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Oxidative stress state and detoxification of un-conjugated bilirubin in patients with sickle cell anemia

A Thesis

Submitted to the Council of the College of Medicine, University of Kerbala, in Partial Fulfilment of the Requirements for the Degree of Master in Clinical Chemistry

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Dedication

To

My Father

My Mother

My Husband

My Family

My Supervisors

The Patients

All my Friends

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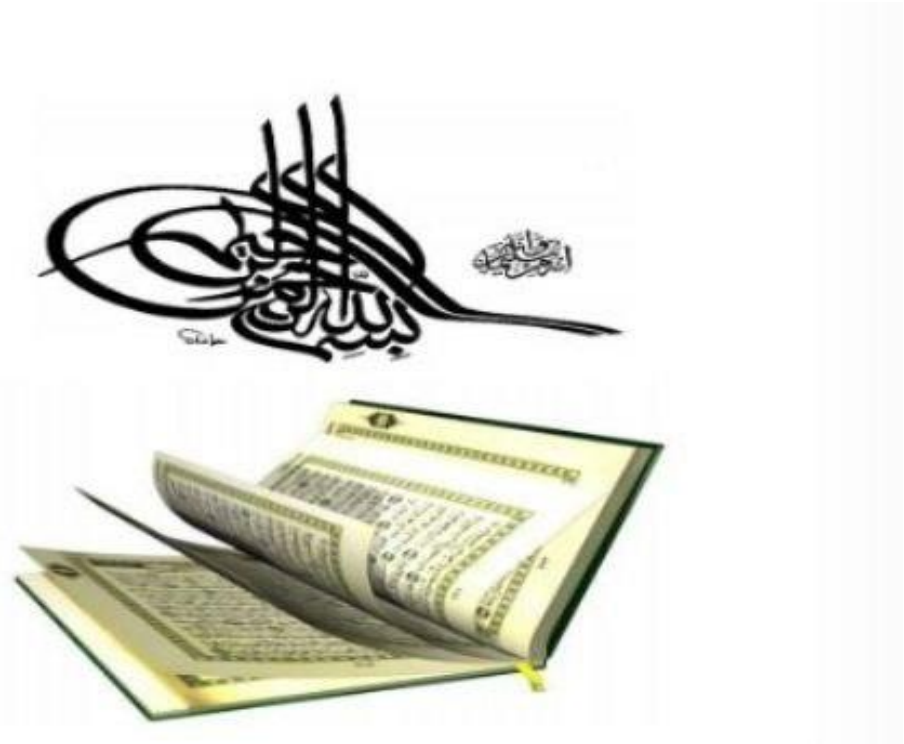

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صدق الله العلي العظيم

(سورة يوسف - الآية 76)

Summary

Sickle cell anemia (SCA) is the most common hereditary disorder of hemoglobin (Hb), which affects approximately a million people worldwide. It is characterized by a single nucleotide substitution in the β-globin gene, leading to the production of abnormal sickle hemoglobin (HbS) with multi-system consequences.

While oxidative stress plays a significant role in the pathophysiology of hemolysis, vessel occlusion, and the subsequent organ damage in sickle cell patients, SCA is also characterized by red cell hemoglobin's propensity to polymerize and transform the red cell from a biconcave disk shape into a sickle shape, resulting in a typical vaso-occlusive episode and accelerated hemolysis, which in turn affects multiple organ systems in the body. The hepatobiliary system in the digestive tract is most frequently impacted by SCA. The spectrum of acute clinical presentations is frequently referred to as sickle cell hepatopathy, and the manifestations range from benign hyperbilirubinemia to overt liver failure.

The objective of this study is to synthesize a new nanocomposite composed of Prussian blue coated magnetite nanoparticles and investigate their capacity to remove precipitate bilirubin.

This case-control study was done between (May, 2022 and Feb., 2023), at Kerbala Teaching Hospital for pediatrics / thalassemia center and the advanced postgraduate laboratories of the chemistry and biochemistry department in the College of Medicine at university of Kerbala.

There were 100 participants with age range between 15-60 years, that were divided into two groups: 50 patients with sickle cell anemia, whose diagnosis was confirmed by a consultant and 50 healthy people who served as healthy controls.

The exanimation serum levels of antioxidants including catalase(CAT), glutathione peroxidase (G-Px) and reduced glutathione (GSH)were detected using a clinical

chemistry analyzer, in addition to measuring the serum level of lipid peroxidation end product malonedialdehyde (MDA) in sickle cell anemia patients.

On the other hand, the chemical co-precipitation method was used to synthesis a Prussian blue-iron oxide nanocomposite that was used as un adsorbent to remove bilirubin because it is a well-documented toxic compound that leads to vessel occlusion and organ damage and through multiple pathways contribute to liver failure diseases. UV Visible Spectrophotometer, zeta-potential, X-Ray diffraction, and transmission electron microscope were used to characterize the nanocomposite.

The results of the study showed a decrease in the levels of serum G-Px and GSH with highly significant differences ($P \leq 0.001$) and an increase in the level of MDA with significant differences ($P \geq 0.05$) in patients compared to the control group; while there are no significant differences in levels of CAT function tests in patients as compared with the control group .

The performance of iron oxide@Prussian blue nanocomposites (Fe_3O_4 @PBNPs) for the removal of bilirubin from blood serum samples were tested in batch mode (pH: 7, the dose of adsorbent: 1mg/mL, and contact time: 30 min, initial concentration: 0.25 mg/mL, Temp. 37.7 ºC) and the result showed the maximum adsorption capacity of bilirubin was 81%.These findings discern the presence of high amounts of precipitate bilirubin in the blood, that may contribute to the development of the spectrum of acute clinical complications including sickle cell hepatopathy. The results of the present work show that Fe_3O_4@PBNPs have great potential to utilize as a nonhazardous and bio adsorbent for effective adsorption of bilirubin in aqueous solution, blood, and patient serum pool.

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List of Abbreviations

Abbreviation	Meaning
AC	Activated carbon
ACS	Acute coronary syndrome
ASC	Auto sample changer
AVN	Avascular necrosis
BMI	Body Mass Index
CAT	Catalase
CBC	Complete Blood Count
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic Acid
DTNB	Dithiobis(2 - nitrobenzoic acid)
GPx	Glutathione peroxidise
GSH	Glutathione
GSTs	Glutathione *S*-transferases
Hb	Hemoglobin

HbS	Hemoglobin S
HPLC	High Performance Liquid Chromatography
HU	Hydroxyurea
IAA	Indole acetic acid
MDA	Malondialdehyde
MNPs	Magnetic nanoparticles
NADH	Nicotinamide adenine dinucleotide+H
NADPH	Nicotinamide adenine dinucleotide phosphate(Reduced)
OS	Oxidative stress
PB	Prussian blue
PBF	peripheral blood smear
PBNPs	Prussian blue nano-particles
PCR	Polymerase Chain Reaction
RBC	Red Blood Cell
RNS	Reactive nitrogen species
ROS	Reactive Oxygen Species
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease

SD	Standard Deviation
SEM	Scanning Electron Microscopy
SOD	superoxide dismutase
TCA	Trichloroacetic acid
TEM	Transmission Electron Microscopy
UDP	Uridine-diphosphate
WHO	World Health Organization
XRD	X-Ray Powder Diffraction
VOC	Volatile organic compound
VOE	Vasoocclusive episode

Chapter One

Introduction and Literature Review

1. Introduction

Sickle cell anemia (SCA) is the most common type of hereditary anemia and genetic disorder worldwide. Cerebrovascular disease is one of its most devastating complications, with consequent increased morbidity and mortality **(Alakbarzade *et al.*, 2023).** Sickle cell hemoglobin (HbS) is an example of a genetic variant of human hemoglobin where a point mutation in the globin gene results in the substitution of glutamic acid for valine at the sixth position of the globin chain (**Mandal *et al.*, 2020)**. Oxidative stress plays a significant role in the pathophysiology of hemolysis, vessel occlusion, and the subsequent organ damage in sickle cell patients. SCD is also characterized by red cell hemoglobin's propensity to polymerize and transform the red cell from a biconcave disk shape into a sickle shape, resulting in a typical vaso-occlusive episode and accelerated hemolysis, which in turn affects multiple organ systems in the body **(Aich *et al.*, 2021).** The hepatobiliary system is most frequently impacted by SCD within the digestive tract. The spectrum of acute clinical presentations is sometimes referred to as sickle cell hepatopathy, and the symptoms vary from benign hyperbilirubinemia to overt liver failure. Iron oxide @prussian blue magnetic nanocomposite possesses both the properties of prussian blue as a good adsorbent as well as iron oxide as a good magnetic material that has high separation efficiency to remove bilirubin that has precipitated by using an external magnetic field.

1.1. Anemia

Anemia is defined as a reduction in red blood cell count (RBC) and/or hemoglobin (Hb) level is below the normal for the respective age, sex, and environment leading to reduced oxygen carrying capacity of blood **(Talukdar *et al.*, 2023).**Human hemoglobin (Hb) is a tetrameric protein consisting of α and β globin chains in

duplicate, HbA ($\alpha_2\beta_2$)as shown in **Fig.(1.1),**the point mutation in the globin chain results in genetic variants of human hemoglobin such as HbS, HbD, HbE and HbJ. Each of these chains is associated with a hemeprosthetic group, atetrapyrrole ring (protoporphyrin IX) containing a central ferrous atom (Fe^{2+}),which can reversibly bind to a molecule of O_2, being, therefore, it is responsible for its transport from the lungs to the tissues**(Lakkakula and Pattnaik, 2021).**

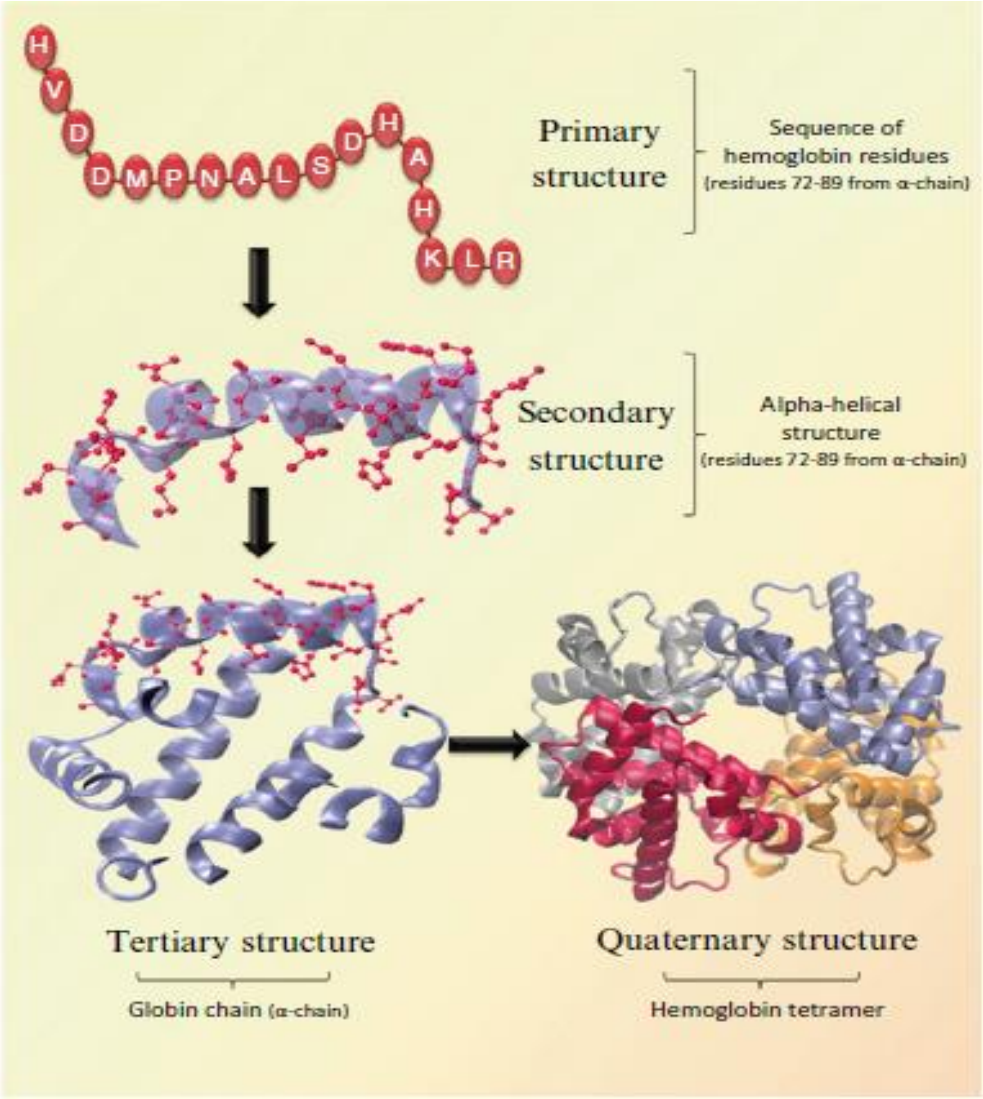


Fig. (1.1): Hemoglobin formation (Abdennour and Abboud, 2016).

According to the World Health Organization (WHO), anemia is defined as hemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men. However, normal Hb distribution varies not only with sex but also with ethnicity and physiological status **(Domenica Cappellini and Motta, 2015).**

1.2. Types of Anemia

Anemia is a condition that may recognize several pathophysiological mechanisms and often is multifactorial**(Milovanovic *et al.*, 2022).**For practical purposes, it can be classified on the basis of either the mean cellular volume , or the underlying pathological mechanism (hypo-proliferative/maturation abnormalities/increased destruction of red cells) or on the patient clinical history (acquired or congenital, acute or chronic) **(Luzzatto *et al*., 2011).**

1.2.1. Nutritional Anemia

a) **Pernicious anemia**: Pernicious anemia is a relatively rare autoimmune disorder that causes diminishment in dietary vitamin B12 (cobalamin) absorption, resulting in B12 deficiency **(Rodriguez and Shackelford, 2022).** Patients with pernicious anemia also have higher incidence of co-occurring autoimmune disorders, including diabetes mellitus type 1, autoimmune thyroid disease, and vitiligo**(Ortet and Vale Costa, 2022).** Furthermore, 5 genome-wide significant signals for pernicious anemia have been identified, providing evidence for genetic risk factors **(Laisk *et al.*, 2021).**
b) **Iron-deficiency anemia**: Iron deficiency anemia is a type of anemia that occurs when there is not enough iron to make the hemoglobin in red blood cells **(Auerbach, 2021)**. The main causes of iron deficiency anemia in adults

are bleeding and conditions that block iron absorption in the intestines. Iron deficiency anemia can be mild or severe **(Elstrott *et al.*, 2020).**

1.2.2. Inherited Anemia

a) **Sickle cell anemia**: Sickle cell anemia(SCA)changes the red blood cells' shape, turning round flexible discs into stiff and sticky sickle cells that blockage blood flow**(Almosfer, 2022),** resulting in artery obstruction cardiovascular complications which are the leading cause of early mortality in patients with sickle cell anemia (SCA)**(Morin *et al*., 2023)**.
b) **Diamond-Blackfan anemia**: This inherited disorder keeps the bone marrow from making enough red blood cells**(Gundry and Sankaran, 2023).**The production of new blood cells is bone marrow's primary job. The bone marrow malfunctions in DiamondBlackfan anemia and is unable to produce enough red blood cells, which transport oxygen to the body's tissues**(Upadhyay *et al*., 2023)**.

1.2.3. Anemia Caused by Abnormal Red Blood Cells

a) **Hemolytic anemia**: In this anemia, the red blood cells break down or die faster than usual **(White, 2018).** The normal lifespan of red blood cells (RBCs) is 120 days. In hemolyticanemia, the period is shortened to a few days due to their destruction. The hemolysis may occur when RBCs are targeted by anti-RBC membrane autoantibodies leading to induction of autoimmune hemolyticanemia**(Al-Kuraishy *et al*., 2022).**
b) **Aplastic anemia**: (AA) is this a rare anemia happens when stem cells in the bone marrow don't make enough red blood cells**(Bhadra and Deb, 2020).**It results in peripheral cytopenia with trilineage bone marrow (BM) aplasia.

Anemia, bleeding, infection and several other clinical symptoms are usually the first presentations of AA. It may occur at any age, however young individuals (age 10–25 years) and the elderly (>60 years) are the most prone**(L. Wang and Liu, 2019).**

1.3. Sickle Cell Anemia

Sickle cell disease (SCD) is the most common genetic diseases, it is an inherited hemoglobinopathy it is first described in 1910 by James Herrick **(Pagare *et al*., 2022)**, where it is caused by a point mutation in the gene encoding the beta globin chain (HBB).It results in an abnormality in the oxygen-carrying protein hemoglobin found in red blood cells. This leads to a rigid, sickle-like shape of red blood cells**(Instructions, 2019)**as shown in **fig.(1.2).**

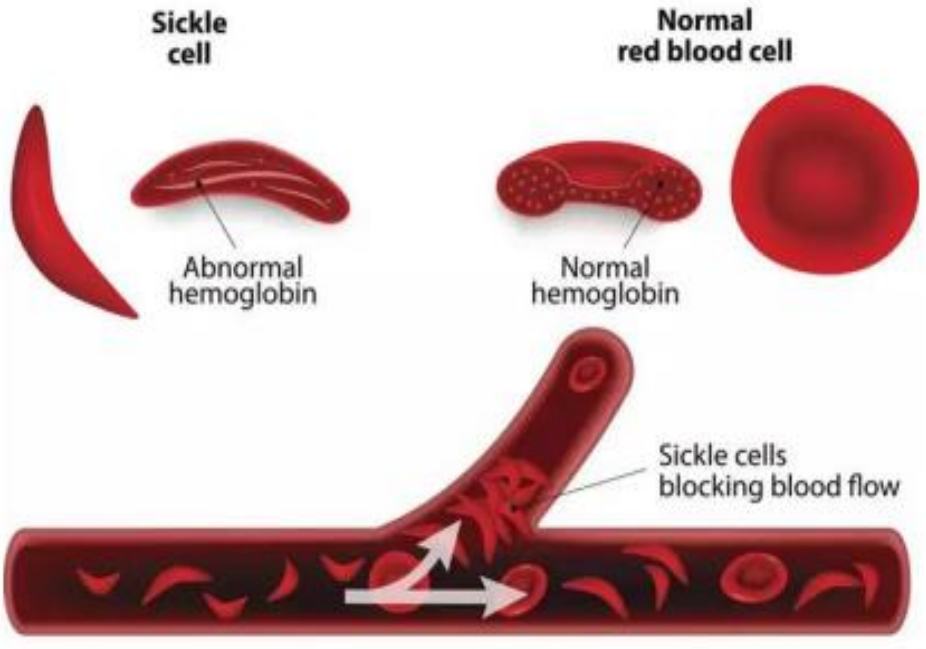


Fig. (1.2): Blood flow of sickle cell (Yeruva *et al*., 2021)

1.3.1. Pathophysiology of Sickle Cell Anemia

The pathogenesis of sickle cell disease revolves around the loss of red blood cell flexibility. The sickling process is cyclical initially, where sickle erythrocytes oscillate between the normal biconcave shape and the abnormal crescent shape **(Kato, Piel, *et al.*, 2018) .**

However, there comes a time when the change becomes irreversible, and the sickle erythrocytes develop a permanent sickle shape increasing the risk for hemolysis and VOC. Low oxygen tension increases red blood cell sickling in sickle cell disease, and repeated episodes of sickling damage the cell membrane and reduce the cell's flexibility as shown in **Fig.(1.3).** When normal oxygen tension is restored, these cells do not revert to their original shape **(Mangla *et al.*, 2021).**

As a result, as these hard blood cells move through tiny capillaries, they are unable to soften, resulting in artery obstruction and ischemia. Because of their form, hemolysis, or the breakdown of red cells, it is the actual cause of the illness's anemia. The bone marrow tries to compensate by producing new red cells, but it can't keep up with the pace of destruction. Healthy red blood cells endure 90–120 days on average, but sickled cells only last 10–20 days **(Talukdar *et al.*, 2023).**

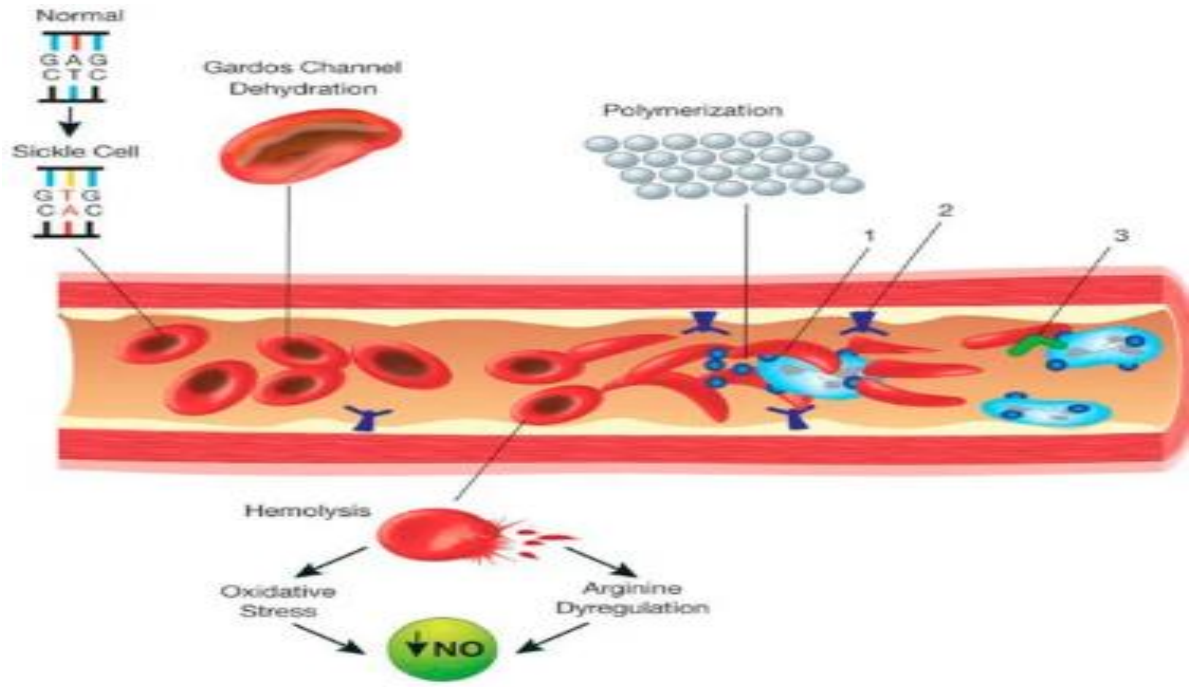


Fig. (1.3):Schematic representation of the pathophysiology of sickle cell anemia (Dosunmu-Ogunbi *et al.*, 2019).

