Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Science for Women

Chemistry Department

Relationship Between Some Trace Elements and Antioxidant in Beta-Thalassemia Major in Hilla City,Iraq

A Thesis
Submitted to the Council of the College of science for women ,
University of Babylon as a Partial Fulfilments of the
Requirements for the Degree of Master of Science /Chemistry

By

Rana Salah Noori Marzoq Al-Saegh

(B.Sc. Chemistry ,College of Science , Babylon University, 1998)
(Diploma in Forensic Evidence, College of Science , Babylon University, 2018)

Supervised by

Assist. Prof. Dr.

Mohamed Abdul-Ridha Ismael

2021 A.D. **1443 A.H.**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

شَهِدَ ٱللَّهُ أَنَّهُۥ لَآ إِلَٰهَ إِلَّا هُوَ وَٱلْمَلَٰٓئِكَةُ وَأُوْلُواْ ٱلْعِلْمِ قَآئِمًۢا بِٱلْقِسْطِ ۚ لَآ إِلَٰهَ إِلَّا هُوَ ٱلْعَزِيزُ ٱلْحَكِيمُ

الآية (١٨) : سورة ال عمران

اللهم صلِ على محمدٍ وآل محمد

Supervisor Certification

I certify thesis entitle (**Relationship Between Some Trace Elements and Antioxidant in Beta-Thalassemia Major in Hilla city**, **Iraq**) Was prepared under my supervision at the University of Babylon / College of Science for Women as a partial Requirement for the degree of Master in Chemistry.

Signature

Dr. Mohamed Abdul-Ridha Ismael
Scientific Order: Assist. Prof.
Address: University of Babylon / College of
Science for Women
Date: / /2022

Recommendation of Head of Chemistry Department

According to the available recommendation , I forward this thesis for Discussion.

Signature :

Dr. Hazim Yahya Mohammed Ali
Scientific order : Assist. Prof.
Address : Head of Chemistry Department
University of Babylon / College of
Science for Women
Date: / /2022

Certification of the Examining Committee

We certify that we have read this thesis entitled (**Relationship Between Some Trace Elements and Antioxidant in Beta-Thalassemia Major in Hilla City,Iraq**) and as examining committee examined the Master student (**Rana Salah Noori**) in its contents , we find that it is adequate to award her degree of Master in Chemistry with degree (**Excellent**).

Committee Chairman:
Signature:
Name**: Dr. Talat Tariq Khalil**
Scientific order: Prof.
Address: University of Babylon, Science College for Women
Date: **/ /2022**

Committee Member
Signature:
Name: **Dr. Hanaa Addai Ali**
Scientific order: Prof.
Address: Kufa University, College of Science
Date : / /**2022**

Committee Member
Signature :
Name :**Dr. Ban Mahmood Shaker Aljoda**
Scientific order : Assist . Prof.
Address : University of Babylon \ College of Medicine
Date : / /**2022**

Committee Member(Supervisor)
Signature :
Name: **Dr. Mohamed Abdul-Ridha Ismael**
Scientific order :Assist . Prof. .
Address :Babylon University, Science College for Women
Date : / /2022

Date of examination : 22/ 12/ 2021

Deanship authentication of Science College for Women
Approved for the college committee of graduate studies

Signature :
Name :Dr. Faez Ali Rashid Al-Mammori
Scientific order : Professor
Address :Dean of College of Science for Women\Babylon University
Date : / /2022

Dedication

To my father and mother...may God have mercy on them

To my beloved grandmother..... who spent her life raising me and watching over my comfort

*To my husband..... the companion of the struggle and the difficult circumstances, who spared no time or effort to help me complete my studies, and to my daughters and sons (**Hawraa, Rawan ,Abdullah ,Ali and Al-Mustafa**)*

To all my brothers and my family

I dedicate my scientific thesis to you

RANA

Acknowledgements

Praise be to Allah of the worlds , who helped me and gave me the strength, patience, and endurance to complete this thesis. I would like to extend my special thanks and appreciation to **Assist. Prof. Dr. Mohamed Abdul-Ridha Ismael** , for him suggestion and providing him continuous advice and valuable guidance, without him guidance and close supervision of the work, when it is never achieved.

I also express my thanks and gratitude to the deanship of the college of Science **Prof. Dr. Faiz Ali Rashed Al-Hamad** and the head of the department of chemistry **Assist. Prof. Dr. Hazim Yahya Mohammed Ali**, I also would like to extend my sincere thanks and appreciation to **Prof. Dr. Dakhel Ghani Omran** and **Assist. Prof. Dr.Ali Talib** and **Prof. Dr. Talat Tariq Khalil** and **Asist. Prof. Dr. Sadiq Abedul-Hussain** for his continuous support and constant encouragement. and all staff whose helped me and provided their valuable pieces of advice and comments.

I would like to extend my great thanks and gratitude to the employees of the Center for Genetic Blood Diseases(Thalassemia center) and the Biochemical Testing Laboratory at Al-Hilla Maternity and Children Hospital, especially the chemists **Zainab Hamid Muhammad and Lamia Salman** for their provision and assistance in collecting and examining blood and serum samples.

Finally, I would like to express my sincere thanks and appreciation to the members of my family, especially, my dear husband who did not skimp on me and encouraged me until I reached this stage .

RANA

Summary

The thalassemia is hereditary anemia caused by mutations that affect the synthesis of the globin and the protein component of the hemoglobin , Thalassemia produces massive public health problems in many parts of the world especially the Mediterranean countries and coastal cities. The study conducted on beta-thalassemia major patients, serum collected amounted to (60 (30 Males and 30 Females)) patients and (30 control group) 15 Males and 15 Females) , Body mass index (BMI) for patients as (17.31%) compared with healthy (18.5-25%) in the Thalassemia Center(Genetic Blood Diseases) and the laboratories of Al-Hilla Teaching Hospital of the Maternity and children , for the age groups from (1 – 35) years for the period from November 2020 till June 2021, Hematological parameters were measured on these patients by a comprehensive blood test that included (amount of hemoglobin Hb, packed cells volume (PCV), number of red blood cells**(**RBCs**)**, corpuscular volume ratio MCV, corpuscular hemoglobin ratio MCH, corpuscular hemoglobin concentration MCHC, Red cell distribution width RDW, White blood cells WBCs and Platelets counts PLT . All of these blood parameters showed a clear decrease in thalassemia patients, except for RBCs and platelets counts, which showed a significant increase.

The results of biochemical test have reveal lowest significant mean zinc in males and females were (96.121), (83.199) µgm\dl respectively. In patients group compared in control group (119.344), (111.376) µgm\dl., respectively and highest significant mean copper in males and females were (203.80), (197.13) µgm \dl. respectively. In pa-

tients group compared in control group (105.00), (115.67) µgm \dl. respectively. As well as highest significant mean iron in males and females were (224.479), (215.639) µgm \dl. respectively. In patients group compared in control group (113.40), (103.33) µgm \dl. respectively. But there are lowest mean albumin in males and females were (4.033), (3.934) gm\dl. respectively in patients group compared in control group (4.506) , (4.10) gm \dl. respectively. There are increase in uric acid in males and females were (46.964), (46.779) mg\L. respectively in patients group compared in control group (38.066), (37.733) mg\L. , respectively.

Concentration of allantoin detected by HPLC technique for serum patients that reveal highest values in males and females were (61.556), (56.916) mg\L., respectively in patients group compared in control group (2.448), (1.477) mg\L., respectively. Whereas the concentration level of total antioxidant capacity(TAOC) in serum of studied groups. Shows decrease with TAOC in males and females were (18.682), (14.469) U\ml. ,respectively in patients group compared in control group(85.660), (80.660) U\ml. ,respectively . As well as there are decrease in concentration level of super oxide dismutase (SOD) in serum of studied groups in males and females were decreased (95.633), (88.429) (U\ml.) ,respectively in patients group compared in control group (208.623), (190.413) (U\ml.),respectively.

The results of the current study revealed all relationships its negative correlation except the following correlation its positive correlation:-

1- zinc and total antioxidant capacity(TAOC)

2- copper and (TAOC)

3- iron and uric acid

4- albumin and(TAOC)

5- allantoin and (TAOC)

6- (TAOC) and SOD enzyme.

This indicates the presence of free radicals generated in thalassemia patients, which are counteracted by some enzymes under study, such as TAOC and SOD that revealed with copper and zinc concentrations and total antioxidants capacity whose concentration is reduced, evidence of their response to these radicals, in addition to some large molecules such as albumin and small ones such as uric acid, whose concentration decreased and increase respectively as a result of oxidation and turned into allantoin.

Concluded from the current study that the fluctuation in the rise of trace elements and iron is due to the complications of thalassemia and blood transfusions. As for the decrease in some molecules such as albumin and uric acid, this is due to the exposure of these molecules to free radicals formed as a result of the breakdown of red blood cells or their secretion by the large number of white blood cells that Its number has increased with the current study.

List of Contents

Chapter three : Results and Discussion

List of Tables

List of Figures

List of Abbreviations

Abbreviations.	Full name
A_{RBL}	Reagent blank
A/U ratio	Allantoin\Uric acid
α- thalassemia	Alpha- Thalassemia
β-thalassemia	Beta Thalassemia
BIL.	Bilirubin
CAT	Catalase
C°	Centigraite
ΔAs	Change of samples
ΔAstd	Change of standard
CBC	Complete Blood Picture
Conc.	Concentration
Cu	Copper
DFX	Deferasirox
DFP	Deferiprone
DFO	Deferoxamine
DNA	Deoxy-ribonucleic acid
EDTA	Ethylene Diamine Tetraacetate
d.f.	dilution factor of sample before test
FT-IR	Fourier Transform Infrared
GPX	Glutathione Peroxidase
GSH	Glutathione Reductase
gm\dl	Gram\deciliter
g\ dl	Gram\deciliter
HbA	Hemoglobin A
HbA2	Hemoglobin A2
Hb	Hemoglobin concentration.

Abbreviations.	Full name
HbF	fetal haemoglobin
HNE	4- hydroxy-2-noneal
HPLC	High Performance Liquid Chromatoghraphy
H.S	Highly significants
$OH^{\cdot}$	Hydroxyl radicals
L.S	Low significants
MDA	Malondialdehyde
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MEL.	Melatonin
µ gm\dl	Microgram\deciliter
mg\L.	Milligram\ liter
N.S	Non-significant
OD	Optical density
PCV	Packed cells volume
PPM	Part per Million
PBS	Phosphate buffer solution
pgm	Pictogram
PLT	Platelets counts
pH	Potential of hydrogen
P	Probability
R^2	Correlation coefficient
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RBCs	Red Blood Corpuscles Counts
RDW	Red cell distribution width

Abbreviations.	Full name
Rt	Retention time
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
$\dot{O}_2^-$	Super oxide anion free radical
SOD	Super Oxide Dismutase
TAOC	Total antioxidant capacity
TIBC	Total iron-binding capacity
UV–Vis	Ultraviolet-Visible Spectroscopy
U\ml.	Unit \ milliliter
UA	Uric acid
WBCs	White blood cells.
WR	Working reagent

List of Appendices

Introduction and

Literatures Review

1.1.General Introduction

Mediterranean anemia (thalassemia) is an inherited hemoglobinopathy caused by a defect in the formation of globin chains **(Behrman *et al.*, 2004).** This disease is characterized by the production of an insufficient amount of globin chains (alpha, Beta, or both) and depending on the type of globin chain affected, anemia can be classified. Mediterranean type Alpha and Beta type anemia. Mediterranean anemia results in the Beta type of decreased or absent production of the Beta-globin chain as a result of a mutation in the Beta-globin gene located on the chromosome (11), and if this mutation occurs in the Beta-globin gene, then the disease is called Mediterranean anemia. symmetric Beta-type or Mediterranean-type major beta-anemia.

Anemia of the major Mediterranean type beta It is the most severe type that was described by pediatrician Thomas Cooley in 1925, which he called anemia Cooley's anemia (**Afshin *et al.*, 2009**). Beta leads to the accumulation of single chains of alpha globin, which leads to a decrease in hemoglobin in blood cells. red blood cells, which leads to severe anemia; Therefore, patients need regular and frequent blood transfusions Throughout their lives, and one of the complications caused by blood transfusion is the deposition of iron in the tissues, which is the cause the main cause of damage to many organs such as the heart, liver and endocrin-glands **(Weatherall and Clegg, 2001).**

The disease is associated with hemolysis in the surrounding circulation excess iron is deposited in the tissues **(Yesilipek, 2007).** Iron removal therapy is responsible for extending patients' life and delaying tissue damage caused by iron overload, as treatment has been shown to prevent damage to the liver and heart and allowed patients, especially children, to grow and develop sexually in a way as well as increasing their life span, **(Rund and Rachmilewitz, 2005).** And on this

from the use of iron removal therapy, but the patients suffer over time from serious complications .To the accumulation of iron in their bodies such as growth, endocrine insufficiency, blood percentage as a result of a defect in the pancreas, liver diseases and splenomegaly, and patients are exposed to transmissible diseases viral hepatitis **(Borgna-Pignatti and Galanello, 2004)**.

It was noted through studies that were conducted on patients with Mediterranean anemia in Babylon province that: Most of the patients belong to the beta-medial anemia category and one patient belongs to the β-amatenic group .The average is alpha type. Therefore, the study was conducted on patients with Beta-mediated anemia only. According to the stats The Iraqi Ministry of Health shows that the number of people with Mediterranean anemia has reached 15,000, as number has reached 15,000 (Babylon province 395 patients, of whom 283 were diagnosed with Mediterranean great Beta anemia **(Al-Saray, 2012).**

Although Mediterranean major anemia is a beta type of common genetic disease, it is the most severe type of disease. But there is a little previous study on the hematological variables and biochemical changes and oxidative stress and study of existence of allantoin in infected patients serum by HPLC technique that occur in patients with this disease in Babylon Governorate, the current study planned to study these changes.

1-1: History of Thalassemia

Thalassemia is a term derived from Greek origin; (Thalassa) means Sea, and the syllable (Hemia) means blood, and the term refers to a disorder related to a defect in making units secondary from the globin protein in hemoglobin, which are Beta and alpha globin, as a result of mutations in genes responsible for the sequence of amino acids that make up the protein (**Eliezer and Patricia, 2011**) . The

severe type of Mediterranean anemia was described firstly by the pediatrician, Thomas Cooley (1925) when he It's called Cooley's anemia.

The first cases of the disease were recorded in Italy and the states It was found that important symptoms such as an enlarged spleen and bone deformities were discovered (**Afshin *et al.*, 2009**).In those days, it was rare for patients to survive in the first decade of life until they were admitted Ordinary blood transfusion systems were introduced by the scientist Orsini in 1960, and thus patients were able to survive in the decade two and three years old. Later in the discovery and naming of the disease, the two scientists Whipple and Bradford rename this disease to Mediterranean anemia; Because it was discovered in the sea area (**Weatherall and Clegg, 2001**).

The disease was discovered under the misconception that the disturbances are limited to the Mediterranean region, but later it was discovered that it is one of the most genetic disorders are common and have a wide spread throughout the countries of the world, but it is more common in the Mediterranean, Southern Asia and West Africa (**Weatherall, 2001**).

The spread of the disease around the world is attributed to the migration of people from cities where the disease is spread to other cities ,the disease is less prevalent, and due to population migration and to some extent the slave trade, the disease has become anemia of the sea ,the average is nowadays spread across Northern Europe, North and South America, and Australia as well (**Mok *et al.*, 2011**).

1-1-1: Mediterranean anemia Thalassemia

A type of anemia that results from a genetic effect that affects the production of hemoglobin. If not ,the body produces a sufficient amount of globin chains (alpha and beta), the red blood corpuscles are not formed properly nor you can

carry enough oxygen, and the result is anemia that begins in early childhood and continues throughout life.

If damage occurs in one of the genes responsible for the production of globin, for example, one of beta genes may fail will produce a normal amount of beta chain protein, and the four alpha genes will work to produce a normal amount of alpha chain protein, and then an imbalance will appear between the amount of beta chain protein and the amount of alpha chain protein .Inside the cell and this is an important feature of the disease. A patient with mediterranean anemia does not produce hemoglobin insufficient HbA (α2β2) in adults, because cells cannot produce all protein chains, they produce ,as for the alpha or Beta strings (**Marengo-Rowe, 2007**).

Main hemoglobin in patients with anemia the intermediate beta type is hemoglobin (HbA2) which is a pair of alpha chains and a pair of Delta α chains (α2 δ2), which is formed in the normal adult human (2 - 4%), while the form of adult hemoglobin (HbA)) 90% of the total hemoglobin in a normal human. Mediterranean anemia is classified as low in content Hemoglobin in red blood corpuscles , Hypochromia is also classified as small red blood corpuscles (Microcytosis) (**Clarke and Higgins, 2000**).

1-2: Hemoglobin Structure

Hemoglobin (in Latin) is an iron-carrying protein, which is an ingredient the chief of red blood corpuscles, R.B.Cs, is symbolized by the symbol Hb or Hgb, and it transports oxygen from lungs and delivers it to tissues to maintain the life of the body, which allows these tissues to breathe aerobic to equipped with the energy needed to carry out the specific functions of each organ in a process called metabolism. Consists and must, β and the other beta α hemoglobin of two identical pro-

teins bound together; one of the alpha presence of both proteins in hemoglobin; To be able to transport and give oxygen to the cells of the body (**Mckenzie, 2010**).

It is not before birth, but there is another protein that takes its place called gama which is not produced by the beta protein it is found only in the fetal stages and serves as a substitute for Beta protein until the time of birth. HbF . fetal hemoglobin it is the main component of hemoglobin for the life of the fetus and at birth, and it constitutes about 80% of the total hemoglobin of the newborn.

Fetal hemoglobin is produced in the sixth week of pregnancy continuing through the remainder of the fetus's life, fetal hemoglobin production declines after birth gradually It is replaced by HbA (**Birgens and Ljung, 2007**). Hemoglobin is made the embryo is made up of two alpha chains and two gamma chains (α2γ2) and each chain contains 141 and 146 amino acids, respectively, and that the same alpha chains are present in adult hemoglobin HbA (α2β2) and HbA2 (α2 δ2) b β is characteristic of fetal hemoglobin and differs from γ-Beta chains, while the gamma 39 chains are amino acid , HbF fetal hemoglobin differs from adult HbA hemoglobin functionally; for having the ability to the familiarity with oxygen is slightly more than that of an adult hemoglobin, and this property makes the oxygen delivery about ,the placenta travels faster and gives the fetus better chances of receiving oxygen from the mother through the bloodstream (**Schechter ,2008**).

Measurement of fetal hemoglobin (HbF) is clinically useful in the study and diagnosis of some The important genetic errors in the globin protein (**Platt, 2008**). The plans for the manufacture of hemoglobin are stored within DNA α is like any other protein. Humans have four genes that control the manufacture of the alpha chain γ has two extra genes that control the production of the gamma chain (β) while two more genes control the manufacture of the Beta chain embryo), and

the alpha and Beta chains are produced in the same quantity despite the different number of genes (**Perutz, 1978**).

The production of hemoglobin requires coordination between the production of heme and globin, and (heme) is the part that helps to Binding oxygen with hemoglobin, as it constitutes 4% of the weight of hemoglobin, or globin, which is the protein that It surrounds and protects the heme molecule and consists of four chains (two alpha chains and two Beta chains), resembling coiled worms. Each globin chain contains a small heme group, and heme is composed of a round-shaped organic compound Porphyrin, which contains iron, is called Fe, and the latter is bonded with one oxygen atom. Meaning that each hemoglobin molecule is able to transport four oxygen atoms to body tissues (**Siems, 2018**) .

The production of hemoglobin requires coordination between the production of heme and globin, and (heme) is the part that helps to Binding oxygen with hemoglobin, as it constitutes 4% of the weight of hemoglobin, or globin, which is the protein that it surrounds and protects the heme molecule and consists of four chains (two alpha chains and two Beta chains), resembling coiled worms. Each globin chain contains a small heme group heme is a round-shaped organic compound porphyrin, which contains iron, is called Fe, and the latter is bonded with one oxygen atom. Meaning that each hemoglobin molecule is able to transport four oxygen atoms to body tissues(**Siems, 2018**). Because the genes encoding hemoglobin are similar in all humans, the hemoglobin structure hemoglobin is the same for everyone. Genetic changes occur over time as a result of nature or other reasons, these mutations in genes are very rare and have occurred over millions of years due to human evolution it is inherited, it means that the gene that has mutated and produces abnormal hemoglobin in a particular individual is transmitted to his children and produce children with abnormal hemoglobin similar to the father most

of the hemoglobin production genes .The mutant does not cause any problem and hemoglobin does its job well, and infection can only be known from during the DNA examination where the patient does not have clinical symptoms, but any change in the protein will change its characteristics and behavior.

There are mutations that occur in the hemoglobin production genes and cause a group among the genetic diseases called hemoglobinopathies, one of these diseases is sea anemia, the medium that produces abnormal hemoglobin due to mutations that occur in the genes responsible for the formation of protein globin, and then lead to severe anemia (**Marbut *et al.*,2020**).

1-3: Types of Thalassemia

The different types of Mediterranean are classified according to the type of globin chain that is produced in low quantities than the normal limit due to mutations, the most dangerous of which is sea anemia the average Beta type, and can be classified as follows:

1-3-1: Alpha Thalassemia

A normal human receives two genes responsible for producing the two alpha chains from both parents and writes the pattern the genotype contains two alpha chains, αα / αα, and produces two α chains. The disease results from deletion of one or more of the genes responsible for the synthesis of the two alpha chains, so the severity of the disease changes,

(**Marengo-Rowe, 2007**). They can be classified according to the number of mutations in the four alpha genes into:

1-3-2:Alpha Thalassemia Silent

The person carrier of the mutant gene (carrier) and does not have any visible symptoms.

1-3-3:Alpha Thalassemia Trait

when occurs a defect in two genes of from four alpha globin genes results in mild alpha-type Mediterranean anemia a person who carries these genes will have very simple symptoms, and they may not be apparent, but they can be detected by blood test.

1-3-4:Hemoglobin H Disease

When the defect is in three of the alpha globin genes the four produce hemoglobin H disease, as the patient suffers from anemia and the symptoms he suffers range from the patient is between moderate to severe, and in the analysis of the blood of the patient with this condition, small red blood corpuscles appear and the patient may develop an enlarged spleen and deformation in the bones due to the increased activity to replace corpuscles damaged red blood corpuscles, (**Higgs and Bowden, 2001**).

1-3-5:Hydrops Fetalis

When a defect occurs in the four constituent genes of the two alpha chains, a serious disorder called hydrocephalus can be diagnosed before birth by ultrasound and doppler, the fetus suffers in this case from severe anemia that can be treated with blood transfusion For the fetus while it is inside its mother's womb, and in cases of delayed childbirth, the fetus dies during or immediately after birth due to lack of oxygen inside the uterus. (**Sinha & Gupta , 2018**).

1-3-6:Beta Thalassemia

More than 180 mutations occur in the beta-globin gene, which we observe in patients with anemia of the disease the medium is beta type, and from these mutations results in partial or complete deletion of the beta globin gene, as some of

them cause Mediterranean anaemia, and some others cause a lack of beta + β production and a low production of beta globin, and it is then called globin and thus called β˚ Mediterranean anaemia (**Amit and Tiwari, 2011**). They are classified into three types:

1-3-7:Thalassemia Minor

It is the most common type of Mediterranean beta anaemia, also called trait Thalassemia. Patients with this type have the beta globin allele is normal and the allele for Mediterranean anaemia is either β˚ Mediterranean anaemia or + β anaemia Mediterranean blood (**Quek and Thein, 2007**). Patients do not have obvious symptoms and are detected the disease was detected by routine blood testing, but some newly diagnosed patients had anaemia mild and small erythrocyte size (**Kaiser, 2009**).

1-3-8:Thalassemia Intermedia

The patient suffers from this the type of anaemia is moderate and most cases do not require a blood transfusion. The clinical symptoms of this type of disease It mediates between the symptoms of Mediterranean anaemia minor and major, including delayed growth and sexual maturity and deformity bones and spleen enlargement due to damaged red blood cells with the risk of excess iron in the body is due to partially to increase intestinal absorption (**Harteveld *et al.*, 2008**; **Taher *et al.*, 2008**).

1-3-9:Thalassemia Major

Also called as Cooley's anemia it is the most severe form of Mediterranean anemia, because mutations occur in both genes responsible for producing chains beta globin. The cells that make up erythrocytes are destroyed, making the erythro-

cytes produced ineffective, Patients have a risk of severe anemia and reduced oxygen transport in the body (**Uda *et al.*, 2008**). Symptoms begin during the first year when the gamma chains stop forming and production begins the beta chains in which the defect has occurred, and the infection in the newborn develops gradually, so the child becomes pale and has two nutritional problems, diarrhea, frequent bouts of fever, and an increase in stomach size due to an enlarged spleen (**Galanello and Origa, 2010**).

The death rate from heart attacks is in patients with Mediterranean anemia the greatest is up to 71% (**Borgna-Pignatti and Galanello, 2004**). The most important symptoms experienced by a person with Mediterranean anemia in some developing countries are: Delayed growth, jaundice, spleen enlargement, and structural changes resulting from the expansion of the bone marrow to block the body's need for red blood cells long bone deformities, facial bones, and jaw enlargement the upper MM makes the teeth visible and the patient's eyes look like the eyes of a patient with down syndrome, (**Galanella and Origa, 2010**). Patients who have two types of genes mutated in the alpha and beta chains, the severity of the disease is they have lighter; (**Erejuwa & Ab Wahab, 2013**).

1-4:Diagnosis of Thalassemia

Family history is very important in the diagnosis of Mediterranean anemia; because it is caused by a genetic mutation in the globin gene, as well as the patient's appearance and symptoms of severe anemia, moderate jaundice, enlargement of the spleen and these symptoms appear during the first two years of life for people with Mediterranean anemia it appears at an older age for people with central Mediterranean anemia or for those with anemia they do not show any symptoms and the disease is determined by a routine blood test complete blood count (CBC) (**Alemu *et al*.,2006)**).

1-4-1:Electrophoresis of Hemoglobin

The best way to separate and identify hemoglobin depends on the migration of hemoglobin molecules in a buffer relay when an electric current is passed (**Sayani *et al.*, 2009**). The purpose of this method is to know abnormal forms of hemoglobin, so we can diagnose diseases related to hemoglobin damage hemoglobinopathies, people with micro-american anemia have an increased HbA2 on the contrary of those with Mediterranean anemia, (**Guler *et al.*, 2008**).

1-4-2:Prenatal Diagnosis

This diagnosis is made by analyzing the DNA of the fetus, which is extracted from cells the fetus is in the fifteenth to eighteenth week of pregnancy, but this test is dangerous for pregnant women therefore, chorionic villus sampling is performed in the eleventh week of pregnancy for exposed couples to have children with Mediterranean anemia after the consent of both partners. Both causative alleles must be identified Of disease prior to this test (**Mavrou *et al.*, 2007**)

1-4-3:Postnatal Diagnosis

If prenatal testing is not performed during pregnancy for couples at risk of having poor children Mediterranean blood A postnatal test for the newborn should be performed in order to diagnose early and refer the child to hematology center; To monitor him and to receive the necessary treatment, and these tests are either molecular tests and that because they are done at any age, or blood tests, but their timing depends on the type of hemoglobin abnormality (**Woolf *et al.*, 2003**).

1-5:Treatment of Thalassemia

The definitive treatment that recovers the patient is a bone marrow transplant after a suitable donor is found it is related to the patient, and the most com-

mon treatment is regular and frequent blood transfusions for the patient taking Medicines to get rid of excess iron, and remove the spleen if necessary.

1-5-1:Blood Transfusion

The goal of blood transfusion is to make the patient resist anemia to survive, and the decision to transfuse is made for the patient after a confirmed diagnosis of Mediterranean anemia in patients with severe anemia hemoglobin (less than 7 g / dL) for more than two weeks, and other symptoms including changes in the face and growth weak, enlarged bones, enlarged spleen, and most of all, the genetic history of the family. should not delaying blood transfusions beyond three years because the red blood cells become multi-antibodies this makes it difficult to find suitable donors (**Borgna-Pignatti and Galanello, 2004**). The blood transfusion is repeated every two to four weeks the amount of blood given to the patient depends on the patient's weight and the goal of increasing the level of hemoglobin and the size of blood cells PCV conglomerate, but the blood volume should not exceed 20 ml / kg per day and a maximum of 5 ml / kg hourly to avoid a rapid increase in blood volume, as there is an equation and a graph to calculate the amount of blood to be transfused for the patient, the patient must be monitored when transfusion of blood by recording some information such as the rate of hemoglobin Hb, PCV and RBCs count and amount iron before and after blood transfusion and to know the rate of daily hemoglobin decrease during the rest period (**Thalassemia International Federation, 2008**) Although a blood transfusion saves a patient's life But it causes many complications that expose the patient to many risks.

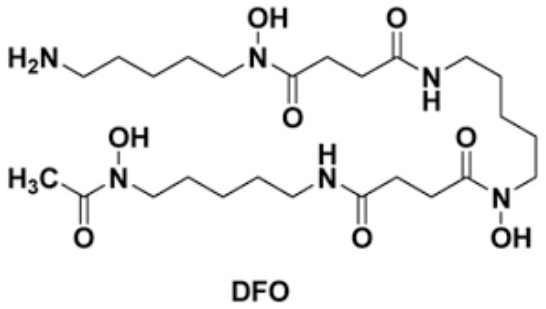
1-5-2:Iron Chelating Therapy

Patients continue to receive blood regularly, but over time they develop serious clinical symptoms because of excess iron resulting from blood transfusion. The serum ferritin concentration is an indicator for evaluation clinical symptoms

related to appropriate treatment. Sequential measurements of ferritin in serum it is reliable and is the easiest way to evaluate iron excess and the effectiveness of iron removal therapy (**Angelucci *et al.*, 2000**). Excess iron is removed by using iron-binding materials that allow by excreting iron through urine or feces, and these drugs are given to patients who receive frequent blood or who have high blood levels their serum ferritin level is above 1000 µg/ml (**Thalassemia International Federation, 2008)**

***Deferoxamine (DFO):($C_{25}H_{48}N_6O_8$)**It is the first drug that was used to get rid of excess iron in the body, no it is absorbed when taken orally, so it is slowly injected under the skin for 8-12 (hourly) hours 6 days at a daily dose of (20-40) mg/kg of body weight for children and (30-50) mg/kg of Adult body weight**(Hershko, 2002**).

Thus there is a decline in all of the drug's action its effectiveness and the continued suffering of patients due to serious complications caused by excess iron in the body (**Cunningham *et al.*, 2004**).



DFO

Figure (1-1): Chemical structure of deferoxamine (DFO). $C_{25}H_{48}N_6O_8$

***Deferiprone(DFP):**($C_7H_9NO_2$) It is an effective treatment given orally at a rate of (75-100) mg/kg per day. It is used in dangerous cases and in order to increase the effectiveness of the drug in removing iron, before it is the combination treatment consisting of Deferoxamine DFO (and Deferiprone DFP),) is shown which

excretes iron through feces and urine Combined therapy is used to achieve secretion levels Iron that cannot be achieved with either treatment without increasing toxicity (Galanello, 2007).

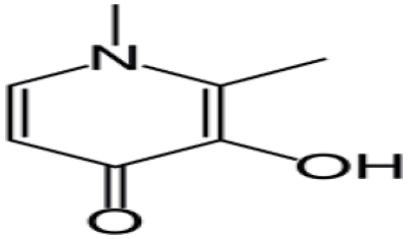


Figure (1-2): Chemical structure of Deferiprone | $C_7H_9NO_2$

Deferasirox (DFX): (**$C_{21}H_{15}N_3O_4$**) to be given orally once a day at 20 mg / kg per day. This depends on the number of blood transfusions and the goal of treatment, which may be to lower iron levels or to maintain iron levels in the body, this medicine has been shown to be effective in children and adults(**Galanello and Origa, 2008**)

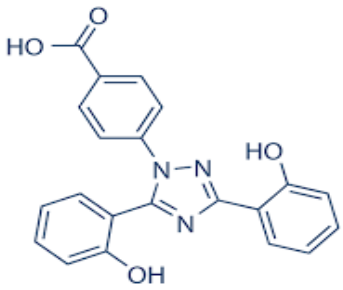


Figure (1-3): Chemical structure of Deferasirox | $C_{21}H_{15}N_3O_4$

The side effects of this treatment are mild digestive disturbances, skin rashes and no discontinuation is required take treatment; Because the side effects disappear automatically. Creatinine levels increase (within the upper limit). normal) in approximately 21% of patients, but in most patients creatinine levels return to normal spontaneously to a normal extent. The use of the treatment for more than

three years causes an increase in levels of creatinine is within the upper limit of normal, according to a study conducted on more than 1000 patients (**Cappellini and Taher, 2008**).

Desazadesferrithiocin-polyether-(S)-3'(OH).

Is the magnesium salt given once? Oral daily, which excretes excess iron through the feces, and this drug has been used in United States for the treatment of chronic iron overload in patients with Mediterranean anaemia dependent on blood transfusion(**Angelucci *et al.*, 2008**).

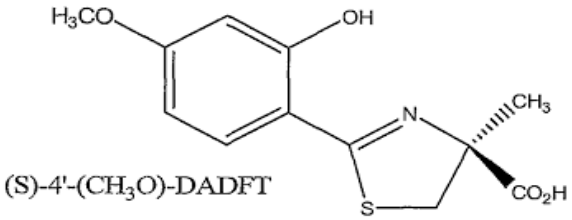


Figure (1-4): Chemical structure of Desazadesferrithiocin-polyether-(S)-3'(OH).

(Folic Acid) : Because their red blood cells are ineffective **(Borgna-Pignatti *et al.*, 2004**). The microcytosis in erythrocytes megaloblastic anemia may obscure the hematological advantage of folic acid deficiency i.e. megaloblastic anemia, and a blood test for folic acid is not a test available routine; Therefore, folic acid deficiency is simply ignored, and the patient may suffer from other complications arising from folate deficiency (**Galanello *et al.*, 2001**).

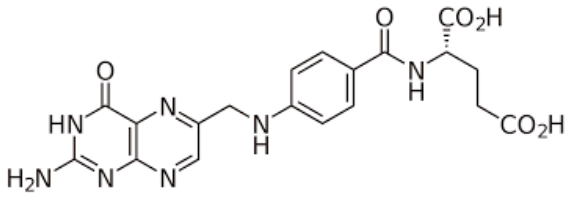


Figure (1-5): Chemical structure of Folic acid structure.

1-5-3:Splenectomy

The spleen is removed when the patient needs a blood transfusion of (180-200) mg / kg body weight the patient will have an annual (**Thalassemia International Federation, 2008**).Excessive activity occurs as it leads to a decrease in white blood cells and platelets, and then an enlargement spleen; When an enlarged spleen is diagnosed, it is removed immediately; Because its size increases rapidly, it causes pain in the stomach, the abdomen, or the cause of an enlarged spleen is the result of frequent blood transfusions that reduce the process of formation of blood cells within bone marrow, which leads to a decrease in the number of red blood cells that do not have a function and that are needed to be shattered within the spleen tissue of(**Panigrahi and Marwaha, 2007**).

Perhaps the purpose of eradicating the spleen is to avoid complications related to frequent blood transfusions. The risks associated with spleen removal are:

1- The patient is susceptible to infection with bacteria such as tuberculosis ,therefore, patients should be immunized with a pneumococcal vaccine every five years and an annual vaccination against influenza virus.
2- The spleen is the main storehouse of iron from transfused blood. When the spleen is removed, it is stored iron accumulates in the liver, causing cirrhosis and cirrhosis of the liver.
3- Serum ferritin levels are significantly elevated in patients with splenectomies compared to others of patients; Because the spleen has an important effect on iron metabolism.

1-5-4:Bone Marrow Transplantation

The critical treatment for patients with Mediterranean anemia is a red bone marrow transplant, and an operation has been performed marrow transplantation for the first time in 1981 for more than 1500 cases. Patients should be monitored

before the operation marrow transplantation mediated by clinical signs, including: Liver examination to see if patients suffer from enlargement and cirrhosis of the liver, the severity of iron accumulation, the history of iron removal from the body, and the operation is performed on patients after making sure that they do not have the previous risk factors Successful bone marrow transplantation for a patient mediterranean anemia is a radical treatment for the disease and predicts a normal life for the patient (**Roselli *et al.*, 2006** ; **Lucarelli *et al.*, 2011**).

Another successful treatment related to bone marrow transplantation is umbilical cord blood transplantation cord blood transplantation One of the advantages of this treatment is that cord blood contains stem cells undifferentiated primitives of the different blood cells and therefore does not necessarily require matching between the donor and the recipient. (**Pinto and Roberts, 2008**)

1-5-5:Complications of Blood Transfusion

A single unit of packed red blood cells used in a transfusion system contains approx. 200 mg of iron, as the body does not have a self-sufficient way to get rid of excess iron (**Porter, 2005**). Patients with Mediterranean Beta anemia major from excess iron in their bodies as a result of the dissolution of globules. they have red blood and to receive blood frequently. Excess iron in the body makes a lot of serious complications (**Peters *et al.*, 2012**) including the following :

Dysfunction of the pituitary gland that results in impaired growth, especially in children. Hypothyroidism A defect in the pancreas gland that results in diabetes mellitus hypogonadism and Liver disorders such as cirrhosis and liver fibrosis. Cardiomyopathy.

