/\mu l$) respectively, The results are shown that in table (3-16).

Table (3-15): CBC value in healthy and β-thalassemia major patients according to gender and group

Variable	Gender	Controls group	Patients group
RBC×106/µL	Female	4.88±0.69[a]	3.36±0.41[b]
	Male	5.80±0.62[a]	3.15±0.53[b]
	Total	5.34±0.80[a]	3.26±0.49[b]
HCT%	Female	38.66±4.75[a]	25.98±3.54[b]
	Male	47.70±3.55[a]	23.66±4.10[b]
	Total	43.18±6.17[a]	24.83±3.98[b]
HGB (g/dL)	Female	12.79±1.31[a]	8.64±1.11[b]
	Male	15.31±1.43[a]	7.76±1.21[b]
	Total	14.05±1.86[a]	8.20±1.23[b]
MCV(fL)	Female	84.76±5.70[a]	75.55±9.21[b]
	Male	83.11±6.85[a]	75.93±4.74[b]
	Total	83.94±6.29[a]	75.74±7.29[b]
MCH(pg)	Female	27.82±2.61[a]	26.47±2.64[b]
	Male	26.15±2.76[a]	25.48±2.86[b]
	Total	26.98±2.79[a]	25.98±2.79[b]
MCHC(g/dl)	Female	32.15±2.20[a]	34.25±1.99[b]
	Male	31.76±0.96[a]	33.37±2.62[b]
	Total	31.96±1.69[a]	33.81±2.36[b]
RDW-SD (fL)	Female	42.07±2.07[a]	50.68±13.19[b]
	Male	42.98±4.64[a]	49.71±11.01[b]
	Total	42.53±3.59[a]	50.19±12.09[b]
RDW-CV %	Female	13.24±1.01[a]	19.61±6.18[b]
	Male	14.27±1.73[a]	19.09±5.14[b]
	Total	13.76±1.50[a]	19.35±5.66[b]
WBC ×10^3/µL	Female	7.06±1.59[a]	15.40±15.54[b]
	Male	7.39±2.09[a]	12.16±8.31[b]
	Total	7.22±1.84[a]	13.79±12.51[b]
LYM%	Female	33.16±11.52[a]	44.07±16.57[b]
	Male	36.68±6.38[a]	42.20±12.76[b]
	Total	34.92±9.36[a]	43.14±14.74[b]
LYM#×10^3/µl	Female	2.31±0.73[a]	7.09±11.05[b]
	Male	2.64±0.88[a]	3.05±1.62[b]
	Total	2.47±0.82[a]	5.07±8.11[b]

* Note: Value represented mean ± : SD (standard deviation).

The different letters refer to significant difference (between the control and patients groups) at the level ($p < 0.05$).

The similar letters refer to non-significant difference (between the control and patients groups).

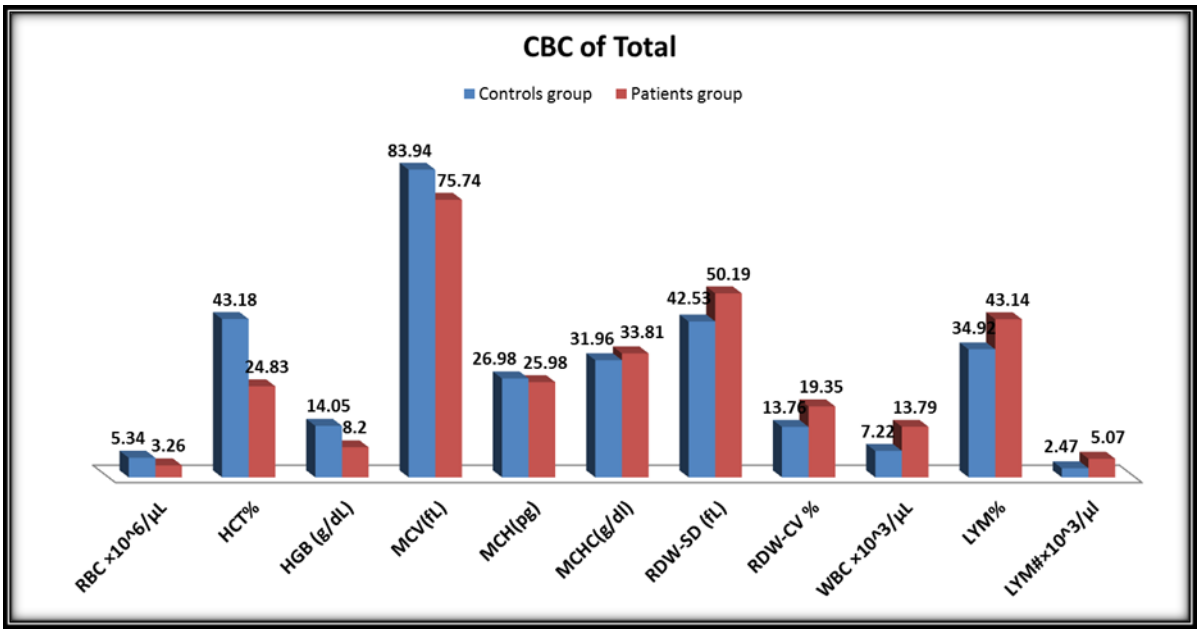

Fig. (3-15): CBC in healthy and β-thalassemia major patients according to group

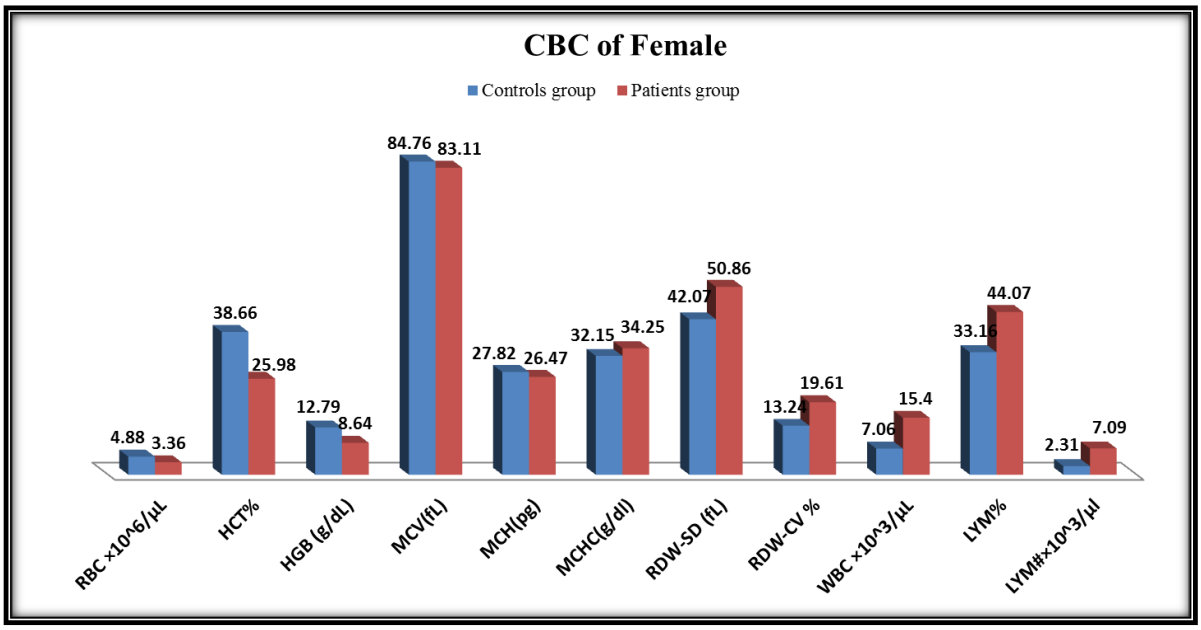

Fig. (3-16): CBC in healthy and β-thalassemia major patients according to gender female