1.3.2. Classification of Sickle Cell Anemia

The most common form of SCA is homozygous HbS, the proportion of which varies depending on the place of origin American Society of Haematology, 2015). The co-inheritance of HbS and HbC, also known as HbSC, it is the second most frequent form of SCD and beta^{+} is known as thalasimia **(Adio *et al.*, 2022).**

1.3.3. Causes of Sickle Cell Anemia

Sickle cell is an inherited disease caused by a single nucleotide substitution mutation in the sixth codon of the betahemoglobin gene that alters the DNA sequence from GAG to GTG **(Mwita *et al.*, 2023).** The mutation changes the sixth amino acid in the beta globin subunit of adult hemoglobin A from glutamic acid to valine, resulting in hemoglobin S (HbS), where a person who inherits just one gene is healthy and

said to be a carrier of the disease **(Cisneros and Thein, 2021)**. A carrier has an increased chance of having a child with sickle cell disease if he or she has a child with another carrier. For parents who are both carrier of a sickle cell gene, there is a 1 in 4, or a 25 % chance of having a child with sickle cell disease**(Ranque *et al.*, 2022).**

1.3.4. Risk Factors

For a baby to be born with sickle cell anemia, both parents must carry a sickle cell gene. Sickle cell anaemia most commonly affects people of African, Mediterranean and Middle Eastern descent **(Naik *et al.*, 2021).** According to data from the World Health Organization (WHO) around 5% of the world's population lives with characteristic genes for haemoglobin disorders, mainly SCD and thalassemia **(Lakkakula and Pattnaik, 2021).**

1.4. Frequency and Distribution of Sickle Cell Gene among Arabs

Geographically, Middle Eastern Arabs can be looked at as the following:

1. The Arabian Peninsula occupying the South West of Asia includes Yemen, Saudi Arabia and other members of Gulf Co-operation Council (Kuwait, Qatar, Bahrain, United Arab Emirates, and Oman).

2. The Northern region of Arabian Peninsula that occupies the North West of Asia and includes Palestine, Jordon, Syria, Lebanon, and Iraq as shown in **Fig. (1.4).**

3. The Arab countries of North Africa that includes Egypt, Libya, Tunis, Algeria, and Morocco**(Steinberg *et al.*, 2009).**

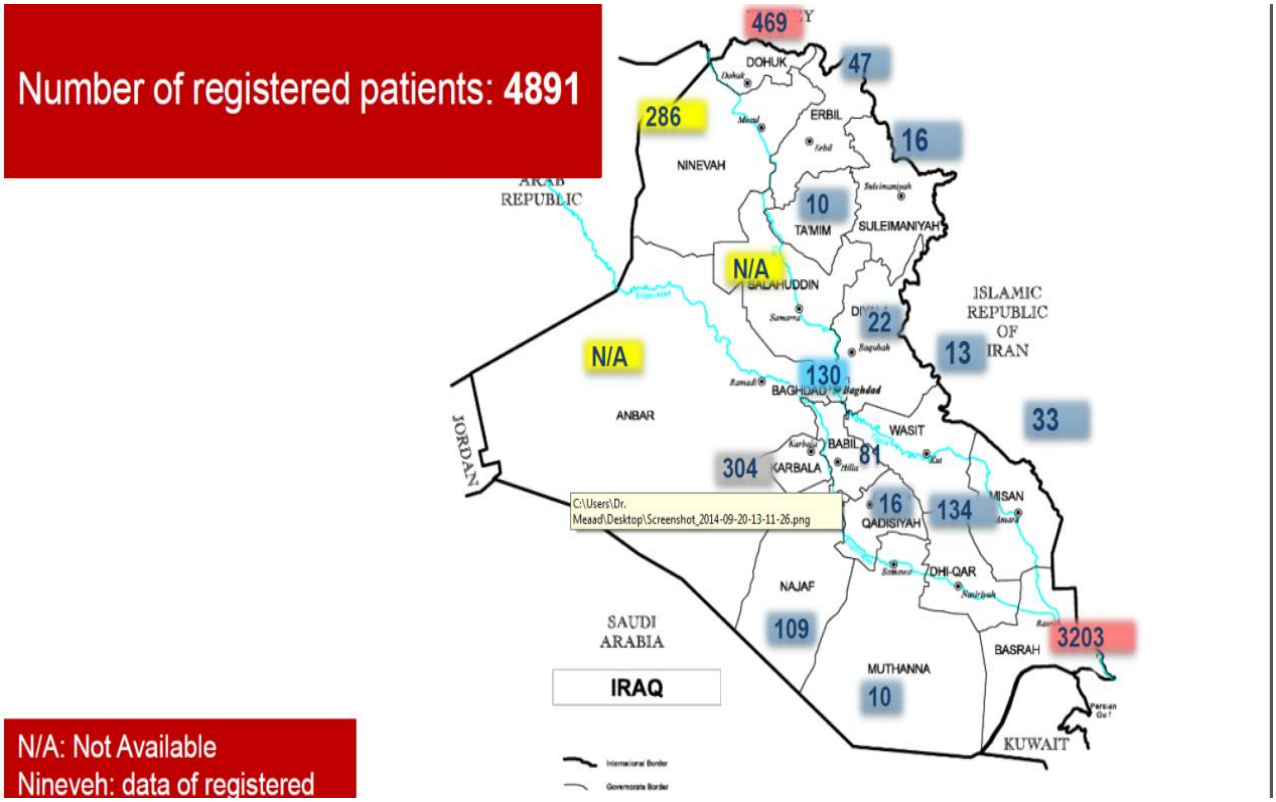


Fig.(1.4): Number of registered SCD patients in Iraq for 2013 (Rassul and Al-musawi, 2020).

1.5. Clinical Symptoms of Sickle Cell Anemia

Signs of sickle cell anemia and symptoms typically begin at the age of 5 months. They differ and alter with time from individual to individual. Symptoms and signs can involve:

1.5.1. Episodes of Pain

The RBC prevents blood supply into narrow blood vessels as called an acute vasoocclusive episode (VOE) as in joints, abdomen, and lungs which is one of the main signs of sickle cells**(Tanabe *et al*., 2019)**,it is a new onset of severe pain that persists for at least four hours for which there is no explanation other than vasoocclusion. VOEs are the primary source of acute pain requiring medical

attention in SCD. They account for most acute pain episodes experienced by patients with this condition. VOEs can be treated with parenteral opioids, ketorolac, or other non-steroidal anti-inflammatory drugs **(Lanzkron *et al.*, 2010).**

1.5.2. Distressing Swelling of Hands and Feet

RBC inhibits the blood supply of hands and feet because of the sickle cell involvement in the body and may pain and swelling from infarctions in the hands and feet**(Platt *et al.*, 1991).** These may be the first SCD-related complications they experience other common locations for VOE pain in patients of all ages include the chest, abdomen, back, and long bones. SCD genotype, older age, and lower fetal Hb levels are risk factors for VOE. Patients may have specific triggers for pain, such as extremes in temperature, dehydration, menstrual cycle changes, or stress **(Hassell *et al.*, 1994).**

1.5.3. Frequent Infections

All SCD genotypes put patients at increased risk for severe infection such as including infection with penicillin-resistant organisms. This increased susceptibility is largely due to splenic and immune dysfunction. Young children with SCA in particular are at elevated risk for pneumonia, septicemia, and meningitis **(Kato *et al.*, 2018).** Incidence of these infections is lower in patients with HbSC and HbSβ+-thalassemia genotypes because splenic function is typically normal or only minimally impaired in infancy. The risk, however, is present in later childhood and adulthood **(Zarrouk *et al*., 2006).**

1.5.4. Vision Problems

RBC prevents sickle cell blood flow through tiny blood vessels. Such cells do not supply the eyes with oxygen, which may contribute to retinal injury. It creates trouble with the vision **(Yeruva *et al*., 2021).**The degree of ocular involvement is not necessarily based on the severity of the systemic disease, where the ocular manifestations in the retina is considered as the most important frequency and visual impairment. Sickle cell retinopathy may affect up to 42% of individuals during the second decade of life **(Al-Jafar *et al.*, 2020)**. The occurrence of retinopathy in patients suffering from severe anemia is well known. The most frequent signs are retinal hemorrhages, soft exudates, retinal neovascularization and tractional retinal detachment, all that may be caused by related to retinal hypoxia**(Lee *et al*., 2018).**

1.6. Clinical Complications of Sickle Cell Anemia

SCD is a condition with mild to severe anemia, painful crises, frequent infections, hand and foot syndrome, and stroke. Some patients require frequent blood transfusions, while others may never need single transfusion. In the severe form, patients may have retarded growth, bone defects, multiple organ dysfunction, and other complications due to frequent transfusion requirements. Patients with mild disease may reach average height and have no multiple organ abnormalities **(Magzoub *et al*., 2022).**

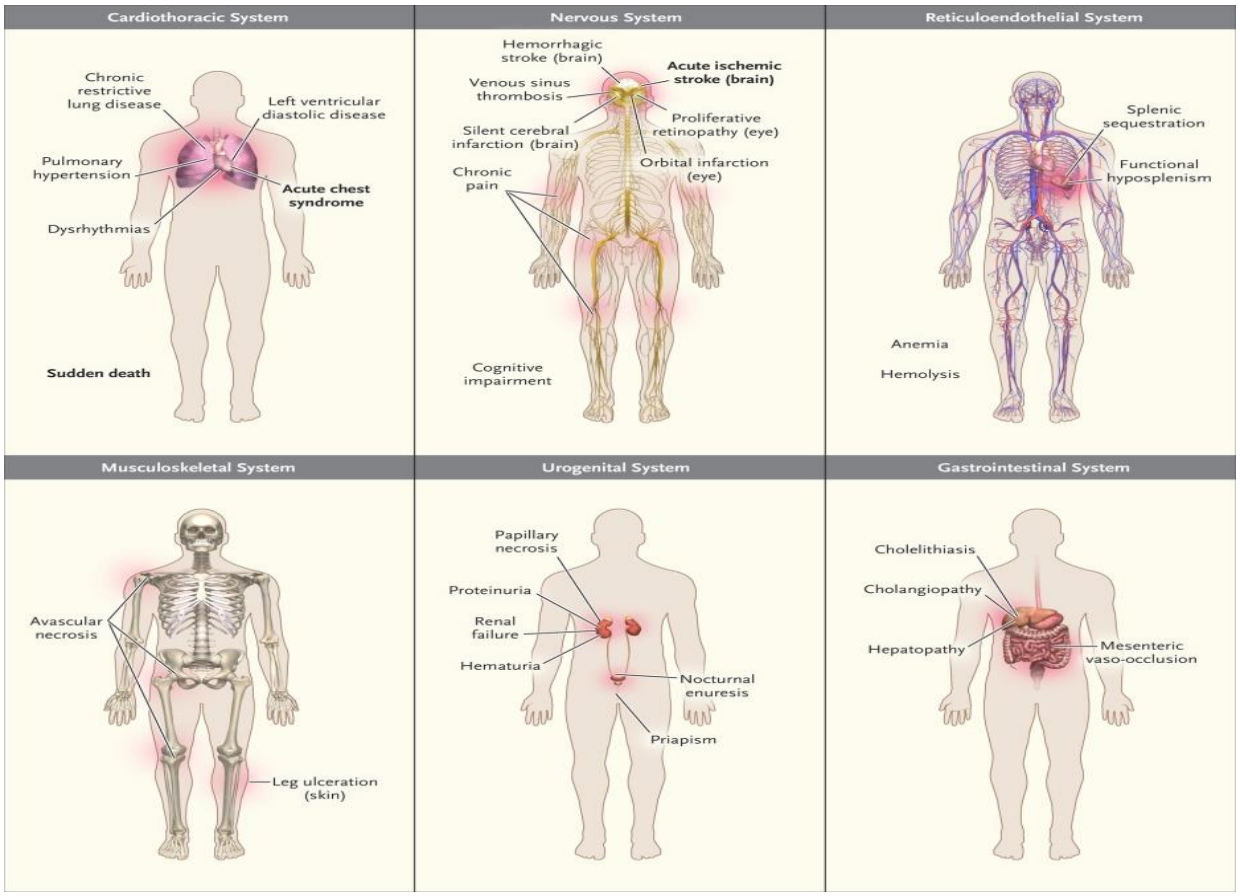


Fig.(1.5): Common Clinical Complications of Sickle Cell Disease(Distelmaier *et al.*, 2020)

1.6.1. Fever

Fever in subjects with sickle cell anaemia is a medical emergency, requiring prompt medical evaluation and delivery of antibiotics because of the increased risk of bacterial infection and subsequent high mortality rate**(Baskin *et al.*, 2013).**

Observation after antibiotic administration is important, because subjects who have sickle cell anemia treated with ceftriaxone can develop severe, rapid, and life-threatening immune hemolysis(**Shankar *et al.*, 2021)**.

1.6.2. Splenic Sequestration

Acute splenic sequestration is a life-threatening complication occurring primarily in infants and young children with sickle cell anemia. Patients with the SC and Sβ+-thalassemia types of sickle cell disease can have acute splenic sequestration events throughout adolescence and adulthood**(Brousse *et al.*, 2012).**

1.6.3. Avascular Necrosis

Avascular necrosis (AVN) occurs at a higher rate among patients with sickle cell disease than in the general population and is a source of both acute and chronic pain and most often the femoral head is affected **(Mahadeo *et al*., 2011).**

AVN of the hip may cause limp and leg-length discrepancy. Other sites affected include the humeral head and mandible. Opioids are often used but usually can be tapered after the acute pain has subsided **(Hernigou *et al.*, 2020).**

1.6.4. Neurologic Complications

Neurologic complications associated with sickle cell disease are varied and complex, ranging from acute ischemic stroke with focal neurologic deficit to clinically silent abnormalities found on radiologic imaging **(Hines *et al.*, 2011).**

Before the development of transcranial Doppler ultrasonography to screen for stroke risk among subjects with sickle cell anemia, approximately 11% experienced an overt stroke and 20% a silent stroke before age 18 yea **(Terrell *et al.*, 2020).**

1.6.5. Pulmonary complications

Lung disease in sickle cell disease patients is the 2nd most common reason for hospital admission and is associated with significant mortality. Acute chest syndrome refers to a life-threatening pulmonary complication of sickle cell disease defined as a new radio density on chest radiography plus any 2 of the following (Fever, Respiratory distress, Hypoxia, Cough, and Chest pain)**(Atagа*et al.*, 2017).**

1.6.6. Pulmonary Hypertension

Cardiopulmonary complications, including cardiomyopathy, diastolic dysfunction, pulmonary hypertension (PH), and sudden cardiac death are the most common causes of morbidity and mortality **(Sachdev *et al.*, 2021).**

Where optimal strategies for screening at risk, patients have not been identified (echocardiogram results are not supported by right-sided heart catheterization results demonstrating elevated pulmonary artery pressures). Attempts to identify targeted therapeutic interventions to alter the natural history of pulmonary hypertension in adults have been unsuccessful**(Mehari *et al.*, 2012).**

1.6.7. Renal Disease

Renal disease among patients with sickle cell disease is a major co morbid condition that can lead to premature death. Sickle cell disease nephropathies have been identified(Nephrotic syndrome, Hyposthenuria, Pyelonephritis, Renal medullary carcinoma) **(Yousefichaijan *et al*., 2016).**

1.6.8. Liver and Gall Bladder Involvement in Sickle Cell Diseases

Liver involvement in patients with sickle cell anemia can have hepatic dysfunction due to acute or chronic viral hepatitis, hemosiderosis from frequent transfusion therapy, hepatic crises related to severe intrahepatic cholestasis, sequestration **(Al-Musawi and Jasim, 2018).**

Sickle cell intrahepatic cholestasis manifests as hepatomegaly, abdominal pain, hyperbilirubinemia, and coagulopathy and they can progress to acute liver failure, leaving transplantation as the only therapeutic option **(Walker *et al.*, 2013).**

1.7. Diagnostic of Sickle Cell Anemia

Several techniques and assays are used for the detection and monitoring of the sickle disease. These techniques can be divided into two main categories: currently used

methods in the diagnosis of SCD; and innovative techniques which are mostly still in the research stage**(Arishi *et al.*, 2021).**

1.7.1. Complete Blood Cell Count

The complete blood count (CBC) is a primary test to characterize the different types of anemia. However, the hemoglobin mutation will affect the hematological parameters, showing a variable change in HbS reveals hemoglobin levels in the 6–8 g/dL range, as well as a high reticulocyte count (as the bone marrow compensates for the destruction of sickled cells by producing more red blood cells) **(Alapan *et al.*, 2016).**

1.7.2. Peripheral Blood Smear

The peripheral blood smear (PBF) is usually done after spotting abnormality in the automation counts and it is considered a landmark of any hematological evaluation. PBF examines the morphology of the blood cell and evaluates any microscopic changes. In sickle cell anemia, moderate to severe an isopoikilocyte is seen with a variable number of elongated sickle cells, which is best observed when the red blood cells are deprived of oxygen .The preparation of these blood smear slides is relatively simple, rapid, and inexpensive **(de Haan *et al*., 2020).**

1.7.3. Hemoglobin Electrophoresis

Electrophoresis is a type of chromatography technique, and it is considered one of the important tests used to detect Hb variants. An alkaline electrophoresis is a diagnostic tool that has been used to detect thalassemia and sickle cell anemia at pH 8.4. First, a hemolysate is prepared from the red blood cells; then, it is added to a cellulose strip and run-in buffer at a constant voltage in an electrophoresis chamber **(Kumar and Derbigny, 2019).**

As a result, the different hemoglobin types with different net charges are separated into various bands depending on their mobility. Hemoglobin electrophoresis can differentiate between HbS and HbC, which are the most clinically significant variants. However, electrophoresis does not distinguish between hemoglobin variants with the same electrical charges and gives the same migration patterns, such as HbD and HbG, which migrate with HbS; HbE and Hb0-Arab have similar migration to the HbC molecules **(Rentapalli *et al*., 2017).**

1.7.4. High Performance Liquid Chromatography

HPLC is documented to separate the hemoglobin fractions as they have different interactions with the stationary phase. HPLC detects different types of hemoglobin based on the retention time and shape of the peak **(Joutovsky *et al.*, 2004).**

Where HPLC is used to detect and quantify HbF, Hb A2, HbS, HbC, Hb barts, and other Hb variants .Developing a fully automated HPLC would be useful in testing a large number of samples accurately. HPLC shows better sensitivity in separation of hemoglobin variants than electrophoresis**(Arishi *et al.*, 2021).**

1.7.5. Genetic Studies

The genetic study is important for the precise detection of the various types of sickle cell disease, based on the detection of β-globin mutations that lead to sickle cell disease development **(Clark and Thein, 2004).**Polymerase chain reaction (PCR) is one of the most powerful diagnostic techniques, where special enzymes are used to amplify specific parts of the genetic materials to millions of copies, using specific primers **(Abdelsalam *et al.*, 2023).**Where digital PCR can be used to determine the genotype of fetuses at risk for sickle cell anemia, the PCR program involves denaturation, annealing, and elongation, which is repeated for 20–40 thermal cycles. Then, the result can be detected by gel electrophoresis, sequencing, melting curve

analysis, or monitoring the change in the fluorescence. PCR sensitivity and specificity have revolutionized the prenatal and neonatal diagnostic field**(Yue *et al.*, 2014).**

1.8. Therapeutic Considerations

1.8.1. Folic Acid

Folic acid is essential for erythropoiesis in SCD patients, as RBC count is lower and folate stores are depleted. Folate intake reduces symptoms of anemia and replenishes depleted folate stores, preventing hyper homocysteinemia **(Patel and Bhaumik, 2019)**

1.8.2. Hydroxyurea

Hydroxyurea (HU) is a myelosuppressive agent that reduces acute pain episodes in adults and infants with sickle cell anemia. A safety feasibility trial showed that HU was safe and well tolerated in children >5 yr of age **(Israa *et al.*, 2016).**

1.8.3.Red Blood Cell Transfusions

RBC transfusions are used to both treat and prevent acute or recurrent complications. Short term transfusions are used to prevent progression of acute complications such as ACS, aplastic crisis, splenic sequestration, and acute stroke, as well as surgery-related ACS **(Merritt *et al.*, 2006and Abboud*et al.*, 2011).**

1.8.4.Hematopoietic Stem Cell Transplantation

The only cure for sickle cell anemia is transplantation with HLA-matched hematopoietic stem cells from a sibling or unrelated donor. However, its use is limited due to lack of suitable donors and decreased application in older patients with morbidity. Myeloablative, HLA-identical sibling transplantation offers

excellent long-term survival rates of 95% and 92%, respectively **(Kassim and Sharma, 2017).**

1.9.Oxidants, Antioxidants and Oxidative Stress

Continuous reactive oxygen species (ROS) production in individuals with sickle cell anemia (SCA) may alter their overall redox status and cause tissue damage **(Aslan and Canatan, 2008)**.Antioxidant defense systems work together to protect against oxidative stress caused by free radical production. Endogenous defense mechanisms are supported by dietary antioxidants, such as vitamin E and ascorbic acid. Vitamin A and C are also important cellular antioxidants**(Gizi*et al.*, 2011).**

1.9.1. Oxidants

Oxygen reduction involves the formation of superoxide anion radicals and hydroxyl radicals, which can occur in alkyl or peroxyl radicals, nitric oxide, and radiation. ROS, RNS, and RSS include free radicals and non-radical derivatives of oxygen, nitrogen, and sulfur that can oxidize substrates under appropriate conditions **(Ali *et al.*, 2020).**

1.9.1.1. Free Radicals

Sickle erythrocytes are the major pro-oxidant sources in SCD, generating two-fold more superoxide, hydrogen peroxide, HO• and lipid oxidation products than HbA containing erythrocytes. This is due to unstable HbS and increased metabolic turnover **(Al-Naama *et al.*, 2015).**

1.9.1.1.1. Reactive Oxygen Species

Oxidative stress (OS) is a disruption in the balance between reactive oxygen species and antioxidant defenses, leading to diseases such as free radical cleavage **(Ge *et al*., 2022).** ROS are unstable, reactive oxygen derivatives created as a by-product of normal metabolic processes. They act as second messengers in cell signaling and are essential for various biological processes in normal and cancer cells**(Chio and Tuveson, 2017).**

1.9.1.1.2. Reactive Nitrogen Species

NO is a free radical with high reactivity with oxygen, superoxide anion, nitrogen derivatives, and transition metals. It can be converted into nitrogen dioxide, trioxide, nitrate, and other RNS **(Lamattina *et al.*, 2003).** ONOO is a stronger oxidant and more stable than O2 and NO, which can modify zinc finger motifs, thiols, and iron-sulfur clusters, leading to a variety of diseases **(Pacher *et al.*, 2007).**

1.9.1.1.3. Reactive Sulfur Species

Reactive Sulfur Species (RSS) is a broad group of sulfur containing reactive species that includes both radical and non-radical sulfur based moieties**(Giles *et al.*, 2017)**.Unlike other reactive oxygen and nitrogen species. RSS are capable of both oxidation and reduction, Sulfur reacts with peptides, proteins, and enzymes, making it an important part of the redox field **(Dhawan, 2014)**.