There are serious diseases that are transmitted with the blood transfused to the patient's body, including the human immunodeficiency virus HIV and hepatitis

C virus. The risk of liver cancer is increased for people with hepatitis virus and those with iron overload **(Muncie and Campbell, 2009)**.

1-6:Oxidative Stress

It is known as an imbalance between the mechanisms that lead to the production of free radicals (prooxidant) and the mechanisms that work to get rid of them or the so-called antioxidant, and this imbalance may be due either to the activation of the first mechanisms or to the inhibition of the second mechanisms or both together, and lead all of these Cases of free radical accumulation that are characterized by a high capacity to damage cellular components of tissue (**Dikalov & Dikalova, 2019**). Oxygen free radicals interact easily with DNA, proteins and fats in the cell envelope, and induce conformational changes to cellular structures and lead to disruption and dysfunction. Oxidative stress is an essential feature in many diseases such as cancer, arthritis, aging and diabetes (**Stulce *et al.*, 2019**).

Reactive oxygen species (ROS) formed during multiple natural processes in tissues and cells have been indicated for the pathogenesis (**Piña-Vázquez *et al.*, 2012**). The inflammation stimulates inflammation by activating mast cells and by producing cytokines that stimulate inflammatory mediators. Reverse oxidative reactions resulting from an inflammatory reaction lead to oxidative damage to uninfected cells during which oxidative damage is produced and some free radicals are released from ROS and NOS. Which have an important role in tissue damage (**Ashkani *et al.*, 2014**).

Many of researchers reveals that the infection impairs the antioxidant system. GSH, the main endogenous antioxidant, associated with lipid peroxidation, a sign of cellular oxidative stress, has long been confirmed as a major factor. Sequences of oxidative damage in different diseases (**Tang *et al.*, 2017**).

1-6-1: Free Radical

Oxidants are defined as chemical elements (atoms or molecules) that possess one or more electrons in the outer orbit, and the presence of a single free electron makes these species unstable and more active with a short half-life (**Jomova & Valko, 2011**). They can carry a positive or negative charge or be neutral (**Zalba *et al.*, 2006**) cells produce free radicals and active species in low concentration during metabolic processes, and this production is controlled by an antioxidant defense system (**Franco & Martinez, 2019**).

Free radicals are produced in the mitochondria and white blood cells and through the interference of detoxification mechanisms due to the effect of exposure to some toxic substances or radiation, and because they are of high activity, they attack cellular components causing them serious damage that can lead to cell death, which works to attack the membrane phospholipids and disrupt the activity of the membrane Cellular, as damage to proteins causes an imbalance of enzymatic functions, as well as for free radicals the ability to change the nature of DNA, which affects the construction of proteins (**Valko *et al*., 2006**).

Neutrophilic cells and macrophages play an important role in host defenses. These cells are able to generate large amounts of highly toxic molecules, such as reactive oxygen species (ROS), including peroxide (O_2) radicals, hydrogen peroxide (H_2O_2) and hydroxyl radicals. ($^{\bullet}OH$), and reactive nitrogen species (RNS), including nitric oxide, where bacteria, parasites, and tumor cells activate macrophages in quantities of NO, with NO having cytotoxic effects on these steroids (**Bogdan *et al.* , 2000**). However, ROS and RNS are able to break down many biomolecules, including DNA, carbohydrates, and proteins, ROS and RNS can attack the polyunsaturated fatty acids of membrane lipids, causing lipid peroxidation and disorganization of cytoskeletal function, as lipid peroxidation leads to the for-

mation of many degradation products, including malondialdehyde (MDA) .There was some evidence that the oxidation system Antioxidants are altered in thalasemiasis (**Vural *et al*., 2004**). The production of ROS may be due to the activity of certain enzymes such as Oxidase, Cyclo-oxygenase, Lipoxygenase, Dehydrogenase and Peroxidase, In addition, ROS can be triggered by the immune response as part of the immune response. (**Muller, 2002**).

1-6-1-1: Reactive Oxygen Species (ROS)

Macrophages are capable of generating large amounts of highly toxic molecules, such as reactive oxygen species (ROS), and include peroxide (O_{2-}) radicals, hydrogen peroxide (H_2O_2) and hydroxyl (OH) radicals, reactive nitrogen species (RNS), and universal nitric oxide. (NO) (**Tsamesidis *et al*., 2017**). Parasites stimulate macrophages to synthesize large quantities of nitric oxide (NO), which has cytotoxic effects on these steroids, as ROS (Reactive oxygen species) and RNS (Reactive nitrogen species) are able to break down many biological molecules. Including DNA, carbohydrates and proteins, moreover, the types of oxygen and active nitrogen ROS and RNS can attack the polyunsaturated fatty acids of membrane lipids that cause lipid peroxidation and disrupt cell building and functions (**Carneiro *et al*., 2011**). Lipid peroxidation is a well-known marker of oxidative stress in cells and tissues (**Terra *et al*., 2019**). High levels of lipid peroxidation products have been associated with a variety of chronic diseases with parasitic infections (**Kilic *et al*., 2003**).

There are mechanisms by which defense cells kill microorganisms, including serum redistribution of basic trace elements Fe, Zn, Cu with increased synthesis of acute phase proteins (such as cereloplasmin) that occur in most infections and changes are part of the organism’s defense strategies and are a result of hormone-like substances, interleukin-1 and tumor necrosis factor alpha-TNF, are re-

lease immunomodulators in a dose-dependent mode, mostly via activated macrophages (**Mastousek *et al*., 2009**).

Many antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are able to break down the yield by *In vivo* and these enzymes require trace minerals for example Cu-Zn SOD is an important intracellular enzyme. It requires both Cu and Zn for normal enzyme activity , Zinc stabilizes the enzyme and copper is necessary for stimulation. It acts as an antioxidant by oxidizing a superoxide anion O_2 root, and copper is an essential component in red blood cells (**Mohammed *et al*.,2020**). GSH-Px, an enzyme that recycles glutathione, stimulates the oxidation of glutathione, catalase reduces the oxidation of glutathione by H_2O_2 and other hydroperoxides to form oxidized glutathione and water and requires selenium Se for serum activity and Fe, Zn, Cu in thalassemia activity (**Lal *et al.*, 2013**). . CAT is a quaternary protein that stimulates the dissociation of H_2O_2 into water and molecular oxygen (**Moustafa, 2015**).

1-6-1-2: Antioxidants Enzymes

Aerobic organisms have antioxidant defense systems, which have the ability to attack active forms of oxygen and reduce their effects on the body. Many antioxidants found in fruits, vegetables and grains and most medicinal herbs contain many vitamins and minerals that show the activity of antioxidants (1-6), so the antioxidants were divided into two parts, enzymatic and non-enzymatic antioxidants (**Al-Helaly & Ahmed, 2014**).

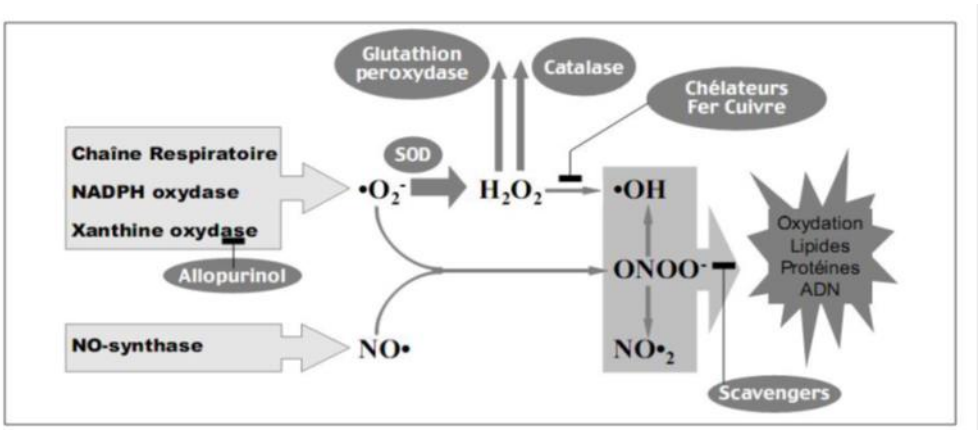


Figure (1-6): Represents the role of antioxidants in countering active forms of oxygen (**Fontaine *et al.*, 2002**).

1-7: Enzymatic antioxidants

1-7-1: Superoxide Dismutase (SOD)

It is a mineral protein found in all plant and animal organelles and in all aerobic primitive organisms, where this enzyme catalyzes the conversion of a radical superoxide O_2 to H_2O_2 and that this reaction takes place automatically and does not require an auxiliary factor or energy, as the SOD enzyme is the first line of defense Anti-damage from free radicals, as the hydroxyl radical prevents the release of the anion over oxide (**Liu Chi & Gu, 2015**). Varies (Zn-Cu, SOD) is cellular outside of that is intracellular with relatively much higher molecular weight and possesses a bound carbohydrate fraction (**De-Leo et al., 2002**). Extracellular SOD is a specific restricted in forms of diverse cells and tissues such as lung, heart, kidney, plasma, lymph, abdominal serum fluid and cerebrospinal fluid (**Weydert & Cullen, 2010**).

$$\bullet O_2^- + \bullet O_2^- + 2H^+ \xrightarrow[\text{Cu/Zn Mn}]{\text{SOD}} H_2O_2 + O_2$$

There are SOD antioxidants associated with copper, zinc and manganese elements in most prokaryotes and eukaryotes. On the other hand, SOD superoxide dismutase was discovered accompanying the iron element in prokaryotes and eukaryotes (**Johnson, 2001**). The significance of the role of superoxide SOD in microorganisms were demonstrated In vitro during the interaction between the host cell, and the regulation of the host and and any disorders superoxide dismutase was verified during the interaction between thalassemia disorders and the host cells, indicating its role in parasite survival in macrophages (**Mohammed *et al.*,2020**).

In addition, the disease deficient in repair of superoxide dismutase displayed reduced survival in rat macrophages, indicating that this enzyme could be a limiting factor (**Ghosh *et al*., 2003**). Generally, because the SODs conjugated with iron and the disease induce of breaking down O_2 into H_2O_2 and O_2; Moreover, other antioxidant enzymes present in disease reduce H_2O_2 to hydration (**Bodgan *et al*., 2000**). It also showed that the interaction between thalassemia and the host SOD can modulate cell response through acquired immunogenicity (**Marieb & Hoehn, 2007**).

1-7-2: Glutathion Peroxidase

Glutathion peroxidase(GPx) is spread in many cells, as it is concentrated in mitochondria, cystol and most mammalian tissues, and it is considered one of the most important antioxidant enzyme systems (**Sonet *et al*., 2018**). GPx is an enzyme that contains Selenium in the form of sélenocysteine, which detoxifies both hydrogen peroxide (H_2O_2) and organic peroxide (ROOH). Most animal tissues contain both the enzyme glutathione peroxidase GPx (Figure 2-3). Those who have the role to get rid of H_2O_2, as the enzyme glutathione peroxidase GPx works to remove H_2O_2 formed in the endoplasmic reticulum or cytosol and mitochondria, while cat-

alase works to remove H_2O_2 formed in peroxisomes (**Sonet *et al.*, 2018**), as in Figure (1-7):

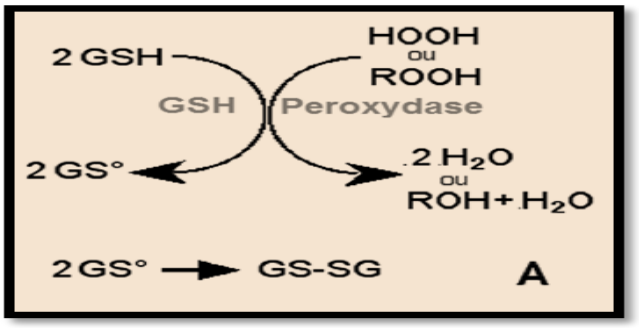


Figure (1-7): Mechanism of action of the enzyme glutathione peroxidase (Sonet *et al.*, 2018)

The non-specific products that are generated during respiratory blast by micoorganisms-containing macrophages are H_2O_2, the active anti micoorganisms molecule as the micoorganisms generates increased quantities of hydroperoxides and prevents damage formed by killing micoorganisms as a host defense strategy, associated with a deficiency. (**Sinha & Gupta , 2018**)

1-7-3: Glutathione Reductase

It is an enzyme that converts the oxidative form of GSSG to the reduced form of Glutathione GSH in the presence of the enzyme glutathione Reductase (**McMahon & Gunnlaugsson, 2012**) which is the following :-

$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

1-7-4: Catalase

Catalase is present in the liver in the form of peroxysomes and erythrocytes and plays an important role in preventing high levels of oxidative stress and protecting cells from H_2O_2 (**Lopes-Neto *et al.*, 2016**). infection with thalassemia acts

through neutrophil and phagocytic cells an important role in the defense of the host from the effects of infection with the infection with thalassemia and an increase in ROS and higher levels of MDA. This leads to lower levels of catalase activity in patients with thalassemia compared to healthy subjects (**Serarslan *et al.*, 2005**).

Abdulla (2018) showed that the incidence of infection with thalassemia reduced the levels of catalase and glutathione peroxidase levels, which play a role in converting the hydrogen peroxide radical, and that the decrease enables hydrogen peroxide to remain in the medium for long periods and high concentrations and increase the NO and its derivatives has a toxic effect too much like mixing nitrite and nitrate with peroxide and thus producing more NO.

1-8: Non-enzymatic antioxidants

Non-enzymatic antioxidants are represented by molecules that are characterized by the ability to inactivate free radicals and rapidly oxidize them. These include GSH (oxidizing GSSG and reduced GSH), uric acid UA, MEL melatonin, bilirubin BIL, dietary vitamins (A, E, B, C), and iron-bound proteins Ferritin, Transfarrin) and albumin, adenosine, carotenoids, etc (**Mirończuk-Chodakowska *et al.*, 2018).**

1-9: The main trace factors in the blood serum

The term trace elements refers to the chemical elements present in a natural substance in very small quantities , In analytical chemistry, a trace is an element in a sample with an average concentration of less than 100ppm measured in atomic number or less than 100 µ\gm , and in biochemistry, The trace element is the nutri-

ent mineral necessary for the body in very fine quantities for growth, development and physiology in the organism (**Al-Fartusie & Mohssan, 2017**).

Trace elements have several important roles in human bodies, some of which are essential for enzyme reactions as they attract and facilitate the conversion of large molecules into specific final products, and some of them give or receive electrons in redox reactions that are of fundamental importance in the generation and use of metabolic energy, and some of them have structural roles and are responsible for Stability of important biological molecules For example, iron that can bind, transport and release oxygen in the body (**Calderón *et al*, 2019**). Indeed, although trace elements are essential components of biological activities, excessive levels of these elements can be toxic to the health of the body and may lead to many fatal diseases, such as cancers (**Bhattacharya *et al.*, 2016**) The body's ability to withstand oxidative stress depends on the state and activities of the antioxidant molecules, including the integrity of several enzymes that require an adequate supply of trace minerals such as zinc, copper, cobalt, selenium and manganese (**Evans & Halliwell, 2001**). The indirect loss of essential body nutrients, resulting from accelerated metabolism or consumption, was confirmed during the course of many infections (**Chaudhuri *et al*., 2008**).

Trace elements are an integral part of the cellular antioxidant system and elements such as zinc, selenium and copper participate in the cellular defense against oxidants (**Klotz *et al.*, 2003**). The cells of the immune system require an adequate supply of trace elements for the structure and function of mineral proteins that are involved in protecting cells against highly toxic ROS (e.g., zinc and copper for superoxide dioxide and iron for catalase (**Munoz *et al.*, 2007**).

1-9-1: Iron (Fe)

Iron that is highly absorbed is stored in the liver, where it is associated with a protein called ferritin. The remaining 25% of the body's stores, and the body loses an amount of iron per day ranging between 1-2 mg, through urine, feces, sweat, and crusted cells from the skin and intestinal lining, and through bleeding especially menstrual blood in women (**Hoffbr & Steensma, 2019**).

Iron is another essential trace element present in almost all cells of the body. Human body requires iron for the synthesis of oxygen carrying protein called haemoglobin found in red blood cells, and myoglobin which is also a protein found in muscles. It also takes part in the production of other important proteins in the body such as for DNA synthesis and cell division. Furthermore, iron is used in the connective tissues in our body, some of the neurotransmittersin our brain, and to maintain the immune system.

Iron is transported through the blood by the serum protein, called transferrin. Transferrin is normally 30% saturated with iron. The total iron-binding capacity (TIBC) reflects the status of iron in the body and is defined as the amount of iron needed for 100% transferrin saturation. The levels of TIBC are raised when the levels of iron are low thus will be helpful in the diagnosis and monitoring of iron deficiency anaemia. When iron is present in excess amounts in the body it will lead to hemochromatosis, which may be primary or secondary. Primary hemochromatosis is a genetic disorder characterized by increased iron absorption and consequent iron overload in the body. Secondary hemochromatosis occurs in diseases like thalassemia due to iron overload especially in thalassemia major where repeated blood transfusions are required. Beta thalassemia major patients require frequent blood transfusions which lead to iron overload in the absence of effective chelation therapy **(Hoffbr & Steensma, 2019**).

This iron deposits in thalassemia patients can exceed from the storage and detoxification capacity of ferritin and also fully saturates transferrin and leads to the formation of free iron which accumulates in blood and tissues , this free iron will cause the formation of very harmful compounds, such as hydroxyl radical (OH). The hydroxyl radicals are highly reactive and attacks lipids to form lipid peroxides which contribute to oxidative stress (**Raghuveer *et al.*, 2009)**.

Regular blood transfusions along with chelation therapy in Beta thalassemia patients drastically improve the quality and duration of life to third and fourth decades. Iron overload is serious complication of long-term blood transfusion. It requires adequate treatment in thalassemia so that the early deaths especially from iron-induced cardiomyopathies will be prevented. It has been shown that the cardi-ovascular involvement in Beta thalassemia major patients without cardiac iron overload **(Stakos *et al.*, 2009).**

1-9-2: Zinc (Zn)

Zinc is an essential trace element that works as a cofactor for 200 enzymes involved in the process of metabolism and cell growth, as a component of many enzymes, and zinc participates in the metabolism of proteins, carbohydrates, fats and energy, zinc has a vital role for the healthy work of many reactions of the body's systems (**Osredkar & Sustar, 2011**)

As it plays an essential role in many biochemical pathways and is especially important for healthy skin and is essential for a healthy immune system and infec-tion resistance, zinc plays an important role in growth and cell division, as it re-quires protein and DNA synthesis, insulin activity and carbohydrate consumption, and enters In gene expression, ovarian and testicular metabolism, and liver func-tion (**Bhattacharya *et al.*, 2016**), In the blood plasma, zinc binds to and transports albumin (60%) and transporter (10%). The concentration of zinc in the blood

plasma remains relatively constant regardless of zinc intake. (**Cummings & Kovacic, 2009**).

The inflammatory products have a role in regulating the zinc balance from the release of interleukins from activated macrophages, which causes a decrease in zinc levels, resulting from increased synthesis of metallothionine in the liver and other tissues, and that the large negative associations of zinc and copper in the serum with MDA lead to a deficiency of trace elements, which may Impairs host protection against oxidative stress (**Pasa *et al.*, 2003**).

The essential trace element present in the body is zinc. It takes part in various important body functions including protein synthesis, DNA synthesis, and cellular growth. It is found almost in every cell and plays a vital role in body's immune system affecting innate and acquired immunity(**Zekavat et al.,2018**). Zinc also has significant antioxidant properties thereby protecting the cells from damage due to free radicals. It is the active site for a number of metalloenzymes which are required for nucleic acid synthesis and also important for other host defence mechanisms like production of monocytes and macrophages and chemo-taxis of granulocytes (**William, 2004**).

Zinc is absorbed from small intestine and found in the blood bound to albumin. Impaired growths, alopecia, loss of weight are few of the associated complications due to deficiency of zinc which is one of the factors responsible for growth and puberty disorders in thalassemia patients **(Shamshirsaz *et al*.,2003)**.

Frequent blood transfusions can lead to iron overload which may result in various endocrine abnormalities. They have studied two hundred twenty patients with Beta thalassemia major on chelation therapy. They found that there is an association between the duration of chelation therapy and abnormalities in lumbar bone mineral density (BMD).

1-9-3: Copper (Cu)

Copper is the other essential trace element present in our bodies. It mostly forms metalloprotiens which act as enzymes. Copper is the major component of hemoglobin which is a protein responsible for oxygen transport in blood cells., Copper is a central component of the antioxidant superoxide dismutase molecule and also helps in the formation of protein called ceruloplasmin thereby protecting the cells from free-radical injury **(Sinha & Gupta , 2018**).

Copper is also required for the production of hormones like nor adrenaline and prostaglandins which are hormone-like chemicals involved in the regulation of blood pressure, pulse, and healing. Deficiency of this trace element will lead to anemia, neutropenia, and growth impairment, abnormalities in glucose and cholesterol metabolism, and increased rate of infections. On the other hand, an accumulation of copper in body leads to Wilson's disease with copper accumulation and cirrhosis of liver. **(El, 2012).**

Copper binds as a catalyst with the enzymatic antioxidant superoxide Cu / Zn superoxide demutase, which removes toxins from the superoxide anion and converts it into hydrogen peroxide and oxygen, completing the enzyme catalase by converting it into oxygen and water by (**Pant *et al.*, 2018**) so the main problem in the biology of copper feed is how organisms accumulate enough copper to function as a cofactor for all cellular enzymes dependent on copper, while carefully controlling the accumulation of copper to prevent the generation of highly harmful oxygen species. In fact, organisms use many levels of regulation. Cellular level to regulate the fine balance of copper adequacy and copper increase, including regulation at the level of copper-dependent gene transcription .As the copper uptake represents the first stage in which cells encounter copper ions, this is a critical step that

must be tightly regulated to control copper accumulation (**Piña-Vázquez *et al*., 2012**).

The researcher **Zardkhoni *et al*.(2021)** indicated that zinc deficiency may contribute to treatments and Susceptibility to infection with intracellular pathogens by causing a decrease in IFN-γ production.

1-10:The Role of Iron in Oxidative Stress:

There has been considerable interest in the role of iron in the promotion of oxidative stress, particulary as it relates to genetic disorders associated with iron storge (**Siems, 2018**).

Several transition metal salts react with H_2O_2 to form ÓH, but in term of the possibility of ÓH generation *In vivo*, most attention has been paid to iron (**Sinha & Gupta , 2018**). Ferrous salts react with H_2O_2 to form ÓH by the so called Fenton reaction which is usually written as

$$Fe^{2+} + H_2O_2 \longrightarrow \acute{O}H + {}^{-}OH + Fe^{3+} \text{ ----- (1}$$

In fact Fenton reaction is far more complex. Thus the initial product of reaction may be an Oxo-iron complex, possibly ferryl, that then decomposes to from ÓH (**Erejuwa & Ab Wahab, 2013**).

$$Fe^{2+} + H_2O_2 \longrightarrow FeOH^{3+} \text{ (or } Fe^{2+}O) \longrightarrow \acute{O}H + Fe^{3+} \text{ ----(2}$$

Different ligands to the iron II may stabilize this intermediate, thus iron-EDTA are good sources of ÓH in the presence of H_2O_2 Fenton reaction generate Fe II . Most ferric complexes react more slowly with H_2O_2 than do Fe II complex, so that reducing agent stimulate Fenton reaction. This can occur with ascorbate.

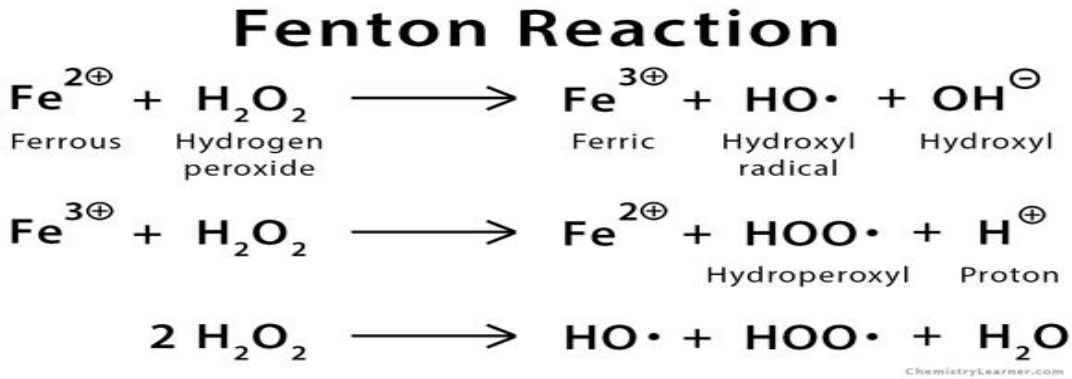
Fe^{3+} + ascorbate——— Fe^{2+} + semidehydro ascorbate ---- **(3** Hence, iron salt-ascorbate – H_2O_2 mixture are good sources of ÓH radical indeed, they have been used to generate ÓH for determination of reaction rate constants.

Superoxide anion can reduce certain ferric chelates. Reaction of Fe^{3+} with $\bar{O}2$; appears to proceed via a perferryl intermediates

$Fe^{3+} + \bar{O}2$ —— $[Fe^{3+}\text{-}\bar{O}2$—— Fe^{2+} - O2] —— Fe^{2+}+ O2**….. (4**

The sum of reaction 2 and 4, ignoring the oxo iron intermediate is

$\bar{O}2 + H_2O_2 \xrightarrow{Fe}$ —— ÓH +ŌH + O2 **….(5**



reaction often called the iron-catalysed Haber-Weiss reaction, or sometimes the superoxide driven Fenton reaction .

This reaction appears to account for as part of the damage that is caused to living cell by excess generation of ROS (**Sinha & Gupta , 2018**) Reaction (5) can be inhibited by using several chelating agents, the best known example being des-ferroxamine (**Marbut *et al.*, 2020).**

Although ferrous salts generally react faster than ferric with H_2O_2 the later reaction can or ignored Gutteridge demonstrated, using the deoxyribose assay to measure ÓH, that some ferric chelates react with H_2O_2 to form ÓH in process in-

volving $\dot{O}2^{-}$, The importance of iron ion in mediating oxidative damage naturally leads to the question as to what forms of iron might be available to catalyze radical reactions *In vivo*(**Mohammed and Abd-Elrasoul, 2020**).

Organisms take great care in the handling of iron, using both transport (such as transferrin) and storage (such as ferritin) proteins so as to minimize the amount of "free" iron within cells and in extracellular fluids (**Mohammed *et al*.,2020**).

However oxidative stress can itself provide iron for free radical reactions Thus $\dot{O}_2^{-}$, can mobilize iron from ferritin (**Zekavat *et al.,* 2018**). Although the amount of superoxide-releasable iron is small and so ferritin-bound iron is much safer than an equivalent amount of free iron. H_2O_2 can degrade heme proteins to release iron, thus, if no catalytic metal ions are available, then $\dot{O}_2^{-}$ and H_2O_2 at physiologic concentration may have limited (if any) damaging effects (**Marbut *et al.,* 2020**).

1-11: Albumin

Serum albumin, often referred to simply as blood albumin, is an albumin (a type of globular protein) found in vertebrate blood. Human serum albumin is encoded by the ALB gene(**Zunszain *et al.,* 2003**) Other mammalian forms, such as bovine serum albumin, are chemically similar.

Serum albumin is produced by the liver, occurs dissolved in blood plasma and is the most abundant blood protein in mammals. Albumin is essential for maintaining the oncotic pressure needed for proper distribution of body fluids between blood vessels and body tissues; without albumin, the high pressure in the blood vessels would force more fluids out into the tissues. It also acts as a plasma carrier by non-specifically binding several hydrophobic steroid hormones and as a transport protein for hemin and fatty acids. Too much or too little

circulating serum albumin may be harmful. Albumin in the urine usually denotes the presence of kidney disease. Occasionally albumin appears in the urine of normal persons following long periods of standing called postural albuminuria (**Zekavat *et al*., 2018**).

1-11-1: Structure and Function of Albumin

The general structure of albumin is characterized by several long α helices allowing it to maintain a relatively static shape, which is essential for regulating blood pressure. Serum albumin contains eleven distinct binding domains for hydrophobic compounds. One hemin and six long-chain fatty acids can bind to serum albumin at the same time (**Sugio *et al*.,2009**)

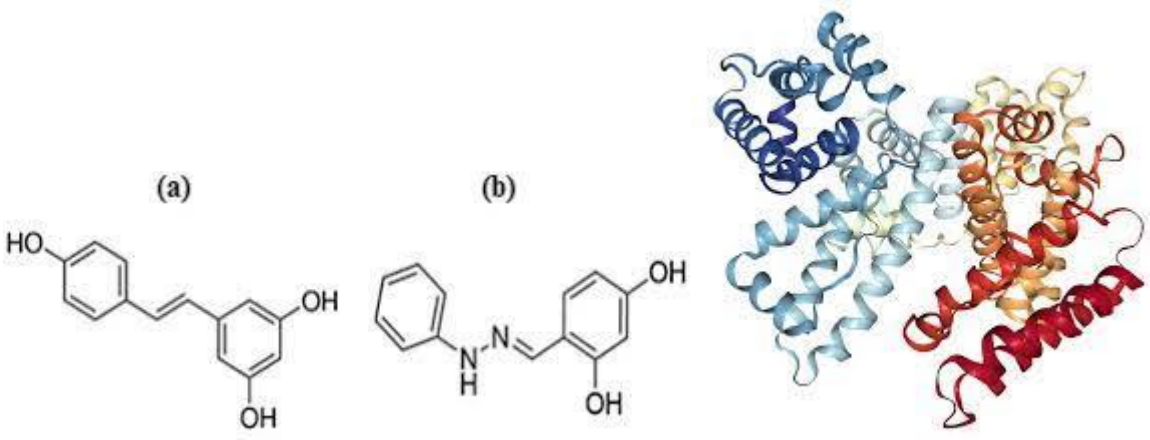


Figure (1-8): Chemical structure of Serum albumin structure.

The function of albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones in the blood and plays a major role in stabilizing extracellular fluid volume by contributing to oncotic pressure (known also as colloid osmotic pressure) of plasma. Because smaller animals (for example rats) function at a lower blood pressure, they need less oncotic pressure to balance , and thus need less albumin to maintain proper fluid distribution.(**Sugio *et al*.,2009; Munira *et al* ., 2020).**

1-12:Uric acid (Structure and clinical significant)

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $C_5H_4N_4O_3$. It forms ions and salts known as urates and acid urates, such as ammonium acid urate. Uric acid is a product of the metabolic breakdown of purine nucleotides, and it is a normal component of urine. High blood concentrations of uric acid can lead to gout and are associated with other medical conditions, including diabetes and the formation of ammonium acid urate kidney stones **(Lieberman *et al.*, 2007).**

In human blood plasma, the reference range of uric acid is typically 3.4–7.2 mg per 100 mLl (200–430 μmol/l) for men, and 2.4–6.1 mg per 100 ml for women (140–360 μmol/l).[29] Uric acid concentrations in blood plasma above and below the normal range are known as, respectively, hyperuricemia and hypouricemia. Likewise, uric acid concentrations in urine above and below normal are known as hyperuricosuria and hypouricosuria. Uric acid levels in saliva may be associated with blood uric acid levels (**Zhao and Huang ,2015**).

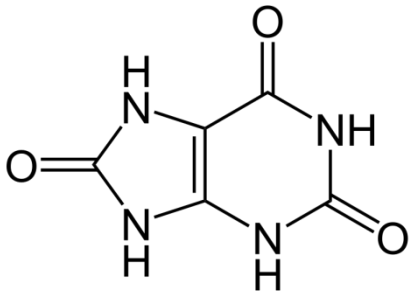


Figure (1-9): Chemical structure of uric acid

1-13: Allantoin Compound

Allantoin is a chemical compound with the chemical formula $C_4H_6N_4O_3$. It is also called 5-uridohydantoin or glyoxylidoride , allantoin is a major mediator of metabolic processes in most living organisms including animals, plants and bacteria. It is produced from uric acid, which is produced by the dissolution of nitrogenous bases by the enzyme uricase or urate oxidase.

Allantoin was first isolated in 1800 by the Italian physician Michele Francesco Boniva (1761-1834) and the French chemist Louis Nicolas Vauquelin, who erroneously believed that it was present in the amniotic fluid. In 1821, the French chemist Jean-Louis Lassaigne found it in the fluid in the allantoic. Two German scientists, Friedrich Waller and Justus Liebig, synthesized it from uric acid and renamed it allantoin.

Allantoin is found in plant extracts from erennial plants and in the urine of most mammals. Chemically manufactured allantoin blocks are chemically identical to natural allantoin in being safe, non-toxic, cosmetic raw materials compatible, and American Personal Care Products Council specifications. More than 10,000 patents refer to allantoin.

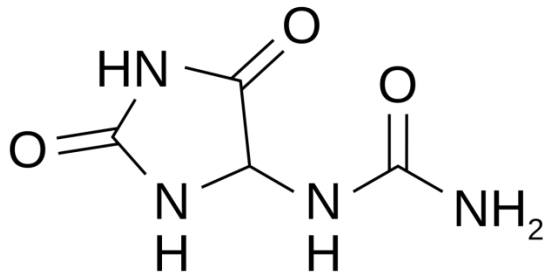


Figure (1-10): Chemical structure of Allantoin structure.

1-13-1:Pharmaceuticals of allantoin

Allantoin is often found in toothpaste, mouthwash, and other oral health products, in shampoos, lipsticks, acne-fighting products, sunscreens, purifying moisturizers and many different cosmetics and creams, and other pharmaceuticals.

1-13-2: As a Biomarker of Oxidative Stress

In humans, allantoin is formed by nonenzymatic oxidation of urate; it may, therefore, be useful in assessing oxidative stress . Since uric acid is the end product of purine metabolism in humans, and therefore only non-enzymatic processes with reactive oxygen species will produce allantoin, it is therefore a suitable biomarker for measuring oxidative stress in chronic disease and aging, Urate is the terminal product of purine metabolism in primates, including humans. Urate is also an efficient scavenger of oxidizing species and is thought to be an important antioxidant in human body fluids. Allantoin, the major oxidation product of urate, has been suggested as a candidate biomarker of oxidative stress because it is not produced metabolically. (**Kand'ár and Záková ,2008**).

1-14: The Aim of the Current Study :

The current study aims to apply the following objectives :-

1-Study of hematological variables for patients and comparing them with healthy (control).

2- The relationship between some trace elements such as **Iron, Copper, Zinc** in the blood serum of patients with Beta-thalassemia and their relationship to non-enzymatic antioxidants such as some organic molecules (**Uric acid** and **Albumin and Allantoin**) As well as determination of total antioxidant capacity and enzymatic antioxidant like super oxide dismutase (SOD).

3- Preparation of allantoin from uric acid in laboratory and then calibrate standard curve for different concentrations of allantoin , Then the allantoin level was measured in the serum samples of patients and compared with the control group by using HPLC technique.