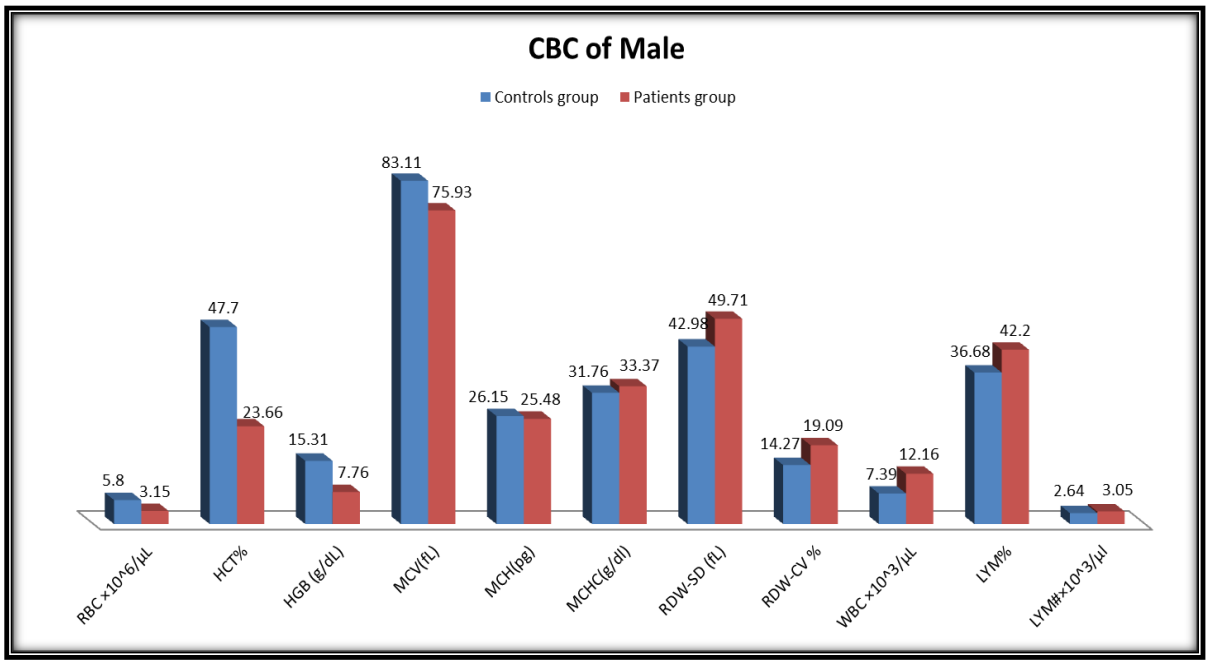

Fig. (3-17): CBC in healthy and β-thalassemia major patients according to gender male

Table (3-16): CBC value in healthy and β-thalassemia major patients according to gender and age

Variable	Gender	Age	Controls group	Patients group
RBC×10^6/µL	Female	10 - 17	5.14±0.73	3.33±0.32
		18 - 25	4.62±0.57	3.39±0.50
	Male	10 – 17	5.77±0.80	3.06±0.47
		18 - 25	5.82±0.39	3.24±0.59
HCT%	Female	10 - 17	37.94±4.42	25.87±2.90
		18 - 25	39.37±5.14	26.09±4.15
	Male	10 – 17	45.97±3.44	22.92±3.18
		18 - 25	47.70±3.55	24.38±4.79
HGB (g/dL)	Female	10 - 17	13.100±1.01	8.58±0.97
		18 - 25	12.49±1.54	8.70±1.25
	Male	10 – 17	14.58±1.30	7.62±1.07
		18 - 25	16.05±1.19	7.90±1.34
MCV(fL)	Female	10 - 17	84.24±3.64	77.01±3.91
		18 - 25	85.27±7.34	74.09±12.38
	Male	10 – 17	81.30±8.97	75.14±4.33
		18 - 25	84.93±3.19	76.72±5.07
MCH(pg)	Female	10 - 17	28.05±2.43	26.59±3.04
		18 - 25	27.59±2.86	26.35±2.23
	Male	10 – 17	25.30±3.61	25.42±2.81
		18 - 25	27.00±1.11	25.54±2.98
MCHC(g/dl)	Female	10 - 17	32.89±2.29	34.02±2.13
		18 - 25	31.41±1.90	34.47±1.86
	Male	10 – 17	31.77±1.23	33.48±2.51
		18 - 25	31.76±0.65	33.26±2.78
RDW-SD (fL)	Female	10 - 17	41.97±1.35	47.23±13.17
		18 - 25	42.17±2.67	54.12±12.52
	Male	10 – 17	40.96±2.98	50.25±11.06
		18 - 25	45.00±5.21	49.16±11.15

RDW-CV %	Female	10 - 17	13.20±0.92	17.81±5.94
		18 - 25	13.29±1.13	21.42±5.99
	Male	10 – 17	14.26±1.89	19.44±5.54
		18 - 25	14.28±1.64	18.75±4.81
WBC×10^3/µL	Female	10 - 17	6.70±1.21	13.95±16.39
		18 - 25	7.41±1.87	16.86±14.84
	Male	10 – 17	7.97±2.48	11.57±9.19
		18 - 25	6.81±1.49	12.74±7.49
LYM%	Female	10 - 17	33.62±7.44	41.69±15.94
		18 - 25	32.70±14.86	46.44±17.16
	Male	10 – 17	37.10±6.45	40.85±11.47
		18 - 25	36.26±6.38	43.54±14.04
LYM#×10^3/µl	Female	10 - 17	2.42±0.61	5.76±10.77
		18 - 25	2.21±0.86	8.42±11.38
	Male	10 – 17	2.94±1.10	3.02±1.27
		18 - 25	2.36±0.53	3.08±1.93

One of the clinical signs that distinguish patients is a decrease in the number of red blood cells in the bloodstream, leading to lack of oxygen transported to the tissues of the body, in addition, red blood cells of thalassemia patients are smaller than normal size[208]. Since the beginning of the seventies of last century, it was proposed to examine the CBC to infer the indicators of thalassemia[(209)].

Anemia is characterized by a decrease in the number of red blood cells, and there is a lot of clinical evidence that indicates severe complications in the heart and blood vessels. Many therapeutic interventions aimed at increasing RBC by transfusion are not effective for stimulating RBC production by the bone marrow[(210,211)].

The destruction of red blood cell precursors in the bone marrow leading to the formation of ineffective red blood cells, as well as oxidation that leading to

hemolysis[148]. The accumulation of unbound alpha globin, the accumulation of non-heme iron in cells and the decrease in Hemoglobin concentration are all due to oxidative damage, which is the most important factor causing organ dysfunction and cell damage in patients with beta thalassemia major[146,212].

The abnormalities of red blood cells in thalassemia patients lead to a shortening of the life of red blood cells, as most patients suffer from chronic hemolytic anemia as a result of the destruction of RBC in the bone marrow and spleen at a later time, so many studies have suggested eradicating these ineffective cells in the bone marrow[213].

Thalassemia patients suffer from anemia, low Hb level and varying degrees of splenomegaly [214]. We found that the level of HGB and hematocrit (HCT) in the serum in patients with thalassemia major is less than in normal people and this is consistent with many studies, that the decrease in the level of hemoglobin in the blood is a result of hemolysis in cells due to the synthesis of abnormal red blood cells, and on the other hand, as a result of the formation of free radicals and the destruction of cell membranes Red blood cells[215]. The lack of hemoglobin can lead to hypoxia, which is associated with delayed growth as well as maturation in patients with thalassemia.

Significantly high Red blood cell distribution width **(**RDW), as shown in Table (3-15), indicates that there is a difference in the size of red blood cells, and this indicates that their size is smaller than the normal size. This is what is characterized by red blood cells in Thalassemia patients, so they do not perform their work normally.

The high Red cell distribution width (RDW) is explained by several factors such as the abundance of red blood cells, the presence of target cells, and high reticulocytes, all of which are present in cases of thalassemia[216,157].

The presence of a weak immune system in thalassemia patients, in addition to the presence of a systemic inflammatory condition, contributes to an increase in the number of white blood cells, neutrophils, and the number of lymphocytes(LYM) in patients[217]. Immune abnormalities in thalassemia patients can be caused by the disease itself or attributed to iron chelation and iron overload treatments and splenectomy and may be the result of functional abnormalities in the immune system due to oxidative stress and inflammation. All of these causes lead to an elevated level of lymphocytes[218,219].

Conclusion

&

Recommendations

Conclusions:-

1. It was found that patients with thalassemia major suffer from a significant increase in (LDH, sTfR, Cu, TP, Cl^{-}, Ferittin, MCHC, RDW-CV, WBC, and LYM) compared to healthy controls.
2. There was a significant significant decrease in the level of (Zn, RBC, HCT, HGB, MCV, and MCH) compared to the healthy controls.
3. In addition, the gender and age of the patient have a significant and noticeable effect on these factors in the blood.
4. It is considered a way to infer the disease by biochemical method, where the abnormal levels of the studied factors showed acceptable sensitivity and specificity in predicting the disease.
5. Vital body organs are affected by the imbalance of the factors that have been studied.
6. The accumulation or deficiency of minerals plays a role in causing many side diseases in addition to the main effects of thalassemia patients.