1.9.1.2. Non-Free Radicals

Non-radical systems consist of PS, carbon catalysts, and electron rich pollutants, making them more selective for electron-rich target organic pollutants. They can also work effectively in a wider pH range, unlike the traditional Fenton process **(Wang *et al*., 2021).**

1.9.2. Antioxidants

Antioxidants are agents and molecules which stabilize or deactivate free radicals. Properties of biologically functional antioxidants include the ability to efficiently scavenge free radicals and chelate redox metals at physiologically relevant levels **(Deshmukh and Gaikwad, 2022).**This definition however was later modified as any substance that delays,prevents or removes oxidative damage to a target molecule to include the molecules that repair the oxidative damage to the system**(Halliwell and Gutteridge, 1995).**

Oxidative/nitrosative stress is caused by a disequilibrium in the oxidant-antioxidant balance, leading to increased reactive oxygen and/or RNS production. Effective antioxidants can delay oxidation reactions and reduce hydroperoxides into stable compounds **(Kurutas, 2015).**

The body's defense mechanisms against oxidative damage involve two main systems: removal of free radicals and scavenging of free radicals by enzymes and electron donors **(Devasagayam*etal.*, 2004)**, (figure 1.6). Antioxidants in foods help to prevent oxidative reactions, but their health effect depends on their systemic bioavailability, concentration, and function **(Ali *et al*., 2020).**

ROS play physiological functions, such as killing infectious bacteria in monocytes and signal transduction of many physiological functions. A balanced balance

between ROS generation and antioxidant systems is necessary for physiological health, preventing oxidative stress**(Yang *et al.*, 2018).**

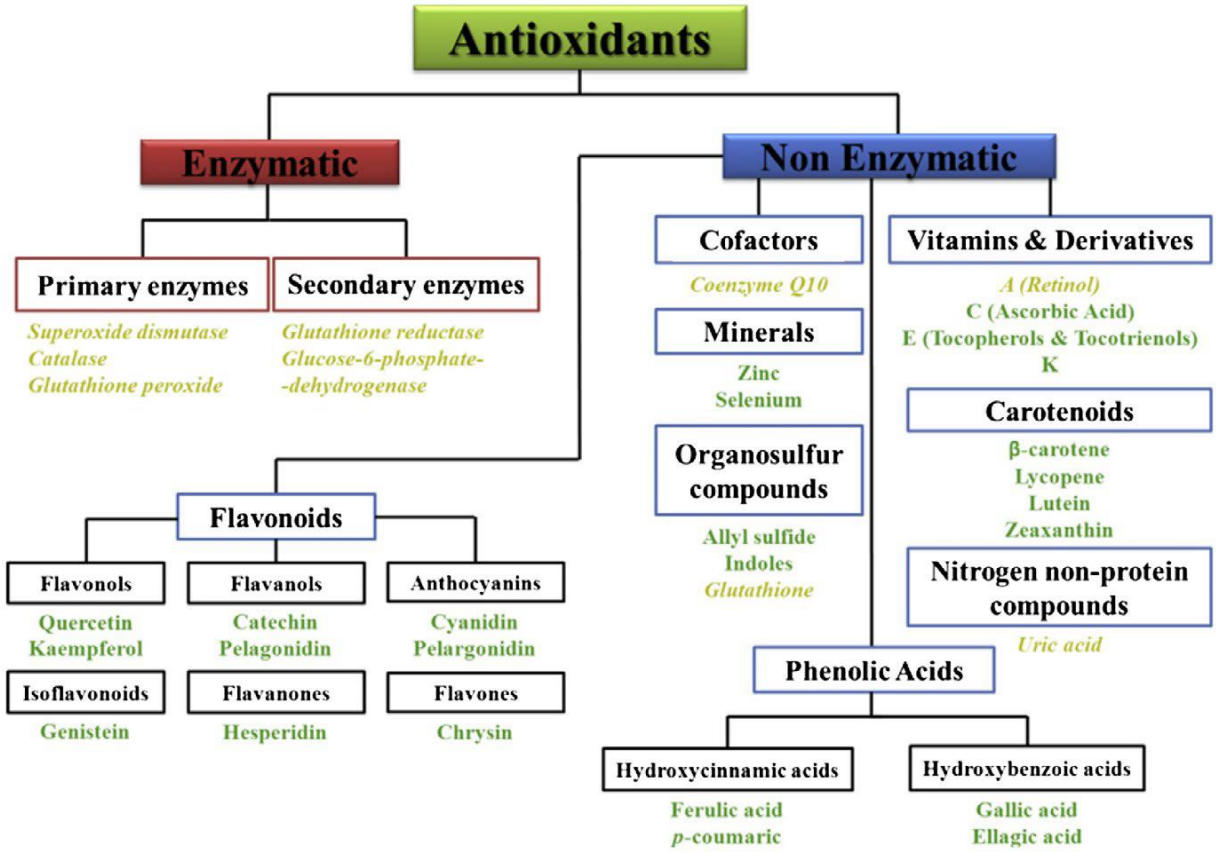


Fig.(1.6):Different classes of antioxidants (Carocho and Ferreira, 2013).

1.9.2.1. Non-Enzymatic Antioxidants

Non-enzymatic antioxidants are synthetic supplements that work as defense against free radicals and non-radical oxidants. The most abundant cellular antioxidant is tripeptide glutathione reductase GSH, which prevents oxidation of thiols groups inside the cell. These antioxidants work as a defense barrier against free radicals and non-radical oxidants, protecting DNA, proteins, lipids, and other molecules from oxidants **(Bansal and Bilaspuri, 2011).**

Where glutathione is the major intracellular non-protein antioxidant and is present in virtually all mammalian tissues, it plays a crucial role in the detoxification of free radicals **(Nobili *et al*., 2005)**. It is found in almost all types of cell compartments: cytosol, mitochondria, and vacuoles at millimolar concentration. GSH scavenges many ROS such as H_2O_2, $O_2^{\bullet-}$and$^{\cdot}$ $HO^{\bullet}$**(Misak*etal.*, 2018)**.

One of the basic role of GSH as an antioxidant is its ability to restore ascorbic acid via the as corbate-GSH cycle. It plays a pivotal role in reducing oxidative stress, maintaining redox balance**(Minich and Brown, 2019)**, enhancing metabolic detoxification as enhance hepatic conversion and excretion of compounds such as mercury and persistent organic pollutants such as precipitate bilirubin in the plasma due to inflammatory diseases of the hepatocyte, or biliary obstruction such as in sickle cell anemia patients**(Roy-Chowdhury *et al.*, 2022).**

1.9.2.2. Enzymatic antioxidants

Human system possesses a battery of enzymes that neutralize the reactive species formed. The important ones include CAT, SOD, and G-Px. The activity of SOD, CAT, and G-Px constitute the first line of antioxidant defense which plays a key role in the total defense mechanisms of the host biological system**(Ighodaroand Akinloye, 2018).**

1.9.2.2.1. Catalase

Catalase (CAT) stands out as one of the most efficient natural enzymes when catalysing the split of H_2O_2 into H_2O and O_2; H_2O_2 is one of the reactive oxygen species (ROS) involved in oxidative stress, a process closely related to aging and several health disorders or diseases**(Rubio-Riquelme*et al*., 2020)**.

Catalase is expressed in all major bodily organs (especially in the liver and kidneys) and in erythrocytes, where it plays an essential role in cell defense against oxidative stress. The importance of the enzyme can be gauged from the fact of its direct and indirect involvement in many diseases and infections. An attempt has been made to correlate the role of catalase with the pathogenesis and progression of oxidative stress-related diseases**(Rakotoarisoa *et al.*, 2019).** A brief account of catalase, its iso forms, structure, reaction mechanism, and its relation with some common important disorders The reaction for which catalase is the best known is the catalytic reaction, in which H_2O_2 oxidizes the heme iron of the resting enzyme to form an oxyferryl group **(Nandi *et al.*, 2019).**

1.9.2.2.2. Glutathione Peroxidase

Glutathione system includes glutathione, glutathione reductase, glutathione peroxidase and glutathione S-transferase. This system is found in animals, plants and microorganisms**(Huang and Zhong, 2012).**G-Pxcatalyses the reduction of organic and inorganic H_2O_2 to H_2O and corresponding alcohols, using GSH as a cofactor**(Birben*et al.*, 2012)**. It plays an important role in the protection of cell membrane polyunsaturated fatty acids, where it functions as a multicomponent antioxidant defence system. The G-Px can be classified into two forms selenium dependent and selenium independent. G-Pxs also play an important role in the detoxification of ROS and it was found to be first enzyme activated under high levels of ROS**(Duggett*et al.*, 2016).**One of the important functions of G-Rx is to maintain the ratio of GSH/GSSG. When the concentration of GSSG (oxidized glutathione) increases inside the cell it often leads to DNA breakage, protein denaturation, and lipid peroxidation**(Zitka*et al.*, 2012).**

1.9.3. Lipid Peroxidation End Products

Oxidative stress has been linked to the etiopathogenesis of several chronic diseases. It can damage specific molecular targets such as lipids, proteins, and carbohydrates, resulting in cell dysfunction or cell death, where is Membranes of sickle shaped erythrocytes are high in polyunsaturated fatty acids, making them more susceptible to endogenous free radical-mediated oxidation**(Damanhouri*et al.*, 2015).** ROS degrade polyunsaturated lipids, forming malondialdehyde (MDA) as a by product which is said to be the biomarker of increased oxidative stress. MDA is an end product of the radical initiated oxidative decomposition of polyunsaturated fatty acids, and therefore, it is a frequently measured biomarker of oxidative stress **(Baliga *et al.*, 2018).**

1.9.4. Oxidative Stress

Oxidative stress has been implicated in the pathogenesis of many chronic diseases including the inflammatory process. Oxidative stress and inflammation are closely related pathophysiological processes and both processes are simultaneously found in many pathological conditions**(Hussain *et al.*, 2016)**. During inflammatory process the activated. Phagocytic cells like neutrophils and macrophages produce large amounts of ROS, RNS, etc. superoxide, H_2O_2, hydroxyl free radical, NO, ONOO, and HOCl to kill the invading agents **(Li *et al.*, 2015).** Under pathological inflammatory conditions , there may be exaggerated generation of reactive species and some of those reactive species diffuse out of the phagocytic cells and thus they can induce localized oxidative stress and tissue injury **(Ahmad & Ahsan, 2020).**

Therefore oxidative stress is an important element in the pathophysiology of SCD. Chronic and systemic oxidative stress in SCD can result via repetitive polymerization and depolymerization of hemoglobin (Hb) in RBC, activated leukocytes, platelets, ECs, and plasma enzymes **(Vona *et al*., 2021).** At subcellular levels, ROS generation by mitochondria is one of the major contributors to elevated oxidative stress in various tissues in SCD. In SCD RBC, free radical production is linked to auto-oxidative unstable HbS, dissociated heme, membrane-bound hemichrome, heme free iron, NADPH oxidase **(Dosunmu-Ogunbi *et al*., 2019).**

1.10. Detoxification of Toxic Compounds

Detoxification is the physiological or medicinal removal of toxic substances from a living organism, including the human body, which is mainly carried out by the liver and where the different tissues detoxify in varying ways **(Pal *et al.*, 2020).**

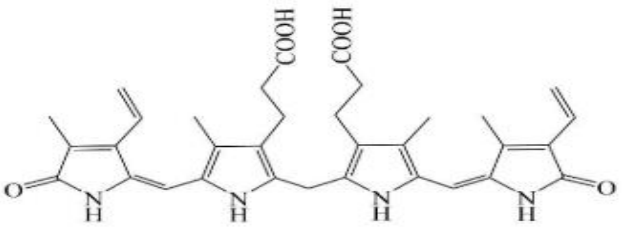
A. **Lungs** can detoxify by removing gases (gas an esthetics are removed from the body by the lungs).
B. **Skin** can detoxify by reducing the penetration of toxic substances (toxins in water don't get in through the skin well; however, toxins in oils do penetrate easily).
C. **Digestive System** can detoxify by eliminating toxic foods, by either vomiting or diarrhea.
D. **Kidneys** detoxify by secreting toxins or filtering toxins out of the blood into urine.
E. **Liver** detoxifies by changing the chemical nature of many toxins.

The choice of which strategy to use for detoxification depend on many factors, involving clinical judgment, the user's personal preference and circumstances,

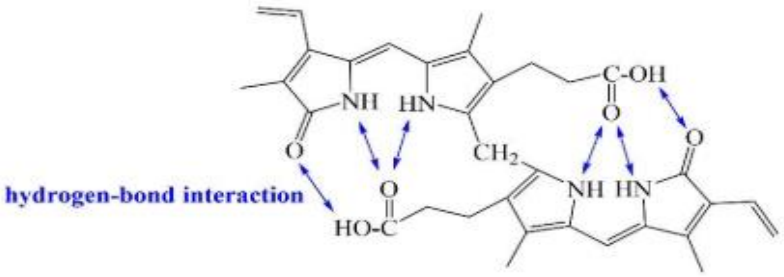
lifestyle and expectations, degree of dependence and concomitant health problems.Detoxification of toxic compound refer to a decrease in biological activity of a drug after is metabolized in the body**(Kanti Das, 2021)**

1.10. Removal of Bilirubin

Bilirubin is a catabolic product of the porphyrin ring, derived from heme.This compound can exist in plasma as bilirubin anion (monoanion or dianion form) or as free bilirubin. Bilirubin dianion binds actively to albumin.Thedianion is not highly soluble in lipid or nonpolar solvents as show in figure (1.8A,B) , but in view of the two polar carboxyl groups and oxidipyrryl (lactam) groups, it is not surprising that the anionic forms of bilirubin are relatively soluble in polar solvents **(Perlman and Volpe, 2017).**



(A) Molecular formula of bilirubin



(B) Molecular configuration of bilirubin

Fig. (1.8): Bilirubin structure.

1.11.1. Bilirubin Metabolism

Normal bilirubin metabolism is considered best in terms of the following sequential events: (1) production, (2) transport, (3) hepatic uptake, (4) conjugation, (5) excretion, and (6) entero-hepaticcirculation .

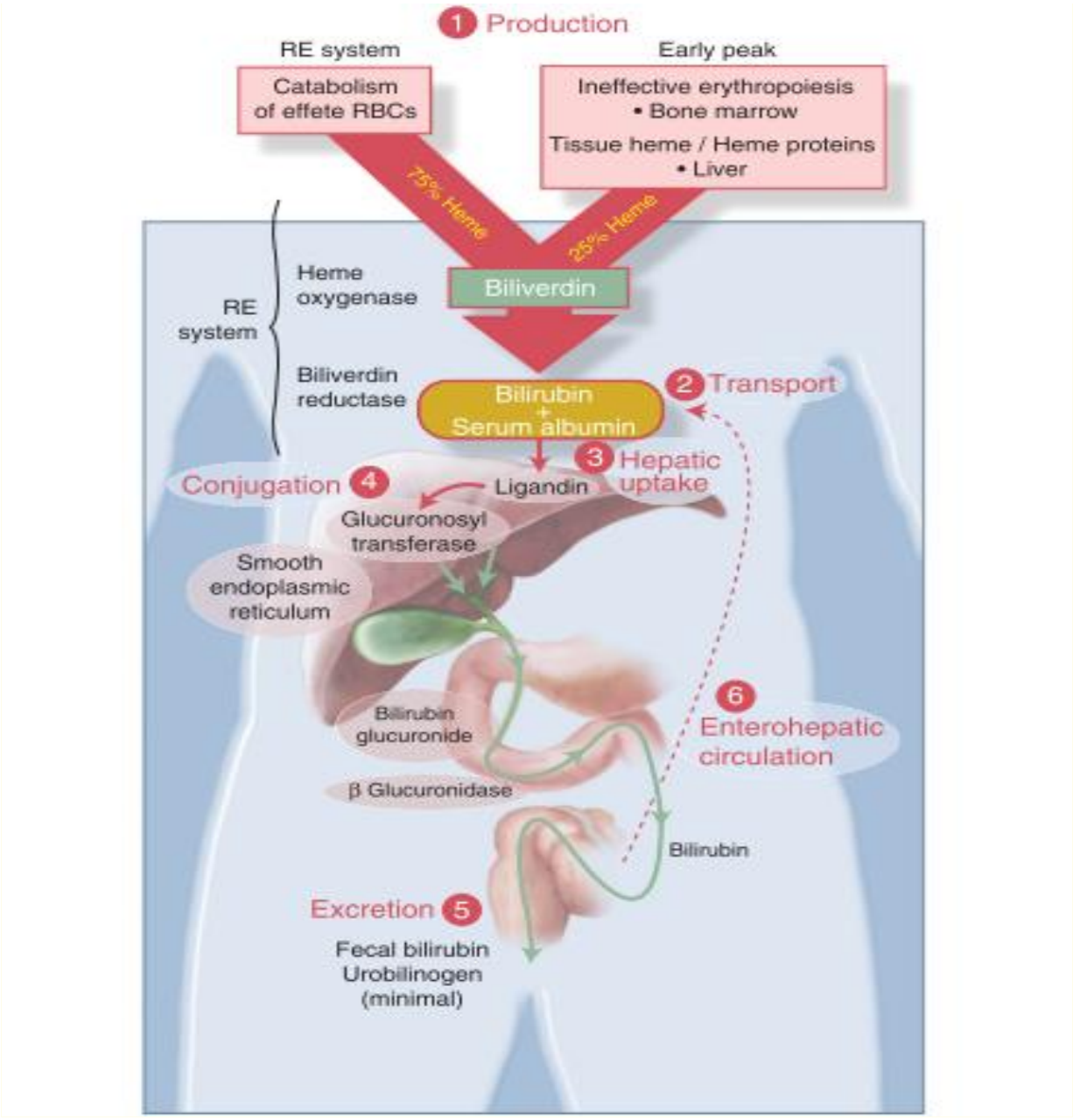


Fig. (1.9): Bilirubin metabolism (Maisels, 2005)

1.11.1.1.Bilirubin Biosynthesis

Bilirubin is the end product of the catabolism of heme, the major source of which is circulating hemoglobin. In the newborn infant, the normal destruction of circulating red blood cells in the reticuloendothelial system accounts for approximately 75% of the daily production of bilirubin**(Rubaltelli, 1993).** The conversion of heme moiety to bilirubin requires two enzymes, hemeoxygenase and NADPPH-dependent biliverdinreductase. 25% of the daily production of bilirubin in the newborn is derived from sources other than senescent red blood cells, with two major components: nonerythropoietic and erythropoietic (Wong et al., 2007)

1.11.1.2. Transportation of Bilirubin

Bilirubin leaves the site of production in the reticuloendothelial system and it is transported in plasma bound to albumin. Human albumin has a single, tight, high-affinity (or primary) binding site for bilirubin and one or more (probably two) weaker, lower-affinity binding sites **(Watchko and Tiribelli, 2013).**

1.11.1.3. Hepatic Uptake of Bilirubin

Hepatocytes have a selective and highly efficient system for removing un-conjugated bilirubin from plasma. This mechanism requires several different organic anion transport proteins. In the hepatocyte, the transported bilirubin is bound to ligandin, a cytosolic protein, that facilitates transfer to the endoplasmic reticulum, the site of bilirubin conjugation **(Cashore, 1998).**

1.11.1.4. Conjugation

Conversion of bilirubin to monoconjugates and diconjugates is carried out primarily by the microsomal enzyme uridine-diphosphate (UDP)–glucuronyltransferase (A1 isoform). The disconjugate accounts for approximately 90% of total bilirubin glucuronide conjugates.

1.11.1.5. Excretion

The conjugated bilirubin is excreted into the bile. Because this event occurs across a concentration gradient, an energy-dependent active transport system is involved. The conjugated bilirubin is then transported to the small intestine, where it is primarily further degraded by intestinal bacteria and excreting in the stool.

1.11.1.6.Entero-hepatic Circulation

Enterohepatic circulation also occurs. Intestinal beta- glucuronidase hydrolyzes the conjugated bilirubin, thus releasing free bilirubin, is then reabsorbed and transported by the portal circulation to the liver **(Perlman and Volpe, 2017).**

1.11.2. Disorders of Bilirubin Metabolism

Increased bilirubin production and abnormalities in any of the four distinct but interactive steps of hepatic bilirubin throughput namely, uptake from the circulation, intracellular binding or storage, conjugation, and biliary excretion may result in hyperbilirubineia. Many clinical disorders, such as hepatitis or cirrhosis, affect multiple steps of this process.