Chapter two

Materials and Methods

2：Materials and Methods

2-1: Materials (Experimental Part)

2-1-1: Instruments

The following devices have been used to perform hematological, biochemical, as in Table (2-1), which shows the devices used by origin and company:

Table (2-1): Equipment used by origin and company :

No.	Equipment	Company	Origin(Country)
1	Biochemical measuring device (Cobas c111)	Roche	Germany
2	Büchner funnel	LAB.	Germany
3	Capillary tube	Afco-Dispo	Jordan
4	Centrifuge	Memmert	Germany
5	EDTA tube	C.S.M.D.	China
6	Ependrof tubes (1.5 ml, 2 mL, 5 mL)	C.S.M.D.	China
7	Filter paper	BROCHE	Turkey
8	FTIR-8400S(Fourier Transform Infra-red Spectrophotometer)	Shimadzu	Japan
9	Hotplate stirrer	LabTech	Korea
10	HPLC model	SYKAMN	Germany
11	Humacount Auto Analyzer	Human	Germany
12	Incubator	Biomerieux	France
13	Mechanical stirrer	LAB.	Germany
14	Melting point apparatus(SMP30)	Stuart	English
15	Micropipettes from	Dragon	China

	(50µl-1000 µl)		
16	Mixer Vortex	Labtech	Korea
17	Motor vacuum	LAB.	Germany
18	Plain tube	C.S.M.D.	China
19	Refrigerator	Concord	China
20	Rotary evaporator (Laborotary 4000 efficient)	Heidolph	Germany
21	Rround-bottomed flask	LAB.	Germany
22	Sensitive Imbalance	Mettler Toledo	Switzerland
23	Spectrophotometer	Emc-lab.	Germany
24	Tips (10 ul., 200 ul., 1000 uL),	Co.ltd	China
25	Water bath	Memmert	Germany

2-1-2: Chemicals and Laboratory Supplies:

The following laboratory for blood tests materials and supplies has been used the blood and biochemical tests as in Table (2-2), which shows materials and laboratory supplies used by origin and company:

Table (2-2): Laboratory materials and supplies used by origin and company: -

No.	Equipment	Company	Origin(Country)
1	Double distilled water,	Roche	Germany
2	Glacial acetic acid (137 gm(2.2 moles)	TCI	aldrich
3	Ionized distilled water	Roche	Germany
4	Manganese dioxide	CDH	aldrich
5	Norite	CDH	China
6	Normal saline (0.9% NaCl),	Biomerieux	France
7	of commercial sodium hydroxide (80 gm (2 moles)	CDH	aldrich
8	Phasphate Buffer Saline (0.01 M, pH 7.4)	Memmert	Germany
9	Potassium EDTA(k_3) tube	Plastic LAB	S.A.R.
10	Potassium permanganate **(3-** 50 gm (0.32 mole).	CDH	aldrich
11	Sodium Heparin tube	BDH	China
12	Uric acid(100 gm (0.595 mole)	BDH	aldrich

2-1-3: Kits that used in the present study

Table (2-3): Kits that used in the present study

No.	Kits	Company	Origin(Country)
1	Copper (Colometric test with Dibrom-PAESA)	Spectrum	Germany
2	Zink (Colometric test with 5-Promo-PAPS)	Spectrum	Germany
3	Iron- Direct method (Ferene)	Biolabo	France
4	Albumins method BCG	Biolabo	France

5	Uric acid Uricase method	Biolabo	France
6	Total antioxidant capacity (T-AOC) Colometric Assay kit	Elabscience	USA
7	Super oxide dismutase(SOD)	Elabscience	USA

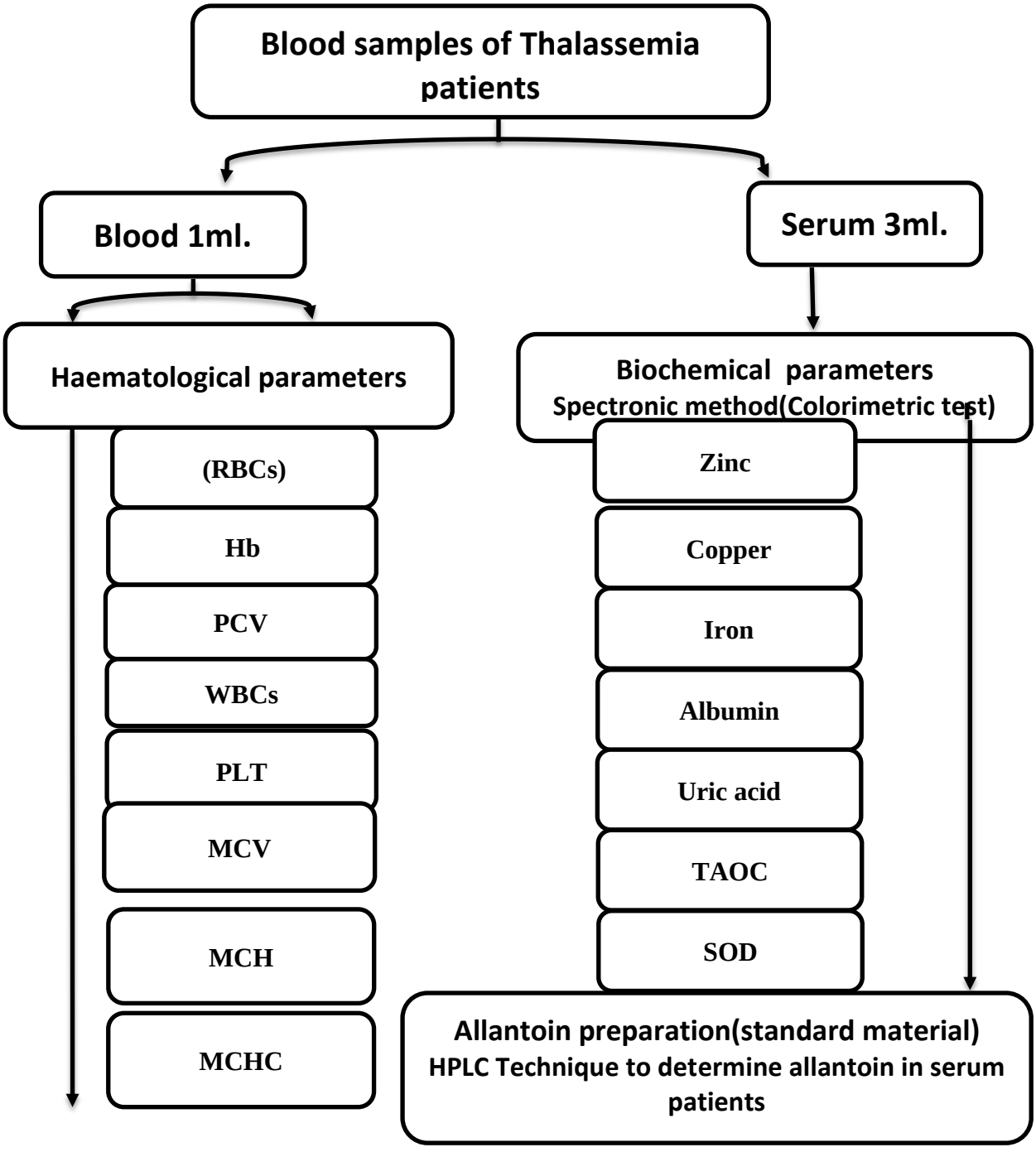
2-2: Subjects

2-2-1: Experimental Design :

The research was carried out in the organic chemistry and Animal Physiology Laboratory for Postgraduate Studies in the College of Science for women - Department of chemistry and biology in Babylon University, in cooperation with the Thalassemia Center(Genetic Blood Diseases) and the laboratories of Al-Hilla Teaching Hospital of the Maternity and children, It was confirmed that the patients had thalassemia major through the periodic review of them to the Thalassemia Center and through the diagnosis of the specialist doctor for the severe deficiency of blood parameters (amount of hemoglobin, the packed cells volume (PCV), the number of red blood cells, MCV corpuscular volume ratio, MCH corpuscular hemoglobin ratio, MCHC corpuscular hemoglobin concentration... etc) .

Blood samples were taken from patients with Beta-Mediterranean major anemia who attend the Thalassemia Center for the purpose of obtaining a blood transfusion and their disease has been diagnosed in advance. These patients suffer from an excess of iron in their bodies due to the blood transfusion, which leads to complications, including chronic hepatitis and an enlarged spleen. The study samples, which amounted to (60 (30 Males and 30 Females)) patients and (30) 15 Males and 15 Females) control group , for the age groups from (1) year to 40 years for the period from November 2020 till June 2021 Figure(2-1). and all the

patients provided with questionnaire paper , Appendix (1) and Athetical paper Appendix (2).



Figure(2-1): Scheme diagram illustrated experimental design of present study .

2-2-1-1: Stability and Storage and Specimen collection and handling of kits that used in biochemical test .

Parameters	Stability and Storage	Specimen collection and handling
Zinc	stored refrigerated at 2-8 °C.	Serum Zinc is stable in serum for. 72 hours at 2-8°C. 6 months at 20°C.
Copper	vial is stable for 3 months at 2-8 °C.	Serum Copper is stable in serum for. 72 hours at 2-8°C. 6 months at 20°C.
Iron	Store at 2-8°C, well recap in the original vial and away from light	Do not use EDTA, oxalate or citrate. Serum iron is stable in specimen for: • 4 days at room temperature. • 1 week stored 2-8°C.
Albumin	Store at 2-8 C°**,** Discard reagent if cloudy or if absorbance at 630 nm > 0.300.	Serum albumin is stable in serum for. 72 hours at 2-8°C. 6 months at 20°C.
Uric acid	Store at 2-8°C**,** Discard any reagent if cloudy or if absorbance at 520 nm > 0.100.	1 week at 2-8°C. • 6 months freeze at-20°C. Add NaOH to keep urine alkaline and to prevent uric acid precipitation.
TAOC	Store at 2-8°C	Serum **TAOC** is stable in serum for. 72 hours at 2-8°C. 6 months at 20°C.
SOD	Store at 2-8°C	4 days at room temperature. • 1 week stored 2-8°C.

2-2-2 : Blood Samples Collection :

Four (4) ml of venous blood were drawn from each patients before receiving a transfusion quantity of healthy people (control) to compare them, and the samples were divided into two part as the following :-

1-Putting 1 ml of blood into an EDTA tube, for the purpose of a blood test for haematological assay.

2- Putting 3 ml of blood in gel tube to getting the serum , for biochemical tests.

2-3 : Haematological Assay

The blood test includes the following tests (red blood cell count, hemoglobin, PCV agglutinated corpuscle volume, MCV corpuscular volume ratio, MCH corpuscular hemoglobin ratio, MCHC corpuscular hemoglobin concentration, percentage of erythrocyte distribution width RDW, number White blood cells (WBCs) and platelets (PLT) were examined using . Humacount Auto Analyzer after adding (1) ml of blood into an EDTA tube and placing the tube in the specified place on the device and then gave the start instruction, as the device reads the results automatically and when it appears .The results have been given a directive (print) for the device to print it, according to what the German company Human brought Origin.

2-3-1:Complete Blood Counts(CBC).

The hematological diagnosis shows anemia due to microcytic anemia mediterranean minor anemia is characterized by a decrease in both the mean MCV and MCV levels corpuscular hemoglobin (MCH), as hemoglobin level (7-10) gm/dl, MCV level (50-80) vemtoliters, and hemoglobin level MCH (16-24) pgm. Whereas for mediterranean anemia, the hemoglobin level is less than . 7 g/dL, MCV level (50-70) vemtoliters, and hemoglobin level MCH (16-24 picograms) (**Galanello *et al.*, 2001**).

2-4 : Biochemical Assay

Putting 3 ml of blood in a gel tube for biochemical tests . And then transferred the serum (2 ml) in appendrof tube after centrifugation 3000rpm for 5 minutes for these assays the following :-

2-4-1: Determination of serum zinc concentration (Colorimetric test with 5-Bromo-PAPS)

Assay Principle

Zinc forms with 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N- sulfopropyl-amino)-phenol a red chelate complex. The increase of absorbance can be measured and is proportional to the concentration of total zinc in the sample.

Reagents

Standard (st.) 200 ug/dl (30.6 umol/) , Reagent (R)

5-Br-PAPS	0.02 mmol/L
Bicarbonate buffer pH 9.8	200 mmol/L
Sodium Citrate	170 mmol/L
Dimethylglyoxime	4 mmol/L
Detergent	1%

For further information, refer to the zinc reagent material safety data sheet

Reagent Preparation

Spectrum zinc reagents are supplied ready-to-use.

Procedure

	Blank	Standard	Sample
Reagent (R)	1 ml	1 ml	1 ml
Standard(St.)	/	50µl	/
Sample	/	/	50µl
Mix and incubate for 10 min at 25 °C or 5 min at 37 °C. Measure the absorbance of the sample .As and the absorbance of standard A st. against reagent blank.			

Calculation

Zinc Concentration (µg/dl)= A specimen /A standard ×200

Zinc Concentration (µmol/l)= A specimen /A standard ×30.6

2-4-2: Determination of serum Copper concentration (Colorimetric test with Dibromo-PAESA)

Principle Assay

Copper forms with 4-(3,5-dibromo-2-pyridylazo)-N-ethyl- sulfopropy-laniline a chelate complex. The increase of absorbance of this complex can be measured and is proportional to the concentration of total copper in the sample.

Reagents

Standard (ST) 100 ug/dl	**15.7 µmol/L**
R (Monoreagent) **Acetate buffer pH 5**	 **0.2 mmol/L**
4-(3,5-dibromo-2-pyridylazo)- N-ethyl-sulfopropylaniline	**0.02 mmol/L**
For further information, refer to the Copper reagent material safety data sheet.	

Reagent Preparation,

Warning: The reagent could precipitate during refrigerate storage.t suggested to let it to dissolve at room temperature before use (15 minutes).Mix well after dissolving. Spectrum Copper reagent is supplied ready-to-use and stable up to the expiry date labeled on the bottles, once opened, the opened vial is stable for 3 months at 2-8 °C.

Procedure

1- Determination of copper in serum

	Blank	Standard	Sample
Reagent (R)	1 ml	1 ml	1 ml
Standard(St.)	/	50µl	/
Sample	/	/	50µl
Mix and incubate for 5 min. at 37 °C. Measure the absorbance of the sample .As and the absorbance of standard Ast. against reagent blank.A_{RBL}			

$\Delta As=As- A_{RBL}$

$\Delta Astd=Astd- A_{RBL}$

Calculation

Copper Concentration (µg/dl)= **$\Delta As/\Delta Astd \times 100$**

Copper Concentration (µmol/l)= **$\Delta As/\Delta Astd \times 100$** ×15.7

2-4-3: Determination of serum Iron concentration direct method (Ferene)

PRINCIPLE

After dissociation of iron-transferrin bound in acid medium, ascorbic acid reduces Fe iron into Fe^{+2} iron. Fe^{+2} iron then form a coloured complex with 3-(2-Pyridyl) -5, -6-difuryl-1, -2, 4-triazine-disulfonate (Ferene). The absorbance thus measured at 600 nm (580-620) is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent to prevent the copper interference.

REAGENTS COMPOSITION

Vial R1 (Reductant)	
Citric acid	150 mmole/L
Ascorbic acid	30 mmole/L
Thiourea	27 mmole/L
Vial R2 (Chromogen)	
Ferene	600 µmole/L
Vial R3 Standard	
Iron	200 µg/dl(35.8 µmole/L)

REAGENTS PREPARATION

Prepare working reagent as follows: RI (50 volumes) + R2 (1 volume). Use carefully cleaned material with HCI 0.1 N and well rinsed with distilled water. Give a special care to the quality of water, reagents and/or specimens. Some automated instrument requires special preparation.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature. Prepare sets of tubes according to following boards:

Blank tubes	Blank	Standard	Assay
Reagent R1	1 mL	1 mL	1 mL
Specimen			200 µL
Standard		200 µL	
Distilled water	200 µL		
Mix gently, Let stand for at least 3 minutes at room temperature. Record A1 absorbance at 600 nm (580-020) against blank. Colour is stable in 1 hour.			
Assay tubes	Blank	Standard	Assay
Working reagent	1 mL	1 mL	1 mL
Specimen			200 µL
Standard		200 µL	
Distilled water	200 µL		
Mix gently, Let stand for at least 5 minutes at room temperature. Record A2 absorbance at 600 nm (580-020) against blank. Colour is stable in 1 hour.			

Calculation

Calculate the result as follows :

Result= (A2-A1)Assay/(A2-A1)Standard × Standard concentration

2-4-4: Determination of serum Albumin concentration BCG Method

PRINCIPLE

In buffered solution at pH 4.2, bromocresol green binds albumin to from a coloured compound which absorbance, measured at 630 nm (620-640) is proportional to the albumin concentration in the specimen.

REAGENTS COMPOSITION

Vial R1 BROMOCRESOL GREEN	
Succinic acid	83 mmole/L
Bromocresol green (BCG)	167 µmole/L
Sodium hydroxide	50 mmole/L
Polyoxyethylene monolauryl ether Preservative	100 g/L
Vial R2 (Standard)	
Bovine albumin	5.0 g/dl. (725 umol/L)

REAGENT PREPARATION

Reagents are ready for use .

Manual Procedure

Let stand reagents and specimens at room temperature

Procedure n°1: Specimen volume 10 µL

Pipette into well identified test tube	Blank	Standard	Assay
Reagent	2 mL	2 mL	2 mL
Demineralized water	10 µL		

Specimen			10 µL
Standard		10 µL	
Mix well. Record absorbance at 630 nm (620-640) within 3 minutes against reagent blank or better after exactly 1 minute (note 2).			

Procedure n°2: Specimen volume 10 µL

Pipette into well identified test tube	Blank	Standard	Assay
Reagent	2.5 mL	2.5 mL	2.5 mL
Demineralized water	5 µL		
Specimen			5 µL
Standard		5 µL	
Mix well. Record absorbance at 630 nm (620-640) within 3 minutes against reagent blank or better after exactly 1 minute (note 2).			

CALCULATION

calculate the result as follows:-

result= Abs (Assay) / Abs Standard × Standard concentration

2-4-5: Determination of serum Uric Acid concentration (Uricase Method)

PRINCIPLE

Uricase acts on uric acid to produce allantoin, carbon dioxide and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with a chromogen (amino-antipyrine and dichloro- hydroxybenzen sulfonate) to yield quinoneimine, a red coloured complex. The absorbance

measured at 520 nm (490-530) is proportional to the amount of uric acid in the specimen.

Vial R1 Enzymes	
Potassium hexacyanoferrate (II)	42 µmole/L
Peroxidase	≥450 U/L
Amino-antipyrine	0.150 mmole/L
Uricase	≥120 U/L
Vial R2 BUFFER	
Dichlorohydroxybenzen sulfonate	2 umol/L
Tris pH 8.0 at 25°C Preservative	50 umol/L
Vial R3 STANDARD	
Uric acid	10 mg/dL (595 umol/L)

REAGENTS PREPARATION

Vial R1 .use a non-sharp instrument to remove Aluminum cap. Add promptly the contents of vial R1 (Enzymes) into vial R2 (Buffer). Mix gently until complete dissolution before using reagent (approximately 2 minute.

MANUAL PROCEDURE

Pipette into well identified test tube	Blank	Standard	Assay
Working solution	1 mL	1 mL	1 mL
Specimen(Note1)			25 µL
Standard		25 µL	
Demineralized water	25 µL		
Mix. Let stands for 5 minutes at 25°C. Record absorbance at 520 nm (490-530) against reagent blank. Colour is stable for 30 minutes.			

1. Serum, plasma, or urines diluted (1+9) with demineralized water. **2.** Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support. **3.** Specimen: a 20 ul volume may be used (increased linearity but slightly decreased sensitivity).

CALCULATION

Calculate the result as follows:

result =Abs (Assay) /Abs (Standard) x Standard concentration
Diluted urines (1+ 9): Multiply the above result by dilution factor 10.

2-4-6: Determination of serum Total Antioxidant Capacity concentration (T-AOC) Colorimetric Assay

Detection principle

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species. The total level reflect the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe to Fe" and Fe" can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 nm.

A Kit components & storage

Item	Component	Specification	Storage
Reagent 1	**Buffer Solution**	**60 ml×2 vials**	**2-8 C° , 6 Months**
Reagent 2	**Chromogenic agent**	**Powder×2 vials**	**2-8 C° , 6 Months,shading light**
Reagent 3	**Ferric salt stock**	**1.5ml×2 vials**	**2-8 C° , 6**

	solution		**Months,shading light**
Reagent 4	**Ferric salt diluent**	**60 ml×1 vials**	**2-8 C° , 6 Months**
Reagent 5	**Stop solution**	**24 ml×1 vials**	**2-8 C° , 6 Months**
Reagent 6	**clarificant**	**24 ml×1 vials**	**2-8 C° , 6 Months**
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with go pee If you have any problem, please contact our Technical Service Center for help, Phone: 240-252-7358USA) Fax 240-252-7376(USA) Email techsupportgelabscence.com Please kindly provide us the kit number (on the outside of the box) of the kit for more efficient service**.**			

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4).

Pre-assay Preparation(A Reagent preparation)

1. Preparation of Reagent 2 working solution dissolve a vial of Reagent 2 with 120 mL. double distilled water fully (it can be dissolved by incubating in 80-90°C water bath). It can be used after cooling to room temperature.

2. Preparation of Reagent 3 working solution Dilute the Reagent 3 with Reagent 4 at the ratio of 1:19. Prepared the working solution before use.
3. Reagent 6 will be freeze in cold weather, dissolve by incubating in 37C water bath till clarification before experiment.

Dilution of sample

Is recommended to take 2-3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.62-145.2 U/mL).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	520 nm

Assay protocol (For serum, plasma or other liquid sample).

Operating steps

1- Sample tube. Add 1.0 ml of reagent 1 to 5 mL EP tube.

Control tube: Add 1.0 ml. of reagent 1 to 5 ml EP tube.

2- Sample tube Add (0.1 ml) of sample to the tube.

Control tube: Add nothing.

3- Add 2.0 mL of Reagent 2 working solution and 0.5 ml of Reagent 3 working solution to each group

4-Mix fully and incubate the tubes at 37C for 30 min.

5-Add 0.1 ml of Reagent 5 to each tube.

1- Sample tube: Add nothing.

Control tube: Add A" ml of sample to the tube.

2- Mix fully and stand for 10 min at room temperature. Set to zero with double distilled water and measure the OD value of each tube at 520 nm with 1 cm optical path quartz.

A Calculation

For serum, plasma or other liquid sample.

Definition: Al 37 C, the OD value of the reaction system was increased 0.01 by 1 ml of sample per minute is defined as a unit of total antioxidant capacity.

T-AOC activity= $\Delta A/0.01 \div 30 \times V1/V2 \times f$

Note: AA: OD_{sample}- $OD_{control}$

*: The reaction time, 30 min.

V1: The total volume of reaction, mL.

V2: The volume of sample added to the reaction, mL.

f : Dilution factor of sample before tested.

2-4-7: Determination of serum Total Superoxide Dismutase (T-SOD) Activity Assay Kit (Hydroxylamine Method)

Detection principle

The superoxide anion free radical (O2•-) can be produced by xanthine and xanthine oxidase reaction system,O2•- oxidize hydroxylamine to form nitrite, it turn to purple under the reaction of developer. When the measured samples containing SOD, the SOD can specifically inhibit superoxide anion free radical (O2•-). The inhibitory effect of SOD can reduce the formation of nitrite, the absorbance value of sample tube is lower than control tube. Calculate the SOD of sample according to the computational formula.

Kit components & storage

Item	Component Specification Storage	Component Specification Storage	Component Specification Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	2-8℃, 6 months
Reagent 2	Nitrosogenic Agent	12 mL × 1 vial	2-8℃, 6 months
Reagent 3	Substrate Solution	12 mL × 1 vial	2-8℃, 6 months
Reagent 4	Enzyme Stock Solution	0.6 mL × 1 vial	-20℃, 6 months
Reagent 5	Enzyme Diluent	12 mL × 1 vial	2-8℃, 6 months
Reagent 6	Chromogenic Agent A	Powder × 1 vial	2-8℃, 6 months
Reagent 7	Chromogenic Agent B	Powder × 1 vial	2-8℃, 6 months
Reagent 8	Chromogenic Agent C	60 mL × 1 vial	2-8℃, 6 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

Reagents

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Pre-assay preparation

Reagent preparation

1. Preparation of reagent 1 working solution:

Dilute the reagent 1 with double distilled water at a ratio of 1:9 before use. Prepared solution can be stored at 2-8℃ for 3 months.

2. Preparation of reagent 4 working solution:

Dilute reagent 4 with reagent 5 at a ratio of 1:19. Prepare the fresh solution before use. Unused reagent can be stored at 2-8℃ for 3 days.

3. Preparation of reagent 6 application solution:

Dissolve a vial of powder with 70-80℃ double distilled water to a final volume of 90 mL. It can be store at 2-8℃ with shading light for 3 months.

4. Preparation of reagent 7 application solution:

Dissolve a vial of powder with double distilled water to a final volume of 90 mL. It can be store at 2-8℃ with shading light for 1 months.

5. Preparation of chromogenic agent:

Prepare chromogenic agent at ratio of reagent 6 application solution: reagent 7 application solution: reagent 8 =3:3:2. Prepare the fresh solution before use and the prepared chromogenic agent can be stored at 2-8℃ in the dark.

Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

The samples should not contain SDS, Tween 20, NP-40, Triton X-100 and other detergents, and should not contain DTT, 2-mercaptoethanol and other reducing reagents

Determination of optimal sampling volume

1. The optimal sampling volume are different for different species, the SOD also are different for different samples. It is recommended to take 2~3 samples to do a pre-experiment to determining optimal sampling volume before formal experiment.
2. The Inhibition ratio of this kit is 15-55%, the optimal inhibition ratio is 25- 45%. When the inhibition ratio is 25-45%, the corresponding sampling volume is the optimal sampling volume.

Inhibition ratio= ODcontrol – Odsample \ ODcontrol × 100%

If inhibition ratio > 55%, need to dilute the sample or decrease the sampling volume than take the test. If inhibition ratio < 15%, need to increase the sampling volume.

Sample type	Dilution factor	The volume of sample
HepG2 supernatant	**1**	**50 µL**
HepG2 cell	**8-10**	**25 µL**
Mouse serum	**3-5**	**20 µL**
10% Mouse liver tissue homogenate	**40-60**	**20 µL**
10% Rat kidney tissue homogenate	**15-20**	**20 µL**
Human urine	**1**	**25 µL**

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	550 nm

Operating steps

1. Sample tube: add 1 mL of reagent 1 working solution and a* mL sample to the sample tubes.

Control tube: add 1 mL of reagent 1 working solution and a* mL double distilled water to the control tubes.

2. Add 0.1 mL of reagent 2, 0.1 mL of reagent 3, 0.1 mL of reagent 4 working solution successively into the tubes of Step 1.

3. Mix fully with a vortex mixer, incubate for 40 min at 37 ℃ .

4. Add 2 mL of chromogenic agent into the tubes of Step 3.

5. Mix fully and stand for 10 min at room temperature.

6. Set to zero with double distilled water and measure the OD value of each tube at 550 nm with 1 cm optical path quartz cuvette.

Note: If the optimal sampling volume (a*) is the same, only one control tube need to be assay.

	Sample tube	**Control tube**
Reagent 1 working solution (mL)	**1.0**	**1.0**
Sample (mL)	**a***	
Double distilled wa-		**a***

ter(mL)		
Reagent 2 (mL)	**1.0**	**1.0**
Reagent 3 (mL)	**1.0**	**1.0**
Reagent 4 working solution (mL)	**1.0**	**1.0**
Mix fully with a vortex mixer, incubate for 40 min at 37 ℃ .		
Chromogenic agent	**2.0**	**2.0**
Mix fully and stand for 10 min at room temperature. Set to zero with double distilled water and measure the OD value of each tube at 550 nm with 1 cm optical path quartz cuvette.		

Calculation

1. For serum (plasma), culture cell supernatant and other liquid samples:

Definition: The amount of SOD when the inhibition ratio reaches 50% in 1 mL reaction solution is defined as 1 SOD activity unit (U).

T-SOD activity (U/mL)=i÷50%×V1\V2 ×f

Note: i: inhibition ratio, i= ODcontrol – Odsample\ ODcontrol × 100%

V1: the total volume of reaction solution, mL.

V2: the volume of sample added, mL.

f: dilution factor of sample before test.

Cpr: the concentration of protein in sample, mgprot/mL

2-5 : Preparation of Allantoin from Uric acid by oxidation as (standard material)

Procedure(depend on Czauderna and Kowalczyk (1997) :

1- One hundred grams of uric acid (0.595 mole) and 4.5l. of hot water (70–85°) are placed in a 12-l. round-bottomed flask equipped with a mechanical stirrer. The stirrer is started, and a solution of 80 gm (2 moles) of commercial sodium hydroxide in 120 ml. of water is added. Stirring is continued until the uric acid is in solution .

2- after which the solution is cooled by means of a stream of water directed against the flask. When the temperature has fallen to 25–30 C°,

3- 50 gm(0.32 mole). of potassium permanganate is added all at once to the vigorously stirred solution. Stirring is continued for 15 - 20 min.

4- The mixture is filtered at once through a 19-cm. Büchner funnel. The first fraction of the filtrate contains a small amount of manganese di-oxide. This fraction must be collected separately and returned to the funnel.

5-As soon as the filtrate becomes clear it is collected in a 12-l. round-bottomed flask which contains 130 cc. (137 gm, 2.2 moles) of glacial acetic acid. The filtrate is tested with litmus to be sure that it is acid, and evaporated to a volume of 1.5–2l. on a steam bath under reduced pressure (20–30 mm.).

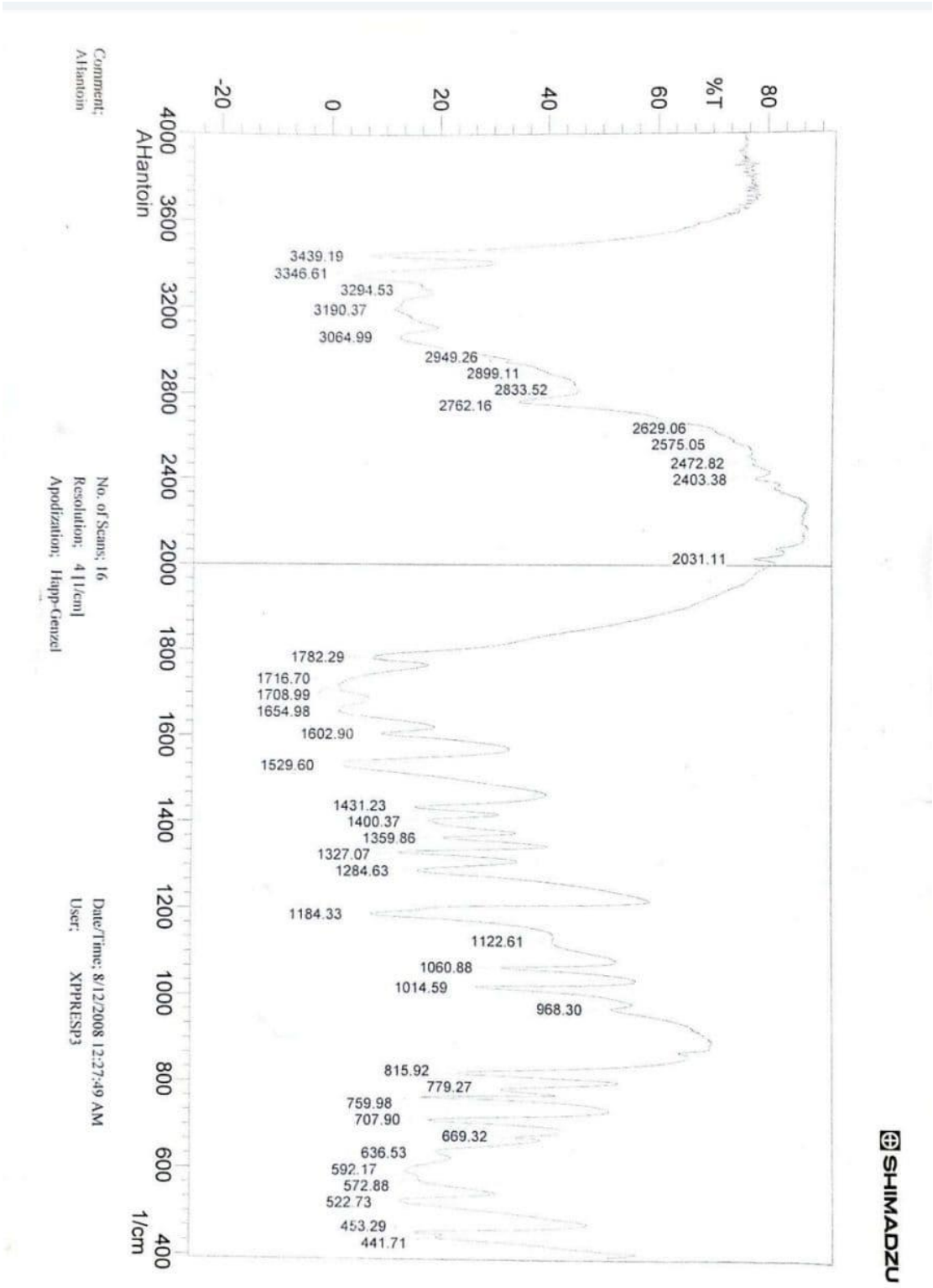
6-The solution thus obtained is allowed to stand in a cool place over-night, and the allantoin which crystallizes is filtered on a 9-cm. Büch-ner funnel.

7- The allantoin is dissolved in 800–900 ml. of boiling water, treated with 5 gm. of Norite, and filtered rapidly through a fluted filter paper in a steam funnel. The filtrate is allowed to stand in a cool place overnight,

and the white crystals of allantoin are separated by filtration with suction.

8-The yield of product melting at 230–231° is 60–71 gm (64–75 %) of the theoretical amount). If the filtrate from the purification liquors is concentrated to 100 ml., there is obtained an additional 3–5 gm of allantoin. (and then tested some gram of allantoin in Melting point apparatus(SMP30) to ensure that this material its allantoin that melting at 230–231° .

10-by FTIR-8400S(Fourier Transform Infrared Spectrophotometer) put the sample and then by this device and from peaks revealed in (Figure 2-1) known that this samples return to allantoin , U.V. visible of uric acid its equal 294.46 nm and λmax**= 380 nm).** Whereas the U.V. visible its 175-800 nm and **λmax** = 299.01 nm (**Czauderna and Kowalczyk, 1997).**



Figure(2-2) : FTIR-8400S(Fourier Transform Infrared Spectrophotometer) for detecting allantoin compound.

2-5-1:Determination of Allantoin level concentration in serum patients and control groups by High Performance Liquid Chromatography technique (HPLC).

2-5-1-1:Sample preparation :

A serum sample (100 µm) was mixed with 400 µm of solvent C (KH_2PO_4/H_3PO_4-buffer with 50 mmol/1 phosphate, pH 4.60) then filtered through a membrane filter with a pore diameter of 0.22 µm (Germany). An aliquot (50 µm) of the filtrate was directly injected into the injector of the HPLC device. The quantification of is based on the peak areas calculated for the wavelength .

2-5-1-2:Preparation of standard solutions :

2 mg of each standard was taken and placed in a volumetric flask **(25 ml)** and the volume was supplemented with methanol (HPLC 99.9 %) to the mark where the stock solution concentration (80 ppm). By using the dilution law **C 1 V 1 = C 2 V 2** , the concentrations that injected into the HPLC were prepared.

2-5-1-3:Diagnostics of separated compounds:

Before starting to diagnose the separated compound, we must know how to prepare the standard material. standard (We often hear about the unit of concentration which is) ppm , part per-million.) ppm = mg/L, µg/ml, µg/gm. That is, when we take a weight, for example (0.01 gm) of any substance and it is dissolved in a known volume (200 ml) ,from any solvent. 0.01 gm/200ml x (103)= 10 mg/200ml x (103)= 10000µg/200ml = 50 µg/ml (50ppm), 0.1 mg/200 ml x (103) = 100µg/200ml = 0.5µg/ml (0.5ppm).

After preparing the standard material (st), the working method is now prepared, which includes ,(Mobile Phase, Column, Detectors, Flow Rate, Run time) ,It is based on a source of work so that the results are a reliable reference.

2-5-1-4:Diagnostics of separated compounds:Injection process:

After turning on the device and leaving it for a while to settle, the solvent that dissolved the substance is injected, then the standard substance is injected with a known concentration, and then the solvent is injected again to ensure that there is no residue of the standard material, then the samples are successively injected.

When conducting the analysis of the standard material, it will appear in the form of a peak (peak) written at the top of this the peak is the retention time (Rt) , which is the time it takes for the material to exit from the detector started from the injection process, which is the descriptive function of the substance under the proven working conditions.

After re-injecting the standard substance more than once to fix its retention time, the sample is injected. Note: After each injection of the standard sample, a solvent is re-injected to ensure that there is no residue of previous sample. Then the first sample is injected and the retention time (Rt) of the standard substance is matched with the retention time separated materials. Whichever is identical with the retention time of the standard material will be the same Standard article. This is called a descriptive diagnosis **.**

2-5-1-5:HPLC Condition :

High Performance Liquid Chromatography HPLC model SYKAMN (Germany) It was used to analyses add detection of Vincristine and vinblastine . The mobile phase was an isocratic acetonitrile– 0.1M phosphate buffer containing 0.5% glacial acetic acid (30 : 70) at flow rate at 1.2 mL/min , column was C18 – ODS (25 cm * 4.6 mm) and the detector UV- 360 nm .