Recommendations:-

1. More studies on thalassemia patients from a biochemical point of view to know the effect of the disease and its causes on many chemical elements in particular and the vital organs of the body in general.
2. As well as expanding and studying these factors on a bone marrow biopsy for thalassemia patients and noting the difference to know the causes and influencing factors.
3. Make many laboratory tests periodically, such as (Cu, ZN, Cl^{-}, and TP…et.al.) to reduce its rise or fall in preventive ways, such as taking some nutritional supplements.
4. A specific diet should not be followed, but thalassemia patients should have a balanced diet according to the individual's need.
5. Increasing awareness of the disease to limit its widespread spread through pre-marital and prenatal diagnosis.

References

The references:-

1. Angastiniotis M, Lobitz S. Thalassemias: An overview. *Int J Neonatal Screen*. 2019;5(1):1-11.

2. Bernini LE, Harteveld CL. α-Thalassaemia. *Baillieres Clin Haematol*. 1998;11(1):53-90.

3. Anwar WA, Khyatti M, Hemminki K. Consanguinity and genetic diseases in North Africa and immigrants to Europe. *Eur J Public Health*. 2014;24:57-63.

4. Masoudi GR, Rezaie Kykha R, Poor MS, Naderi M, Zareban I. The effect of halth belief model-based training on preventing major thalassemia in thalassemia carrier couples. *J Res Heal Soc Dev Heal Promot Res Cent*. 2015;5(2):211-219.

5. Kong Y, Zhou S, Kihm AJ, et al. Loss of α-hemoglobin-stabilizing protein impairs erythropoiesis and exacerbates β-thalassemia. *J Clin Invest*. 2004;114(10):1457-1466.

6. Cooley, T. B. and Lee P. Series of cases of splenomegaly and peculiar changes in the bones. *Trans Am Pediatr Soc*. 1925;37:29–30.

7. Cooley, T. B.; Witwer, E. R. and Lee P. Anemia in children: With splenomegaly and peculiar changes in the bones report of cases. *Am J Dis Child*. 1927;3(34):347-363.

8. Whipple, G.H. and Bradford WL. Mediterranean disease—‖—thalassemia‖ (erythroblastic anemia of Cooley); associated pigment abnormalities simulating hemochromatosis. *J Pediatr*. 1936;9:279–311.

9. W eatherall, D.J. & C legg JB. The Thalassaemia Syndromes 2nd edition. Published online 1972.

10. Weatherall DJ. The Thalassemias: The Role of Molecular Genetics in an Evolving Global Health Problem. *Am J Hum Genet*. 2004;74(3):385–392.

11. Marengo-Rowe A. The thalassemias and related disorders. *Proc (Bayl Univ Med Cent)*. 2007;20(1):27–31.

12. Steinberg MH, Forget BG, Higgs DR WD. Disorders of hemoglobin: genetics, pathophysiology, and clinical management. *Cambridge Univ Press*. Published online 2009.

13. Weatherall DJ. The thalassemia: Disorders of globin synthesis. In: William Hematology 9th edidition. Published online 2016:725-758.

14. Marengo-Rowe AJ. The thalassemias and related disorders. *Proc (Bayl Univ Med Cent)*. 2007;20(1):27–31.

15. Tari, K.; Ardalan, P.V.; Abbaszadehdibavar, M.; Atashi A. J, A. and Gheidishahran M. Thalassemia an update: molecular basis, clinical features and treatment. *Int J Biomed Public Heal*.

2018;1(1):48-58.

16. Gibbons, R.; Higgs DR., Olivieri, N. F. and Woods W. The beta thalassemia. 4th ed. *Oxford, Engl Blackwell Sci*. Published online 2001:287-356.

17. Giardina, P.J. and Rivella S. Thalassemia Syndromes. In: Hematology Basic Principles and Practice.6th edition. *Elsevier Heal Sci Saunders, Philadelphia*. Published online 2013:Chapter 38.

18. Sharma, D.C.; Arya, A.; Kishor, P.; Woike, P. and Binda J. Overview on Thalassemia. *Med Res Chronicles*. 2016;4(3):325-337.

19. Weatherall, D. J. . Kaushansky K, Lichtman MA, Prchal JT, Levi WP, Press OW, Burns LJ CM. The thalassemia: Disorders of globin synthesis.9th ed. *McGraw-Hill Educ USA*. Published online 2018:725-758.

20. Turgeon ML. Clinical Hematology: Theory and Procedures; 5 th edition. *Lippincott Williams Wilkins, a Wolters Kluwer business, Philadelphia USA*. 2019;(13):220-224.

21. Howard PR. Blood banking and transfusion practices. 5th ed. *Elsevier lnc*. Published online 2021:300-410.

22. Ho, P.J. and Thein S. Gene regulation and deregulation: a β globin

perspective. *Blood Rev*. 2000;14(2):78-93.

23. Weatherall DJ. The thalassemia: Disorders of globin synthesis.9th ed. *McGraw-Hill Educ USA*. Published online 2016:725-758.

24. Sharma, D.C.; Arya, A.; Kishor, P.; Woike, P. and Binda J. Overview on Thalassemia: A Review Article. *Med Res Chronicles*. 2017;4(3):325-337.

25. Taher, A.; Vichinsky, E.; Musallam, K.; Cappellini MD., Viprakasit, V. and Weatherall D. Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT). *Thalass Int Fed Nicosia, Cyprus*. 2013;13:978-9963.

26. Viprakasit, V. and Ekwattanakit S. .Clinical Classification, Screening and Diagnosis for Thalassemia. *Hematol Oncol Clin North Am*. 2018;32:193–211.

27. Weatherall, D.J. & Clegg J. Inherited hemoglobin disorders: an increasing global health problem. *Bull World Heal Organ*. 2000;79:704-712.

28. Borgna, C. and Galanello R. Thalassemias and related disorders: quantitative disorders of hemoglobin synthesis.13th ed. *Wolters Kluwer, Lippincott Williams Wilkins Heal Philadelphia, USA*. Published online 2014:862-913.

29. Sankaran, V.G.; Nathan, D.G. and Orkin SH. Thalassemia.8th ed.

Elsevier Heal Sci Saunders, Philadelphia, USA. Published online 2015:715-769.

30. De Sanctis, V.; Kattamis, C.; Canatan, D.; Soliman AT. E, H.; Karimi, M.; Daar, S.; Wali, Y.; Yassin, M.; Soliman N. S, P.; Al Jaouni, S.; El Kholy, M.; Fiscina, B. and Angastiniotis M. B-thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. *Mediterr J Hematol Infect Dis*. 2017;9(1):3-14.

31. Sharma, D.C.; Arya, A.; Kishor, P.; Woike, P. and Binda J. Overview on Thalassemia. *Med Res Chronicles*. 2017;4(3):325-337.

32. Sherif AA, G'Medhin A, Tadesse F, Tsegaye T. Thalassemia syndrome. *Ethiop Med J*. 2014;52(1):43-47.

33. Hamamy, H. A. and Al-Allawi NA. Epidemiological profile of common haemoglobinopathies in Arab countries. *J Community Genet*. 2013;4(2):147-167.

34. Kadhim KA, Baldawi KH, Lami FH. Prevalence, Incidence, Trend, and Complications of Thalassemia in Iraq. *Hemoglobin*. 2017;41(3):164-168.

35. Khaled M. Musallam| Rayan Bou-Fakhredin| Maria Domenica Cappellini|, Taher AT. 2021 update on clinical trials in β-

thalassemia. Published online 2021.

36. Fosburg, M.T. & Nathan D. Treatment of Cooley's anemia. *Blood*. 1990;76:435-444.

37. Borgna-Pignatti, C. & Galanello R. Thalassemias and related disorders: quantitative disorders of hemoglobin synthesis.11th ed. *Lippincott Williams Wilkins Philadelphia, p.p*. Published online 2004:1319-1365.

38. Hamidi Z. Endocrine Disorders in Thalassemia Major Patients: A Review. *Kuwait Med J*. 2016;48(1):4-11.

39. De Sanctis V. Endocrine complications. *Thalass Reports*. 2018;8:33-36.

40. Nita prasannan COA and DHR. Medicine for MRCP. *oxford Univ*. Published online 2020:chapter 9.

41. Qari MH, Wali Y, Albagshi MH, et al. Regional consensus opinion for the management of Beta thalassemia major in the Arabian Gulf area. *Orphanet J Rare Dis*. 2013;8(1):1-12.