In contrast, in several inherited disorders, a specific step of bilirubin throughput may be involved. From the view- point of bilirubin metabolism, these disorders may be classified into those that cause predominantly unconjugated hyper bilirubinemia and those that are characterized by elevation in levels of both conjugated and un-conjugated bilirubin in plasma **(Roy-Chowdhury and Roy-Chowdhury, 2012).** The extra bilirubin could accumulate and cause mental retardation, hepatic coma, deafness, hearing loss, epilepsy, permanent brain damage, or even result in death in severe cases, and thus various adsorbents for the removal of bilirubin from blood stream have been developed, such as activated carbon (AC), mesoporous silica, bilirubin imprinted particles, nanomaterials**(Chai *et al.*, 2021).**

1.12. Nanotechnology and Nanomedicine.

Nanobiotechnology, which is related to data science and cognitive abilities, is anticipated in opening new doors in the next decades in several fields, including agriculture, the environment, medicine and different commercial sectors **(Odda, Alhaideri, *et al.*, 2022).** Nanomedicine is the application of nanotechnology to medicine **(Boisseau and Loubaton, 2011).** Nanomedicine is defined as the application of nanotechnology to health. It exploits the improved and often novel physical, chemical, and biological properties of materials at the nanometricscale. Nanomedicine has potential impact on the prevention, early and reliable diagnosis and treatment of diseases.

The field of nanomedicine is the science and technology of diagnosing, treating and preventing disease and traumatic injury of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body**(ESF and An, 2005).**

1.12.1. Detoxification by Nanomaterials

Nanomaterials can be classified into carbon-based nanomaterials (such as carbon nanotubes and graphene) and inorganic nanomaterials that include metallic nanoparticles such as metal oxide nanoparticles, including zero-valent iron (ZVI), iron-oxide based magnetic nanomaterial's. These nanocomposites have been extensively researched and are used for the treatment of organic dyes, inorganic compounds, heavy metals from different fluids. In addition, various nanomaterials with different phases have been developedas shown in figure(1-6) **(Yaqoob *et al.*, 2020)**

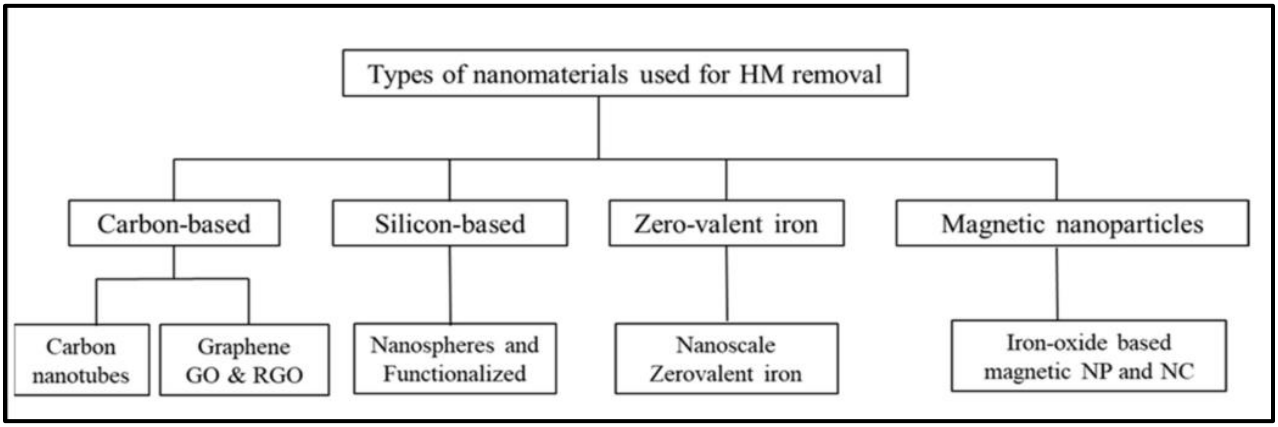


Fig. (1.7):Nanomaterials for heavy metal and toxic compound remediation in aqueous media.

Nanomaterials have been widely applied in industry, commerce, medicine, and environmental remediation **(Cai *et al.*, 2019).**Due to the potential properties of nanomaterials, such as high specific surface, porosity, surface functionalities, ion binding capabilities, and their nano-scale dimensions (ranging from 1 to 100 nm), they show some unique physical, chemical, and biological properties. These properties result in the modification of their structure and specific surfaces **(Theron *et al.*, 2008).**Among these magnetic nanoparticles (MNPs) based adsorbents are the more convenient technologies for removal of heavy metals from the aqueous system**(Dave and Chopda, 2014).**

1.12.2. Blood Purification

Blood purification is an important technology for removing toxins in the blood of patients with liver failure, adult respiratory distress syndrome, and other diseases in clinical settings. As reported, there is an increase in the number of patients that require blood purification in the world. As the most common small molecular toxin for patients with liver failure, bilirubin is a pathogenic toxin that is metabolized from porphyrins. The metabolism disorders of bilirubin may cause yellow discoloration of the skin, called hyperbilirubinemia**(Liu *et al.*, 2017).**

Therefore, the blood purification is not an etiological treatment; it may provide patients with numerous opportunities for organ transplantation or other cases. For example, before and after liver transplantation, the body's toxic substance such as bilirubin and bile acids will extremely increase in short time, which threatens the patient's life seriously. Therefore, detoxification through blood purification therapy is required, which benefits the recovery of organs after surgery **(Ikegami *et al.*, 2012).**

1.12.3. Blood Purification by Nanotechnology

Since filter and stationary adsorbent based extracorporeal detoxification systems are oftentimes mass-transfer limited, magnetic nanoparticle-based blood purification offers an attractive alternative for the efficient cleansing of body fluids**(Lattuada*et al.*, 2016).**Freely accessible nanoparticles uniformly dispersed in the fluid offer an increased surface area for binding and smaller diffusion distances. In extracorporeal blood purification, the residence time of blood in the extracorporeal circuit is typically limited to a few minutes in order to ensure sufficient throughput **(Anthis *et al.*, 2019).**Magnetic separation-based blood purification is especially attractive for

the removal of high molecular weight compounds, which are poorly removed by conventional hemo adsorption.

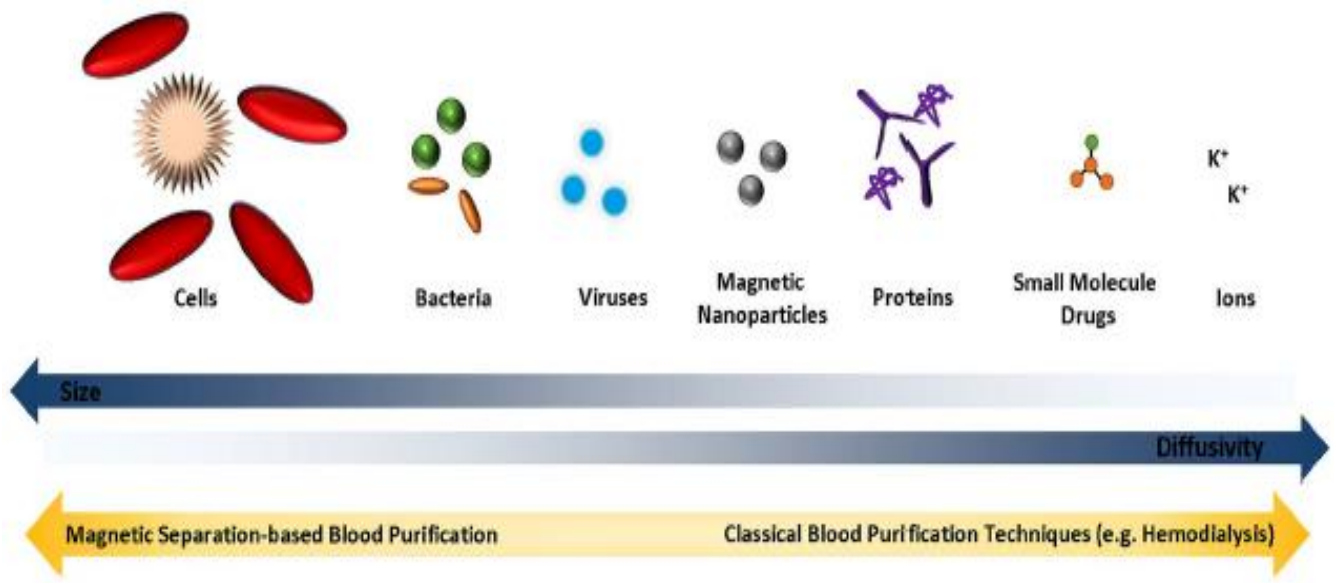


Fig. (1.8): Size and diffusivity of various biologically relevant target compounds for blood purification(Vanholder*et al.*, 2008).

The small size, the high surface-to-volume ratio and the high mobility of nanoparticles allow short diffusion distances and hence increased binding efficiencies even for high molecular weight compounds.

A range of chemically diverse target compounds, including heavy metal ions uranyl ,lead and cadmium ions ,small molecule drugs (diazepam ,proteins bacteria and bacterial compounds) have been successfully removed ex *vivo* from whole blood in the past decade **(Herrmann *et al.*, 2013 and Cai *et al.*, 2011).**

Both charcoal and resin-based cartridges, including anion exchange resins have been successfully used in preclinical and clinical settings for the treatment of liver failure. Recently, the successful removal of bilirubin, a toxic degradation product of hemoglobin, has been achieved by magnetic particle sorbent, nitrogen-doped porous carbon, or magnetic multiwalled carbon nanotubes**(Anthis*et al.*, 2019),**where it

highlights the role of nanoparticles in delivering a chemotherapeutic medication, targeted treatment, and the mechanisms of targeting as in the study**(Alfuraiji *et al.*, 2021).**

1.12.4. Magnetic Nanoparticles.

As a novel method, nanotechnology offers a class of promising adsorbents that are ultra-fine with a large surface area and possess magnetic properties to facilitate efficient separation within a short time by applying an external magnetic field**(Attallah*et al.*, 2016).**During the last two decades, micro and nanoscaled magnetic particles have attracted attention as adsorbents for eliminating biological molecules such as bilirubin, and heavy metal ions from different solutions**(Tamjidi*et al.*, 2019).**It is reported that MNPs provide target specificity and cost-effectiveness as compared to conventional treatment methods**(Odda, Cheang, *et al.*, 2022).**Moreover, the biological synthesis of MNPs is proven to be eco-friendly and aids in sustainable development(**Shukla *et al.*, 2021)**.The major advantage of magnetic nano-materials lies in their easy recovery after exhaustion from the treated solution by applying an external magnetic field**(Vojoudi*et al.*, 2017)** as shown in figure (1-10).

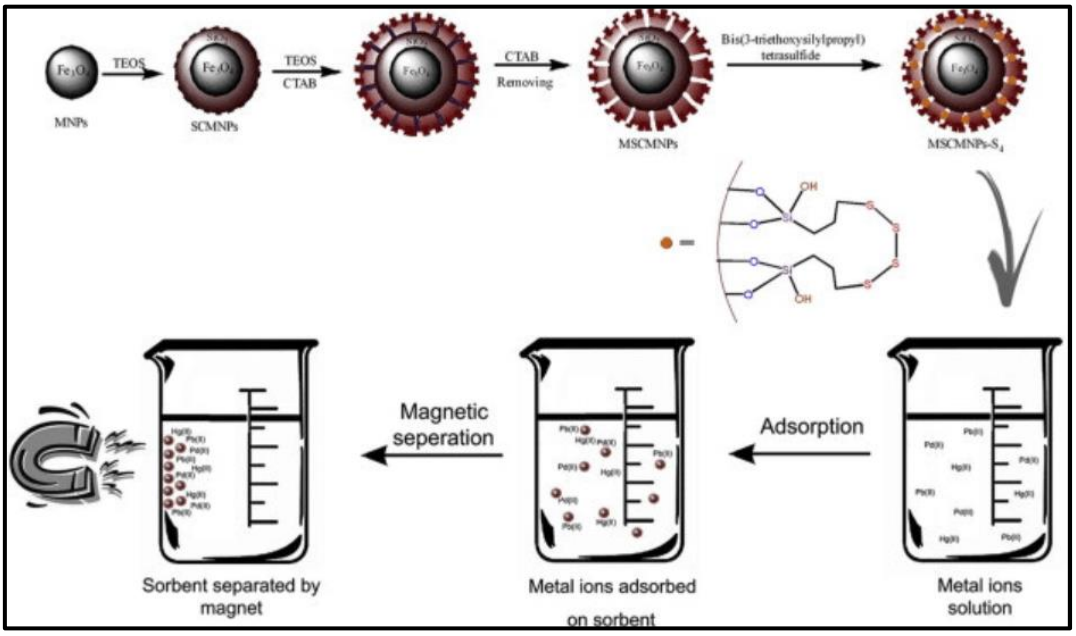


Fig.(1-10): Schematic showing the mechanism of the magnetic nanoparticles in the removal of toxic compound and heavy metals(Vojoudi*et al.*, 2017)

1.12.5. Magnetic Iron-Oxide Nanoparticles

Among the magnetic materials, magnetic iron-oxide nanoparticles (Fe_3O_4) has widely biochemical application due to their biocompatibility, high magnetic susceptibility, chemical stability, innocuousness, high saturation magnetization, and inexpensiveness**(Ganapathe *et al.*, 2020).** There are different experimental methods available for the preparation of Fe_3O_4such as micro-emulsion, thermal decomposition, precipitation, hydrothermal electrochemical process, sol-gel synthesis, sono-chemical synthesis, and laser pyrolysis **(Maiyong *et al.*, 2017).** Among all the synthetic methods, most of the researchers focused on the co-precipitation method for iron oxide because of its less time to prepare, large scale production, simple operation, and good yield.

The properties of Fe_3O_4 are well-known as size, morphology, surface charge status and functional group that have significant impacts on its biocompatibility. Strategies

to improve some properties like biocompatibility, poor biodegradability and chemical instability in a physiological environment rely on surface modification of MNPs**(Janko *et al.*, 2019).**

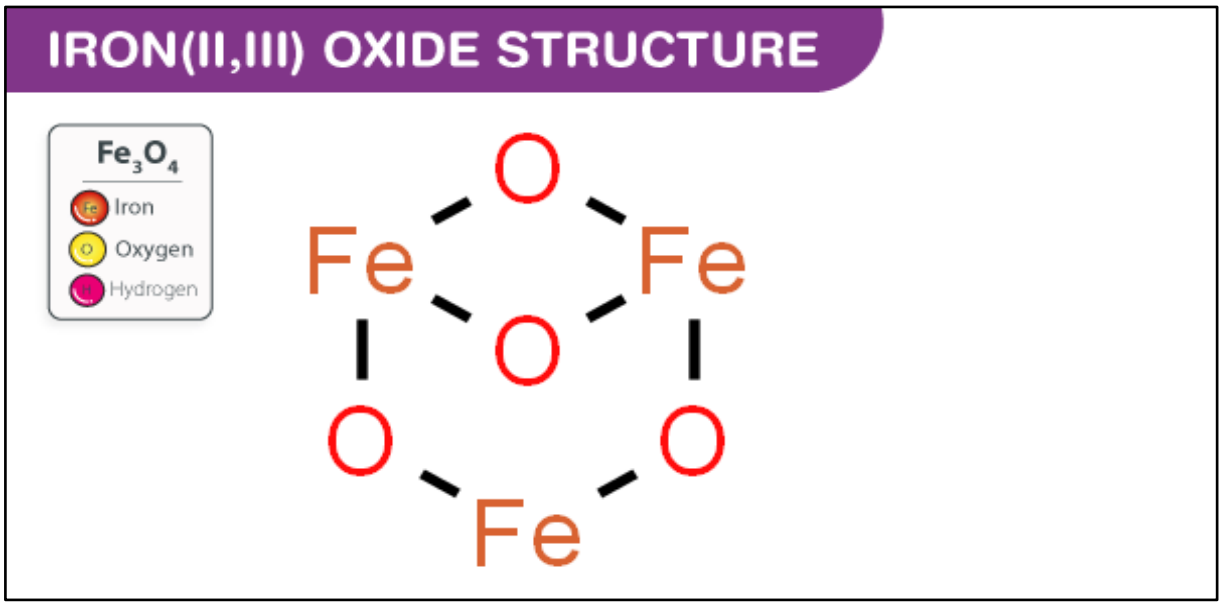


Fig.(1-11): Chemical structure of magnetite iron oxide(Fe_3O_4)(Fe_3O_4 - Iron (II, III) Oxide Structure, Molecular Mass, Properties, Uses, n.d.)

The surface modification of polymers on Fe_3O_4 has had a great impact on higher adsorption capacities toward heavy metal ions. This is achieved by covalently binding MNPs with biodegradable and biocompatible polymers such as polysaccharides and linoleic acid in order to selectively bind to target toxins and then subsequently separate under external magnetic fields**(Mylkie*et al.*, 2021).**

1.12.6. Prussian Blue-Based Iron Oxide Nanoparticles

The Prussian Blue-Based Iron Oxide Nanoparticles was referred as [Fe_3O_4@PB]. The Prussian Blue (PB) is defined as Berlin blue, was discovered by the Berlin artist Diesbach during the early 18th century and was the first pigment obtained

synthetically. A mixed-valence iron (II and III) hexacyanoferrate with an empirical chemical formula $Fe4[Fe(CN)_6]_3$ .x H_2O **(Li *et al.*, 2019)**.The blue color of PB results from a charge transfer (CT) transition of intervalent electrons from iron (II) to iron (III). Depending on the exact stoichiometry and conditions of preparation, PB can be divided into insoluble PB ($FeIII_4[FeII(CN)_6]_3$ .x H_2O, where x = 14–16) and soluble PB (AFeIII $[FeII(CN)_6]\cdot xH_2O$, where x = 1–5 and A is a monovalent cation, such as K^+, Na^+ or NH^{4+}). Soluble PB contains K+ inclusions that can dissociate in aqueous media, leading to net negative ionization on the crystal surface that allows a stable dispersion to be formed if the crystals are sufficiently small**(Busquets and Estelrich, 2020).**

Insoluble PB presents some defects because of interstitial water molecules, which can be divided into two types according to their coordination: water molecules coordinated to Fe (II) sites (coordinative water) and those inside cavities that do not coordinate to metal sites (zeolitic water) **(Li *et al.*, 2019).** For soluble PB, alkali metal ions replace water molecules in the cavities to balance the charge. Prussian blue is assembled into nano-sized architectures through cyano-bridged ligands: Prussian blue nanoparticles(PBNPs) ,were have interesting properties, including solubility, stability, flexible molecule structure, porosity , and adjustable physical and chemical properties **(Guari and Larionova, 2019**).Prussian blue nanoparticles have been widely used in biomedical fields, such as targeted drug delivery. It becomes an ideal molecular carrier for diagnosis and therapy to improve the efficiency of drug delivery for targeted therapy by adjusting its morphology **(Qin *et al.*, 2018).**Moreover, the excellent biosafety and biocompatibility of PBNPs provide a strong basis for its application as a nano drug carrier and imaging agent**(Z. Wang *et al.*, 2020).** In 2003, the US Food and Drug Administration approved PBNPs as a treatment for acute and chronic Thallium poisoning ,which will have good prospects

in the development of nano drugs for disease diagnosis and treatment **(Li *et al.*, 2022**).Due to its good solubility in water, its use approaches the greener synthesis of Fe_3O_4**(Rana *et al.*, 2019).**The ferricyanide complex of $K_3[Fe(CN)_6]$ or $K_4[Fe(CN)_6]$ ions is usually used as the precursor, and Fe^{3+}or Fe^{2+} ions can be slowly released in acidic solution, either reduced or oxidized to Fe^{2+}/Fe^{3+}. Compared with the two-precursor synthesis, the reaction time of the single precursor is longer. In the double precursor synthesis process, the equal molar amount of an iron salt (Fe^{3+}/Fe^{2+}) with an alkali hexacyanoferrate $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ is directly mixed to form the PBNPs. The advantages of this synthesis method are short reaction time without requirement of reducing agent, but its dispensability, stability and morphology control need to be further improved **(Li *et al.*, 2022).**

Recently, iron oxide-based composites have been getting a lot of attention because they can be used to make a lot of superparamagnetic hybrid nanoparticles in a simple one-step process. Their superparamagnetic properties offer the possibility of using them for targeted delivery of active molecules as well as adsorbents because of the ease of separation under the influence of a magnetic field **(Bustamante-Torres *et al.*, 2022).**

Fig. (1-12):Preparation of PB encapsulated Fe_3O_4 NPs. (Fu *et al.*, 2014).

Aims of the study:

The main objectives of this study were indicated below:

1.Evaluation of some biomarkers such as Catalase activity(CAT) ,glutathione reductase activity (GSH) as protective factors agnist the effect of risk sickle cell anemia.

2. Studying the correlations between various data obtained.

3.Studying the effectiveness of Prussian blue dye -coated magnetite iron oxide for removing precipitate bilirubin from the patient serum pool with the maximum adsorption capacity of bilirubin was 81%.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Study design

A case-control study was conducted from May, 2022 to Feb., 2023 at Kerbala Teaching Hospital for pediatrics, Kerbala Medical City, Kerbala Health Directorate / Kerbala - Iraq and the advanced postgraduate laboratories of the chemistry and biochemistry department in the College of Medicine at University of Kerbala / Kerbala - Iraq.

2.2. Subjects

2.2.1. Patients

Fifty participants were diagnosed with Sickle cell anemia, with an age ranging from 15 to 60 years were included in this research. The samples were collected from the inherited hematological center in Kerbala Teaching Hospital for pediatrics . The following information was obtained from patients and labeled with precise details such as (name, age, height, weight, body mass index, smoking, family history, sporting time of sample collection).

2.2.2. Apparently Healthy Control

Fifty people with age ranged between 15 - 60 years whom apparently healthy and selected as the control group according to inclusion criteria from the general population who’s attended the hospital for a checkup and from the attendants of patients.

2.2.3. Approval of the Ethical Committee

The protocols of the study were approved by the ethical committee after a verbal written informed consent for participation and for taking the blood for investigations from everyone enrolled in this study.

2.3. Materials

2.3.1. Chemicals and Diagnostic Kit

All chemicals and kits that were used in this study are listed in table (2-1).

Table: (2-1): Chemicals and diagnostic kits used with their origin.

No.	Chemicals and Diagnostic Kit
1	Ammonium metavanadate
2	DTNB reagent
3	EDTA
4	EDTA- Na_2
5	Hydrogen peroxide
6	Na_2HPO_4
7	Phosphate buffer
8	Reduced giutathione
9	Sodium azide
10	Sodium phosphate buffer
11	Sulfuric acid
12	Trichloroacetic acid (TCA)
13	Tris EDTA buffer
14	Tris-Hcl

15	Barium-sulfate
16	Fe Cl_3
17	Ionized water
18	Iron oxide
19	$K_4[Fe(CN)_6]$
20	Prussian Blue
21	Tetramethoxyproane
22	Thiobarbituric acid (TBA)
23	Total bilirubin kit

2.3.2. Apparatuses and equipment

Table (2-2) shows the apparatuses and equipment's that were used in this study.