08/08/2021 12:52 م Calibration F:\allantoin.CAL Page 1 of 2

Clarity - Chromatography SW
DataApex
www.dataapex.com

Calibration : F:\allantoin
Description :
Created : 22/02/2021 09:40:00 ص
By : Administrator
Modified : 08/08/2021 12:51:59 م

Calculation	: ESTD	Mode	: Calibrate
Calibrate	: Automatic	Recalibration Type	: Replace
Change Response	: Enable	Weight	: 0.25
Update Reten. Time	: Enable	Search Criteria	: 0.00%
Deviation Limit	: Not Used	Correlation Limit	: Not Used
Default Injected Volume	: Not Used		

Calibration Summary Table (ESTD - F:\allantoin - Signal 1)

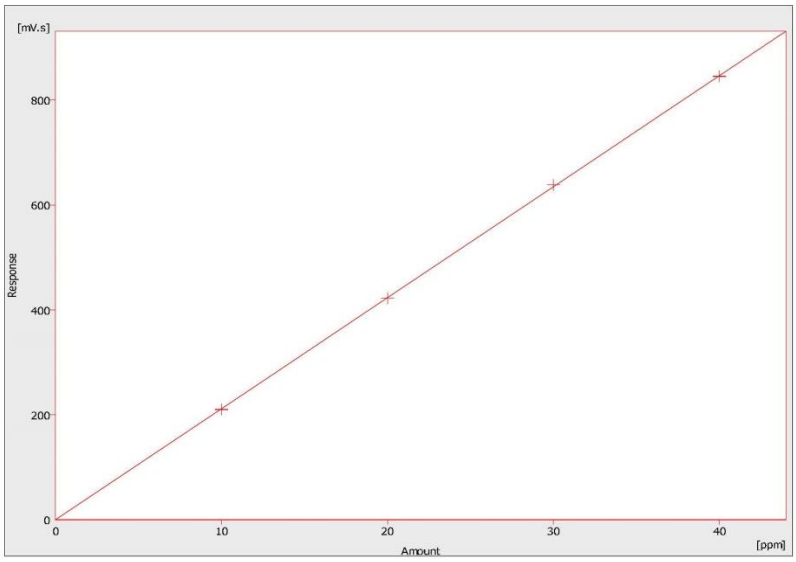
Used	Compound Name	Reten. Time	Left Window	Right Window	Peak Type	Peak Color	LOD	LOQ	RB	Resp. Factor
☒	allantoin	15.100	0.200 min	0.200 min	Ordnr		0.000	0.000	A	0.0000

08/08/2021 12:52 م Calibration F:\allantoin.CAL Page 2 of 2

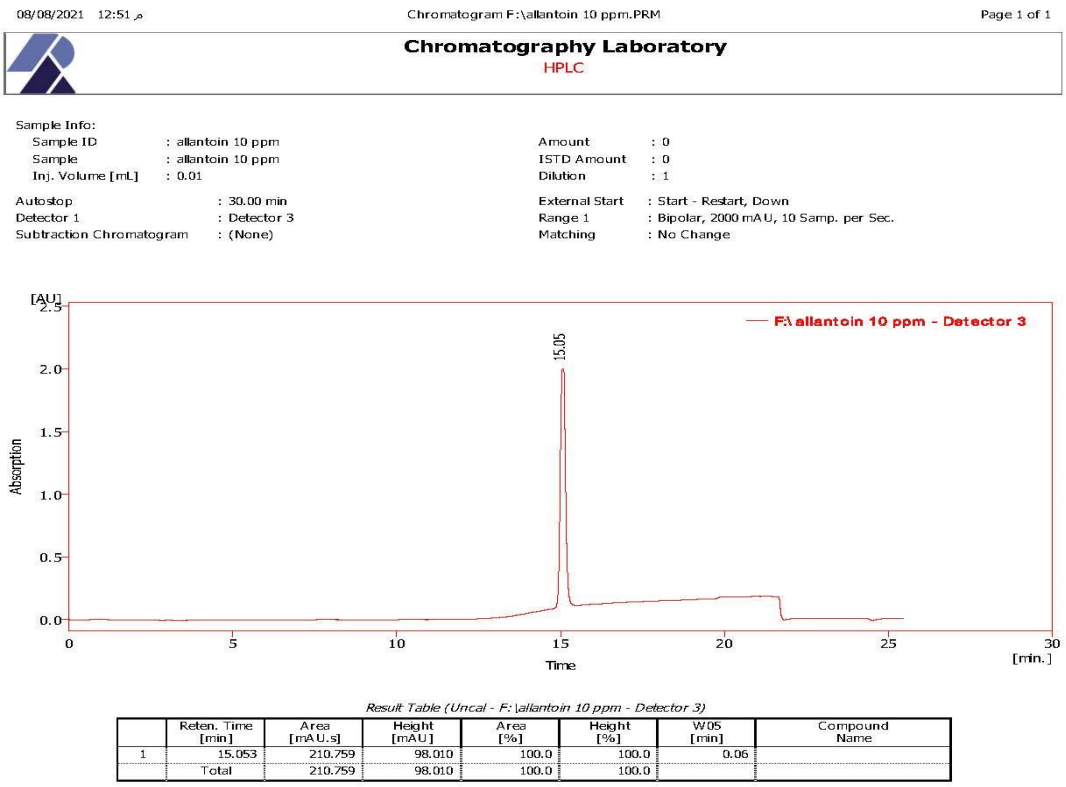
allantoin - Signal 1 - 15.1 min.

Compound Type	: Ordnr
Left Window	: 0.2 min
Right Window	: 0.2 min
Response Base	: Area
Curve Fit Type	: Linear
Origin	: Curve passes through Origin
Weighting Method	: None
Equation	: Y = 21.16*X
Correlation Factor	: 0.9999722
Residuum	: 1.8 [mV.s]
Linearisation X	: None
Linearisation Y	: None

	Response	Amount	Resp. Factor	Rec No.	Used
1	210.0000	10.0000	0.0476	1	☒
2	422.0000	20.0000	0.0474	1	☒
3	638.0000	30.0000	0.0470	1	☒
4	845.0000	40.0000	0.0473	1	☒
5	0.0000	0.0000	0.0000	1	☒
6	0.0000	0.0000	0.0000	1	☒
7	0.0000	0.0000	0.0000	1	☒
8	0.0000	0.0000	0.0000	0	☒
9	0.0000	0.0000	0.0000	0	☒
10	0.0000	0.0000	0.0000	0	☒
11	0.0000	0.0000	0.0000	0	☒
12	0.0000	0.0000	0.0000	0	☒
13	0.0000	0.0000	0.0000	0	☒
14	0.0000	0.0000	0.0000	0	☒
15	0.0000	0.0000	0.0000	0	☒
16	0.0000	0.0000	0.0000	0	☒
17	0.0000	0.0000	0.0000	0	☒
18	0.0000	0.0000	0.0000	0	☒
19	0.0000	0.0000	0.0000	0	☒
20	0.0000	0.0000	0.0000	0	☒
BL	0.0000	Blank	0.0000	0	☒



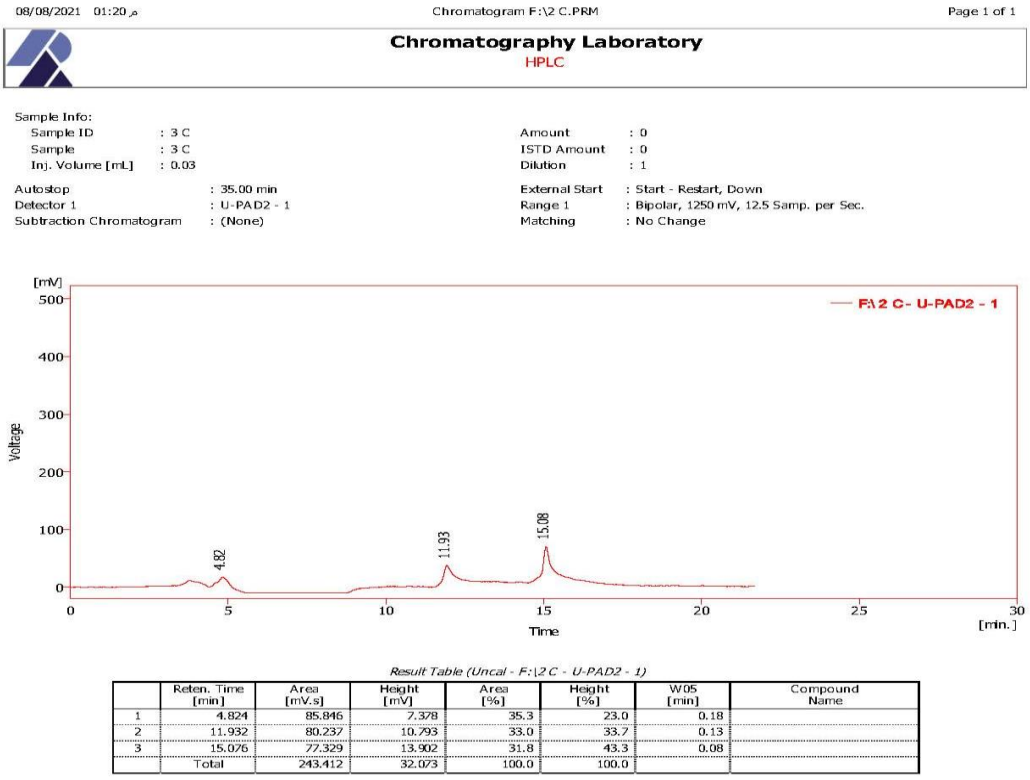
Figure(2-3): Standard curve for determination of allantoin concentration



Result Table (Uncal - F:\allantoin 10 ppm - Detector 3)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	15.053	210.759	98.010	100.0	100.0	0.06	
	Total	210.759	98.010	100.0	100.0		

Figure(2-4): Typical chromatogram of prepared allantoin (standard material) that used in HPLC technique



Result Table (Uncal - F:\2 C - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.824	85.846	7.378	35.3	23.0	0.18	
2	11.932	80.237	10.793	33.0	33.7	0.13	
3	15.076	77.329	13.902	31.8	43.3	0.08	
	Total	243.412	32.073	100.0	100.0		

Figure(2-5) : Typical chromatogram of allantoin in control (healthy) serum

11/08/2021 01:27 م Chromatogram F:\1 M.PRM Page 1 of 1

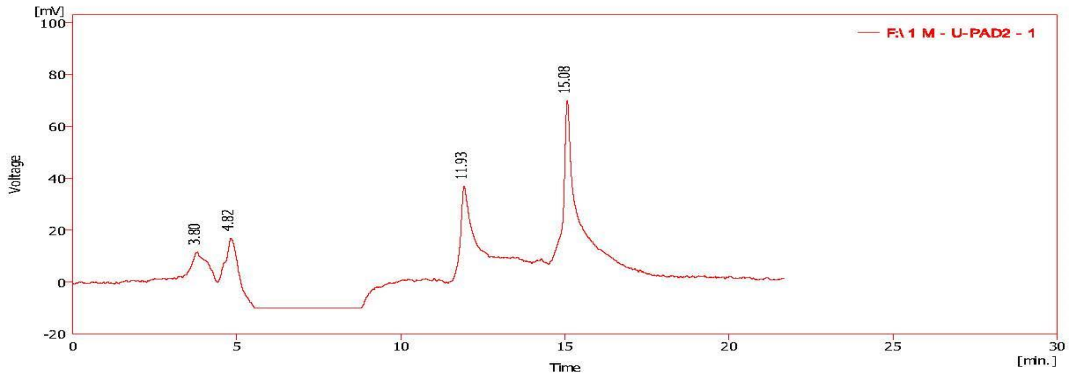
Chromatography Laboratory
HPLC

Sample Info:
Sample ID : 1 M
Sample : 1 M
Inj. Volume [mL] : 0.03

Amount : 0
ISTD Amount : 0
Dilution : 1

Autostop : 35.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\1 M - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.804	47.723	4.088	2.7	4.2	0.18	
2	4.824	208.202	12.870	12.0	13.3	0.26	
3	11.932	232.191	20.018	13.3	20.7	0.21	
4	15.076	1251.769	59.865	71.9	61.8	0.22	
	Total	1739.885	96.842	100.0	100.0		

Figure(2-6): Typical chromatogram of allantoin in male patient serum.

12/08/2021 12:42 م Chromatogram F:\1 F.PRM Page 1 of 1

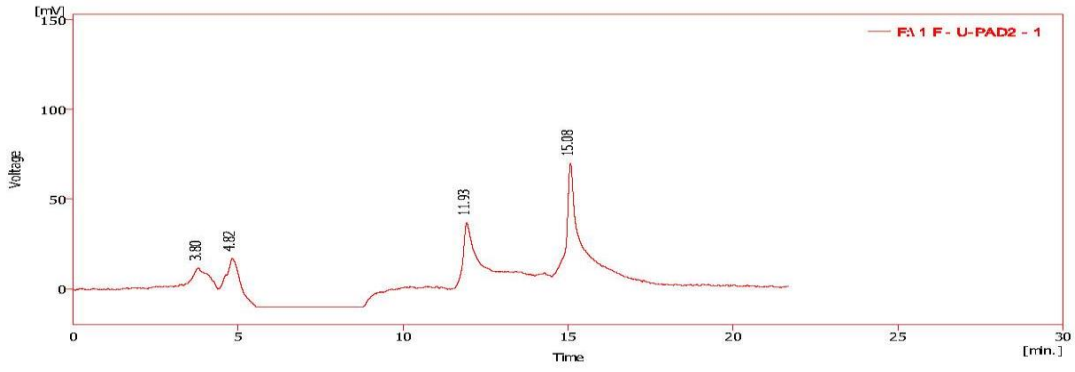
Chromatography Laboratory
HPLC

Sample Info:
Sample ID : 1 F
Sample : 1 F
Inj. Volume [mL] : 0.03

Amount : 0
ISTD Amount : 0
Dilution : 1

Autostop : 35.00 min
Detector 1 : U-PAD2 - 1
Subtraction Chromatogram : (None)

External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12.5 Samp. per Sec.
Matching : No Change



Result Table (Uncal - F:\1 F - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.804	94.384	5.792	10.0	7.3	0.30	
2	4.824	90.989	8.452	9.7	10.7	0.19	
3	11.932	146.884	16.241	15.6	20.5	0.18	
4	15.076	609.095	48.869	64.7	61.6	0.18	
	Total	941.353	79.354	100.0	100.0		

Figure(2-7): Typical chromatogram of allantoin in female patient serum

The concentration of the substance in the serum samples was calculated by the following method:-

Material Concentration= ((Standard substance concentration × Sample area \ Standard material area)) × (Dilution factor \ Sample volume)

2-6: Statistical analysis

The SPSS program version 24 was used to detect the results are expressed as frequency, percentage, and whenever possible as mean + SD (SE) of number of observations. The data are analyzed by using Student's "t"-test and simple correlation tests taking ($P \leq 0.05$) as the lowest limit of significance and Qi-Square test ($P \leq 0.05$) (SPSS ,2012).

Chapter Three

Results and Discussion

CHAPTER THREE

3.Results and Discussion

β- thalassemia major (BTM) is the most prevalent, requires regular transfusion therapy to maintain hemoglobin levels of at least 9 to 10 gm\ dl and to reduce hepatosplenomegaly due to extramedullary hematopoiesis , Both haemolysis and transfusional iron overload cause excessive generation of free radicals (through the Fenton reaction), and, consequently, iron-chelation therapy is largely responsible for doubling the life expectancy of patients with BTM (**Rund and , Rachmilewitz , 2005**)

3-1: Frequency of current β- thalassemia with age groups of Patients and body mass index(BMI)

The present study shows (Table **3-1**) its (P≤0.05) Highly significant that the largest proportion (91.67%) of the infected are in the age groups from (**1-20**)year, and the percentage decreases(8.33%) in the age groups from (**20-35**) year, and this present study agree with results of **Al-Haddad (2012) in** Gaza governorate (Palestine) and study **Elias (2016)** in Wasit province that conducted on β- thalassemia patients , may attributed due to the increase in the mortality rate as the patient gets older due to complications that the patient suffers from after receiving blood on a continuous and regular basis, which , It leads to an excess of iron in the body, and thus serious fatal diseases such as chronic hepatitis as well for blood-borne diseases such as AIDS and hepatitis C **(Peters *et al.*, 2012).**

BMI measured according to the following equation BMI = $Wt/(Ht)^2$ where **weight = weight (kg), $(Ht)^2$= box height $(m)^2$** (**Hamood *et al.*,2018**). The results showed that thalassemia has a signifi-

cant effect on the BMI as (17.31%) compared with healthy (18.5-25%) Almost similar findings were shown by another study that thalassemia major patients had lower rate of growth and lower BMI which was attributed to low hemoglobin, high ferritin levels, and suboptimal iron chelation are thought to be the causes of growth defects (**Al-Mosawy ,2017**) and these results agree with **Hamood *et al.*(2018)**

Table(3-1): Percentage of infected with β- thalassemia, distributed by age groups

Age groups (years)	Infected No.	(%)
1-20	55	91.67*
21-35	5	8.33
Total	60	100
X^2=50 X^2=3.841 P=1.54E-12 (P≤0.05) *Highly significant		

3-2: Hematological parameters changes in β- thalassemia

Table (**2-3**) shows decreased in the average values for the number of RBCs**(×10^6/mm^3**) of males and females In patients group (3.02±0.0456 ; 2.69±0.0496), respectively compared with the average values for the number of RBCs**(×10^6 /mm^3**) of males and females In healthy group(control) (**4.56**±0.0403 ; 4.19±.0293), respectively. Whereas Hb concentration (**gm. /dl**), the average values of males and females In patients group (8.154±0.1008; 7.8796±0.125), respectively and the average values for the Hb concentration(**gm. /dl**) of males and females In healthy group(control) (14.32±0.034; 14.106±0.051), respectively .

Packed cell volume PCV**(%)** registered decreased average values of males and females In patients group (24.304±0.313; 23.583±0.232),

respectively compared with the average values for the PCV**(%)** of males and females In healthy group(control) (41.591±0.285 ; 40.93±0.229), respectively.

There are increased average values for the number of **WBCs (×10^3 /mm^3**) of males and females In patients group (**14.54±**0.233; 13.48±0.248), respectively compared with the average values for the number of RBCs**(×10^3 /mm^3**) of males and females In healthy group(control) (7.934±0.184; 7.134±0.165), respectively. Whereas increased average values of Platelets counts(PLT) **×10^3\mm^3**) of males and females In patients group (**323.379±**8.978; 307.539±8.935), respectively compared with the average values for the number of RBCs**(×10^3 /mm^3**) of males and females In healthy group(control) (218.636±11.67; 210.082±10.65), respectively.

Mean corpuscular volume (MCV) and Mean corpuscular hemoglobin(MCH) for all patients decreased with a significant difference ($p < 0.05$) for all patients (males and females). Compared with healthy (control males and females). As for the average Mean corpuscular hemoglobin concentration (MCHC), there is Significant difference between patients and healthy people ($p < 0.05$). To display the Red cell distribution width (RDW) for patients increases with a significant difference ($p > 0.05$) compared to the healthy group for all patients(males and females), increased within the upper limit of normal compared to the healthy group , it was found that .The percentage of red cell distribution width RDW for males and females (16.330±.106; 15.772±.104),respectively and (12.446±.056; 12.113±.036),respectively for healthy group (Table 2-3).

Table(3-2): Haematological changes in the blood of patients with β- thalassemia and healthy control , distributed according to gender .

Parameters / Gender / Control	(RBCs)× (10^6\mm^3) (Mean±SE)	(Hb) (gm\dl) (Mean±SE)	(PCV) (%) (Mean±SE)	(WBCs) (10^3\mm^3) (Mean±SE)	(PLT) (10^3\mm^3) (Mean±SE)	(MCV) µm^3 (Mean±SE)	(MCH) Pictogram (Mean±SE)	(MCHC) (gm\dl) (Mean±SE)	(RDW) (%) (Mean±SE)
Males	3.02±0.0456	8.154±0.1008	24.304±0.313	**14.54**±0.233	**323.379**±8.978	81.772±0.167	27.39±0.112	33.621±.148	**16.330**±.106
Control	**4.56**±0.0403	**14.32**±0.034	**41.591**±0.285	7.934±0.184	218.636±11.67	**91.094**±0.468	**31.47**±0.285	**34.586**±.270	12.446±.056
Females	2.69±0.0496	7.8796±0.125	23.583±0.232	13.48±0.248	307.539±8.935	80.126±0.119	26.33±0.108	31.802±.148	15.772±.104
Control	4.19±.0293	14.106±0.051	40.93±0.229	7.134±0.165	210.082±10.65	89.509±0.386	30.24±0.281	33.584±.268	12.113±.036
	0.000 (H.S)	0.000 (H.S)	0.000 (H.S)	0.000 (H.S)	0.000 (H.S)	0.000 (H.S)	0.000 (H.S)	0.000 (H.S)	0.000 (H.S)

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.

(RBCs)= Red Blood Corpuscles Counts.

Hemoglobin concentration.= (Hb)

(PCV)= Packed cells volume .

White blood cells. =(WBCs)

(PLT)= Platelets counts.

(MCV)= Mean corpuscular volume.

(MCH)= Mean corpuscular hemoglobin.

(MCHC)= Mean corpuscular hemoglobin concentration.

(RDW)= Red cell distribution width.

3-2-1: Discussion of The Hematological Parameters

The results of the study showed in Table **(3-2)** and Figure **(3-1)** a significant decrease in the number of red blood cells RBCS and hemoglobin Hb in patients with beta-mediated major anemia compared .This can be attributed to the decrease in the formation of beta-globin chains in the molecule of healthy individuals (control). hemoglobin for patients that lead to changes in the structure of hemoglobin molecules, and as a result, Red blood cell is described as an increase in the dysregulated globin protein in the cell membranes and this makes it vulnerable to destruction by phagocytic cells in the bone marrow that have the ability to distinguish and destroy abnormal cells and this leads to A large number of red blood cells are destroyed during the process of their formation , Since the volume of the aggregated blood cells PCV depends on the number and size of erythrocytes and is also reduced **(Alemu *et al*., 2006)** . These results are consistent with many other studies **Pavlova *et al.*(2007) ;Ali (2008); Al-Saray (2012); Al-Haddad (2012); Al-Mousawi (2014), and Elias (2016).**

The results also showed a significant decrease in the level of the average red blood cell volume MCV in patients; This is due to the small size of the microcytic erythrocyte due to a deficiency in the production of the beta chain globin resulting from a mutation in the beta globin gene, the results also showed a significant decrease in the level of average weight MCH globular hemoglobin due to a defect in the production of globin protein, which results in a decrease in hypochromic hemoglobin content, these findings are consistent with other studies **Pavlova *et al.*(2007) Taher *et al.* (2008) ; Al-Saray (2012) ; Al-Haddad (2012); Elias (2016).** The average MCHC concentration is It was normal and did not change in patients compared to the healthy, and the results were consistent with the findings **Hussain *et al.* (2005) and Study of Pavlova *et***

***al.*(2007) study of Al-Haddad (2012) and Elias (2016)** who noted a decrease in the level of (Hb, PCV, MCV, MCH) and the MCHC criterion was found to be within normal limits in patients with minimal Mediterranean anemia. **(Mohammed *et al*.,2020).**

The presence of small-sized globules with uncompensated and unequal deficiency for beta-globin chains reflected by an increase in the value of RDW **(Aslan *et al*., 2002; Al-Haddad (2012); Elias (2016)),** this result is consistent with as pointed out by (**Mankad *et al.* 2013**). As for the significant increase in blood platelets **(Wandersee *et al.*, 2001).** The spleen produces abnormal cells that have a short lifespan and quickly degrade, and they are concentrated in the spleen, , which reveals the important role that the spleen plays in removing platelets ,These results are consistent with those of **Eldor and Rachmilewitz (2002) ; Al-Haddad (2012); Elias (2016).**As for the significant elevation in the number of WBCs in patients, its cause is due to the presence of a large number of red blood cells Immature RBCs, containing nuclei that may be incorrectly counted as white blood cells, These results are consistent with the results of **Pavlova *et al*.(2007) ; Elhams (2010); Al-Haddad (2012) ; Al-Saray (2012) ; Al-Mousawi (2014), and Elias (2016).**

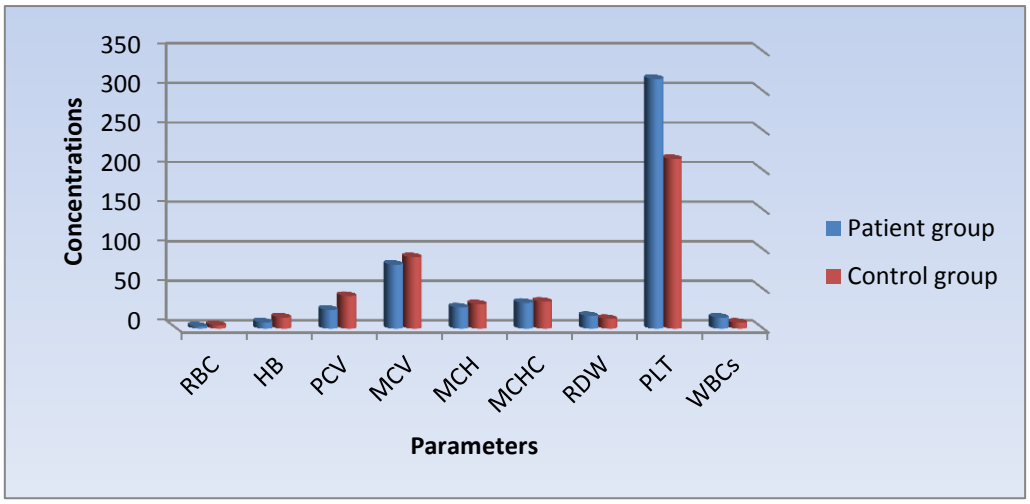


Figure (3. 1): Distribution of heamatological aspect in studied groups

3-3: Biochemical parameters change in β- thalassemia

3-3-1: Concentration level of Zinc in serum of studied groups.

Table **(3-3)** Figure **(3-2)** show that the lowest mean zinc in males and females were **(96.121), (83.199) µgm\dl** respectively, in patients group compared in control group **(119.344), (111.376) µgm\dl**. respectively ,There is a highly significant differences between studied groups (patients and control), at P-value ≤ 0.05. Regarding to differences between gender in each group, the result show a non-significant differences between gender in patients group and control group.

Table (3-3): Concentration level of Zinc in β- thalassemia patients(Males and Females) and control groups

Study groups					
Statistical Standards	**Patients (n=60) µgm\dl**		**Control (n=30) µgm\dl**		
	Male (n=30)	**Female (n=30)**	**Male (n=15)**	**Female (n=15)**	*P-value
Mean	**96.121**	**83.199**	**119.344**	**111.376**	0.000 (H.S)
Std. Error	1.1957	9.710	5.178	4.039	
Std. Deviation	6.549	53.185	20.057	15.643	
Minimum	182.872	106.329	72.600	70.6	
Maximum	213.670	293.670	127.0	114.0	
***P-value**	0.193 (N.S)		0.321 (N.S)		

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.

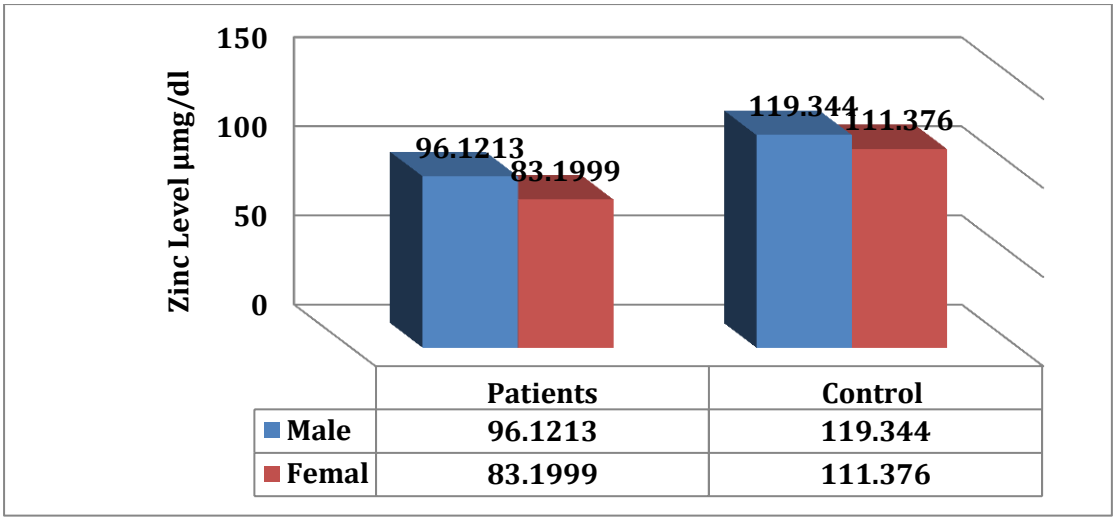
Zinc is an essential trace element found in the human body. Protein synthesis, DNA synthesis, and cellular development are just a few of the crucial activities it plays in the body. It is found practically everywhere in the body and plays a critical function in the immune system, influencing both innate and acquired immunity. Zinc also has substantial antioxidant qualities, which protect cells from free radical damage. , It is the active site for several metalloenzymes that are essential for nucleic acid synthesis as well as other host defense mechanisms such as monocyte and macrophage production and granulocyte chemotaxis (**William, 2004**). **Al-Samarrai *et al.* (2008), Moafi *et al*. (2008),** and **Bekheirnia *et al.* (2008)** agreement with present study whose observed a serum zinc deficit in beta thalassemia patients, which was linked to hyperzincuria caused by the release of Zn from hemolyzed red cells (**William, 2004**).

Although 16 % of participants exhibited a zinc shortage, the mean serum zinc level was not poor.. In disagreement with studies conducted by **Mehdizadeh *et al*.(2008)** and **Rashidi *et al*.(2011)**, and a study by **Zardkhoni *et al.*(2021),** mean serum zinc levels were significantly higher in the thalassemia group. They also noted that zinc deficiency is rare in thalassemia and did not report zinc deficiency in beta thalassemia patients. Regular transfusion therapy in both males and females may have resulted in the absence of predominant zinc deficiency in patients. Report of **Reshadat *et al.*(2006)** showed that 77% of thalassemia patients have normal serum zinc level and remainder less than normal. They emphasize that medical treatment of these patients is not appropriate, so the value of zinc administration should be more decreased . Present study showed that decrease the level of zinc of thalassemia patients have hypozincemia. The causes of zinc sufficiently in these patients may be related to competence amount of zinc in daily meals, abnormality in

urinary absorption of zinc, kidney dysfunction, urinary secretion of zinc, disorder in zinc metabolism and higher level of zinc excretion in sweat.

Low serum zinc and copper was observed in 79.6% and 68% of the study population, respectively. There is significant association of serum zinc levels with lumbar but not femoral BMD.

Another study was carried out to evaluate the serum copper and zinc in Jordanian thalassemia patients. 24 patients with β-thalassemia major on periodical blood transfusion and Deferoxamine were included in this study (**Kamal *et al*.,2009**). Forty age- and gender-matched healthy controls were included in the study. The results indicate that copper and zinc levels were significantly increased in Beta thalassemia major patients compared with controls. These finding may be explained by the decreasing rate of glomerular filtration of zinc seen in chronic hemolysis and the disturbance in the metabolism of zinc and copper in thalassemia patients due to the increasing serum zinc. The high level of copper could be due to increase absorption of copper from gastrointestinal tract. It is shown that a case control prospective study including 100 Beta thalassemia major patients with heights within 3rd to 10th percentile (**Faranoush *et al*.,2008**). They randomly divide patients in two groups ach comprising of 50 patients. Group 1 was given oral zinc supplements while group 2 is a control group with no zinc supplements. The patients were observed for 18 months. They found out that there is no significant difference in height between the two groups after 18 months of observation and concluded that oral zinc sulphate has no significant effect on linear growth of Beta thalassemia major patients.



Figures (3-2): Concentration of zinc in studied groups(males and females) in thalassemia patients and control.

3-3-2: Concentration level of Copper in serum of studied groups.

Table **(3-4)** Figure **(3-3)** show that the highest mean copper in males and females were **(203.80), (197.13) µgm \dl.** respectively, in patients group compared in control group **(105.00), (115.67) µgm \dl**. respectively ,There is a highly significant differences between studied groups at P-value ≤ 0.05. Regarding to differences between gender in each group, the result show a non-significant differences between gender in control group and patient group.

Table (3-4): Concentration level of Copper in β- thalassemia patients (Males and Females) and control groups

Study groups					
Statistical Stand-ards	**Patients (n=60) µgm\dl**		**Control (n=30) µgm\dl**		
	Male (n=30)	**Female (n=30)**	**Male (n=15)**	**Female (n=15)**	*P-value
Mean	203.80	197.13	105.00	115.67	0.000 (H.S)
Std. Error	1.166	1.371	5.774	6.053	
Std. Deviation	6.386	7.510	22.361	23.442	
Minimum	190	180	70	80	
Maximum	219	212	140	155	
***P-value**	0.001 (H.S)		0.213 (N.S)		

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.

The other vital trace element found in our bodies is copper. It mostly produces metalloprotiens, which are enzymes. Copper is a major compo-nent of hemoglobin, a protein that transports oxygen in blood cells. It also works with vitamin C to produce elastin, a protein that keeps the skin, blood vessels, and lungs supple. It's antimicrobial and contains a lot of antioxidants(**Shazia *et al.*,2012**).

Copper is a key component of the antioxidant superoxide dismutase molecule and also aids in the synthesis of ceruloplasmin, a protein that protects cells from free radical damage., Copper is also essential for the formation of hormones such as noradrenaline and prostaglandins, which

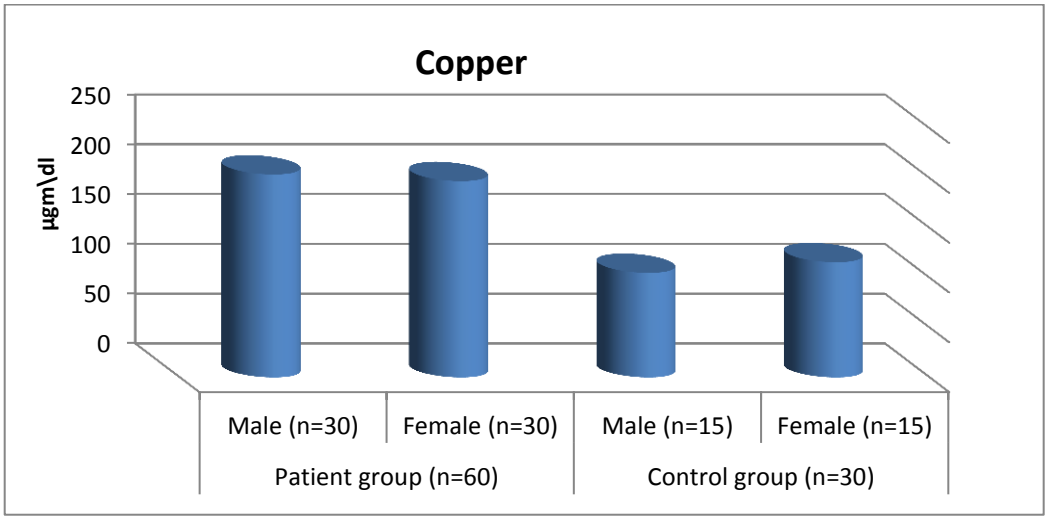
are hormone-like substances that regulate blood pressure, pulse, and healing. Anaemia, neutropenia, and growth retardation, as well as anomalies in glucose and cholesterol metabolism and an increased prevalence of infections, are all symptoms of this trace element deficiency. Wilson's disease with copper build up and liver cirrhosis, on the other hand, is caused by an accumulation of copper in the body. The serum levels of zinc and copper in beta thalassemia major children were determined in a prospective research **(Mahyar *et al*.,2010; Shazia *et al*.,2012).**

The present study is consistent with the results of the study of **Al-Samarrai *et al*.(2008)** that revealed the increase of copper concentration in serum patients and some studies have reported elevated the level of copper like attributed to the Hypercupremia occurs in acute and chronic infections and hemochromatosis, which is a principal complication of thalassemia , The amount of copper consumed in the daily diet, intestinal uptake of copper, iron accumulation, renal function, copper to zinc ratio, and administration of desferal all influence the serum copper content in individuals with major thalassemia, **(Mohammed *et al.* (2020)** Thankfully, none of our thalassemia patients were deficient in copper. This demonstrates that the factors that affect copper levels are in check.

A prospective study was performed to determine the serum levels of zinc and copper in Beta thalassemia major children (Mahyar *et al.*, 2010). This cross-sectional study revealed that hypozincemia is common in thalassemia patients, but there is no copper deficiency.

Another study was carried out to evaluate the level of some essential elements in one hundred and five thalassemia blood-transfusion-dependent patients and 54 healthy controls (**Al-Samarrai *et al.*, 2010**). They found lower serum zinc and magnesium levels and higher copper

and potassium levels in thalassemia major patients as compared to controls. Zinc deficiency may be due to hyperzincuria resulted from the release of zinc from haemolysed red cells while hypercupremia occurs in acute and chronic infections and hemochromatosis which is the principal complication of thalassemia. A study done on status of thyroid function and iron overload in patients with Beta thalassemia major on Deferoxamine in Jordan concluded that there is significantly high ($P < 0.05$) levels of serumferritin, FT3, zinc, and copper in patients with Beta thalassemia major as compared to controls (**Irshaid and Mansi, 2009**).



Figures (3-3): Concentration of copper in studied groups(males and females) in thalassemia patients and control.