42. Ganz T, Aronoff GR, Gaillard CAJM, et al. Iron Administration, Infection, and Anemia Management in CKD: Untangling the Effects of Intravenous Iron Therapy on Immunity and Infection Risk. *Kidney Med*. 2020;2(3):341-353.

43. Singh, P. and Seth A. Growth and endocrine issues in children with thalassemia. *Pediatr Hematol Oncol J*. 2017;2:98-106.

44. Schaible, U.E. & Kaufmann S. Iron and microbial infection. *Nat Rev Microbiol*. 2004;2(12):946-953.

45. Kadiiska, M.B., Burkitt, M.J., Xiang, Q.H., & Mason R. Iron supplementation generates hydroxyl radical in vivo. An ESR spin-trapping investigation. *J Clin Invest*. 1995;3:1653-1657.

46. Crisponi G, Nurchi VM, Lachowicz JI. *Iron Chelation for Iron Overload in Thalassemia*. Vol 19.; 2019.

47. Amaral MSA, Reis ACMB, Pauna HF, et al. Current Topics in Medicine and Medical Research Vol. 6. *Curr Top Med Med Res Vol 6*. 2020;(September).

48. Herbert V, Jayatilleke E, Shaw S, et al. Serum ferritin iron, a new test, measures human body iron stores unconfounded by inflammation. *Stem Cells*. 1997;15(4):291-296.

49. Kell DB, Pretorius E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics*. 2014;6(4):748-773.

50. Serai SD, Trout AT, Miethke A, Diaz E, Xanthakos SA, Dillman JR. Putting it all together: established and emerging MRI techniques for detecting and measuring liver fibrosis. *Pediatr*

Radiol. 2018;48(9):1256-1272.

51. Nojiri S, Kusakabe A, Fujiwara K, et al. Noninvasive evaluation of hepatic fibrosis in hepatitis C virus-infected patients using ethoxybenzyl-magnetic resonance imaging. *J Gastroenterol Hepatol*. 2013;28(6):1032-1039.

52. Lang J, Zhao X, Wang X, et al. Targeted co-delivery of the iron chelator deferoxamine and a HIF1α inhibitor impairs pancreatic tumor growth. *ACS Nano*. 2019;13(2):2176-2189.

53. Wright SW, Valento M, Mazor SS, Chen BC. Severe iron poisoning treated with prolonged deferoxamine infusion: a case report. *Toxicol Commun*. 2018;2(1):6-9.

54. Anderson, L.J., Westwood, M.A., Holden, S., Davis, B., Prescott, E. et al. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. *Br J Haematol*. 2004;127(3):348-355.

55. Galanello R, Origa R. Open Access REVIEW BioMed Central Beta-thalassemia. *Orphanet J Rare Dis*. 2010;5:11.

56. Small SA, Mather RS. Beta-Thalassemia mini review. *Int J Pharmacol Res*. 2009;3(2):71-79.

57. Taher AT, Porter JB, Kattamis A, Viprakasit V, Cappellini MD.

Efficacy and safety of iron-chelation therapy with deferoxamine, deferiprone, and deferasirox for the treatment of iron-loaded patients with nontransfusion-dependent thalassemia syndromes. *Drug Des Devel Ther*. 2016;10:4073-4078.

58. Taher AT, Cappellini MD. How I manage medical complications of b-thalassemia in adults. *Blood*. 2018;132(17):1781-1791.

59. Kushner JP, Porter JP, Olivieri NF. Secondary iron overload. *Hematology Am Soc Hematol Educ Program*. Published online 2001:47-61.

60. Assessed P, Mri W, Patients YT. Correlative Study of Iron Accumulation in Liver ,. 2006;28(5):311-315.

61. AL-Zuhairy SH, Darweesh MA, Othman MAM. Relation of Serum Ferritin Level with Serum Hepcidin and Fucose Levels in Children with β-Thalassemia Major. *Hemoglobin*. 2021;45(1):69-73.

62. De Sanctis V, Soliman AT, Elsedfy H, et al. Gonadal dysfunction in adult male patients with thalassemia major: an update for clinicians caring for thalassemia. *Expert Rev Hematol*. 2017;10(12):1095-1106.

63. Backe MB, Moen IW, Ellervik C, Hansen JB, Mandrup-Poulsen T. Iron Regulation of Pancreatic Beta-Cell Functions and Oxidative Stress. *Annu Rev Nutr*. 2016;36(April):241-273.

64. Sood R, Rastogi P, Bansal D, et al. An Autopsy Case of β-Thalassemia Major Illuminating the Pathological Spectrum of the Disease. *Hemoglobin*. 2021;45(3):180-185.

65. Donker AE, van der Staaij H, Swinkels DW. The critical roles of iron during the journey from fetus to adolescent: Developmental aspects of iron homeostasis. *Blood Rev*. 2021;50(July):100866.

66. Vichinsky E. Non-transfusion-dependent thalassemia and thalassemia intermedia: Epidemiology, complications, and management. *Curr Med Res Opin*. 2016;32(1):191-204.

67. Galanello, R. and Origa R. Beta-thalassemia. *Orphanet J Rare Dis*. 2010;5(1):11.

68. Anurathapan U, Hongeng S, Pakakasama S, et al. Hematopoietic stem cell transplantation for homozygous β-thalassemia and β-thalassemia/hemoglobin e patients from haploidentical donors. *Bone Marrow Transplant*. 2016;51(6):813-818.

69. Rostami T, Kasaeian A, Maleki N, et al. The effect of bone marrow-derived mesenchymal stem cell co-transplantation with hematopoietic stem cells on liver fibrosis alleviation and survival in patients with class III β-thalassemia major. *Stem Cell Res Ther*. 2021;12(1):1-12.

70. Mohamed SY. Thalassemia Major: Transplantation or Transfusion

and Chelation. *Hematol Oncol Stem Cell Ther*. 2017;10(4):290-298.

71. Gujja P, Rosing DR, Tripodi DJ, Shizukuda Y. Iron overload cardiomyopathy: Better understanding of an increasing disorder. *J Am Coll Cardiol*. 2010;56(13):1001-1012.

72. Liao Y, Geyer MB, Yang AJ, Cairo MS. Cord blood transplantation and stem cell regenerative potential. *Exp Hematol*. 2011;39(4):393-412.

73. Agata Forkasiewicz1, Maja Dorociak1, Kamilla Stach2, Piotr Szelachowski1 RT and KA. The usefulness of lactate dehydrogenase measurements in current oncological practice. Published online 2020.

74. Adeva-Andany M, López-Ojén M, Funcasta-Calderón R, Ameneiros-Rodríguez E, Donapetry-García C, Vila-Altesor M R-SJ. Comprehensive review on lactate metabolism in human health. *Mitochondrion*. 2014;17:76-100.

75. Feng Y, Xiong Y, Qiao T, Li X, Jia L HY. Lactate dehydrogenase A: A key player in carcinogenesis and potential target in cancer therapy. Published online 2018.

76. Mercer RM, Corcoran JP, Porcel JM. CMJv19n3-Mercer.indd. 2019;19(3):19-23.

77. Tokinoya K, Ishikura K, Yoshida Y, Ra SG, Sugasawa T, Aoyagi A, Nabekura Y, Takekoshi K OH. LDH isoenzyme 5 is an index of early onset muscle soreness during prolonged running. *J Sport Med Phys Fit*. 2020;60(7):1020-1026.

78. Mohammad AM, Dawad MM, Kashmoola MA, Al-Allawi N. Doppler-defined pulmonary hypertension in β-thalassemia major in Kurdistan, Iraq. *PLoS One*. 2020;15(12 December):1-11.

79. Galila M. Mokhtar, Amira A.M. Adly, Mohsen S. El Alfy LMT &Ahmed TK. N-Terminal Natriuretic Peptide and Ventilation-Perfusion Lung Scan in Sickle Cell Disease and Thalassemia Patients with Pulmonary Hypertension. *Hemoglobin*. 2010;34(1):78-94.

80. Shahnawaz Khan M, Mabood Husain F, Alhumaydhi FA, et al. Exploring the molecular interactions of Galantamine with human Transferrin: In-silico and in vitro insight. *J Mol Liq*. 2021;335:116227.

