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Clinical Study for β-Thalassemia Major Patients through Bone Morphogenetic Protein2 (BMP2), Interleukin8 (IL-8) and Oxidant-Antioxidant Status

A thesis

Submitted to the Council of College of the Science University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biochemistry

By

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

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صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

CERTIFICATION

I certify that this thesis was prepared under my supervision at the department of Chemistry, College of Science, University of Babylon, in partial requirements for the Degree of Master of Science in Biochemistry and this work has never been published anywhere.

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Dedication

To my refuge from a hardship ... my mother.

To the pure heart ... my father.

To whoever supports and encourages me ... my beloved wife ... Meshkat.

To the apple of my eyes and the fruit of my heart ... my daughter Masa.

To my dear brothers and sister ... Ameer, Haider, Zahraa, Hassan.

I dedicate this humble effort to you, my dear ones.

Mohammed

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Summary

Thalassemia causes poor hemoglobin synthesis. Thalassemia is caused by a DNA mutation in hemoglobin-producing cells, which is inherited. This disorder needs regular blood transfusions to replenish patients' blood volume. This causes a buildup of iron in their bodies, which increases oxidative stress and causes osteoporosis, an enlarged spleen, irregular pulse, liver illness, and delayed development.

This study describes some precise immunological parameters which are: Bone Morphogenetic Protein2 (BMP-2), is a protein composed of two polypeptide chains containing 114 amino acids linked by a single disulfide bond. It induces bone and cartilage formation. It plays a key role in osteoblast differentiation. Also describes Interleukin8 (IL-8). It has anti-inflammatory activity, which has been established in various models of infection, and inflammation. A variety of medical subspecialties are now using interleukin-8, a promising marker for a wide range of clinical diseases.

On the other hand describes the changes that occur to the most important oxidative factors in the body, which are: the determination of Total Oxidant Status (TOS) and determination of Malondialdehyde (MDA) levels. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. The total oxidant status (TOS), is used to determine the overall level of oxidation that has occurred inside the body.

Also describes the most important antioxidant factors in the body, which are: the determination of the Total Antioxidant Capacity Assay (TAC), determination of Tocopherol (Vitamin E) Levels, and determination of Glutathione (GSH) Levels.

Ninety people were taken for the study, their ages ranged from (5-20 years). The samples were taken for patients groups in Thalassemia Center, Al-Zahraa Teaching Hospital for Maternity and Children, Najaf Governorate, Iraq, for those who visited the Thalassemia Center from September to December 2021, when the study was done. And samples for control groups were taken from volunteers during the same period. They are categorized into groups. (PG1) Thirty male patients, (PG2) thirty female patients, (CG1) fifteen male controls, and (CG2) fifteen female controls.

When compared to the levels of bone morphogenetic protein2 (BMP-2) found in the control group (1.88 ± 1.04), there was a statistically significant increase detected in the levels of bone morphogenetic protein2 (BMP-2) found in the group of patients (7.04 ± 4.40), ($p<0.05$). This morale rise indicates that this protein performs its aforementioned function.

The levels of interleukin-8 (IL-8) were found to be significantly higher in the group of patients (348.8 ± 174.1) when compared to the levels reported in the control group (348.8 ± 174.1), with a significance level of ($p<0.05$). This indicates that this interleukin rises as an anti-inflammatory.

A significant increase was found for the group of patients in the levels of total oxidative stress (TOS) (2.85 ± 0.67) relative to the control group (2.85 ± 0.67), where ($p<0.05$). A significant increase was found for the group of patients in the levels of Malondialdehyde (MDA) (3.35 ± 0.60) relative to the control group (1.05 ± 0.12), where ($p<0.05$).

On the other hand, there was a significant decrease in the levels of total antioxidant capacity Assay (TAC) (0.81 ± 0.32), relative to the control group (2.98 ± 1.08), where ($p<0.05$). And tocopherol (Vitamin E) (8.42 ± 1.05), relative to the control group (12.69 ± 1.27), where ($p<0.05$). While glutathione (GSH) (1.61 ± 0.23), in the patients as compared to the levels in the control groups (3.18 ± 0.27), where ($p<0.05$). Oxidative stress is the culprit in all of this.

On the other hand, the patients themselves were divided into two other groups: (the splenectomy group) which numbered twenty and the (no-splenectomy) which numbered forty. It was noticed that there is a significant difference between them for all parameters that were measured in this study, where ($p<0.05$).

All that happened is due to oxidative stress caused by periodic blood transfusions, which in turn causes iron accumulation in body tissues and complications.

The results showed a correlation between the studied parameters. This leads to the possibility of predicting any of these parameters with the knowledge of the other.

Contents

List of Figures

List of Tables

List of Abbreviation	
Abbreviation	**The Meaning**
ACS	Absorbable collagen sponge
ALAS	Aminolevulinate synthase
BMPs	Bone morphogenetic proteins
BMP-2	Bone morphogenetic protein-2
CBC	Complete blood count
Cp	Ceruloplasmin
DcytB	Duodenal cytochrome B
DFO	Deferoxamine
DFP	Deferiprone
DFX	Deferasirox
DMT1	Divalent metal transporter 1
DNA	Deoxyribonucleic acid
DTNB	5,5-dithio-bis-(2-nitro benzoic acid)
EDTA	Ethylene diamine tetraacetic acid
ELISA	The enzyme-linked immunosorbent assay
EPL	Erythrophagolysomes
FPN1	Ferroportin 1
GP	Glutathione peroxidase
GSH	Glutathione
GSSG	Glutathione disulfide
Hb	Hemoglobin
HbA	Adult hemoglobin
HbA2	Minor adult hemoglobin
HbF	Fetal hemoglobin
HCP1	Heme carrier protein 1
Heph	Hephaestin
Hox	Heme oxygenase-1
HPG	Haptoglobin
HPx	Hemopexin
HPx-Heme	Hemopexin-bound heme
HRP	Horseradish peroxidase
IFN	Interferons

ILs	Interleukins
IL-8	Interleukin8
IRE	Iron-responsive element
IRP1	Regulatory proteins1
IRP2	Regulatory proteins2
MDA	Malondialdehyde
MSC	Mesenchymal stem cell
NADPH	Nicotinamide adenine dinucleotide phosphate
Nc	Neucuproine (2,9- dimethyl- 1,1-phenathroline)
NTBI	Haptoglobin
OD	Optical Density
RBCs	Red blood cells
RES	Reticuloendothelial system
ROS	Reactive oxygen species
RNA	Ribonucleic acid
SPSS	Statistical Package for the Social Sciences
SMADs	Small mothers against decapentaplegic
STEAP3	Six-transmembrane epithelial antigen of the prostate3
TAC	Total antioxidant capacity assay
TBA	ThioBarbituric acid
TBARS	ThioBarbituric acid reactive substance
TCA	Trichloroacetic acid
TGF	Transforming growth factor
Tf	Transferrin
TSI	Transferrin saturation
TOS	Total oxidant status
VLDL	Very Low Density Lipoprotein

1. Introduction

1.1. Hemoglobin

Erythrocytes, or RBCs, are the cells of the blood that contain the complex aqueous solution of hemoglobin, non-hemoglobin proteins, lipids and glucose with other elements [1]. Hemoglobin is the main part of the cytoplasm of red blood cells. It makes up about 90% of the dry-weight elegant mature cell. It has heme and globin in it [2]. Figure 1-1.

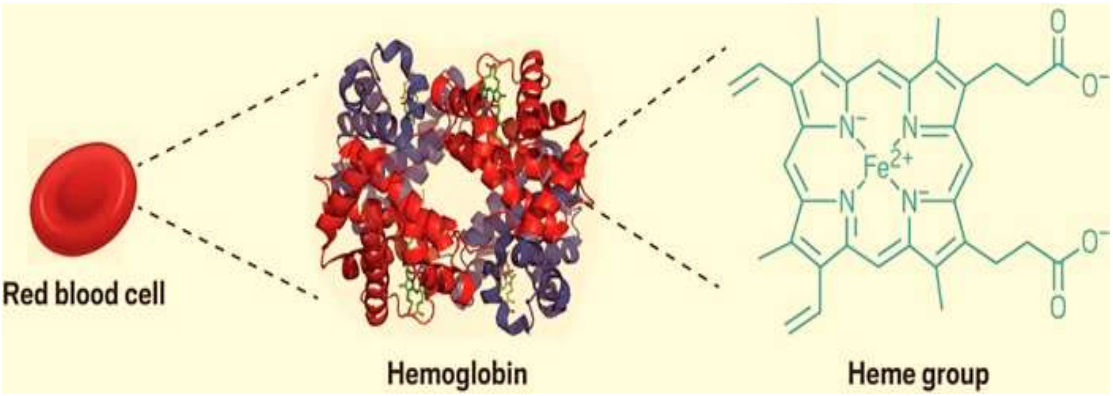


Fig. 1-1: Structure of Hemoglobin Protein [3].

Two β-globin polypeptides and two α-globin polypeptides, each containing a heme group, make form the heterotetramer hemoglobin A (HbA). This multisubunit protein transport oxygen from the lungs to the rest of the body by binding it cooperatively (one oxygen per heme molecule) [4]. The gene that tells the body how to make α-globin chains is on chromosome 16, while the gene that tells the body how to make β-globin chains is on chromosome 11 [2]. Figure 1-2.

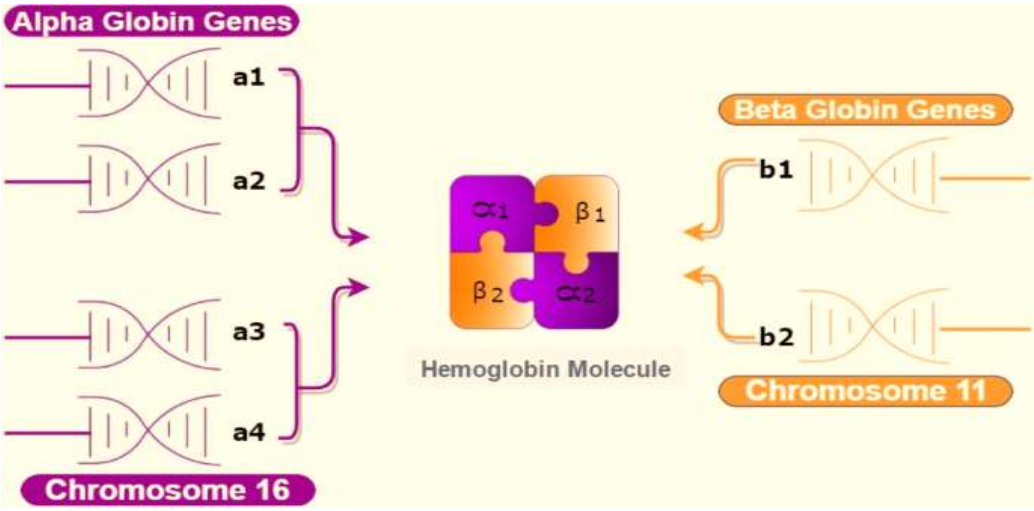


Fig. 1-2: composition of hemoglobin [5].

1.2. Degradation and Remodeling of Hemoglobin

Heme is a porphyrins that has iron in it and is needed for most biochemical processes that involve molecules of oxygen [6]. Porphyrins are a group of organic compounds called heterocyclic macrocycles. They consist of four altered pyrrole subunits linked by methane bridges at their carbon atoms [7]. Figure 1-1.

Hemolysis is the release of "free" hemoglobin and the subsequent breakdown of hemoglobin into bile pigments and their derivatives during phagocytic processes, which occur mostly in the liver and spleen. About 120 days after the erythrocyte is produced, this happens in humans on average [8]. Bilirubin is produced when hemoglobin is broken down in the reticuloendothelial system; the liver, spleen, and bone marrow all have enzymes that can convert bilirubin into its conjugate form, hence most bilirubin in the bloodstream originates from these three organs [9].

A result of heme degradation is bilirubin (part of hemoglobin, myoglobin, cytochromes, peroxidase, etc.). The main source of bilirubin is hemoglobin in RBCs. Heme catabolism is a process that involves the reticuloendothelial system (spleen and liver) [10].

Then, in the pro-erythroblasts, the synthesis of hemoglobin begins and continues until the red blood cells reach the reticulocyte stage. Reticulocytes continue to produce modest quantities of hemoglobin for another day or two after they leave the bone marrow and enter the circulation, maturing into erythrocytes [11]. Figure 1-3.

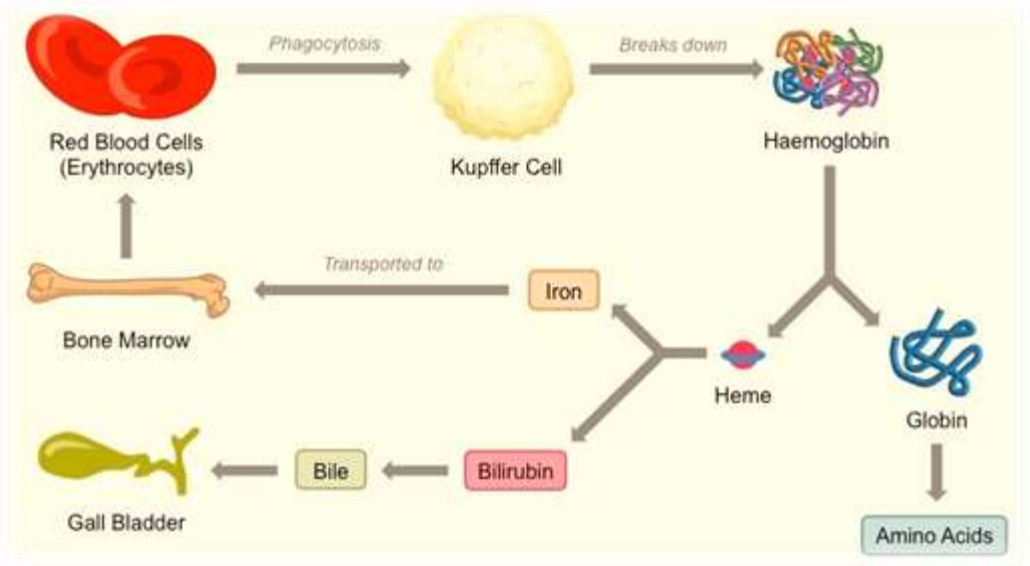


Fig. 1-3: Process of Erythrocyte and Hemoglobin Recycling [12].

1.3. Hemoglobin Types

Hemoglobin is a protein that is important for delivering oxygen from the lungs to the tissues throughout the body. Each red blood cell contains around 300,000,000 molecules of hemoglobin. Hemoglobin molecules are composed of four globin chains, each of which is made up of two identical sub-units. and split into two categories [13]:

- Two alpha-chains (α) consisting of 141 different amino acids [14], the cluster of alpha globin, which is composed of both the zeta (ζ) and alpha (α) globin chains [13].

- Two beta-chains (β) consisting of 146 different amino acids [14], the globin cluster denoted by the letter, which includes the globin chains denoted epsilon (ε), gamma (γ), delta (δ), and beta (β) [13].

In order to meet each group's unique oxygen needs, different hemoglobins are produced in the embryo, fetal and adult. They are all tetrameric, with one heme molecule and two distinct pairs of globin chains connected to each other [15].

1- "**Embryonic**" hemoglobins, which can be found from the third to the tenth week of pregnancy and represent ζ2ε2 (Hb Gower 1), α2ε2 (Hb Gower 2), ζ2γ2 (Hb Portland 1); and ζ2β2 tetramers (Hb Portland 2).
2- "**Fetal**" hemoglobin (HbF), in which it plays a major role as a transporter of oxygen during pregnancy and is a α2γ2 molecule.
3- "**Adult**" hemoglobin (HbA α2β2), which takes the place of (HbF) soon after birth.
4- **A minor adult component**, (HbA_2 α2δ2) [16].

Even though (Hb A_2) isn't important to an adult's health, its level may go up if the alpha or beta chains get shorter[15].

The specific types of hemoglobin were made, but "hemoglobin switching," a term that refers to how ethnic groups change over time, is known to have ended. About 97-98% of an adult's red blood cells are made up of (Hb A), and 2% to 3% are made up of (Hb A_2) or traces of it (Hb F) [16]. Figure (1-4) and Table 1-1.

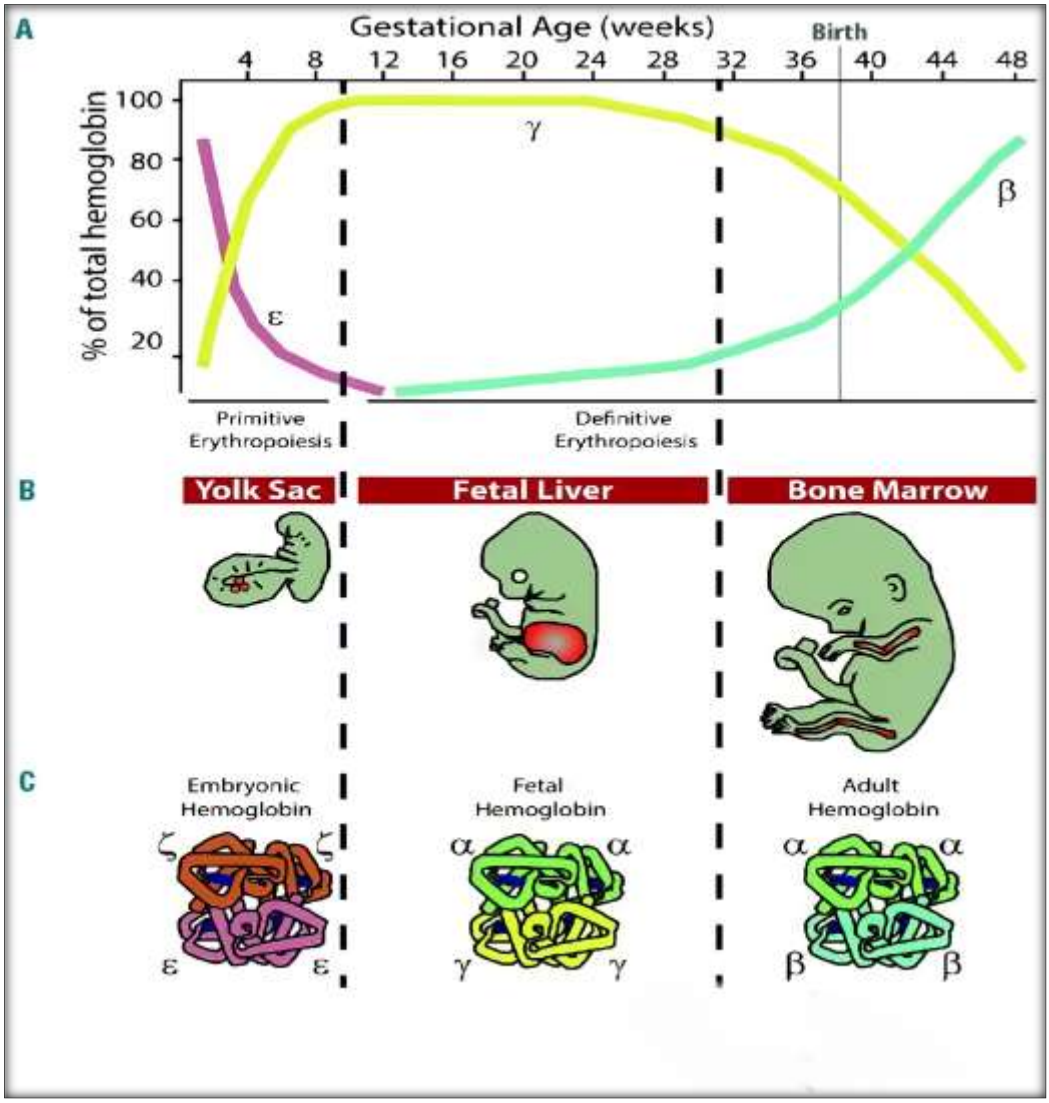


Fig. 1-4: Human erythropoiesis and developmental pattern of hemoglobin expression [17].

(A) Hemoglobin switching in man. Around week 6 of gestation, embryonic globin (ε) is silenced and fetal globin (γ) starts to be expressed. Perinatally the switch to adult globin (β) occurs. For the α-like globins, a single switch from the embryonic (ζ) to adult (α) globin occurs.

(B) Developmental hematopoietic sites. In the first 8 weeks of pregnancy, erythropoiesis begins in the yolk sac blood islands, then the fetal liver, eventually the bone marrow. The spleen is a temporary erythropoietic organ around birth.

(C) Structure of the main human hemoglobins expressed during development. Embryonic globin (ζ2 ε2 ; HbE Gower-1); fetal hemoglobin (α2 γ2 ; HbF) and adult hemoglobin (α2 β2 , HbA).

Table 1-1: comparison between the different types of hemoglobin [18].

Hemoglobin	Symbol	Structure	% of Normal Adult Hb
Embryonic hemoglobin	[Hb Gower 1]	ζ2 ε2	**~ 0 %**
	[Hb Gower 2]	α2 ε2	
	[Hb Portland 1]	ζ2 γ2	
	[Hb Portland 2]	ζ2 β2	
Fetal hemoglobin	[Hb F]	α2 γ2	< 1%
A minor adult hemoglobin	[Hb A2]	α2 δ2	~ 3 %
Adubloodstreamn	[Hb A]	α2 β2	> 96%

1.4. Thalassemia

The word "thalassemia" refers to a group of diseases in which one or more globin chains are made less or not at all. Beta thalassemia is caused by not making enough beta globin chains, which makes more alpha globin chains than normal. These extra alpha globin chains are not soluble and build up inside the red cell. This can cause a number of clinical symptoms [19].

First identified clinically in 1925 by Dr. Thomas Cooley, who reported anemia with microcytic erythrocytes as a condition. Then it became known as Cooley's anemia. Wipple and Bradford later dubbed this condition "Thalassemia." [20].

1.4.1. Thalassemia Classification

Reduced production of one or more globin chains causes thalassemia. The most significant categories are those that influence either alpha or beta chain synthesis, and they include the following [19]:

1.4.1.1. Alpha Thalassemia

A clinical phenotype of alpha-thalassemia may range from nearly asymptomatic to having fatal hemolytic anemia, and it is passed as an autosomal recessive illness with microcytic hypochromic anemia as one of its main features [21]. The alpha globin genes, which encode the alpha globin chains, are duplicated and located on chromosome 16 [22]. There are several forms of alpha thalassemia. The most common forms are:

1- Silent Carrier Alpha Thalassemia:

(Low Bart's): If only one alpha gene is affected, the other three genes can almost completely make up for it, and only a low level of Bart's is found [23]. Except for rare low red blood cell indices, patients are hematologically healthy [19].

2- Alpha Thalassemia Trait:

People with this trait have two functional copies of the alpha globin gene. Normal blood tests will reveal that their red blood cells are small, and they may also have a mild form of anemia. There is no need for treatment [24].

3- Hb H Disease:

Hemoglobin H illness is caused by abnormalities in three of the four alpha genes that are present in persons who are healthy. HbH is compatible with life and often manifests itself in a manner that is similar to that of thalassemia intermedia [25]. Patients often exhibit severe anemia, splenomegaly, icterus, and abnormal red blood cell indices when they are first diagnosed with this condition [19].

4- Alpha Thalassemia Major:

Hemoglobin Bart's, also known as "Hydrops fetalis," is a disorder in which no alpha genes are present in the patient's DNA, leading to the creation of hemoglobin with four gamma-globin chains by the developing fetus [14]. Unless an intrauterine blood transfusion is performed, the baby will likely die soon after birth due to hydrops fetal [19].

1.4.1.2. Beta Thalassemia

Beta-thalassemia is a set of inherited blood illnesses defined by abnormalities in the synthesis of hemoglobin's beta chains, resulting in a wide range of phenotypes from severe anemia to clinically asymptomatic persons. Beta-thalassemia is caused by mutations or deletions in the beta globin gene on chromosome 11, resulting in reduced or absent hemoglobin beta chains [22]. There are several forms of beta thalassemia:

1- Silent carrier Beta Thalassemia or Beta Thalassemia Minor:

This kind of beta thalassemia is caused by a defect in one of the beta-globin genes, but it does not often interfere significantly with the hemoglobin protein's ability to perform its normal functions [14]. Those who have the thalassemia minor gene are mostly clinically asymptomatic, however they may sometimes experience moderate anemia [26].

2- Beta Thalassemia Trait or Beta Thalassemia Intermediate:

Patients with this syndrome have two faulty genes yet are still able to produce some beta-globin. disease characterized by severe anemia, fatigue, weakness, shortness of breath, bone deformities, moderate jaundice, and an enlarged spleen due to a lack of beta polypeptide in the hemoglobin [14].

3- Beta Thalassemia Major

A person is diagnosed with beta thalassemia major, commonly known as Cooley anemia, if the synthesis from both genes is significantly impaired or missing altogether. Because HbF is present at birth, people who have beta thalassemia major usually never have any symptoms when they are first born. However, by the age of six months, symptoms typically start to appear [27]. In untreated patients, this illness is marked by transfusion-dependent anemia, large splenomegaly, bone abnormalities, growth retardation, and a distinctive facial appearance [19].

From the above, a summary can be made to compare the aforementioned beta thalassemia types. Table 1-2.

Table 1-2: Brief comparison of beta-thalassemia types [28]**.**

Beta Thalassemia Major	Beta Thalassemia intermedia	Beta Thalassemia Minor
• It occurs when there is a mutation in both beta globin genes. • The level of hemoglobin in the blood drops to less than 7 g per 100 ml. • The patient needs a periodic blood transfusion every 2-4 weeks.	• It occurs when there is a mutation in both beta globin genes. • The level of hemoglobin in the blood decreases to from 7 to 10 g per 100 ml. • The patient may need a blood transfusion, but not periodically.	• It occurs when there is a mutation in one beta globin gene. • The percentage of hemoglobin in the blood ranges from 10 to 12 g per 100 ml. • The patient does not need a blood transfusion.

1.4.2. Epidemiology

The average incidence rate of beta-thalassemia at birth for Mediterranean nations is 31.0 per 100,000 infants. However, the distribution of health services and monitoring tools, as well as the epidemiological statistics, differ significantly across nations [29].

Most people with alpha thalassemia syndrome come from Southeast Asia or China, while African Americans are not immune. Beta thalassemia typically affects people of Mediterranean heritage (Italian, Greek) and, to a lesser degree, Chinese, other Asians, and blacks [19].

1.5. The Importance of Iron in the Human Body

Iron is one of the essential elements required by the human body for metabolism [30]. Iron has various biological functions. About 65-80% of the body's iron is in hemoglobin. Hemoglobin distributes oxygen to human tissues. Myoglobin, which oxygenates muscle cells, needs iron. Iron contributes to energy-producing bodily responses [31].

The storing iron molecules ferritin and hemosiderin are mostly found in the reticulo-endothelial system. Ferritin, a protein, comprises an outer shell and an interior core that holds the iron deposited. This protein's life cycle is short a few days, resulting in constant breakdown and resynthesize of an easily available iron pool [30]. Ferritin is a protein-iron complex with a protein cage and a mineral core comprising thousands of ferric ions [32].

Despite being the most prevalent metal on earth, live cells only contain minimal amounts of iron. All of the human body's cells need iron to function. To create energy, every cell in the body requires iron [33].

1.6. Iron Absorption

Heme iron and non-heme iron are the two types of edible iron that may be consumed by humans. Heme iron is the more common kind. The non-heme iron originates from plant-based sources, while heme iron comes

from animal sources. Heme iron has a faster absorption rate and is absorbed in a different way than non-heme iron [34].

Compared to animal-based sources, plant-based sources of iron provide a broader variety of possible health advantages. The chemical structure of non-heme iron in food has a significant impact on how bioavailable iron is in the diet [32].

The level of iron in the body, the kind of food consumed, the consumption of vitamin C, and other dietary components all have an impact on iron absorption. People who don't have enough iron reserves will absorb more iron than those who do. This is the body's attempt to defend itself against iron toxicity while maintaining proper quantities of iron [31].

There is absolutely no iron absorption that takes place in the mouth, esophagus, or stomach. On the other hand, the stomach does produce some hydrochloric acid. This not only assists in the removal of iron that is attached to proteins via the process of protein denaturation, but it also assists in the solubilization of iron through the reduction of its ferric state to its ferrous state [35].

The duodenum absorbs iron from meals. Absorbable iron may be retained in enterocytes or circulated with liver-produced transferrin (Tf). It's absorbed by the tissues, where it's used for things like red blood cell creation in the bone marrow, myoglobin synthesis in the muscle, and oxidative metabolism in respiring cells [36].

1.7. Iron Homeostasis

Around 3500–4000 mg of iron are found in total in a 70 kg man, which equates to an average concentration of 50–60 mg of iron per kg of

body weight. The hemoglobin of erythrocytes contains the majority (2300 mg, or 65%) of the iron in the body. Myoglobin in muscle, enzymes, and cytochromes in other tissues each contain about a tenth of the body's total iron (350 mg). About 500 mg of the remaining iron is found in reticuloendothelial system (RES) macrophages, 200–1000 mg is stored as ferritin in hepatocytes, and 150 mg is found in the bone marrow [36].

1.8. Major Cells for Systemic Iron Homeostasis

Systemic iron homeostasis has been found to depend on four key cell types or tissues [37]:

1.8.1. Enterocyte:

The small intestine regulates iron absorption. Duodenal cells absorb dietary iron [16]. Hypoxia-inducible factor (HIF)-2alpha, a transcription factor regulated by oxygen and iron, plays a major role in regulating intestinal iron absorption by directly targeting the three main intestinal iron transporters: divalent metal transporter 1 (DMT1), duodenal cytochrome b (Dcytb), and ferroportin [21].

Ferric iron (Fe^{3+}) from food is reduced to ferrous iron (Fe^{2+}) by an apical ferric reductase called duodenal cytochrome b (DcytB). This reduced ferrous iron is then delivered into the enterocyte by an apical iron transporter called divalent metal transporter-1 (DMT1) [38].

In order for absorbed heme-bound iron to join elemental iron's pathway, cytosolic heme oxygenase must free the iron [39].

After having been transported into the cytoplasm by the divalent metal transporter 1 (DMT1), the iron is next processed by the iron exporter

ferroportin (FPN), which is located on the basolateral side of the enterocyte. Either the iron is stored in the cell as ferritin or it is exported from the cell via FPN [38].

After the iron is exported via the FPN, it is oxidized by the ferroxidases hephaestin and ceruloplasmin (Cp) to return to its ferrous condition. After that, the iron is loaded onto transferrin (Tf), which subsequently causes it to circulate throughout the body [40]. Figure 1-5.

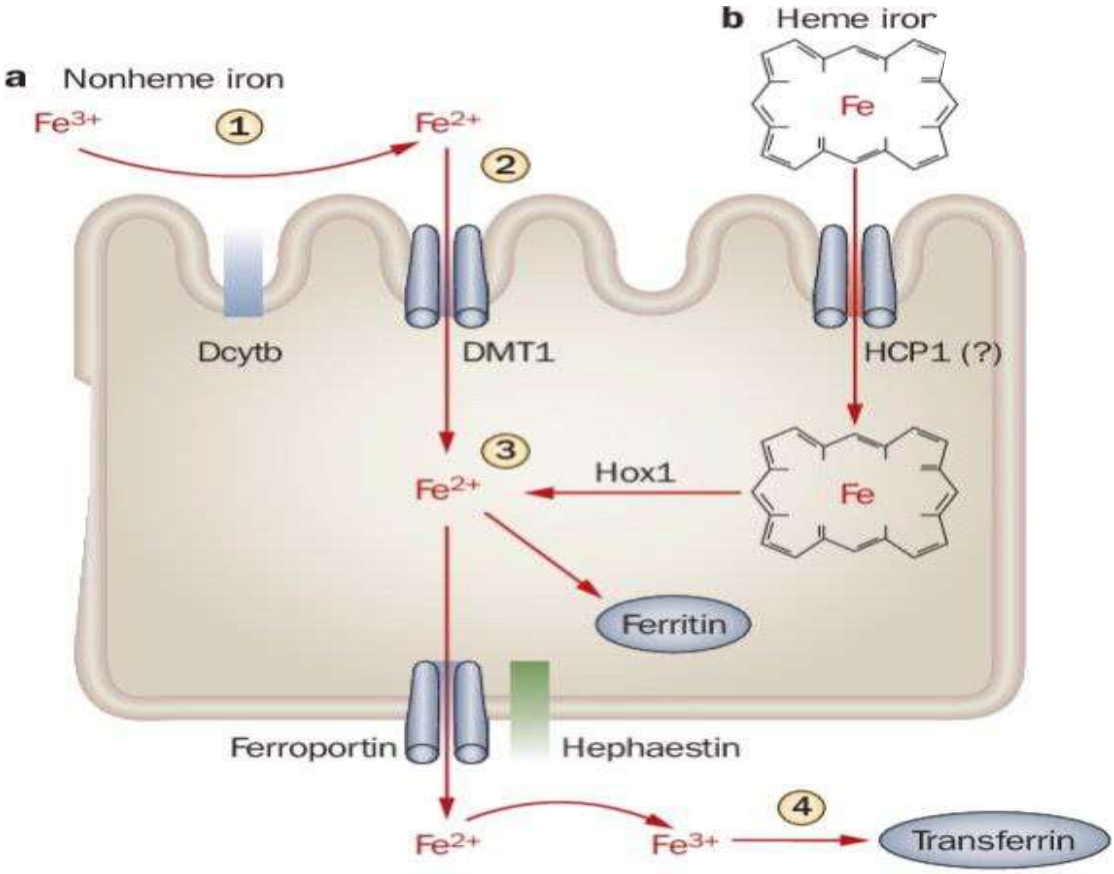


Fig 1-5: Mechanisms of duodenal absorption of heme and non-heme iron [41]**.**

(a) Non-heme iron absorption.

1- Non-heme iron is reduced from Fe^{3+} to Fe^{2+} either chemically (by gastric acid) or through the action of Dcytb.

2- Fe^{2+} is taken up into mucosal cells via DMT1.