3-3-3: Concentration level of Iron in serum of studied groups.

Table **(3-5)**and Figure **(3-4)** show that the highest mean iron in males and females were **(224.479), (215.639) µgm \dl.** respectively, in patients group compared in control group **(113.40), (103.33) µgm \dl**. respectively ,There is a highly significant differences between studied groups at P-value ≤ 0.05. Regarding to differences between gender in each group, the result show a non-significant differences between gender in control group and patient group.

Table (3-5): Concentration level of Iron in β- thalassemia patients (Males and Females) and control groups.

Study groups					
Statistical Standards	**Patients (n=60) μgm\dl**		**Control (n=30) μgm\dl**		
	Male (n=30)	**Female (n=30)**	**Male (n=15)**	**Female (n=15)**	*P-value
Mean	224.479	215.639	113.40	103.33	0.000 (H.S)
Std. Error	3.085	3.227	10.08	10.37	
Std. Deviation	16.898	17.675	39.039	40.163	
Minimum	190.914	161.875	65.00	50.000	
Maximum	264.514	247.441	175.00	170.000	
***p-value**	0.052 (N.S)		0.492 (N.S)		

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.

Patients with β-thalassemia are mainly exposed to oxidative stress due to iron overload (**through the Fenton reaction**). Therefore evaluation and maintenance of antioxidant defence can be useful in protecting β-thalassemia patients from more serious complications of the disease (**Bazavand *et al.*,2011**).

$$Fe^{2+} + H_2O_2 \longrightarrow \acute{O}H + \bar{O}H + Fe^{3+} \text{ ----- (1}$$

The present study is consistent with the results of the study of **Traez *et al.* (2007)** ; **Al-Samarrai *et al.*(2008)** and study of; **Piga *et***

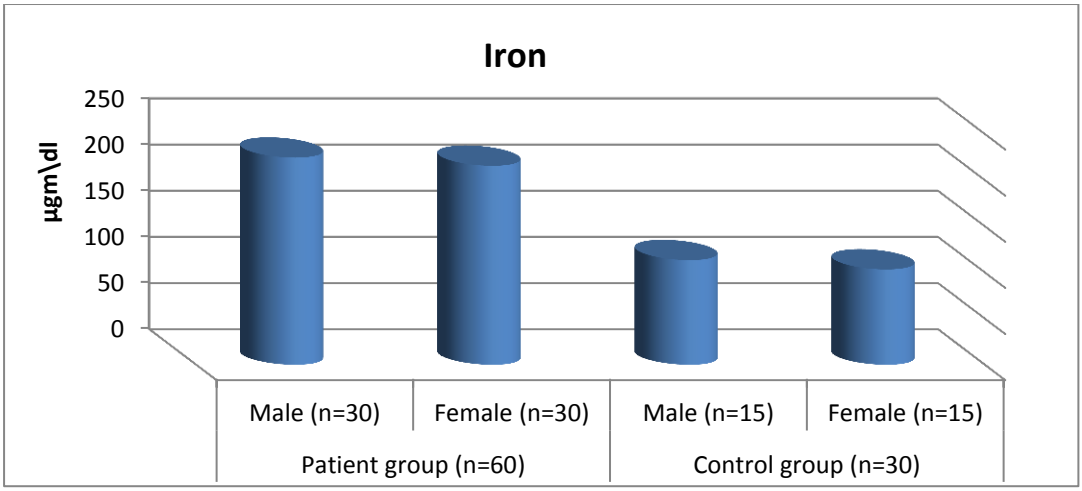
***al*.,2009; Kuppusamy and Tan(2011) and study of Abdulla (2018)**that revealed the increase of iron concentration in serum patients Some studies **(Kassab-Chekir *et al*.,2003) ;(Kuppusamy and Tan,2011); (Saud ,2012).** have reported elevated the level of iron like attributed to the Hemochromatosis is caused by an excess of iron in the body, which can be either primary or secondary. Primary hemochromatosis is a condition in which the body's iron stores are depleted. Increased iron absorption is a symptom of a hereditary disease. As a result, the body is overloaded with iron. Secondary , due to disorders like thalassemia, hemochromatosis develops. Iron overload is common in thalassemia major, where it occurs on a regular basis, There will be a need for blood transfusions. major beta thalassemia Patients require frequent blood transfusions, which can result in complications. In the absence of adequate chelation therapy, iron overload can occur.

This iron accumulation in thalassemia individuals might exceed ferritin's storage and detoxifying capability, entirely saturating transferrin and resulting in the generation of free iron in the blood and tissues. The development of very damaging chemicals, such as hydroxyl radicals, will be caused by this free iron Hydroxyl radicals ($OH^{\cdot}$) are extremely reactive, attacking lipids and forming lipid peroxides, which contribute to oxidative stress (**Al-Mashhedy (2007) ; Raghuveer *et al*..2009**).

The blood transfusion is every (2-4) weeks to treated severe anemia which result iron overload in various tissue including the liver, heart and endocrine tissue. The kidneys are another site of iron accumulation in thalassemia, unlike in the other organs; it is unclear whether kidney affection results solely from intravascular hemolysis, chronic transfusion or as a complication of iron chelation therapy **(Traez *et al*.,2007)** .

The significant increase of serum iron and ferritin in Iraqi patients indicated an existing iron overload.. The repeated transfusion of blood causes

iron overload in the β-thalassemia without chelation therapy that leads to many complications such spleenomegaly and influence on liver function (**Piga *et al*.,2009**). But if free iron was available, it reacted with H_2O_2 to form hydroxyl radicals which were extremely reactive species leading to depolymerisation of polysaccharide, DNA strand breakage, inactivation of functional proteins etc.. (**Kassab-Chekir *et al*.,2003) ;Al-Mashhedy (2007); Kuppusamy and Tan(2011) and Saud (2012).**



Figures (3-4): Concentration of iron in studied groups(males and females) in thalassemia patients and control.

3-3-4: Concentration level of Albumin in serum of studied groups.

Table **(3-6)** and Figures **(3-5)** show that lowest mean albumin in males and females were **(4.033), (3.934) gm\dl.** respectively in patients group compared in control group **(4.506) , (4.10) gm \dl**. respectively. There is a significant differences between studied groups at P-value ≤ 0.05. Regarding to differences between gender in each group, the result show a significant differences between gender in control group and patient group.

Table (3-6): Concentration level of Albumin in β- thalassemia patients (Males and Females) and control groups.

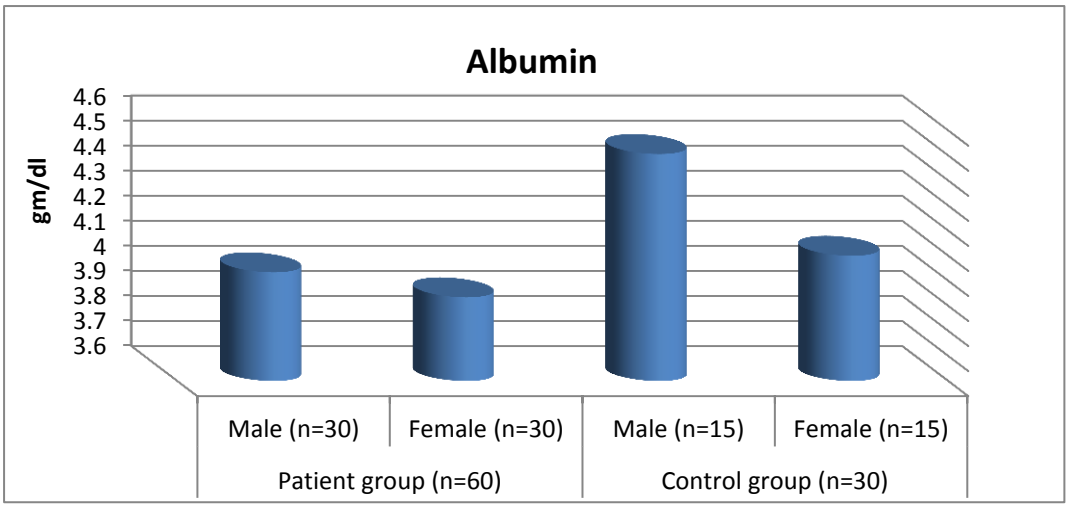
Study groups					
Statistical Standards	**Patient group (n=60) gm\dl**		**Control group (n=30) gm\dl**		
	Male (n=30)	**Female (n=30)**	**Male (n=15)**	**Female (n=15)**	*P-value
Mean	**4.033**	**3.934**	**4.506**	**4.10**	0.003
Std. Error	0.0383	0.034	0.139	0.115	(Sig)
Std. Deviation	0.2098	0.187	0.539	0.447	
Minimum	3.401	3.350	3.700	3.4	
Maximum	4.500	4.163	5.500	4.8	
***P-value**	0.058 (N.S)		0.033 (Sig)		

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.

Antioxidants play an essential role in protection of the cells from oxidative damage. They include several agents such as enzymes (glutathione peroxidase, superoxide dismutase, catalase), large molecules (ferritin, albumin), and small molecules (uric acid, glutathione, bilirubin, ascorbic acid, α tocopherol, and vitamin E). Their defence mechanism in biological system involves chain breaking (SOD) and preventive (Vitamin E) mechanisms (**Bazvand *et al.*,2011**). Another study also revealed that oxidative stress in males is slightly greater than in females, which may be explained by the compensatory antioxidants rise in males

in our study. On the other hand, this finding maybe due to the significant rise of albumin in male in comparison with female, As a result of the increase in free radicals formed due to the increase in iron in the blood, the albumin molecules attack these free radicals and their concentration decreases, and this is what was found in the current study (**Sverko *et al*.,2004**). As indicated in the present results, there was a general decrease in albumin profile. However, this decrease was significant for albumin. Similar results were documented by **Malik *et al*. (2010)** and study of **Murtadha (2011)** and study of **Al-Haddad (2012).**

The possible cause of decreased serum total protein and albumin is due to secondarily decreased synthesis of protein by the liver. It was reported that protein measurements can reflect nutritional state, kidney disease and liver disease, Therefore, disturbance of liver function observed in thalassemia patients do confirm the decrease in protein synthesis by the liver. In addition, elevation of liver protein enzymes in thalassemia patients further support this view and the lack of protein in the diet, which may indicate malnutrition, Infection in the kidney, Celiac disease, in which the immune system attacks any foods containing Gluten digestive disorders, such as Crohn's disease **(Abbass and Defer, 2011).**



Figures (3-5): Concentration of albumin in studied groups(males and females) in thalassemia patients and control.

3-3-5: Concentration level of Uric acid in serum of studied groups.

Table **(3-7)** and Figures **(3-6)** show that the mean uric acid in males and females were **(46.964), (46.779) mg\L.** , respectively in patients group compared in control group **(38.066), (37.733) mg\L.** respectively. There is a significant differences between studied groups at P-value ≤ 0.05. Regarding to differences between gender in each group, the result show a significant differences between gender in control group, while there is a non-significant differences between gender in patient group.

Table (3-7): Concentration level of Uric acid in β- thalassemia patients (Males and Females) and control groups.

Study groups					
Statistical Standards	**Patient group (n=60) (mg\L.)**		**Control group (n=30) (mg\L.)**		
	Male (n=30)	**Female (n=30)**	**Male (n=15)**	**Female (n=15)**	*P-value
Mean	**46.964**	**46.779**	**38.066**	**37.733**	0.006 (Sig)
Std. Error	0.26741	0.211	2.975	2.849	
Std. Deviation	1.46469	1.157	11.525	11.037	
Minimum	37.45	37.90	36.00	26.00	
Maximum	43.33	43.00	72.00	60.00	
***P-value**	0.117 (N.S)		0.019 (Sig)		

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.

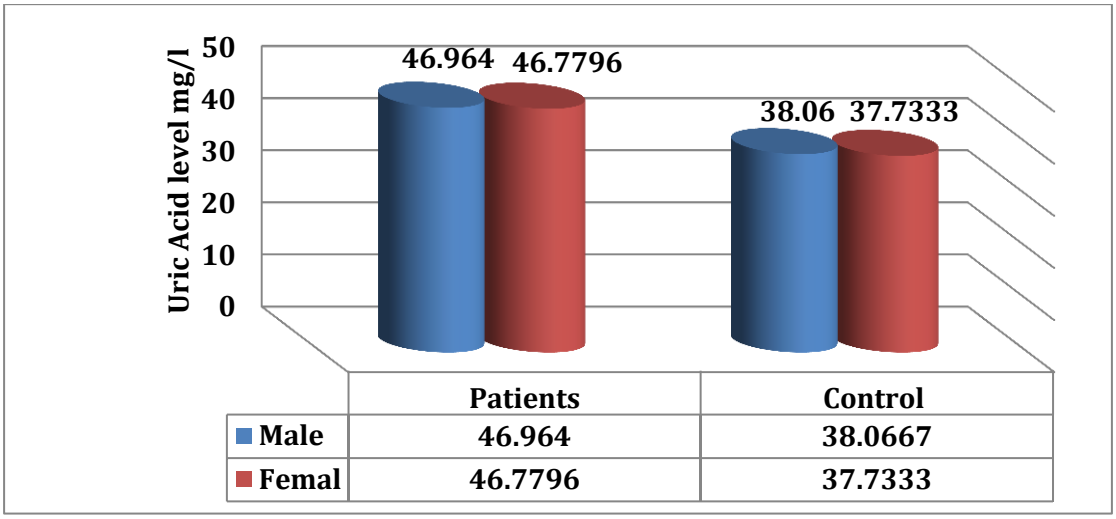
However serum urea concentration show significant change in patients. Such findings are in disagreement with that obtained by **Aldudak *et al*.(2000)** ,**Hamed and ElMelegy, (2010), and Bazvand *et al.* (2011)** Creatinine is a waste product that is normally filtered from the blood and excreted with the urine. Uric acid has been thought to be metabolically inert end product of purine metabolism in red blood cells. The observed increase of serum uric acid concentrations in thalassemic patients can be explained by highly erythrocyte turnover in combination with decreased reabsorption of filtered uric acid from the possible damaged renal tubules **(Hamed and ElMelegy, 2010).**

In the present study, the mean serum uric acid levels were significantly changed in males and females the patients **(46.964), (46.779) mg\L.** respectively whereas there are significant differences in the controls group (males and females) **(38.066), (37.733) mg\L.** respectively, Figures **(3-6)**. In addition to rapid erythrocyte turnover, proximal tubular damage may lead to hyperuricemia in these patients, because the filtered uric acid can be reabsorbed from the proximal tubules (**Al-Haddad ,2012** ; **Rasool *et al*.,2016).**

The increase level of uric acid in the current study for the group of patients compared to the control group may be due to the presence of free radicals (ROS), as uric acid have little interacts with it and generates from this oxidation the compound allantoin, whose concentrations were high for the group of thalassemia patients , Uric acid is the end product of purine metabolism.

Measurement of the product (e.g. allantoin) of the interaction of reactive oxygen metabolites with uric acid (as an antioxidant) is considered as a marker of oxidative damage (**Ismael,2001**). And another reason for assessment of oxidative stress its finger print assays: When the ROS react with the biological molecule it a produce a chemical change for

example of hydroxyl radical on DNA produce a pattern of chemical change to all four purine and pyrimidine bases that seem characteristic of ÓH. Other oxygen-derived species either do not attack the DNA bases at all ($\dot{O}_2$, and H_2O_2) or they modify only guanine (singlet oxygen) (**Ismael,2001**).



Figures (3-6): Concentration Uric acid in studied groups (males and females) in thalassemia patients and control.

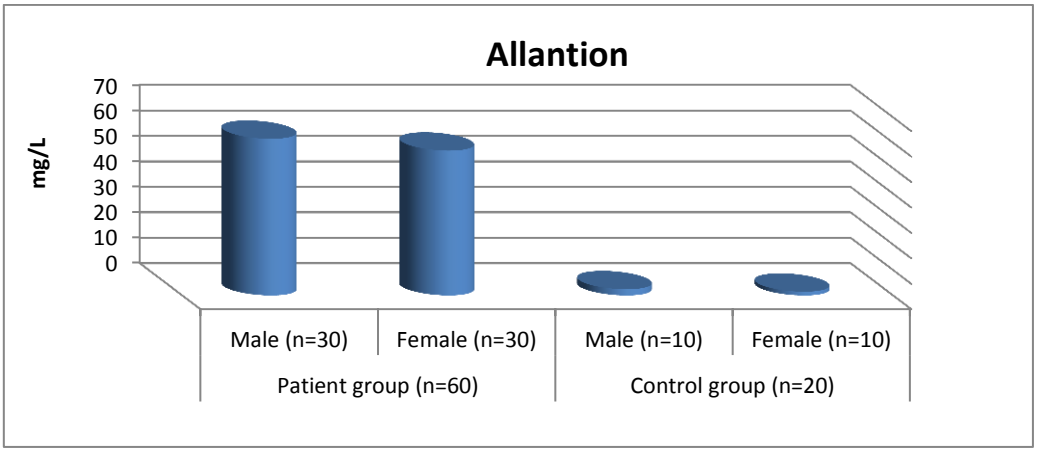
3-3-6: Concentration level of Allantoin in serum of studied groups.

Table **(3-8)** and Figure **(3-7)** shows that the mean allantoin in males and females were **(61.556), (56.916) mg\L. or PPM** ,respectively in patients group compared in control group **(2.448), (1.477) mg\L. or PPM** ,respectively. There is a highly significant differences between studied groups at P-value ≤ 0.05. Regarding to differences between gender in each group, the result show a highly significant differences between gender in patient group, while there is a non-significant differences between gender in control group.

Table (3-8): Concentration level of Allantoin in β- thalassemia patients (Males and Females) and control groups.

Study groups					
Statistical Standards	**Patient group (n=60) (mg\ L.)or PPM**		**Control group (n=20) (mg\ L.)or PPM**		
	Male (n=30)	**Female (n=30)**	**Male (n=10)**	**Female (n=10)**	*P-value
Mean	**61.556**	**56.916**	**2.448**	**1.477**	0.000 (H.S)
Std. Error	0.331	0.519	0.428	.263	
Std. Deviation	1.817	2.843	1.353	.832	
Minimum	59.00	50.10	0.00	0.00	
Maximum	65.50	60.60	3.84	2.40	
***P-value**	0.001 (H.S)		0.131 (N.S)		

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.

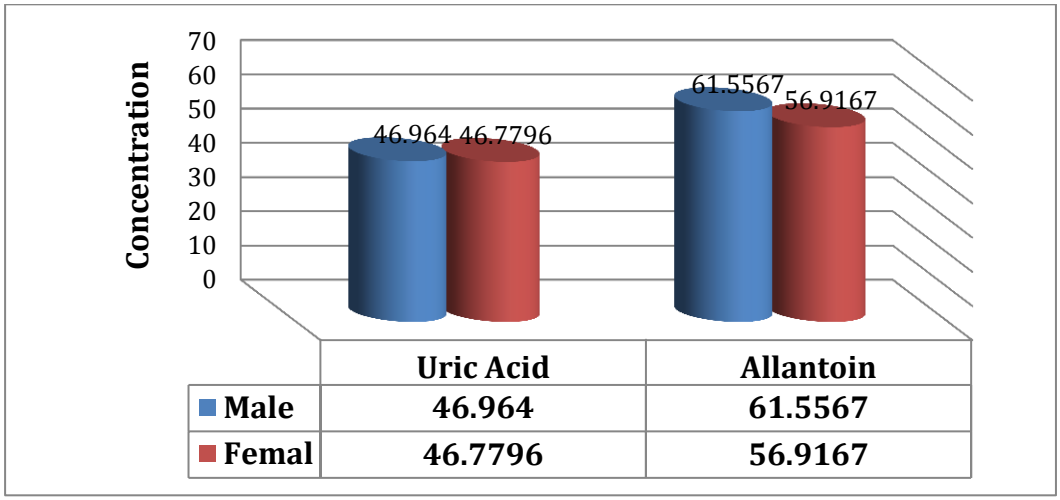


Figures (3-7): concentration allantoin in studied groups(males and females) in thalassemia patients and control.

3-3-6-1: Estimation of scavenger system

uric acid- allantoin pathway, uric acid is generated in the human body by the oxidation of purines, but no enzymes is present to oxidize it further The levels of uric acid and allantoin were significantly reduced and elevated in patients with male and female as compared with corresponding levels of controls in **(Table 3- 7 and 3-8 and Fig(3-8).** This difference is observed in patients serum there is non- significant negetive correlation between U/A ratio(**R^2 = -0.059) as shown in Fig (3-29) and Fig (3-33) .** This observation is of great importance since it demonstrated that scavenger system is greatly depleted with generation of RNS and ROS in thalassemia patients (males and females) and also it indicated that ROS is the major contributor of oxidative stress in male thalassemia patients than females. Since the estimation of uric acid - allantoin pathway is conducted for the first time in thalassemia patients, the literatures concerning this topic are completely unavailable. Uric acid is a known water soluble scavenger which proved to exert an important anti-oxidant activity **(Ismael,2001;Mohammed and Abd-Elrasoul (2020**). It is of great importance to mention here the specifications of metabolic pathway of uric acid: **1-**The synthesis of uric acid is conducted via the activation of xanthine dehydrogenase under physiological condition. In pathological condition (particularly oxidative stress), the xanthine oxidase enzyme is activated by ROS leading to formation of uric acid. Therefore most probably the uric acid level in this work is related to activation of xanthine oxidase enzyme. **2-**In human being the responsible enzyme for uric acid degradation is uricase which is not available under physiological conditions. This enzyme as well as its end product from uric acid notably allantoin is not detected in biological fluids (**Zekavat *et al.*,2018**). Therefore, the results reported in this work showed the that oxidative stress of in thalassemia patients is counteracted by uric acid - allantoin

pathway as it reflected by depletion of uric acid and increase in allantoin level. Further analysis revealed that the activity of uricase-like enzyme is decreased in in thalassemia patients by 1/4 fold of that of controls .



Figures (3-8): Relatioship between uric acid and allantoin in studied groups(males and females) in thalassemia patients and control.

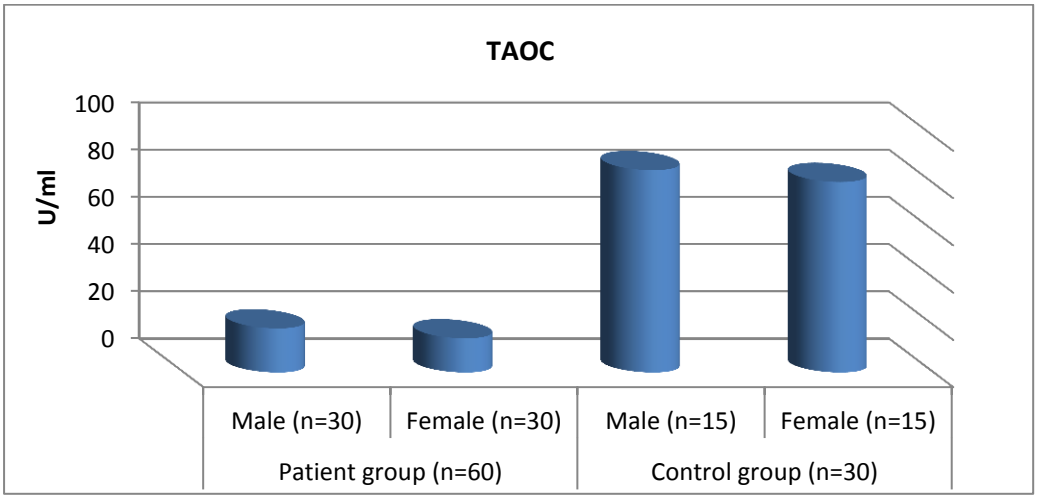
3-3-7: Concentration level of Total antioxidant capacity(TAOC) in serum of studied groups.

Table **(3-9)** and Figure **(3-9)** show that the mean **TAOC** in males and females were **(18.682), (14.469) U\ml.** ,respectively in patients group compared in control group**(85.660), (80.660) U\ml.** ,respectively. There is a highly significant differences between studied groups at P-value ≤ 0.05. Regarding to differences between gender in each group, the result show a highly significant differences between gender in patient group, while there is a non-significant differences between gender in control group.

Table (3-9): Concentration level of Total antioxidant capacity(TAOC) in β- thalassemia patients (Males and Females) and control groups.

Study groups					
Statistical Standards	**Patient group (n=60) (U\ml.)**		**Control group (n=30) (U\ml.)**		
	Male (n=30)	**Female (n=30)**	**Male (n=15)**	**Female (n=15)**	*P-value
Mean	18.682	14.469	85.660	80.660	0.000 (H.S)
Std. Deviation	0.2959	0.333	0.439	0.294	
Minimum	1.620	1.828	14.984	14.984	
Maximum	15.796	11.1	68.30	63.300	
Std. Error	22.056	19.843	121.30	116.30	
***P-value**	0.001 (H.S)		0.389 (N.S)		

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.



Figures (3-9): Concentration of total antioxidant capacity(TAC) in studied groups(males and females) in thalassemia patients and control.

The depletion of total antioxidant capacity(TAOC) induced by oxidative stress is eliminated by release and stock organ antioxidant, mainly from liver and adipose tissue and the induction or activation of

antioxidant enzymes and this agreement with our study and previous study like study of **Popa-Wagner *et al*.(2013); Al-Mashhedy , (2007).**

β-thalassemia major and minor are characterized by an overproduction of free radicals, i.e. when the antioxidant defense of an organism is overwhelmed or are established when a deficit of defenses of the organism against oxidation occurs. The primary defense against oxidative stress in extracellular fluids results from a number of low molecular weight antioxidant molecules either water – (ex. ascorbic acid) or lipid-soluble (ex.Vitamin E). These antioxidants can also be generated during normal metabolism (ex. uric acid, bilirubin, albumin, thiols) or introduced in the body by the consumption of dietary products rich in antioxidants (olive oil, fruits and vegetables, tea, wine, etc **(Stakos *et al*.,2009)** .

At a later phase of oxidative stress, The TAC falls due to depletion of antioxidant, low molecular weight antioxidants penetrate specific locations in the cell where oxidative stress may occur and protect against free radicals**(Kinnunen *et al*.,2005)** .

The sum of endogenous and food-derived antioxidants represents the total antioxidant activity of the extracellular fluid. In addition, the levels of these antioxidants are suitable not only as a protection against oxidation, but could also reflect their consumption during acute oxidative stress states. The cooperation among different antioxidants provides a greater protection against attack by reactive oxygen or nitrogen radicals, than any single compound alone Thus, the overall antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual parameters, as it considers the cumulative effect of all antioxidants present in serum and body fluids(**Ghiselli *et al*., 2000**).

3-3-8: Concentration level of super oxide dismutase(SOD) in serum of studied groups.

Table **(3-10)** and Figure **(3-10)** show that there are decrease in the mean **SOD** in males and females were **(95.633), (88.429) (U\ml.)** ,respectively in patients group compared in control group **(208.623), (190.413) (U\ml.)** ,respectively. There is a highly significant differences between studied groups at P-value ≤ 0.05. Regarding to differences between gender in each group, the result show a highly significant differences between gender in control group and patient group.

Table(3-10): Concentration level of Super oxide dismutase (SOD) in studied groups

Study groups					
Statistical Standards	**Patient group (n=60) U\ml**		**Control group (n=30) U\ml**		
	Male (n=30)	**Female (n=30)**	**Male (n=15)**	**Female (n=15)**	*P-value
Mean	**95.633**	**88.429**	**208.623**	**190.413**	0.000 (H.S)
Std. Deviation	0.42250	0.491	4.039	1.313	
Minimum	2.31412	2.691	15.643	5.088	
Maximum	126.50	100.50	94.70	89.76	
Std. Error	135.90	114.80	145.39	105.32	
***P-value**	0.001 (H.S)		0.000 (H.S)		

*t- test, H.S= highly significance, Sig= significance, N.S= no significance, P-value ≤ 0.05.

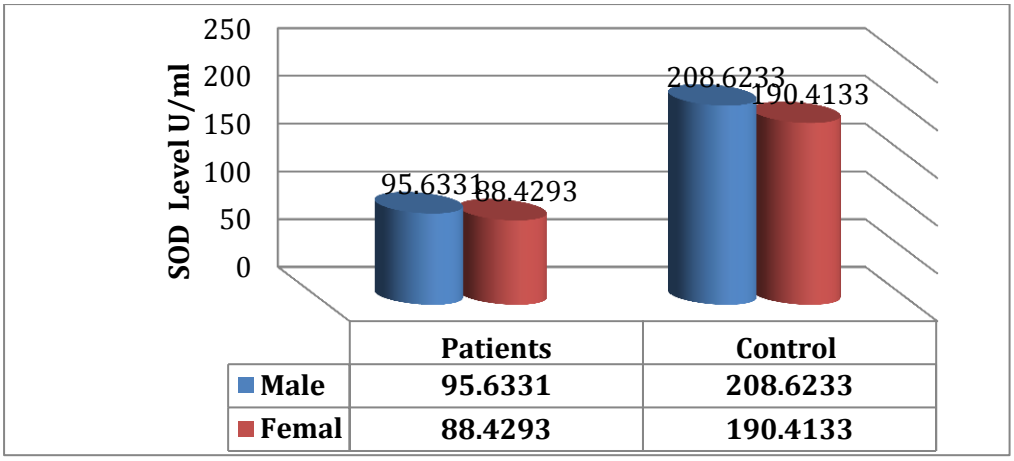


Figure (3-10): Concentration of Super oxide dismutase(SOD) in studied groups(males and females) in thalassemia patients and control.

The results are in agreement with those of **Dhawan *et al.* (2005)** ,who found that the mean SOD enzyme activity was at least 1.5 times lower in the thalassemia than in controls. The findings pertaining to SOD enzyme activity reported by other investigators are varied They ranged from high SOD activity to no difference in patients and controls. **Simsek *et al.* (2005)**.

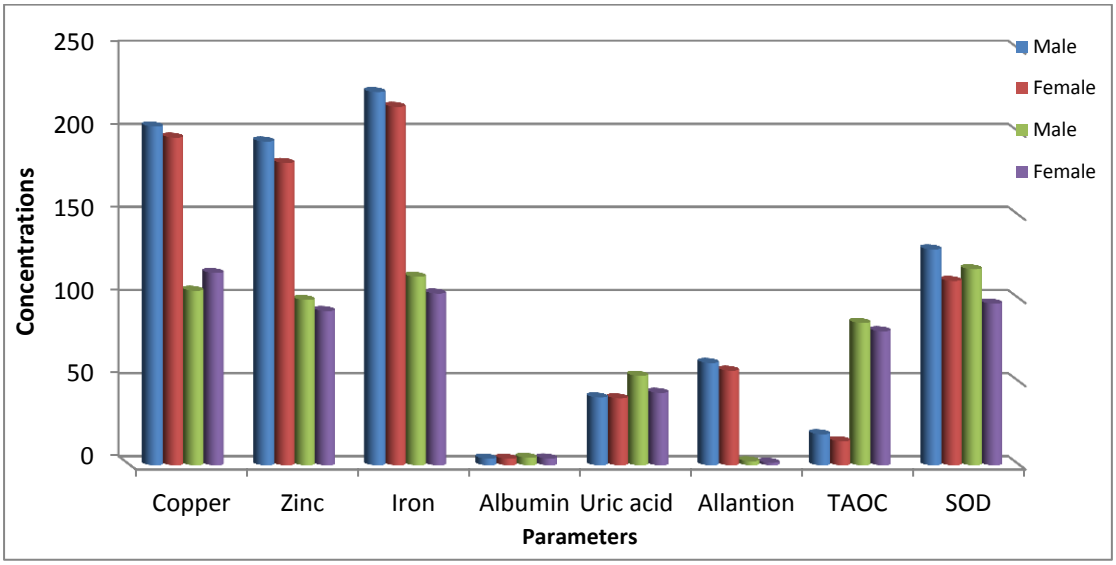
There are agreement of the present study and correlate of with study of **Patne *et al.* (2012)** also who studied on 50 β- thalassemia major and 50 healthy controls and found that serum superoxide dismutase and glutathione activities were significantly decreased in thalassemia patients as compared to healthy individuals. These results also goes with the **Faiza Waseem *et al.(*2011)** who also found the levels of these enzymes were significantly lowered ($P<0.001$) in thalassemia patients compared to control groups. And study of **Choudhary *et al.*(2017)** disgreement with present study . The significant decrease of SOD in current study may suggest that with longer disease duration, SOD induction and consequently its activity progressively decrease, since nonenzymatic glycation, the other cause of hydrogen peroxide production, later predominates and further inhibition of Cu/Zn SOD occurs. the

formation of hydrogen peroxide which activates SOD **(Alemu *et al*.,2006)).** Therefore, the accumulation of hydrogen peroxide may be one of the explanations for decreased activity of SOD in these patients. The primary catalytic cellular defence that protects cells and tissues against potentially destructive reactions of superoxide radicals and their derivatives is the Cu/Zn-SOD. It has been observed that SOD can be rapidly induced in some conditions when cells or organisms are exposed to oxidative stress **(Abdulla , 2018).**

Another previous study revealed disagreement with present results that the superoxide dismutase activity (SOD) was significantly increased in β-thalassemia patients compared to the controls (967.43±115.6 U/ml vs. 170.7±40.2 U/ml), while catalase was significantly less than control (144.77±17.3 U/ml vs. 194.95±47.2), that disagreement with current study that may be attributed to the increased activity of SOD in β-thalassemia may be involved in scavenging the superoxideradical (O_2^-), thereby producing more hydrogen peroxide in the erythrocytes **(Vaculin *et al*.,2010)**. In addition, the decrease of intracellular antioxidant enzymes might be hypothesized to be a direct effect of increased intracellular iron on gene expression **(Kassab-Chekir *et al*.2003).** During the course of metabolism, superoxide anion was converted to H_2O_2 by ubiquitous enzyme superoxide dismutase. Normally H_2O_2 was converted to innocuous compounds by the action of catalase and peroxidase (**Ghone *et al*.2008**) **.**SOD values in thalassemia patients have previously been explained as a reaction to, or compensation for, the decreased production of superoxide radicals**(Hossain ,2013).**

Oxidative stress is the result of an imbalance between free radical production and reduced degradation (**Vaculin *et al*.,2010**), An increased oxidant stress and a decreased antioxidant status promote peroxidative damage to cell and organelle membranes. It is well documented that dis-

turbances of oxidant-antioxidant balance occur in hemoglobinopathies, especially in thalassemia and sickle cell diseases (**Şimşek *et al*.,2005**).Removal of toxic oxygen metabolites is the putative function of antioxidant enzymes such as SOD and GPX. It has already been demonstrated that oxidative stress induces antioxidative enzymes, including SOD and GPX (**Kessab Chekir *et al*.,2003**).



Figures (3-11): Distribution of biochemical aspects in studied groups.

3-4:Correlation and association between biochemical parameters in patient group.

The results of the current study revealed all relationships its negative correlation except the following correlation its positive correlation:-

1- zinc and total antioxidant capacity(TAOC)
2- copper and (TAOC)
3- iron and uric acid
4- albumin and(TAOC)
5- allantoin and (TAOC)
6- (TAOC) and SOD enzyme.

This indicates the presence of free radicals generated in thalassemia patients, which are counteracted by some enzymes under study, such as TAOC and SOD that revealed with copper and zinc concentrations and total antioxidants capacity whose concentration is reduced, evidence of their response to these radicals, in addition to some large molecules such as albumin and small ones such as uric acid, whose concentration decreased and increase respectively as a result of oxidation and turned into allantoin.

Table (3-11): Association between biochemical aspects in patient group.

		Cu	Zinc	Iron	Albumin	Uric acid	Allantoin	TAOC	SOD
Cooper	R2		.268*	.306*	.015	-.043-	.173	.377**	.091
	Sig		.038	.017	.907	.745	.186	.003	.488
Zinc	R2			.202	.340**	.130	.430**	.256*	.337**
	Sig			.121	.008	.322	.001	.048	.009
Iron	R2				.052	.261*	-.022-	.139	-.015-
	Sig				.691	.044	.869	.290	.908
Albumin	R2					.134	.145	.236	.150
	Sig					.307	.269	.069	.251
Uric acid	R2						.035	-.033-	.121
	Sig						.790	.800	.358
Allantoin	R2							.577**	.394**
	Sig							.000	.002
TAOC	R2								.409**
	Sig								.001
SOD	R2								
	Sig								

R2= square person correlation

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

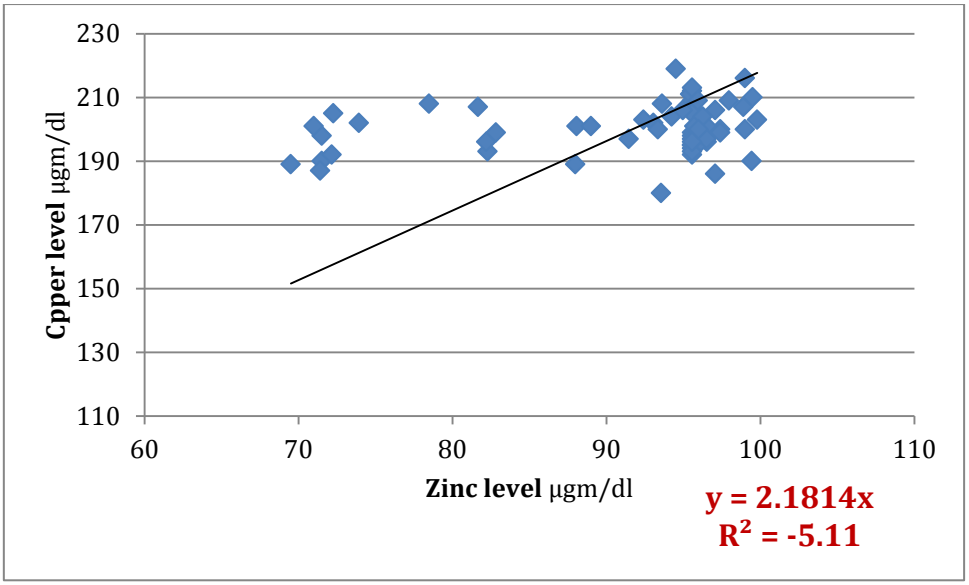


Figure (3-12): Correlation between Zinc and Copper in patient group.