81. Khalil S, Holy M, Grado S, et al. A specialized pathway for erythroid iron delivery through lysosomal trafficking of transferrin receptor 2. *Blood Adv*. 2017;1(15):1181-1194.

82. Speeckaert MM, Speeckaert R, Delanghe JR. Biological and clinical aspects of soluble transferrin receptor. *Crit Rev Clin Lab*

Sci. 2010;47(5-6):213-228.

83. Dignass A, Farrag K, Stein J. Limitations of Serum Ferritin in Diagnosing Iron Deficiency in Inflammatory Conditions. *Int J Chronic Dis*. 2018;2018(Table 1):1-11.

84. Dedoussis GVZ, Kyrtsonis MC, Andrikopoulos NE, Voskaridou E, Loutradis A. Inverse correlation of plasma leptin and soluble transferrin receptor levels in β-thalassemia patients. *Ann Hematol*. 2002;81(9):543-547.

85. Skarmoutsou C, Papassotiriou I, Traeger-Synodinos J, et al. Erythroid bone marrow activity and red cell hemoglobinization in iron-sufficient β-thalassemia heterozygotes as reflected by soluble transferrin receptor and reticulocyte hemoglobin content. Correlation with genotypes and HB A2 levels. *Haematologica*. 2003;88(6):631-636.

86. Huang Y, Lei Y, Liu R, et al. Imbalance of erythropoiesis and iron metabolism in patients with thalassemia. *Int J Med Sci*. 2019;16(2):302-310.

87. Z. Ozturk, G.E. Genc SG. Minerals in thalassaemia major patients: An overview. *Trace Elem Med Biol*. 2017;41:1-17.

88. Deshpande J, Joshi M, Giri P. Zinc: The trace element of major importance in human nutrition and health. *Int J Med Sci Public*

Heal. 2013;2(1):1.

89. Ploysangam A, Falciglia GA, Brehm BJ. Effect of marginal zinc deficiency on human growth and development. *J Trop Pediatr*. 1997;43(4):192-198.

90. Fung EB, Kwiatkowski JL, Huang JN, Gildengorin G, King JC, Vichinsky EP. Zinc supplementation improves bone density in patients with thalassemia: A double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr*. 2013;98(4):960-971.

91. Erdoĝan E, Canatan D, Örmeci AR, Vural H, Aylak F. The effects of chelators on zinc levels in patients with thalassemia major. *J Trace Elem Med Biol*. 2013;27(2):109-111.

92.] S. Bartakke, S.B. Bavdekar, P. Kondurkar, M.N. Muranjan MVM, R. Sharma. Effect of deferiprone on urinary zinc excretion in multiply transfused children with thalassemia major. *Indian Pediatr*. 2005;42(2):150–154.

93. Galanello R. Deferiprone in the treatment of transfusion-dependent thalassemia. *Clin Risk Manag*. 2007;3(5):795–805.

94. O.R. Zekavat, A. Bahmanjahromi, S. Haghpanah, S. Ebrahimi NC. The Zinc and Copper Levels in Thalassemia Major Patients, Receiving Iron Chelation Therapy. *Pediatr Hematol*. 2018;40(3):178–181.

95. G.E. Genc, Z. Ozturk, S. Gumuslu AK. Mineral levels in thalassaemia major patients using different iron chelators. *Biol Trace Elem Res*. 2016;170(1):9–16.

96. Nimkarn N, Songdej D, Dumrongwongsiri O, Sirachainan N, Chuansumrit A. Age as a major factor associated with zinc and copper deficiencies in pediatric thalassemia. *J Trace Elem Med Biol*. 2021;68(July).

97. Er EÖ, Öz E, Zeynep Y, Bak S. Sodium , Magnesium , Calcium , Manganese , Iron , Copper , and Zinc in Serums of Beta Thalassemia Major Patients. Published online 2020.

98. Swain S, Rautray TR. Estimation of Trace Elements, Antioxidants, and Antibacterial Agents of Regularly Consumed Indian Medicinal Plants. *Biol Trace Elem Res*. 2021;199(3):1185-1193.

99. Bjørklund G. The role of zinc and copper in autism spectrum disorders. *Acta Neurobiol Exp (Wars)*. 2013;73(2):225-236.

100. Taylor AA, Tsuji JS, Garry MR, et al. Critical Review of Exposure and Effects: Implications for Setting Regulatory Health Criteria for Ingested Copper. *Environ Manage*. 2020;65(1):131-159.

101. Squitti R, Faller P, Hureau C, Granzotto A, White AR, Kepp KP. Copper Imbalance in Alzheimer's Disease and Its Link with the Amyloid Hypothesis: Towards a Combined Clinical, Chemical, and

Genetic Etiology. *J Alzheimer's Dis*. 2021;83(1):23-41.

102. Michalak M, Pierzak M, Kręcisz B, Suliga E. Bioactive compounds for skin health: A review. *Nutrients*. 2021;13(1):1-31.

103. Kajanachumpol S, Tatu T SW. Zinc and copper status of thalassemia children. *Southeast Asian J Trop Med PublicHealth*. 1997;28(4):877-880.

104. Bashir NA. Serum zinc and copper levels in sickle cell anaemia and β-thalassaemia in North Jordan. *Ann Trop Paediatr*. 1995;15(4):291-293.

105. Al-Samarrai AH, Adaay MH A-TK. Evaluation of some essential element levels in thalassemia major patients in Mosul district, Iraq. *Saudi Med J*. 2008;29(1):94–7.

106. Claster S, Wood JC, Noetzli L, et al. Nutritional deficiencies in iron overloaded patients with hemoglobinopathies. *Am J Hematol*. 2009;84(6):344-348.

107. Anwer A, Alwash M. Biomarkers and trace elements in beta thalassemia major. *Iraqi J Cancer Med Genet*. 2013;(April).

108. Shamshirsaz AA, Bekheirnia MR, Kamgar M, et al. Metabolic and endocrinologic complications in beta-thalassemia major: A multicenter study in Tehran. *BMC Endocr Disord*. 2003;3:2-7.

109. Eshghi P, Alavi S, Ghavami S, Rashidi A. Growth impairment in β-thalassemia major: The role of trace element deficiency and other potential factors. *J Pediatr Hematol Oncol*. 2007;29(1):5-8.

110. Buzanovskii VA. Determination of proteins in blood. Part 1: Determination of total protein and albumin. *Rev J Chem*. 2017;7(1):79-124.

111. Moshage HJ, Janssen JAM, Franssen JH, Hafkenscheid JC, Yap SH. Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. *J Clin Invest*. 1987;79(6):1635-1641.

112. Asadi MS, Mirvaghefei AR, Nematollahi MA, Banaee M, Ahmadi K. Effects of Watercress (Nasturtium nasturtium) extract on selected immunological parameters of rainbow trout (Oncorhynchus mykiss). *Open Vet J*. 2012;2(1):32-39.

113. Tracy Morrison.Ronald A. Booth.Kristin Hauff. Chapter One - Laboratory assessment of multiple myeloma. *Adv Clin Chem*. 2019;107:1-303.

114. Ichiki A, Hashimoto N, Ueda T, et al. IgG4-related disease with bone marrow involvement. *Intern Med*. 2016;55(16):2295-2299. doi:10.2169/internalmedicine.55.6749

115. Qu C, Wang L, He J, et al. Carbon nanotubes provoke inflammation by inducing the pro-inflammatory genes IL-1β and

IL-6. *Gene*. 2012;493(1):9-12.

116. Grünberg W, Donkin SS, Constable PD. Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base, calcium, and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows. *J Dairy Sci*. 2011;94(2):727-745.

117. Bayejid Hosen M, Fazlul Karim M, Al Mahmud R. Association of AST, ALT, ALB and Total Protein with Beta-thalassemia in Bangladeshi Population Association of vitamin D and vitamin D binding protein (DBP) gene polymorphism with susceptibility of type 2 diabetes mellitus in Bangladesh View project Comput. *Artic Int J Adv Res*. 2015;(January).

118. Azza A.G. Tantawy, Nagham El Bablawy, Amira A.M. Adly and FSEE. Early Predictors of Renal Dysfunction in Egyptian Patients with β-Thalassemia Major and Intermedia. *Hematol Infect Dis*. Published online 2014.

119. Awadallah SM, Atoum MF, Nimer NA, Saleh SA. Ischemia modified albumin: An oxidative stress marker in β-thalassemia major. *Clin Chim Acta*. 2012;413(9-10):907-910.