3- Once inside the intestinal epithelial cell, iron either binds to ferritin or is directly released by means of ferroportin. Hephaestin oxidizes Fe^{2+} to Fe^{3+}.

4- Fe^{3+} binds to transferrin.

(b) Heme iron absorption. Heme iron is liberated from its porphyrin framework by Hox1 and enters a common pathway with non-heme iron.

Abbreviations: (HCP1): Haem carrier protein 1. (Hox): Heme oxygenase-1 (HO-1), it is responsible for the oxidative cleavage of heme groups leading to the generation of biliverdin, carbon monoxide, and release of ferrous iron. (DMT1): divalent metal transporter 1, DMT1 is a member of the Nramp (natural resistance associated macrophage protein) family of membrane-bound divalent cation transporters. (Dcytp): Duodenal cytochrome B.

1.8.2. Erythroblast:

It is possible that hemoglobin contains around two thirds of the body's entire iron concentration. Because of this, and due to the fact that erythropoiesis occurs in the bone marrow, the bone marrow is the major tissue in the body that utilizes iron[36]. Erythroblasts derive from hematopoietic stem cells (HSCs), which are the primary source of these cells [42].

Transferrin-bound iron, also known as TBI, is taken up by the cell via a process known as receptor-mediated endocytosis. In the beginning, iron-bound transferrin will bind to TfR1 at the cell membrane. This will result in the development of the iron-transferrin/transferrin receptor complex, also known as Fe_2-Tf-TfR1. iron erythroblast uptake is increased by increasing TfR production and reducing ferritin production, and vice versa [36].

Ascorbic acid is a reducing agent that has the capacity to both free iron from the protein ferritin and mobilize iron from the reticuloendothelial system into transferrin. Both of these processes are necessary for optimal iron metabolism [21].

Relapsed Fe^{3+} ions are reduced to Fe^{2+} by the STEAP3 protein (reductase six-transmembrane epithelial antigen of the prostate3). Ferrous iron is carried out of the endosome by DMT1 and stored in ferritin [36].

At the level of translation, iron regulatory protein (IRP) and iron-responsive element (IRE) both regulate iron absorption as well as iron transport and storage. Both IRP1 and IRP2 are RNA-binding proteins, and they interact with (IRE) to control the stability and translation of ferritin, Fpn, and transferrin receptor mRNA (TfR mRNA). iron erythroblast uptake is increased by increasing TfR production and reducing ferritin production, and vice versa [43].

Known as erythropoiesis, this is the process in which hematopoietic stem cells (HSCs) in the bone marrow proliferate and develop into a sequence of more confined myeloid progenitors, erythroid-restricted progenitors, and erythroid precursor cells. Erythrocytes are the cells that have reached the last stage of differentiation during the process of erythropoiesis [44]. Figure 1-6.

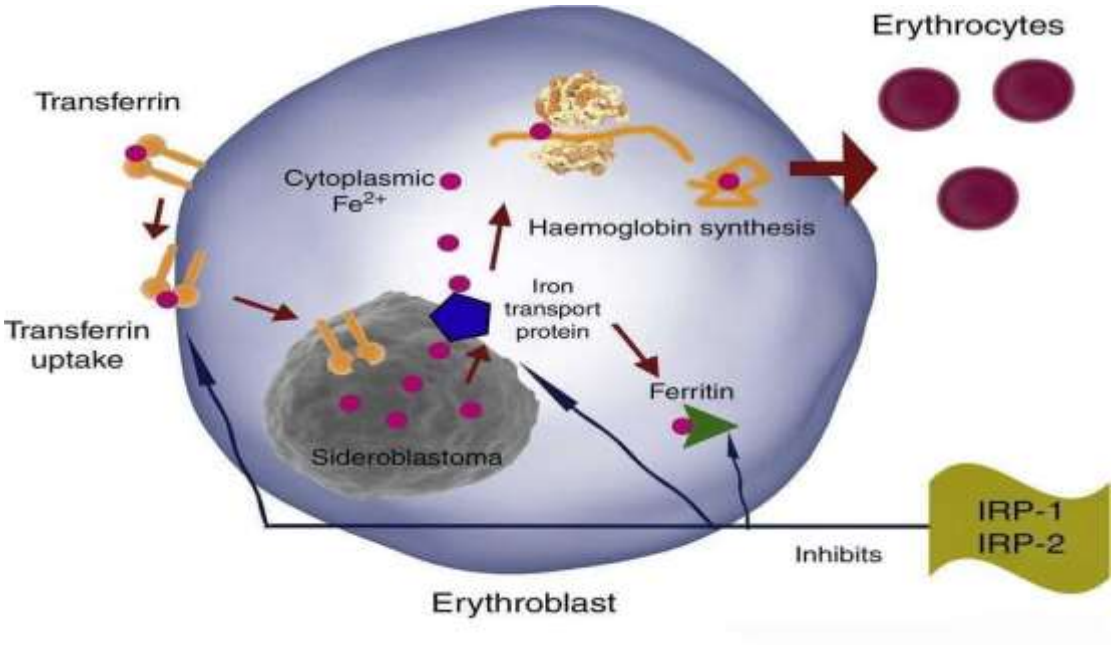


Fig. 1-6: Iron absorption and metabolism pathways in the erythroblast [45]**.** Iron receptor protein1: IRP-1, Iron receptor protein2: IRP-2, Transferrin: Tf.

1.8.3. Macrophage:

Every day, the body generates 200 billion new red blood cells, and the reticuloendothelial macrophages are responsible for removing an equal amount of those cells in order to keep the body's red cell mass in a balanced state and prevent conditions such as anemia and erythrocytosis [46].

The transferrin receptor increases the uptake of iron that has been coupled to transferrin (TfR or CD71). Direct acquisition of non-transferrin iron occurs through divalent metal transporter 1 (DMT-1), which is linked to cytochrome B in the duodenum (DcytB). DMT1 transports iron over the endosomal membrane and into the cytosol after it has been reduced by the endosomal reductase six-transmembrane epithelial antigen of the prostate 3 (STEAP3). The resulting intracellular free iron is then transferred to the labile iron pool (LIP), where it is either utilised by cellular processes or stored in ferritin. Ferroportin 1 (FPN1), which is assisted by the ferroxidase ceruloplasmin (Cp), allows iron to escape the cell. Iron is then integrated into transferrin to aid in its transport to the target cells [47]. Figure 1-7.

The process of preventing mitochondria and other organelles, proteins, and nucleic acids from oxidative damage depends on macrophages' antioxidant response, despite the fact that they create oxidants in a variety of ways. In macrophages, the flow that produces NADPH in the pentose phosphate pathway is more active. NADPH plays a key role in the synthesis of antioxidants like glutathione (GSH) and thioredoxin, which prevent oxidative damage, as well as oxidants like superoxide anion during the respiratory burst [46].

Plasma transferrin is the most efficient transporter of ferric iron, and erythropoiesis relies on transferrin-bound iron [48].

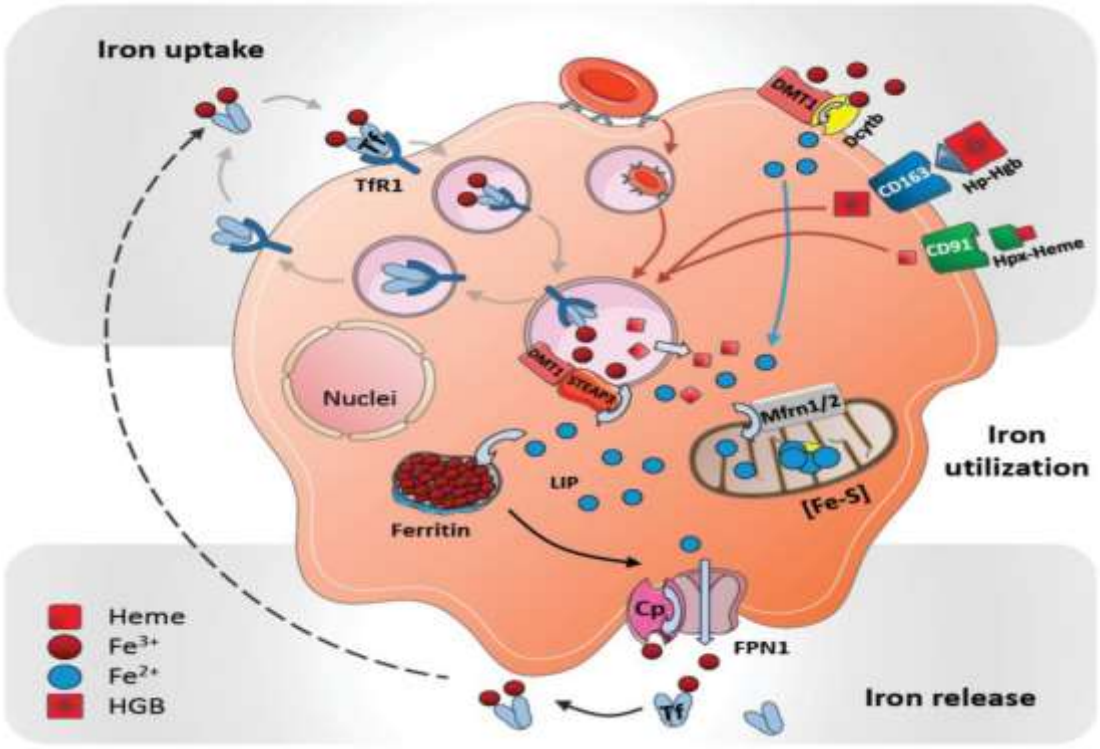


Fig. 1-7: Iron metabolism by macrophages[47]**.**

The transferrin receptors (TfR or CD71) absorb the iron that is coupled to transferrin (TfR or CD71). Transferrin receptor complex and empty apo-transferrin are recycled once again (grey arrows). Through the divalent metal transporter 1 (DMT-1), which is linked to duodenal cytochrome B (DcytB), non-transferrin iron may be directly absorbed (blue arrow). Hemopexin-bound heme (Hpx-Heme) iron is scavenged by CD91 (LRP1) receptors, whereas haptoglobin-hemoglobin (Hp-Hgb) complex is taken up by CD163 receptor (red arrows).

1.8.4. Hepatocyte:

Since the liver is responsible for the synthesis of a significant portion of the proteins that are involved in iron metabolism, it plays an essential and essential part in maintaining iron homeostasis. Most importantly, the liver is the primary location for the production of hepcidin, the primary regulator of iron homeostasis. Hepcidin is the primary hormone responsible for regulating iron homeostasis. It does this by bonding to FPN1, which leads to its ubiquitination [36].

Hepcidin activity relies on interactions with ferroportin. Hepcidin regulates ferroportin expression. Hepcidin binds to ferroportin, causing it to be internalized and destroyed in endolysosomes. When iron stores are large, increased hepcidin expression lowers intestinal iron absorption and macrophage iron release. Iron deficiency reduces hepcidin production [49]. Figure 1-8.

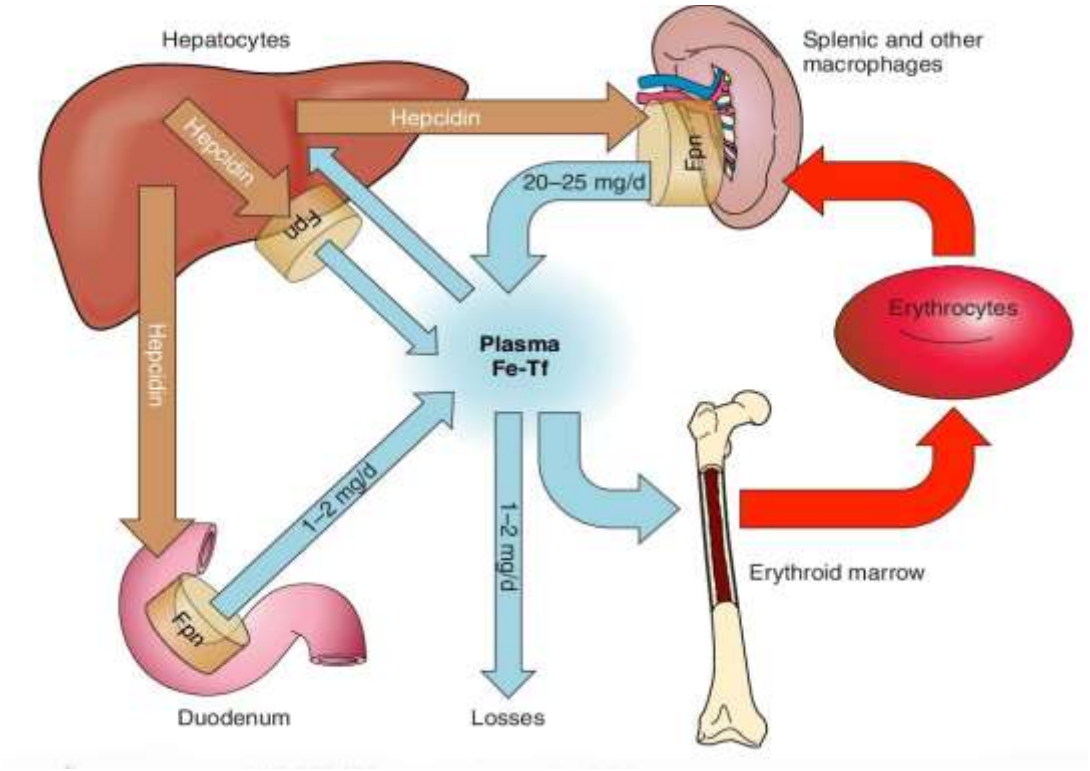


Fig. (1-8): The regulation of major iron flux by hepcidin and ferroportin [50]**.**

Iron found in transferrin is represented by the color blue, whereas iron found in erythrocytes is shown by the color red. Through activating the endocytosis and proteolysis of the iron exporter ferroportin, hepcidin is able to regulate the amount of iron that enters the plasma (brown).

The downregulation of ferroportin and intracellular iron retention in macrophages are effects of hepcidin-producing conditions. Conversely, a decrease in hepcidin leads to an increase in ferroportin-mediated export into the plasma [51].

Before hepcidin was discovered, the liver was considered the principal site of iron buildup and iron toxicity. This attitude continued after hepcidin's discovery. Since the liver is the major source of hepcidin, iron overload may be induced by the loss of hepcidin-producing hepatic mass and hereditary and acquired factors that impede hepcidin synthesis in the liver. Low-grade excess iron may exacerbate liver disease or other chronic illnesses through oxidative stress [52].

In general, enteroduodenal cells are the ones responsible for the absorption of iron from the food. This is important for maintaining iron homeostasis. Iron, once absorbed, is transported by the protein transferrin throughout the body, where it is taken up by various tissues for use. The reticuloendothelial system, which includes the macrophages in the spleen, is responsible for recycling the iron that is found in erythrocytes that have reached their end of life [36].

The creation, activity, and storage of protein are all under tightly coordinated regulation in order to maintain intracellular iron homeostasis. When there is not enough iron inside the cell, the cell must get extra iron from its store or from the plasma [35]. Figure 1-9.

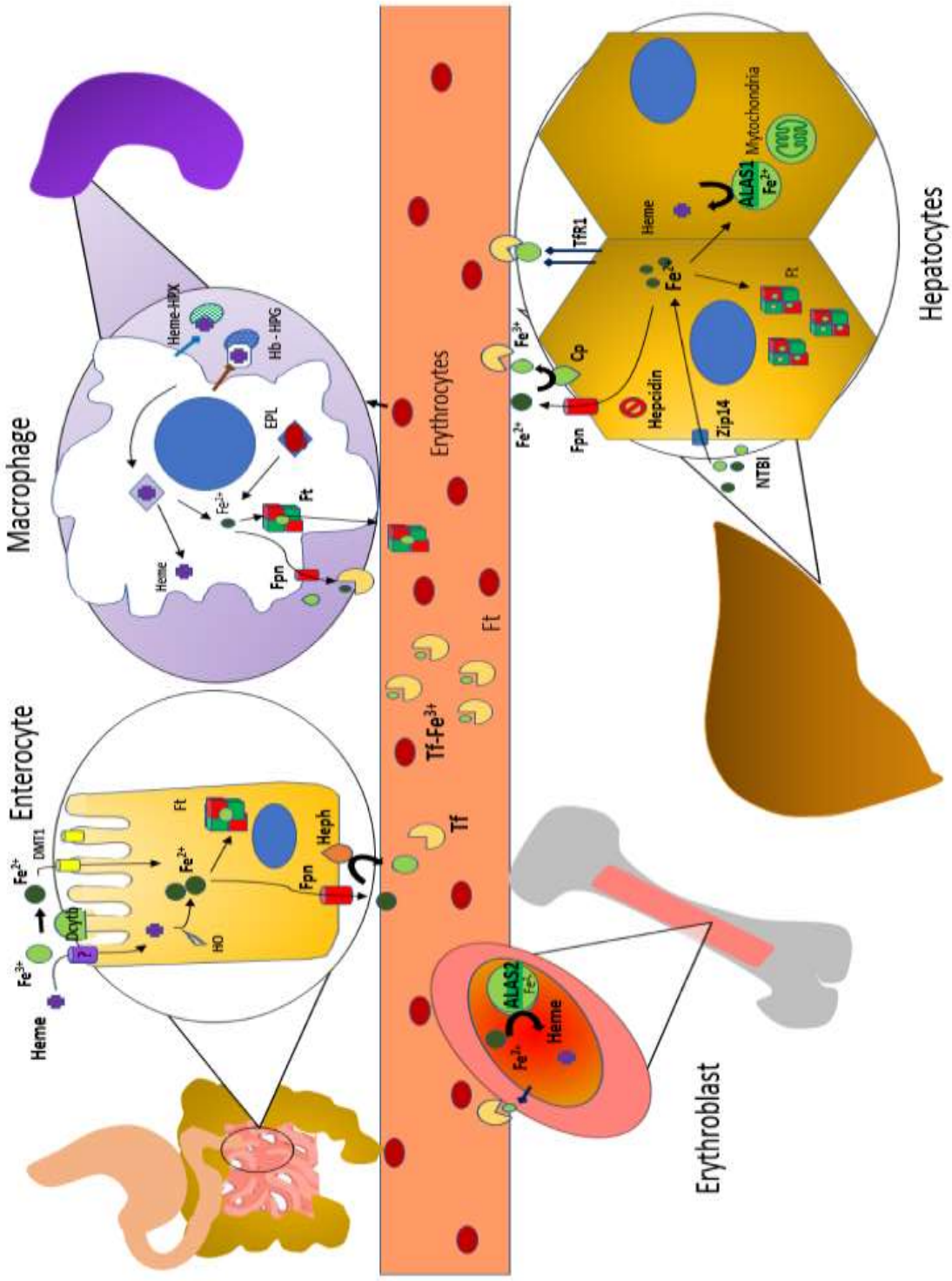


Fig. 1-9: Metabolism of Iron in the Human Body[53]**.**

Abbreviations: DMT-1, divalent metal transporter 1; Dcytb, duodenal cytochrome B; HO: heme oxygenase; Ft, ferritin; Fpn, ferroportin; Heph, hephaestin; Tf, transferrin; EPL, erythrophagolysomes; Cp, ceruloplasmin, ferroxidase activity; Hb, hemoglobin; HPX, hemopexin; HPG, haptoglobin; NTBI, non transferrin-bound iron; TfR1, transferrin receptor 1; ALAS, aminolevulinate synthase.

1.9. Iron Overload in Beta Thalassemia Major Patients

Iron overload is a frequent consequence of thalassemia syndromes and may cause organ damage and death [54]. Within a year of initiating frequent transfusions, iron deposition develops in tissue areas [55].

Most of the iron in the body is in the form of ferritin. Small amounts of ferritin are made by the body and put into the plasma. In the absence of inflammation, the amount of this plasma (or serum) ferritin is linked to the amount of iron in the body as a whole. Normal levels of ferritin depend on age and gender. Concentrations are high at birth, rise during the first two months of life, and then fall throughout later infancy [56].

Concentrations begin to grow once again at around one year of life and continue to do so all the way until adulthood[55]. Chronic blood transfusions cause iron overload because humans can't eliminate extra iron. Untreated iron excess causes morbidity and death[57]. Antioxidant capability is also shown to be reduced in these cases [58].

1.10. Haber–Weiss Reaction

The Haber–Weiss reaction is a kind of chemical reaction that results in the production of a hydroxyl radical ($\cdot OH$) from hydrogen peroxide (H_2O_2) and a peroxide radical ($\cdot O_2^-$). Because this event takes place inside of cells, we may assume that it is a source of oxidative stress. Iron serves as the catalyst for this very slow reaction.

- The first step involves **reducing the ferric ion to the ferrous ion**:

$$Fe^{3+} + \cdot O_2^- \rightarrow Fe^{2+} + O_2$$

- The second step is **the Fenton reaction**:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$$

The yield of reaction is (Haber–Weiss):

$$\cdot O_2^- + H_2O_2 \rightarrow OH^- + \cdot OH + O_2$$ [59].

Although this reaction may also be catalyzed by other transition metal ions, the iron-catalyzed Haber-Weiss reaction, which employs Fenton chemistry, is now thought to be the primary mechanism by which the extremely reactive hydroxyl radical is produced in biological systems [60].

1.11. The Oxidant and Antioxidant Status in β-Thalassemia Major Patients

Antioxidant substances, such as albumin, bilirubin, uric acid, vitamins C and E, glutathione , and antioxidant enzymes, such as superoxide dismutase or glutathione peroxidase, are shielding the cells from the potentially damaging effects of oxidant agents [61].

A thalassemia patient may have cardiac, endocrine, and liver dysfunctions. Causes include hemolysis and high iron[62]. In individuals with beta-thalassemia major, the status of iron overload as well as iron-induced oxidative stress has been examined on a number of occasions [63].

The word "oxidative stress" refers to a scenario in which the system of oxidants and antioxidants is out of balance, resulting in the development of more oxidizing agents and an imbalance in the number of reactive oxygen species (ROS) [64]. Figure 1-10.

Highly reactive molecules called reactive oxygen species are produced during the metabolism of oxygen. Radicals and non-radicals are both possible. These (ROS) include the well-known superoxide anion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$) [65].

ROS have physiological functions (e.g., cell signaling) and are routinely produced as byproducts of oxygen metabolism. Despite this, environmental stressors (e.g., UV, ionizing radiations) and xenobiotics (e.g., antiblastic medicines) substantially boost ROS production, resulting in an imbalance that causes cell and tissue damage (oxidative stress) [66]. Reactive oxygen species assist signaling. ROS may both benefit and harm signal transduction pathways [58].

Vitamin E is a fantastic free radical defense for the body since it is one of the most fat-soluble antioxidants, breaking chains and so protecting cell membranes from free radical damage [66].

The principal role of tocopherol is to scavenge the lipid peroxyl radical before it attacks the target lipid. Two peroxyl radicals are trapped for every tocopherol molecule oxidized. Tocopherol itself becomes a relatively stable free radical that does not propagate the radical chain [67]. Figure 1-11.

In addition, glutathione (GSH) is an antioxidant that is well-known for its capacity to lessen the effects of oxidative stress [67]. The oxidized form of glutathione is called glutathione disulfide (GSSG). When NADPH is present, the glutathione reductase converts it to GSH (GR). Hydrogen peroxide is converted to water by glutathione peroxidase (GP) [68]. Figure 1-12.

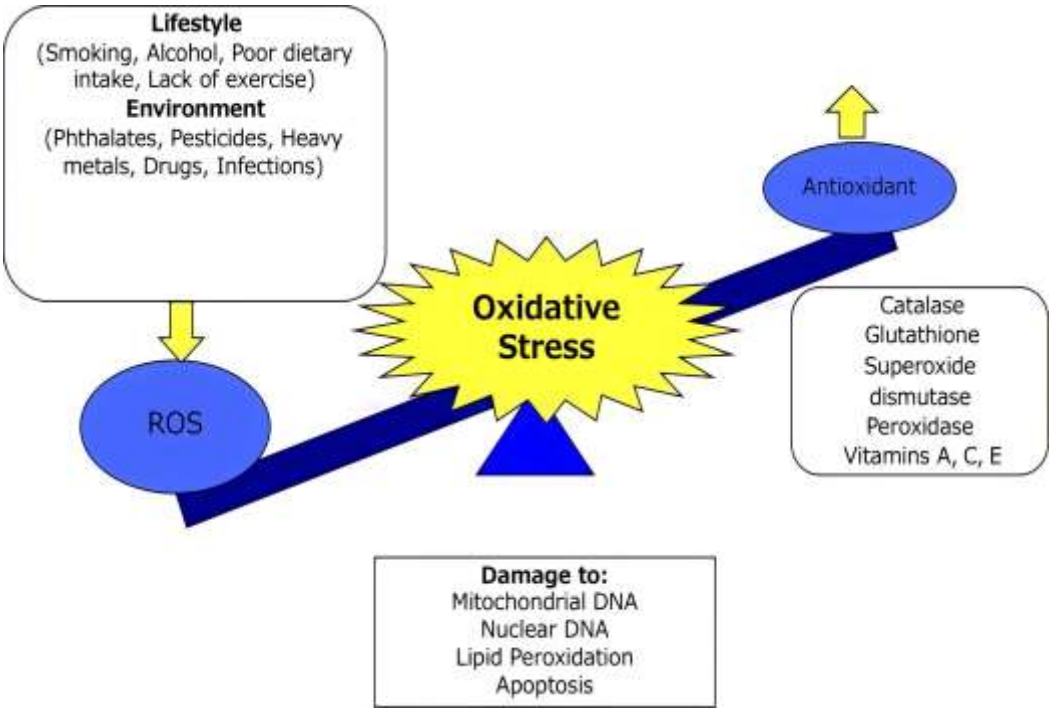


Fig. 1-10: Factors that can cause oxidative stress[69]**.**

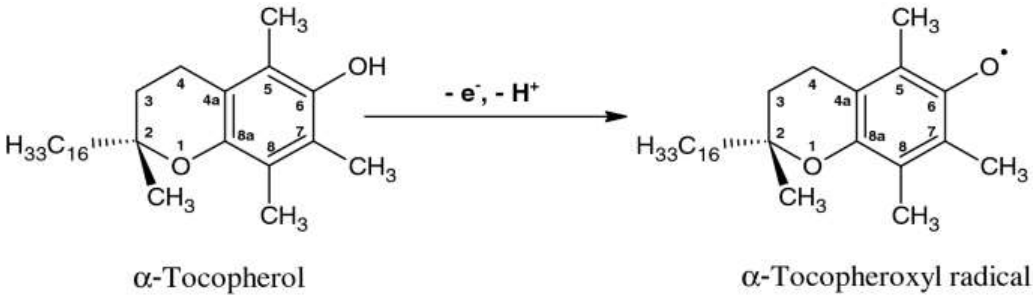


Fig. 1-11: Peroxyl trapping by tocopherol[70]**.**

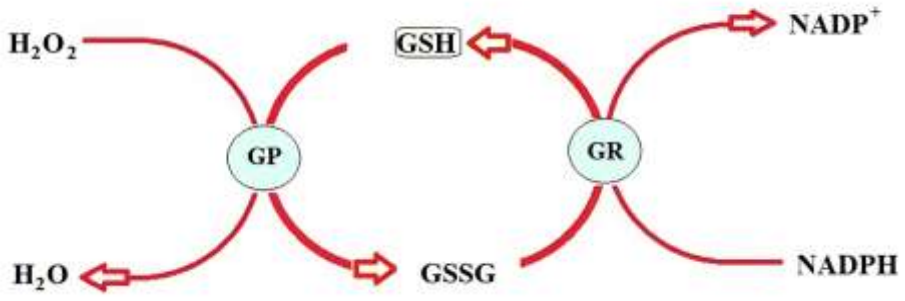


Fig. 1-12: Glutathione's redox cycle[71]**.**

1.12. Bone Morphogenetic Proteins (BMPs)

Bone Morphogenetic Proteins, or BMPs, are a class of potent and adaptable growth factors that were first identified in 1965 by Urist and colleagues3 as a subfamily of the TGF-β (transforming growth factor beta) superfamily [72]. There are few types of major subfamilies within this large family of proteins: the TGF/activating branch and the BMP/growth and differentiation factor branch [73].

Transforming growth factors are involved in a broad variety of biological processes, including those that are connected to the proliferation and differentiation of cells. In general, TGF-α promotes cell proliferation while TGF-β may stimulate or inhibit proliferation depending on the cell type and growth factor environment [74].

BMPs are growth factors and cytokines that promote bone and cartilage formation. At initially, seven proteins were discovered. Six of them, BMP-2 through BMP-7, are TGF proteins. Following then, 13 more BMPs were identified, bringing the total to 20 [60]. BMPs are involved in numerous cellular physiological processes. These include cell division, differentiation, growth inhibition, and maturation. Physiological processes are impacted by cellular surroundings [75].

The fact that all of the more than 30 growth factors identified to date signal by binding and hetero-oligomerization (a chemical process that converts monomers to macromolecular complexes through a finite degree of polymerization) of a very limited set of transmembrane serine-threonine kinase receptors, which can be classified into two subgroups termed type I and type II, is a distinguishing feature of the TGF protein family. There are just seven type I and five type II receptors for all 30 plus TGF members, indicating a high level of ligand-receptor promiscuity [76].

SMADs are proteins that are involved in the regulation of cell proliferation and differentiation. They are activated by the signaling of transforming growth factor (TGF- β), as well as BMPs [73].

The active type II receptors in the ligand-receptor complex are responsible for phosphorylating and activating the type I receptors. Type I receptors are responsible for the phosphorylation of the receptor-regulated SMADs (R-SMADs). TGF and activation signaling are engaged with SMAD2 and SMAD3, while BMP signaling is with SMAD1, SMAS5, and SMAD8. R-SMADs that have undergone phosphorylation are produced in a heterotrimeric complex with SMAD4, a special common partner SMADs. The complexes then go to the nucleus where they cooperate with other transcription factors and transcriptional coactivators to either activate or suppress gene expression [77]. Figure 1-13.

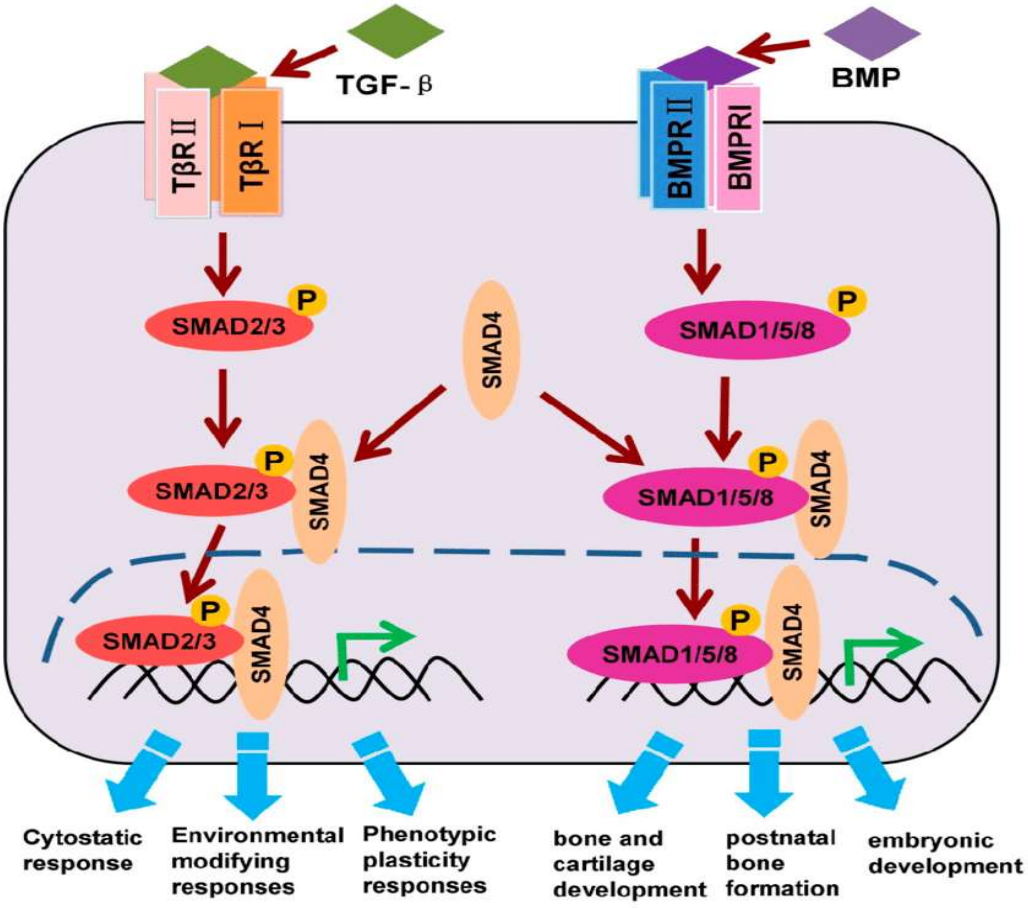


Fig. 1-13: Signals transduction of TGF-β and BMP [78].

1.12.1 Bone Morphogenetic Protein2 (BMP-2)

BMP2 protein was extracted from inclusion bodies, purified, and then altered using the redox method. One of the two osteoinductive growth factors now employed in medical devices to promote bone resorption is bone morphogenetic protein2 (BMP-2). The majority of the time, this protein is obtained from the sector in economic at absurdly expensive rates and in paltry amounts that fall short of therapeutic needs [79].

Bone morphogenetic protein-2 (BMP2) orchestrates the growth of osteoblasts and governs the survival, maturation, and activation of osteoclasts through type I (BMPR I) and type II (BMPR II) transmembrane receptors. When BMPs attach to BMPR II, this causes BMPR II to recruit BMPR I, which then phosphorylates BMPR I in its GS domain and activates it (GS domain consisting of multiple glycine and serine residues) [80].

It is important to note that BMP-2 is a potent osteogenic agent that promotes the differentiation of mesenchymal stem cells into fibroblasts and chondroblasts. Cell division, the activity of alkaline phosphatase (ALP), and the production of collagen are all boosted by its presence. Matrix (Gla) protein, which is found in high quantities in bone and cartilage, limits the potential of these tissues to induce mineralization [81].