Table (2-2): Apparatuses and equipment

No.	Apparatuses and Equipment	Company	Origin
1	Balance sensitive	A and D	Japan
2	Centrifuge	Hettich	Germany
3	Deep freezer	Fisher Scientific	USA
4	Distillator (Water distiller)	Gfl	Germany
5	ELISA instrument system	Biotek	USA
6	Mechanical stirrer	Labtech	Korea
7	Micropipettes	Bioasic	Canada
8	Oven	Binder	USA
9	PH meter	Inolab	Germany
10	Refrigerator	Concord	Lebanon

11	Ultra-sonication bath	Labtech	Korea
12	UV-spectrophotometer	Shimadzu	Japan
13	Vortex mixer	Gemmy	Taiwan
14	Water bath	Memmert	Germany

2.3.3. Glassware

The glassware used in this study is listed in table (2-3).

Table (2-3): Glassware used in the study

No.	Glassware	Manufactured by	Origin
1	Conical Flask 10mL+500mL	Glassco	China
2	Disposable Plain Tube 10mL	Arth AL-Rafidin	China
3	Watch glass	Arth AL-Rafidin	China
4	Gel Tube 6mL	Arth AL-Rafidin	China
5	Hitachi Cups	Arth AL-Rafidin	China
6	Pipette Tips	Arth AL-Rafidin	China
7	Syringe 5mL	Arth AL-Rafidin	China

2.4. Methods

2.4.1. Sample collection, preparation, and analytical methods

Five milliliters of venous blood were obtained by vein puncture using 5mL disposable syringes from each individual (patient and healthy control). A blood sample was placed in a gel tube and allowed to clot on the bench for 20 min, then centrifuged for 10 min to get the serum. Then it was separated into three eppendrof tubes. These eppendrof tubes with serum were tored at -20 ˚C until they were used

for assays of biomarkers by using the multiplayer reader technique used to analyze biochemical tests such as (CAT, GPX, MDA, GSH) and total bilirubin

2.4.2. Measurement of Body Mass Index

The body mass index (BMI) is the metric currently in use for defining anthropometric height/weight characteristics in adults and for classifying (categorizing) them into groups as shown in table (2-4) according to WHO. The following equation was used to calculate body mass index (BMI), **(Kuang *et al.*, 2022).**

BMI, in kg/m^2=Weight (kg) / Height in $(meter)^2$

Table (2.4): The category of BMI in adult

BMI (Kg/m^2)	Weight status
<18.5	Abnormal weight
25	normal weight
30	Obesity

2.4.3. Biochemical Tests

2.4.3.1. Assay of Reduced Glutathione

More than one type of analytical methods used to determine serum glutathione (GSH) depends on the action sulfhydryl methods include photometric .

Principle:

Dithiobis (2 - nitrobenzoicacid) (DTNB) is adichromogen that is readily reduce by sulfhydryl groupof GSH to produce an intensely yellow Compound. Reduce chromogen has maximum absorbance at 412 nm and is directly proportional to GSH concentration, Fig (2-1)

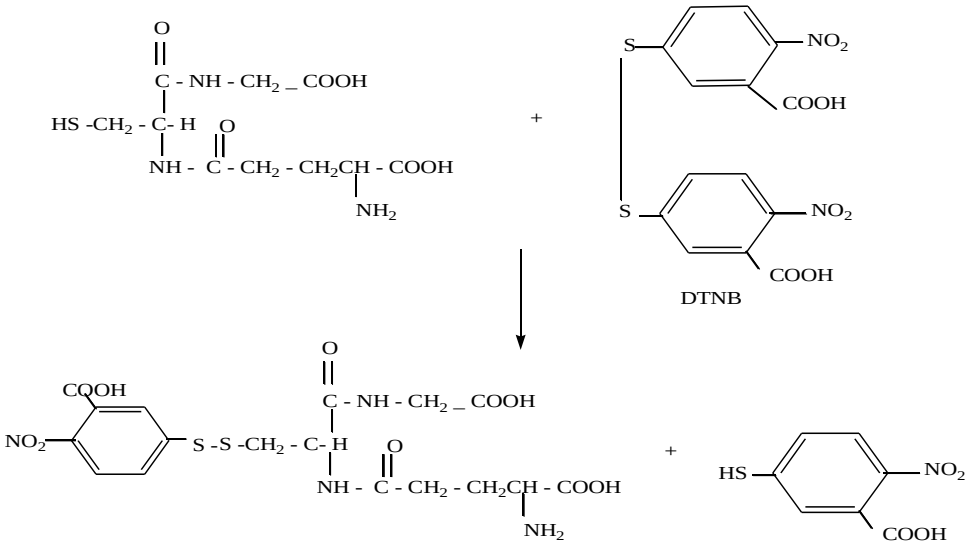


Fig. (2.1): Reaction between GSH and DTNB

Procedure

Sample GSH was determined by using a modified procedure utilizing Ellman's reagent (DTNB), which is summarized as the following. Duplicates of each standard and sample test tubes are prepared then pipette into test tubes.

Reagents	Sample µL	Reagent black µL	Standard µL
Sample	100	-----	-----
Standard	-----	----	100
DDW	800	900	100
TCA	100	100	100

The concentration of GSH is obtained from the calibration curve in µM (fig.2-2)

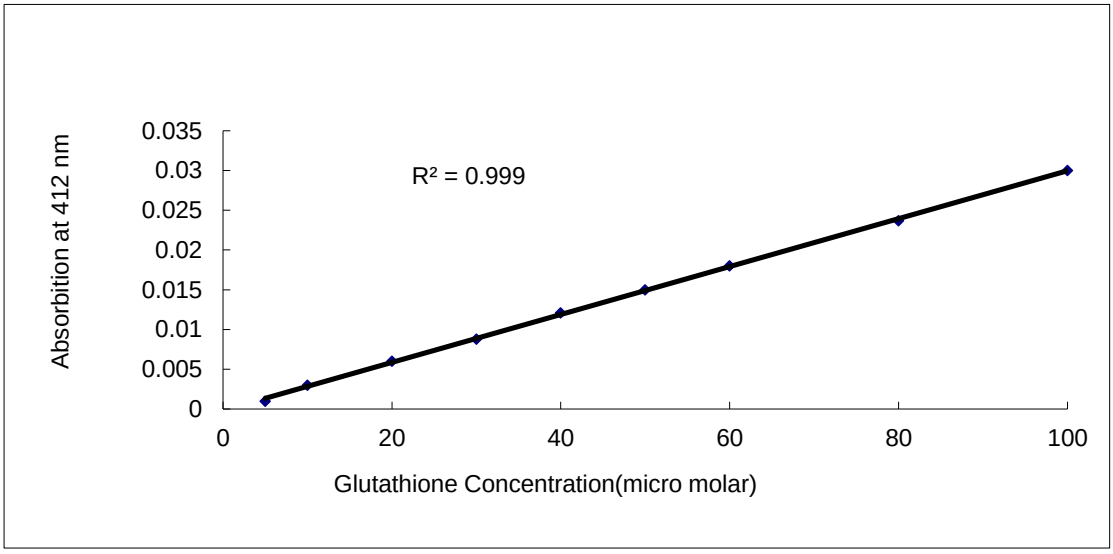


Fig.(2.2): The standard curve of glutathione concentration

2.4.3.2. Assay of Glutathione Peroxidase Activity

Principle:

Glutathione peroxidase G-Px was prepared by reagents:

Reagent A: [Sodium phosphate buffer (0.15M) pH=7.0]: (0.81153g) Na_2HPO_4, (11.1420g) NaH_2PO_4, (1.68g) Na_2-EDTA was dissolved in one litter D.W. **Reagent B:** (0.025g) of NADPH-Na_4 was dissolved in (10mL) D.W. (freshly prepared).

Reagent C: (0.0005g) of GSSG-Red (type III) was dissolved in (10mL) phosphate buffer. Reagent (0.039g) of NaN_3 was dissolved in (10mL) D.W.

Reagent E: (0.000921g) of GSH was dissolved in (10mL) D.W. (freshly prepared).

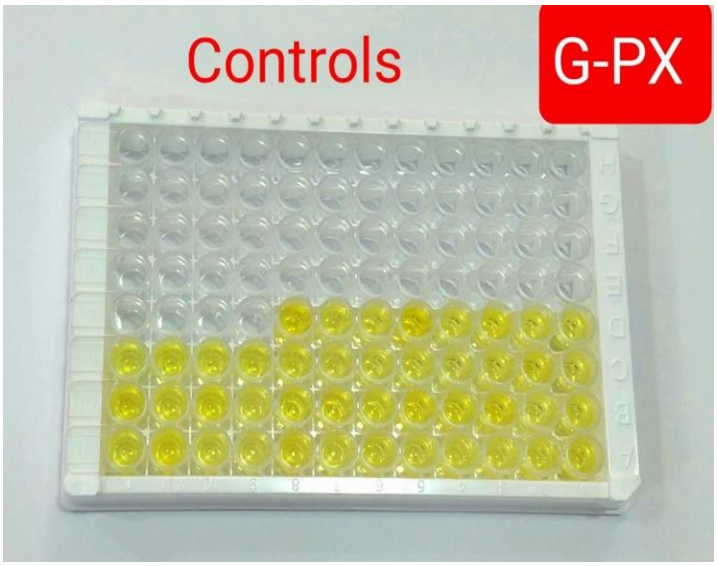
Reagent F: (Double- strength Drabkin's reagent): One gram of $NaHCO_3$, (0.2 g) $K_3[Fe(CN)_6]$, (0.052 gm) KCN was dissolved in (500mL) deionised water.

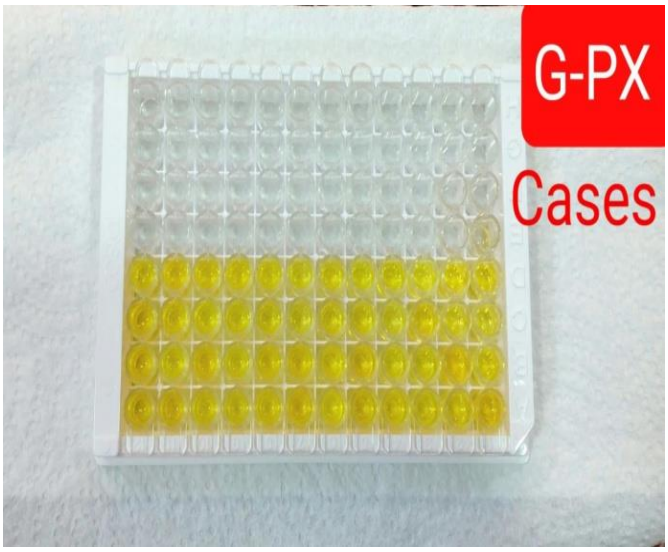
Reagent G: (0.008mL) of (30%) H_2O_2 solution was added to (100mL) D.W. (prepared immediately before assay).

Glutathione peroxidase catalyzes the following reaction:

$$\mathbf{2GSH + LOOH \xrightarrow{\text{G-PX}} GSSG + LOH + H_2O}$$

The decrement of reduced glutathione concentration can be monitored by Ellman's reagent [5,5'-Dithio-bis-(2-nitobenzoic acid) (DTNB)].





2.4.3.3. Determination of Catalase activity

Principle

The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as unstable intermediate. The acetate produced is measured colorimetrically at 610 nm. This was assayed by the method described by Sinha**(Ogbuagu*et al.*, 2022).**

Procedure

Table (2-5): Procedure for assessments of catalase activity.

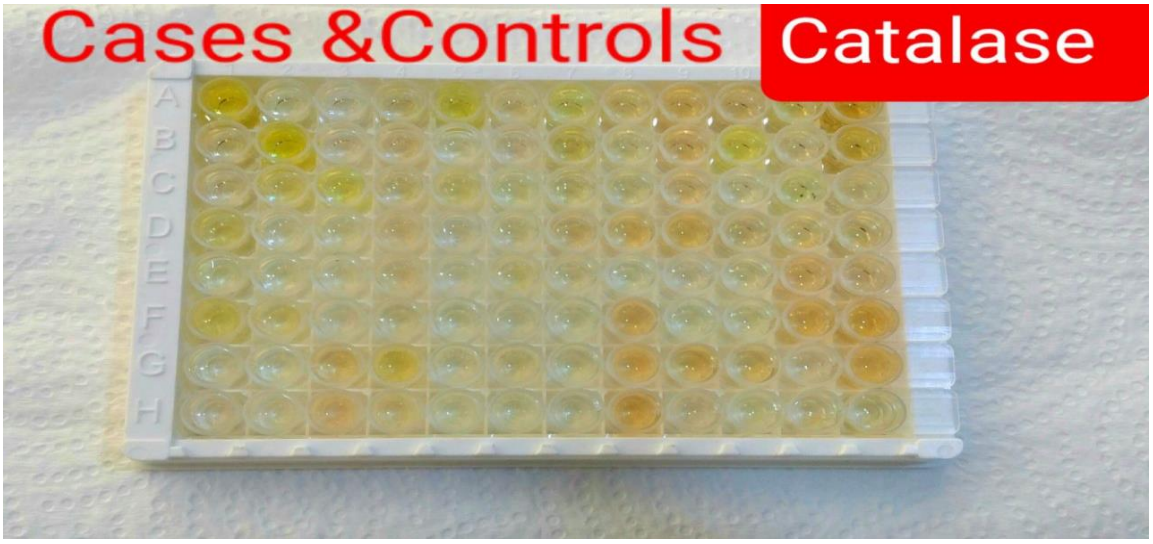
Reagents	Test	Standard	Blank
Sample	100 µl	------	-----
Phosphate buffer	900 µl	1000 µl	3000 µl
Hydrogen peroxide	2000 µl	2000 µl	----
Mixed with vortex and incubate at 37 °C for 2 min, after that, add:			
Vanadium reagent	2000 µl	2000 µl	2000 µl
After that, the tubes were kept at 25 °C for 10 min. the changes in absorbance were recorded at 452 nm against the reagent blank.			

Calculation

Enzyme activity procedure was elucidated in (table 1). The rate constant (k) of the first-order reaction equation for catalase activity was calculated using the following formula:

Catalase Activity , U/L = (2.303 / t) x log (S^o / S) ……(1)

where t is time, S^0 is the absorbance of the standard solution, and S is the absorbance of the sample.



2.4.3.4. Determination of Serum Malondialdehyde

Principle

Lipid peroxidation in sera was evaluated by thiobarbituric acid reactive substances (TBARS).The TBARS test gives a basic, reproducible, and standardized tool for measuring lipid peroxidation in serum. The MDA-TBA adduct designed by the response of MDA and 1,3-Diethyl-2-thiobarbituric acid (DETBA) under high temperature (90-100°C) at acidic conditions is measured colorimetrically at 530-540 nm or fluorometrically at an excitation wavelength of 515 nm and an emission

wavelength of 555 nm. This reaction has a much higher sensitivity when measured fluorometrically.

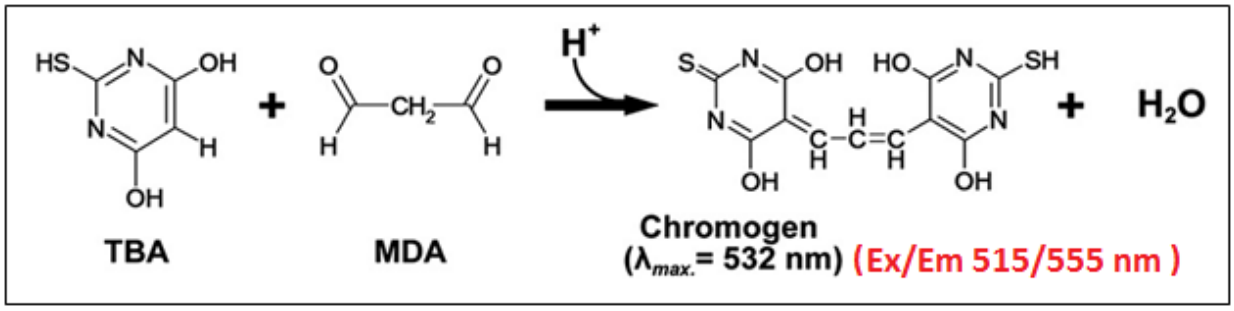


Figure : (2.5) Scheme of the adduct MDA–$(TBA)_2$.

Procedure:

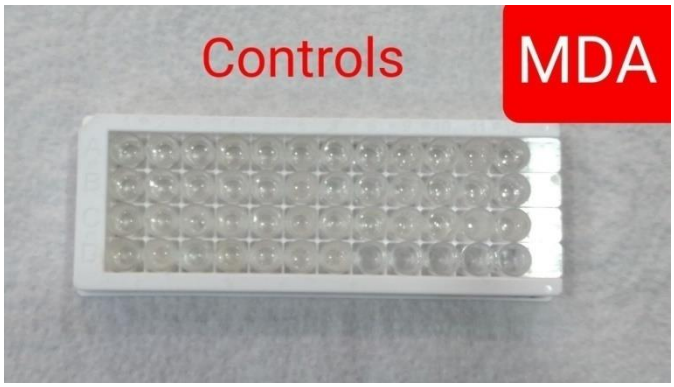
1. Added 100 µL of sample to the test tube ,and 2ml of working solution which prepares as following :

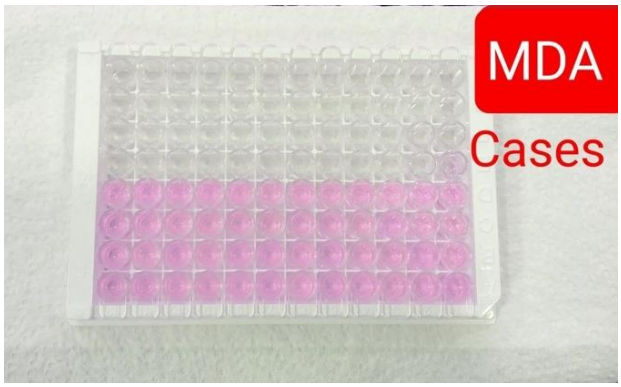
0.514 of TBA , 25 g of TCA and 0.5 ml of 1M HCl was mixed with 190 ml of D.W. Then we added 1 g of SDS and completed the volume to 200 ml .

2. The sample was vortexes and heated in a 90º C water bath for 50 min, and then allowed to cool.

3.The sample was centrifuged for 5 min at 5000 rpm then measure the absorbance spectrophotometrically of the supernatant at the wave length 532 nm against a reagent blank. The preparation of reagent blank was the same procedure above except change the sample with DW.

MDA Conc. = (Abs / d . E) x Dilution factor (D.F.)





2.4.5. Synthesis and characterization of Fe_3O_4@prussian blue (PB) nanocomposite

In this research, a new nanocomposite based on PB-coated magnetic nanoparticles was synthesized and used to remove bilirubin from blood. This nanocomposite possesses both the properties of PB as a good adsorbent as well as iron oxide as a good magnetic material.

2.4.5.1. Synthesis of magnetic iron oxide nanoparticles (Fe_3O_4)

The Fe_3O_4 are easy to make using chemical methods like the co-precipitation method, which is considered the best traditional way to make MNPs because it uses a non-toxic solvent, has a high yield, and is easy to reproducibility. The co-precipitation process involves ferric (Fe^{3+}) precipitation and ferrous (Fe^{2+}) salts aqueous solutions by adding a base. A general scheme is depicted in depicted in **fig.(2.6).**

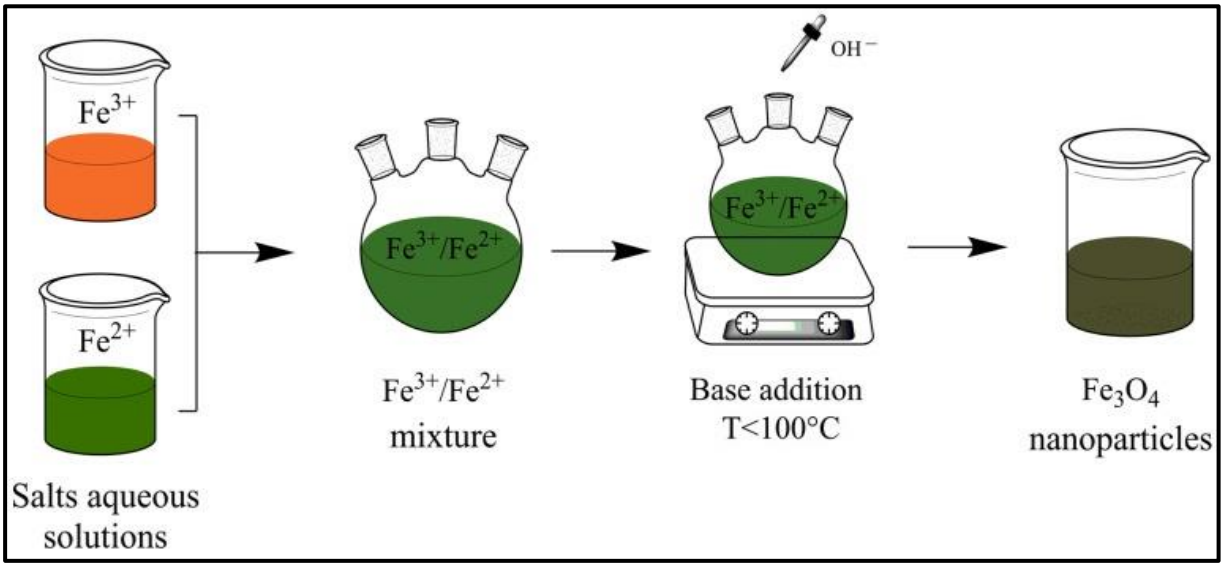


Figure (2.6): Schematic representation of the synthesis of Fe_3O_4 nanoparticles by the co-precipitation method.

Procedure

A 50 mL solution containing a 1:2 molar ratio of ferric chloride ions ($Fecl_3$) and ferrous chloride ions ($FeCl_2$) were used to synthesize iron oxide nanoparticles according to **(Hui & Salimi, 2020)**.Both were dissolved in distilled water and the resulting mixture was made basic (pH > 9) by adding sodium hydroxide solution drop wise using a syringe till the solution became completely black indicating the formation of magnetite. Then, the solution was kept overnight for the particles to settle down. The supernatant liquid was decanted and the black precipitate was collected by a permanent magnet, washed with distilled water till pH was 7, and the particles were dried in hot air oven at 65 °C for 1 h to obtain Prussian blue –coated iron oxide nanoparticles.