120. Abd-Ali M-N, Abd-Reza M. Clinical study of β-thalassemia major patients. *J Pharm Sci Res*. 2019;11(3):909-911.

121. Malik AM, Malik EM, Al-Shammaa NMJ, Al-Rubaei ZM. A. M. Malik, E. M. Malik, N. M. J. Al-Shammaa, and Z. M. Al-Rubaei, "A Comparative Biochemical Study of Proteins Profile in Iraqi Children and Adolescent with β–Thalassemia," Iraqi J Pharm Sci, vol. 19, no. 2, pp. 19–23, 2010. *Iraqi J Pharm Sci*. 2010;19(2):19-23.

122. Murtadha MK. Determination of sialic acid and biochemical parameters level in β -thalassemic patients. 2011;9(1):177-183.

123. Wilde WS. Role of potassium in human and animal nutrition. *Role Potassium Agric*. 2015;(July):203-220.

124. Lu YP, Huang JW, Lee IN, et al. A Portable System to Monitor Saliva Conductivity for Dehydration Diagnosis and Kidney Healthcare. *Sci Rep*. 2019;9(1):1-9.

125. Grodin JL, Verbrugge FH, Ellis SG, Mullens W, Testani JM, Tang WHW. Importance of Abnormal Chloride Homeostasis in Stable Chronic Heart Failure. *Circ Hear Fail*. 2016;9(1):1-7.

126. Arnaud MJ. Mild dehydration: A risk factor of constipation? *Eur J Clin Nutr*. 2003;57:S88-S95.

127. Omar AK, Ahmed KA, Helmi NM, et al. The sensitivity of Na+, K+ ATPase as an indicator of blood diseases. *Afr Health Sci*. 2017;17(1):262-269.

128. Karim MF, Ismail M, Hasan AM SH. Hematological and biochemical status of Beta-thalassemia major patients in Bangladesh: A comparative analysis. *Int J Hematol Oncol Stem Cell Res*. 2016;10:7-12.

129. Anemia C, Tosteson BDC, Shea E, Darling RC. Potassium and sodium of red blood cells in sickle cell anemia. *J Clin Invest*. 1952;31:406-411.

130. Nnodim JK, Meludu SC, Dioka CE, Onah C, Chilaka UJ, Obi PC. Altered membrane potential and electrolyte in sickle cell anemia. *J Krishna Inst Med Sci Univ*. 2014;3(1):70-73.

131. Bayejid Hosen M, Shamimul Hasan M, Farhad Azim M, Sarder R, Mesbah Uddin M. Evaluation of Renal Function in Beta-Thalassemia Patients in Bangladesh. *an Open Access J*. 2015;6(1):11-14.

132. EC T. Ferritin protein nanocages – the story. *Nanotechnol Percept*. 2012;8(1):7-16.

133. Beck G, Ellis TW HG. Evolution of the acute phase response: iron release by echinoderm (Asterias forbesi) coelomocytes, and cloning of an echinoderm ferritin molecule. *Dev Comp Immunol*. 2002;26(1):11–26.

134. Rujeerapaiboon N, Tantiworawit A, Piriyakhuntorn P, et al.

Correlation Between Serum Ferritin and Viral Hepatitis in Thalassemia Patients. *Hemoglobin*. 2021;45(3):175-179.

135. Tamaddoni A, Ramezani MS. Comparison between deferoxamine and combined therapy with deferoxamine and deferiprone in Iron overloaded thalassemia patients. *Iran Red Crescent Med J*. 2010;12(6):655-659.

136. Habeb, A. M.; Al Hawsawi, M.; Morsy, M.; Al-Harbi AM., Osilan, A. S.; Al-Magamsi, M. S. and Zolaly MA. Endocrinopathies in beta thalassemia major. *Saudi Med J*. 2013;34(1):67-73.

137. Majeed MS. Evaluation of some Biochemical and Endocrine Profiles in transfusion dependent Iraqi major β - thalassemia patients. *Iraqi J Sci*. 2017;58(2):639-645.

138. Abdulzahra, M. S.; Al-Hakeim, H. K. and Ridha MM. Study of the effect of iron overload on the function of endocrine glands in male thalassemia patients. *Asian J Transfus Sci*. 2011;5(2):127-131.

139. Yenzeel, J.H. and Salih L. Effect of Beta-Thalassemia Major on Reporodutive Hormones Levels and Ovarian Status in Sample of Iraqi Female. *Int J Sci Nat*. 2017;8(3):500-504.

140. Shamshirasaz, A.A.; Bekheirnia, M.R.; Kamgar M. Metabolic and Endocrinologic Complications in Beta Thalassemia Major: A Muticentre Study in Tehran. *BMC Endocr Disord*. 2003;3(1):4.

141. Papadopoulos T, Abrahim A, Sergelidis D, Bitchava K. A cross-sectional study of metabolic and endocrine complications in beta-thalassemia major. 2011;2(January):119-123.

142. Eshaqh-hosseini, S. K.; Jafari-Koshki, T.; Arsang-Jang S and, Shapouri J. Endocrinopathy complications and the role of serum ferritin as a marker of endocrinopathy prediction in patients with beta-thalassemia major. *Adv Hum Biol*. 2018;8(3):169.

143. Lee JS, Cho SI, Park SS, Seong MW. Molecular basis and diagnosis of thalassemia. *Blood Res*. 2021;56(April):39-43.

144. Valiathan R, Ashman M, Asthana D. Effects of Ageing on the Immune System: Infants to Elderly. *Scand J Immunol*. 2016;83(4):255-266.

145. Gluba-Brzózka A, Franczyk B, Rysz-Górzyńska M, Rokicki R, Koziarska-Rościszewska M, Rysz J. Pathomechanisms of immunological disturbances in β-thalassemia. *Int J Mol Sci*. 2021;22(18).

146. Kuhn V, Diederich L, Keller TCS, et al. Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. *Antioxidants Redox Signal*. 2017;26(13):718-742.

147. Kalpravidh RW, Siritanaratkul N, Insain P, et al. Improvement in oxidative stress and antioxidant parameters in β-thalassemia/Hb E

patients treated with curcuminoids. *Clin Biochem*. 2010;43(4-5):424-429.

148. Rachmilewitz EA, Weizer-Stern O, Adamsky K, et al. Role of iron in inducing oxidative stress in thalassemia: Can it be prevented by inhibition of absorption and by antioxidants? *Ann N Y Acad Sci*. 2005;1054:118-123.

149. Rutjanaprom W, Kanlop N, Charoenkwan P, et al. Heart rate variability in beta-thalassemia patients. *Eur J Haematol*. 2009;83(5):483-489.

150. Sirachainan N, Chuansumrit A, Kadegasem P, Sasanakul W, Wongwerawattanakoon P, Mahaklan L. Normal hemostatic parameters in children and young adults with α-thalassemia diseases. *Thromb Res*. 2016;146:35-42.

151. Çil B, Ayyıldız H, Tuncer T. Discrimination of β-thalassemia and iron deficiency anemia through extreme learning machine and regularized extreme learning machine based decision support system. *Med Hypotheses*. 2020;138(January).

152. Goss C, Giardina P, Degtyaryova D, Kleinert D, Sheth S, Cushing M. Red blood cell transfusions for thalassemia: Results of a survey assessing current practice and proposal of evidence-based guidelines. *Transfusion*. 2014;54(7):1773-1781.

153. Okada H, Kobune F, Sato TA, et al. Extensive lymphopenia due to apoptosis of uninfected lymphocytes in acute measles patients. *Arch Virol*. 2000;145(5):905-920.

154. Elshami EH AA. Automated diagnosis of thalassemia based on datamining classifiers. *Int Conf Informatics Appl*. Published online 2012:4-440.

155. Setsirichok D, Piroonratana T, Wongseree W, et al. Classification of complete blood count and haemoglobin typing data by a C4.5 decision tree, a naïve Bayes classifier and a multilayer perceptron for thalassaemia screening. *Biomed Signal Process Control*. 2012;7(2):202-212.

156. Masala GL, Golosio B, Cutzu R PR. A two-layered classifier based on the radial basis function for the screening of thalassaemia. *Comput Biol Med*. 2013;43:31-1724.

157. AlFadhli SM, Al-Awadhi AM, AlKhaldi D. Validity assessment of nine discriminant functions used for the differentiation between Iron deficiency anemia and thalassemia minor. *J Trop Pediatr*. 2007;53(2):93-97.