By using a modified BMP-2 plasmid, it is possible to boost the transcription and translation of the BMP-2 protein (A plasmid is a circular DNA segment containing thousands of nitrogenous bases. Its primary function is to transmit genetic information to neighboring cells), which, in turn, leads to earlier and higher levels of BMP-2 protein production as well as increased calcium deposition. It is possible to increase the rate of protein synthesis as well as the amount of protein produced, as well as to expand

the capabilities of the gene-activated scaffold (it is materials that have been engineered to cause desirable cellular interactions to contribute to the formation of new functional tissues for medical purposes) [82].

The BMP-2 protein is a significant factor in all organs. BMP-2 and/or its signaling pathways are now being researched for their use in the development of potential therapies for osteoporosis, osteopenia, osteoarthritis, and coronary artery disease. To this day, the Food and Drug Administration (FDA) has only granted approval for the use of BMP-2 in cranioplasties, anterior lumbar interbody spinal fusions, and maxillofacial reconstructive surgery [83].

By changing one or more of the following parameters - the quantity of osteoblasts, their activity, the quantity of osteoclasts, their activity, or any combination of these parameters- it is possible to promote the formation of new bone tissue. Each mesenchymal stem cell (MSC) has the capacity to differentiate into different kinds of cells. One of the main growth factors that controls stem cells' fate is the bone morphogenetic protein2 (BMP2) [84]. Figure 1-14.

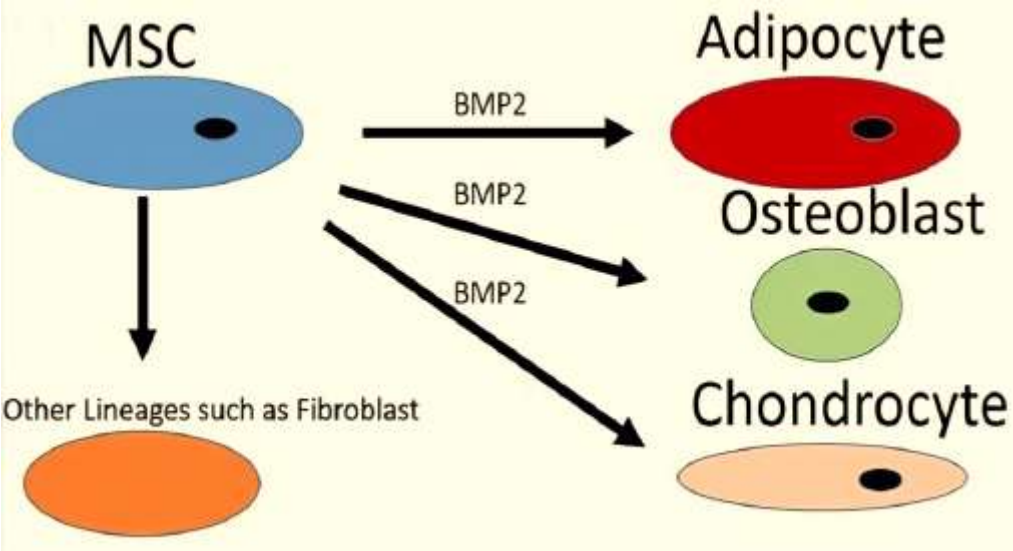


Fig. 1-14: The role of (BMP-2) with stem cells in building osteocyte and chondrocyte and adipocyte cells [84].

1.13. Cytokines

Cytokines are proteins or glycoproteins with a low molecular weight that exert their hormone-like effects at very low concentrations (pico molar and femto molar levels) by binding to receptors in a manner similar to that of hormones [85].

Cytokines may activate the immune system in a way that is not antigen-specific, thus it is important to control this process to prevent unwarranted reactions that might be harmful to the host's health [86].

Cytokines are categorize according to their type to:

- Interleukins; IL (IL-1, IL-2 and others).
- Interferons; IFN (IFN-α, β and γ).
- Tumor Necrosis Factors; TNF (TNF-α and TNF-β and others) [87].

1.14. Interleukins

Interleukins are a class of cytokines initially identified as being generated by leucocytes. Interleukins are one of the mechanisms through which leukocytes communicate with one another. Dr. Vern Paetkau from the University of Victoria coined the phrase for it in 1979. Interleukins (IL) have the ability to stimulate the development and differentiation of cells, in addition to their functional activation. Interleukins were discovered as a consequence of a study that was conducted to find a solution to the question of how various types of cells communicate with one another [88].

Since the discovery of monocyte IL (also referred to as IL-1) and lymphocyte IL (often referred to as IL-2), the effects of ILs have significantly grown; more than 40 cytokines are now labeled as ILs [89].

1.14.1. Interleukin 8 (IL-8)

During the inflammatory process, the chemotactic factor known as interleukin 8 (IL-8) is responsible for drawing in neutrophils, basophils, and T-cells. It is comparable to IL-6, but its half-life is much longer. IL-8 is a cytokine that is secreted by several cell types in response to inflammation. These cell types include monocytes, macrophages, neutrophils, as well as cells from the gut, kidney, placenta, and bone marrow. IL-8 plays a role in the activation of neutrophils. Mitogenesis, the suppression of angiogenesis, inflammation, chemotaxis, neutrophil degranulation, leukocyte activation, and calcium homeostasis are among processes that are influenced by IL-8 [90].

The nomenclature of human interleukin-8 (IL-8) has been changed to chemokine (C-X-C motif) ligand 8, and the gene symbol for it has been accepted as CXCL8. Its receptors alpha and beta are now designated as CXCR1 and CXCR2, respectively [91].

The causes of tissue damage that might result in inflammation include infection, trauma, neoplasm, and autoimmune disease, to name just a few. Different types of leukocytes are guided in their movement by chemokines, which are small molecules that bind with transmembrane receptors. Most inflammatory pathways depend on interleukin (IL)-8, a crucial chemokine. In non-pathological tissue, IL-8 levels are undetectable significantly; nevertheless, they are raised 10 to100 fold in response to pro-inflammatory cytokines such tumor necrosis factor (TNF) and interleukin-1 (IL-1), bacterial or viral virulence factors, and oxidative stress [92].

1.15. Thalassemia Treatment

1.15.1. Regular Blood Transfusions

Regular transfusions will be helpful if the kid is developing poorly, has facial or other bone deformities, and/or when Hb levels are less than 7 g/dL. Transfusion treatment must be administered concurrently with addressing any confounding conditions that might increase the severity of anemia, such as folic acid insufficiency, severe febrile illness, blood loss, or inherited glucose-6-phosphate dehydrogenase deficiency. Transfusions are given monthly throughout infancy and thereafter at intervals of two to four weeks when a pretransfusion Hb level of 9-10 g/dL is reached [93].

A blood transfusion is a potentially dangerous treatment that should be undertaken only when the therapeutic advantages exceed the hazards, which include acute hemolytic responses and infections transferred by blood transfusions. Certain steps must be taken to make sure that the right blood is given and that any bad reactions are dealt with quickly [94].

1.15.2. Chelation Therapy:

The purpose of this treatment is to eliminate the extra iron in the blood. The cumulative iron load is an unavoidable result of continued transfusion treatment when each RBC unit contains less than 200 mg of iron. The rate of transfusional and Gastrointestinal (GI) tract iron buildup in people with major and moderate thalassemia is typically 0.3-0.6 mg/kg per day [93].

Hepcidin is a protein that controls iron absorption from the GI tract and increases release of recycled iron from macrophages. Severe anemia and infectious endocarditis both down-regulate the synthesis of hepcidin, which results in an increased amount of iron being absorbed from the GI

tract. This can lead to an increased amount of iron being absorbed from the GI tract [95]. To date, there are 3 major classes of iron chelators:

- Hexadentate: ((deferoxamine [DFO], Desferal)), in which 1 atom of iron is bound to 1 DFO molecule. Figure 1-15.
- Bidentate: ((deferiprone, L1 [DFP], Kelfer)), in which 1 atom of iron is bound to 3 DFP molecules. Figure 1-16.
- Tridentate: ((deferasirox [DFX], Exjade)), in which 1 atom of iron is bound to 2 DFX molecules [96]. Figure 1-17.

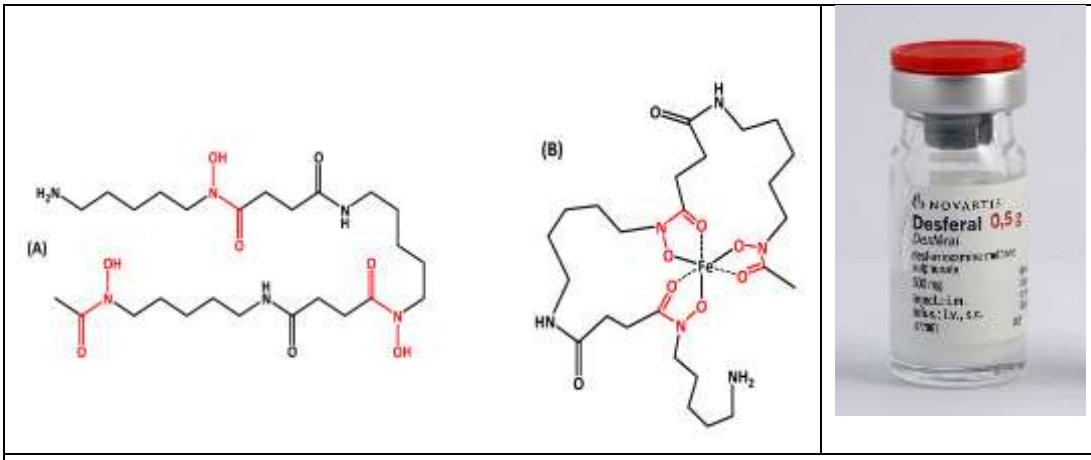


Fig. 1-15: Chemical structure of (A) deferoxamine , and (B) feroxamine. Hydroxamic groups, responsible for metal chelation, are evidenced in red [97]**.**

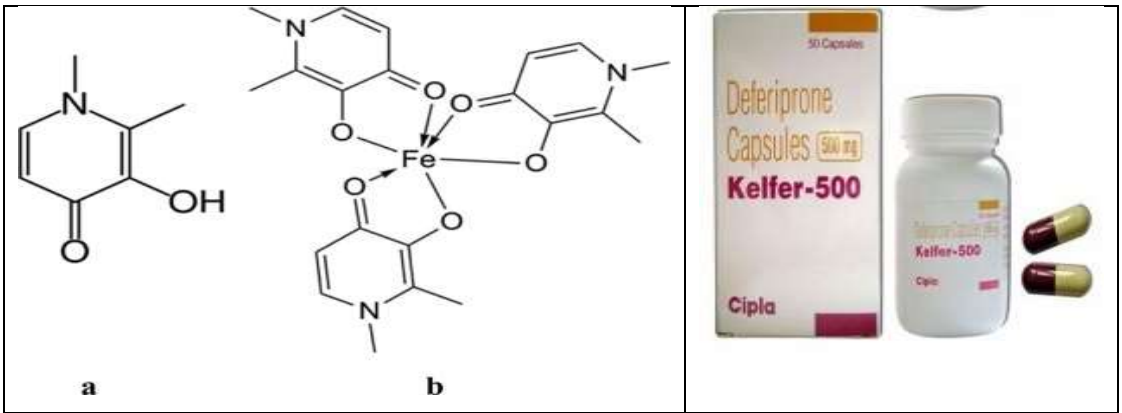


Fig. 1-16: (a) Chemical structure of ((deferiprone)) Hydroxy-1,2-dimethyl-4(1H)-pyridone. (b) Three molecules of deferiprone chelated with an iron atom [98]**.**

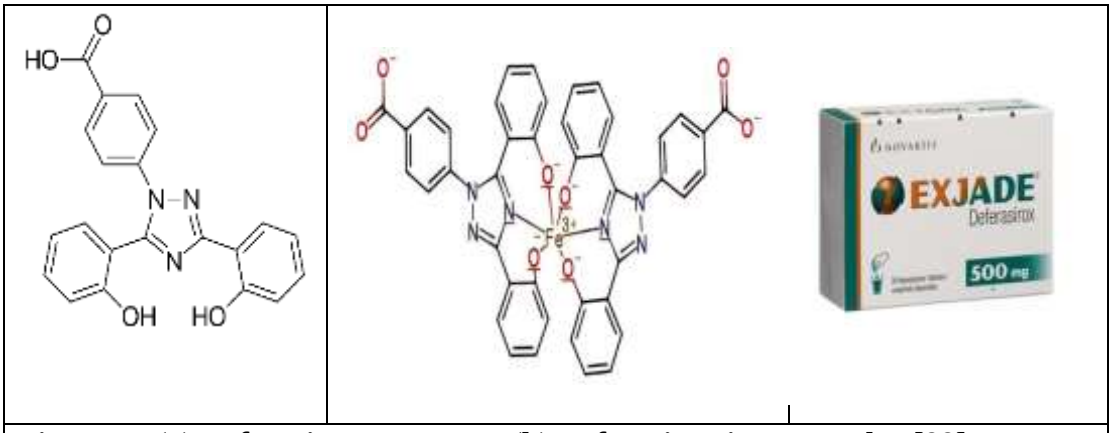


Fig. 1-17: (a) Deferasirox structure. (b) Deferasirox iron complex [99]**.**

1.15.3. Stem Cell Transplantation:

When a patient has stem cell replacement therapy, sometimes referred to as a bone marrow transplant, healthy stem cells are given to them to replace stem cells that have been lost to illness, heavy doses of chemotherapy, and/or radiation therapy. This treatment can also be used to treat other conditions in which stem cells are needed to treat the condition. Annually, there are more than 17,500 transplantation operations conducted in the United States using stem cells [100]. Figure 1-18.

Over 18,000 transplantations are carried out each year throughout Europe at more than 350 different hospitals [101].

Stem cell transplantation has become a safer process, and patient survival rates continue to rise as a result of ongoing improvements in the methods and practices of supportive care after transplantation [100].

Hematopoietic stem cells are the original, undeveloped cells from which all mature blood cells develop, including erythrocytes, monocytes, and platelets. The process of making new blood cells is known as hematopoietic. These cells are very immature and have yet to reach maturity. These cells share a common origin but can differentiate into any

blood cell type the body needs at the time. There are many stem cells in the bone marrow. They reproduce there to generate brand new blood cells. When red blood cells have completed their maturation process, they travel from the bone marrow to the circulation [102].

The human body's capacity to produce blood cells, especially healthy red blood cells and hemoglobin free of thalassemia, is restored via the growth of healthy stem cells in the bone marrow. The extremely immature cells known as stem cells mature and grow into platelets, white blood cells, and red blood cells. The body's red blood cells, or erythrocytes, transport oxygen to other cells. Leukocytes, or white blood cells, combat infection. Blood clotting is aided by platelets (thrombocytes). These cells all mature from stem cells [103].

Because of their potential to differentiate into a variety of lineages, their capacity for self-renewal in the repair of damaged organs and tissues in vivo, and their ability to generate tissue constructs in vitro, stem cells are a natural choice for use in cellular therapy. Stem cells also have the ability to generate tissue constructs in vitro. For cellular therapies to be successful, it is very necessary to crack the code on how to force stem cells to differentiate into a specific lineage in an effective manner [104].

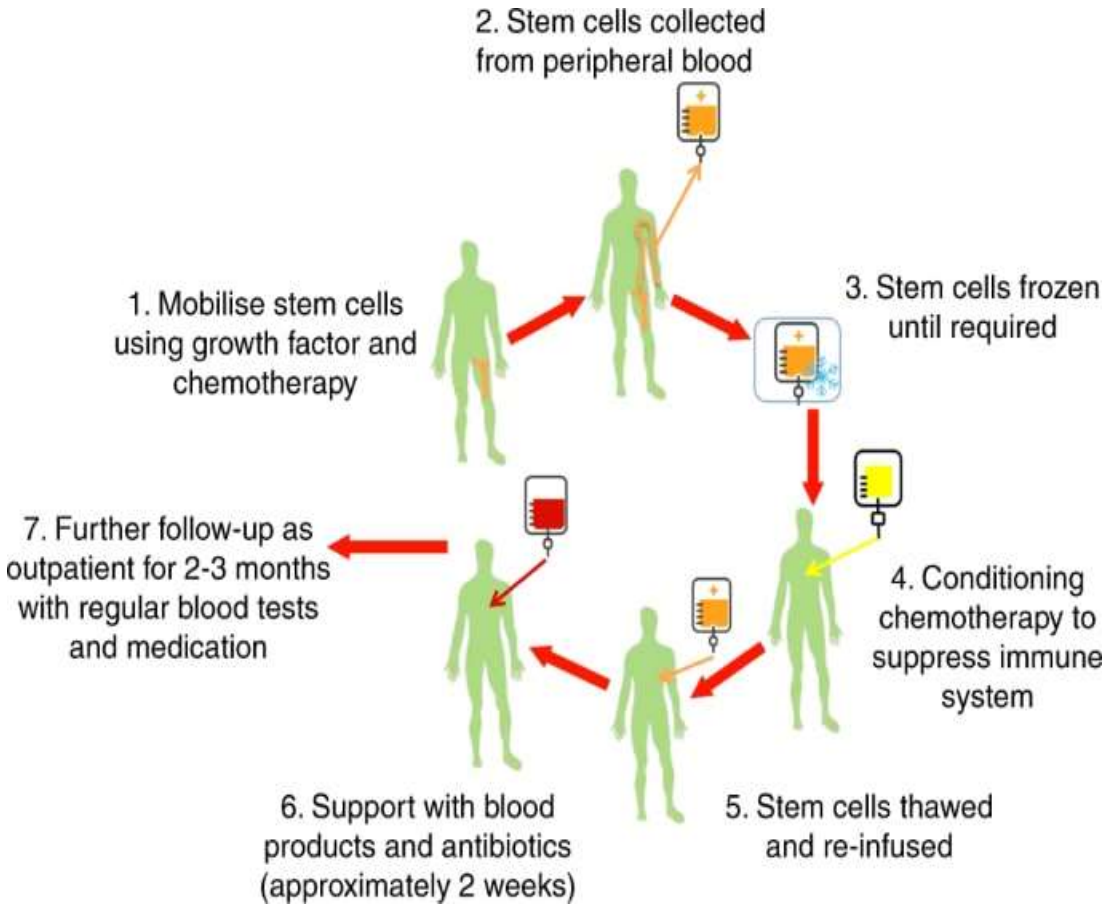


Fig. 1-18: Summary of stem cell transplantation procedure, and follow-up of cases [105]**.**

The Aims of the Study

The objective of the study are included:

1- Explain of the effect of blood transfusions for patients with beta thalassemia major on the increase or decrease of:

- Oxidizing substances in the body: such as (MDA) and (TOS).
- Antioxidant factors in the body: such as (TAC), (Vitamin E) and (GSH) in patients compare with healthy groups.

2- Measure the level of bone morphogenetic protein 2 (BMP2) and its effect by oxidative stress in patients compare with healthy groups.

3- Measure the level of interleukin-8 (IL-8), and showing the effect of oxidative stress on it patients compare with healthy groups.

Comparing patients who underwent splenectomy with no-splenectomy, and the effect of this on iron accumulation in different parts of the body and its consequences in the patients group.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and Kits

In this research study, the chemicals and kits are utilized in their raw, store-bought forms rather than having any further purifying work done on them. This eliminates the requirement for the chemicals and kits to be refined in any way. This research made use of a variety of different kits and chemicals, all of which are included in the inventory Table 2-1.

Table 2-1: Chemicals and kits

No.	Chemicals and Kits	Formula or Symbol	Purity %	Company and/or Country
1	Na_2-EDTA (Ethylenediamine tetra acetic Acid, Disodium Salt)	$C_{10}H_{14}N_2O_8Na_2.2H_2$	99%	Fluka
2	2,2–bipyridyl,n-propanol.	$C_{10}H_8N_2O$	99%	Fluka
3	Ammonium sulfate	$(NH_4)_2SO_4$	99.9%	Fluka
4	Cupper Chloride hydrate	$CuCl_2.2H_2O$	98%	Fluka
5	DTNB 5,5-dithio-bis-(2-nitro benzoic acid)	$C_{14}H_8N_2O_8S_2$	98%	Fluka
6	EDTA Ethylene diamine tetraacetic acid	$[CH_2N(CH_2CO_2H)_2]_2$	99%	Fluka
7	Ethanol	C_2H_6O	99.9%	Fluka
8	Ferric chloride	$FeCl_3$	99%	Fluka
9	Ferrous ammonium sulfate	$(NH_4)_2.Fe(SO_4)_2$	99%	BDH
10	Glutathione (GSH)	$C_{10}H_{17}N_3O_6S$	99%	Biochemical
11	Glycerol	$C_3H_8O_3$	99%	Fluka

12	Human Bone Morphogenetic Protein2 ELISA Kit	BMP2	-------	BT LAB. China
13	Human Interleukin8 ELISA Kit	IL-8	-------	BT LAB. China
14	Hydrochloric Acid	HCl	39%	Aldrich
15	Hydrogen Peroxide	H_2O_2	35%	Alderich
16	Methanol	CH_3OH	99%	Fluka
17	Neucuproine (2,9-dimethyl- 1,1-phenathroline) (Nc)	$C_{14}H_{12}N_2$	99.9%	Sigma-Aldrich Inc.
18	O-dianisidine dihydrochloride	$C_{14}H_{16}N_2O_2.2HCl$	99%	Sigma-Aldrich Inc.
19	Sodium Chloride	NaCl	99%	Sigma-Aldrich Inc.
20	Sodium Hydroxide	NaOH	99%	Gmbh
21	Sulphuric Acid	H_2SO_4	99%	Fluka
22	ThioBarbituric Acid (TBA)	$C_4H_4N_2O_2S$	99%	Fluka
23	TriChloroacetic Acid (TCA)	$C_2HCl_3O_2$	98%	Fluka
24	Tris (hydroxyl methyl) amino methane	$(HOCH_2)_3CNH_2$	95%	Fluka
25	Uric Acid	$C_5H_4N_4O_3$	99%	CDH
26	Xylene (extra pure)	C_8H_{10}	99.9%	Fluka
27	Xylenol Orange	$C_{31}H_{32}N_2O_{13}S$	99%	Sigma-Aldrich Inc.
28	α-Tocopherol (Vitamine E)	$C_{28}H_{48}O_2$	99%	Fluka

2.1.2. Instruments and Equipments

Table 2-2 contains an inventory of all of the resources that were used during the course of this study.

Table 2-2: Instruments and Equipments.

No.	Instruments	Company and/or Country
1	Brown and transparent glass bottles	Gelsonlab
2	Can tubes	HONGQUE
3	Centrifuge	Hermle labortechnic
4	Eppendorf tubes	OEM Brand
5	Gel tube	OEM Brand
6	Incubator	Hearson (England)
7	Micropipette 100-1000 µL	Slamed (Germany)
8	Micropipette 10-100 µL	Slamed (Germany)
9	Micro-plate-reader	Bio Tek (USA)
10	plane tubes	HONGQUE
11	plastic Rack for Micro Centrifuge Tubes	CE, ISO Material
12	Sensitive balance	Stanton 461 AN
13	Test tubes rack	CE, ISO Material
14	U.V-visible spectrophotometer (UV-1100)	EMCLAB (Germany)
15	Urine cups	OEM Brand
16	Vortex mixter	Karlkole (Germany)
17	Water bath	Karlkole (Germany)

2.1.3. Methodology

2.1.3.1. Subject

The samples were taken for patients groups in Thalassemia Center, Al-Zahraa Teaching Hospital for Maternity and Children, Najaf Governorate, Iraq, for those who visited the Thalassemia Center from September to December 2021, when the study was done. This was done under the postal book (Mission facilitation) (Appendix1).

A samples for control groups were taken from volunteers during the same period. The search was performed using data collection criteria in terms of age, sex, presence of disease and splenectomy, medication, intake of iron chelate treatments, and duration of blood transfusion.

The necessary information for each person within this study was recorded in a form for each of them as shown in (Appendix 2).

2.1.3.2. Study Design

This study was design as case control study. In this study, which included (90) people, age (5-20) years, they were divided as follows; and show in figure 2-1.

- The first group: (PG1) includes 30 males patient.
- The second group: (PG2) includes 30 females patient.
- The third group: (CG1) includes 15 males control.
- The fourth group: (CG2) includes 15 females control.

After that, the patients, whether male or female, were classified into:

- The (splenectomy group) includes 20 patient.
- The (no-splenectomy group) includes 40 patient.

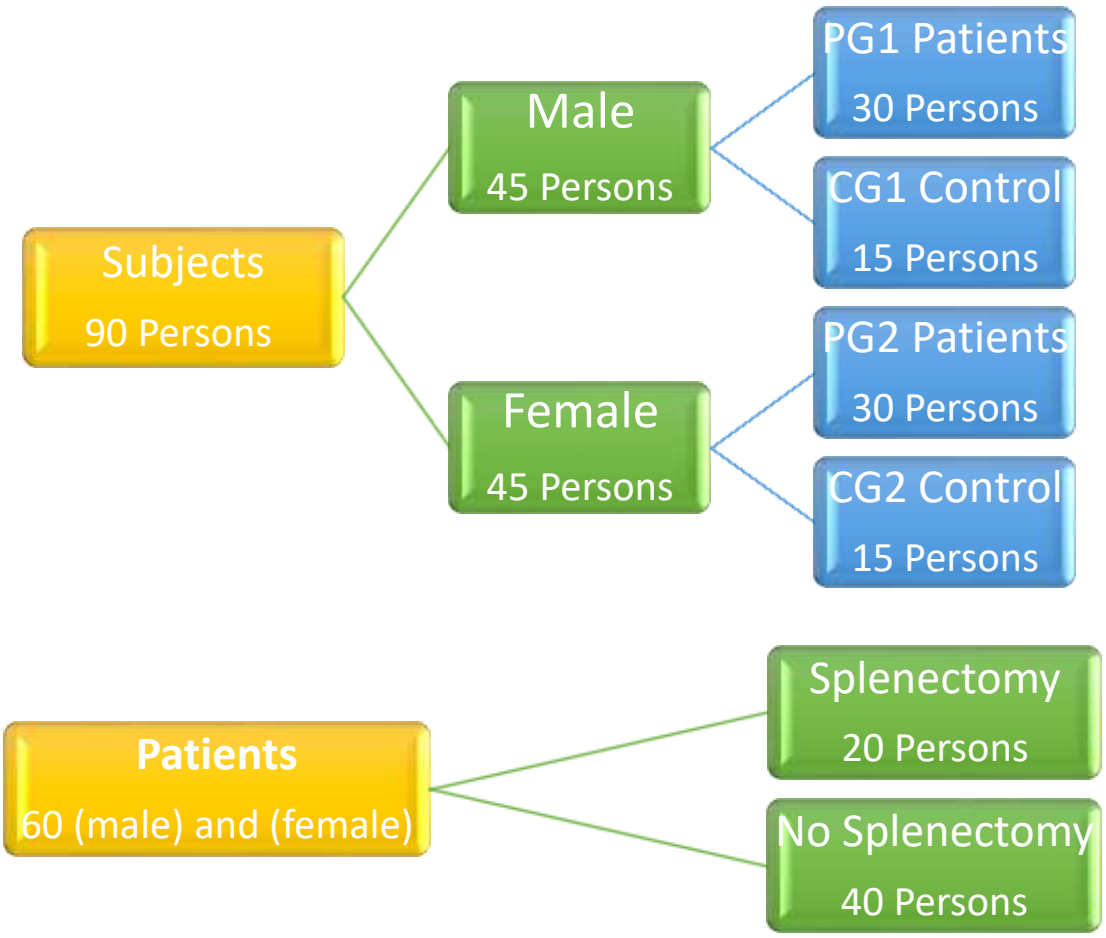


Fig. 2-1: study design to the groups.

2.1.4. An Overview of the Classified Groups

2.1.4.1. Patient Groups (PG)

The patient group included sixty persons. All patients were diagnosed with a complete blood count (CBC), as well as a center Hemoglobin electrophoresis. They were divided into the first group (PG1), which consisted of thirty males, and the second group (PG2), which consisted of thirty females. The most prominent symptoms recorded on them are pallor of the skin, jaundice, enlargement of the spleen and liver, fragility and deformation of the bones, and the accumulation of iron in the body as a result of repeated blood transfusions, which leads to hormonal disorders, some problems in the thyroid gland, heart and liver.

2.1.4.2. Control Groups (CG)

The control group included (thirty) apparently healthy people. None of them have any signs and symptoms of the anemia disease. The control elements consist of the first group (CG1), which consists of fifteen males, and the second group (CG2), which also consists of fifteen females.

2.1.5. Excluded Criteria

Within the scope of our study, we did not take into consideration any of the following states:

- Heart disease.
- Iron overload due to causes other than beta thalassemia.
- Chronic liver disease.
- Immunodeficiency diseases
- Those with thalassemia minor and middle or alpha thalassemia.

2.1.6. Blood Sample Collection

Blood samples are collected using sterile disposable syringes and needles. Blood samples 5 ml from the patients and the control group were taken and put in empty tubes of gel tubes after being allowed to clot at room temperature. The tubes were then centrifuged at 1000 xg for 10 minutes. In Eppendorf tubes, sera are separated and split into parts before being frozen until use.

2.2. Methods

2.2.1. Determination of Bone Morphogenetic Protein2 (BMP-2) (ELISA Kit)

2.2.1.1. Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human BMP2 antibody. BMP2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human BMP2 Antibody is added and binds to BMP2 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated BMP2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human BMP2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

2.2.1.2. Reagent Provided

Table 2-3: Components of reagent provided

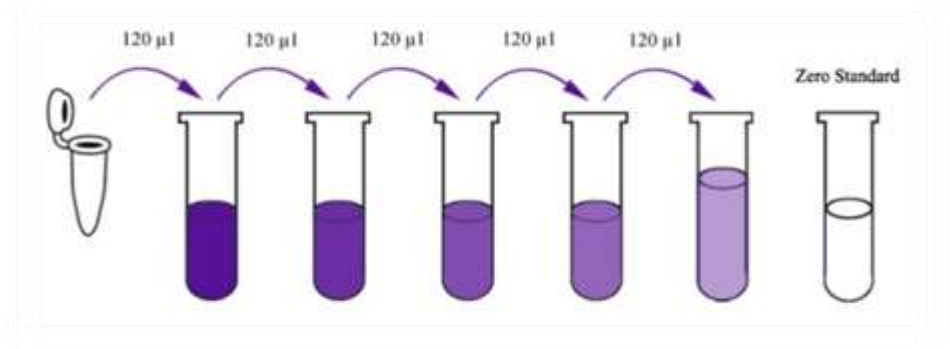
Components	Quantity (96T)
Standard Solution (16ng / ml)	0.5 mL x 1
Pre - coated ELISA Plate	12 * 4 well strips x 1
Standard Diluent	3 mL x 1
Streptavidin - HRP	3 mL x 1
Stop Solution	3 mL x 1
Substrate Solution A	3 mL x 1
Substrate Solution B	3 mL x 1
Wash Buffer Concentrate (25x)	20 mL x 1
Biotinylated Human BMP - 2 Antibody	1 mL x 1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

2.2.1.3. Reagent Preparation

- All reagents were brought to room temperature before use.
- A volume of 120 µL of standard diluent (16 ng/mL) was reconstituted with 120 µL of standard diluent to generate an 8ng/mL standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points are prepared by serially diluting the standard stock solution (8ng/mL) 1:2 with standard diluent to produce 4 ng/mL, 2 ng/mL, 1 ng/mL and 0.5 ng/mL solutions. Standard diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20 ° C and used within one month. Dilution of standard solutions suggested are as follows:

Table 2-4: Dilution of standard solutions in BMP2 Kit

8 ng/mL	Standard No.5	120 µL Original Standard + 120 µL Standard Diluent
4 ng/mL	Standard No.4	120 µL Standard No.5 + 120 µL Standard Diluent
2 ng/mL	Standard No.3	120 µL Standard No.4 + 120 µL Standard Diluent
1 ng/mL	Standard No.2	120 µL Standard No.3 + 120 µL Standard Diluent
0.5 ng/mL	Standard No.1	120 µL Standard No.2 + 120 µL Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
16 ng/mL	8 ng/mL	4 ng/mL	2 ng/mL	1 ng/mL	0.5 ng/mL

- Wash buffer was done by diluting 20 ml of concentrated wash solution 25 x in deionized or distilled water to produce 500 ml of 1 x wash buffer. If crystals form in the center, they are gently mixed until the crystals are completely dissolved.

2.2.1.4. Assay Procedure:

1- All reagents, standard solutions and samples were prepared according to the instructions. All reagents are brought to room temperature before use. The examination is performed at room temperature.

2- The number of slides required for the assay was determined. The strips were inserted into the tires for use. Unused strips should be stored at 2-8°C.

3- A standard 50 µl were added to the standard well. NOTE: No biotinylated antibody was added to the standard well because the standard solution contains a biotinylated antibody.

4- A volume of 40 µl sample were added to sampling wells then 10ul of BMP-2 antibody was added to sampling wells, then 50 µl of streptavidin-HRP was added to sampling wells and standard wells (not empty wells to control). It was mixed well. The panel is covered with a sealant. Incubate 60 min at 37 °C.

5- The sealant was removed and the plate was washed 5 times with washing solution. Wells are soaked in 300 µL wash solution for 30 seconds to 1 minute per wash. For automated washing, each well was decanted or decanted and washed 5 times with washing solution. The plate was filtered on paper towels or another absorbent material.

6- A volume of 50 µL of substrate solution A was added to each well and then 50 µL of substrate solution B was added to each well. The plate covered with new sealant is incubated for 10 minutes at 37 °C in the dark.

7- A volume of 50µl of stop solution was added to each well, the blue color will change into yellow immediately.

8- Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

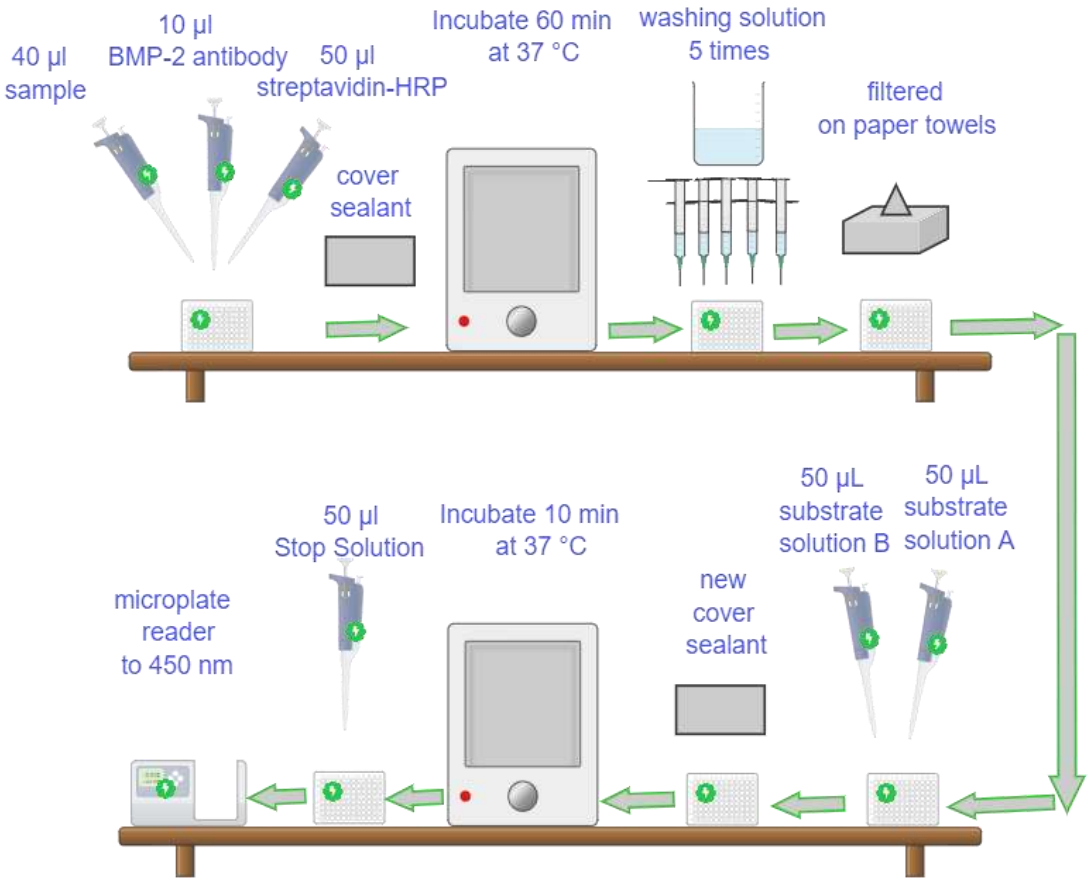


Fig. 2-2: Diagram showing procedures for measuring (BMP-2) levels.

2.2.1.5. The Brevity of the Procedures

1- All reagents, samples and standards are prepared.
2- A sample and ELISA reagent are added to each well. Incubation is done for 1 hour at 37°C.
3- The dish is washed 5 times.
4- Substrate solution A and B are added. It is incubated for 10 minutes at 37°C.
5- Stop solution is added and color develops.
6- The OD value is read within 10 minutes.

2.2.1.6. Calculation

A standard curve was created by plotting the average optical density (OD) for each standard on the vertical (Y) axis versus the concentrations on the horizontal (X) axis and plotting a best-fit curve through the points on the graph. The calculations were made using the computer by using Excel and (SPSS) programs, and the best fit line was determined by determining the regression. A curve for all assays was found to be similar to the standard curve.

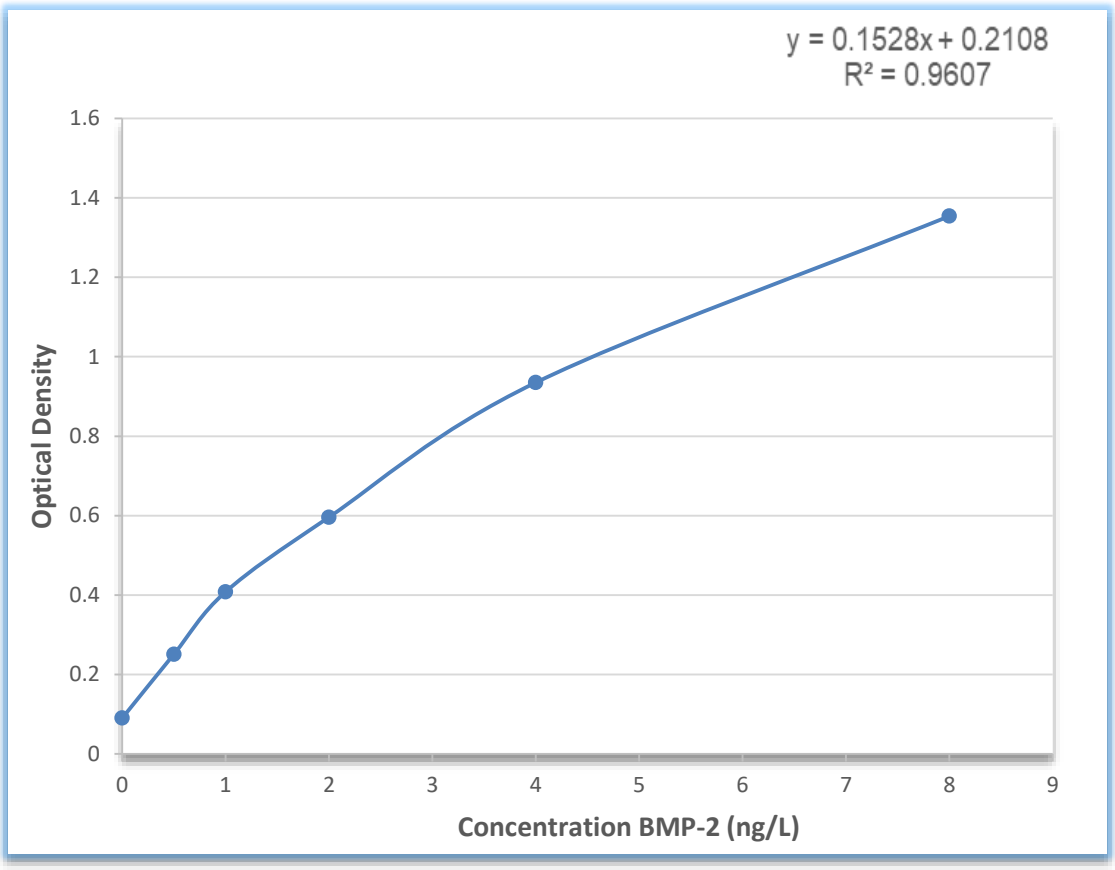


Fig. 2-3: Standard curve between optical density and concentration for Bone Morphogenetic protein2 [115].

2.2.2. IL-8 Determination of Interleukin-8 (IL-8) (ELISA Kit)

2.2.2.1. Assay Principle:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL8 antibody. IL8 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human IL8 Antibody is added and binds to IL8 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated IL8 antibody. After incubation unbound Streptavidin-HRP is washed away

during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL8. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

2.2.2.2. Reagent Provided

Table 2-5: Components of reagent provided

Components	Quantity (96T)
Standard Solution (1280ng / L)	0.5 x 1
Pre - coated ELISA Plate	12 * 8 well strips x 1
Standard Diluent	3 mL x 1
Streptavidin - HRP	6 mL x 1
Stop Solution	6 mL x 1
Substrate Solution A	6 mL x 1
Substrate Solution B	6 mL x 1
Wash Buffer Concentrate (25x)	20 mL x 1
Biotinylated Human IL - 8 Antibody	1 mL x 1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

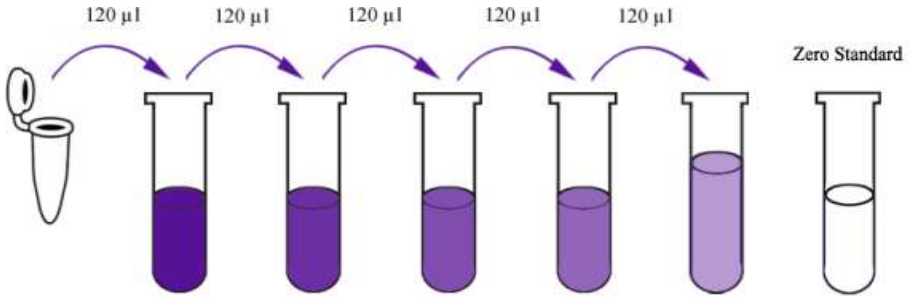
2.2.2.3. Reagent Preparation

- All reagents were brought to room temperature before use.
- A volume of 120 µL of the standard (1280 ng/L) was reconstituted with 120 µL of standard diluent to generate a 640ng / L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points are prepared by serially diluting the standard stock solution (640 ng/L) 1:2 with standard diluent to produce 320 ng/L, 160 ng/L, 80 ng/ L and 40 ng/L solutions. Standard diluent serves as the zero standard (0 ng/L). Any remaining solution

should be frozen at -20 °C and used within one month. Dilution of standard solutions suggested are as follows:

Table 2-6: Dilution of standard solutions in Interleukin-8 Kit

640 ng/L	Standard No.5	120 µL Original Standard + 120 µL Standard Diluent
320 ng/L	Standard No.4	120 µL Standard No.5 + 120 µL Standard Diluent
160 ng/L	Standard No.3	120 µL Standard No.4 + 120 µL Standard Diluent
80 ng/L	Standard No.2	120 µL Standard No.3 + 120 µL Standard Diluent
40 ng/L	Standard No.1	120 µL Standard No.2 + 120 µL Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
1280 ng/L	640 ng/L	320 ng/L	160 ng/L	80 ng/L	40 ng/L

- Wash buffer was done by diluting 20 ml of concentrated wash solution 25 times in deionized or distilled water to obtain 500 mL of 1x wash buffer. If crystals form in the center, they are gently mixed until the crystals are completely dissolved.

2.2.2.4. Assay Procedure:

1- All reagents, standard solutions and samples were prepared according to the instructions. All reagents are brought to room temperature before use. The examination is performed at room temperature.

2- The number of slides required for the assay was determined. The strips was inserted into the tires for use. Unused strips should be stored at 2 to 8 °C.

3- A standard 50 µL was added to the standard well. Note: The biotinylated antibody is not added to the standard well because the standard solution contains the biotinylated antibody.

4- A volume of 40 µL sample was added to sample wells then 10 µL anti-IL-8 antibody was added to sample wells, then 50 µL streptavidin-HRP was added to sample wells and standard wells (not control blank well). It was mixed well. Cover the panel with a sealant. Incubate 60 min at 37 °C.

5- The sealant was removed and the plate was washed 5 times with a washing solution. Wells were soaked in 300 µL wash solution for 30 seconds to 1 minute per wash. For automated washing, each well was withdrawn or decanted and washed 5 times with washing solution. The plate was halved on paper towels or other absorbent material.

6- A volume of 50 µL of substrate solution A was added to each well and then 50 µL of substrate solution B was added to each well. The plate covered with fresh plug was incubated for 10 minutes at 37°C in the dark.

7- A volume of 50 µL of stop solution was added to each well, it will turn blue to yellow immediately.

8- The optical density (OD value) of each well is determined immediately using a microplate reader set to 450 nm within 10 min after the addition of the stop solution.

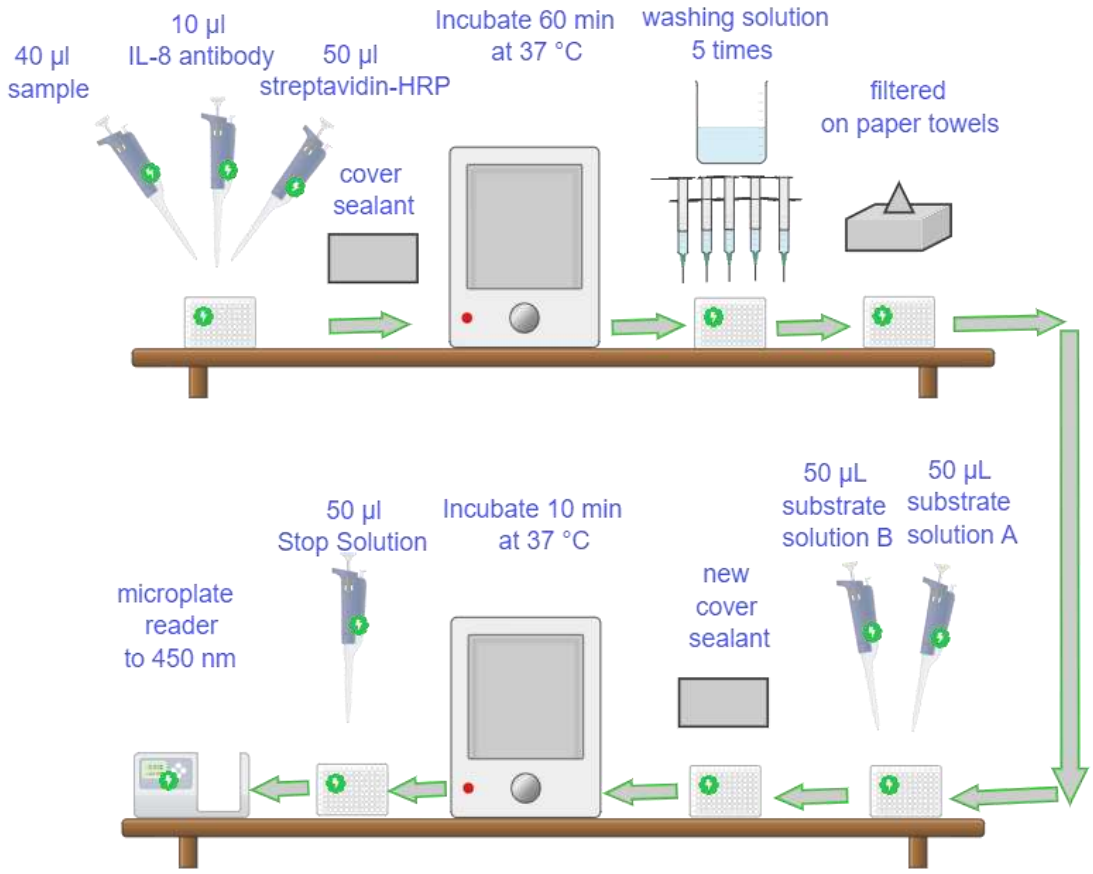


Fig. 2-4: Diagram showing procedures for measuring (BMP-2) levels.

2.2.2.5. The Brevity of the Procedures

1- All reagents, samples and standards are prepared.

2- Sample and ELISA reagent are added to each well. Incubate for 1 hour.

3- The dish is washed 5 times.

4- Substrate solution A and B are added. It is incubated for 10 min. at 37°C.

5- Stop solution is added and color develops.

6- The value of the optical density (OD) is read within 10 minutes.

2.2.2.6. Calculation

A standard curve was generated by plotting the average optical density (OD) for each standard on the vertical (Y) axis versus the focus on the horizontal (X) axis. The calculations were performed on the computer using Excel and (SPSS). The best fit curve is plotted by the points on the graph. A curve for all assays was found to be similar to the standard curve.

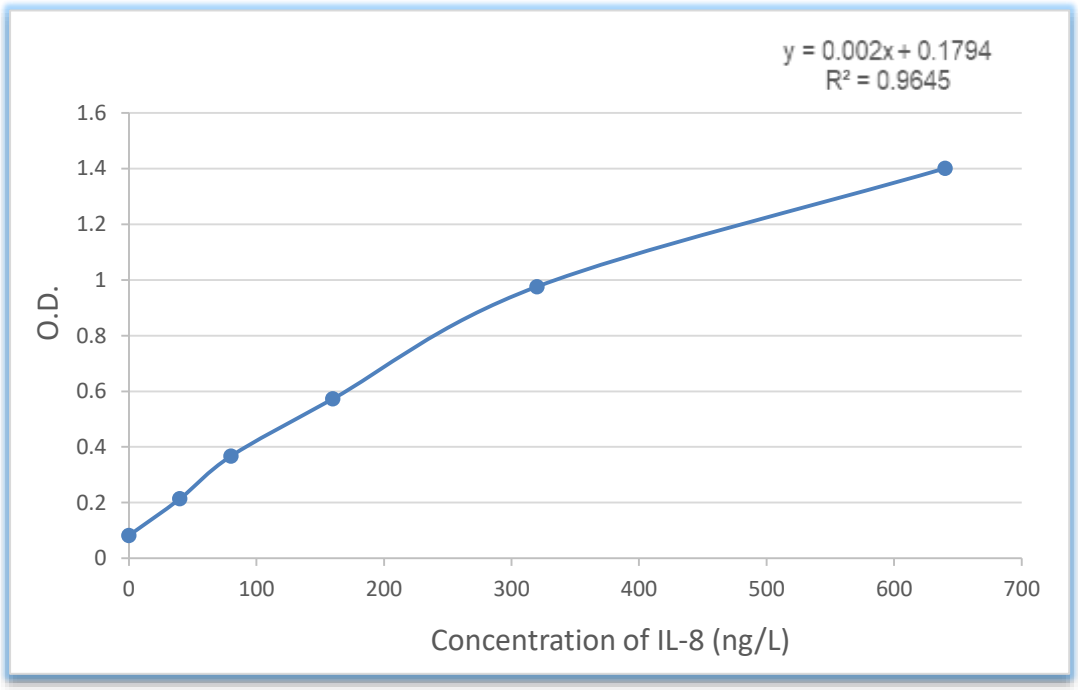


Fig. 2-5: Standard curve between optical density and concentration for interleukin 8. [116].

Pictures from the practical side when estimating with ELISA kits in the laboratory are shown in Appendix 3.

2.2.3. Determination of Malondialdehyde (MDA) Levels

2.2.3.1. Principle

One of the important oxidation products is known as malondialdehyde (MDA). Thio barbituric acid (TBA) is reacted with MDA, which is resulting in a colour compound, which can be determined spectrophotometrically [106]. Figure 2-2.

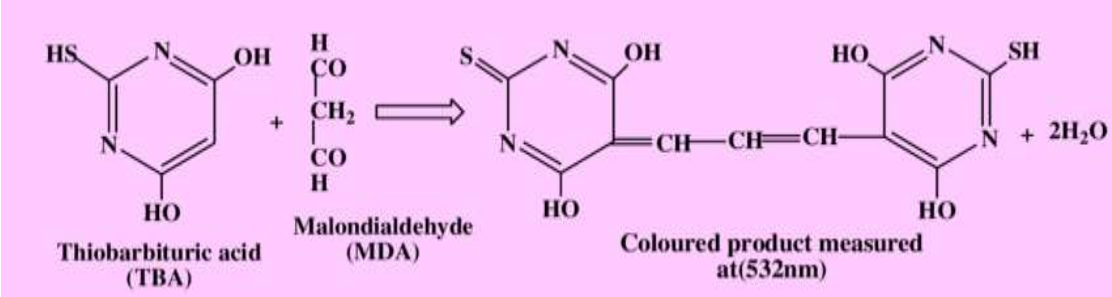


Fig. 2-6: Reaction of malondialdehyde with thiobarbituric acid to form a colored product [107]**.**

2.2.3.2. Reagents:

1. **Thiobarbituric acid (TBA) 0.6%,** produced by dissolving 0.6 g in 100 mL D.W.
2. **Trichloroacetic acid (TCA) 17.5%,** produced by dissolving 0.6 g in 100 mL D.W.
3. **Trichloroacetic acid (TCA) 70%,** produced by dissolving 70 g in 100 mL D.W.

2.2.3.3. Procedure

The approach included adding components to a set of three tubes that represented a sample, a reagent, and a blank in a methodical manner. Table 2-3.

Table 2-7: the details of determination of Malondialdehyde

Reagents	Test	Blank
Sample (Serum)	150 µL	---------
TCA (17.5%)	1000 µL	1000 µL
TBA (0.6%)	1000 µL	1000 µL
All tubes were mixed by vortex, incubated in boiling water bath ($80^{\circ}C$) for 15minutes and then left to cool (at $25^{\circ}C$)		
TCA (70%)	1000 µL	1000 µL
D.W.	---------	150 µL

After 20 minutes of remaining at room temperature, the solution was centrifuged at 450 xg for 15 minutes. A spectrophotometer was used to measure the absorbance of each tube at 532 nm in centrifuge tube [108].

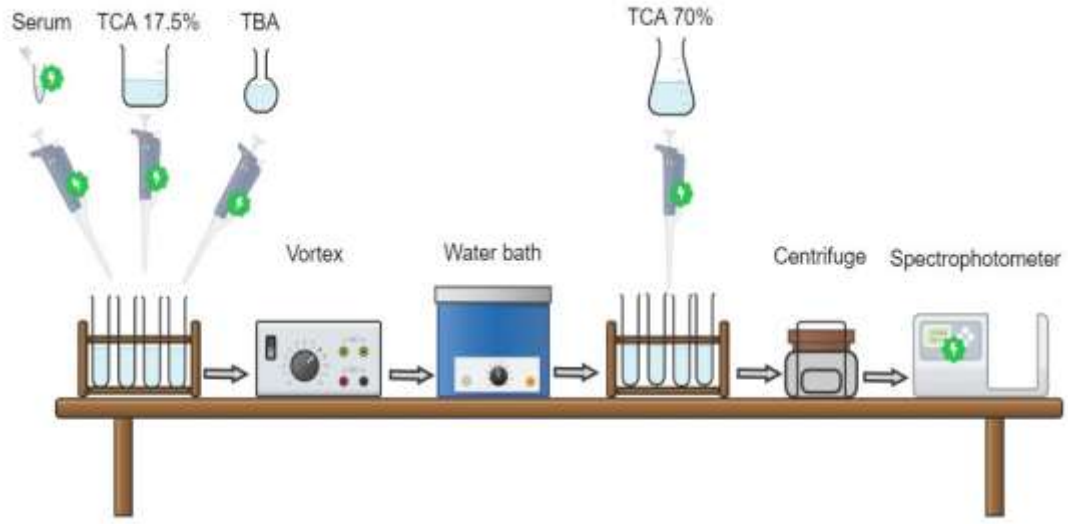


Fig. 2-7: Diagram showing procedures for measuring malondialdehyde levels.

Calculation

$$\textbf{The concentration of MDA } {}^{\textbf{m mole}}/_{\textbf{L}} = \frac{\textbf{A sample}}{\textbf{L} * \boldsymbol{\varepsilon}} * \textbf{D}$$

Where:

L = light path (1cm)

ε = Molar extinction coefficient (1.56 * 10^5 $M^{-1}\,cm^{-1}$)

D = Dilution factor.

Where: D $= \frac{1\ ml\ (volume\ used\ in\ reference)}{0.15\ ml\ (volume\ used\ in\ sample)} = \frac{1}{0.15} = 6.7$

2.2.4. Oxidant-Antioxidant System

2.2.4.1. The Determination of Total Oxidant Status (TOS)

Principle

Erel developed a unique automated measuring technique that was used to measure the TOS of serum (2005). The ferrous ion-o-dianisidine complex is oxidized to ferric ions by oxidants in the sample. Glycerol molecules in the reaction medium enhance oxidation. In an acidic media, the ferric ion combines with xylenol orange to form a colorful complex. Total oxidant molecules in a sample correlate with color intensity, which may be measured spectrophotometrically. The findings are given in terms of micromolar hydrogen peroxide equivalent per liter (mol H_2O_2 Eq./L), and the test is calibrated using hydrogen peroxide [112].

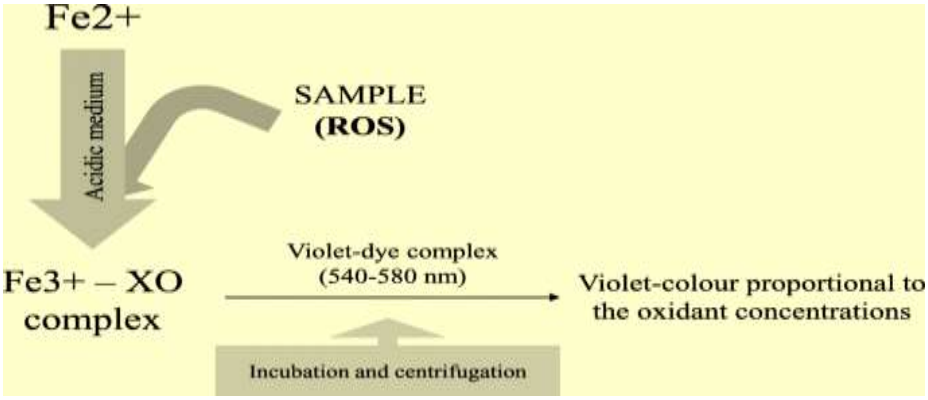


Fig. 2-8: An overview of the total oxidant status measurement based on ferrous ion–O-dianisidine complex (TOS-dianisidine) reaction [113]**.**

Reagents

Reagent 1: Prepared by mixing 100 ml of 25 mM H_2SO_4 solution with 1.96 g of ferrous ammonium sulfate and 3.17 g of O-dianisidine dihydrochloride. The final reagent included 10 mM O-dianisidine dihydrochloride and 5 mM ferrous ammonium sulfate.

Reagent 2: prepared by mixing 8.18 g of sodium chloride and 114 mg of xylenol orange in 900 ml of 25 mM H_2SO_4 solution. The solution received 100 milliliters of glycerol. The final reagent included 1.35 M glycerol, 140 M NaCl, and 150 M xylenol orange. The chemical had a pH of 1.7. At 4ºC, this reagent is stable for a minimum of six months.

Hydrogen peroxide (STD): (100 mol/L) was newly diluted, and the standardization was done on a regular basis using a molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$ at 240 nm.

Procedure

The necessary additions for the determination of the Total Oxidant Status (TOS) were made as shown in Table 2-5.

Table (2-8): The specifics of the TOS procedure

Reagents	Blank	S.T.D	Test
D.W	25 µL	-------	-------
Serum	-------	-------	25 µL
H_2O_2	-------	25 µL	-------
R1	1000 µL	1000 µL	1000 µL
Test tubes was mixed by vortex, then add:			
R2	250 µL	250 µL	250 µL

Each tube addition is well blended, and let to remain for 30 minutes at room temperature, and then read spectrophotometrically at 560 nm [114].

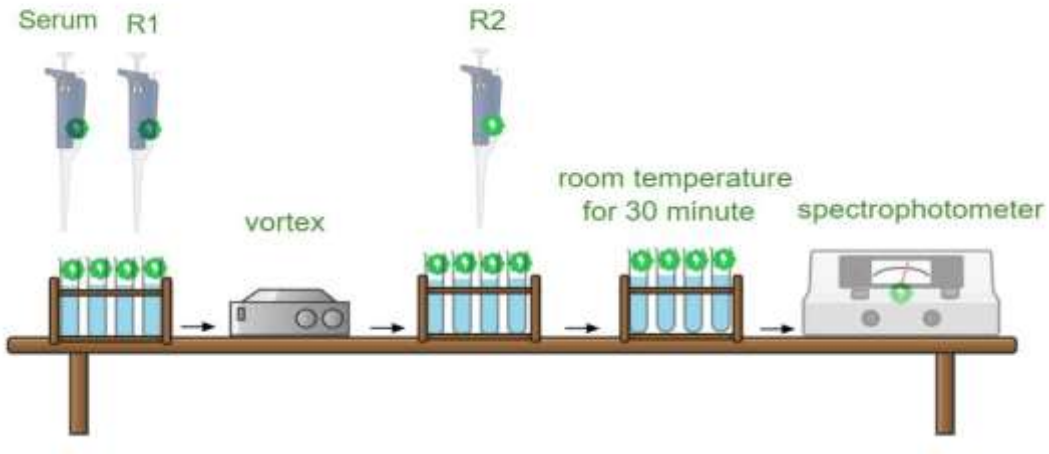


Fig. 2-9: Diagram showing procedures for measuring (TOS) levels.

Calculation

$$\textbf{Total Oxidants levels} = \frac{\mathbf{A_{.test}}}{\mathbf{A_{.STD}}} * \textbf{Conc. of STD} \left(\mu \frac{\mathbf{mol}}{\mathbf{L}}\right)$$

2.2.4.2. Total Antioxidant Capacity Assay (TAC) (The CUPRAC Method)

Principle

Total antioxidant capacity (TAC) is a common analyte used to determine the antioxidant status of biological samples and may measure how well the body fights off free radicals caused by a particular diseases [109]. Fugger 2-4.

$$\boldsymbol{Antioxidant + Cu^{2+} \rightarrow Cu^{+}}$$

$$\boldsymbol{Cu^{+} + 2,9\text{–}dimethyl\text{–}1,10\text{–}phenanthroline \rightarrow Complex}$$

$(at\ 450\ nm)$

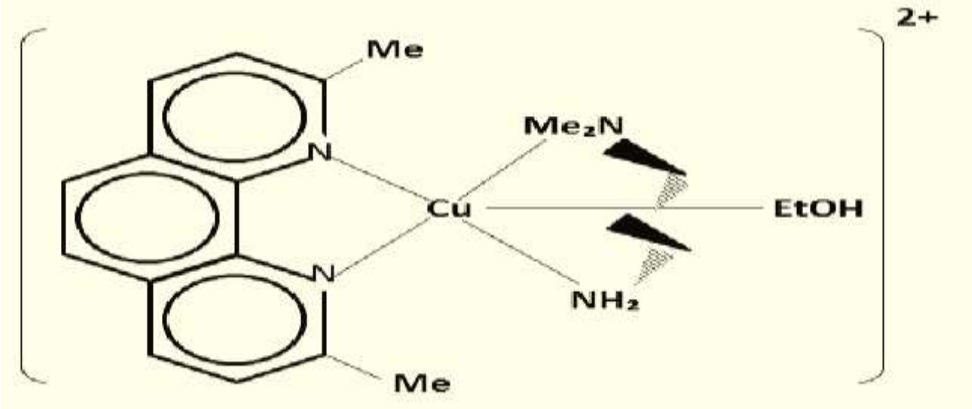


Fig. 2-10: Structure of the complex: [Cu(2,9-dimethyl-1,10-phenanthrolineN,N-dimethyltrimethylene-diamine)(EtOH)$(NO_2)_2.2H_2O$] [110]**.**

Reagents

1- **Copper (II) chloride solution (concentration of 10^{-2} M):**

This solution was prepared from 0.4262 g of $CuCl_2.2H_2O$, which was dissolved in distilled water and completed 250 ml of distilled water.

2- **Ammonium acetate buffer (NH4Ac), pH=7.0 :**

This solution was prepared from 19.27 g of $CH_3COO\ NH_4$ which was dissolved in distilled water and completed the volume to 250 ml.

3- **Neocuproine solution (Nc) (2.9-dimethyl-1,1-phenanthroline) (concentration of 7.5 * 10^{-3} M) :**

This solution was prepared by dissolving 0.04 g of (Nc) in 96% ethanol, then the volume was completed the volume to 25 ml ethanol.

4- The standard Uric acid ($C_5H_4N_4O_3$) solution (1mM) :

This solution was formed by dissolving 16.81 mg of Uric acid in 100 ml of deionized water. A few drops of sodium hydroxide are added to Uric acid in to completely dissolve it.

Procedure

According to the particular protocol, the essential additions were performed in order to calculate the total antioxidant capacity assay. Table (2-4).

Table 2-9: The features of the CUPRAC procedure

Reagents	Test	S.T.D	Blank
$CuCl_2.2H_2O$ Solution	1000 µL	1000 µL	1000 µL
Serum	50 µL	-------	-------
Uric acid Solution	-------	50 µL	-------
Distilled water	-------	-------	50 µL
Neocuproine (Nc) Solution	1000 µL	1000 µL	1000 µL
Ammonium Acetate (NH_4Ac) buffer	1000 µL	1000 µL	1000 µL

Test tubes were mixed by vortex-mixer and incubated at 37 degrees Celsius for 30 minutes before being centrifuged at 1000 xg for two minutes. The absorbance was then measured using a spectrophotometer at 450 nm [111].

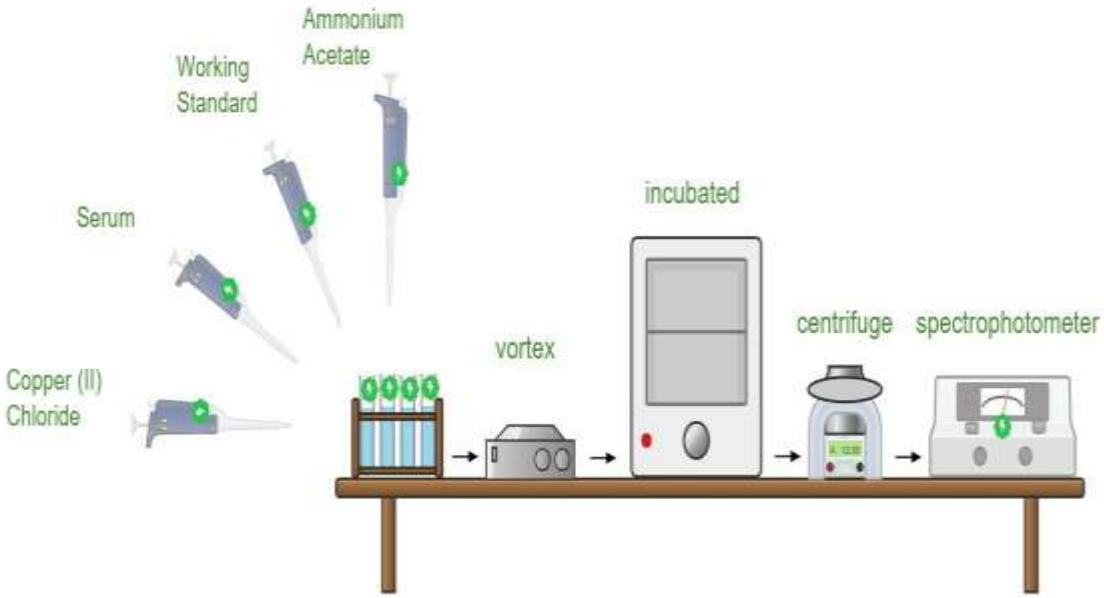


Fig. 2-11: Diagram showing procedures for measuring (TAC) levels.