These nanocomposites were observed to be stuck on the magnet bar which confirmed their paramagneticity as show in Figure (2-18).

Figure (2-7): Photograph of a magnet attracting of Fe_3O_4in water.

2.4.5.2. Preparation of Fe_3O_4@PB Nanocomposite

The Fe_3O_4@PB NPs were synthesized by an improved shell-growing procedure according to the literature. Briefly, 10 mL Fe_3O_4 NPs aqueous dispersion was added drop wise into 20 mL aqueous K4[Fe- (CN)6] solution (2.0 mM, pH = 3.0) under vigorous mechanical stirring at room temperature. The colour of the Fe_3O_4 NP aqueous dispersion gradually changed to light green during the addition process. Then, 20 mL aqueous $FeCl_3$ solution (2.0 mM, pH = 3.0) was added drop wise into the above mixed solution. The obtained Fe_3O_4@PB NPs were separated from the aqueous solution and washed three times with deionized water using an external magnetic field. The obtained composite NPs were finally re-dispersed in deionized water to form the aqueous dispersion of the Fe_3O_4@PB NPs.

2.4.5.3. Characterizations of PB-coated iron oxide nanoparticles

UV –Visible Spectrophotometer, Zeta potential and XRD have been used for PB-coated iron oxide nanoparticles characterization.The UV- visible absorbance spectral analysis was done by using UV-VIS spectrophotometer (SHIMADZU1900, Japan) after diluting a small amount of the sample into distilled water at wave length 540 nm.The phase and crystallinity were analyzed using powder X-ray diffraction (XRD) analysis obtained by an X-ray diffractometer (pw1730,philips, Holland) in the range of 2θ=10–80°. The mean crystallite size of g-Fe_2 O_3 nanoparticles was quantitatively determined from XRD data by employing Debye Scherer's equation:

$$\mathbf{D = k\lambda/\beta cos\theta}$$

where D is the average crystallite size, K=0.9 is the shape factor, λ is the X-ray wavelength (in nm), θ is the Bragg diffraction angle, and β is the high peak at half maximum (FWHM) of the intense peak (in radians).

The surface zeta potentials of the magnetic nanoparticles and nanocomposite were measured using a (Zetasizer Nano-ZS DLS particle size analyzer, Malvern). Transmission electron microscope (TEM) technique is usedto investigate the structure and measure the particle size of the nanocomposite. (TEM, Zeiss-EM10C-100 KV, Germany) was used.

2.4.6. Adsorption of bilirubin from aqueous solution

The adsorption properties of the prepared Fe_3O_4@PB nanocomposites for bilirubin. The efficiency of the adsorption process depends on different factors such as temperature, contact time, pH of the sample solution, adsorbent amount, and bilirubin concentration. The optimized conditions of these factors were performed according to the Brunauer–Emmett–Teller (BET) nitrogen adsorption method**(Kou & Wang, 2022**). Stock solution (10 mg/mL) of bilirubin was prepared in deionized water. Stock aqueous solution of bilirubin was prepared (10mg) of adsorbent Fe_3O_4@PB nanocomposites and prepared from Stock Solution five dilute solutions(1mg/mL, 0.5mg/mL, 0.25mg/mL,0.125 mg/mL, 0.0625 mg/mL) by dilution equation at room temperature (25 ± 1 °C):

$$\mathbf{M1V1=M2V2}$$

After that added 1 ml of stock solution of bilirubin for five tubes with mini magnetic bars in each tube firstly measured adsorption before reaction for 0 min at 540 nm by UV-VIS spectrophotometer (SHIMADZU1900, Japan).

The removal efficiency (%R) of bilirubin was obtained from the following equation:

$$\mathbf{RE\ (\%) = C_0 – C_e\ /C_0 \times 100}$$

where RE (%) represents the removal efficiency, C_0 (mg. L^{-1}) is the initial concentration of ions, and C_e (mg. L^{-1}) is the concentration of ions at equilibrium.The second step the tubes were placed on a magnet for (15, 30,60 ,120 minutes) and after each limited time ,applied procedure of Linear total bilirubin kit

Principle of kit:

Bilirubin is photometrically measured after being converted to coloredazobilirubin by diazotized sulfanilic acid. Only the bilirubin glucuronide fraction reacts directly in serum, while the free bilirubin bound to albumin fraction reacts after being displaced from protein by an accelerator. The difference between two measurements, total bilirubin (with accelerator) and direct bilirubin (without accelerator), allows

indirect bilirubin to be calculated. The terms direct and indirect bilirubin only refer to the reaction characteristics in the presence or absence of an accelerator or solubilizer, and they are only approximate equivalents of the bilirubin fractions.

Reagent preparation :

Working reagents:Prepared by mixing (1 mL) RN and (4 mL)RT ,stable for 8 days at 2-8ºC.Calibrator :reconstitute the vial by adding exactly (1.0 mL) of distilled water, mixed carefully and let stand for 5-10 minutes before use.

Procedure :

TUBES	Reagent Blank	Sample Blank	Sample	CAL
Distilled water	100 µL	–	–	–
Sample	–	100 µL	100 µL	–
CAL	–	–	–	100 µL
RT	–	1.0 mL	–	–
Working reagent	1.0 mL	–	1.0 mL	1.0 mL

The four tubes were mixed and let the tubes stand for 2 minutes at room temperature , after that read the absorbance (A) of the sample blanks at 540 nm against distilled water, and then read the absorbance (A) of the samples at 540 nm against the reagent blank.

Finally, by absorbance data were calculated concentrations of TB by

$$\frac{A_{Sample} - A_{Sample\ blank}}{A_{Cal}} \times C_{Cal} = \text{mg/dL total or direct bilirubin}$$

Where normal range up to 1.0 mg/dL.

2.4.7. Removing Bilirubin from Blood

In this method, serum samples of patients were mixed in pool and tacked(1 ml) from it and was mixed with(1 ml) of NPs. Under optimum conditions (Temp.37.7, concentration of NPs. 0.25 mg /mL, time 30 min.) was measured the absorbance to calculate concentration of T.B. All that was compared with serum pool of controls under same conditions.

2.5. Statistical Analyses

Information from the questionnaire and all test results from study groups samples were entered a data sheet. The data analysis for this work was generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA) and the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copyright (2013 – 2020)

Descriptive statistics was performed on the data of each group. Values were illustrated by n (%) for categorical, Scale variables were presented by mean ± standard deviation. Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant.

Chapter Three

Results

and

Discussion

3. Results and Discussion

3.1. Demographic and Clinical Characteristics

The clinical demographic characteristics of both study group were summarized in table (3) table and figure (3.1)illustrated the blood groups of participants which was mostly within O group.

Table (3): Descriptive of the Demographic and of the study population (n= 100)

Variable		Patient N (%)	Control N (%)
blood Group	O	23	26
	A	14	13
	B	8	7
	AB	5	4
BMI Category	Normal	46	41
	Abnormal	4	9
Nutrition	Normal	50	49
	Abnormal	0	1
Sporting	Yes	0	2
	No	50	48
Status	Single	24	21
	Married	26	29
chronic daises	Yes	12	0
	No	38	50
Family history	Yes	26	0
	No	24	50
Gender	Male	23	26
	Female	27	24

Gender distribution among the studied groups were shown female, more than male in patients' group as shown in figure (3.3), participants in both study groups were within normal BMI as presented in figure (3.2) . Out of total patients, two thirds of the participants were not suffering from any chronic diseases or family history.

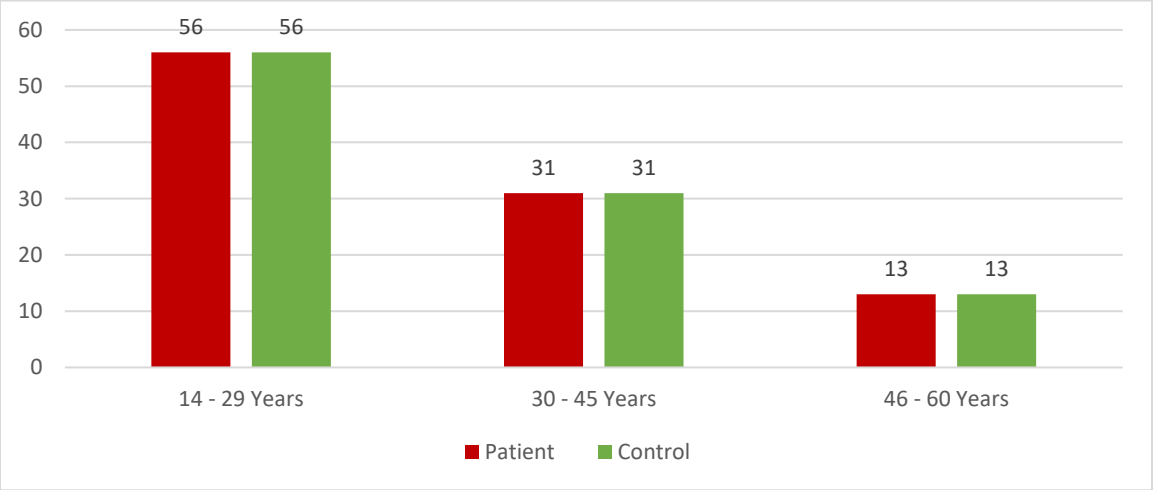


Fig. 3.1: Frequency of the study population in cases of sickle disease compared to control group according to age groups (N%).

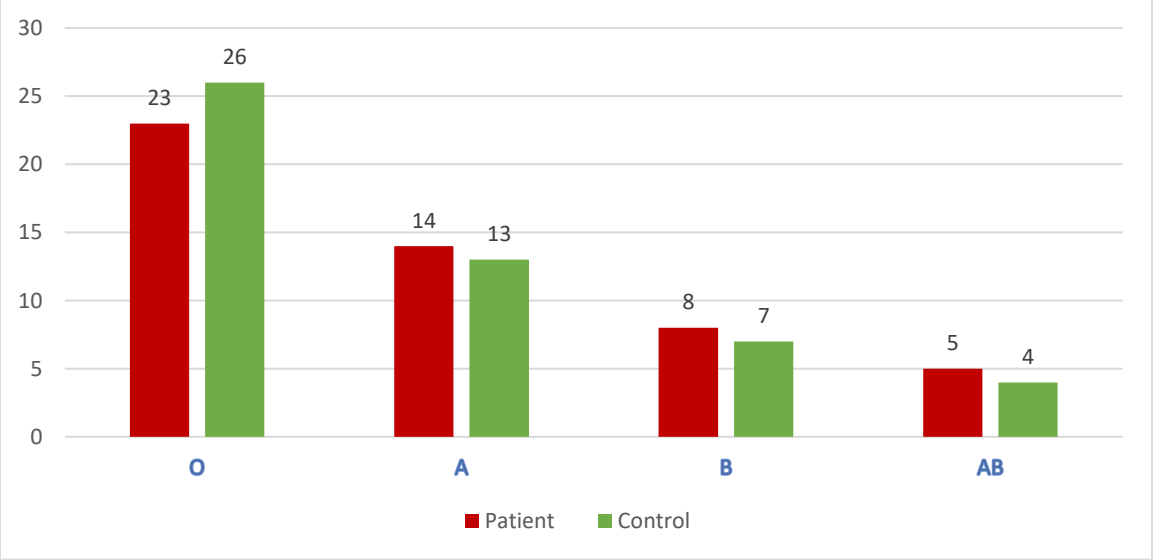


Fig. 3.2: Frequency of the study population in cases of sickle disease compared to control group according to blood groups (N= 100)

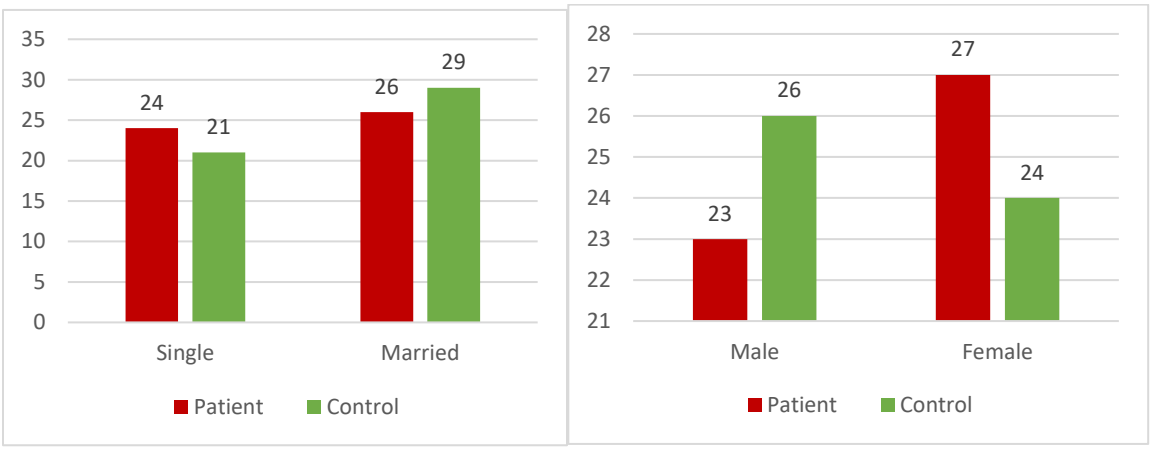
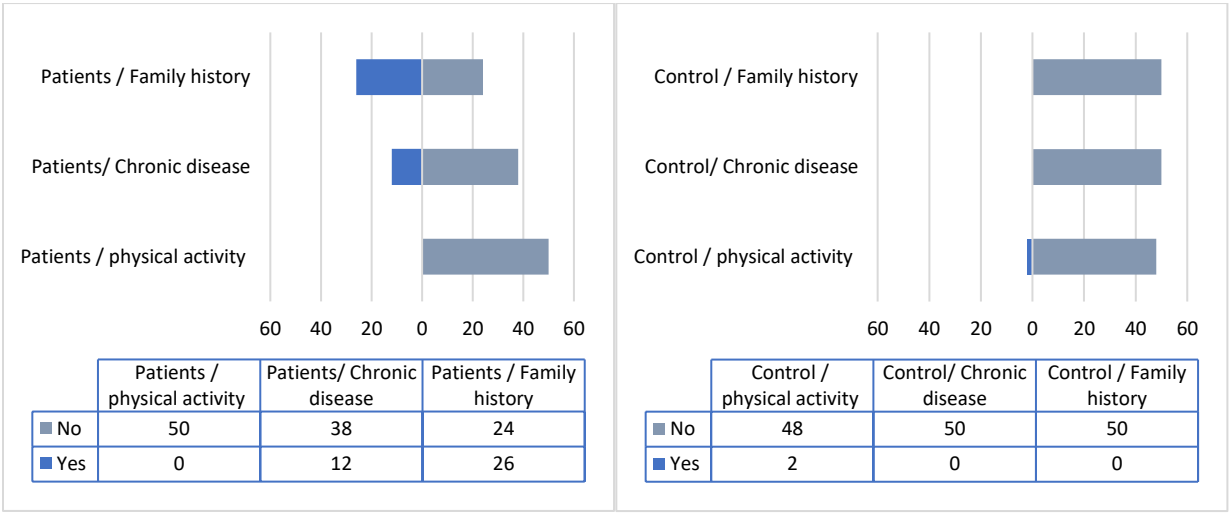


Fig. 3.3: Frequency of the study population in cases of sickle disease compared to control group according to marital status and gender (N= 100)

About 76% of the patients' groups were reported to have a family history. Also 48% were pointed to chronic disease and no one of the patient's group were reporting any physical activity.



	Patients / physical activity	Patients/ Chronic disease	Patients / Family history
No	50	38	24
Yes	0	12	26

	Control / physical activity	Control/ Chronic disease	Control / Family history
No	48	50	50
Yes	2	0	0

Fig. 3.4: Frequency of family history, chronic disease and physical activity of the study population in (A)cases of sickle disease compared to (B) control group (N= 100).

3.2. Biochemical Assays

3.2.1. Evaluation of antioxidants status of studied groups

Table 3.1: Mean differences of biomarkers increases of sickle disease as compared to control group.

Variable	Patient (N=50) Mean± SD	Control (N=50) Mean ±SD	P value
CAT, U/L	0.33±0.13	0.36±0.14	0.204[NS]
GPx, U/L	334.41±112.63	400.51±72.53	**<0.001[S]**
MDA, mmol/L	2.16±1.50	1.44±0.44	**0.002[S]**
GSH,mmol/L	18.85±2.56	22.93±2.80	**<0.001[S]**
Results are presented as mean ± SD, p≤ 0.05 considered significantly different, [S]= Significant, [NS]= non-significant– test			

In Table (3.1) a significant difference was observed among the study group in the levels GPx, MDA, GSH (P values were<0.001, respectively). In the sickle cell patients, the reduction of antioxidant defence mechanisms can lead to damage of cellular organelles and enzymes and cause an increase of lipid peroxidation **(Ama Moor *et al.*, 2016)**.

Level of G-Px activity was significantly decreased activity in SCD subjects as compared to healthy individuals. Additionally, the level of GSH was significantly decreased activity in SCD subjects as compared to healthy subjects. which is consistent with the study of **(Biswal *et al.*, 2019)**, that may be due to excessive production of ROS and it was leaded that the decreased activity of G-Px activity, GSH may be a defense mechanism in response to increased oxidative stress causing H_2O_2 build-up. However, H_2O_2 was produced via two electron transfer or due to

sickling, and it was removed by two main antioxidants such as G-Px and CAT activity levels.

Results of present study were further confirm previous conclusions, which suggest that the reduced the level of GSH and G-Px activity because increased oxidative stress. While MDA level was significantly increased in the serum of patients with SCA compared to healthy controls. The increase in MDA levels in the present study could be attributed to enhanced ROS formation in SCA patients where the MDA levels therefore gives an idea of the extent of oxidative damage in the cells, which is consistent with the study of **(Saka *et al.*, 2023)and (Luo *et al*., 2020)**.On the other hand, other studies have recognized a statically significant MDA decreased in older patients with SCA. Also revealed a relation in which there was no age correlation between the patients with sickle cell anaemia and healthy patients **(Bahal *et al.*, 2022).**

Table 3.2: Mean differences of biomarkers increases of sickle disease among patient group based on gender

Variable	Male(N=23) Mean± SD	Female (N=27) Mean ±SD	P value
CAT activity, U/L	0.32±0.13	0.36±0.13	0.135[NS]
G-Px activity, U/L	363.00±102.22	371.74±98.50	0.664[NS]
MDA, mmo/L	2.10±1.27	1.51±0.97	**0.011[S]**
GSH, mmol/L	20.89±3.49	20.89±3.28	0.997[NS]
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= non-significant. T– test			

Table (3.2) based on gender groups, only MDA levels was shown a significant difference, the increase in the oxidative stress level especially MDA in sickle cell homozygous patients based on gender confirms where that oxidative stress is

involved in the pathogenesis of the sickle cell disease, and p value was 0.01and also consistent with the study of **(Ama Moor *et al*., 2016)**.

Table 3.3: Mean differences of biomarkers in cases of sickle disease among patient group based on age

Variables	14 – 29 Years	30 – 45 Years	46 – 60 Years	P value
CAT activity, U/L	0.30±0.13	0.36±0.12	0.34±0.14	0.377
G-Px activity, U/L	387.51±59.44	376.21±105.14	357.96±104.61	0.535
MDA, mmol\L	1.53±0.64	1.82±1.14	1.85±1.27	0.656
GSH, mmol/L	19.33±2.72	21.10±3.21	21.14±3.54	0.203
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant, [NS]= non-significant. ANOVA test				

while based on age groups, no significant differences were found in the levels of measured biomarker p values were >0.05 as presented in above table .

3.2.2.Correlation coefficient between the different biomarkers

Table (3.4): Correlations of the Biochemical parameters among patients' groups

Variables	CAT activity	GPx activity	MDA	GSH
CAT activity, U/L	1	**r=0.3** **P=0.003**	r=-0.91 P=<0.001	**r= 0.4** **P=<0.001**
GPx activity, U/L	**r=0.3** **P=0.003**	1	r= - 0. 1 P= 0.540	**r= 0. 2** **P=0.017**
MDA, mmol/L	r= - 0.2 P=0.119	r=0. 1 P=0.540	1	r=0.1 P=0.998
GSH, mmol/L	**r=0.4** **P=<0.001**	**r=0. 2** **P=0.017**	r=0.1 P=0.998	1
Results are presented as mean ± SD, p<0.05 considered significantly different .				

Considering the important role of the measured biomarkers in the progression of case study, the multivariable linear regression model was used to analyse the response relationship between parameters. Serum CAT activity levels was positively significant correlated to their G-Px activity and GSH this may be due to the consumption of these substances by pro-oxidants in SCA. This therefore place SCA patients at increase risk of oxidative stress and injury, the GPX and CAT activities were significantly lower (P< 0.001) . The oxidative stress may contribute to the sickling process with formation of dense cells, the development of vaso- occlusion and shortened red blood cells survival to biological macromolecules while it was negatively correlated with MDA levels which is consistent with the studies of **(Antwi-Boasiako *et al.*, 2020)** ,**(Shahabi *et al*., 2007)**. In addition, GPX was weakly significantly correlation CAT activity and GSH levels. On the other hand, serum GSH levels was also significant positively correlated to CAT and GPx activity levels (all P < 0.05). The relationship between the parameters was presented in above table .

Table (3.5):Estimation the Associated of analysed markers increases of sickle disease

Biomarkers	**Angiography Intervention**	
	OR (95% CI)	**p value**
CAT activity, U/L	0.043 (0.00 – 7.471)	0.232[NS]
G-Px activity, U/L	1.015 (1.005 – 1.024)	**0.002[S]**
MDA mmol/L	4.487 (1.660 – 12.128)	**0.003[S]**
GSH, mmol/L	0.503(0.380 – 0.667)	**<0.001[S]**

Results are presented as numbers and percentage, p<0.05 considered significantly different, [S]; Significant, [NS]; Non significant

Binary logistic regression was performed and forward logistic regression was adopted to analyse the results. Results were indicated that CAT activity and GSH in cases of sickle disease patient that were shown to be a protective markers (OR: 0.043 and 0.503; 95% CI: (0.00 – 7.471) (0.380 – 0.667)respectively and also consistent with the study of **(Silva et al., 2013)**,while G-Px activity and MDA(OR: 1.015 and 4.487, 95% CI: (1.005 – 1.024) (1.660 – 12.128) were independent risk factors for sickle disease patient , as shown in above table .