158. RW N. Red Blood Cell Distribution Width in Pediatric Microcytic Anemias. *Pediatrics*. 1987;80:4-251.

159. Hoffmann JJML, Urrechaga E. Role of RDW in mathematical

formulas aiding the differential diagnosis of microcytic anemia. *Scand J Clin Lab Invest*. 2020;80(6):464-469.

160. Al-Numan AH, Al-Obeidi RA. The value of the red cell distribution width index versus other parameters in the differentiation between iron deficiency anemia and beta thalassemia trait among children in Mosul, Iraq. *Expert Rev Hematol*. 2021;14(4):401-406.

161. Roberts GT, El Badawi SB. Red blood cell distribution width index in some hematologic diseases. *Am J Clin Pathol*. 1985;83(2):222-226.

162. Burk M, Arenz J, Giagounidis AA et al. Erythrocyte indices as screening tests for the differentiation of microcytic anemias. *Eur J Med Res*. 1995;1:7-33.

163. Farmakis D, Giakoumis A, Angastiniotis M, Eleftheriou A. The changing epidemiology of the ageing thalassaemia populations: A position statement of the Thalassaemia International Federation. *Eur J Haematol*. 2020;105(1):16-23.

164. Listowsky A and. Iron Transport and Storage Proteins. *Annu Rev Biochem*. 1980;49:357-393.

165. Al KAT serum protein. KA et. Total serum protein. *Princeton*. Published online 1984:1316-1324 and 418.

166. Kato GJ, McGowan V, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood*. 2006;107(6):2279-2285.

167. Bernaudin F, Verlhac S, Chevret S, et al. G6PD deficiency, absence of α-thalassemia, and hemolytic rate at baseline are significant independent risk factors for abnormally high cerebral velocities in patients with sickle cell anemia. *Blood*. 2008;112(10):4314-4317.

168. Barcellini W, Fattizzo B. Clinical Applications of Hemolytic Markers in the Differential Diagnosis and Management of Hemolytic Anemia. *Dis Markers*. 2015;2015.

169. Chowdhury SF, Anwar S. Management of Hemoglobin Disorders During the COVID-19 Pandemic. *Front Med*. 2020;7(June):1-6.

170. Connes P, Lamarre Y, Hardy-Dessources MD, et al. Decreased hematocrit-to-viscosity ratio and increased lactate dehydrogenase level in patients with sickle cell anemia and recurrent leg ulcers. *PLoS One*. 2013;8(11):6-10.

171. Randi ML, Bertozzi I, Santarossa C, et al. Prevalence and causes of anemia in hospitalized patients: Impact on diseases outcome. *J Clin Med*. 2020;9(4).

172. ElAlfy MS, Khalil Elsherif NH, Ebeid FSE, et al. 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*. 2018;103(December 2017):65-70.

173. Benites BD, Bastos SO, Baldanzi G, et al. Sickle cell/β-thalassemia: Comparison of Sβ0 and Sβ+ Brazilian patients followed at a single institution. *Hematology*. 2016;21(10):623-629.

174. Taher AT, Musallam KM, Karimi M et al. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. *Blood*. 2010;115:92-1886.

175. Ricchi P, Ammirabile M, Costantini S, Spasiano A, Di Matola T, Verna R, Pepe A, Cinque P, Saporito C, Filosa A PL. Soluble form of transferrin receptor as a biomarker of overall morbidity in patients with non-transfusion-dependent thalassaemia: a cross-sectional study. *Blood Transfus*. 2016;14(6):538-540.

176. Ricchi P, Ammirabile M CS et al. A useful relationship between the presence of extramedullary erythropoeisis and the level of the soluble form of the transferrin receptor in a large cohort of adult patients with thalassemia intermedia: a prospective study. *Ann Hematol*. 2012;91:9-905.

177. Hambidge M, Cousins RJ, Costello RB. Zinc and health: Current status and future directions: Introduction. *J Nutr*. 2000;130(5 SUPPL.):1360-1366.

178. Prasad AS, Bao B, Beck FWJ, Kucuk O, Sarkar FH. Antioxidant effect of zinc in humans. *Free Radic Biol Med*. 2004;37(8):1182-1190.

179. Andreini C, Banci L, Bertini I, Rosato A. Counting the zinc-proteins encoded in the human genome. *J Proteome Res*. 2006;5(1):196-201.

180. S PA. Discovery of human zinc deficiency: 50 years later. *J Trace Elem Med Bio*. 2012;26:66–9.

181. Gerwien F, Skrahina V, Kasper L, Hube B, Brunke S. Metals in fungal virulence. *FEMS Microbiol Rev*. 2018;42(1):1-21.

182. Shabbir Z, Sardar A, Shabbir A, et al. Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere*. 2020;259:127436.

183. Barber RG, Grenier ZA, Burkhead JL. Copper toxicity is not just oxidative damage: Zinc systems and insight from wilson disease. *Biomedicines*. 2021;9(3).

184. Guo H, Ouyang Y, Wang J, et al. Cu-induced spermatogenesis disease is related to oxidative stress-mediated germ cell apoptosis

and DNA damage. *J Hazard Mater*. 2021;416(April):125903.

185. Muchenditsi A, Yang H, Hamilton JP, et al. Targeted inactivation of copper transporter Atp7b in hepatocytes causes liver steatosis and obesity in mice. *Am J Physiol - Gastrointest Liver Physiol*. 2017;313(1):G39-G49.

186. Petukhov VI, Baumane LK, Kalvinsh IY, et al. Chronic hepatitis C: Quantitative EPR analysis of nitrogen oxide and copper in patients' blood. *Bull Exp Biol Med*. 2008;146(6):875-877.

187. Hosseini, M.J., Shaki, F., Ghazi-Khansari, M., Pourahmad J. Toxicity of Copper on Isolated Liver Mitochondria: Impairment at Complexes I, II, and IV Leads to Increased ROS Production. *Cell Biochem Biophys*. 2014;70:367–381.

188. Saeidnia M, Nowrouzi-Sohrabi P, Erfani M, Fazeli P, Tamaddon G, Karimi M. The effect of curcumin on serum copper, zinc, and zinc/copper ratio in patients with β-thalassemia intermedia: a randomized double-blind clinical trial. *Ann Hematol*. 2021;100(3):627-633.

189. Malavolta M, Giacconi R, Piacenza F, et al. Plasma copper/zinc ratio: An inflammatory/nutritional biomarker as predictor of all-cause mortality in elderly population. *Biogerontology*. 2010;11(3):309-319.

190. Faber S, Zinn GM, Kern JC, Skip Kingston HM. The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. *Biomarkers*. 2009;14(3):171-180.

191. Dàvila E, Parés D, Cuvelier G, Relkin P. Heat-induced gelation of porcine blood plasma proteins as affected by pH. *Meat Sci*. 2007;76(2):216-225.

192. Hooijberg EH, Miller M, Cray C, Buss P, Steenkamp G, Goddard A. Serum protein electrophoresis in healthy and injured southern white rhinoceros (Ceratotherium simum simum). *PLoS One*. 2018;13(7):1-17.

193. Moore C, Stefanovski D, Luethy D. Clinical performance of a commercially available thymidine kinase 1 assay for diagnosis of lymphoma in 42 hospitalized horses (2017-2020). *J Vet Intern Med*. 2021;35(5):2495-2499.

194. Fatkullin RR, Ermolova EM, Kosilov VI, Matrosova Y V., Chulichkova SA. Biochemical Status of Animal Organism Under Conditions of Technogenic Agroecosystem. 2018;151:182-186.

195. Ma B, Zhang L, Li J, Xing T, Jiang Y, Gao F. Heat stress alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers. *Poult Sci*. 2021;100(1):215-223.

196. Punzo F, Tortora C, Argenziano M, Casale M, Perrotta S, Rossi F. Iron chelating properties of Eltrombopag: Investigating its role in thalassemia-induced osteoporosis. *PLoS One*. 2018;13(12):1-11.

197. Mansoor F, Bai P, Kaur N, et al. Evaluation of Serum Electrolyte Levels in Patients With Anemia. *Cureus*. 2021;28(5):691-696.

198. Rhoda M, Apovo M, Beuzard Y, Giraud F. Ca2+ permeability in deoxygenated sickle cells. *Blood*. 1990;75(12):2453-2458.

199. Hannemann A, Weiss E, Rees DC, Dalibalta S, Ellory JC, Gibson JS. The properties of red blood cells from patients heterozygous for HbS and HbC (HbSC Genotype). *Anemia*. 2011;2011.