2.3.1.2.4. Calculation

$$\textbf{Total antioxidants levels} = \frac{A_{.test}}{A_{.STD}} * \textbf{Conc. of STD} \left(\text{m}\frac{\text{mol}}{\text{L}}\right)$$

2.2.5. Determination of Tocopherol (Vitamin E) Levels

2.2.5.1. Principle

Vitamin E is one of the most essential antioxidants associated with disease regulation[117]. As an intermediary, -tocopherol's free-radical scavenging processes include the α-tocopheroxyl radical. The coupling of a suitable free radical with the α-tocopheroxyl radical may produce a non-radical product if a sufficient free radical is available [118]. Figure 2-12.

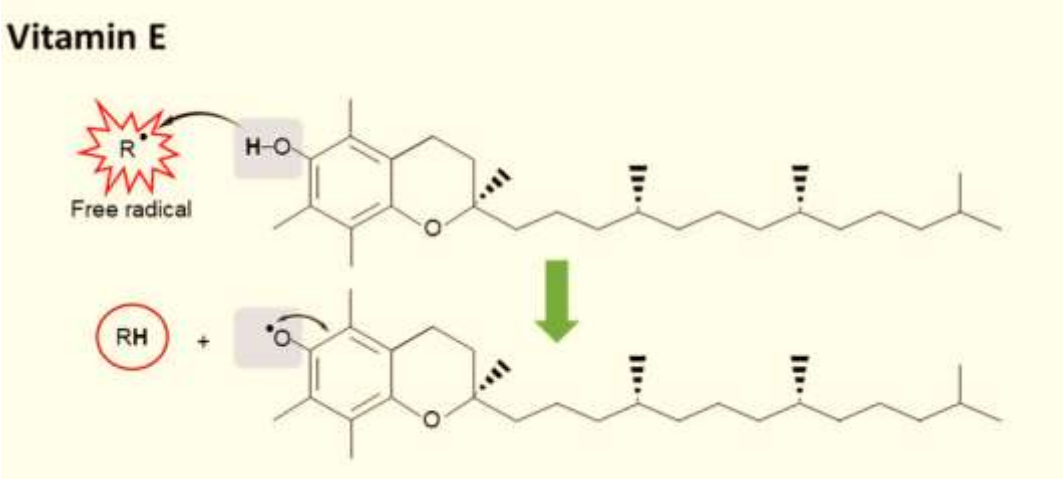


Fig. 2-12: (vitamin E) as an Antioxidants [119]**.**

Vitamin E exerts its antioxidant effects by quenching free radicals. The hydroxyl group on chromanol ring can donate its hydrogen atom to a free radical resulting in a delocalized and stabilized unpaired electron, vitamin E radical.

2.2.5.2. Reagents

- **(Reagent 1) Stock standard of α-tocopherol (0.27 %, w/v):**
  270 mg of α -tocopherol acetate diluted in 100 mL ethanol (aldehyde free) and mixed thoroughly.
- **(Reagent 2) 2,2-Bipyridyl (0.12 % , w/v):**
  120 mg 2,2-bipyridyl dissolved and made the volume up to 100 mL with n-propanol and kept in a brown bottle.
- **(Reagent 3) Ferric chloride (0.12 %, w/v):**
  120 mg $FeCl_3.6H_2O$ dissolved in 100 mL ethanol (aldehyde free) and also kept in a brown bottle. All these solutions are stable at room temperature (25 ° C ± 2 ° C).

2.2.5.3. Preparation of Standard Curve

- **Working standard of α -tocopherol (27 µ g/mL):**

Volume 1 mL of stock standard solution was taken and made the volume up to 100 mL with ethanol (aldehyde free) to obtain a concentration of 27 µg/mL. This solution is stable at room temperature. Six centrifuge tubes were taken and labeled as B (blank) and S 1, S 2, S 3, S 4, and S 5 for standard solution. In these tubes, 0 (zero), 150, 300, 450, 600, and 750 µL of working standard solution (27 µ g/mL) were added, respectively, and made the volume up to 750 µL by adding ethanol (aldehyde free).

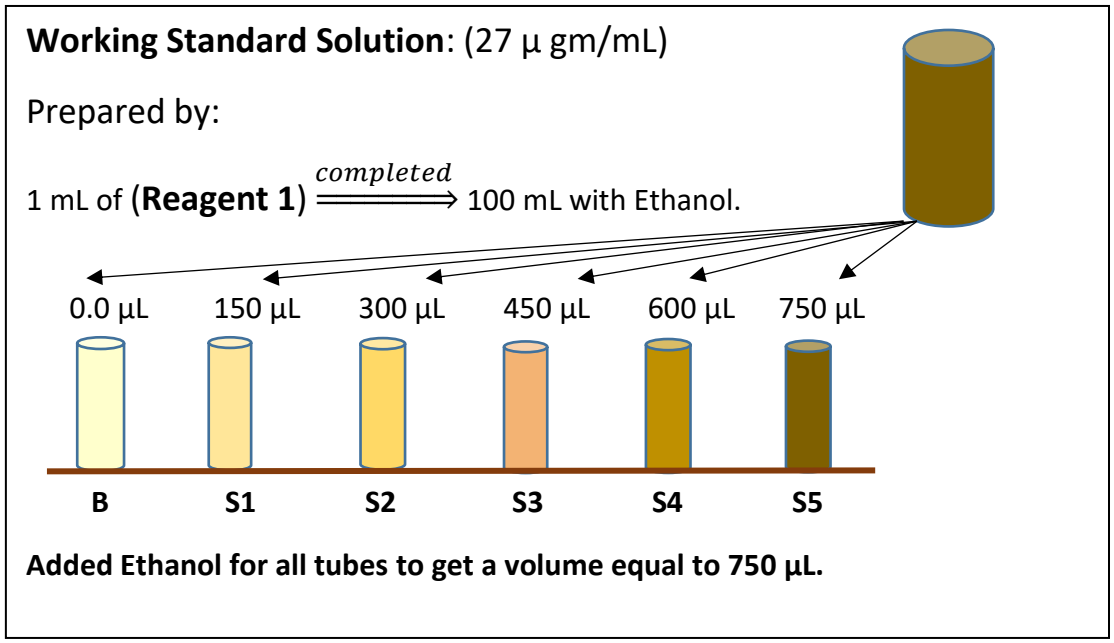


Fig. 2-13: Prepare the standard working solution in six tubes and add ethanol to it and reach the specified standard concentrations in order to take the absorption.

- **Measurement Procedures Completed:**

These solutions (S1 - S5) are equivalent to 4, 8, 12, 14, 16, and 20 µg/mL α-tocopherol concentration, respectively.

Taken 200 µL of each of the above solutions (including the blank) by micropipette, and the absorbance is measured, by placing an ELISA

micro plate it contains 96 wells (not coated with an antibody) in an ELISA reader (Micro plate reader) at 492 nm and reading it.

A standard curve is plotted with absorbance concentration (nm) (Y-axis) versus α-tocopherol concentration (µg/ml) (X-axis).

2.2.5.4. Analysis of α-Tocopherol in Serum

Additives and steps are used to measure vitamin E level.

Step 1: **Table 2-10: step one for analysis of vitamin E in serum.**

Reagents	Test	Blank
EtOH	750 µL	750 µL
Serum	750 µL	-------
D.W.	-------	750 µL
All the tubes were covered tightly by wrap paper and shaken vigorously for at least 30 s.		
Xylene	750 µL	750 µL
All the tubes were covered tightly by wrap paper and shaken vigorously for at least another 30 s and then centrifuged for 10 min at 3000 Xg. A floating layer of xylene will clearly separate		

Step 2: **Table 2-11: step two for analysis of vitamin E in serum.**

Reagents	Test	Blank
Xylene layer (supernatant)	500 µL	500 µL
2,2-bipyridyl solution (Reagent 2)	500 µL	500 µL
Ferric chloride solution (Reagent 3)	100 µL	100 µL
Waited for 2 minutes.		

Step three:

- The solutions **(200 µ L) from each of these tubes** were transferred to a plain ELISA microplate (non-antibody coated).
- The primary wavelength was set at 492 nm and the absorbance of all the samples (including blank) were measured.
- The ELISA reader (Micro plate reader) was set in 'rapid measure' mode.

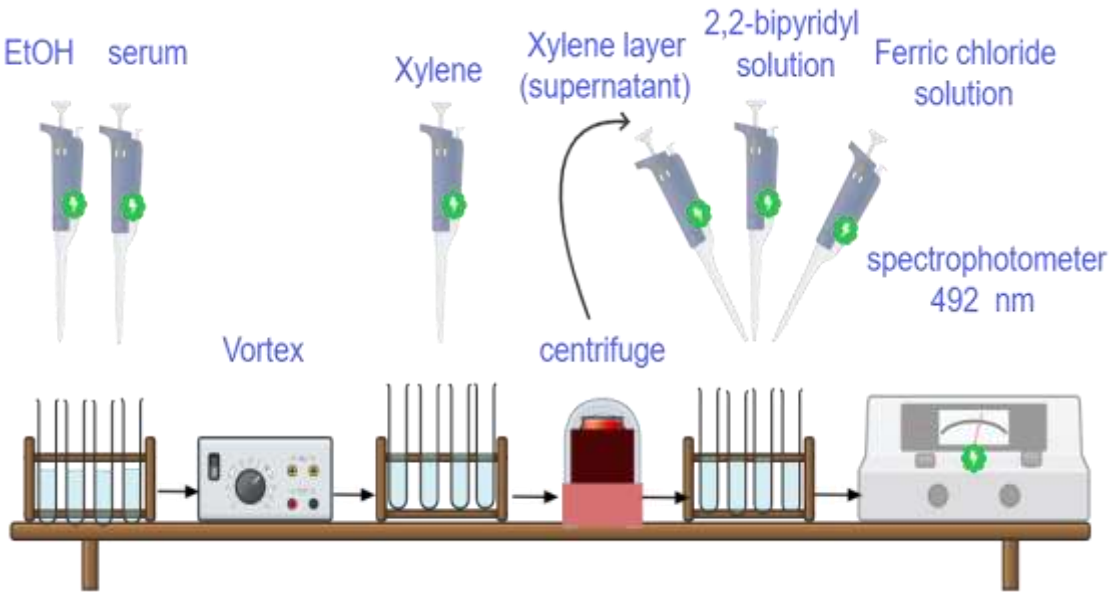


Fig. 2-14: Diagram showing procedures for measuring (BMP-2) levels.

2.2.5.5. Calculation

The serum α -tocopherol concentration of each the sample was obtained by using the standard curve prepared earlier. Figure 2-15.

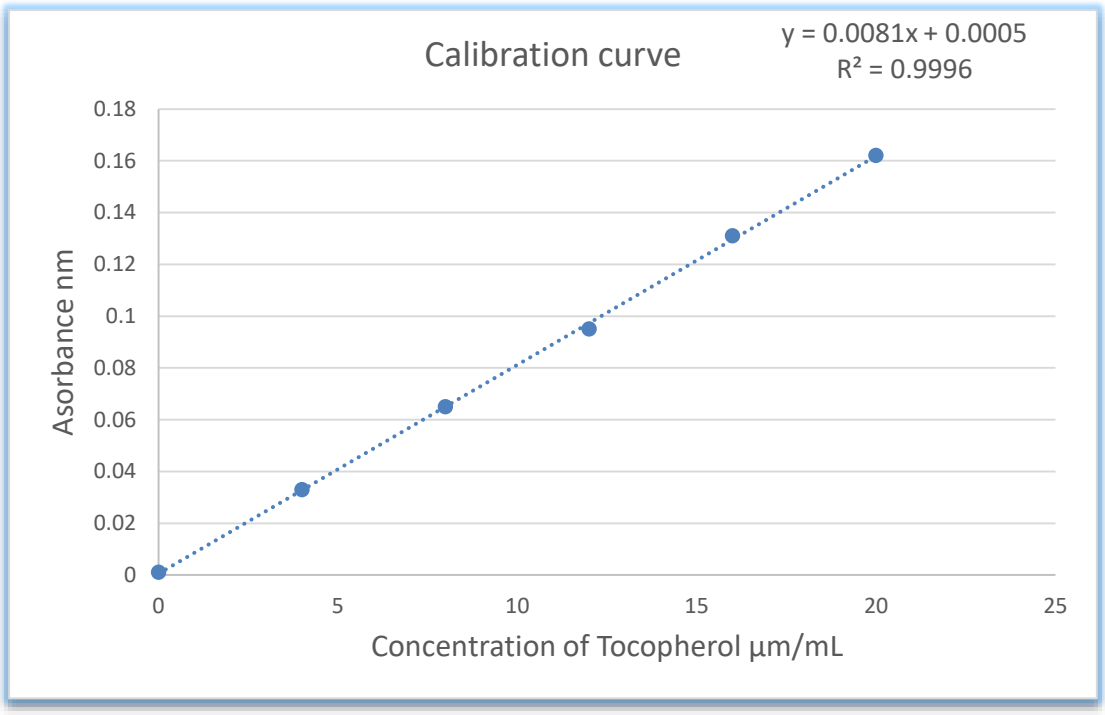


Fig. 2-15: The standard curve between absorbance and concentration of tocopherol. [117].

2.2.6. Determination of Glutathione (GSH) Levels

2.2.6.1. Principle

The compound 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) is a disulfide chromogen That is readily reduced by sulfahydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm, which is directly proportional to the GSH concentration [120].

2.2.6.2. Reagents

1- **(R1) Precipitating solution (50 % TCA)**:

Fifty grams of TCA were dissolved in a little volume of distilled water and then completed to a volume of 100 ml with distilled water.

2- **(R2) Disodium-ethylenediamine tetra acetic acid (Na_2 -EDTA) (0.2M)**:

Na_2-EDTA (3.722 g) was dissolved in a final volume of 50 ml with distilled water.

3- **(R3) Tris-EDTA buffer (0.2M) pH 8.9**:

Tris (hydroxyl methyl) amino methane, or known during medical use as tromethamine (0.4845 g) was dissolved in 8 ml of distilled water. One ml of 0.2 M Na_2- EDTA solution was added (R2). The pH was adjusted to 8.9 by the addition of 1 M HCl and then the final volume of the solution was brought to 10 ml by the addition of distilled water. This solution is stable for at least 10 days.

4- **(R4) 5,5-dithiobis (2-nitro benzoic acid) DTNB reagent (0.01 M)**:

DTNB (0.0396 g) was dissolved in absolute methanol, and brought to a final volume of 10 ml. This solution is stable for at least 13 weeks at 4 ° C.

5- **(R5) Reduced glutathione (GSH) standard solution (0.001M)**:

Stock standard solution (0.001M) was prepared by dissolving 0.0156 g of GSH in sufficient volume of Tris-EDTA (Tris -(hydroxyl methyl) amino methane) and completed to total volume of 50 ml of 0.2M EDTA solution. Serial dilution was made with EDTA solution to 5 , 10 , 20 , 30 , 40 , 50 , 60 , 70 , 80 , 90 , 100 µM of the stock . These working standard solutions should be prepared daily.

2.2.6.3. Procedure

Serum GSH is determined by using a modified producer utilizing Ellman's reagent (DTNB), which is summarized as follows: Standard and sample test tube were prepared then pipetted into test tubes. Table (2-8).

Table 2-12: Procedures for estimating serum glutathione levels

Reagents	Sample	Blank	STD
Serum	100 µL	-------	-------
Standard (R5)	-------	-------	100 µL
D.W	800 µL	900 µL	800 µL
TCA 50% (R1)	100 µL	100 µL	100 µL
Tubes were in vortex mixture intermittently for 5-10 minutes, and centrifuged for 15 minutes at 3000 X g, then the **supernatant** pipetted into test tubes.			
Supernatant	400 µL	400 µL	400 µL
Tris-EDTA (R3)	800 µL	800 µL	400 µL
Buffer DTNB (R4)	20 µL	20 µL	20 µL

The spectrophotometer was adjusted with reagent blank to read zero absorbance (A) at 412 nm, and the absorbance of standards and sample was read within 5 minutes of the addition of DTNB.

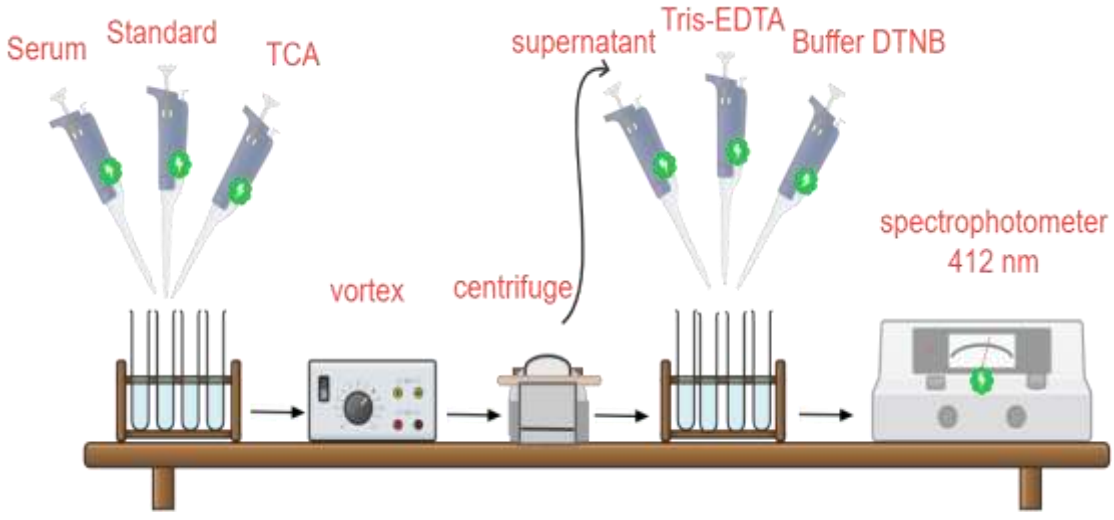


Fig. 2-16: Diagram showing procedures for measuring (Vitamine E) levels.

2.2.6.4. Calculations

The standard curve of glutathione was drawn between the absorbance of figure 2-17, then the different concentrations of glutathione solutions. Figure 2-17.

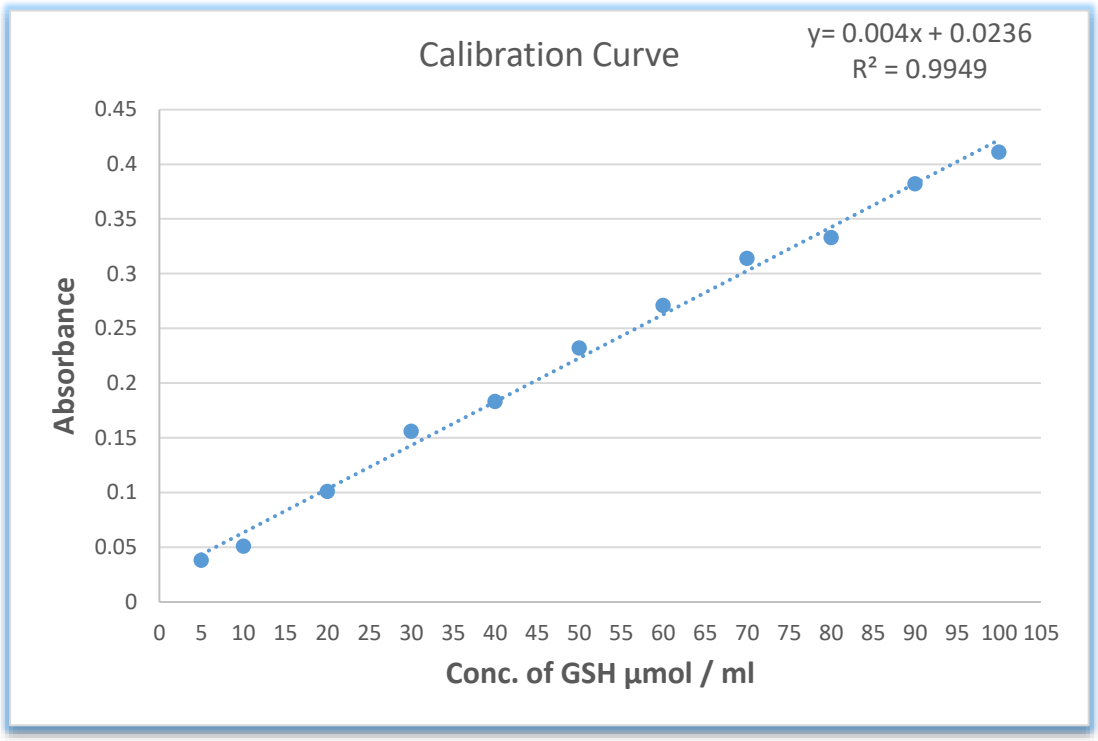


Fig. 2-17: Standard curve between Absorbance and concentration for glutathione (µM) [121].

2.3. Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version V.26.0, for Windows. Data were expressed as the mean values ± standard deviation. Student's t-test was used for normally distributed variables. Relationships between variables were analyzed by Pearson correlation analysis according to the distribution type of parameters. It was considered ($p < 0.05$) to be statistically significant.

3. Results and Discussion

3.1. Characteristics of Patients Group and Control Group

Table 3-1 demonstrates the base line characteristics of the study, which includes the data of the patients group and the control group. It contains the arithmetic results for the measurements of the levels of each of: bone morphogenetic protein2 (BMP-2), interleukin8 (IL-8), malondialdehyde (MDA), total antioxidant capacity assay (TAC), total oxidant status (TOS), , tocopherol (vitamin E) and glutathione (GSH) in the body. In this study the subjects 90 persons (5 - 20) years involve patients groups (60 patient) and its Control groups (30 control).

Table 3-1: Clinical Measurements of the Samples Population

Variables	Group	No.	Mean ± SD	95% confidence interval for Mean		Sig. value
				Upper	Lower	
BMP-2 ng / L	Thalassemia Patient	60	7.04 ± 4.40	8.15	5.92	0.0001
	Control	30	1.88 ± 1.04	2.25	1.51	
IL-8 ng / L	Thalassemia Patient	60	348.8 ± 174.1	392.9	304.8	0.0001
	Control	30	158.5 ± 63.5	181.2	135.7	
MDA mmol / L	Thalassemia Patient	60	3.35 ± 0.60	3.50	3.20	0.0001
	Control	30	1.05 ± 0.12	1.10	1.01	
TAC mmol / L	Thalassemia Patient	60	0.81 ± 0.32	0.89	0.73	0.0001
	Control	30	2.98 ± 1.08	3.36	2.59	
TOS µmol / L	Thalassemia Patient	60	2.85 ± 0.67	3.02	2.68	0.0001
	Control	30	1.35 ± 0.46	1.51	1.18	
Vitamin E (µg / mL)	Thalassemia Patient	60	8.42 ± 1.05	8.69	8.16	0.0001
	Control	30	12.69 ± 1.27	13.15	12.24	
GSH (µg / mL)	Thalassemia Patient	60	1.61 ± 0.23	1.66	1.55	0.0001
	Control	30	3.18 ± 0.27	3.28	3.08	

The following results were obtained for the parameters that were assessed for both patients and controls after the practical portion of the research was carried out:

The levels of malondialdehyde, often known as MAD, were found to be significantly higher in individuals suffering from beta-thalassemia major than in the control group, where ($p<0.05$).

When the levels of total antioxidant capacity assay (TAC) for patients were compared to those for the control group, there was a significant decrease, where ($p<0.05$).

There is a statistically significant rise in the levels of total oxidant status (TOS) in patients compared to control subjects, where ($p<0.05$).

The results of bone morphogenetic protein2 (BMP2) showed an increase values for patients with β-thalassemia major than control group, with significant positive differences ($p<0.05$).

The results of interleukin8 (IL-8) determination of showed an increase values for patients with β-thalassemia major than control group, with significant positive differences ($p<0.05$).

Vitamin E (Tocopherol), which is one of the antioxidants, its levels were observed to decrease in patients with beta thalassemia major compared to the control group, where there is a significant decrease, where ($p<0.05$). There was a statistically significant decrease in the levels of glutathione (GSH), which is also an antioxidant, in patients with beta-thalassemia major as compared to the control groups in the study where ($p<0.05$).

3.2. Bone Morphogenetic Proteins2 (BMP-2) in Beta-Thalassemia Patients Groups and Control Groups

There is a significant increase in the levels of BMP2 levels (7.80, 6.27) for patients groups than for control groups (1.89, 1.87) for males and females, respectively. These results are attributed to the increase in the amount of iron resulting from the periodic exchange of blood, this causes a rise in reactive oxygen species (ROS), factors that are important in controlling osteoclast differentiation [134]. Table (3-2).

Table 3-2: Comparison of statistics results (BMP2 ng \ L)

Variable	Groups	N	Mean ± SD	95% confidence interval for Mean		significant value
				Upper	Lower	
BMP2	(P G1)	30	7.80 ± 4.69	9.48	6.13	0.0001
	(C G2)	15	1.89 ± 1.19	2.49	1.29	
	(P G2)	30	6.27 ± 4.04	7.71	4.82	0.0001
	(C G2)	15	1.87 ± 0.90	2.33	1.42	

BMP2 are a novel therapeutic target for illnesses associated to bone loss since various research clearly reveal its remarkable effectiveness as an osteogenesis inducer. This makes BMP2 a promising candidate for the treatment of these conditions [80].

Autografts, allografts, xenografts, and artificial bones are used to treat bone deformities. Autologous bone grafts are the gold standard for healing and rebuilding bone abnormalities owing to their osteoconductive and osteoinductive qualities. However, limited donor tissue and subsequent damage hinder their utilization. BMP-2 is one of the most essential BMPs and the greatest osteogenic inducer. BMP-2 is the most promising factor for bone tissue engineering and controls fracture healing [135].

(J. Sundermann et al. 2020) showed that bone morphogenetic protein2 (BMP2) has a high tendency to aggregate at physiological pH and physiological ionic strength. The aggregation behavior in differently concentrated BMP2 solutions was investigated using dynamic and static light scattering. It was found that larger aggregates are formed at higher concentrations, but their size goes down again as the concentration goes down. Albumin made the particles easier to dissolve, so they didn't stick together as much. These results help us learn more about how BMP2 dissolves in water, which will help with future drug research and the development of new ways to speed up bone healing [136].

On the other hand, (*J. Sundermann, et al. 2021*), in which they explained that the chitosan Nano-gel coated polycaprolactone fiber implant prototypes with tailored release of bone morphogenic protein2 (BMP-2) a promising approach to achieve implant mediated bone regeneration. When BMP-2 is measured in a medium that also contains solubilizing chemicals, there is a considerable possibility of aggregation-based matrix effects occurring. These effects may lead to much greater recovery rates. The solubilizing additives are responsible for these effects, which may be traced back to them [137].

Because it aids in the process of osteogenic differentiation, bone morphogenic protein2 was an essential component in the reparation of fractured bones [138].

Further research (*R. L. Huang et al., 2014*) shows that an overly inflammatory environment may reduce BMP-2-induced bone mass, by inhibiting osteoblastogenesis-mediated bone. The

short-term and weak osteoinductive potential of BMP-2 in clinical situations may therefore be explained by these facts. In order to increase the osteoinductive effectiveness of BMP-2 in the future, it is important to carefully evaluate the suppression of the inflammatory response during the time of BMP-2/absorbable collagen sponge (ACS) implantation [139].

3.3. Interleukin (IL-8) in Beta-Thalassemia Patients Groups and Control Groups

The data indicate that there is a significant increase in the values of IL-8 for patients when compared with the values of the controls, as it was found that (162.73) for the male control, and (154.20) for the female control. While the levels increased to (384.20) for male patients, and (313.47) for female patients. Because they have inflammatory conditions associated with their situation. Table 3-3.

Table 3-3: Comparison of statistics results (IL-8 ng \ L)

Variable	Groups	N	Mean ± SD	95% confidence interval for Mean		significant value
				Upper	Lower	
IL-8	(P G1)	30	384.20 ± 199.00	455.41	312.99	0.0001
	(C G1)	15	162.73 ± 65.61	195.94	129.52	
	(P G2)	30	313.47 ± 139.63	363.43	263.50	0.0001
	(C G2)	15	154.20 ± 63.29	186.24	122.16	

A variety of medical subspecialties are now using interleukin-8, a promising marker for a wide range of clinical diseases, to either make a quick diagnosis or to forecast the patient's prognosis. IL-8 levels tend to increase due to the frequency of inflammatory disorders; as a result, knowledge of IL-8 levels is critical for creating a connection with the diagnosis of clinical problems [140].

Experiments including the deletion of amino acids and mutagenesis have shown that the sequence Glu-Leu-Arg (ELR), which comes before the first cysteine at the N terminus of interleukin 8 (IL-8), is necessary for receptor interaction and neutrophil activation [141].

Interleukin-8 (IL-8), also known as CXCL8, is a chemokine of the CXC family that stimulates inflammations that are processed by giving rise to a functionally competent protein of 77 amino acids in the case of IL-8 produced by parenchymal cells, and 72 amino acids in the case of the one produced by monocytes and macrophages [140].

Several different types of cells, including monocytes, macrophages, fibroblasts, lymphocytes, vascular endothelial cells, and epithelial cells, are capable of producing the cytokine IL-8. Expansion and infiltration into the intima of human artery smooth muscle cells are facilitated by 1L-8. Even in the presence of blood flow, IL-8 may cause monocytes to adhere firmly to the vascular endothelium. Myocardial damage may be mediated by IL-8 production, which in turn can encourage neutrophil adherence to myocytes [142].

Serum levels of interleukin-8 (IL-8) in patients were found to be statistically substantially greater than those in the control group ($p<0.01$), according to research by (*A. M. Al-gebori, 2012*) [143].

(*L. Gómez-Quiroz et al. 2003*), the researchers investigated that IL-8 is an essential component in the process of recruiting neutrophils to sites of inflammation. Furthermore, the findings of the in vivo investigation demonstrate that oxidative stress has an effect on the production of IL-8. Together, these findings suggest that oxidative stress plays a role in the recruitment of neutrophils to sites of inflammation. In addition, the findings of the study indicated that antioxidants are capable of preventing the generation of the inflammatory cytokine known as interleukin-8 [144].

Neutrophils are pulled to the site of a problem when there is an infection or damage because macrophages, epithelial cells, or endothelial cells produce IL-8, which then leads neutrophils to be attracted to the affected area. As a consequence of the event, there has been a purging of pathogens and an activation of the vascular response [91].

3.4. Measurement of Malondialdehyde (MDA) in the Serum of Patient and Control Groups.

MDA levels were measured, a comparison was made between the group of male patients (PG1) and the male control group for them (CG1), and on the other hand, the comparison was made between the group of female patients (PG2) and the female control group for them (CG2). Table 3-4.

Table 3-4: Comparison of statistics results (MDA mmol \ L) for the groups

Variable	Groups	N	Mean ± SD	95٪ confidence interval for Mean		significant value
				Upper	Lower	
MDA	(P G1)	30	3.50 ± 0.65	3.74	3.27	0.0001
	(C G1)	15	1.09 ± 0.13	1.15	1.02	
	(P G2)	30	3.20 ± 0.50	3.37	3.02	0.0001
	(C G2)	15	1.02 ± 0.10	1.07	0.97	

When comparing malondialdehyde levels for β-thalassemia major patients with control groups, there is a clear difference in the value of the (MDA) concentration of patients (PG1), (PG2) with a higher than the control groups (CG1), (CG2). This means an increase in their oxidation, due to the accumulation of iron in the body as a result of the periodic exchange of blood, according to Haber–Weiss reaction which is mentioned in the introduction chapter.

MDA is a biomarker of oxidative stress that has garnered interest since it can be found in a wide range of biological matrices, including blood, urine, and exhaled breath condensate[122]. One of the helpful techniques to investigate the levels of oxidative stress is the measurement of MDA in blood plasma or tissue homogenates. MDA is a kind of thiobarbituric acid reactive substance (TBARS), which is a measure of lipid peroxidation. MDA levels in various samples, including serum, plasma, and tissues, are measured using a variety of procedures. The technique most often used to determine MDA is the thiobarbituric acid (TBA) test [123].

The level of malondialdehyde (MDA), is a helpful biomarker for oxidative stress. In conclusion, MDA estimates may be used as a trustworthy instrument for determining the amounts of oxidative stress that a system is under and determining how these levels are related to a variety of illness patterns [124].

While when comparing the group of male patients (PG1) with the group of female patients (PG2), there are no significant moral differences. The same applies to the comparison between the control groups (CG1) and (CG2).

3.5. Oxidant-Antioxidant System

3.5.1. Measurement of Total Antioxidant Capacity Assay (TAC) in the Serum of Patient and Control Groups.

Table (3-3) illustrate there was a significant decrease of total antioxidants in the groups of patients with beta thalassemia (PG1) and (PG2) than in the control groups (CG1) and (CG2).

While it was found that there is no significant relationship of total antioxidants Assay between groups of male patients (PG1) with female patients (PG2). The same result to the male control groups (CG1) with women (CG2), and there is no significant relationship between them.

Table 3-5: Comparison of statistics results (TAC mmol \ L)

Variable	Groups	N	Mean ± SD	95٪ confidence interval for Mean		significant value
				Upper	Lower	
TAC	(P G1)	30	0.77 ± 0.33	0.88	0.65	0.0001
	(C G1)	15	2.79 ± 1.08	3.34	2.24	
	(P G2)	30	0.86 ± 0.31	0.98	0.75	0.0001
	(C G2)	15	3.17 ± 1.07	3.71	2.63	

The term "total antioxidant capacity," or TAC, refers to a number of different compounds that work together to shield biological systems against harmful reactive oxygen species (ROS). Enzyme systems (such as catalase, glutathione peroxidase, and superoxide dismutase, among others), small molecules (such as ascorbate, uric acid, glutathione, vitamin E, and others), and proteins are the three groups that make up antioxidant species (albumin, transferrin, etc.) [125].