3.2.3. Receiver operating characteristics of biomarkers level in patients

Table (3.6): AUC, optimal threshold, Sensitivity and specificity of GSH marker obtained by the ROC curves for prediction of sickle disease

Test Variable	GSH
AUC(Area under Curve)	85.5%
Sensitivity %	76%
Specificity %	84%
You den index	0.6
Cut-off points	21.1213
CI(Confidence interval) (95%)	0.781 – 0.928
P value	**<0.001**

Results of the receiver operating curve (ROC) curve and AUC analysis for the GSH were performed as a possible diagnostic parameter. GSH level was shown a good diagnostic performance for prediction of cases of sickle disease Patients compared to control group, data are presented in figure (3.5) and table (3.6).

The sensitivity was 76%, specificity 84%at a level = 21.12 , p-values of the AUC for GSH were <0.001 and statistically significant

Table 3.7: Distribution of patients according to the GH cutoff value in the studied groups

GSH, U/L	**Patient**	**Control**
<21.1213	38	8
>21.1213	12	42
Total	50	50

A accordingly, the distribution of patients using GSH cutoff values was presented in above table .

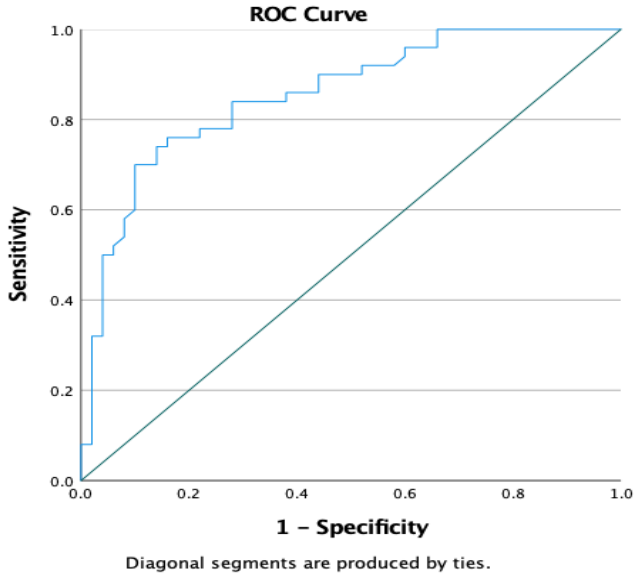


Figure 3.5: Receiver operating characteristics (ROC) curve analysis of GSH levels The area under ROC curve: 85.5%. CI 0.781- 0.928; p value <0.001

A box plot was used to visually show the distribution of data through displaying the data quartiles and averages. Figure (3.6) demonstrated a across distribution of serum level of CAT, G-Px, MDA, GSH in cases of sickle disease.

Patients and healthy control group. Throughout the results, the quartiles and range levels of G-Px and CAT were decreased markedly in patients, while MDA was estimated to have great variability in patients compared to control. The range levels in patients groups were shown in Figures 3.6.

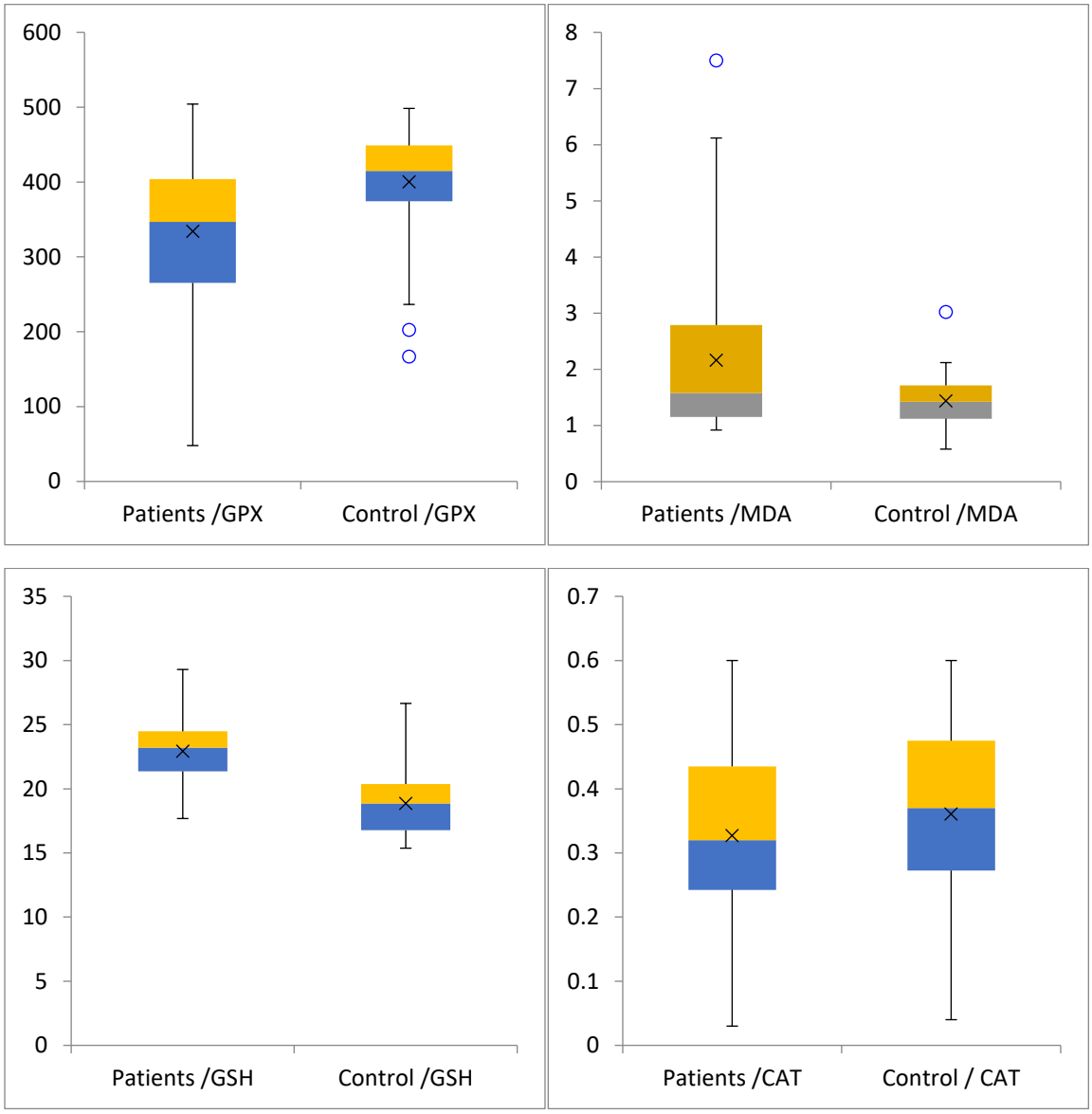


Figure (3.6): Boxplot for distribution G-Px, MDA, GSH and CAT(U/L) levels in patient samples compared to control.

3.3. Characterization of Fe_3O_4@PB nanoparticles

3.3.1. Synthesis and characterization of Fe_3O_4@PB nanocomposites

Initially, Uniform Fe_3O_4Nanoparticles were prepared using hydrolytic reaction based on in situco-precipitation of ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions under alkali conditions according to the previous study (**Wang *et al.*, 2020)**.

Then, The Fe_3O_4@PB nanocomposites were combined by growing PB nanoshells around superparamagnetic Fe_3O_4nanocoresthrough a shell-growing procedure as illustrated in **Scheme 1**.Aqueous Fe^{3+} and $[Fe(CN)_6]^{4-}$ were added dropwise to aqueous dispersions of Fe_3O_4 NPs under acidic condition.

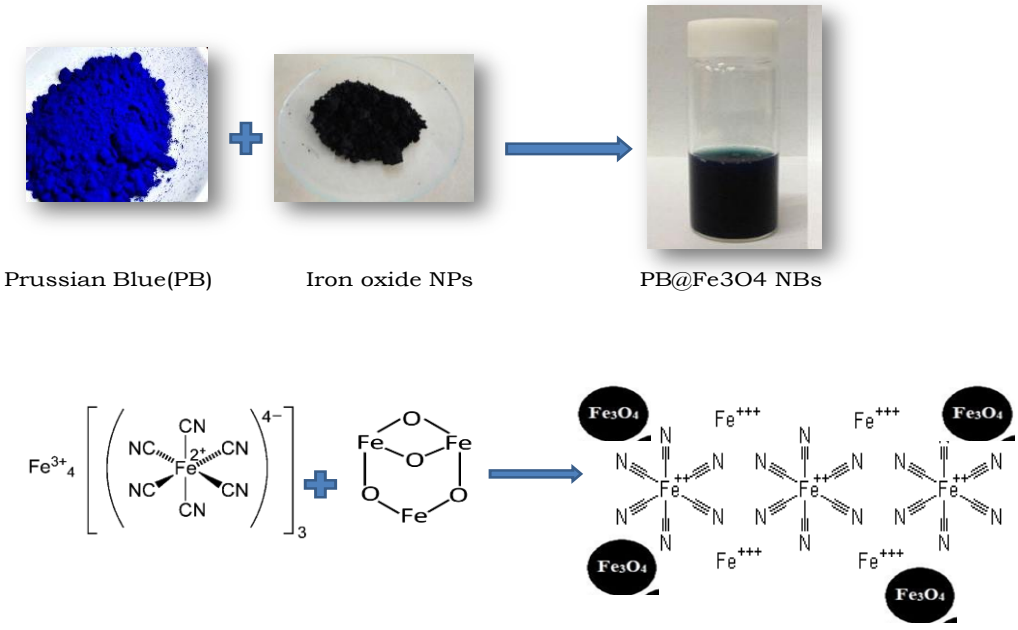


Figure (3.7): Schematic representation the preparation of Fe_3O_4@PB .

The morphology and structure of the as-prepared nanoparticles were characterized by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).The SEM images are well shown that the synthesized Fe_3O_4coresexhibited a smoothly spherical shape as shown in **figure (3.8, a).**

After *in situ* growing PB on the surface of Fe_3O_4NPs,a sphere-like structure is maintained well with an enlarged in the diameter, revealing the existence of the coating layer. The resulting Fe_3O_4@PB spherical external layer and the cube-like nanoparticles around the sphere, which is the common morphology of PB **figure (3.8, b)**.

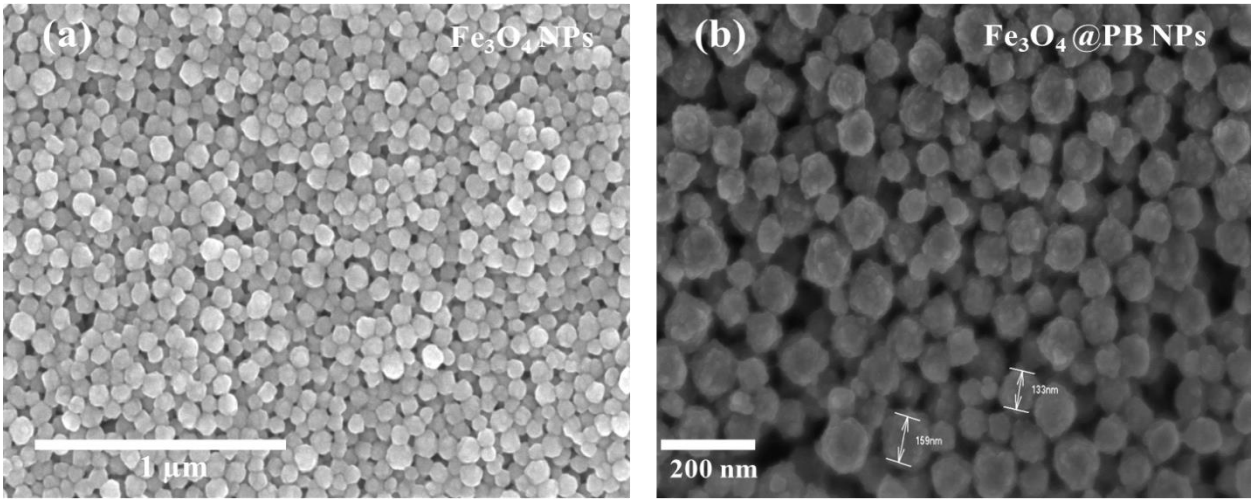


Fig. (3.8): SEM images of (a) Fe_3O_4NPs, (b)Fe_3O_4@PB nanocomposites.

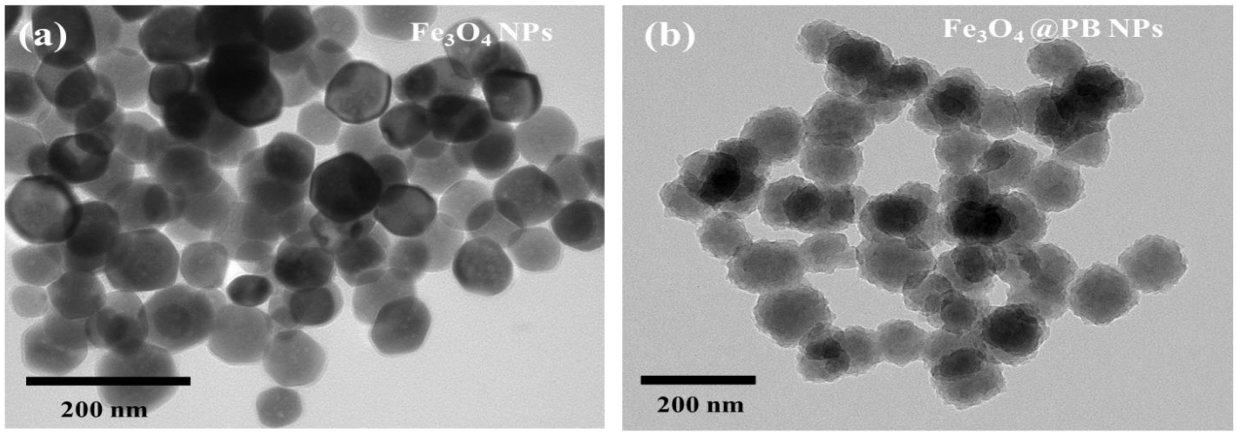


Fig. (3.9): TEM images of (a) Fe_3O_4NPs, (b) Fe_3O_4@PB nanocomposites.

Furthermore, the TEM images well demonstrated the uniform spherical-shaped structure of the as-prepared Fe_3O_4 NPs with an irregular average particles size of 70 nm, meanwhile after the *in situ* formulation PB around the Fe_3O_4NPs, a fused Fe_3O_4@PB nanocomposites are resulted with an increasing of particle size to be around 160 compering to the pristine Fe_3O_4 NPs, **figure (3.9)**.

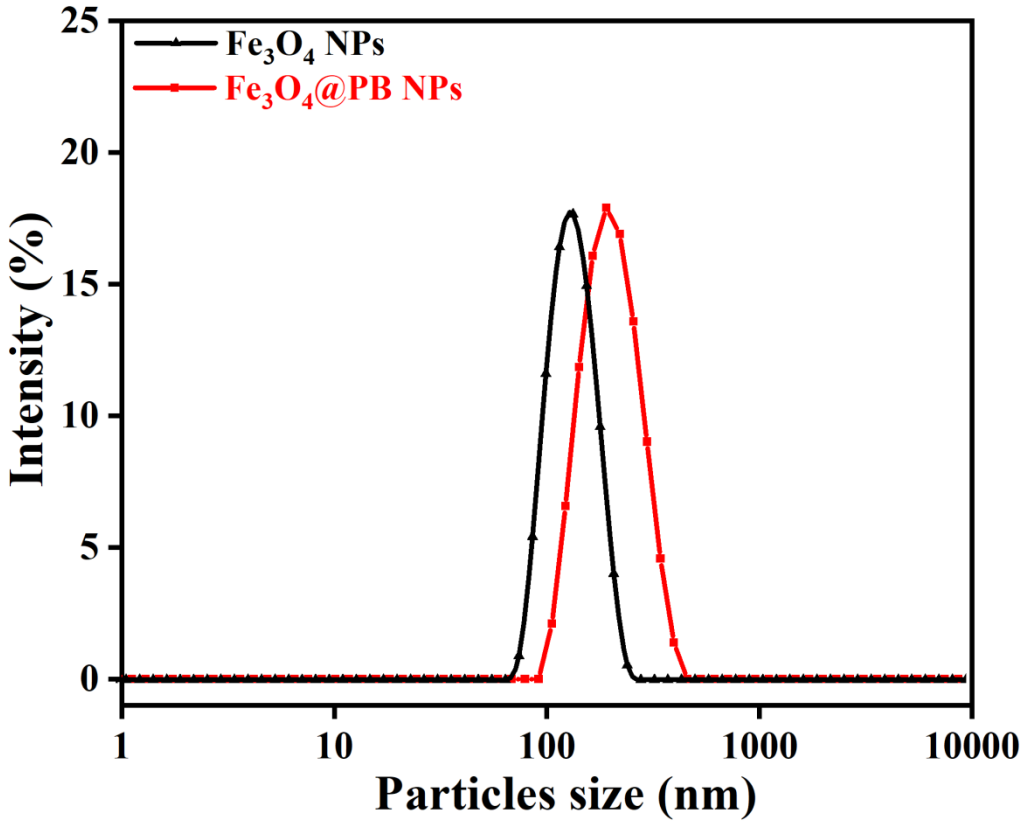


Fig. (3.10): DLS results of Fe_3O_4NPs (black line), Fe_3O_4@PB nanocomposites (red line).

The particle sizes of the as-made nanomaterials were also further examined using dynamic laser scattering (DLS) measurements, and it appears to be larger than those determined by TEM. The diameters of Fe_3O_4NPs were ~100 nm while, after fusing with the prussian blue, an increase in the average size from ~100 nm to ~250 nm was observed, as shown in **figure(3.10)**, demonstrating the successful formulation of Fe_3O_4@PB nanocomposites.

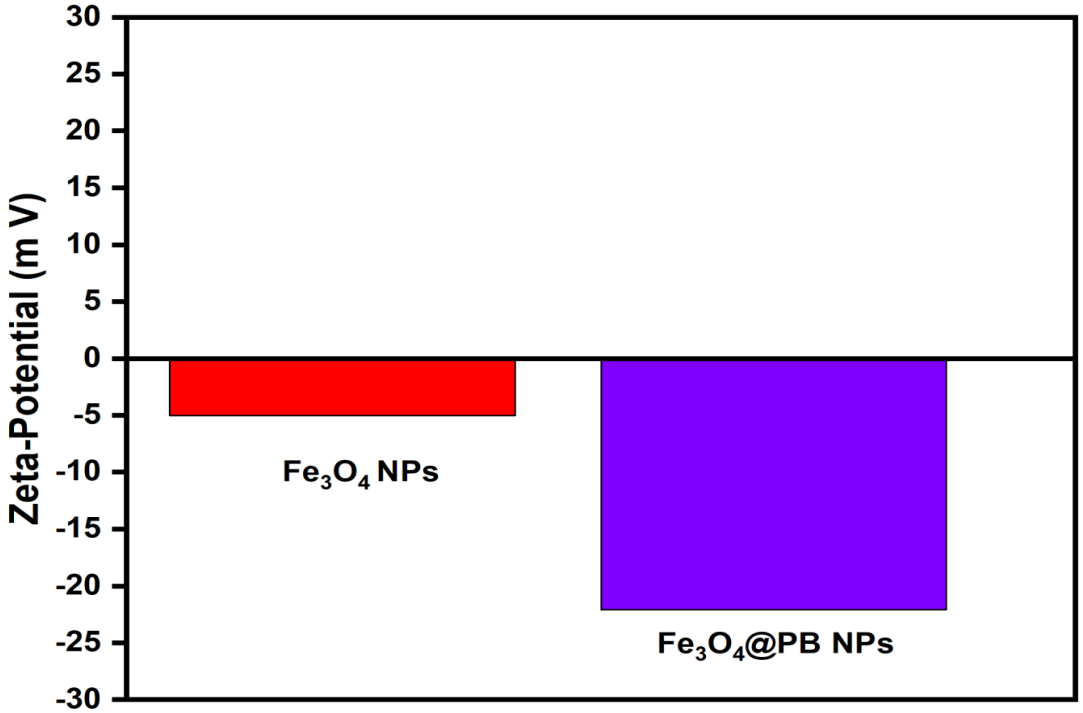


Fig.(3.11): Zeta-potentials of Fe_3O_4NPs, Fe_3O_4@PB NPs

Furthermore, the corresponding zeta potentials of the as-prepared nanoparticles were evaluated; as observed in **Figure(3.11)**, later being formulation with PB, the Fe_3O_4@PB NPs surface charges, and the zeta potentials transformed from -5.2 to -20.1 mV. The negative shift indicated the presence of negatively charged cyanide ions (CN^-) within the PB structure.