200. O'grady SM. Oxidative stress, autophagy and airway ion transport. *Am J Physiol - Cell Physiol*. 2019;316(1):C16-C32.

201. Luthra MG S DA. Increased Ca++, Mg++, and Na++ K+ ATPase activities erythrocytes of sickle cell anemia. *Blood*. 1982;60:1332-1336.

202. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica*. 2004;89(10):1187-1193.

203. Yassin MA, Soliman AT, De Sanctis V, et al. Jadenu ® substituting Exjade ® in iron overloaded β-thalassemia major

(BTM) patients: A preliminary report of the effects on the tolerability, serum ferritin level, liver iron concentration and biochemical profiles. *Mediterr J Hematol Infect Dis*. 2018;10(1):1-6.

204. Belhoul KM, Bakir ML, Kadhim AM, Dewedar HES, Eldin MS, Alkhaja FA. Prevalence of iron overload complications among patients with β-thalassemia major treated at Dubai Thalassemia Centre. *Ann Saudi Med*. 2013;33(1):18-21.

205. Gardenghi S, Grady RW, Rivella S. Anemia, ineffective erythropoiesis, and hepcidin: Interacting factors in abnormal iron metabolism leading to iron overload in β-thalassemia. *Hematol Oncol Clin North Am*. 2010;24(6):1089-1107.

206. Leecharoenkiat, K.; Lithanatudom, P.; Sornjai W and S, D.R. Iron dysregulation in beta-thalassemia. *Asian Pac J Trop Med*. 2016;9(11):1035–1043.

207. Palareti G, Legnani C, Cosmi B, et al. Comparison between different D-Dimer cutoff values to assess the individual risk of recurrent venous thromboembolism: Analysis of results obtained in the DULCIS study. *Int J Lab Hematol*. 2016;38(1):42-49.

208. Old J, Harteveld CL, Trarger-Synodinos J, Petrou M, Angastiniotis M, Galanello R. *Prevention of Thalassaemias and Other*

Haemoglobin Disorders. Volume 2: Laboratory Methods. Vol 2.; 2004.

209. Infirmary RV. TRAIT BY ROUTINE BLOOD-COUNT patient for whom I applied the simplified discriminant. Published online 1972:1971.

210. Ducrocq G, Puymirat E, Steg PG, et al. Blood transfusion, bleeding, anemia, and survival in patients with acute myocardial infarction: FAST-MI registry. *Am Heart J*. 2015;170(4):726-734.e2.

211. Jolicœur EM, O'Neill WW, Hellkamp A, et al. Transfusion and mortality in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention. *Eur Heart J*. 2009;30(21):2575-2583.

212. Hershko C, Link G CI. Pathophysiology of iron overload. *Ann NY Acad Sci*. 1998;850:191–201.

213. Pattanapanyasat K, Noulsri E, Fucharoen S, et al. Flow Cytometric Quantitation of Red Blood Cell Vesicles in Thalassemia. *Cytom Part B - Clin Cytom*. 2004;57(1):23-31.

214. Aessopos A, Farmakis D, Deftereos S, et al. Cardiovascular effects of splenomegaly and splenectomy in β-thalassemia. *Ann Hematol*. 2005;84(6):353-357.

215. Dissayabutra T, Tosukhowong P, Seksan P. The benefits of vitamin C and vitamin E in children with beta-thalassemia with high oxidative stress. *J Med Assoc Thai*. 2005;88 Suppl 4(October).

216. Jones KW. Evaluation of cell morphology and introduction to platelet and white blood cell morphology. *Clin Hematol Fundam Hemost*. Published online 2009:93-116.

217. Elsayh, K.I.; Mohammed, W.S.; Zahran, A.M.; Saad K. Leukocytes apoptosis and adipocytokines in children with beta thalassemia major. *Clin Exp Med*. 2016;16:345–350.

218. Gharagozloo M, Karimi M, Amirghofran Z. Double-faced cell-mediated immunity in β-thalassemia major: Stimulated phenotype versus suppressed activity. *Ann Hematol*. 2009;88(1):21-27.

219. Scholz EMB, Kashuba ADM. The Lymph Node Reservoir: Physiology, HIV Infection, and Antiretroviral Therapy. *Clin Pharmacol Ther*. 2021;109(4):918-927.

الخلاصة

أجريت هذه الدراسة للفترة تشرين الثاني (نوفمبر) 2020 الى حزيران (يونيو) 2022 , تم اخذ 150 عينة دم مؤلفة من (100 مريض و50 اصحاء) مجموع المرضى كان 50 أناث و50 ذكور من المصابين بمرض الثلاسيميا الكبرى (فقر دم البحر الابيض المتوسط) وبمدى اعمار10- 25 سنة من المرضى المراجعين لمركز ميسان لفقر دم البحر الابيض المتوسط ,اما مجموعة الأصحاء كانوا متوافقين بالعمر والجنس .

وتم تقسيم مجموعتي المرضى والأصحاء الى مجموعتين اعتمادا على العمر: المجموعة الاولى من 10-17 سنة والمجموعة الثانية 18-25 سنة .

هدفت هذه الدراسة الى معرفة المتغيرات الكيميائية والاختلافات في تراكيز (نازعة هيدروجين اللاكتات (LDH) والكلور (Cl^-) والزنك (Zn) والنحاس (Zn) والبروتين الكلي (TP) ومستقبلات الترانسفيرين القابلة للذوبان (sTfR) وفيريتين) في مصل الدم , وتم دراستها من حيث الجنس ومجاميع كليه وكذلك من ناحية العمر .

اظهرت النتائج زيادة معنوية ($P<0.005$) في مستوى (Cl^-,LDH ,TP, sTfR, Cu, وفيريتين) لدى مرضى الثلاسيميا الكبرى مقارنة بالأصحاء, وكذلك زيادة في المستوى لدى الذكور مقارنة بالإناث بالإضافة الى تأثير العمر حيث لوحظ ارتفاع واضح في المستوى ($P<0.005$) لدى مرضى الثلاسيميا مقارنة مع الأفراد الاصغر سنا. اما عنصر الزنك (Zn) كان منخفض لدى مرضى الثلاسيميا بشكل ملحوظ مقارنة بالأصحاء وكذلك كانت مستوياته منخفضة عند الإناث مقارنة بالذكور ومع تقدم مرضى الثلاسيميا في السن ينخفض تركيز الزنك في مصل الدم .

اما بالنسبة لتحليل العدّ الدمويّ الشامل (CBC) فقد تم دراسة هذه العوامل (RBC, HCT, HGB, MCV, #MCH, MCHC, RDW-SD, RDWCV, WBC, LYM%, LYM) وقد وجد انخفاض واضح احصائيا ($P>0.005$) في مستوى كل من (RBC, HCT, HGB, MCH, MCV) مقارنة لدى الاصحاء ، بالمقابل وجد هناك زيادة في مستويات كل من (MCHC, RDW-SD, RDWCV, WBC, #LYM%, and LYM) لدى المرضى مقارنة بالأشخاص الاصحاء . اما من ناحية الجنس فقد وجد ان هذه العوامل (RBC, HCT, HGB, MCH, MCHC, RDW-SD, RDWCV, WBC,LYM%,

#LYM) في النساء المصابات بالثلاسيميا كانت مرتفعة مقارنه بالذكور المرضى على نقيض من عامل(MCV)الذي وجد منخفض لدى النساء المصابات بالمرض .

وفقا للعمر فقد ارتفع كل من (#RBC, HCT, HGB, RDWCV, WBC, LYM%, and LYM) مع تقدم العمر على عكس من (MCVوMCH) الذي لوحظ انخفاض مستواهم مع تقدم العمر، اما بالنسبة لمستويات كل من (MCHCوRDW-SD) فقد لوحظ انخفاضه مع تقدم العمر لدى المرضى الذكور على عكس الإناث من نفس النوع .

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِير)

صدق الله العلي العظيم

سوره المجادلة اية 11

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة ميسان
كلية العلوم
قسم الكيمياء

قياس بعض الدوال البايو كيميائية المختلفة لمرضى ثلاسيميا بيتا الكبرى في ميسان

رسالة مقدمة الى

كلية العلوم/ جامعة ميسان جزء من متطلبات نيل

شهادة الماجستير في الكيمياء

من الطالبة

كوثر حكيم جاسم الساعدي

بكالوريوس علوم كيمياء /جامعة ميسان (2017)

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