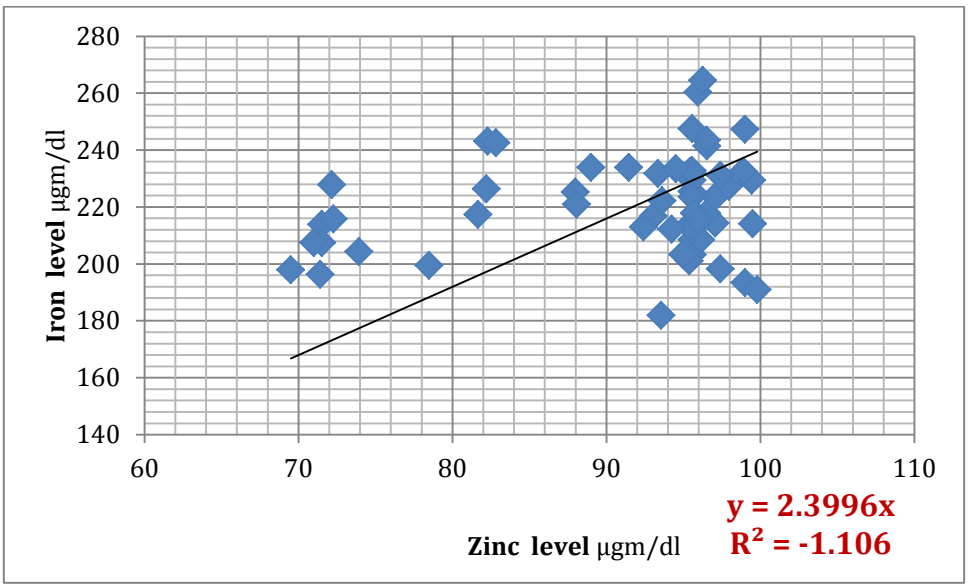
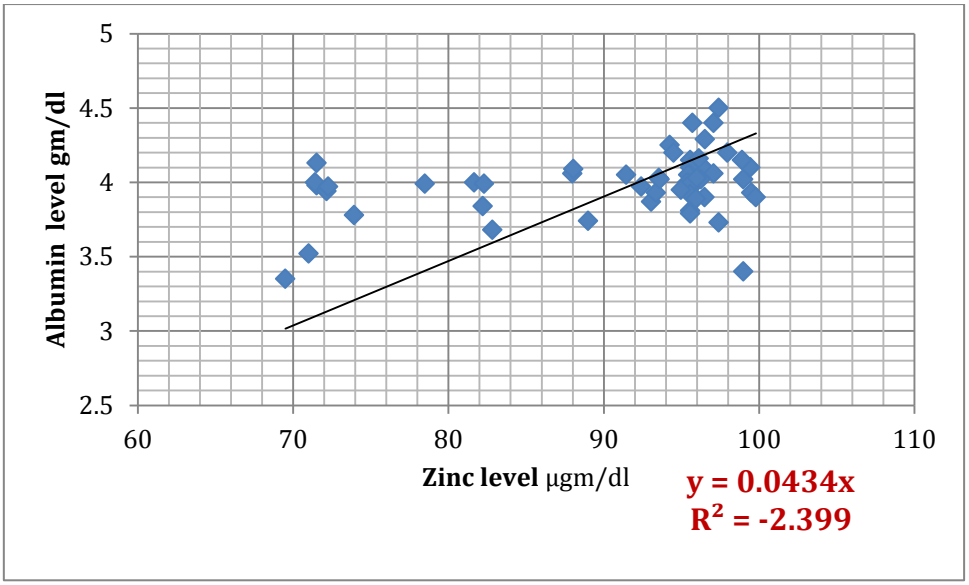
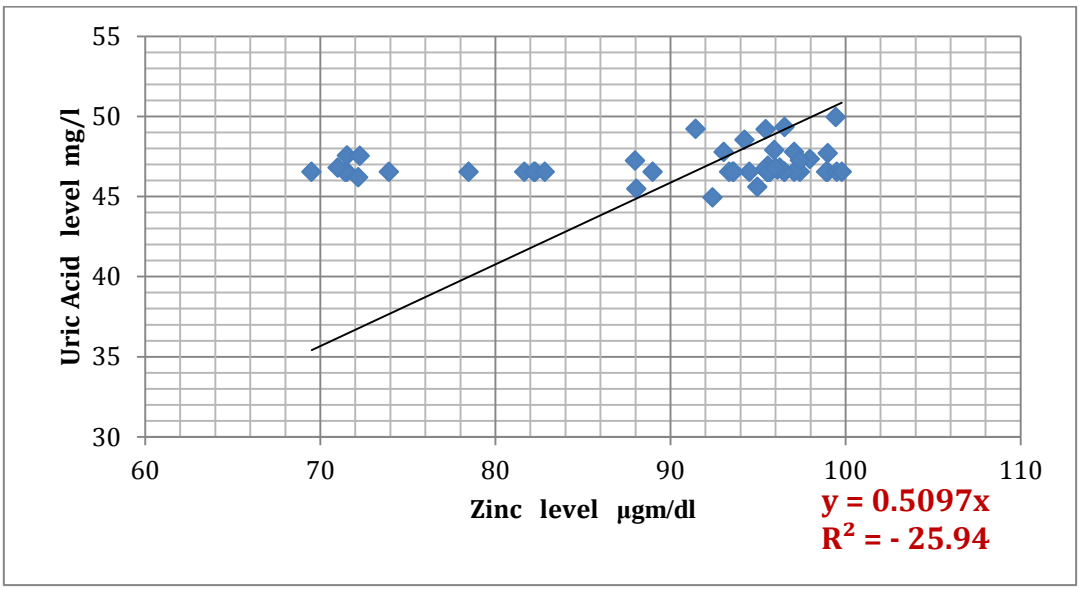


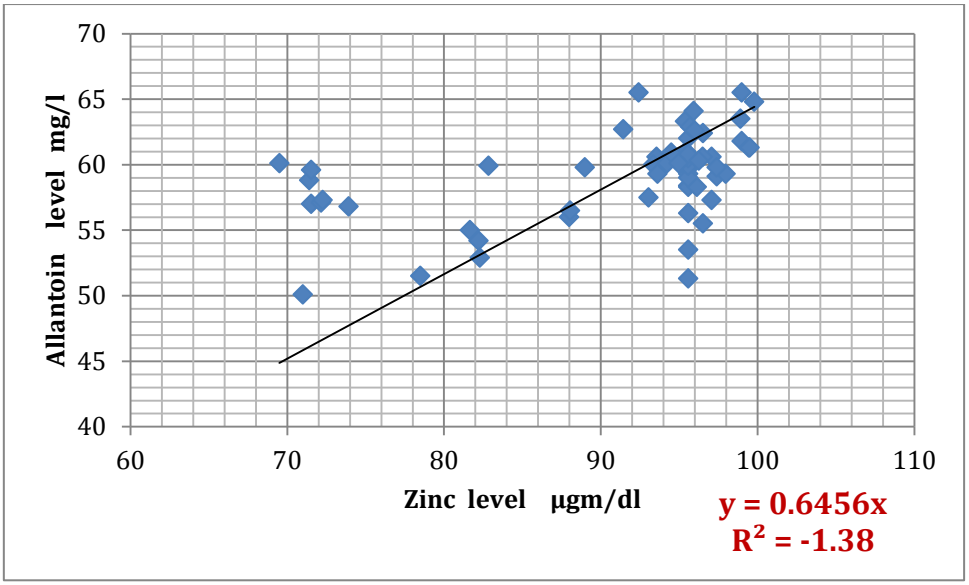
Figure (3-13): Correlation between Zinc and Iron in patient group.



Figures (3-14): Correlation between Zinc and Albumin in patient group.



Figures (3-15): Correlation between Zinc and Uric acid in patient group.



Figures (3-16): Correlation between Zinc and Allantoin in patient group.

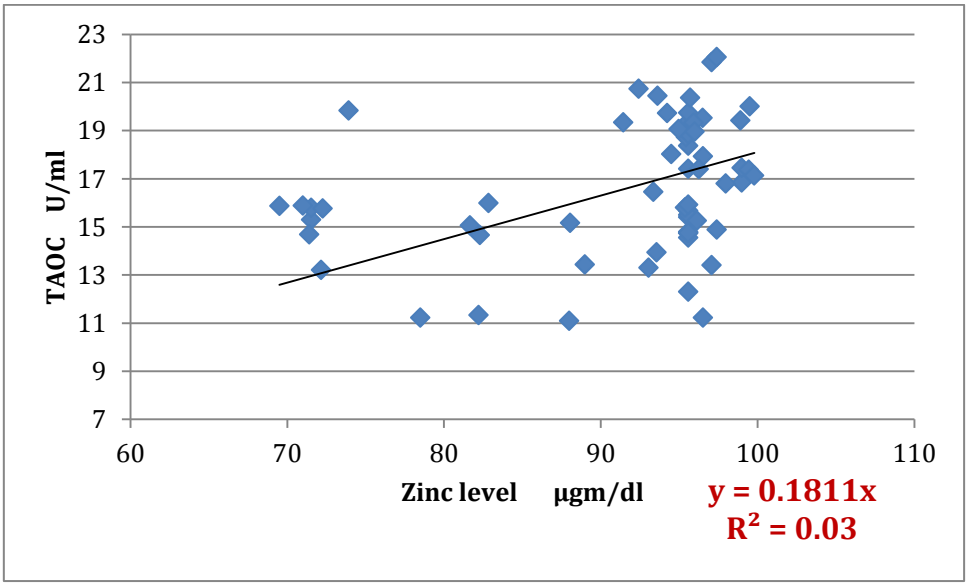
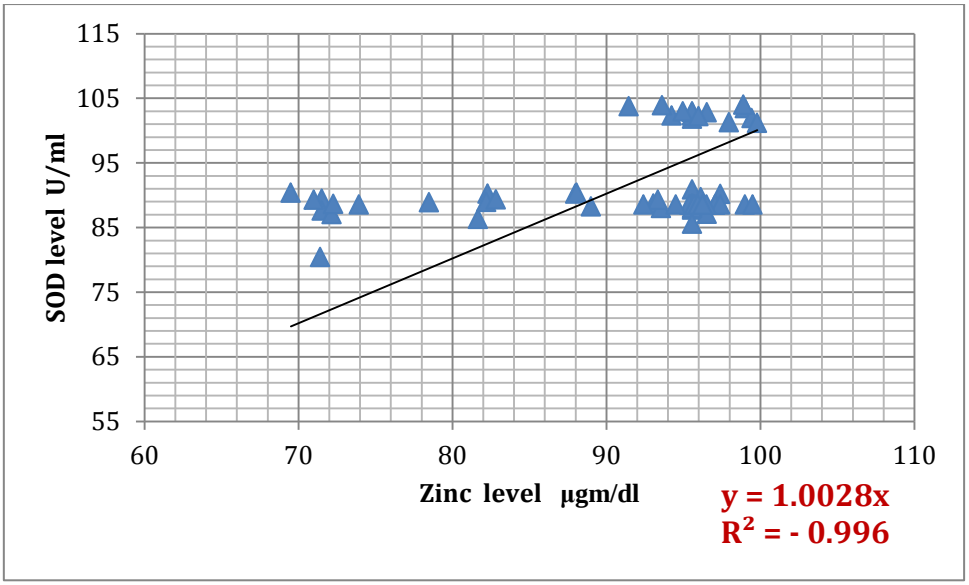
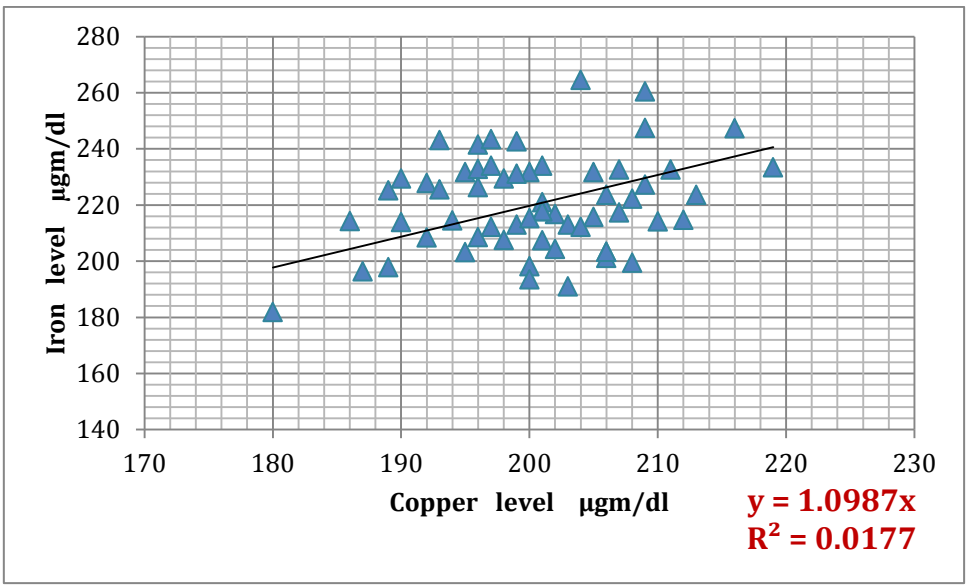


Figure (3-17): Correlation between Zinc and TAOC in patient group.



Figures (3-18): Correlation between Zinc and SOD in patient group.



Figures (3-19): Correlation between Copper and Iron in patient group.

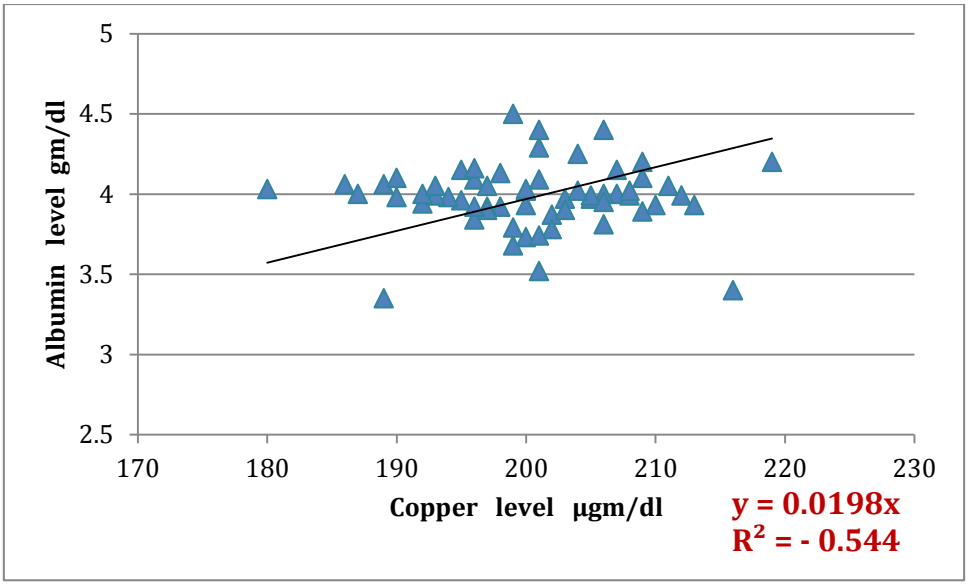
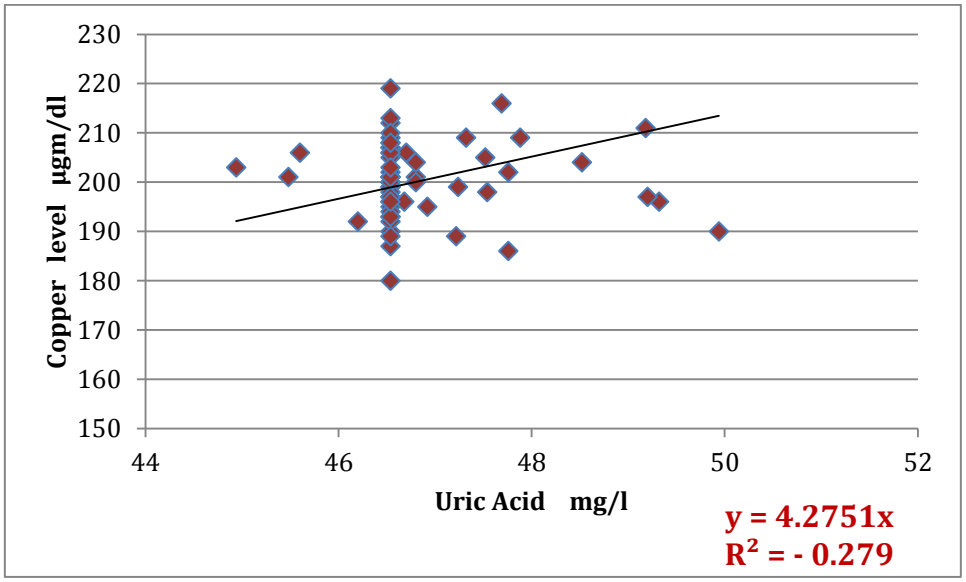
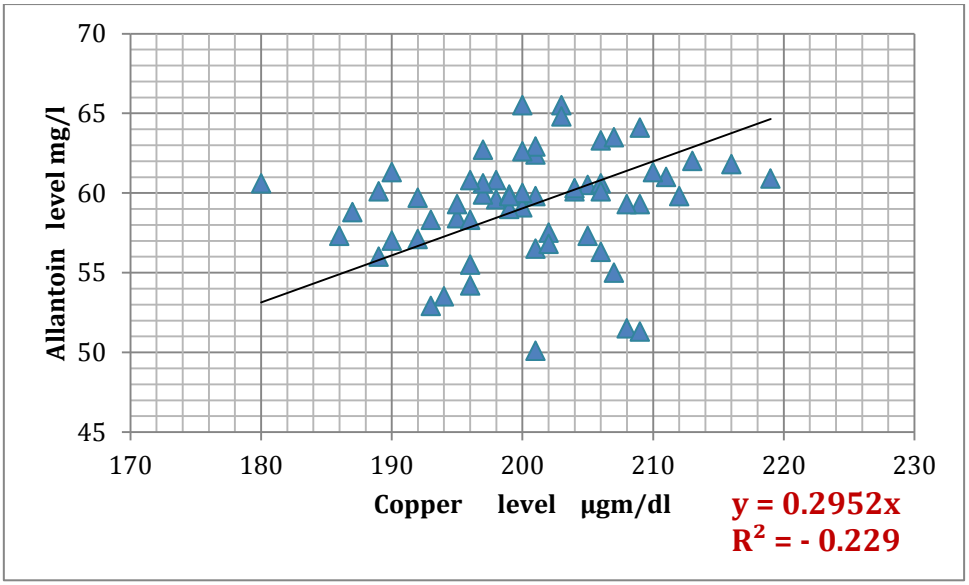


Figure (3-20): Correlation between Copper and Albumin in patient group.



Figures (3-21): Correlation between Copper and Uric acid in patient group.



Figures (3-22): Correlation between Copper and Allantion in patient group.

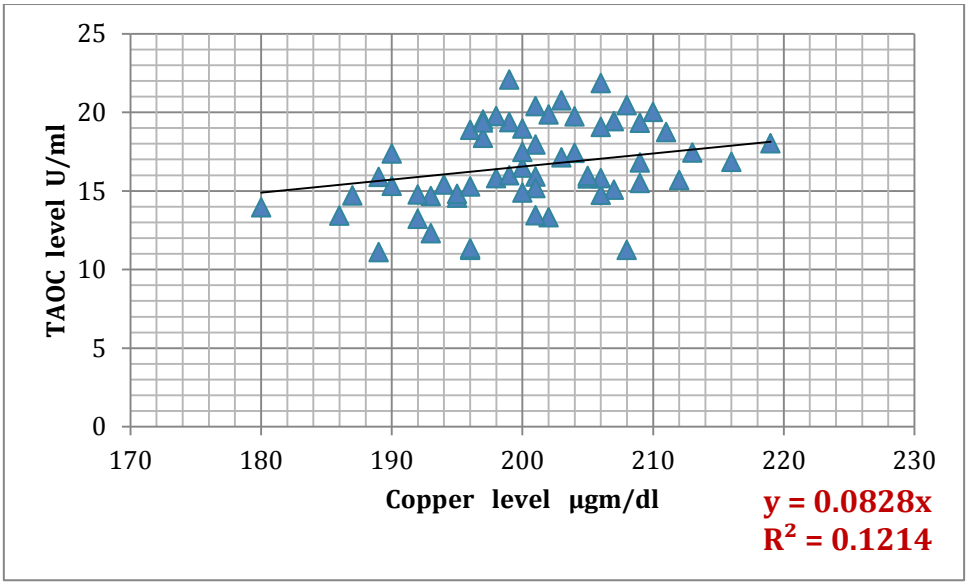


Figure (3-23): Correlation between Copper and TAOC in patient group.

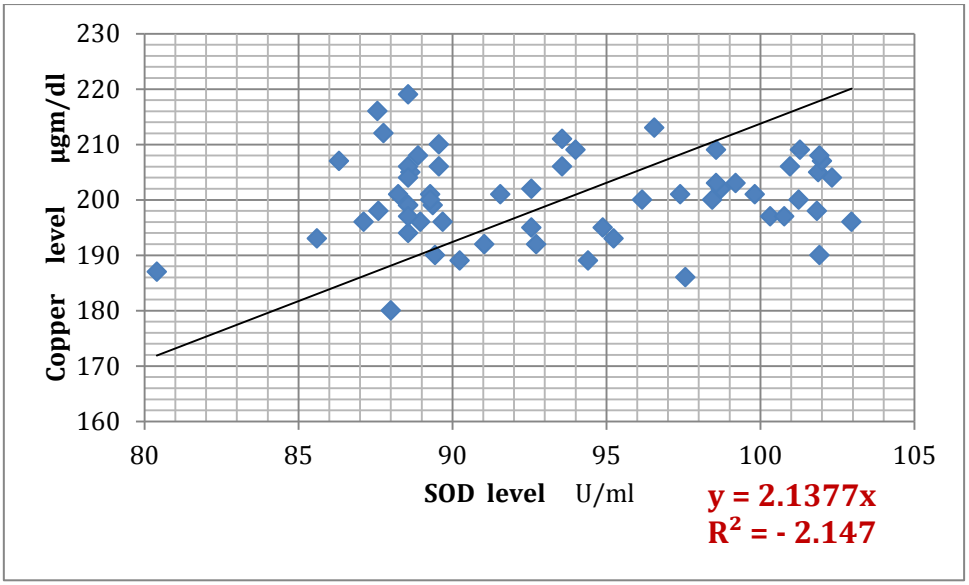


Figure (3-24): Correlation between Copper and SOD in patient group.

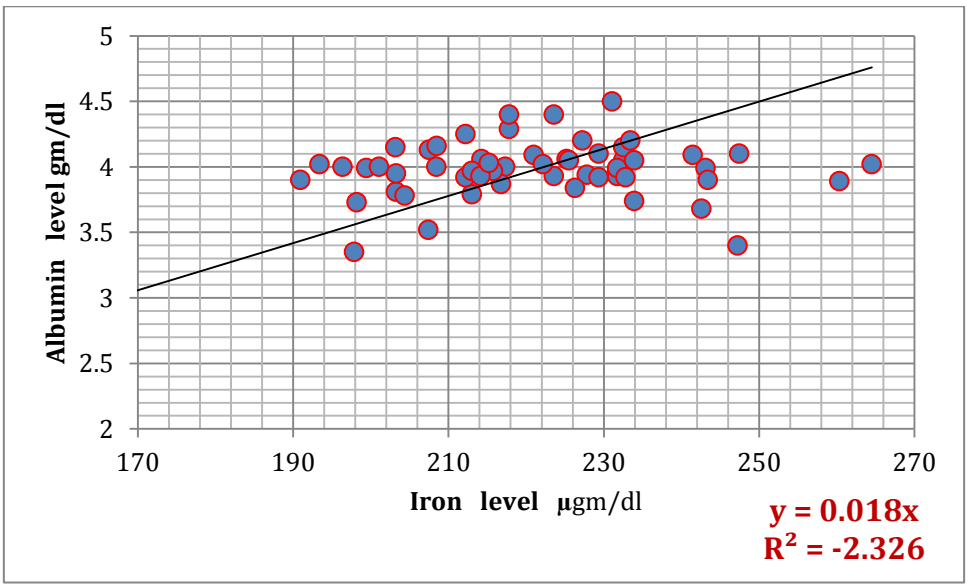


Figure (3-25): Correlation between Iron and Albumin in patient group.

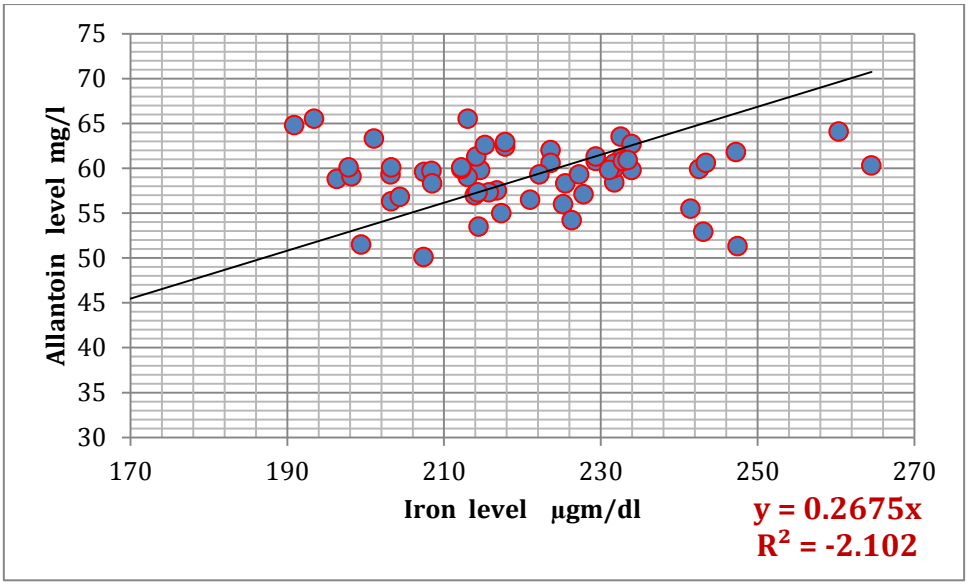


Figure (3-26): Correlation between Iron and Allantoin in patient group.

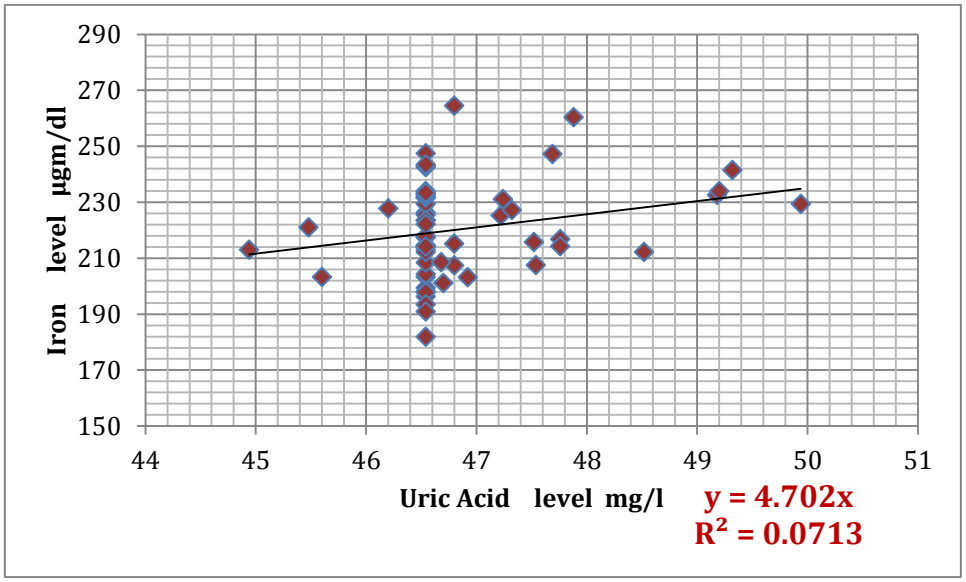
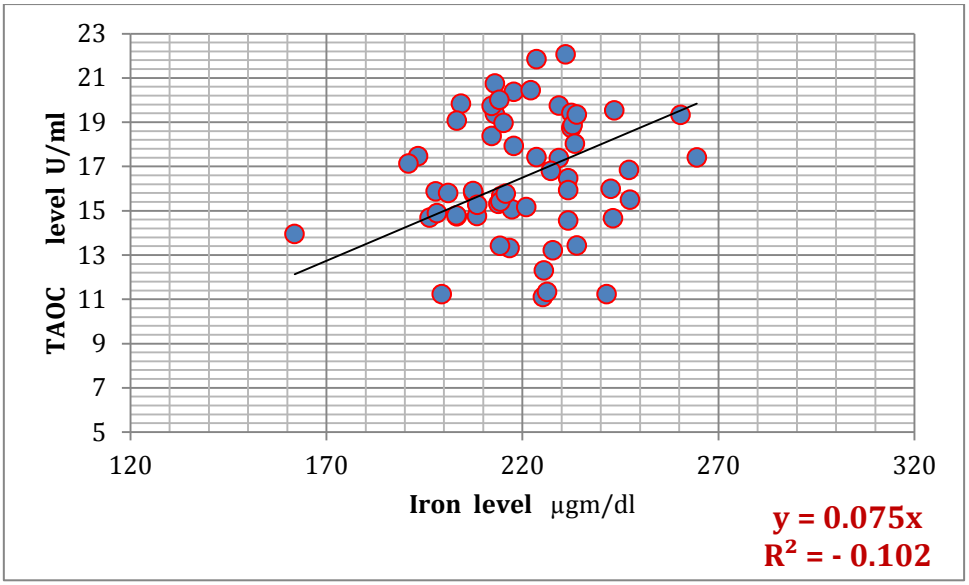
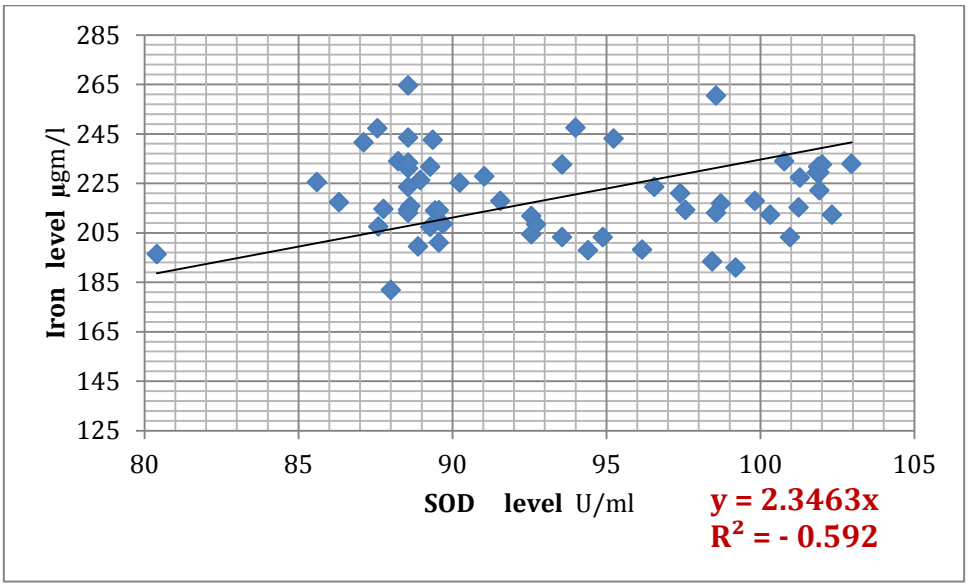


Figure (3-27): Correlation between Iron and Uric acid in patient group.



Figures (3-28): Correlation between Iron and TAOC in patient group.



Figures (3-29): Correlation between Iron and SOD in patient group.

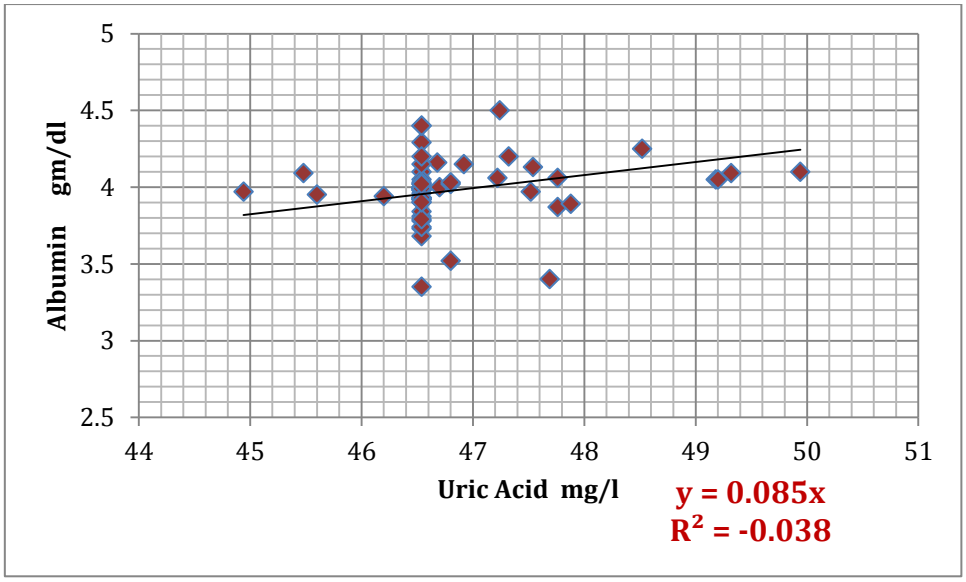


Figure (3-30): Correlation between Albumin and Uric acid in patient group.

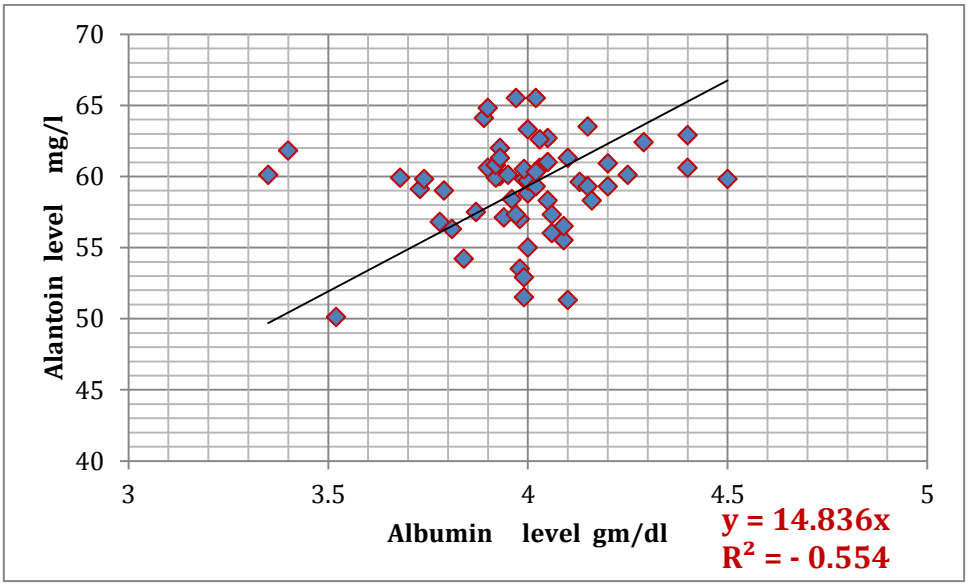


Figure (3-31): Correlation between Albumin and Allantoin in patient group.