A study introduced by (*E. Soeizi et al. 2017*) demonstrated that the pathophysiology of beta-thalassemia major was characterized by iron overload as well as severe oxidative stress. They discovered that the total antioxidant capacity (TAC) of the control group was 2.08±0.35 nmol/l [126].

Another team consisting of (*I. Tsamesidis et al. 2017*) showed that beta thalassemia patients' TAC levels were lower compared to the control groups according to a study conducted in Italy and Greece. In each of the two mentioned countries, the values for patient groups compared to control

groups were (1.83 mmol/l vs. 2.7 mmol/l) and (2.42 mmol/l vs. 3.2 mmol/l), respectively [127].

According to the findings of a group of researchers (*A. Hadzovic-Dzuvo et al. 2011*), a number of different diseases have been associated with an imbalance in the oxidant and antioxidant levels of cells (oxidative stress) [128].

3.5.2. Measurement of Total Oxidant Status (TOS) in the Serum of Patient and Control Groups

There are significant changes in the levels of total oxidative status (TOS) between groups of patients (PG1) and (PG2) and between groups of controls (CG1) and (CG2).

There is no significant correlation when comparing groups of male patients (PG1) with female patients (PG2). Also, there was no significant correlation when comparing the male control group (CG1) with the female control group (CG2).

Table 3-6: Comparison of statistics results (TOS μmol \ L)

Variable	Groups	N	Mean ± SD	95% confidence interval for Mean		significant value
				Upper	Lower	
TOS	(P G1)	30	2.90 ± 0.74	3.17	2.64	0.0001
	(C G2)	15	1.34 ± 0.45	1.57	1.12	
	(P G2)	30	2.79 ± 0.60	3.01	2.58	0.0001
	(C G2)	15	1.35 ± 0.48	1.59	1.11	

The total oxidant status, also known as TOS, is often used to determine the overall level of oxidation that has occurred inside the body, while the total antioxidant capacity, also known as TAC, is utilized to

determine the overall level of antioxidant activity. In addition, the oxidative stress index (OSI), which measures the ratio of total oxidant status (TOS) to total antioxidant capacity assay (TAC), is thought to be a more accurate indicator of oxidative stress [129].

In a study conducted by (*M. Asif et al. 2015*), Individuals beta-thalassemia had values for their total oxidant status that were considerably greater compared to those of healthy children ($P<0.01$) [130].

In another study conducted by (*A. Cakmak et al. 2010*), It was found that those who had a severe form of beta thalassemia had significantly elevated levels of total oxidant status (TOS) ($P<0.0001$) [131].

Oxidative stress, which is defined as an imbalance between pro-oxidant reactive species and antioxidant molecules, both endogenous and exogenous, has been linked to a number of non-communicable diseases, including insulin resistance and diabetes, atherosclerosis, autoimmune diseases, various cancers, and aging as a physiological process. Numerous clinical studies were developed to examine various ways for oxidative status improvement in afflicted persons, such as various treatment approaches to the underlying disorders or vitamin therapy. Due to the widespread interest in oxidative stress and antioxidants, various commercial test kits for assessing oxidative stress become available [132].

The total oxidant status (TOS) was significantly higher in patient cases compared to healthy controls. This parameter expresses the total oxidative content in the biological material and may indicate increased formation of free radicals in patients [133].

3.6. Vitamin E in Beta-Thalassemia Patients Groups and Control Groups

There was a significant decrease in the levels of tocopherol (vitamin E) in patients with beta-thalassemia major than in the control groups. Table 3-7.

Table 3-7: Comparison of statistics results (Vitamin E µg / mL)

Variable	Groups	N	Mean ± SD	95% confidence interval for Mean		significant value
				Upper	Lower	
Vitamin E	(P G1)	30	8.18 ± 1.20	8.61	7.75	0.0001
	(C G1)	15	12.35 ± 1.23	12.97	11.72	
	(P G2)	30	8.66 ± 0.83	8.96	8.37	0.0001
	(C G2)	15	13.04 ± 1.24	13.67	12.41	

The fat-soluble antioxidant vitamin E, also known as alpha-tocopherol, is an important micronutrient that has been hypothesized to have a role in protecting tissues against excessive lipid peroxidation. This vitamin also plays a crucial role in the protein function and gene modulation processes. The metabolism of vitamin E is dependent on hepatic binding proteins, which preferentially retain dietary alpha-tocopherol for incorporation into nascent VLDL and tissue distribution coupled with esters cholesterol and triglycerides [145].

Vitamin E is an essential antioxidant that plays a significant role in preventing peroxidation of unsaturated fatty acids that are found in lipid membranes [45].

Because it interacts with peroxyl radicals more quickly than the molecules of polyunsaturated fatty acids, the lipid-soluble antioxidant vitamin E plays a significant role in the mitochondrial membranes. As a result, the mitochondrial membranes are shielded from excessive oxidative damage [146].

Antioxidants, such as vitamins C and E, are essential for good health because they play a vital role in alleviating the detrimental effects of stressors caused by both biotic and abiotic agents [147].

Enzymatic antioxidants like catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase, as well as non-enzymatic antioxidants like reduced glutathione (GSH) and vitamins A, C, and E, are very good at getting rid of ROS that are made in the body tissue. In order to stop the cellular damage brought on by free radical-modified lipid peroxidation, antioxidants are crucial. There is a stable equilibrium in normal metabolism between free radical production and the antioxidant defense system [148].

NADPH provides electrons to many of the mitochondrial antioxidants that detoxify ROS, including glutathione and thioredoxin that further donate electrons to peroxiredoxins, vitamin E and vitamin C [149]. Figure 3-1.

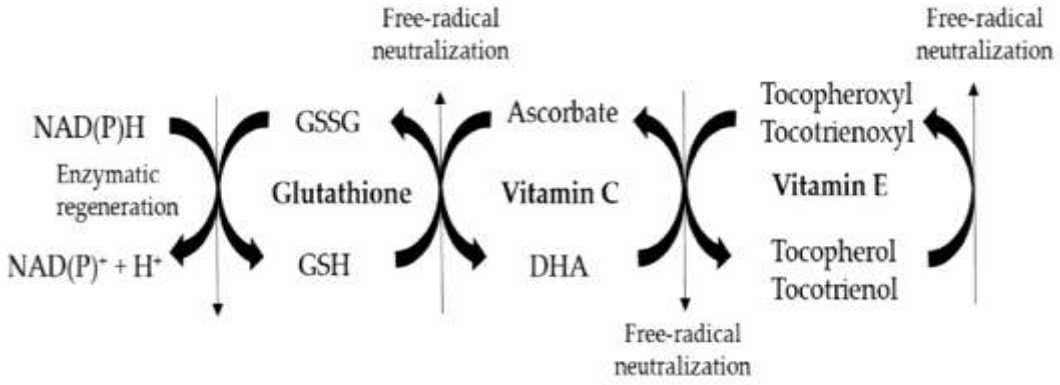


Fig. 3-1: Schematic illustration of the antioxidant network including vitamin E, vitamin C, and glutathione in the neutralization of free radicals. GSSG: glutathione disulfide; GSH: reduced glutathione; DHA: dehydroascorbate (reduced vitamin C) [150].

3.7. Glutathione (GSH) in Beta-Thalassemia Patients Groups and Control Groups

As can be seen in the table below, the levels of glutathione in patients suffering from beta thalassemia major were found to be considerably lower (in a negative sense) than those in the control cases. These findings were shown in the table 3-8.

Table 3-8: Comparison of statistics results Glutathione (GSH µg / mL)

Variable	Groups	N	Mean ± SD	95% confidence interval for Mean		Significant value
				Upper	Lower	
GSH	(P G1)	30	1.62 ± 0.23	1.71	1.54	0.0001
	(C G1)	15	3.21 ± 0.27	3.35	3.08	
	(P G2)	30	1.59 ± 0.23	1.67	1.51	0.0001
	(C G2)	15	3.00 ± 0.28	3.14	2.86	

All organisms need glutathione, a crucial chemical, in both their main and secondary metabolism. The cellular redox state may be determined by looking at the glutathione redox status. Cysteine, glutamic acid, and glycine make up the tripeptide known as reduced glutathione (GSH), which is essential for controlling signaling, detoxification, and a number of other cellular functions [71].

The key function of GSH molecules is the maintenance of protein structure and function, the regulation of protein synthesis and degradation, the maintenance of immune function, protection against oxidative damage, and detoxification of reactive chemicals. GSH molecule also plays role in immune function. Increasing the level of intracellular GSH increases the level of TAC [68].

Due to the presence of the sulfhydryl (-SH) group, the tripeptide thiol glutathione, also known as GSH, has the ability to readily donate

electrons. Glutathione is a vital water-phase antioxidant that is also a necessary component for antioxidant enzymes. It not only protects mitochondria from endogenous oxygen radicals, but it also protects other parts of the cell from free radicals. Because of its high electron-donating capacity and its high intracellular concentration, GSH has a significant amount of reducing power. This power is used to manage a complicated thiol-exchange system (—SH ⇄ S-S—), which involves the exchange of thiols [151].

Glutathione is a part of the enzymatic antioxidant system and plays an important role in a number of fundamental physiological processes. These processes include the detoxification of endogenous compounds and xenobiotics; the transport and storage of cysteine, which modulates immune function; and the synthesis, repair, and expression of DNA [152].

In a study conducted by (*A. H. Alta'ee et al. 2015*), it was shown that the levels of glutathione in the serum were considerably lower ($p<0.05$) in patients of both sexes suffering from a variety of disorders that are caused by oxidative stress [153].

The data of a research study that was carried out by (*M. Ibraheem et al. 2019*) showed that there was a significant increase in the GSH level for the group of patients when compared to the group that served as the control group, where ($p\leq0.01$) [154].

In a study that was carried out by (*C. Arana et al. 2017*), the researchers observed that the ratio of GSSG to GSH in the patients who participated in their research was significantly greater than in the control group that was also being investigated ($p<0.001$) [155].

3.8. Correlation Analysis

Table 3-9 shows correlations between the measured parameters of the samples under study.

Table 3-9: A correlations (r) between the measured parameters

sequence	variables against each other	Correlation (r)
1	MDA vs TAC	-0.88
2	MDA vs TOS	0.87
3	TAC vs TOS	-0.82
4	BMP2 vs MDA	0.84
5	BMP2 vs TAC	-0.77
6	BMP2 vs TOS	0.89
7	IL-8 vs MDA	0.77
8	IL-8 vs TAC	-0.70
9	IL-8 vs TOS	0.76
10	IL-8 vs BMP2	0.84
11	Vit. E vs MDA	-0.90
12	Vit. E vs TAC	0.83
13	Vit. E vs TOS	-0.82
14	Vit. E vs BMP2	-0.82
15	Vit. E vs IL-8	-0.76
16	GSH vs MDA	-0.67
17	GSH vs TAC	0.65
18	GSH vs TOS	-0.64
19	GSH vs BMP2	-0.60
20	GSH vs IL-8	-0.53
21	GSH vs Vit. E	0.80

Correlation values are clearly manifested between each of the studied assays, which means that there are correlations between these parameters.

This study showed that there is an inverse (negative) correlation between the level of MDA and the level of TAC in patients with beta thalassemia major, where (r = -0.88). Figure 3-2 Appendix4. While this study showed that there is a direct (positive) correlation between the levels of MDA and TOS in patients, where (r = 0.87). Figure 3-3 Appendix4.

Also, it was noticed that there is a strong (negative) direct relationship between the conc. of TOS and the conc. of TAC, where (r = - 0.82). As one goes up, the other goes down, and vice versa. Figure 3-4 Appendix4.

There is a positive (positive) correlation between the levels of (BMP2) and (MDA), where (r = 0.84). Figure 3-5 Appendix4. It is clear that there is a strong (negative) direct relationship between the concentration of both BMP2 and TAC (r = -0.77). Figure 3-6 Appendix4. It is clear that there is a strong (positive) direct correlation between Concentration BMP2 and concentration of TOS (r = + 0.89). Figure 3-7 Appendix4.

There is a positive (positive) correlation between the levels of (IL-8) and (MDA), where (r = 0.77). Figure 3-8 Appendix4. While it was noted that there is a strong (negative) direct relationship between the concentration of IL-8 and TAC (r = - 0.70). Figure 3-9 Appendix4. It was noticed that there is a strong (positive) direct relationship between the concentration of IL-8 and TOS (r = + 0.76). Figure 3-10 Appendix4. It was noticed that there is a strong (positive) direct relationship between the concentration of IL-8 and BMP2 (r = + 0.84). Figure 3-11 Appendix4.

There is an inverse (negative) correlation between the levels of (Vitamin E) and (MDA), where (r = -0.90). Figure 3-12 Appendix4. There is a direct (positive) correlation between the levels of (Vitamin E) and (TAC), where (r = 0.83). Figure 3-13 Appendix4. There is an inverse (negative) correlation between the levels of (Vitamin E) and (TOS), where (r = -0.82). Figure 3-14 Appendix4. Also, there is an inverse (negative) correlation between the levels of (Vitamin E) and (BMP2), where (r = -0.82). Figure 3-15 Appendix4. There is an inverse (negative) correlation between the levels of (Vitamin E) and (IL-8), where (r = -0.76). Figure 3-16 Appendix4.

The study found that there is an inverse (negative) correlation between levels of (GSH) and (MDA), where (r = - 0.67). Figure 3-17 Appendix4. The study also found that there is a direct (positive) correlation between the levels of (GSH) and (TAC), where (r = 0.65). Figure 3-18 Appendix4. While the study found that there is an inverse (negative) correlation between the levels of (GSH) and (TOS), where (r = -0.64). Figure 3-19 Appendix4. It was noted that there is an inverse (negative) correlation between concentrations of (GSH) and (BMP2), where (r = -0.60). Figure 3-20 Appendix4.

It was also noted that there is an inverse (negative) correlation between concentrations of (GSH) and (IL-8), where (r = -0.53). Figure 3-21 Appendix4. While it was noted that there is a direct (positive) correlation between concentrations of (GSH) and (Vitamin E), where (r = 0.80). Figure 3-22 Appendix4.

From the above it is clear that each of the studied parameters is related to the other, so it can be said that the levels of one of them depend on each other.

3.9. Effect of Splenectomy on Patients with β-Thalassemia Major

As for the patients themselves, of both sexes, they were divided into two groups, one of which was the splenectomy group and the other was the no splenectomy group. Statistics were performed for the parameters that were measured for them to show the comparison. Table 3-10.

Table 3-10: Comparison between the splenectomy and no splenectomy groups

Variables	Group	No.	Mean ± SD	95% confidence interval for Mean		P value
				Upper	Lower	
MDA	no splenectomy	40	3.01 ± 0.37	3.13	2.90	0.0001
	splenectomy	20	4.02 ± 0.33	4.17	3.88	
BMP2	no splenectomy	40	4.26 ± 1.34	4.70	3.82	0.0001
	splenectomy	20	12.59 ± 2.72	13.78	11.39	
IL-8	no splenectomy	40	249.66 ± 82.78	275.32	224.01	0.0001
	splenectomy	20	547.18 ± 134.05	605.93	488.42	
TOS	no splenectomy	40	2.45 ± 0.38	2.57	2.34	0.0001
	splenectomy	20	3.63 ± 0.36	3.79	3.47	
TAC	no splenectomy	40	0.97 ± 0.24	1.04	0.89	0.0001
	splenectomy	20	0.50 ± 0.21	0.59	0.40	
Vitamin E	no splenectomy	40	8.97 ± 0.71	9.15	8.79	0.0001
	splenectomy	20	7.33 ± 0.72	7.46	7.02	
GSH	no splenectomy	40	1.69 ± 0.20	1.74	1.64	0.0001
	splenectomy	20	1.43 ± 0.18	1.51	1.35	

It is clear that the patients who had their spleen removed had a significant increase in the parameters of (MDA), (BMP2), (IL-8) and (TOS), where ($p<0.05$). While they had a significant decrease in (TAC), (Vitamin E) and (GSH), where ($p<0.05$). It is evident that the presence or absence of the spleen affects the levels of the assays.

The spleen is an intricately divided lymphoid organ with a complex cellular and vascular structure. The spleen performs a number of physiological functions, including as the phagocytosis of old erythrocytes and platelets, iron recycling, inducing an immune response to blood antigens, and protection against pathogenic invaders like bacteria, fungi, viruses, and other infectious agents. Splenomegaly may be brought on by liver disorders, cancer, metabolic problems, infections, and inflammation[156]. The spleen is an unique organ that aids in the efficient phagocytosis of erythrocytes and the recycling of iron, as well as the capture and removal of pathogens and the development of adaptive immune responses [157].

Splenic iron overload is the clinical condition that occurs most often in people who have thalassemia. An excess of iron in the spleen is linked to an overload in the liver and a high serum ferritin level. Splenectomy results in an enlarged liver and may increase the amount of iron that is stored in the heart [158].

Splenectomy generates a chain reaction of hemodynamic changes, most notably a decrease in portal vein blood flow and constriction of that vein. Having a greater platelet count after hypersplenism remits renders one more prone to thrombosis. These routes may contribute to the recurrence of portal hypertensive disorders, affecting the prognosis of patients [156].

There are several reasons to have a splenectomy, from trauma to hematological disorders. Surgical and immunological issues can arise after splenectomy. One of the most dreaded complications with a high death rate is a severe post-splenectomy infection. Patients who have had a spleen removed or who are hyposplenism are more susceptible to a variety of problems, from surgical to major immunological and septic ones [159].

The clinical picture in a number of medical illnesses may become much worse if the spleen is present, whether it is morphologically and physiologically healthy or diseased. Splenectomy, however, should only be done after carefully weighing the patient's possible benefits against the short- and long-term dangers. The underlying splenectomy indication appears to be more responsible for this risk than the splenectomy itself [160].

A splenectomy should not be done on a patient until after the potential benefits for the patient have been thoroughly weighed against the risks, both in the short-term and the long-term [160].

It is now more vital than ever to manage splenic injuries without resorting to surgery because of the knowledge that splenectomy may put patients at risk of developing more serious complications [161].

Conclusions

A clinical study was conducted for the levels of many parameters, some of which were high and some were significantly lower than the control, due to iron accumulation and oxidative stress. It as follows:

1- It was found that the concentration of BMP2 (which is a factor to promote the differentiation of stem cells into fibroblasts and chondrocytes) increases significantly in patients so as to enhance its role.
2- It was found that the level of (IL-8) is much higher in patients than it is in healthy ones, so as to enhance its role, as it is elevated for many inflammatory conditions as an immune signal, it stimulates chemotaxis towards target cells and a potent stimulator of angiogenesis.
3- On the other hand, it was observed that the levels of both malondialdehyde and total oxidant status increased, while total antioxidants capacity assay decreased in patients compared to controls, which indicates the accumulation of iron in them as a result of periodic blood transfusions.
4- Significant decrease in the levels of vitamin E and glutathione, which are antioxidants, in patients than in healthy.
5- There are no significant differences between groups of males with females, whether for patients or for controls.
6- There are correlations between each of the measured parameters, some of which are direct and inverse, which means that the level of any of them is proportional to the level of the other. Thus, one of them can be reliable in guessing the other.
7- It was also found that patients who splenectomy had increased levels of (BMP2), (IL-8), (MDA) and (TOS) and decreased levels of (TAC), (Vitamin E) and (GSH) compared to patients who no splenectomy. Oxidative stress is the culprit in all of this.

Recommendations

- It is necessary for patients to adhere to iron chelating treatments to reduce iron accumulation as much as possible.
- Development of studies towards the use of bone morphogenetic protein2 as a treatment for patients with beta thalassemia, as it promotes the differentiation of stem cells into osteoblasts and chondrocytes.
- Conducting another study on patients with beta thalassemia on other types of bone morphogen proteins, such as (BMP6) as it plays in Controls iron homeostasis via regulation of hepcidin. Or (BMP8a) which is involved in bone and cartilage development.
- Paying attention to the health of the spleen because of its extreme importance in order to avoid the consequences of its complications
- It the necessary done a hematology test before marriage to reduce the spread thalassemia.

References

[1] M. Meuwly and M. Karplus, “The functional role of the hemoglobin-water interface,” *Mol. Aspects Med.*, vol. 84, pp. 1–22, 2022, doi: 10.1016/j.mam.2021.101042.

[2] S. Carne, “Estimation of Haemoglobin,” *Br. Med. J.*, vol. 1, no. 5429, p. 252, 1965, doi: 10.1136/bmj.1.5429.252-c.

[3] S. S. Last, “Iron : Guidelines to improve your child ’ s intake,” pp. 1–6, 2020.

[4] R. C. Hardison, “Globin Genes: Evolution,” *eLS*, pp. 1–7, 2006, doi: 10.1038/npg.els.0005134.

[5] A. Cao and R. Galanello, “Beta-thalassemia,” *Genet. Med.*, vol. 12, no. 2, pp. 61–76, 2010, doi: 10.1097/GIM.0b013e3181cd68ed.

[6] T. Korolnek, I. Hamza, C. C. Philpott, and J. G. Quigley, “Like iron in the blood of the people: the requirement for heme trafficking in iron metabolism,” 2014, doi: 10.3389/fphar.2014.00126.

[7] L. Cook, G. Brewer, and W. Wong-Ng, “Structural aspects of porphyrins for functional materials applications,” *Crystals*, vol. 7, no. 7, 2017, doi: 10.3390/cryst7070223.

[8] J. M. Orten, “Metabolism of hemoglobin and bile pigments.,” *Ann. Clin. Lab. Sci.*, vol. 1, no. 2, pp. 113–124, 1971.

[9] B. H. Billing, “Bilirubin metabolism,” *Postgrad. Med. J.*, vol. 39, no. 450, pp. 176–187, 1963, doi: 10.1136/pgmj.39.450.176.

[10] B. LisaM MD. VanWagner, MSc M Richard M. Green, MD “Estimation of Serum Bilirubin Levels and Related Clinical Cases.”

[11] H. Kamil, “Hemoglobin formation,” *Univ. babylon*, no. Figure 2, pp. 1–

5, 2017, [Online]. Available: http:// www.uobabylon.edu.iq/eprints/publication _11_29502_626.pdf.

[12] P. Slusarczyk and K. Mleczko-Sanecka, “The multiple facets of iron recycling,” *Genes (Basel).*, vol. 12, no. 9, 2021, doi: 10.3390/genes12091364.

[13] M. D. Cappellini, D. Farmakis, J. Porter, A. Taher, and J. Taher, “Guidelines for the Management of Transfusion Dependent Thalassaemia 4th Edition,” 2021.

[14] F. Shafique *et al.*, “Thalassemia, a human blood disorder,” *Brazilian J. Biol.*, vol. 83, pp. 1–8, 2023, doi: 10.1590/1519-6984.246062.

[15] C. Ullrich, “Chapter 1 - Introduction,” *Unlawful Content Online*, pp. 31–58, 2021, doi: 10.5771/9783748927051-31.

[16] V. Cappellini, Cohen A, Porter. J, Taher.A, “Genetic Basis, Pathophysiology and diagnosis of thalassemia,” *Guidel. Manag. Transfus. Depend. Thalass.*, no. 20, pp. 186–190, 2014, [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK269382/.

[17] I. Cantú and S. Philipsen, “Flicking the switch: Adult hemoglobin expression in erythroid cells derived from cord blood and human induced pluripotent stem cells,” *Haematologica*, vol. 99, no. 11, pp. 1647–1649, 2014, doi: 10.3324/haematol.2014.116483.

[18] A. Wahed and A. Quesada, *Hematology and Coagulation: A Comprehensive Review for Board Preparation, Certification and Clinical Practice*. 2019.

[19] M. D. Cappellini, “The Thalassemias,” *Goldman’s Cecil Med. Twenty Fourth Ed.*, vol. 1, no. November 2017, pp. 1060–1066, 2012, doi: 10.1016/B978-1-4377-1604-7.00165-2.

[20] A. A. Sherif, A. G'Medhin, F. Tadesse, and T. Tsegaye, "Thalassemia syndrome," *Ethiop. Med. J.*, vol. 52, no. 1, pp. 43–47, 2014, doi: 10.5005/jp/books/12955_8.

[21] L. E. Bernini and C. L. Harteveld, "α-Thalassaemia," *Baillieres. Clin. Haematol.*, vol. 11, no. 1, pp. 53–90, 1998, doi: 10.1016/S0950-3536(98)80070-X.

[22] R. Galanello and A. Cao, "Alpha-thalassemia," *Genet. Med.*, vol. 13, no. 2, pp. 83–88, 2011, doi: 10.1097/GIM.0b013e3181fcb468.

[23] W. State Department of Health - Newborn Screening Program, "Hemoglobin Bart's and Alpha Thalassemia Fact Sheet - Washington State Dept of Health," [Online]. Available: http://www.doh.wa.gov/Portals/1/Documents/5220/HbBartFactSheet.pdf.

[24] S. Schrier, "Alpha Thalassemia Trait," *ASH Image Bank*, vol. 2002, no. 0409, pp. 100327–100327, 2002, doi: 10.1182/ashimagebank-2002-100327.

[25] S. Ünal *et al.*, "Türkiye'de hemoglobin H hastalığı: Sekiz merkezin deneyimi," *Turkish J. Hematol.*, vol. 33, no. 1, pp. 56–59, 2016, doi: 10.4274/tjh.2014.0461.

[26] R. Galanello and R. Origa, "Open Access REVIEW BioMed Central Beta-thalassemia," *Orphanet J. Rare Dis.*, vol. 5, p. 11, 2010, [Online]. Available: http://www.ojrd.com/content/5/1/11.

[27] H. L. Muncie and J. S. Campbell, "Alpha and beta thalassemia," *Am. Fam. Physician*, vol. 80, no. 4, 2009.

[28] J. W. NYIKAL, "Guidelines for the appropriate use of opioid analgesics among patients with persistent noncancer pain have been developed,"

Inpharma Wkly., vol. NA;, no. 1444, p. 3, 2004, doi: 10.2165/00128413-200414440-00005.

[29] G. Bellis and A. Parant, "Beta-thalassemia in Mediterranean countries. Findings and outlook," *Investig. Geogr.*, vol. 2022, no. 77, pp. 129–138, 2022, doi: 10.14198/INGEO.19079.

[30] F. Jawad, "Iron in the Human Body," *J. Pakistan Med. Assoc.*, pp. 332–334, 1996.

[31] J. Clifford, K. Niebaum, and L. Bellows, "Iron: An Essential Nutrient," *Food Nutr. Ser.*, 2015, [Online]. Available: https://extension.colostate.edu/docs/pubs/foodnut/09356.pdf.

[32] E. C. Theil and J.-F. J.-F. Briat, "Plant ferritin and non-heme iron nutrition in humans," *Harvest. Tech. Monogr. 1*, 2004.

[33] A. Jackson, Scientific Advisory Commitee of Nutrition, "Iron and Health 2010," 2010.

[34] Nutrition Australia, "Fact sheet - Iron," *Nutr. Aust.*, 2014, [Online]. Available: http://www.nutritionaustralia.org/sites/default/files/Iron-2014.pdf.

[35] J. L. Beard, H. Dawson, and D. J. Piñero, "Iron metabolism: A comprehensive review," *Nutr. Rev.*, vol. 54, no. 10, pp. 295–317, 1996, doi: 10.1111/j.1753-4887.1996.tb03794.x.

[36] A. Yiannikourides and G. Latunde-Dada, "A Short Review of Iron Metabolism and Pathophysiology of Iron Disorders," *Medicines*, vol. 6, no. 3, p. 85, 2019, doi: 10.3390/medicines6030085.

[37] Ng Chee Ping, "NIH Public Access," *Bone*, vol. 23, no. 1, pp. 1–7, 2013, doi: 10.1002/cphy.c120016.Iron.

[38] M. P. Mims and J. T. Prchal, "Divalent metal transporter 1,"

Hematology, vol. 10, no. 4, pp. 339–345, 2005, doi: 10.1080/10245330500093419.

[39] N. Scheers, "Regulatory effects of Cu, Zn, and Ca on Fe absorption: The intricate play between nutrient transporters," *Nutrients*, vol. 5, no. 3, pp. 957–970, 2013, doi: 10.3390/nu5030957.

[40] P. Hahn *et al.*, "Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 101, no. 38, pp. 13850–13855, 2004, doi: 10.1073/pnas.0405146101.

[41] C. Wong, "Iron deficiency anaemia," *Paediatr. Child Heal. (United Kingdom)*, vol. 27, no. 11, pp. 527–529, 2017, doi: 10.1016/j.paed.2017.08.004.

[42] G. Keerthivasan, A. Wickrema, and J. D. Crispino, "Erythroblast enucleation," *Stem Cells Int.*, no. January 2011, 2011, doi: 10.4061/2011/139851.

[43] Z. D. Zhou and E. K. Tan, "Iron regulatory protein (IRP)-iron responsive element (IRE) signaling pathway in human neurodegenerative diseases," *Mol. Neurodegener.*, vol. 12, no. 1, pp. 1–12, 2017, doi: 10.1186/s13024-017-0218-4.

[44] P. Ponka, M. J. Koury, and A. D. Sheftel, *Erythropoiesis, Hemoglobin Synthesis, and Erythroid Mitochondrial Iron Homeostasis*, no. April 2016. 2013.

[45] C. Wong, "Iron deficiency anaemia," *Paediatr. Child Heal. (United Kingdom)*, vol. 27, no. 11, pp. 527–529, 2017, doi: 10.1016/j.paed.2017.08.004.

[46] S. Recalcati and G. Cairo, "Macrophages and iron: A special

relationship," *Biomedicines*, vol. 9, no. 11, 2021, doi: 10.3390/biomedicines9111585.

[47] N. Sukhbaatar and T. Weichhart, "Iron regulation: Macrophages in control," *Pharmaceuticals*, vol. 11, no. 4, 2018, doi: 10.3390/PH11040137.

[48] T. Ganz, "Macrophages and systemic iron homeostasis," *J. Innate Immun.*, vol. 4, no. 5–6, pp. 446–453, 2012, doi: 10.1159/000336423.

[49] J. Kwapisz, A. Slomka, and E. Zekanowska, "Hepcidin and Its Role in Iron Homeostasis.," *Ejifcc*, vol. 20, no. 2, pp. 124–8, 2009, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/27683336%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4975279.

[50] T. Ganz, "Systemic iron homeostasis," *Physiol. Rev.*, vol. 93, no. 4, pp. 1721–1741, 2013, doi: 10.1152/physrev.00008.2013.

[51] E. E. Johnson, A. Sandgren, B. J. Cherayil, M. Murray, and M. Wessling-Resnick, "Role of ferroportin in macrophage-mediated immunity," *Infect. Immun.*, vol. 78, no. 12, pp. 5099–5106, 2010, doi: 10.1128/IAI.00498-10.

[52] A. Pietrangelo, "Iron and the liver," *Liver Int.*, vol. 36, no. November 2015, pp. 116–123, 2016, doi: 10.1111/liv.13020.

[53] E. Buzzetti, P. Ventura, and E. Corradini, "Iron in Porphyrias: Friend or Foe?," *Diagnostics*, vol. 12, no. 2, 2022, doi: 10.3390/diagnostics12020272.

[54] R. Mariani, P. Trombini, M. Pozzi, and A. Piperno, "Iron metabolism in thalassemia and sickle cell disease," *Mediterr. J. Hematol. Infect. Dis.*, 2009, doi: 10.4084/mjhid.2009.006.

[55] A. K. Mishra and A. Tiwari, “Iron overload in Beta thalassaemia major and intermedia patients.,” *Maedica (Buchar).*, vol. 8, no. 4, pp. 328–32, 2013, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/24790662%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3968466.

[56] M. Domellö, K. G. Dewey, B. Lö, R. J. Cohen, and O. Hernell, “Community and International Nutrition The Diagnostic Criteria for Iron Deficiency in Infants Should Be Reevaluated 1,” 2002. [Online]. Available: https://academic.oup.com/jn/article/132/12/3680/4712110.

[57] M. D. Cappellini, “Exjade® (deferasirox, ICL670) in the treatment of chronic iron overload associated with blood transfusion,” *Ther. Clin. Risk Manag.*, vol. 3, no. 2, pp. 291–299, 2007, doi: 10.2147/tcrm.2007.3.2.291.

[58] F. Bazvand *et al.*, “Total antioxidant status in patients with major β-thalassemia,” *Iran. J. Pediatr.*, vol. 21, no. 2, pp. 159–165, 2011.

[59] W. H. Koppenol, “The Haber-Weiss cycle - 70 years later,” *Redox Rep.*, vol. 6, no. 4, pp. 229–234, 2001, doi: 10.1179/135100001101536373.

[60] J. P. Kehrer, “The Haber-Weiss reaction and mechanisms of toxicity,” *Toxicology*, vol. 149, no. 1, pp. 43–50, 2000, doi: 10.1016/S0300-483X(00)00231-6.

[61] E. Akyol, Z. Selamoglu, H. Dogan, H. Akgul, and A. Unalan, “Determining the total antioxidant status and oxidative stress indexes of honey samples obtained from different phytogeographical regions in Turkey,” *Fresenius Environ. Bull.*, vol. 24, no. 4, pp. 1204–1208, 2015.

[62] A. A. F. I. M. S. TAMER A. EL-BEDEWY, M.D. and E. H. E.-S. M. D. AMAL HELMY ABD EL-HAMEED, M.D., “The Oxidant and Antioxidant Status in b-Thalassemia Major Patients,” *Med. J. Cairo*

Univ., vol. 86, no. March, pp. 367–374, 2018, doi: 10.21608/mjcu.2018.55135.

[63] J. C. Wood *et al.*, "Relationship between labile plasma iron, liver iron concentration and cardiac response in a deferasirox monotherapy trial," *Haematologica*, vol. 96, no. 7, pp. 1055–1058, 2011, doi: 10.3324/haematol.2010.032862.

[64] L. C. Seaver and J. A. Imlay, "Are respiratory enzymes the primary sources of intracellular hydrogen peroxide?," *J. Biol. Chem.*, vol. 279, no. 47, pp. 48742–48750, 2004, doi: 10.1074/jbc.M408754200.