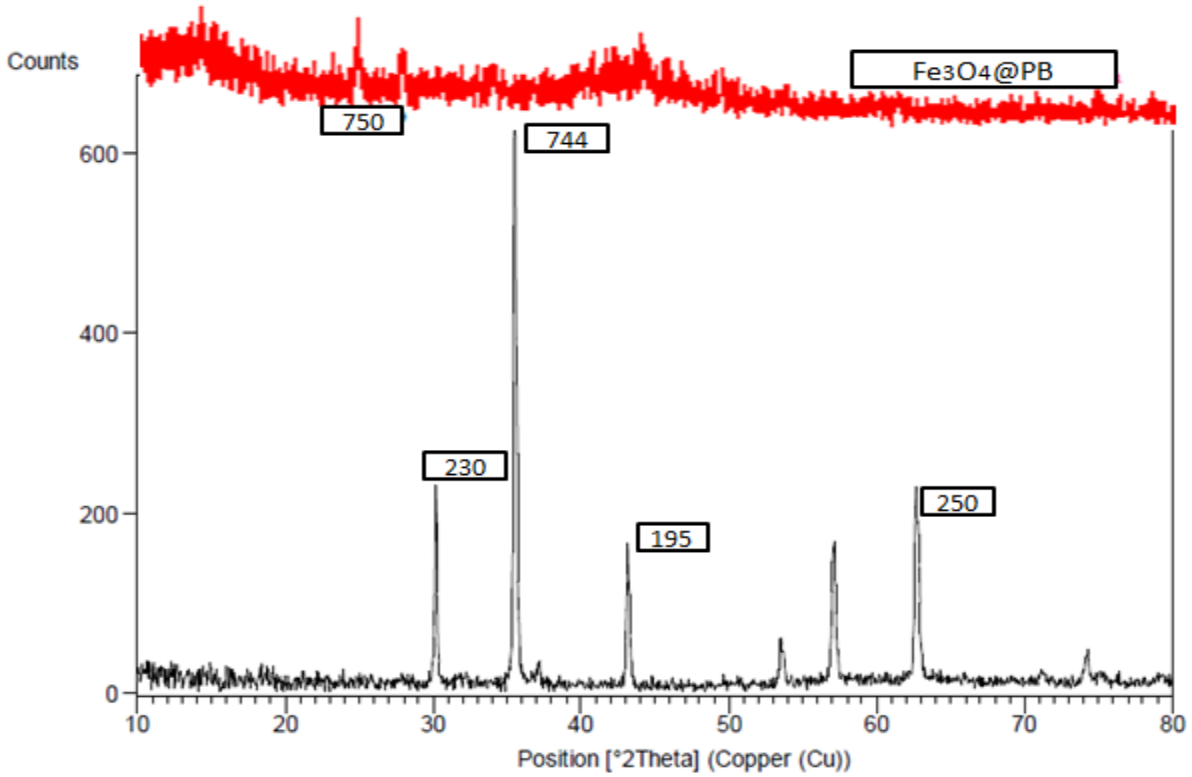


Fig. (3.12): XRD results of (a) Fe_3O_4NPs, (b) Fe_3O_4@PB NPs.

The crystallinity of the as-prepared $Fe_3O_4$and Fe_3O_4@PB samples were studied by X-ray diffraction (XRD) pattern. As shown in Figure (3.12),where X-ray diffraction(XRD)patterns were obtained using a Rigaku Ultima III X-ray diffractometer equipped with a CuKα radiation source. The Fe_3O_4 nanoparticles exhibited sharp and strong diffraction peaks**, (Falak, 2023)** indicating the high crystallinity structure, and in accordance with the standard pattern of the as-preparedFe_3O_4.While, the produced Fe_3O_4@PB sample showed less crystallinity because of the presence of inorganic layers of Prussian blue polymer on the surface of Fe_3O_4 nanocrystals, as well as all diffraction peaks are well at the same position as the Fe_3O_4 crystal. No obvious peak-shifted or crystal transformation can be observed, indicating the successful formation of pure core-shell Fe_3O_4@PB NPs, and we noticed the appearance of very sharp and clear beaks when 598-744 cm-1

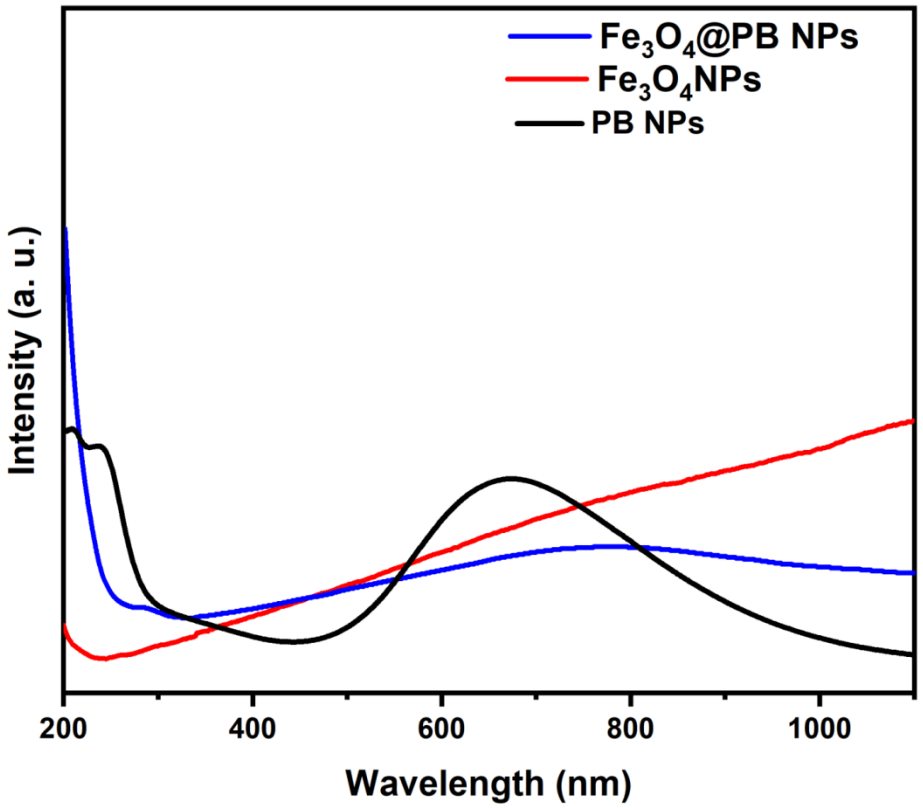


Fig. (3.13): UV−Vis-NIR spectra of Fe_3O_4NPs (red line), PBNPs (black line), and Fe_3O_4@PB NPs (blue line).

Besides, the optical absorption properties of the as-prepared samples were investigated by UV−Vis-NIR spectra, as shown in **figure (3.13)**Fe_3O_4@PB NPs have the characteristic beak of both Fe_3O_4NPs and PB NPs which is clear evidence for the formation of Fe_3O_4@PB NPs.

3.3.2. Removing bilirubin from aqueous solution

To investigate the toxic compound performance of Fe_3O_4@PB NPs, aqueous solutions are firstly employed to investigate their ability to adsorb the bilirubin under optimal condition(Temp. 37.5C^O ,Concentration of NPs 0.25mg/mL, Time 30 min.) ,where noticed decreased concentration of bilirubin after addition NPs with reduced

the absorption also was reduced the concentration with rising temperatures as shown in **table (3-8 a).** The experimental results were analyzed comparison made with the Beer–Lambert equation **(Goh *et al.*, 2014).**

These findings discern the presence of high amounts of precipitate bilirubin in the blood, may contribute to the development of the spectrum of acute clinical complications including sickle cell hepatopathy, and the results of the present work show that Fe_3O_4@ PBNPs have great potential to utilize as a nonhazardous and bio adsorbent for effective adsorption of bilirubin in aqueous solution, blood, and patient serum pool.

Table (3.8a): Estimation concentration of bilirubin after /before NPs

Conc. Bilirubin before reaction mg/mL	Conc. NPs mg/mL	Conc. Bilirubin the rest after reaction mg/mL
3.7	1	3.68
3.7	0.5	3.52
3.7	0.25	2.62
3.7	0.125	2.82
3.7	0.0625	2.89

After selected best concentration of NPs (0.25 mg/mL) ,was been measured different times with all conc. of NPs and the best time was(30 min), finally measured the conc. in (30 min) with different temperatures as shown in **table (3.8b)** .

Table (3.8b) : The effectiveness of temperature at clear away bilirubin

Temp. C°	Conc. Bilirubin after reaction mg/mL
25	3.66
30	2.8
35	2.2
37.5	1.5
40	4.2

When it was found that the absorbance decreased as the temperature increased the concentration of bilirubin inside the cuvette also decreased. In other words, the absorbance varied with temperature, while at large deviations from this temperature, the sensitivity of the spectrometer in use was to blame **(Amir & Javed, 2023) .**

Table (3-8): The effectiveness of Fe_3O_4@PB NPs for remove of bilirubin from aqueous solution, blood and patients serum pool

Fluid type	Precipitate material	C_0	C_e	RE %
Aqueous solution	Bilirubin	1.5371	0.3771	75%
Blood	Bilirubin	1.5590	0.2701	82%
Serum pool	Bilirubin	1.4252	0.2622	81%
RE (%) = removal efficiency, C_0 = is the initial concentration of bilirubin , C_e = is the concentration at equilibrium after 30 min.				

The results of the present work show that Fe_3O_4@PBNPs has great potential to utilize as a nonhazardous and bio adsorbent for effective adsorption of bilirubin in

aqueous solution, blood and patient serum pool. As can be seen in table (3-8) the removal efficiency (RE %) of Fe_3O_4@PBNPs for bilirubin from aqueous solution was 75% while from blood was 82% and for patient serum pool was 81% respectively.

This result agree with previous studies that have indicated magnetic separation exhibits greater potential than classical resin-based blood purification in blood therapy of toxic compounds **(Luo *et al*., 2020),(Guo *et al*., 2019).**

Conclusions and Recommendations

Conclusions

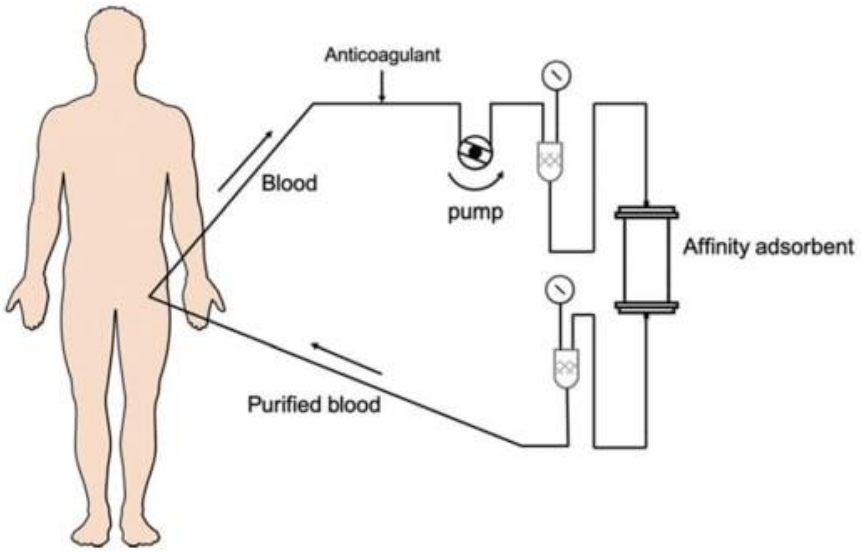
From all results of different variables in the current study, it could be concluded that:

1. There is associated between sickle cell disease and precipitate bilirubin where increased hemolysis from any cause may lead to markedly elevated levels of serum bilirubin. This may lead to the onset and progression of hyperbilirubinemia.
2. According to the antioxidant defence mechanisms analysis, the plasma biomarker, plasma concentration assay had ability to distinguish between sickle cell anemia patients and healthy controls, where noticed the increased MDA level and decreased antioxidants (CAT,-GSH and G-Px)-levels in the patient group compared to the healthy control group.
3. The Prussian blue coated magnetite nanoparticles showed good adsorption capability for the removal of bilirubin from blood and patient serum pool.
4. MDA is an biopredictor for oxidative stress in SDA.

Recommendations:

The current research project has the following recommendations for the future:

1. Increasing understanding of the risks complications of precipitate bilirubin to health where hyperbilirubinemia has a clear role in liver failure
2. It is crucial to develop new techniques and biomarker tests that would allow the detection of presymptomatic individuals.
3. Developing a magnetic nanocapture agent which is a novel therapeutic option with biocompatible safety and high efficiency for bilirubin scavenger in the blood.
4. Preparation different nanocomposites adsorbent conjugated to other carbohydrate biopolymers and studying the removal effectiveness of bilirubin from aqueous solution.
5. Study the effect of other antioxidants parameters, such as Co Q10 and superoxide dismutase (SOD) in patients with SCA.



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Appendix

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Dear Dr. Fadhil Jawad Al-Tu'ma,

The APJCP editorial board is glad to inform you that the manuscript titled "**Design and Development of Fe3O4@Prussian Blue Nanocomposite: Potential Application in the Detoxification of Bilirubin**" has passed the preliminary acceptance criteria for publication in the Asian Pacific Journal of Cancer Prevention. The Manuscript authorship information includes:

Corresponding author: Fadhil Jawad Al-Tu'ma
First Author: Zainab Ali Hadi
Listed Co-Authors: *Zainab Ali Hadi, Atheer Hameid Odda, Ammar Fadhil Jawad, Fadhil Jawad Al-Tu'ma*

You will receive the manuscript's galley proof in later stages for your final approval before being published.

Thank you for your interest in publishing in APJCP.

SA Mosavi Jarrahi, MSPH, Ph.D.
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Serum Antioxidant Status in Sickle Cell Disease Patients: Implications for Oxidative Stress and Disease Severity

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Abstract
Objectives: The main aim of this subject is to determine the oxidative status of Iraqi sickle cell anemic patients and then correlated with various biomarkers.
Methods: In this study, blood samples from 100 sickle cell anemic subjects were analyzed, and then compared with control group which consisting of 50 individuals without sickle cell anemia was established. Various biochemical techniques were employed to measure different oxidative stress markers and inflammatory mediators. Serum samples were collected from blood to determine the levels of antioxidants such as catalase (CAT), glutathione peroxidase (G-Px), reduced glutathione (GSH), and lipid-peroxidation product malondialdehyde (MDA).
Results: The results revealed that the levels of serum antioxidant activity (CAT), GSH, and G-Px were significantly reduced with ($P < 0.05$) in sickle cell anemic patients as compared with apparently control group. In contrast, the MDA level was significantly higher in sickle cell anemic patients than that found in the apparently control group.
Conclusion: In this work, there is an increased oxidative stress in sickle cell anemic patients, which is accompanied by a decrease in antioxidant activity and a rise in lipid peroxidation, leading to the intensification of sickle cell anemic symptoms in patients.
Keywords: Antioxidants, anemia, sickle cell, oxidative stress, reactive oxygen species

Introduction

Sickle cell disease (SCD) is an inherited blood disorder brought on by a mutation in the B-globin gene, also known as hemoglobin subunit beta (HB-beta), which codes for a part of hemoglobin (Hb), the protein complex that makes up 70% of red blood cells (RBCs) and is in charge of carrying oxygen to all body organs.[1,2]

More than 300,000 babies each year were impacted; the United Nations (UN) and the World Health Organization (WHO) classify hereditary blood diseases like SCD as a global health concern. In addition to the United States and Europe, this illness is widespread in most of sub-Saharan Africa, the Middle East, India, the Caribbean, South and Central America, and several Mediterranean nations.[3,4]

Under some circumstances, such as dehydration, illness, or a lack of oxygen, the aberrant, sickled Hb (HbS) in SCD tends to polymerize in RBCs. RBCs are shaped like a sickle or a banana as a result of this process, as seen in Figure 1, which also leads them to become hard and distorted. Sickle cell anemia phenotypic manifestation is a complicated pathophysiologic syndrome with various sources of pro-oxidant mechanisms and resulting chronic and systemic oxidative stress. Erythrocytes live in an environment of constant free radical production in healthy biological systems. To combat ROS, erythrocytes have a self-sustaining activity of antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase(G-Px), reduced glutathione (GSH), and vitamins.[5,6]

Excessive ROS production overwhelms the blood's defenses and destroys biological macromolecules such as proteins, lipids, and DNA, altering the physical properties of RBC membrane, changing membrane permeability, and causing hemolysis.[7] However, reactive oxygen species (ROS) and byproducts of their oxidative processes may be used to predict the severity of SCD.[8] The repeated polymerization and depolymerization of HbS molecules, on the other hand, results in increased levels of reactive oxygen species (ROS), which can cause a cyclic cascade characterized by blood cell adhesion, hemolysis, increased susceptibility to infections, chronic inflammatory diseases, and microvascular damage in organs, resulting in a decrease in quality of life and life expectancy.[9] In SCD patients, sickle erythrocytes are the primary source of pro-oxidants; the unstable, autoxidative HbS and rapid metabolic turnover caused by recurrent HbS polymerizations and depolymerizations stimulate ROS formation.[10]

Oxidative stress plays an important role in the pathophysiology of SCD and its effects.[11] The higher oxidative burden in SCD patients has been linked to a variety of factors. A high level of cell-free hemoglobin, a prolonged inflammatory state, increased HbS auto-oxidation, and iron overload are only a few of the processes.[12] According to previous studies,[13] higher levels of reactive oxygen species (ROS) have been linked to a variety of SCD-related problems. Furthermore, it has been proposed that an altered oxidant/antioxidant balance and increased oxidative stress have a role in the etiology of a variety of disorders in SCD patients.[14] ROS levels rise due to variables such as increased intravascular hemolysis and continuing inflammation.[15] Even though the body has its own system for combating excessive ROS, the antioxidant defense may be overwhelmed by the vast pool of ROS and may not effectively negate their effects in SCD patients.[13]

Despite the fact that various studies have looked at the pathophysiology of SCD in Iraq, there is very little information on oxidative stress. This study was carried out to bridge a knowledge gap and add to the body of knowledge on oxidative stress and antioxidants in SCD patients. The current study's goal was to examine blood antioxidant parameters in healthy

الملخص

فقر الدم المنجلي (SCA) هو الاضطراب الوراثي الأكثر شيوعًا للهيموجلوبين (Hb) ، والذي يصيب ما يقرب من مليون شخص في جميع أنحاء العالم. يتميز باستبدال النوكليوتيدات الفردي في جين بيتا-غلوبين ، مما يؤدي إلى إنتاج الهيموغلوبين المنجلي غير الطبيعي (HbS) مع عواقب متعددة الأنظمة.

حيث يلعب الإجهاد التأكسدي دورًا رئيسيًا في الفيزيولوجيا المرضية لانحلال الدم وانسداد الأوعية الدموية وتلف الأعضاء التالي في مرضى الخلايا المنجلية ، كما يتميز داء الكريّات المنجلية بميل هيموغلوبين الخلية الحمراء إلى بلمرة وتشويه الخلية الحمراء من شكل قرص ثنائي التجويف إلى شكل المنجل ، مما يؤدي إلى انسداد الأوعية الدموية وانحلال الدم المتسارع ، وبالتالي يؤثر على العديد من أجهزة الجسم. داخل الجهاز الهضمي ، يكون الجهاز الصفراوي أكثر شيوعًا في الإصابة بداء الكريّات المنجلية. حيث تتراوح الأعراض من فرط بيليروبين الدم إلى فشل الكبد ، مع مجموعة من الأعراض السريرية الحادة التي يشار إليها غالبًا باسم اعتلال الكبد المنجلي.

حيث ان الهدف من هذه الدراسة هو تصنيع مركب نانوي جديد يتكون من جزيئات أكسيد الحديد الممغنط المطلية بالصبغة البروسية الزرقاء والتحقيق في قدرتها على إزالة البيليروبين المترسب.

تمت دراسة الحالات والشواهد هذه لمدة 10 أشهر من (أيار)2022 و (شباط) 2023 ، في مستشفى كربلاء التعليمي لطب الأطفال ومختبرات الدراسات العليا المتقدمة في قسم الكيمياء والكيمياء الحيوية في كلية الطب في جامعة كربلاء.

حيث كان هناك مجموعه من 100 مشارك تتراوح أعمارهم بين 15 و 60 عامًا ، تم تقسيمهم إلى مجموعتين: 50 مريضًا مصابًا بفقر الدم المنجلي ، وتم تأكيد تشخيصهم من قبل استشاري ، و 50 شخصًا سليمًا كانوا بمثابة ضوابط صحية.

وتم فحص مستويات مصل مضادات الأكسدة بما في ذلك الكاتالاز (CAT) ، الجلوتاثيون بيروكسيديز (GPx) ، الجلوتاثيون المختزل (GSH) وتم اكتشافه باستخدام تجارب تحليلات الكيمياء السريرية ، إضافة لقياس مستوى مصل الدهون بيروكسيد (MDA) في مرضى فقر الدم المنجلي.

من ناحية أخرى ، تم استخدام طريقة الترسيب الكيميائي المشترك لصنع مركب نانو أكسيد الحديد الأزرق البروسي الذي تم استخدامه كمادة ممتزة لإزالة البيليروبين لأنه مركب سام موثق جيدًا حيث

يؤدي إلى انسداد الأوعية وتلف الأعضاء ومن خلال مسارات متعددة المساهمة في أمراض فشل الكبد. كذلك تم استخدام مقياس الطيف المرئي للأشعة فوق البنفسجية ، إمكانات زيتا ، حيود الأشعة السينية ، والمجهر الإلكتروني النافذ لتوصيف المركب النانوي.

أظهرت نتائج الدراسة انخفاضًا في مستويات المصل GPX و GSH مع وجود فروق ذات دلالة إحصائية ($P\leq 0.001$) وزيادة في مستوى MDA مع وجود فروق معنوية ($P\leq 0.05$) في المرضى مقارنة بمجموعة الأصحاء. بينما لا توجد فروق ذات دلالة إحصائية في مستويات اختبارات وظائف CAT في المرضى مقارنة بمجموعة الأصحاء.

تم اختبار أداء المركب النانوي المتكون من أكسيد الحديد الأزرق البروسي لإزالة البيليروبين من عينة الدم في وضع الدُفعات (الرقم الهيدروجيني: 7 ، جرعة الممتزات: 1 مجم / مل ، ووقت التلامس: 30 دقيقة ، التركيز الأولي: 0.25 مجم / مل ، ودرجة الحرارة 37.7 درجة مئوية) وأظهرت النتائج أن قدرة الامتصاص البيليروبين القصوى كانت 81٪وتبين هذه النتائج وجود كميات كبيرة من مادة البيليروبين المترسب في الدم ، وقد تساهم في تطور مجموعة من المضاعفات السريرية الحادة بما في ذلك اعتلال الكبد المنجلي , كما تظهر نتائج العمل الحالي أن Fe_3O_4@PB NPs لديه إمكانات كبيرة تستخدم كمادة ممتزة غير ضارة وحيوي لامتصاص فعال للبيليروبين في المحلول المائي والدم ومصل المريض .

جمهورية العراق
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حالة الاجهاد التأكسدي وازالة سموم البيليروبين غير المقترن في المرضى المصابين بفقر الدم المنجلي

رسالة ماجستير

إلى مجلس كلية الطب/ فرع الكيمياء والكيمياء الحياتية/ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الكيمياء السريرية

من قبل

زينب علي هادي

بكالوريوس علوم كيمياء- كلية العلوم للبنات – جامعة بابل 2013- 2014

باشراف

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1444 **2023**