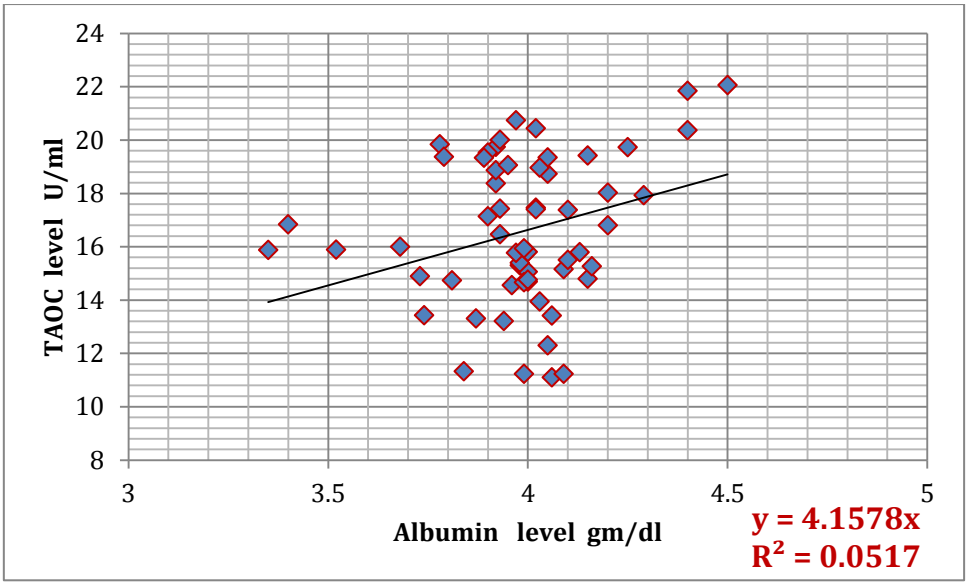


Figure (3-32): Correlation between Albumin and TAOC in patient group.

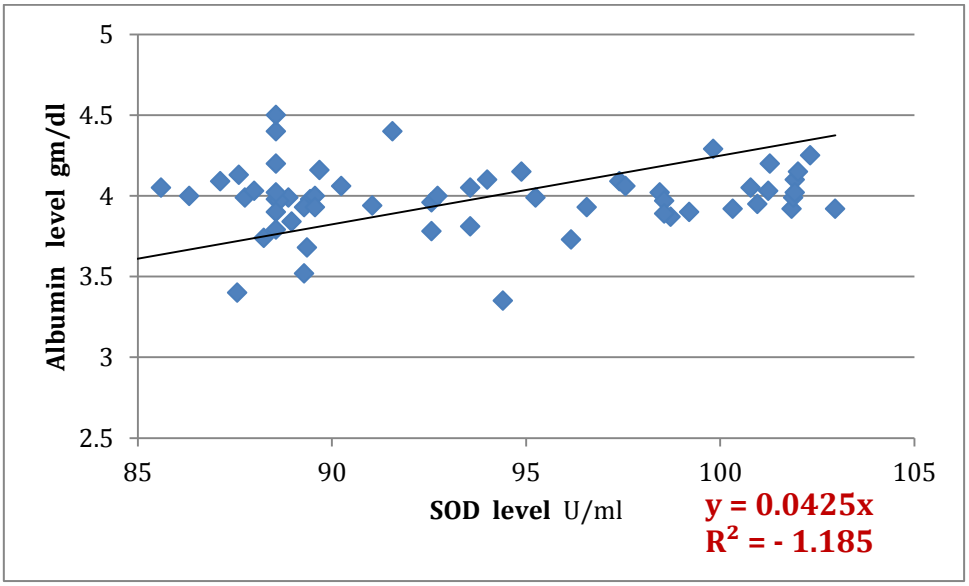


Figure (3-33): Correlation between Albumin and SOD in patient group.

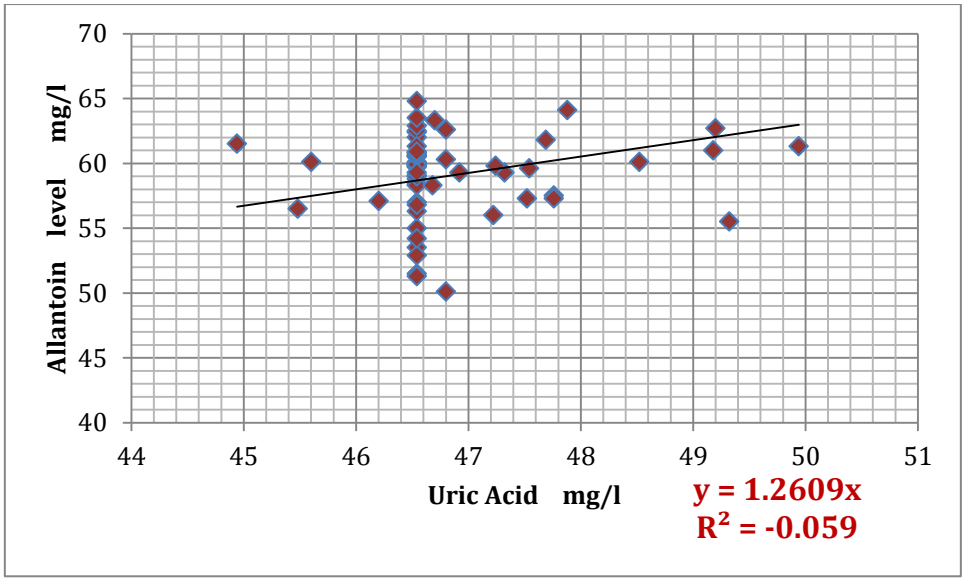


Figure (3-34): Correlation between Uric acid and Allantoin in patient group.

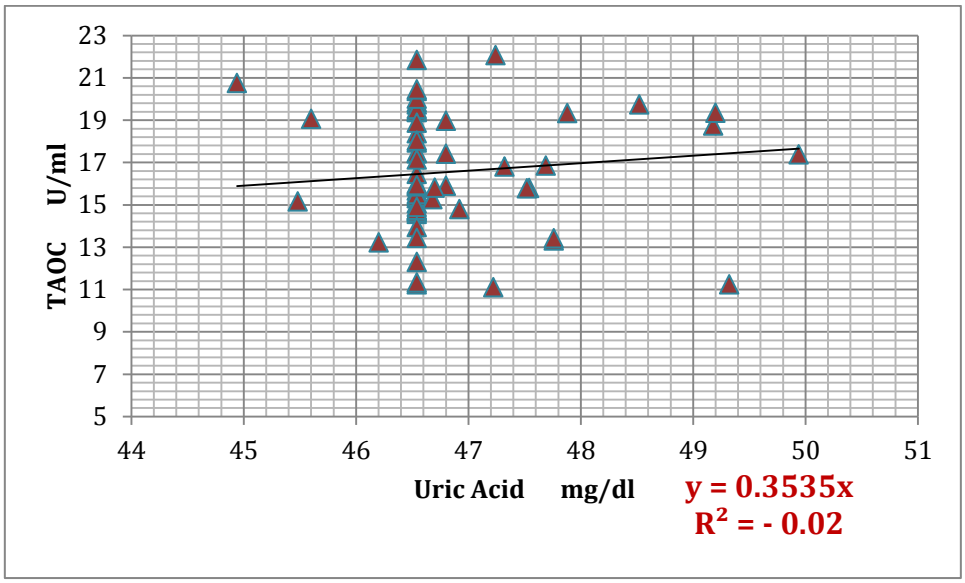
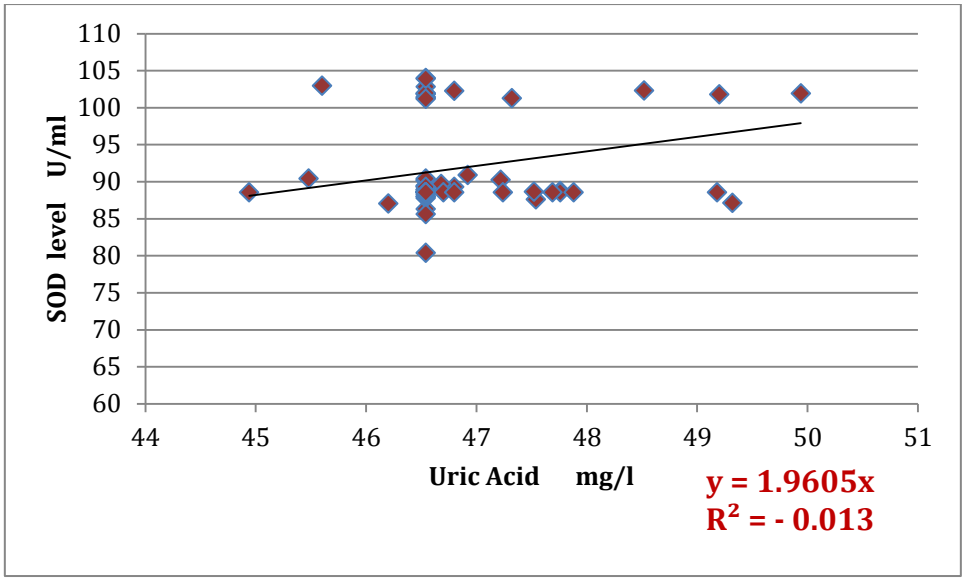


Figure (3-35): Correlation between Uric acid and TAOC in patient group.



Figures (3-36): Correlation between Uric acid and SOD in patient group.

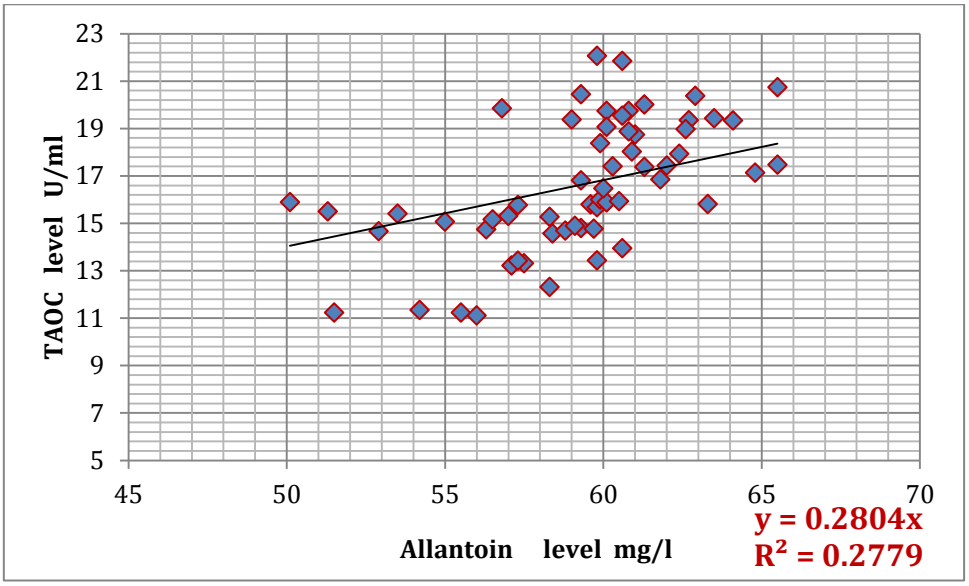


Figure (3-37): Correlation between Allantoin and TAOC in patient group.

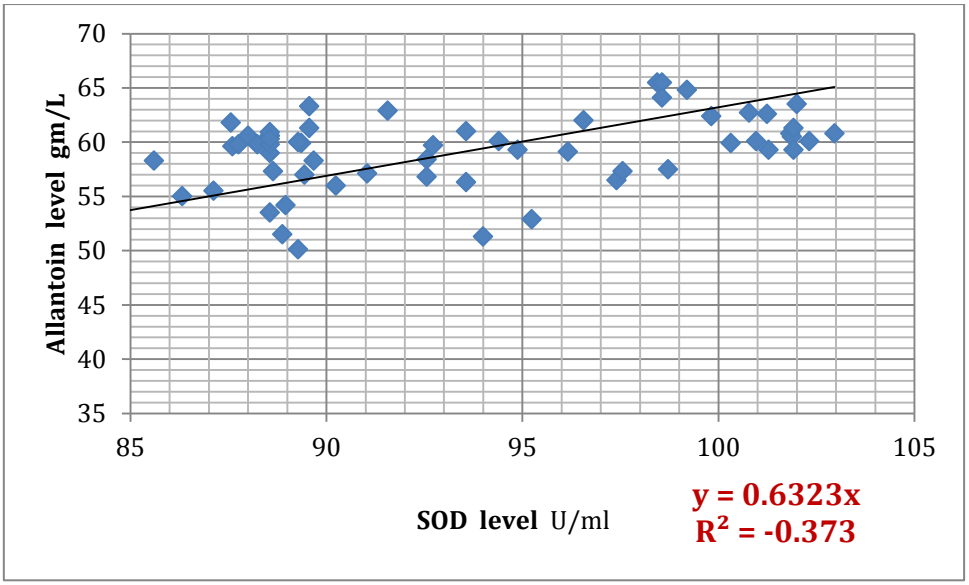
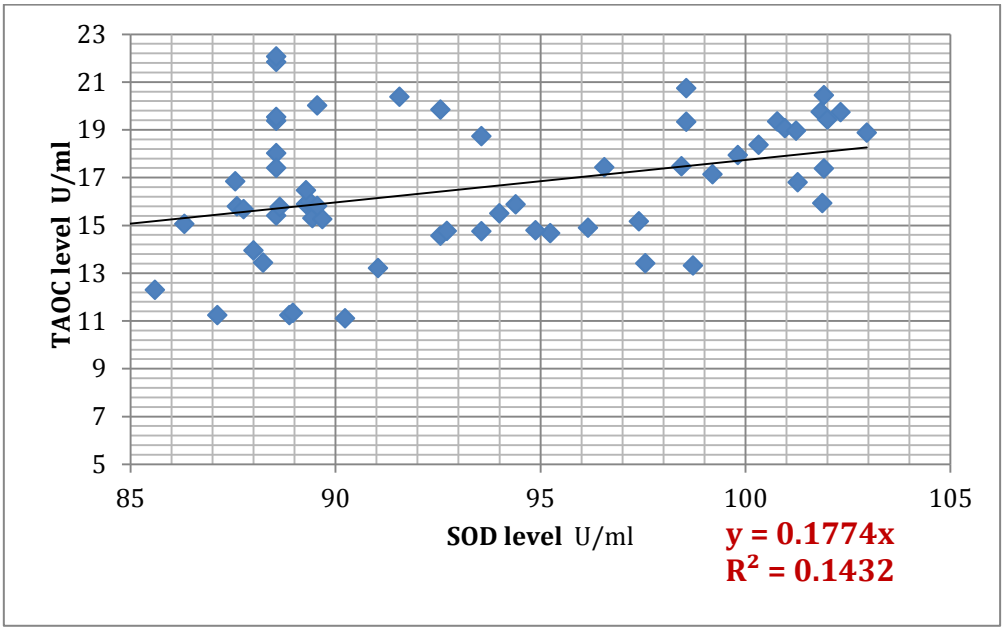


Figure (3-38): Correlation between Allantoin and SOD in patient group.



Figures (3-39): Correlation between TAOC and SOD in patient group.

Conclusions

1- Patients with thalassemia show various clinical signs and symptoms that seem to be unavoidable due to the regular transfusion of blood and its associated physiological as well as pathological outcomes. Having insights into various clinical aspects of thalassemia

2- All of these blood parameters showed a clear decrease in thalassemia patients, except for RBCs and platelets counts, which showed a significant increase.

3- Age group (1-20) years more than affected with thalassemia than the age group (21-35) years.

4- The Males in the current study were more affected than females with significant differences in the criteria (trace elements and antioxidants under current study).

5- A clear decrease in zinc and increase for copper due to repeated blood transfusions for half a month for Thalassemia patients, whether for males or females

6- A clear rise in iron due to destroyed red blood cells for Thalassemia patients, whether male or female.

7- A clear decrease in albumin and increase for uric acid, as they are important substances that interact with free radicals (ROS) formed in Thalassemia patients.

8- The increase level of uric acid in the current study for the group of patients compared to the control group may be due to the presence of free radicals (ROS), as uric acid interacts with it and generates from this oxidation to yield allantoin compound 1\3 (U\A) , allantoin concentrations were high for the group of thalassemia patients .

9- Decreased total antioxidant capacity (TAOS) and decreased of superoxide dismutase enzyme(SOD), evidence of multiple free radical formation as a result of Thalassemia disease.

Recommendations

1- The antioxidative therapy may be more helpful for long survival of patients with thalassemia with conventional iron chelating therapy.
2- Studying interlukin-1 and interlukin-22 because have interaction with people have thalassemia.
3- Study of other trace elements such as selenium and magnesium or other antioxidants such as bilirubin, vitamin C, malondehyde, glutathione peroxidase, ascorbic acid, alphatocopherol and catalase enzyme in Thalassemia patients.
4- Conducting studies at the molecular level for Thalassemia patients to find out the genes responsible for the functional and morphological changes of red blood cells
5- Urging Thalassemia patients to eat foods rich in antioxidants such as fruits and vegetables to reduce the damage caused by the formation of free radicals.
6- Educating citizens in areas outside city centers not to marry relatives for more than one generation one, especially individuals with a family history of Mediterranean anemia.
7- Directing relevant institutions such as the Ministry of Health and courts to make a disease diagnosis test mediterranean anemia before marriage is mandatory.
8- Prenatal diagnosis by DNA analysis For the fetus for couples at risk of having children with Mediterranean anemia, according to a date sick family.

References:

Abbass SAR, and Defer IH , Some Biochemical Parameters In Iraqi Patients With Thalassemia And Related With DM1. Internat. J. Chem. Res. **2011;**1 (5): 46-56.

Abdulla A A, Evaluation of serum antioxidant enzymes in β- thalassemia major patients Internat. J. of ChemTech Res., 2018;11(07): 323-328.

Afshin S , Shohreb M , Abtin H , and Yazdan G , Urine B2 microglobin and other biochemical indices in β-thalassemia major**.** Acta. Med. Iranica., **2009;**47(6) : 443-446 .

Aldudak B, Karabay Bayazit A , Noyan A , Özel A , Anarat A , Sasmaz I , Kilinç Y , Gali E , Anarat R , Dikmen N. Renal function in Paediatric patients with â-thalassemia major. Pediatric Nephrology, **2000**;15 (1-2): 109-112.

Alemu Y, Atomsa A, Sahlemariam Z , (). Hematology. USAID ,Jimma University **2006**;541pp.

Al-Fartusie F S , and Mohssan S N , Essential trace elements and their vital roles in human body. Indian J. Adv. Chem. Sci., **2017;**5(3), 127- 136.

Al-Haddad R M, Molecular, biochemical and hematologicalinvestigations of β-thalassemia children in Gaza governorate. M.Sc. Thesis of Science in Biological Sciences . Medical Technology **2012**;83pp.

Al-Helaly, L A, and Ahmed T Y , Antioxidants and some biochemical parameters in workers exposed to petroleum station pollutants in Mosul City, Iraq. **2014**; 35, 34-62.

Al-Jaouni S K, Survival and disease complication of thalassemia major: experience of 14 years at King Abdulaziz University Hospital, Jeddah, KSA," Medical Science Journal**. 2009**; 17(1) 19–28 .

References

Al- Jaouni S K, "Serum Ferritin is a poor indicator of Myocardial iron Content in Early Stage of Iron Overload in Thalassemia Major," The Egyptian Journal of Haematology.2007; 32 (3): 171–176.

Al-Mashhedy LAM, Total Antioxidant Capacity As Indicative of Oxidative Stress on β-Thalassemia Patients, Medical Journal of Babylon. 2007; 4 (3 & 4):291-295.

Al-Mousawi N H , Assessment of immunological status for Beta- thalassemia major patients in wasit province **.** M.Sc. Thesis in Biology-Immunology, College of Education for Pure Science, Dept. of Biology, University of Thi-Qar, Iraq 2014;132pp.

Al-Mosawy WF, The beta-thalassemia. Sci J Med Res. 2017;1:24-30.

Al-Samarrai AH, Adaay MH, Al-Tikriti KA, et al. Evaluation of some essential element levels in thalassaemia major patients in Mosul district, Iraq. Saudi Med J**.** 2008; 29(1):94-97.

Al-Saray ZA , A study of some physiological changes of thalassemia patients in Wasit Province **.** M.Sc. Thesis in Animal Physiology, College of Science, University of Wasit, Iraq . 2012.

Ali M , Biochemical study for serum oxidant-antioxidant state in pateints with β-thalassemia in Thi-Qar **.** M.Sc. Thesis in Clinical Biochemistry, College of Science, University of Thi-Qar, Iraq. 2008.

Amit K M , and Tiwari A , β-Thalassemia, a fatal blood disorder**.** Inter. J. Rev. Life Sci., 2011; 1(2) : 83-87.

Angelucci E , Barsoi G, and Gamaschella C , Italian society of hematology pracitce guidelines for the management of iron overload in thalassemia major and related disorders**.** Hematol., 2008;93(5) : 742 -742.

Angelucci E, Brittenham GM, McLaren CE, Ripalti M , Baronciani D , and Giardini C , Hepatic iron concentration and total body iron stores in thalassemia major **.** N. Engl. J. Med., 2000;343(5) : 327-331**.**

References

Ashkani-Esfahani S , Zarifi F, Asgari Q, Samadnejad A Z, Rafiee S, and Noorafshan A, Taurine improves the wound healing process in in mice model, based on stereological parameters. Advanced biomedical research, 3. 2014.

Aslan D, Gumruk F, Gurgey A, and Altay C , Importance of RDW value in differential diagnosis of hypochrome anemias **.** Amer. J. of Hematol., 2002;69(1) : 31-33.

Bazvand F, Shams S, Esfahani MB, Koochakzadeh L, Monajemzadeh M, Ashtiani MTH, and Rezaei N, Total Antioxidant Status in Patients with Major β-Thalassemia, Iranian Journal of Pediatrics, 2011; 21(2): 159-165.

Behrman, R. E. ; Kliegman, R. M. and Jenson, H. L. Nelson Textbook Of Pediatrics. 17th edn., Saunders : Philadelphia , 2004 ;(1630-1633) .

Bekheirnia MR, Shamshirsaz AA, Kamgar M, et al. Serum zinc and its relation to bone mineral density in beta-thalassemic adolescents. Biol Trace Elem Res. 2004; 97(3):215-24.

Bhattacharya PT, Misra SR, and Hussain M. , Nutritional aspects of essential trace elements in oral health and disease: an extensive review. Scientifica, 2016; 1-10.

Birgens H, and Ljung R,The thalassemia syndromes **.** Scan J. Clin. Lab. Invest., 2007,67(1) : 11–26 .

Bogdan C, Rollinghoff M, and Diefenbach A, The role of nitric oxide in innate immunity. Immunol. Rev., 2000;173:17–26.

Borgna-Pignatti C, and Galanello R, Thalassemia and related disorders : Quantitative disorders of hemoglobin synthesis *In* "Williams Wintrobe's Clinical Hematology" . Lippincott and Williams (Eds.) . Vol.42. 11th edn., Philadelphia. 2004; 1319-1365 .

Borgna-Pignatti C, Vergine G, Lombardo T, Cappellini M D, Cianciulli P, and Maggio A, Hepatocellular carcinoma in thalassemia syndromes **.** Brit. J. Haematol., 2004; 124(1) : 114-117 .

References

Calderón Guzmán, D , Juárez Olguín H , Osnaya Brizuela N , Hernández Garcia E , and Lndindoro Silva M, (). The use of trace and essential elements in common clinical disorders: roles in assessment of health and oxidative stress status. Nutrition and cancer, 2019;71(1), 13- 20.

Carneiro, P. P., Conceição, J., Macedo, M., Magalhães, V., Carvalho, E. M., and Bacellar, O. The role of nitric oxide and reactive oxygen species in the killing of by monocytes from patients. PloS one, 2016;11(2),:1-10.

Cappellini, M. D. and Taher, A. Long-term experience with deferasirox, a once-daily oral iron chelator, in the treatment of transfusional iron overload **.** Exp. Opin. Pharmacother, 2008;9(13) : 2391-2402.

Carneiro, P. P., Conceição, J., Macedo, M., Magalhães, V., Carvalho, E. M., & Bacellar, O. The role of nitric oxide and reactive oxygen species in the killing of Leishmania braziliensis by monocytes from patients with cutaneous leishmaniasis. PloS one, 2016;11(2) :1-10.

Choudhary M, Vyas, R.K.; Lahoti,A. and Soni, Y. Correlation of oxidative stress with serum antioxidant enzymes level in thalassemia in a tertiary level hospital of western Rajasthan, International Journal of Biotechnology and Biochemistry, 2017;13(2): 155-165.

Chaudhuri, S., Varshney, J., & Patra, R. Erythrocytic antioxidant defense, lipid peroxides level and blood iron, zinc and copper concentrations in dogs naturally infected with Babesia gibsoni. Research in Veterinary Science, 2008;85(1), 120-124.

Clarke, G. and Higgins, T. Laboratory investigation of hemoglobinopathies and thalassemias **.** Beckman Conference Clin. Chemist., 2000;46(8) : 1284-1290 .

Cummings, J. E., and Kovacic, J. P. The ubiquitous role of zinc in health and disease. Journal of veterinary emergency and critical care, 2009;19(3), 215-240.

References

Cunningham, M. J. ; Macklin, E. A. ; Neufeld, E. J. and Cohen, A. R. Complications of beta-thalassemia major in north America**.** Blood., 2004;104(1) : 34-39 .

Czauderna, M. and Kowalczyk, J. Simultaneous measurement of allantoin, uric acid, xanthine and hypoxanthine in blood by high-performance liquid chromatography. Journal of Chromatography B, 1997;704 : 89–98.

De Leo, M. E., Tranghese, A., Passantino, M., Mordente, A., Lizzio, M. M., Galeotti, T. and Zoli, A. Manganese superoxide dismutase, glutathione peroxidase, and total radical trapping antioxidant capacity in active rheumatoid arthritis. J. Rheumatol. 2002;29(10): 2245-2246.

Dhawan, V. Kumar, Kh. R. Marwaha, R. K. Naravan S.and Kamgar, M. Antioxidant Status in Children with Homozygous Thalassemia, Ind. Pedi., 2005;42, 1141-1145 .

Dikalov, S. I. and Dikalova, A. E. Crosstalk between mitochondrial hyperacetylation and oxidative stress in vascular dysfunction and hypertension. Antioxidants & redox signaling. 2019;102pp.

El, H. A. H. M. A. Lipid peroxidation end-products as a key of oxidative stress: effect of antioxidant on their production and transfer of free radicals Lipid peroxidation: Intech Open. 2012;119pp.

Eldor, A. and Rachmilewitz, E. A. The hypercoagulable state in thalassemia **.** Blood., 2002;99(1): 36-43 .

Elhams, S. F. Immunological assessment of β-thalassemic major children aged 5-12 years old attending Abd El-Aziz El-Rantisy hospital in Gaza Strip . Msc. Thesis, Faculty of Science. Gaza University, Palestine 2010.147pp

Elias, R.H.I. Study of some physiological and cytogenetic parameters in patients with β- thalassemia major in Wasit Province. M.Sc. Thesis, College of Science , Wasit University. 2016;89 pp.

References

Eliezer, A. R. and Patricia, J. G. How I treat thalassemia **.** Blood J. Hematol. Library Org., 2011;118(13) : 3479-3488 .

Erejuwa, O. O., Sulaiman, S. A., & Ab Wahab, M. S.. Evidence in support of potential applications of lipid peroxidation products in cancer treatment. Oxidative Medicine and Cellular Longevity, (2013).

Evans, P., & Halliwell, B. Micronutrients: oxidant/antioxidant status. British journal of nutrition, (2001). 85(S2), S67-S74.

Faiza Waseem, Karira A.Khemomal, Raihan Sajid Antioxident status in beta thalassemia major: A single case study, Indian journal of pathology and microbiology , (2011). 54(4):19-28.

Faranoush, M. Rahiminejad, M. S. Karamizadeh, Z. Ghorbani, R.and Owji, S. M. Zinc supplementation effect on linear growth in transfusion dependent Beta thalassemia"، Iranian Journal of Blood and Cancer , (2008). 1(1): 29–32.

Fontaine, E.; Barnoud, D.; Schwebel , C. and Leverve, X. Place des antioxydants dans la nutrition du patient septique. Réanimation, (2002). 11(Issue 411): 4206.

Franco, R., Navarro, G., & Martínez-Pinilla, E. Antioxidant defense mechanisms in erythrocytes and in the central nervous system. Antioxidants, (2019). 8(2): 46-46

Galanello, R. Deferiprone in the treatment of transfusion-dependent thalassemia : a review and perspective **.** Ther Clin. Risk Manag., (2007). 3(5) : 795-805 .

Galanello, R. and Origa, R. Beta thalassemia **.** Orphanet . J. of Rare Dise., (2010). 5 : 11 -11.

Galanello, R. ; Piras, S. ; Barella, S. ; Leoni, G. B. ; Cipollina, M. D. and Perseu, L. Cholelithiasis and gilbert's syndrome in homozygous beta-thalassemia **.** Brit. J. Haematol., (2001). 115(4):926-928.

References

Ghiselli, A.; Serafini, M.; Natella, F. and Scaccini, C. Free Radic Biol Med. (2000). 29:1106-1114.

Ghone, R.A; Kumbar, KM. and Suryakar, A.N. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. Ind. J. Clini. Biochem. (2008). 23: 337-340.

Hamed E.A. and ElMelegy N.T. Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study. Italian Journal of Pediatrics, (2010). 36: 39 1-10.

HAMMOD , H. J. MOKIF , T.A. AL-HARBI,H. J. THE CORRELATION BETWEEN THALASSEMIA WITH BODY MASS INDEX AND BLOOD GROUPS IN CHILDREN AND ADULT PATIENT IN THE PROVINCE OF BABYLON, IRAQ. Asian J Pharm Clin Res, 2018,11(Issue 9): 509-512.

Harteveld, C. L. ; Refaldi, C. ; Cassinerio, E. ; Cappellini, M. D. and Giordano, P. C. Segmental duplications involving the alphaglobin gene cluster are causing beta-thalassemia intermedia phenotypes in beta-thalassemia heterozygous patients **.** Blood Cells Mol. Dis., (2008). 40(3) : 312-316 .

Hershko, C. Role of iron chelation therapy in thalassemia major **.** Turk. J. Haematol., (2002). 19(2) : 121-122 .

Higgs, D. R. and Bowden, D. K. Clinical and laboratory features of the thalassemia syndromes In " Disorders of hemoglobin ; genetics, pathophysiology, and clinical management" . M. H. Steinberg , B. G. Forget , D. R. Higgs , R. L. Nagel (Eds.). Cambridge University Press : Cambridge, United Kingdom . (2001). (431-469).

Hoffbrand, A. V., & Steensma, D. P. Hoffbrand's essential Haematology : John Wiley & Sons, (2019). 350 pp.

Hossain, U.S. Oxidative stress and antioxidant status in beta-thalassemia heterozygotes. Scientific Comments. (2013). 35(6): 378-388.)

References

Hussain , Z. ; Malik, N. and Chughtal, A. S. Diagnostic significance of red cell indices in beta-thalassemia trait**.** Biomedica., (2005). 21(49) : 129-131 .

Irshaid, F. and Mansi, K. Status of thyroid function and iron overload in adolescents and young adults with Beta- thalassemia major treated with deferoxamine in Jordan," Proceedings of World Academy of Science, Engineering and Technology, (2009). 58: 658–663 .

Ismael, M.A.D. A Novel Keynote in Male Infertility as Oxidative Stress Syndrome.Ph.D Thesis . College of Science of Al-Mustansiriya University(2001).:122pp.

Johnson, S. The possible crucial role of iron accumulation combined with low tryptophan, zinc and manganese in carcinogenesis. Chemistry Preprint Archive, 2001;(5):178-190.

Jomova, K., & Valko, M. Importance of iron chelation in free radical-induced oxidative stress and human disease. Current pharmaceutical design, (2011). 17(31), 3460-3473.

Kaiser, J. Beta-thalassemia treatment succeeds, with a caveat **.** Sci. Ge. ther., (2009). 326(5959) : 1468-1469 .

Kamal, M. Talal, A. Moussa, B. and Hamzeh, N. Copper and zinc status in Jordanian patients with β-thalassemia majortreated with Deferoxamine," Research Journal of Biological Sciences, (2009) . 4 (5): 566–572 .

Kand'ár, R., Záková, P. "Allantoin as a marker of oxidative stress in human erythrocytes". Clinical Chemistry and Laboratory,(2008). 15:54-54.

Kessab-Chekir, A.; Laradi, S.; Haj khelil A.; Feki, M.; Amri, F.; Selmi, H.; Bejaoui, M. and Miled A. Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. Clin. Chim. Acta. (2003). 338(1-2): 79-86.

References

Kilic E, Saraymen R, Sahin I. Serum malondialdehyde levels in toxoplasma seropositive patients. Ann Saudi Med. (2003);23(6):413-413.

Kinnunen S., Atalay M., Hyyppa S., Lehmuskero A., HANNINEN O., Oksala N. (2005) .J. of Sports Sci. and Med.; 4: 415-421.

Klotz, L.O.; Kroncke, K.D. and Buchczyk , D.P., Sies H. Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J. Nutr., (2003). 133:1448–1451.

Kuppusamy, U.R. and Tan, JA. Chelation Therapy with Desferrioxamine does not Normalize Ferritin Level but Attenuates Oxidative Damage and Improves Total Antioxidant Level in Malaysian Chinese β-thalassaemia Major Patients West Indian Med. J., (2011). 60(1): 3-3.

Lal, C. S., Kumar, S., Ranjan, A., Rabidas, V. N., Verma, N., Pandey, K., Das, P. Comparative analysis of serum zinc, copper, magnesium, calcium and iron level in acute and chronic patients of visceral leishmaniasis. Journal of Trace Elements in Medicine and Biology, (2013). 27(2), 98-102.

Lieberman, Michael; Marks, Allan D.; Smith, Colleen M.; Marks, Dawn B. Marks' Essential Medical Biochemistry. Philadelphia: Lippincott Williams & Wilkins. **(2007);** 47 pp.

Liu, H., He, J., Chi, C., & Gu, Y. Identification and analysis of icCu/Zn-SOD, Mn-SOD and ecCu/Zn-SOD in superoxide dismutase multigene family of *Pseudosciaena crocea*. Fish & shellfish immunology, (2015). 43(2), 491-501.

Lopes-Neto, B. E.;Santos, G. J. L.; Lima, A. L.; Barbosa, M. C.; dos Santos, T. E. J.; Uchoa, D. C. and Nunes-Pinheiro, D. C. S. Catalase and glutathione peroxidase in dogs naturally infected by *Leishmania infantum*. Acta Scientiae Veterinariae, (2016). 44, 1-6.

Lucarelli, G. ; Polchi, P. ; Galimberti, C. ; Angelucci, E. ; Giardini, C. and Politi, P. Bone marrow transplantation in adult thalassemia patients **.** J. Amer. Soci. of Hematol., (2011). 93(4) : 1164-1167 .

Madan, N. ; Beachler, L. ; Konstantinopoulos, P. ; Worley, S. ; Sun, Z. and Latson, L. A. Red cell indices and discriminant functions in the detection of beta - thalassemia trait in a population with high prevalence of iron deficiency anaemia **.** Pedi. Cardiol., (2010). 31 : 1203-1208 .

Mahyar, A. Ayazi, P. Pahlevan, A. A. Mojabi, H. Sehhat, M. R. and Javadi, A. Zinc and copper status in children with Betathalassemia major, Iranian Journal of Pediatrics, (2010). 20(3): 297–302.

Malik, A.M.; Malik, E.M.; Al-Shammaa, N.MJ.and Al-Rubaei, Z.M. A Comparative Biochemical Study of Proteins Profile in Iraqi Children and Adolescent with â.Thalassemia. Iraqi Journal of Pharmaceutical Sciences, (2010): 19(2): 19-23.

Marieb, E. N., and Hoehn, K. Human anatomy and physiology: Pearson education,**(**2007); 567pp.

Marengo-Rowe, A. J. The thalassemia and related disorders **.** Depart. of Pathology, Bayl. Univ. Med. Cent., Dallas, Texas, (2007). 20(1) : 27-31 .

Mastousek, J.A.; Burguera, L.J.; Burguera, M. and Anez, N..Changes in total concentration, copper and zinc in serum, heart, liver, spleen and skeletal muscle tissues of rats infected with Trypanasoma cruzi. Biol Trace Elements Anal. (2009) 37:51–60.

Mavrou, A. ; Kouvidi, E. ; Antsaklis, A. ; Souka, A. ; Kitsiou Tzeli, S. and Kolialexi, A. Identification of nucleated red blood cells in maternal circulation : a second step in screening for fetal aneuploidies and pregnancy complications **.** Prenat. Diagn., (2007). 27(2) : 150-153 .

References

Mckenzie, S. B. Clinical Laboratory Hematology **.** 2nd edn ., Upper Saddle River, N. J. : Person Education (2010)., 732-761 pp .