[65] A. Agarwal *et al.*, "Oxidative stress in human reproduction: Shedding light on a complicated Phenomenon," *Oxidative Stress Hum. Reprod. Shedding Light a Complicat. Phenom.*, no. May, pp. 1–190, 2017, doi: 10.1007/978-3-319-48427-3.

[66] G. Pizzino *et al.*, "Oxidative Stress: Harms and Benefits for Human Health," *Oxid. Med. Cell. Longev.*, vol. 2017, 2017, doi: 10.1155/2017/8416763.

[67] A. Ecz, "VITAMIN E AS AN ANTIOXIDANT Bilgehan DOĞRU PEKİNER Ankara University , Faculty of Pharmacy , Department of Biochemistry 06100 Tandoğan , Ankara , TURKEY," vol. 32, no. 4, pp. 243–267, 2003.

[68] O. Atakisi *et al.*, "Effects of reduced glutathione on nitric oxide level, total antioxidant and oxidant capacity and adenosine deaminase activity," *Eur. Rev. Med. Pharmacol. Sci.*, vol. 14, no. 1, pp. 19–23, 2010.

[69] T. Takeshima *et al.*, "Oxidative stress and male infertility," *Reprod. Med. Biol.*, vol. 20, no. 1, pp. 41–52, 2021, doi: 10.1002/rmb2.12353.

[70] A. Kumar, A. Prasad, and P. Pospíšil, "Formation of α-tocopherol hydroperoxide and α-tocopheroxyl radical: relevance for photooxidative stress in Arabidopsis," *Sci. Rep.*, vol. 10, no. 1, pp. 1–12, 2020, doi: 10.1038/s41598-020-75634-0.

[71] G. Salbitani, C. Bottone, and S. Carfagna, "Determination of Reduced and Total Glutathione Content in Extremophilic Microalga Galdieria phlegrea," *Bio-Protocol*, vol. 7, no. 13, pp. 2–6, 2017, doi: 10.21769/bioprotoc.2372.

[72] A. Sharma and H. Sharma, "Bone Morphogenetic Proteins: An Overview," *Ann. Appl. Bio-Sciences*, vol. 4, no. 2, pp. R35–R37, 2017, doi: 10.21276/aabs.1336.

[73] H. K. M. Saad *et al.*, "Activation of STAT and SMAD Signaling Induces Hepcidin Re-Expression as a Therapeutic Target for-Thalassemia Patients," *Biomedicines*, vol. 10, no. 1, 2022, doi: 10.3390/biomedicines10010189.

[74] M. Partridge, M. R. Green, J. D. Langdon, and M. Feldmann, "Production of TGF-α and TGF-β by cultured keratinocytes, skin and oral squamous cell carcinomas - Potential autocrine regulation of normal and malignant epithelial cell proliferation," *Br. J. Cancer*, vol. 60, no. 4, pp. 542–548, 1989, doi: 10.1038/bjc.1989.310.

[75] D. Y. Shu and F. J. Lovicu, "Insights into bone morphogenetic protein—(BMP-) signaling in ocular lens biology and pathology," *Cells*, vol. 10, no. 10, pp. 1–26, 2021, doi: 10.3390/cells10102604.

[76] J. Nickel and T. D. Mueller, "Specification of BMP Signaling," *Cells*, vol. 8, no. 12, 2019, doi: 10.3390/cells8121579.

[77] A. Hata and Y. G. Chen, "TGF-β signaling from receptors to smads," *Cold Spring Harb. Perspect. Biol.*, vol. 8, no. 9, 2016, doi:

10.1101/cshperspect.a022061.

[78] F. Xu, C. Liu, D. Zhou, and L. Zhang, "TGF-β/SMAD Pathway and Its Regulation in Hepatic Fibrosis," *J. Histochem. Cytochem.*, vol. 64, no. 3, pp. 157–167, 2016, doi: 10.1369/0022155415627681.

[79] A. Mesa Restrepo, J. Fernando Alzate, and E. B. Patiño Gonzalez, "Bone morphogenetic protein 2: heterologous expression and potential in bone regeneration," *Actual. Biológicas*, vol. 43, no. 114, 2021, doi: 10.17533/udea.acbi.v43n114a01.

[80] C. C. Mandal, F. Das, S. Ganapathy, S. E. Harris, G. G. Choudhury, and N. Ghosh-Choudhury, "Bone morphogenetic protein-2 (BMP-2) activates NFATc1 transcription factor via an autoregulatory loop involving Smad/Akt/Ca2+ signaling," *J. Biol. Chem.*, vol. 291, no. 3, pp. 1148–1161, 2016, doi: 10.1074/jbc.M115.668939.

[81] V. E. Growth *et al.*, "Bone Morphogenetic Protein 2 Foreign Body Reaction and Stem Cell Responses," 2016.

[82] O. Brien, "This is a repository copy of Delivery of the improved BMP-2-Advanced plasmid DNA within a gene-activated scaffold accelerates mesenchymal stem cell osteogenesis and critical size defect repair. White Rose Research Online URL for this paper: Version ," 2018.

[83] D. Halloran, H. W. Durbano, and A. Nohe, "Developmental review bone morphogenetic protein-2 in development and bone homeostasis," *J. Dev. Biol.*, vol. 8, no. 3, pp. 28–30, 2020, doi: 10.3390/JDB8030019.

[84] A. Lisberg *et al.*, "Mathematical modeling of the effects of CK2.3 on mineralization in osteoporotic bone," *CPT Pharmacometrics Syst. Pharmacol.*, vol. 6, no. 3, pp. 208–215, 2017, doi: 10.1002/psp4.12154.

[85] M. C. Cohen and S. Cohen, "Cytokine function: A study in biologic

diversity," *Am. J. Clin. Pathol.*, vol. 105, no. 5, pp. 589–598, 1996, doi: 10.1093/ajcp/105.5.589.

[86] J. Testar, "Cytokines : Introduction Cytokines : Introduction cont .," *Imp. Coll. London,* 2019, [Online]. Available: http://bitesized.immunology.org/receptors-and-molecules/cytokines/.

[87] C. A. Dinarello, "Historical insights into cytokines," *Eur. J. Immunol.*, vol. 37, no. SUPPL. 1, pp. 34–45, 2007, doi: 10.1002/eji.200737772.

[88] A. Khadka, "Interleukins in therapeutics," *PharmaTutor*, vol. 2, no. 4, pp. 67–72, 2014.

[89] M. Akdis *et al.*, "Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases," *J. Allergy Clin. Immunol.*, vol. 138, no. 4, pp. 984–1010, 2016, doi: 10.1016/j.jaci.2016.06.033.

[90] E. Cells, T. Necrosis, F. Alpha, and P. Cytokine, "Interleukin 8 The Use of Biomarkers for Detection of Early- and Late-Onset Neonatal Sepsis Biomarkers in Burn Patient Care Adult and Fetal," vol. 8, 2012.

[91] A. Filimon, I. A. Preda, A. F. Boloca, and G. Negroiu, "Interleukin-8 in melanoma pathogenesis, prognosis and therapy—an integrated view into other neoplasms and chemokine networks," *Cells*, vol. 11, no. 1, 2022, doi: 10.3390/cells11010120.

[92] K. Jundi and C. M. Greene, "Transcription of interleukin-8: How altered regulation can affect cystic fibrosis lung disease," *Biomolecules*, vol. 5, no. 3, pp. 1386–1398, 2015, doi: 10.3390/biom5031386.

[93] E. A. Rachmilewitz and P. J. Giardina, "How I treat thalassemia," *Blood*, vol. 118, no. 13, pp. 3479–3488, 2011, doi: 10.1182/blood-2010-08-300335.

[94] The Blood Safety and Conservation Team, "Blood Transfusion Policy and Procedures," *Oxford Univ. Hosp.*, vol. 4, no. June 2012, pp. 1–32, 2012.

[95] A. Manuscript, "Author Manuscript," pp. 31–35, 2011, doi: 10.1111/j.1749-6632.2010.05585.x.Hepcidin.

[96] S. Sheth, "Iron chelation: An update," *Curr. Opin. Hematol.*, vol. 21, no. 3, pp. 179–185, 2014, doi: 10.1097/MOH.0000000000000031.

[97] D. Bellotti and M. Remelli, "Deferoxamine b: A natural, excellent and versatile metal chelator," *Molecules*, vol. 26, no. 11, pp. 1–21, 2021, doi: 10.3390/molecules26113255.

[98] J. L. Hamilton and J. N. Kizhakkedathu, "Polymeric nanocarriers for the treatment of systemic iron overload," *Mol. Cell. Ther.*, vol. 3, no. 1, 2015, doi: 10.1186/s40591-015-0039-1.

[99] G. J. M. Bruin *et al.*, "Pharmacokinetics, distribution, metabolism, and excretion of deferasirox and its iron complex in rats," *Drug Metab. Dispos.*, vol. 36, no. 12, pp. 2523–2538, 2008, doi: 10.1124/dmd.108.022962.

[100] H. Fernandez, "Blood and Marrow Stem Cell Transplantation," *Leuk. Lymphoma Sosiety*, pp. 1–72, 2018.

[101] N. Schaap, "Stem cell transplantation," *HemaSphere*, vol. 3, no. S2, p. 163, 2019, doi: 10.1097/HS9.0000000000000266.

[102] American Cancer Society, "How Stem Cell and Bone Marrow Transplants Are Used to Treat Cancer," *Cancer.Org*, pp. 1–46, 2020, [Online]. Available: https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/stem-cell-transplant/why-stem-cell-transplants-are-used.html.

[103] A. Transplants, T. Central, and V. Catheter, "FACT SHEET : Thalassemia & Hematopoietic Stem Cell Transplants," pp. 1–4. Children's Hospital & Research Center at Oakland, 2001.

[104] R. H. Fu *et al.*, "Differentiation of stem cells: Strategies for modifying surface biomaterials," *Cell Transplant.*, vol. 20, no. 1, pp. 37–47, 2011, doi: 10.3727/096368910X532756.

[105] H. Jessop *et al.*, "General information for patients and carers considering haematopoietic stem cell transplantation (HSCT) for severe autoimmune diseases (ADs): A position statement from the EBMT Autoimmune Diseases Working Party (ADWP), the EBMT Nurses Group, the EBMT Pati," *Bone Marrow Transplant.*, vol. 54, no. 7, pp. 933–942, 2019, doi: 10.1038/s41409-019-0430-7.

[106] A. Zeb and F. Ullah, "A Simple Spectrophotometric Method for the Determination of Thiobarbituric Acid Reactive Substances in Fried Fast Foods," *J. Anal. Methods Chem.*, vol. 2016, 2016, doi: 10.1155/2016/9412767.

[107] S. Alkass, S. Yousif, M. Hamid, and A. Alhamadany, "Effect of Cyclosporine on the Relevant Biochemical Parameters in Renal Transplantation Patients in Mosul/Iraq," *J. Educ. Sci.*, vol. 26, pp. 210–220, Dec. 2013, doi: 10.33899/edusj.2013.163070.

[108] A. Athab and A. Abbas, "The Effect of Obesity on Lipocalin-2 and Adipolin Levels in Women with Type II Diabetes Mellitus in Babylon Governorate." 2021.

[109] C. P. Rubio, J. Hernández-Ruiz, S. Martinez-Subiela, A. Tvarijonaviciute, and J. J. Ceron, "Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: An update," *BMC Vet. Res.*, vol. 12, no. 1, pp. 1–7, 2016, doi: 10.1186/s12917-016-0792-7.

[110] M. Shamsipur, M. Roushani, S. M. Pourmortazavi, and N. Shahabadi, "Amperometric determination of sulfide ion by glassy carbon electrode modified with multiwall carbon nanotubes and copper (II) phenanthroline complex," *Cent. Eur. J. Chem.*, vol. 12, no. 10, pp. 1091–1099, 2014, doi: 10.2478/s11532-014-0552-0.

[111] I. G. Munteanu and C. Apetrei, "Analytical methods used in determining antioxidant activity: A review," *Int. J. Mol. Sci.*, vol. 22, no. 7, 2021, doi: 10.3390/ijms22073380.

[112] Hasan Karsen, "Serum paraoxonase and arylesterase activities and oxidant status in patients with brucellosis," *African J. Microbiol. Res.*, vol. 5, no. 13, pp. 1–6, 2011, doi: 10.5897/ajmr11.438.

[113] C. P. Rubio and J. J. Cerón, "Spectrophotometric assays for evaluation of Reactive Oxygen Species (ROS) in serum: general concepts and applications in dogs and humans," *BMC Vet. Res.*, vol. 17, no. 1, pp. 1–13, 2021, doi: 10.1186/s12917-021-02924-8.

[114] H. Baser *et al.*, "Serum total oxidant/anti-oxidant status,ischemia-modified albumin and oxidized-low density lipoprotein levels in patients with vitamin D deficiency," *Arch. Endocrinol. Metab.*, vol. 59, no. 4, pp. 318–324, 2015, doi: 10.1590/2359-3997000000055.

[115] H. Bone, M. Protein, and B.-E. Kit, "Human Bone Morphogenetic Protein 2, BMP-2 ELISA Kit," pp. 1–8.

[116] H. Interleukin and I.-E. Kit, "Human Interleukin 35, IL-35 ELISA Kit," pp. 2–7.

[117] J. G. Jargar, S. H. Hattiwale, S. Das, S. A. Dhundasi, and K. K. Das, "A modified simple method for determination of serum α-tocopherol (Vitamin E)," *J. Basic Clin. Physiol. Pharmacol.*, vol. 23, no. 1, pp. 45–48, 2012, doi: 10.1515/jbcpp-2011-0033.

[118] R. YAMAUCHI, “Vitamin E: Mechanism of Its Antioxidant Activity.,” *Food Sci. Technol. Int. Tokyo*, vol. 3, no. 4, pp. 301–309, 1997, doi: 10.3136/fsti9596t9798.3.301.

[119] W. Rungratanawanich, M. Memo, and D. Uberti, “Redox homeostasis and natural dietary compounds: Focusing on antioxidants of rice (oryza sativa L.),” *Nutrients*, vol. 10, no. 11, pp. 1–19, 2018, doi: 10.3390/nu10111605.

[120] F. A. Jabar, “How To Understand The Relationship Between Oxidative Stress , Non Enzymatic Antioxidant And Trace Elements In Diabetic Patients .,” vol. 5, no. 2, 2007.

[121] P. Monostori, G. Wittmann, E. Karg, and S. Túri, “Determination of glutathione and glutathione disulfide in biological samples: An in-depth review,” *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, vol. 877, no. 28, pp. 3331–3346, 2009, doi: 10.1016/j.jchromb.2009.06.016.

[122] V. Turcu *et al.*, “Towards Reference Values for Malondialdehyde on Exhaled Breath Condensate: A Systematic Literature Review and Meta-Analysis,” *Toxics*, vol. 10, no. 5, p. 258, 2022, doi: 10.3390/toxics10050258.

[123] M. Khoubnasab Jafari, K. Ansarin, and A. Jouyban, “Comments on ‘use of malondialdehyde as a biomarker for assesing oxidative stress in different disease pathologies: A review,’” *Iran. J. Public Health*, vol. 44, no. 5, pp. 714–715, 2015.

[124] Z. Singh, I. P. Karthigesu, P. Singh, and R. Kaur, “Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: A review,” *Iran. J. Public Health*, vol. 43, no. 3, pp. 7–16, 2014.

[125] A. Rahnama, S. A. Razavi, M. Hedayati, and N. Razmi, “Total

Antioxidant Capacity Determination with Colorimetric Method and Microplate Reading Format and Comparison with Chemilumin Essence Assay," *Eur. Online J. Nat. Soc. Sci.*, vol. 4, no. 4, pp. 875–881, 2015.

[126] E. Soeizi, M. Rafraf, M. Asghari-Jafarabadi, A. Ghaffari, A. Rezamand, and F. Doostan, "Effects of green tea on serum iron parameters and antioxidant status in patients with β-thalassemia major," *Pharm. Sci.*, vol. 23, no. 1, pp. 27–36, 2017, doi: 10.15171/PS.2017.05.

[127] I. Tsamesidis *et al.*, "Total antioxidant capacity in Mediterranean β-thalassemic patients," *Adv. Clin. Exp. Med.*, vol. 26, no. 5, pp. 789–793, 2017, doi: 10.17219/acem/63746.

[128] A. Hadžović-Džuvo *et al.*, "Serum total antioxidant capacity in patients with multiple sclerosis," *Bosn. J. Basic Med. Sci.*, vol. 11, no. 1, pp. 33–36, 2011, doi: 10.17305/bjbms.2011.2620.

[129] X. F. Du, L. L. Zhang, D. Z. Zhang, L. Yang, Y. Y. Fan, and S. P. Dong, "Clinical significance of serum total oxidant/antioxidant status in patients with operable and advanced gastric cancer," *Onco. Targets. Ther.*, vol. 11, pp. 6767–6775, 2018, doi: 10.2147/OTT.S153946.

[130] M. Asif, Z. Manzoor, M. S. Farooq, S. H. Munawar, A. Aziz, and I. A. Khan, "Status of oxidant, antioxidantand serum enzymes in thalassaemic children receiving multiple blood transfusions," *J. Pak. Med. Assoc.*, vol. 65, no. 8, pp. 838–843, 2015.

[131] A. Cakmak, M. Soker, A. Koc, and N. Aksoy, "Prolidase activity and oxidative status in patients with thalassemia major," *J. Clin. Lab. Anal.*, vol. 24, no. 1, pp. 6–11, 2010, doi: 10.1002/jcla.20361.

[132] E. H. J. M. Jansen and T. Ruskovska, "Comparative analysis of serum (Anti)oxidative status para{cyrillic}meters in healthy persons," *Int. J. Mol. Sci.*, vol. 14, no. 3, pp. 6106–6115, 2013, doi:

10.3390/ijms14036106.

[133] B. Choroma, “Plasma and Urine Total Antioxidant Capacity in Patients With Adrenal Tumors.”, 2020.

[134] T. S. Agidigbi and C. Kim, “Reactive Oxygen Species in Osteoclast Differentiation and Possible Pharmaceutical Targets of ROS-Mediated Osteoclast Diseases,” *Int. J. Mol. Sci.*, vol. 20, no. 14, 2019, doi: 10.3390/ijms20143576.

[135] J. Su, H. Xu, J. Sun, X. Gong, and H. Zhao, “Dual delivery of BMP-2 and bFGF from a new nano-composite scaffold, loaded with vascular stents for large-size mandibular defect regeneration,” *Int. J. Mol. Sci.*, vol. 14, no. 6, pp. 12714–12728, 2013, doi: 10.3390/ijms140612714.

[136] J. Sundermann, H. Zagst, J. Kuntsche, H. Wätzig, and H. Bunjes, “Bone morphogenetic protein 2 (BMP-2) aggregates can be solubilized by albumin—investigation of BMP-2 aggregation by light scattering and electrophoresis,” *Pharmaceutics*, vol. 12, no. 12, pp. 1–20, 2020, doi: 10.3390/pharmaceutics12121143.

[137] J. Sundermann, S. Sydow, L. Burmeister, A. Hoffmann, H. Menzel, and H. Bunjes, “Elisa-and activity assay-based quantification of bmp-2 released in vitro can be biased by solubility in ‘physiological’ buffers and an interfering effect of chitosan,” *Pharmaceutics*, vol. 13, no. 4, 2021, doi: 10.3390/pharmaceutics13040582.

[138] L. C. Ingwersen, M. Frank, H. Naujokat, K. Loger, R. Bader, and A. Jonitz-Heincke, “BMP-2 Long-Term Stimulation of Human Pre-Osteoblasts Induces Osteogenic Differentiation and Promotes Transdifferentiation and Bone Remodeling Processes,” *Int. J. Mol. Sci.*, vol. 23, no. 6, 2022, doi: 10.3390/ijms23063077.

[139] R. L. Huang, Y. Yuan, J. Tu, G. M. Zou, and Q. Li, “Exaggerated

inflammatory environment decreases BMP-2/ACS-induced ectopic bone mass in a rat model: Implications for clinical use of BMP-2," *Osteoarthr. Cartil.*, vol. 22, no. 8, pp. 1186–1196, 2014, doi: 10.1016/j.joca.2014.06.017.

[140] A. Shahzad, M. Knapp, I. Lang, and G. Köhler, "Interleukin 8 (IL-8)-a universal biomarker?," *Int. Arch. Med.*, vol. 3, no. 1, 2010, doi: 10.1186/1755-7682-3-11.

[141] I. Clark-Lewis, B. Dewald, T. Geiser, B. Moser, and M. Baggiolini, "Platelet factor 4 binds to interleukin 8 receptors and activates neutrophils when its N terminus is modified with Glu-Leu-Arg," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 90, no. 8, pp. 3574–3577, 1993, doi: 10.1073/pnas.90.8.3574.

[142] R. H. Zhou, Q. Shi, H. Q. Gao, and B. J. Shen, "Changes in serum interleukin-8 and interleukin-12 levels in patients with ischemic heart disease in a Chinese population.," *J. Atheroscler. Thromb.*, vol. 8, no. 1, pp. 30–32, 2001, doi: 10.5551/jat1994.8.30.

[143] A. M. Al-gebori, "Levels of Interleukine-8 and Some Antioxidants in Serum of Patients with Osteoarthritis .," vol. 11, no. 4, pp. 845–849, 2012.

[144] L. Gómez-Quiroz *et al.*, "Interleukin 8 response and oxidative stress in HepG2 cells treated with ethanol, acetaldehyde or lipopolysaccharide," *Hepatol. Res.*, vol. 26, no. 2, pp. 134–141, 2003, doi: 10.1016/S1386-6346(03)00010-X.

[145] F. Galli *et al.*, "Vitamin E (Alpha-Tocopherol) Metabolism and Nutrition in Chronic Kidney Disease," *Antioxidants*, vol. 11, no. 5, p. 989, 2022, doi: 10.3390/antiox11050989.

[146] G. Napolitano, G. Fasciolo, S. Di Meo, and P. Venditti, "Vitamin e

supplementation and mitochondria in experimental and functional hyperthyroidism: A mini-review," *Nutrients*, vol. 11, no. 12, 2019, doi: 10.3390/nu11122900.

[147] Z. W. Zhang *et al.*, "Vitamin e is superior to vitamin c in delaying seedling senescence and improving resistance in arabidopsis deficient in macro-elements," *Int. J. Mol. Sci.*, vol. 21, no. 19, pp. 1–17, 2020, doi: 10.3390/ijms21197429.

[148] R. Yanardag, O. Ozsoy-Sacan, S. Ozdil, and S. Bolkent, "Combined effects of vitamin C, vitamin E, and sodium selenate supplementation on absolute ethanol-induced injury in various organs of rats," *Int. J. Toxicol.*, vol. 26, no. 6, pp. 513–523, 2007, doi: 10.1080/10915810701707296.

[149] W. M. Curtis, W. A. Seeds, M. P. Mattson, and P. C. Bradshaw, "NADPH and Mitochondrial Quality Control as Targets for a Circadian-Based Fasting and Exercise Therapy for the Treatment of Parkinson ' s Disease," 2022.

[150] L. Pangrazzi, L. Balasco, and Y. Bozzi, "Natural antioxidants: A novel therapeutic approach to autism spectrum disorders?," *Antioxidants*, vol. 9, no. 12, pp. 1–16, 2020, doi: 10.3390/antiox9121186.

[151] P. M. Kidd, "Glutathione: Systemic protectant against oxidative and free radical damage," *Altern. Med. Rev.*, vol. 2, no. 3, pp. 155–176, 1997.

[152] S. Parida and S. H. E. Kaufmann, "tuberculosis Ac ce p te d us cr ip t Ac ce p te d us cr t," pp. 1–39, 2018, doi: 10.1093/asj/sjy179/5059000.

[153] A. H. Alta, "Serum Glutathione as Biomarker for Oxidative Stress in Health and Diseases Serum Glutathione as Biomarker for Oxidative Stress in Health and Diseases," no. February, 2015.

[154] M. Ibraheem, L. Mustaf, and N. Salih, "Measurement of Some Antioxidants in Serum of Infected Pregnant Women with Toxoplasmosis," *J. Educ. Sci.*, vol. 28, no. 4, pp. 108–115, 2019, doi: 10.33899/edusj.1970.163329.

[155] C. Arana *et al.*, "Increased salivary oxidative stress parameters in patients with type 2 diabetes: Relation with periodontal disease," *Endocrinol. Diabetes y Nutr.*, vol. 64, no. 5, pp. 258–264, 2017, doi: 10.1016/j.endinu.2017.03.005.

[156] C. Yang *et al.*, "Effect of splenectomy on the outcomes in patients with cirrhosis receiving transjugular intrahepatic portosystemic shunt," *J. Gastroenterol. Hepatol.*, vol. 36, no. 10, pp. 2893–2902, 2021, doi: 10.1111/jgh.15543.

[157] R. E. Mebius and G. Kraal, "Structure and function of the spleen," *Nat. Rev. Immunol.*, vol. 5, no. 8, pp. 606–616, 2005, doi: 10.1038/nri1669.

[158] M. G. Çetinçakmak *et al.*, "Evaluation of the relationship between splenic iron overload and liver, heart and muscle features evident on T2-weighted magnetic resonance imaging," *Adv. Clin. Exp. Med.*, vol. 29, no. 4, pp. 475–480, 2020, doi: 10.17219/acem/116758.

[159] J. Turek, Z. Krska, M. Pesková, and J. Výborný, "Complications of splenectomy," *Bratisl. Lek. Listy*, vol. 99, no. 12, pp. 689–691, 1998, doi: 10.33545/surgery.2020.v4.i2d.420.

[160] E. P. Weledji, "Benefits and risks of splenectomy," *Int. J. Surg.*, vol. 12, no. 2, pp. 113–119, 2014, doi: 10.1016/j.ijsu.2013.11.017.

[161] A. Cadili and C. de Gara, "Complications of Splenectomy," *Am. J. Med.*, vol. 121, no. 5, pp. 371–375, 2008, doi: 10.1016/j.amjmed.2008.02.014.

Appendixes

Appendix 1

Criteria sheet of the patients

Republic of Iraq
Al-Najaf Al-Ashraf Governorate
Najaf Health Directorate
Training and Human Development Center
No.
Date:

جمهورية العراق
محافظة النجف الاشرف
مركز التدريب و التنمية البشرية
العدد: ٥٤٥١٩
التاريخ ٢٠٢١/١١/٢٥

إلى/ جامعة بابل / كلية العلوم / قسم علوم الكيمياء

م / تسهيل مهمة

تحية طيبة ...

إشارة إلى كتابكم ذي العدد ٤١٤٩ في ٢٠٢١/١٤/١١ بخصوص تسهيل مهمة الباحث طالب الماجستير (محمد قاسم مجدي هاني) للحصول على الموافقة الاخلاقية لإجراء البحث الموسوم:

A clinical study for B-Thalassemia major patients through bone morphogenetic protien , Interleukin 8 (CXCL 8) and oxidant - antioxidant status

حصلت موافقة اللجنة العلمية للبحوث في مركز دائرتنا على إجراء البحث في (مستشفى الزهراء التعليمي / مركز الثلاسيميا) في دائرتنا مع التأكيد على الالتزام الكامل بتعليمات السلامة الحيوية والضوابط الاخلاقية والحصول على موافقة المشاركين قبل الشروع بالبحث والحفاظ على خصوصيتهم وعدم افشاء البيانات او استخدام العينات لغير اغراض البحث العلمي ... على أن لا تتحمل دائرتنا أية تبعات مادية ..للتفضل بالاطلاع ..... مع الاحترام.

ملاحظة :
تم استيفاء أجور جباية البحوث والبالغة (١٠٠٠٠) عشرة الاف دينار بموجب الوصل المرقم (٤٨٠٢٢٤) في ٢٠٢١/٢٢/١١

الدكتور
رضوان كامل الكندي
المدير العام
٢٠٢١ / ١١ /

نسخة منه الى/
- مكتب المدير العام / للعلم مع الاحترام .
- مركز التدريب و التنمية البشرية / مع الأوليات .
- مستشفى الزهراء التعليمي / مركز الثلاسيميا ..... تسهيل مهمة الباحث مع التقدير.

تسلسل الاستمارة ٣٥٨

Appendix 2

Criteria sheet of the patients

Criteria for patients with β-Thalassemia major	
Name	
Sex	
Age	
Urban or Rural	
Splenectomy or No Splenectomy	
Vitamin C regulated	
Folic Acid regulated	
Chelating agent	
Transfusion through	
Another disease	
Weight	
Height	

Appendix 3

Pictures from the practical side when estimating by ELISA kits in the laboratory

Appendix 4

Diagram of the correlation between each of the measured parameters

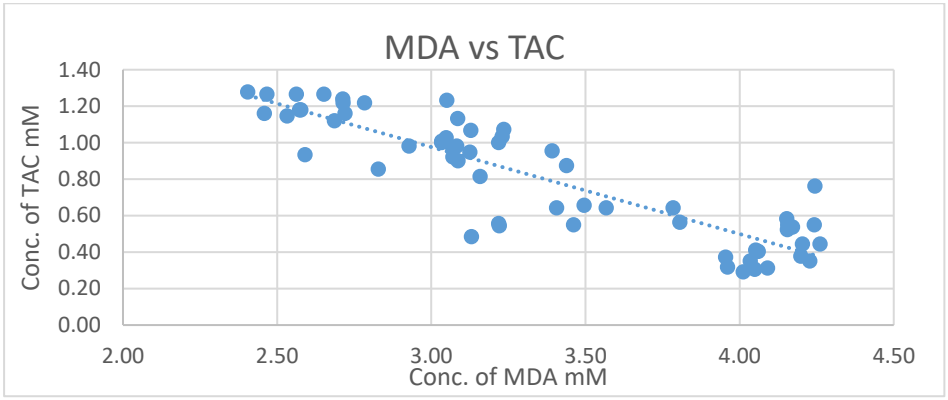


Fig. (3-2): Diagram of the relationship of conc. MDA (mM) to conc. TAC (mM).

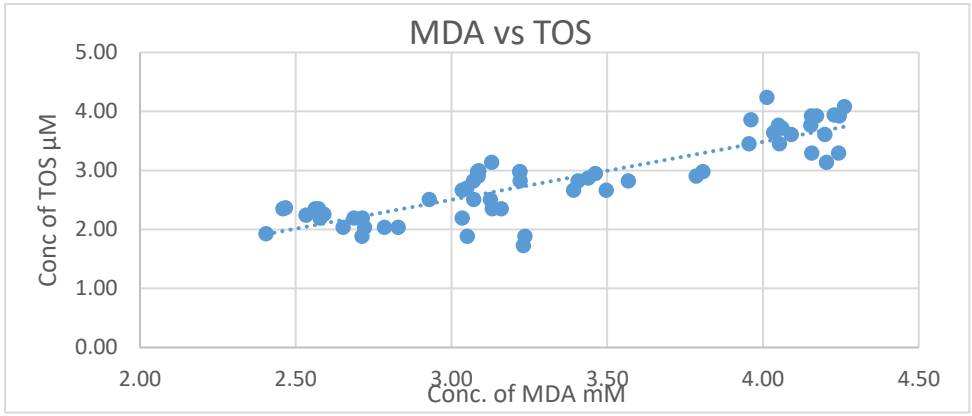


Fig. (3-3): Diagram of the relationship of conc. MDA (mM) to conc. TOS (µM).

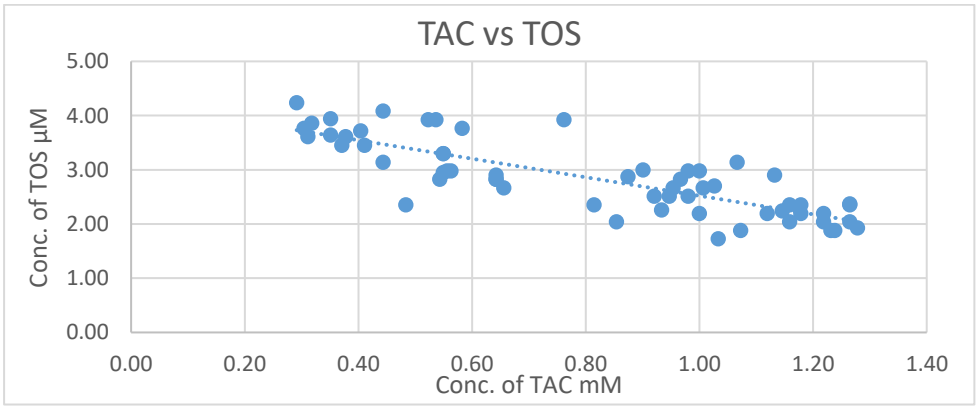


Fig. (3-4): Diagram of the relationship of conc. TAC (mM) to conc. TOS (µM).

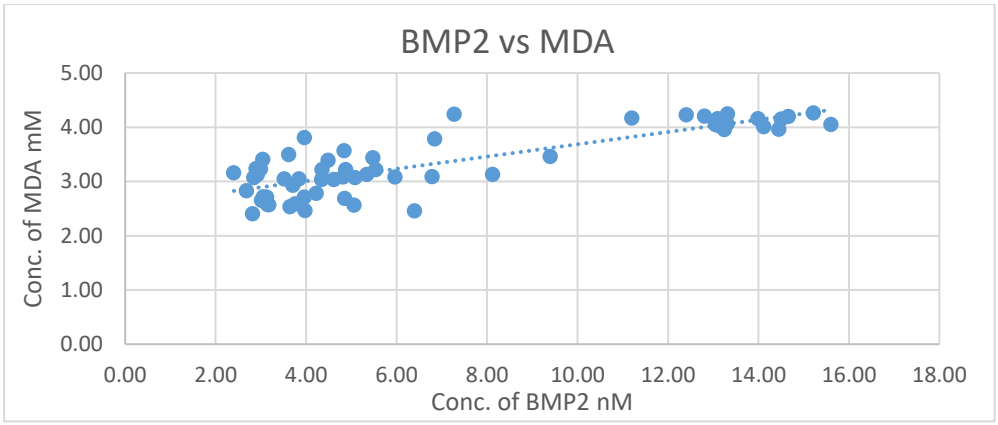


Fig. (3-5): Diagram of the relationship of conc. BMP2 (nM) to conc. MDA (mM).

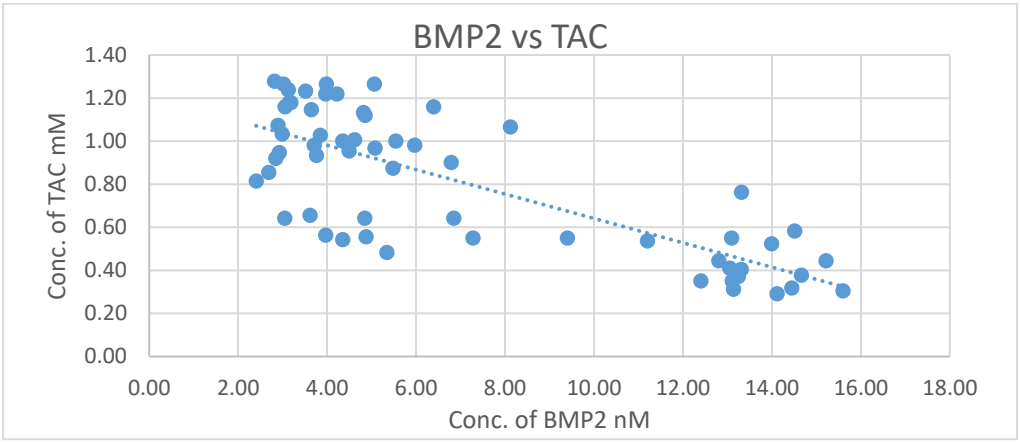


Fig. (3-6): Diagram of the relationship of conc. BMP2 (nM) to conc. TAC (mM).

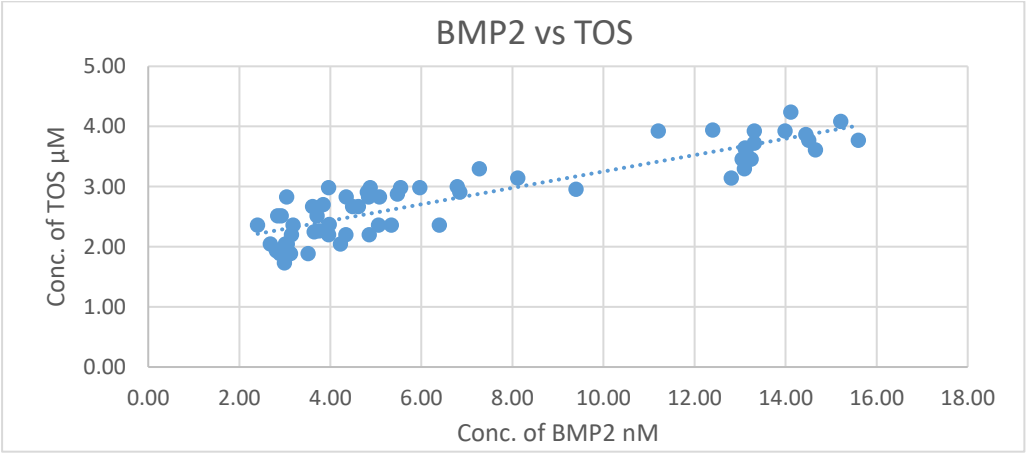


Fig. (3-7): Diagram of the relationship of conc. BMP2 (nM) to conc. TOS (µM).

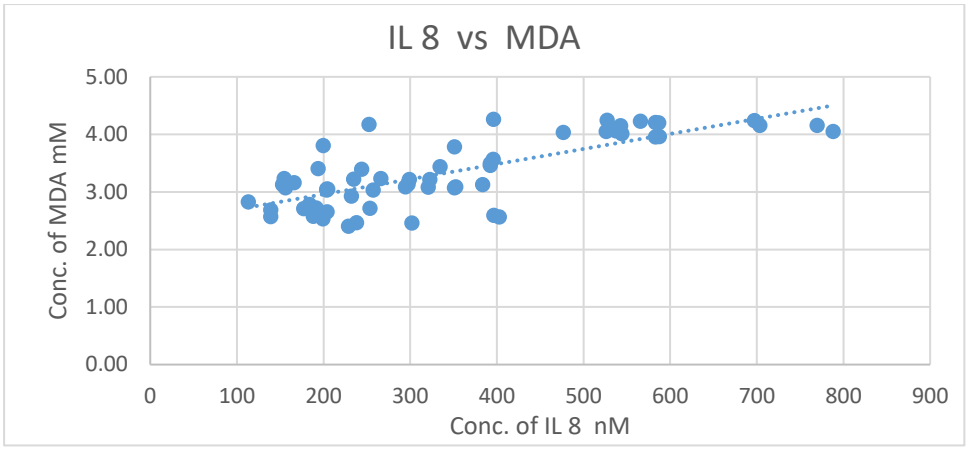


Fig. (3-8): Diagram of the relationship of conc. IL-8 (nM) to conc. MDA (mM).

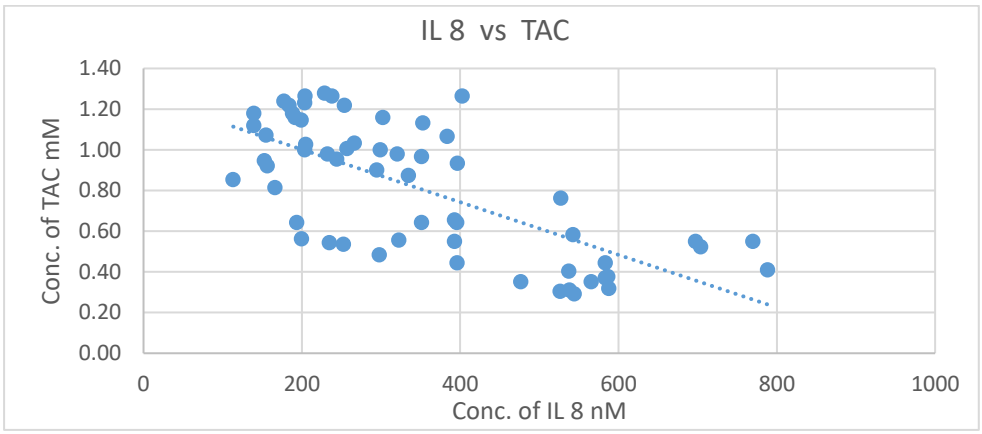


Fig. (3-9): Diagram of the relationship of conc. IL-8 (nM) to conc. TAC (mM).

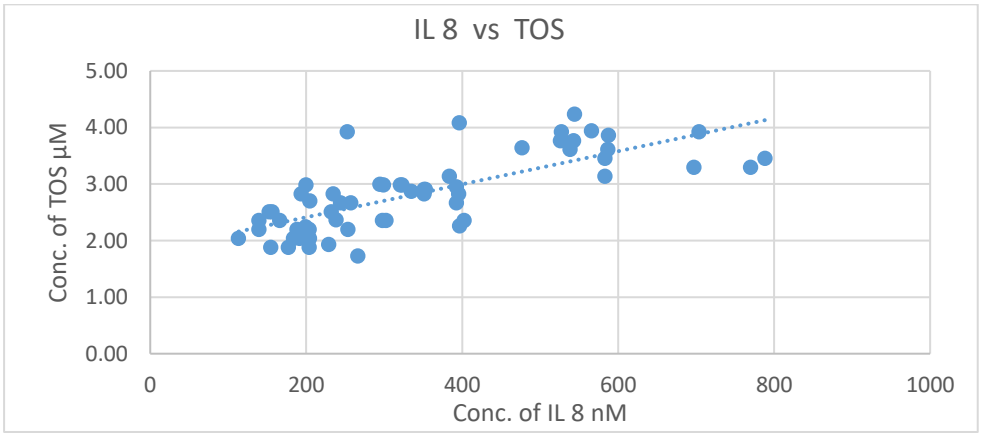


Fig. (3-10): Diagram of the relationship of conc. IL-8 (nM) to conc. TOS (µM).

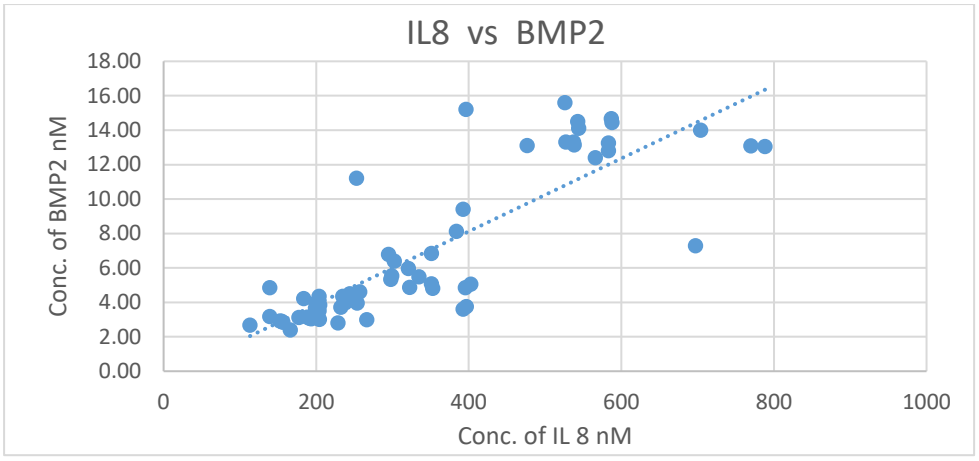


Fig. (3-11): Diagram of the relationship of conc. IL-8 (nM) to conc. BMP2 (nM).

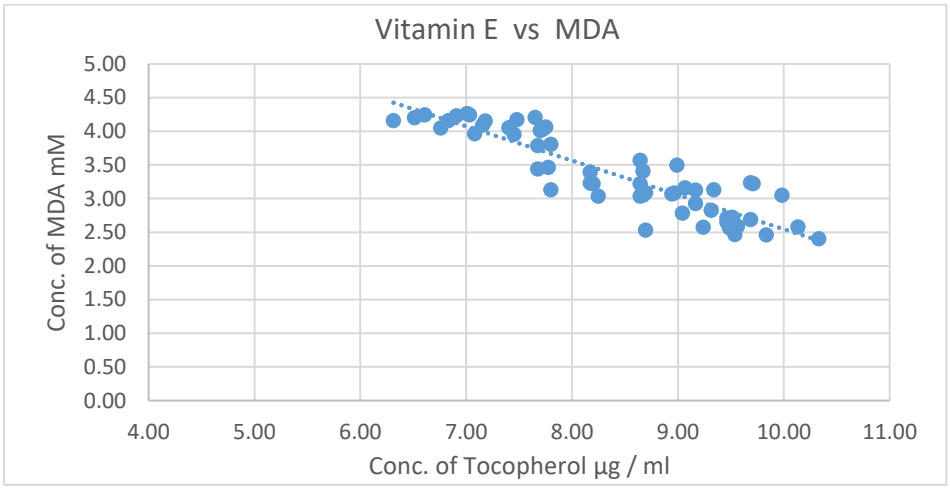


Fig. (3-12): Diagram of the relationship of conc. Vit. E (µM) to conc. MDA (mM).

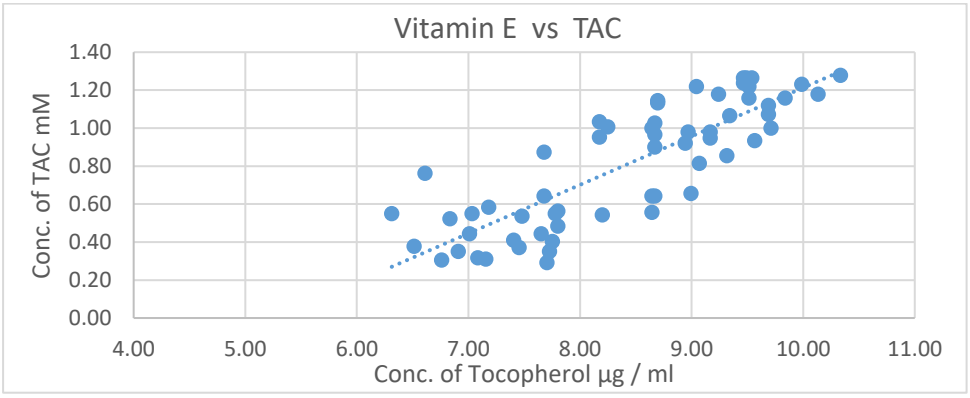


Fig. (3-13): Diagram of the relationship of conc. Vit. E (µM) to conc. TAC (mM).

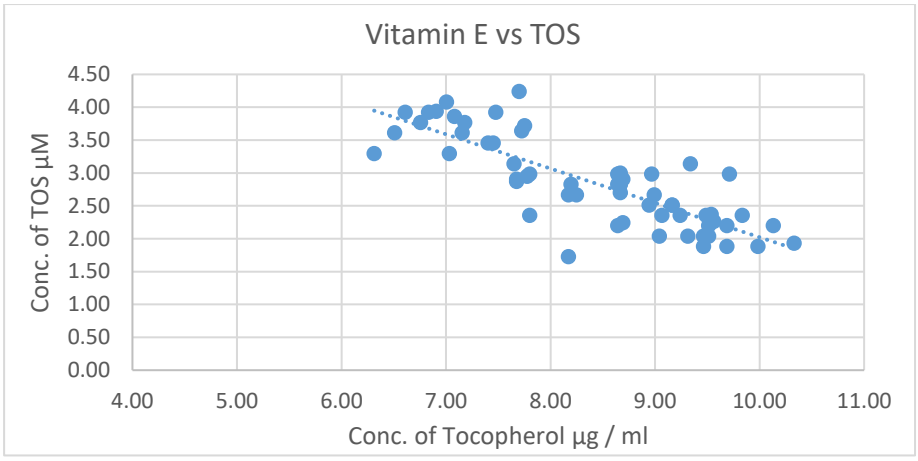


Fig. (3-14): Diagram of the relationship of conc. Vit. E (µM) to conc. TOS (µM).

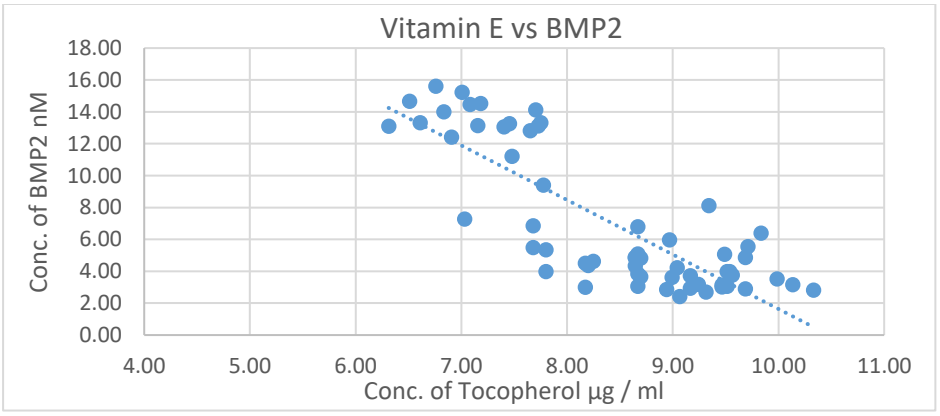


Fig. (3-15): Diagram of the relationship of conc. Vit. E (µM) to conc. BMP2 (nM).

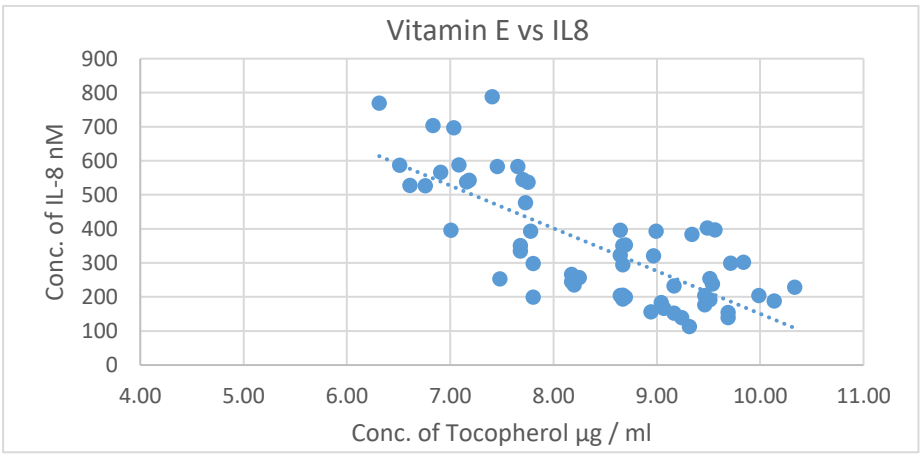


Fig. (3-16): Diagram of the relationship of conc. Vit. E (µM) to conc. IL-8 (nM).

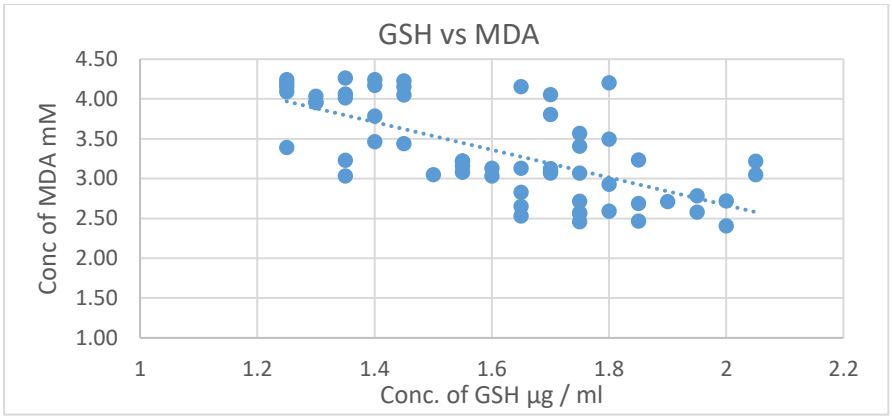


Fig. (3-17): Diagram of the relationship of conc. GSH (µM) to conc. MDA (mM).

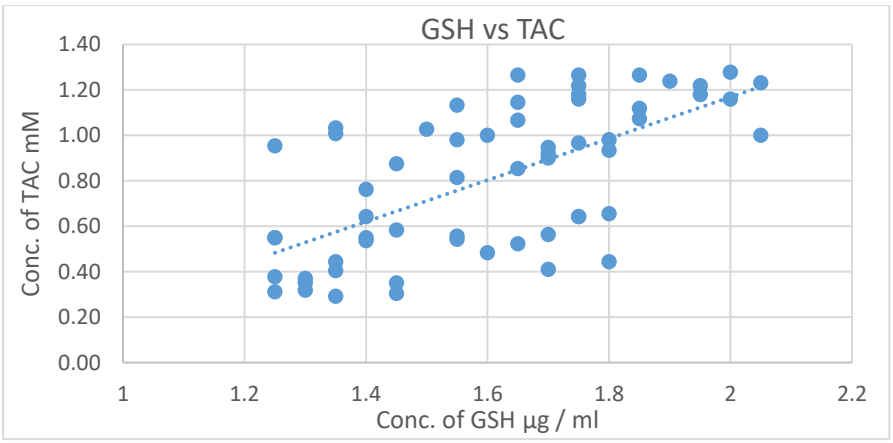


Fig. (3-18): Diagram of the relationship of conc. GSH (µM) to conc. TAC (mM).

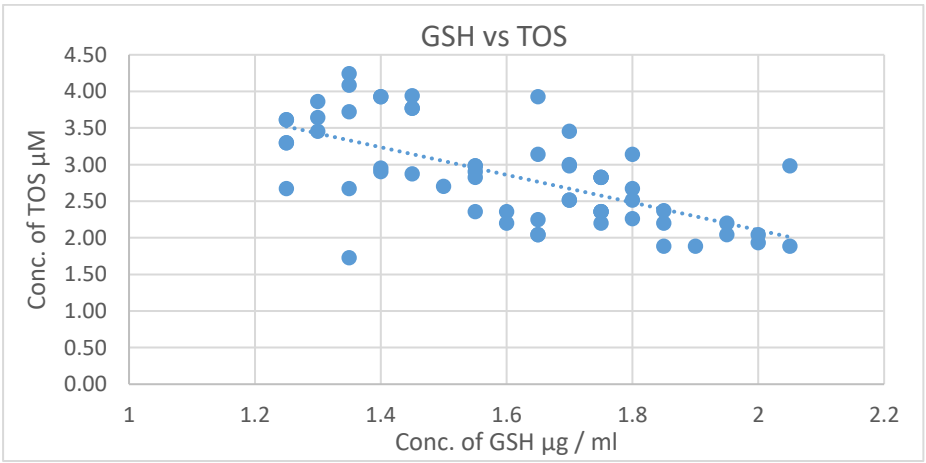


Fig. (3-19): Diagram of the relationship of conc. GSH (µM) to conc. TAC (µM).

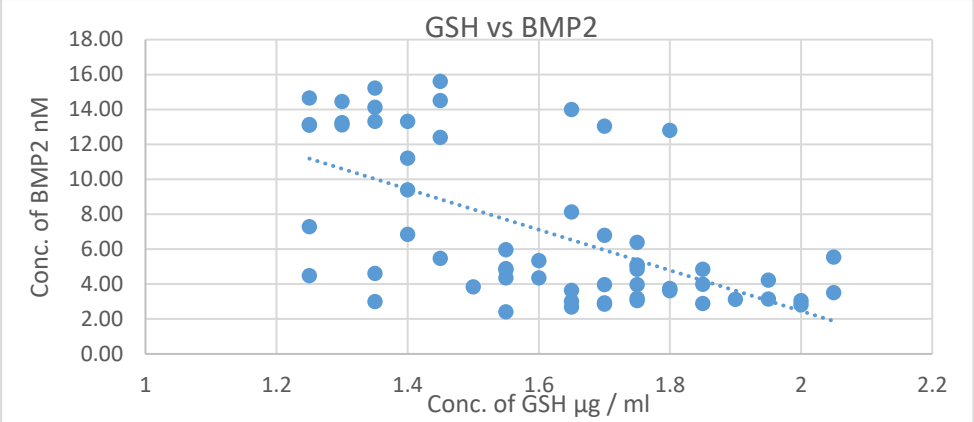


Fig. (3-20): Diagram of the relationship of conc. GSH (µM) to conc. BMP2 (nM).

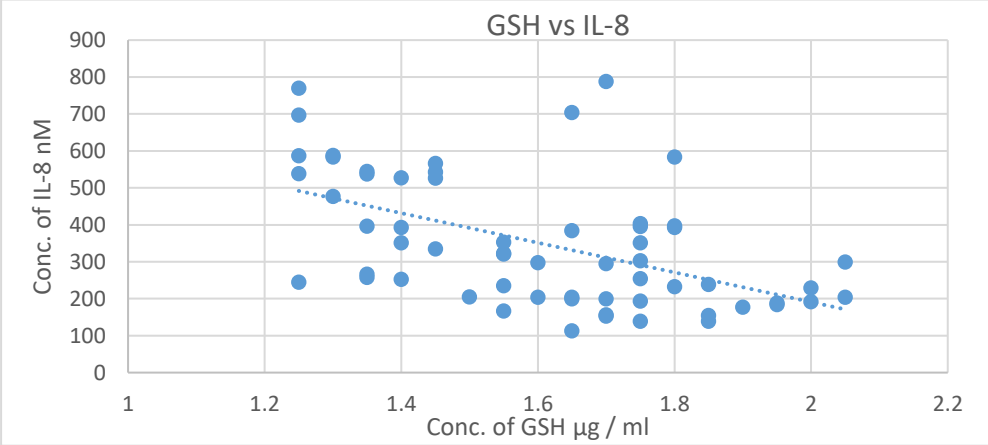


Fig. (3-21): Diagram of the relationship of conc. GSH (µM) to conc. IL-8 (nM).

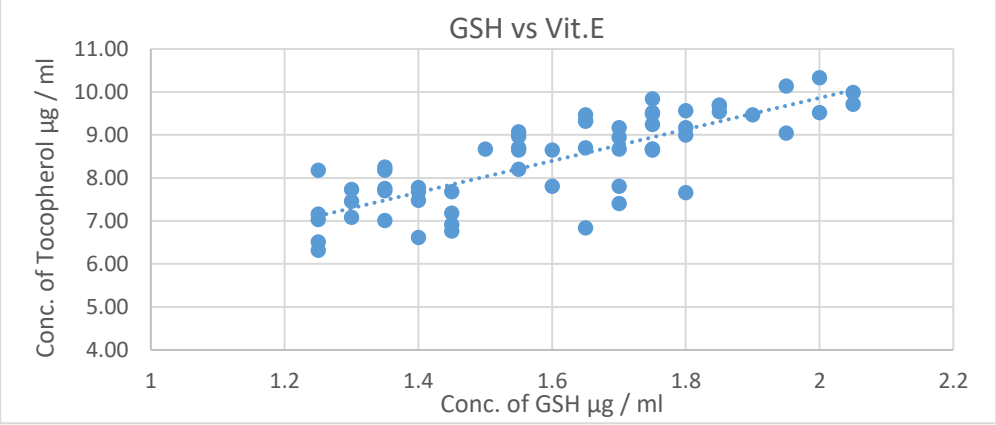


Fig. (3-22): Diagram of the relationship of conc. GSH (µM) to conc. Vit. E (µM).

Appendix 5

Accepting the article for publication

NeuroQuantology
An Interdisciplinary Journal of Neuroscience and Quantum Physics
ISSN 1303 5150

Date: 15. August.2022

Dear author's
Mohammed Qasim Al Badiri [1]
Lamia AM Almashhedy [2]

Chemistry Department, College of Science, University of Babylon, Hilla City, Babylon Governorate, Iraq

Title: Clinical Study for β-Thalassemia Major Patients through Bone Morphogenetic Protein2 (BMP2), Interleukin8 (IL-8) and Oxidant-Antioxidant Status.

We pleased to inform you that your above-mentioned article has been accepted for publication in **NeuroQuantology**, in the forthcoming issue, 2022.

Regards

Editor
NeuroQuantology
https://www.neuroquantology.com
https://www.scopus.com/sourceid/11600154151
ISSN: 1303-5150

Appendix 6

Published manuscript in NeuroQuantology journal within Scopus data

NeuroQuantology
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ISSN 1303 5150

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Clinical Study for β-Thalassemia Major Patients through Bone Morphogenetic Protein2 (BMP2), Interleukin8 (IL-8)and Oxidant-Antioxidant Status

Mohammed Qasim Al Badiri, Lamia AM Almashhedy

Abstract

Thalassemia is caused by a genetic mutation in the DNA of hemoglobin-forming cells, and this mutation is genetically passed from parents to children, causing low levels of hemoglobin production. This study describes the changes that occur to bone morphogen protein2(BMP2) in patients with beta thalassemia major,it also describes changes in their interleukin-8 (IL-8) levels, as well as describing their oxidant-antioxidant Status, and compares all of this to healthy controls, then describing the relationships of the parameters to each other. Ninety people were taken for the study, their ages ranged from (5-20 years). They were classified into groups; (PG1) male patients, (PG2) female patients, (CG1) male controls, (CG2) female controls. A significant increase was found for the group of patients in the level of each (BMP2), (IL-8) and total oxidant status (TOS) where ($p<0.05$) for each of them compared to the control group. While there was a significant deficiency of patients in the level of total antioxidants capacity assay(TAC) where ($p<0.05$) compared to the control group. There is no significant value between the concentrations of the studied parameters between groups of males and females. On the other hand, the patients themselves were divided into two other groups, the splenectomy group and the no splenectomy group It was noted that there is a significant difference between them ($p<0.05$).

Keywords

Bone Morphogenic Protein 2; Interleukin-8; Stem Cells; Total Oxidant Status; Total Antioxidants Capacity Assay; Iron Overload.

Full Text

PDF

الملخص

يتسبب مرض الثلاسيميا في ضعف تخليق الهيموجلوبين. ينتج مرض الثلاسيميا عن طفرة وراثية في الحمض النووي في الخلايا المنتجة للهيموجلوبين. يحتاج هذا الاضطراب إلى عمليات نقل دم منتظمة لتجديد حجم دم المريض. يؤدي هذا إلى تراكم الحديد في أجسامهم ، مما يزيد من الإجهاد التأكسدي ويسبب هشاشة العظام وتضخم الطحال وعدم انتظام النبض وأمراض الكبد وتأخر النمو.

تصف هذه الدراسة بعض المتغيرات المناعية الدقيقة وهي: بروتين العظام المورفوجيني 2 (BMP-2) ، وهو بروتين يتكون من سلسلتين عديد الببتيد تحتويان على 114 من الأحماض الأمينية المرتبطة برابطة ثنائية كبريتيد واحدة. يحفز تكوين العظام والغضاريف. إنها تلعب دورًا رئيسيًا في تمايز بانيات العظم. تصف أيضًا الانترلوكين8 (IL-8) الذي له نشاط مضاد للالتهابات، والذي ثبت في نماذج مختلفة من العدوى والالتهابات. تستخدم الآن مجموعة متنوعة من التخصصات الطبية الفرعية الإنترلوكين 8 ، وهو مؤشر واعد لمجموعة واسعة من الأمراض السريرية.

من ناحية أخرى ، يصف التغييرات التي تحدث لأهم عوامل الأكسدة في الجسم ، وهي: تحديد حالة الأكسدة الكلية (TOS) وتحديد مستويات المالونداياليديهايد(MDA) وهو أحد المنتجات النهائية لأكسدة الأحماض الدهنية المتعددة غير المشبعة في الخلايا. يتم استخدام حالة الأكسدة الكلية (TOS) لتحديد المستوى العام للأكسدة التي حدثت داخل الجسم.

تصف أيضًا أهم العوامل المضادة للأكسدة في الجسم ، وهي: تحديد مقايسة قدرة مضادات الأكسدة الكلية (TAC) ، وتحديد مستويات توكوفيرول (فيتامين هـ) ، وتحديد مستويات الجلوتاثيون (GSH).

تم أخذ تسعين شخصاً للدراسة تراوحت أعمارهم بين (5-20 سنة). وأخذت العينات لمجموعات المرضى في مركز الثلاسيميا بمستشفى الزهراء التعليمي للولادة والأطفال بمحافظة النجف بالعراق لمن زار مركز الثلاسيميا في الفترة من ايلول الى كانون الاول 2021 عند اكمال الدراسة. وأخذت عينات لمجموعات الضبط من المتطوعين خلال نفس الفترة. يتم تصنيفهم إلى مجموعات. (PG1) ثلاثون مريضًا ، (PG2) ثلاثون مريضة ، (CG1) خمسة عشر من الذكور ، و (CG2) خمسة عشر من الإناث.

عند مقارنتها بمستويات البروتين المكون للعظام 2 (BMP-2) الموجودة في المجموعة الضابطة (1.88 ± 1.04) ، كانت هناك زيادة ذات دلالة إحصائية تم اكتشافها في مستويات البروتين المُشكل للعظام 2 (BMP-2) الموجودة في مجموعة المرضى (7.04 ± 4.40) ، ($p < 0.05$). يشير هذا الارتفاع المعنوي إلى أن هذا البروتين يؤدي وظيفته المذكورة أعلاه.

تم العثور على مستويات إنترلوكين 8 (IL-8) لتكون أعلى بشكل ملحوظ في مجموعة المرضى (348.8 ± 174.1) بالمقارنة مع المستويات المذكورة في المجموعة الضابطة (348.8 ± 174.1) ، مع مستوى دلالة ($p<0.05$). هذا يشير إلى أن هذا الإنترلوكين يرتفع كمضاد للالتهابات.

تم العثور على زيادة معنوية لمجموعة المرضـــى في مســتويات الإجهاد التأكســدي الكلي (TOS) (2.85 ± 0.67) مقارنة بالمجموعة الضـــابطة (2.85 ± 0.67) ، حيث (p <0.05). تم العثور على زيادة معنوية لمجموعة المرضـى في مسـتويات المالونداياالديهايد (MDA) (3.35 ± 0.60) بالنسبة للمجموعة الضابطة (1.05 ± 0.12) حيث (p <0.05).

من ناحية أخرى ، كان هناك انخفاض معنوي في مستويات مقايسة السعة المضـادة للأكسدة الكلية (TAC) (0.81 ± 0.32) ، نسبة إلى المجموعة الضـابطة (2.98 ± 1.08) ، حيث (p <0.05). وتوكوفيرول (فيتامين إي) (8.42 ± 1.05) ، نسـبة إلى المجموعة الضـــابطة (12.69 ± 1.27) حيث (p<0.05). بينما الجلوتاثيون (GSH) (1.61 ± 0.23) ، في المرضـى مقارنة بالمستويات في المجموعات الضـابطة (3.18 ± 0.27) ، حيث (p <0.05). الإجهاد التأكسدي هو الجاني في كل هذا.

من جانب اخر، تم تقسيم المرضـى أنفسـهم إلى مجموعتين أخريين: (مجموعة اسـتئصـال الطحال) وعددها عشـرين و(مجموعة عدم اسـتئصـــال الطحال) التي يبلغ عددها أربعين. لوحظ وجود فرق معنوي بينهما لجميع المتغيرات التي تم قياسها في هذه الدراسة حيث (p <0.05).

إن كل ما حدث هو بسـبب الاجهاد التأكسـدي الناجم عمليات نقل الدم الدورية, والتي بدورها تسـبب تراكم الحديد في انسجة الجسم وحدوث المضاعفات.

أظهرت النتائج وجود علاقة ارتباط بين المتغيرات المدروســـة. هذا يؤدي إلى إمكانية التنبؤ بأيٍ من هذه الباراميترات بمعرفة الآخر.

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

دراسة سريرية لمرضى بيتا ثلاسيميا العظمى من خلال بروتين العظام المورفوجيني2 (BMP-2) وانترلوكين8 (IL-8) وحالة الأكسدة ـ مضادات الأكسدة

رسالة

مقدمة الى مجلس كلية العلوم / جامعة بابل كجزء من متطلبات نيل درجة الماجستير في العلوم / الكيمياء الحياتية

من قبل

محمد قاسم مجدي هاني البديري

بكالوريوس علوم الكيمياء – جامعة بابل - 2009

باشراف

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