Mankad, G. P. ; Mankad, B. and Singh, S. P. A study of serological and hematological parameters in thalassemic patients of Gujarat, Western India **.** IOSR J. of Dent. and Med. Sci., (2013). 9(5) : 52-55 .

Marbut, M.M, Abdulazeez, S.S, Salman, H.E, Mahmood, A.A. (2020). Determination of the serum ferritin liver enzymes, zinc and copper concentration in the serum of thalassemia patients. Eurasia J. Biosci.,14: 7371-7374.

McMahon, B. K., & Gunnlaugsson, T. (2012). Selective detection of the reduced form of glutathione (GSH) over the oxidized (GSSG) form using a combination of glutathione reductase and a Tb (III)-cyclen maleimide based lanthanide luminescent 'switch on'assay. Journal of the American Chemical Society, 134(26), 10725-10728.

Mehdizadeh M, Zamani G, Tabatabaee S. (2008). Zinc status in atients with major beta-thalassaemia. Pediatr. Hematol. Oncol. , 25(1):49-54.

Michiels, C., Raes, M., Toussaint, O. and Remacle J.(1994): Importance of Se-glutathione peroxidase, catalase and Cu/Zn SOD for cell survival against oxidative stress. Free Radic Biol Med, 17:235-248.

Mirończuk-Chodakowska, I., Witkowska, A. M., & Zujko, M. E. (2018). Endogenous non-enzymatic antioxidants in the human body. Advances in medical sciences, 63(1), 68-78.

Moafi A, Mobaraki G, Taheri SS, et al. (2008). Zinc in thalassemic patients and its relation with depression. Biol Trace Elem Res;123(1-3): 8-13.

Mohammed,J. I. ; Al-Bajari,S. A. ; AL.Akashe ,M.A. and Totanjee,A.N. (2020). Alterations in Antioxidants and Trace Element

with Interleukin -6 Level in β Thalassemia Major Patients Medico-legal Update, 20(1) :1411─ 1415.

Mohammed,N.A and Abd-Elrasoul , H. (2020). Evaluation of Oxidative Stress and Antioxidant Status in Beta Thalassemia Major Patients: A Single-Center Study Med. J. Cairo Univ ., 88,(5) : 2147─2154.

Mok, S. ; Imwong, M. and Mackinnon, M. J. (2011). "Artemisinin resistance in *Plasmodium falciparum* is associated with an altered temporal pattern of transcription." BMC Genomics.V_{12} article 391.

Moustafa, S. R. (2015). Association of superoxide dismutase, glutathione peroxidase, catalse, and xanthine oxidase with incidence of bladder cancer. Cancer Research Journal, 3(2), 17-27.

Muller, M.(2002). Pyocyanin induces oxidative stress in human endothelial cells and modulates the glutathione redox cycle . Free rad . Biol. Med., 33: 1527-1533.

Muncie , J. R. and Campbell , J. S. (2009). Alpha and Beta- thalassemia **.** Amer. Fam. Physi., 80(40) : 340-344 .

Munira, F.T. ; Begum, S.; Urmi, S.F.H.(2020). Alteration of serum calcium and magnesium level in transfusion dependent thalassemia patients with combined iron chelator therapy J. Bangladesh Soct. Physiol.,15(1): 17-22.

Munoz C, Rios E, Olivos J, Brunser O, Olivares M (2007). Iron, copper and immunocompetence. Br J Nutr 98:24–28.

Murtadha M.K. (2011): Determination of sialic acid and biochemical parameters level in â-thalassemic patients. American Journal of Plant Science 9 (1): 177-183.

Osredkar, J., & Sustar, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. J Clinic Toxicol., 3(2161): 0495-0495.

Panigrahi, I. and Marwaha, R. K. (2007). Mutational spectrum of thalassemia in India **.** Ind. J. Hum. Gen., 13(1) : 36-37 .

Pant, N. C., Tewari, M., Dhoundiyal, R., Pandey, C., Singh, J., & Agrawal, S. (2018). Evaluation of Micronutrients in Fenugreek (Trigonella foenum-graecum L.): A Viable Alternative for Micronutrient Supplementation. Int. J. Curr. Microbiol. App. Sci, 7(5), 2446-2464.

Patne , A.B. Hisalkar , P.J. Gaikwad S.B. and Patil S.V. (2012). International journal of pharmacy and life sciences,Alterations in antioxidant enzyme status with lipid peroxidation in β- thalassemia major patients, 3(10):1-10.

Pavlova, L.E.; Savov, V.M.; Petkov, H.G. and Charova, I.P.(2007). OXIDATIVE STRESS IN PATIENTS WITH β-THALASSEMIA MAJOR .Contributions, Sec. Biol. Med. Sci .,28(1): 145–154.

Pasa, S.; Kargin, F.; Bildik, A.; Seyrek, K.; Ozbel, Y.; Ozensoy, S. (2003). Serum and hair levels of zinc and other elements in dogs with visceral leishmaniasis. Biol Trace Elem Res 94:141–147.

Peters, M. ; Heijboer , H. ; Smiers, F. ; and Giordano , P. C. (2012). Diagnosis and management of thalassemia **.** Brit. Med. J., 344 : 228 .

Piga, A.; Longo, F.; Duca, L.; Roggero, S. and Vinciguerra, T. (2009). High nontransferrin bound iron levels and heart disease in thalassemia major. Am. J. Hematol. 84: 29-33.

Piña-Vázquez, C.; Reyes-López, M.; Ortíz-Estrada, G.; de la Garza, M.;Serrano-Luna, J. (2012). Host-parasite interaction: Parasite-derived and -induced proteases that degrade human extracellular matrix. J.Parasitol. Res, 24. [CrossRef] [PubMed]

Pinto, F. O. and Roberts, I. (2008). Cord blood stem cell transplantation for haemoglobinopathies **.** Brit. J. Hematol., 141(3) : 309-324 .

Platt, O. S. (2008). Hydroxyurea for the treatment of sickle cell anemia **.** N. Engl. J. Med., 358 : 1362-1369 .

References

Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., & Buga, A.-M. (2013). ROS and brain diseases: the good, the bad, and the ugly. Oxidative Medicine and Cellular Longevity .

Porter, J. (2005). Pathophysiology of iron overload **.** Hematol. Oncol. Clin. North Am., 19(1) : 7-12 .

Quek, L. and Thein, S. L. (2007). Molecular therapies in beta-thalassemia**.** Brit. J. Hematol., 136(3) : 353-365 .

Raghuveer, P. Vidya, P.and Prabhu, R. S.(2009). Iron overload in beta Thalasemia—a review, Journal of Bioscience and Technology, 1(1): 20–31.

Raghuveer, P. ;Vidya, P. and Prabhu, R. S. (2009). "Iron overload in Beta Thalasemia-a review," Journal of Bioscience and Technology، 1, (1): 20–31.

Rashidi , **M.; Aboomardani , M.; Rafraf , M.; Arefhosseini ,S.R.; Keshtkar , A. and Joshaghani ,H. (2011).** Effects of Vitamin E and Zinc Supplementation on Antioxidants in Beta thalassemia major Patients. Iran. J. Pediatr., l 21 (1): 8-14.

Rasool, M. ; Malik, A., Jabbar, U. M. l, Begum, I. ; Qazi, M. H. ; Asif, M., Muhammad M.P. Naseer, I. Ansari, S. A. , Jarullah, J. ; Haque, A. and Jamal, M. S. .(2016). Effect of iron overload on renal functions and oxidative stress in beta thalassemia patients. Saudi. Med. J., 37 (11): 1239-1242.

Reshadat, S., Kiani, A. and Iranfar, S.H. (2006). Zinc level of major thalassemic patients in Kermanshah. Behbood.;2(10):157-67.

Roselli, E. A. ; Cesari, R. and Miccio, A. (2006). Gene therapy for Beta- thalassemia : Preclinical studies on human cells **.** Mol. thera., 13(1) : 667 .

Rund, D. and Rachmilewitz, E. (2005) . Beta-thalassemiaRund ,N. Engl. J. Med.,; 353(11):1135-1146.

SPSS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 24th edn., SAS, Inst, Inc. Cary, N.C., USA.

References

Saud, A.M. (2012). Molecular and Biochemical Study on β-Thalassemia Patients in Iraq. Ph.D. Thesis. College of Science. University of Baghdad. Iraq.

Sayani, F. ; Warner, M. ; Wu, J. ; Wong-Rieger, D. ; and Humphrey, K. (2009). Guidelines for the clinical care for patients with thalassemia in Canada **.** Canada : Anemia Institute For Research And Education, Thalassemia Foundation Of Canada (15-44) pp.

Serarslan, G., Yılmaz, H., & Söğüt, S. (2005). Serum antioxidant activities, malondialdehyde and nitric oxide levels in human cutaneous leishmaniasis. Clinical and Experimental Dermatology, Experimental dermatology, 30(3): 267-271.

Shamshirsaz, A. A. Bekheirnia, M. R. Kamgar M. (2003). Metabolic and endocrinologic complications in Beta-thalassemia major: a multicenter study in tehran, BMC Endocrine Disorders, 3 (4) :1-7.

Shazia, Q.; Mohammad, Z. H.; Rahman, T. and Shekhar,H.U. (2012). Correlation of Oxidative Stress with Serum Trace Element Levels and Antioxidant Enzyme Status in Beta-thalassemia Major Patients: A Review of the Literature. Hindawi Publishing Corporation Anemia, 11(55):1-7.

Siems, W. G. (2018). Lipid peroxidation and pharmaceutical drugs. Free Radical Biology and Medicine, 124, 565.

Şimşek, F.; Öztürk G.; Kemahli S.and Lardi, H. (2005). Oxidant and Antioxidant Status in â Thalassemia Major Patients, J Ankara Univ. Fac. Med., 58, 34-38 .

Sinha, A. and Gupta, S.(2018). Lipid peroxidation and its impact on infertility., 10, 2.

Schechter, A. N. (2008). Hemoglobin research and the origins of molecular medicine **.** Blood., 112(10) : 3927-3938 .

Sonet, J., Bierla, K., Bulteau, A. L., Lobinski, R. and Chavatte, L. (2018). Comparison of analytical methods using enzymatic activi-

ty, immunoaffinity and selenium-specific mass spectrometric detection for the quantitation of glutathione peroxidase 1. *Analytica chimica acta*, 1011, 11-19.

Stakos, D. A. ;Margaritis, D. ;Tziakas D. N. and et al., (2009) . Cardiovascular involvement in patients with β-thalassemia major without cardiac iron overload, International Journal of Cardiology, 134(2 207–211.

STULCE, J. M., BIDDLE, C. & VACCHIANO, C.(2019). Low-Flow Domiciliary Oxygen as a Mechanism of Ongoing Oxidative Stress. Respiratory care, respcare. 56(1):8-10.

Sugio S, Kashima A, Mochizuki S, Noda M, Kobayashi K (2009). "Crystal structure of human serum albumin at 2.5 A resolution". Protein Engineering. 12 (6): 439–446.

Sverko, V.; Sobocanec, S.; Balog, T. and Marotti, T. (2004). Age and gender differences in antioxidant enzyme activity: potential relationship to liver carcinogenesis in male mice. Biogerontology; 5:235–242.

Tsamesidis,L. ; Fozza,C. ; Vagdatli, E. ; Kalpaka, A. ; Cirotto, C, , Carmina, M, ; Panataleo, A , ; Turrini, F. ; Grigoriou E, and Lymperaki,E.(2017). Total antioxidant capacity in Mediterranean β-thalassemic patients. Adv Clin Exp Med., 26(5):789–793.

Taher, A. ; Fuad, E. R. ; Hussain, I . ; Suzane, K. ; Adlette, I . and Maria, D. C. (2008). Correlation of liver iron concentration determined by R_2 MRI with serum ferritin in patients with thalassemia intermedia **.** Hematol., 93(10) : 1584-1586 .

Tang, Y.; Choi, E.J.; Han, W.C.; Oh, M.; Kim, J.; Hwang, J.Y.; Park, P.J.; Moon, S.H.; Kim, Y.S.; Kim, E.K. (2017).Moringa oleifera from Cambodia ameliorates oxidative stress, hyperglycemia, and kidney dysfunction in type 2 diabetic mice. J. Med. Food, 20, 502–510. [CrossRef] [PubMed].

Terra, R., Alves, P. J. F., Lima, A. K. C., Gomes, S. M. R., Salerno, V. P., Gonçalves Da-Silva, S. A., & Dutra, P. M. L. (2019). Immunomodulation from moderate exercise promotes control of experimental cutaneous leishmaniasis. Frontiers in cellular and infection microbiology, 9, 115.

Thalassemia International Federation. (2008). Guidelines For The Clinical Management Of Thalassemia **.** 2nd edn., : 3-198 pp .

Traez, M.; Alam, J. and Nath, K. (2007). Physiology and pathophysiology of heme, implication for kidney disease. J. Am. Soc. Nephrol. 18: 414-414 .

Uda, M. ; Galanello, R. ; Sanna, S. ; Lettre, G. ; Sankaran, V. G. and Cao, A. (2008). Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia **.** Proc. Natl. Acad. Sci. USA, 105(5) : 1620-1625 .

Vaculin, S.; Franek M. and Vejrazka M. (2010). Role of oxidative stress in animal model of visceral pain. Neuro. Sci. Lett. 477(2): 82-85.

Valko M., Rhodes C.J., Moncol J., Izakovic M., Mazur M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions, 160 (Issue 1): 1–40.

Wandersee, N. J. ; Lee, J. C. ; Deveau, S. A. and Barker, J. E. (2001). Reduced incidence of thrombosis in mice with hereditary spherocytosis following neonatal treatment with normal hematopoietic cells **.** Blood., 97(12) : 3972-3975.

Weatherall, D. (2001). The Thalassemias *In* "Williams Hematology". B. Beutler, M. A. Lichtman, B. S. Coller, T. J. Kipps and S. Seligsohn (Eds). 6th Ed. New York : McGraw-Hill ,(562–564) .

Weatherall, D. J. and Clegg, J. B. (2001). The Thalassemia Syndromes **.** 4th ed. Oxford, England : Blackwell Sci., 192-236 pp .

Weydert, C. J. and Cullen, J. J. (2010). Measurement of Superoxide Dismutase, Catalase, and Glutathione Peroxidase in Cultured Cells and Tissue. Nat. Protoc. 5(1): 51–66.

William, R. B. (2004).“Zinc and immune system,” in Encyclopaedia of Immunology, , Elsevier, Amsterdam, The Netherlands, 2nd edition, 2515–2516 pp.

Woolf, SH. ; Battista, R. N. ; Angerson, G. M. ; Logan, A. G. and Eel, W. (2003). Canadian force on preventive health care . new grades for recommendations Canadian task force on preventive health care**.** CMAJ., 169(3) : 207-208 .

Yesilipek, M. A. (2007). Stem cell transplantation in hemoglobinopathies **.** Hemoglobin, 31(2) : 251-256 .

Zalba, G.; Fortuno, A. and Diez, J.(2006). Oxidative Stress and Atherosclerosis in Early Chronic Kidney Disease. Oxford University Press .

Zardkhoni,S.Z.; Moghaddam,A.G.; Rad,F.; Ghatee, M.A.; Omidifar,N.; Ghaedi,M. & Etemadfar,P.(2021). Serum Zinc Level in β-Thalassemia Major: A Retrospective Study in Southwest Iran. international journal for hemoglobin research,45(ISSUE 2): 103-106.

Zekavat O.R.; Bahmanjahromi, A.; Haghpanah, S.; Ebrahimi, S. Cohan, N. (2018). The Zinc and Copper Levels in Thalassemia Major Patients, Receiving Iron Chelation Therapy. Journal of Paediatric Haematology/Oncology, 40(3):178-81.

Zhao, J; Huang, Y (2015). "Salivary uric acid as a noninvasive biomarker for monitoring the efficacy of urate-lowering therapy in a patient with chronic gouty arthropathy". Clinica Chimica Acta. 450: 115–20.

Zunszain PA, Ghuman J, Komatsu T, Tsuchida E, Curry S (2003). "Crystal structural analysis of human serum albumin complexed with hemin and fatty acid". BMC Structural Biology. 3: 1-6 .

Appendix(1): Questionnaire that given to thalassemia patients.

Name:……

Application number: .....

Age: .....

Gender: Male Female

Blood transfusion

Do the mother and father have Thalassemia?

Treatment :……

Appendix(2): The ethical paper given to Thalassemia patients to take agree or not to take blood samples

Paper that reveal the agreement of patient before taken the samples as athetics.

Athetics

You are invited (e) to participate in the clinical scientific research that will be conducted at ---------- Please take (j) enough time to read the following information carefully before deciding (j) To participate or not. You can request clarifications or additional information about anything in this form or about this study from your doctor.

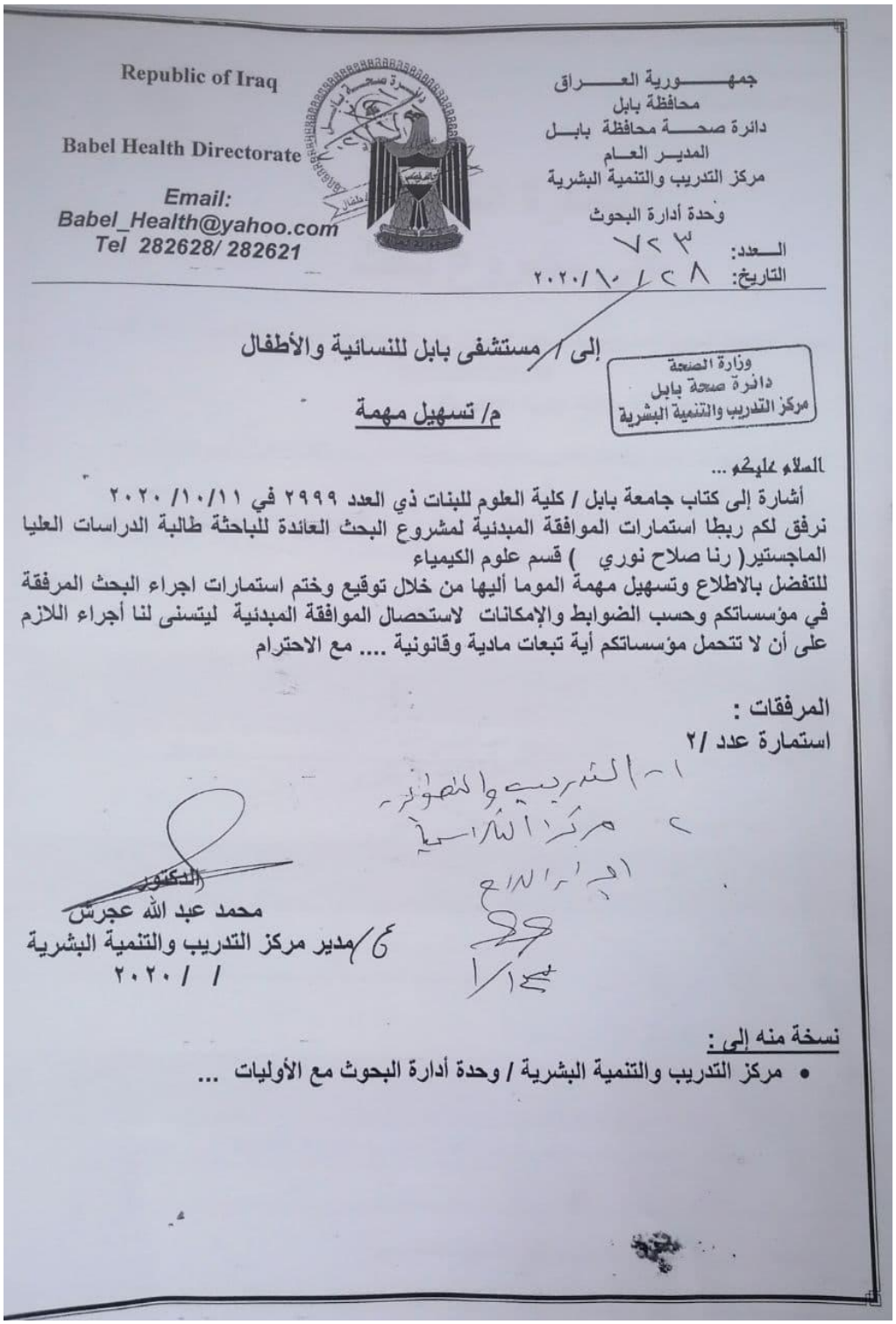
In the event that you agree and accept to participate in this study, your name, address and phone number will remain.

Agree **Not agree**

I have read this acceptance form and know what it is about. All my questions were answered. I authorize the conduct of this study and agree to participate in it,

Subscriber name: Subscriber signature:

Appendix(3): A letter facilitating a task addressed to Babylon Hospital for Maternity and Children (Center for Genetic Blood Diseases).



Republic of Iraq

Babel Health Directorate

Email:
Babel_Health@yahoo.com
Tel 282628/ 282621

جمهـــــورية العـــــراق
محافظة بابل
دائرة صحـــــة محافظة بابـــل
المديـــر العـــام
مركز التدريب والتنمية البشرية
وحدة أدارة البحوث

العـــدد: ٧٢٣
التاريخ: ٢٠٢٠/١٠/٢٨

إلى / مستشفى بابل للنسائية والأطفال

وزارة الصحة
دائرة صحة بابل
مركز التدريب والتنمية البشرية

م/ تسهيل مهمة

السلام عليكم ...

أشارة إلى كتاب جامعة بابل / كلية العلوم للبنات ذي العدد ٢٩٩٩ في ١١/١٠/ ٢٠٢٠
نرفق لكم ربطا استمارات الموافقة المبدئية لمشروع البحث العائدة للباحثة طالبة الدراسات العليا الماجستير(رنا صلاح نوري) قسم علوم الكيمياء
للتفضل بالاطلاع وتسهيل مهمة الموما أليها من خلال توقيع وختم استمارات اجراء البحث المرفقة في مؤسساتكم وحسب الضوابط والإمكانات لاستحصال الموافقة المبدئية ليتسنى لنا أجراء اللازم على أن لا تتحمل مؤسساتكم أية تبعات مادية وقانونية .... مع الاحترام

المرفقات :
استمارة عدد /٢

الدكتور
محمد عبد الله عجرش
م/مدير مركز التدريب والتنمية البشرية
٢٠٢٠/ /

نسخة منه إلى :
- مركز التدريب والتنمية البشرية / وحدة أدارة البحوث مع الأوليات ...

Appendix(4): Approval form for a research project issued by the Iraqi Ministry of Health and Environment.

جمهورية العراق
وزارة الصحة/البيئة

وزارة الصحة العراقية
Iraqi Ministry of Health
Founded 1920

استمارة الموافقة
على مشروع بحث

استمارة الموافقة المبدئية لمشروع بحث يمكن الحصول على النموذج من موقع وزارة الصحة الالكتروني
www.moh.gov.iq

١. عنوان مشروع البحث (باللغة العربية /الانكليزية)

العلاقة بين العناصر النزرة ومضادات الأكسدة في مرضى بيتا ثلاسيميا الكبرى في مدينة الحلة

Relationship between Trace elements and Antioxidant in Major Beta-Thalassemia for patients in Hilla city.

٢. بيانات عن الباحث الرئيسي

الاسم الثلاثي	اللقب العلمي او العنوان الوظيفي	مكان العمل	رقم الجوال	الايميل
رنا طلاح نوري	رئيس مهندسين أقدم	كلية العلوم للبنات / جامعة بابل	٠٧٧٥٠٧٢٤٩٦	alsaegh202@gmail.com

٣. بيانات عن الباحثين المشاركين بالبحث

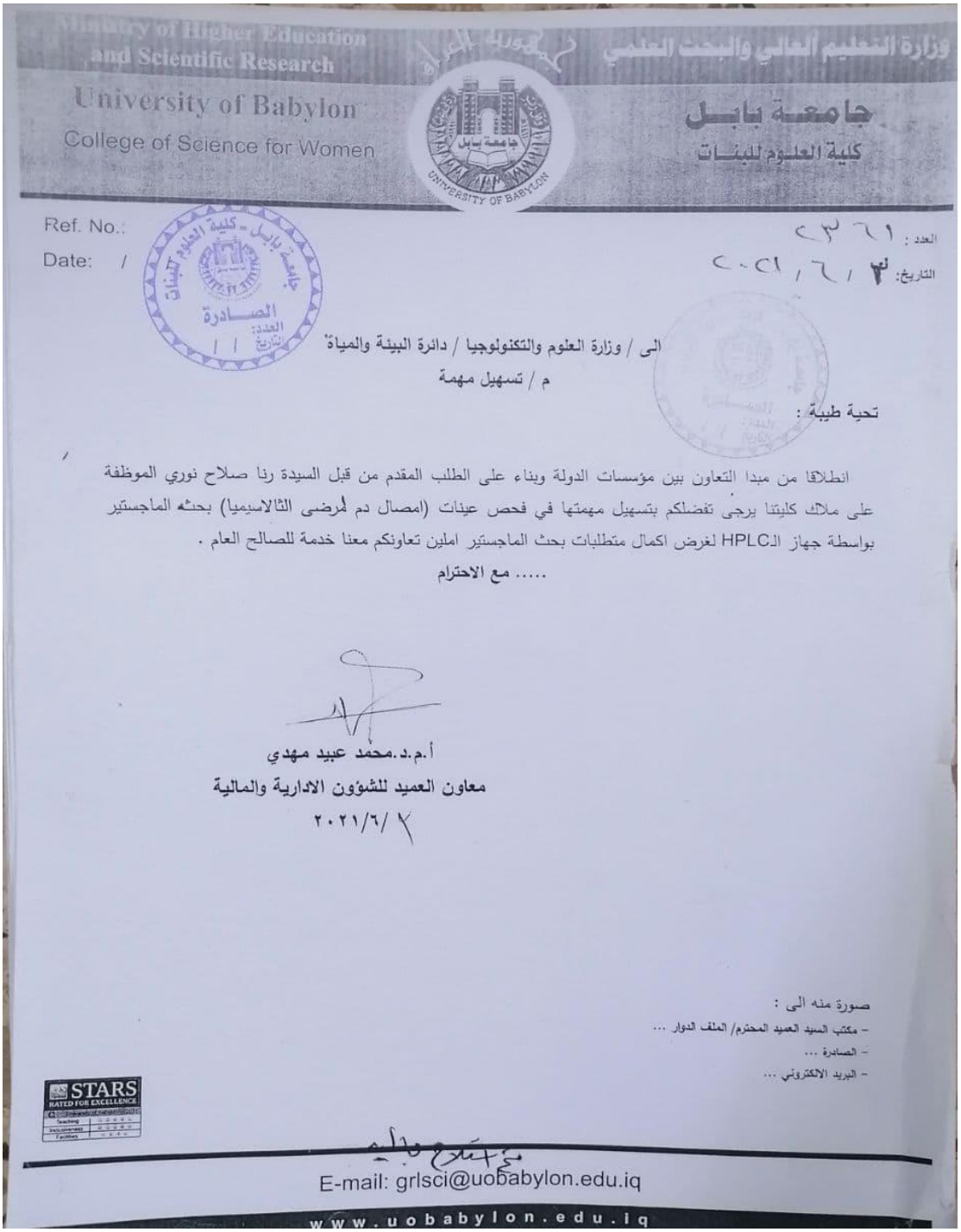
الاسم الثلاثي	اللقب العلمي او العنوان الوظيفي	مكان العمل	رقم الجوال	الايميل

٤. بيانات عن المشرف العلمي ان وجد

الاسم الثلاثي	اللقب العلمي او العنوان الوظيفي	مكان العمل	رقم الجوال	التوقيع
[illegible]	استاذ مساعد	كلية العلوم للبنات	٠٧٨٢٠٧٧٥٤١	

ملاحظة: تملئ هذه الاستمارة الكترونيا ولاتقبل الاستمارة المملوءة يدويا

Appendix(5): A letter facilitating a task to the Ministry of Science and Technology / Department of Environment and Water for the purpose of examining serum samples of Thalassemia patients using the HPLC technique.



Ministry of Higher Education and Scientific Research
University of Babylon
College of Science for Women

وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم للبنات

Ref. No.:
Date: /

العدد : ٤٣٦١
التاريخ: ٣ / ٦ / ٢٠٢١

الصادرة
العدد:
التاريخ: / /

الى / وزارة العلوم والتكنولوجيا / دائرة البيئة والمياة
م / تسهيل مهمة

تحية طيبة :

انطلاقا من مبدا التعاون بين مؤسسات الدولة وبناء على الطلب المقدم من قبل السيدة رنا صلاح نوري الموظفة على ملاك كليتنا يرجى تفضلكم بتسهيل مهمتها في فحص عينات (امصال دم لمرضى الثالاسيميا) بحثه الماجستير بواسطة جهاز الHPLC لغرض اكمال متطلبات بحث الماجستير املين تعاونكم معنا خدمة للصالح العام .

..... مع الاحترام

أ.م.د.محمد عبيد مهدي
معاون العميد للشؤون الادارية والمالية
٢٠٢١/٦/ ٣

صورة منه الى :
- مكتب السيد العميد المحترم/ الملف الدوار ...
- الصادرة ...
- البريد الالكتروني ...

STARS RATED FOR EXCELLENCE

E-mail: grlsci@uobabylon.edu.iq
www.uobabylon.edu.iq

الخلاصة

الثلاسيميا هو فقر دم وراثي ناتج عن طفرات تؤثر على تخليق بروتين الغلوبين في الهيموغلوبين. وينتج عن الثلاسيميا مشاكل صحية عامة جسيمة في أجزاء كثيرة من العالم وخصوصا بلدان البحر الابيض المتوسط والمدن الساحلية . أجريت الدراسة على مرضى الثلاسيميا بيتا العظمى ، بلغ عدد مصول الدم التي تم جمعها (٦٠ (٣٠ ذكور و ٣٠ إناث)) مريض و (٣٠ مجموعة سيطرة) ١٥ ذكور و ١٥ إناث) ، للفئات العمرية من (١-٣٥) سنة. وكان مؤشر كتلة الجسم (BMI) للمرضى بنسبة (١٧.٣١٪) مقابل الأصحاء (١٨.٥-٢٥٪) في مركز الثلاسيميا (أمراض الدم الوراثية) ومختبرات مستشفى الحلة التعليمي للولادة والأطفال، للمدة من تشرين الثاني ٢٠٢٠ حتى اب ٢٠٢١ ، تم قياس المعايير الدموية على هؤلاء المرضى عن طريق فحص دم شامل شمل (كمية الهيموغلوبين Hb ، حجم الخلايا المرصوصة (PCV) ، عدد كريات الدم الحمر (RBCs) ، نسبة حجم الجسم MCV ، الجسم العضلي نسبة الهيموجلوبين MCH ، تركيز الهيموجلوبين في الجسم MCHC ، عرض توزيع الخلايا الحمراء RDW ، خلايا الدم البيض WBCs وعدد الصفائح الدموية PLT ، كل هذه المعايير الدموية أظهرت انخفاضًا واضحًا في مرضى الثلاسيميا ، باستثناء كريات الدم الحمر والصفائح الدموية التي أظهرت زيادة معنوية.

أظهرت نتائج الاختبارات الكيموحيوية أن أعلى متوسط معنوي للزنك عند الذكور والإناث كان (٩٦.١٢١)، ٨٣.١٩٩) ميكروغرام / ديسيلتر على التوالي. في مجموعة المرضى مقارنة بمجموعة السيطرة (١١٩.٣٤٤) ، (١١١.٣٧٦) ميكروغرام / ديسيلتر ، على التوالي. بلغ أعلى متوسط معنوي للنحاس عند الذكور والإناث كان (٢٠٣.٨٠ ، ١٩٧.١٣) ميكروغرام / ديسيلتر. على التوالي ، في مجموعة المرضى مقارنة في مجموعة السيطرة (١٠٥.٠٠ ، ١١٥.٦٧) ميكروغرام / ديسيلتر. وكذلك كان أعلى متوسط معنوي للحديد عند الذكور والإناث (٢٢٤.٤٧٩ ، ٢١٥.٦٣٩) ميكروغرام / ديسيلتر. على التوالي ، في مجموعة المرضى مقارنة في مجموعة السيطرة (١١٣.٤٠ ، ١٠٣.٣٣) ميكروغرام / ديسيلتر ، على التوالي.

أما أدنى متوسط للألبومين في الذكور والإناث فقد بلغ (٤.٠٣٣٨، ٣.٩٣٤) غرام / ديسيلتر. على التوالي في مجموعة المرضى مقارنة في مجموعة السيطرة (٤.٥٠٦ ، ٤.١٠) غرام / ديسيلتر. على التوالي. كما توجد زيادة في حامض اليوريك عند الذكور والإناث كانت (٤٦.٩٦٤) ، (٤٦.٧٧٩) ملغم / لتر على التوالي في مجموعة المرضى مقارنة في المجموعة السيطرة (٣٨.٠٦٦) ، (٣٧.٧٣٣) ملغم / لتر ، على التوالى. على التوالي. في حين تركيز ألالنتوين المكتشف بواسطة تقنية HPLC لمصل المرضى الذي أظهر

قيم عالية عند الذكور والإناث كان (٦١.٥٥٦ ، ٥٦.٩١٦) (ملغم / لتر) على التوالي في مجموعة المرضى مقارنة في مجموعة السيطرة (٢.٤٤٨ ، ١.٤٧٧) (ملغم / لتر.) ، على التوالي.

في حين أن مستوى تركيز القدرة المضادة للأكسدة الكلية (TAOC) في مصل الدم للمجموعات المدروسة. أظهرت النتائج انخفاض TAOC في الذكور والإناث (١٨.٦٨٢ ، ١٤.٤٦٩) (وحدة دولية/مليلتر) ، على التوالي في مجموعة المرضى مقارنة في مجموعة السيطرة (٨٥.٦٦٠ ، ٨٠.٦٦٠) وحدة دولية/مليلتر) ، على التوالي. وكذلك ظهر هناك انخفاض في مستوى تركيز انزيم (SOD) في مصل الدم للمجموعات المدروسة في الذكور والإناث كان ((٩٥.٦٣٣)، ٨٨.٤٢٩) وحدة دولية/مليلتر ، على التوالي في مجموعة المرضى مقارنة في مجموعة السيطرة (٢٠٨.٦٢٣)، (١٩٠.٤١٣) وحدة دولية/مليلتر ، على التوالي .أظهرت نتائج الدراسة الحالية ارتباطها السلبي بجميع العلاقات ما عدا الارتباط التالي كان ارتباطها موجبا معنويا : -

١- الزنك ومضادات للأكسدة الكلية

٢- النحاس ومضادات للأكسدة الكلية

٣- الحديد وحامض اليوريك

٤- الالبومين ومضادات للأكسدة الكلية

٥- آلانتوين و ومضادات للأكسدة الكلية

٦- انزيم SOD مع مضادات للأكسدة الكلية

وهذا يدل على وجود الجذور الحرة المتولدة في مرضى الثلاسيميا ، والتي يتم إبطالها بواسطة بعض الإنزيمات قيد الدراسة ، مثل TAOC و SOD والتي ترتبط مع النحاس والزنك ومضادات للأكسدة الكلية والتي قل تركيزها بالدراسة الحالية ، دليل على استجابتها لهذه الجذور ، بالإضافة إلى بعض الجزيئات الكبيرة مثل الألبومين والجزيئات الصغيرة مثل حامض اليوريك الذي ينخفض تركيزه ويزداد على التوالي نتيجة الأكسدة وتحول إلى مركب آلانتوين.

نستنتج من الدراسة الحالية أن التذبذب في ارتفاع العناصر النزرة والحديد يعود إلى مضاعفات الثلاسيميا ونقل الدم. أما النقص في بعض الجزيئات مثل الألبومين والزيادة في حامض اليوريك فربما يعود ذلك إلى تعرض هذه الجزيئات إلى الجذور الحرة المتكونة نتيجة تكسر كريات الدم الحمر أو نتيجة تكونها بسبب كثرة تحررها من خلايا الدم البيض نتيجة وجود اعتلال وظيفي اذ لوحظ ازدياد اعداد هذه الخلايا بالدراسة

الحالية . واما النقصان في مضادات الاكسدة الكلية (TOAS) وانزيم ال SOD وربما يرجع الى تفاعلها مع الجذور الحرة المتكونة نتيجة الاصابة بمرض الثالاسيميا .

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

العلاقة بين بعض العناصر النزرة ومضادات الأكسدة في مرضى بيتا- ثلاسيميا الكبرى في مدينة الحلة-العراق

رسالة مقدمة الى

مجلس كلية العلوم للبنات / جامعة بابل

وهي جزء من متطلبات نيل درجة ماجستير علوم

في الكيمياء

من قبل

رنا صلاح نوري مرزوك الصائغ

(بكالوريوس علوم ، كيمياء ، بابل ، ١٩٩٨)
(دبلوم عالي ادلة جنائية ،علوم ،علوم الحياة ، بابل، ٢٠١٨)

بأشراف

أ.م.د. محمد عبدالرضا اسماعيل

تشرين الاول 2021 م **ربيع الاول 1443هـ**