University of Kerbala

College of Applied Medical Sciences

Department of Clinical Laboratories

Evaluation the Level of Some Hemolysis Markers Visfatin, sVcam-1 in Steady and Crisis State of Sickle Cell Anemia Patients and Related with Vasculopathy

A thesis

Submitted to the Council of the

College of Applied Medical Sciences – University of Kerbala

In Partial of Fulfillment of the Requirements for the Degree of Master in Clinical Laboratories

Written by

Noor Yahya Abidziad Mohammed

B.Sc. Clinical Laboratories\Applied Medical Sciences-University of Kerbala, 2015

Supervised by

Prof. Dr. Abeer Cheaid Yousif Al-Fatlawi

2023 (August) **1445 (Safer)**

بسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيم

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا)

صدق الله العلي العظيم

«سورة طه: الآية 114»

Dedication

I dedicate my humble effort to Imam Mahdi (Peace be upon him).

To the soul of my martyred grandfather, Abdulwahhab Abdulkazim al-Dari'i.

To those who gave me life and hope, and taught me to ascend the ladder of life wisely and patiently, my dear parents.

To those whom Allah has given me the blessing of their presence, those who have helped me in the journey of this study, my brothers and sisters.

Noor

Acknowledgments

Thank God for making things easier to complete at this stage.

All thanks, appreciation, and gratitude to the supervisor, Prof. Dr. Abeer Cheaid Yousif Al-Fatlawi, who was the best helper, supervisor, and professor throughout the period of work and writing.

Thanks and appreciation to Dr. Israa Mustafa Salih Almusawi, who was the support and the other guide that I am proud to know and work with at the hereditary blood disease center / Karbala Health Directorate.

We express our sincere gratitude to the University of Karbala, the College of Applied Medical Sciences, and its respected Dean D. Jawdat and Head of Clinical Laboratories.

Thanks to All the staff at the hereditary blood disease center for their support and help. After that, I would like to thank all the patients who have cooperated with me. and also the patients' families, I wish God to write about their healing and wellness.

Special Thanks to Ali Mohammad Shaheed, who works as laboratory director at Al Hindiya General Hospital for his support and help.

I would like to thank all of my friends, family, and relatives who helped me.

Noor

Supervisor's certification

I certify the thesis entitled (**Evaluation the Level of Some Hemolysis Markers Visfatin, sVcam-1 in Steady and Crisis State of Sickle Cell Anemia Patients and Related with Vasculopathy**) was prepared under my supervision by **Noor Yahya Abidziad Mohammed** at the department of Clinical Laboratories\ College of Applied Medical Sciences\ University of Kerbala, in partial fulfillment of the requirements for the degree of Master in Clinical Laboratories.

Signature

Prof. Dr. Abeer Cheaid Yousif

Supervisor

/ / 2023

Head of Department Recommendation

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature

Assist. Prof. Dr. Linda Hammed Turki

Head of Clinical Laboratories Department

College of Applied Medical Sciences/ University of Kerbala

/ /2023

Discussion Committee Certification

We, the examining committee, certify that we have read the thesis entitled " **Evaluation the Level of Some Hemolysis Markers Visfatin, sVcam-1 in Steady and Crisis State of Sickle Cell Anemia Patients and Related with Vasculopathy** " and and have examined the student (**Noor Yahya Abidziad Mohammed**) in its content and that in our opinion it is accepted as a thesis for degree of Master of **Clinical Laboratories** with **Excellent** degree.

Signature

Prof. Dr. Hanan Jasim Hammod

(Chairman)

/ / 2023

Signature

Prof. Dr. Narjis Hadi Al-Saadi

(Member)

/ / 2023

Signature

Lecture. Dr. Ruqaya Kareem Mohammed

(Member)

/ / 2023

Signature

Prof. Dr. Abeer Cheaid Yousif

(Member & Supervisor)

/ / 2023

I have certified upon the discussion of the examining committee.

Signature

Prof. Dr. Jawdat Noori Gaaib

Dean of the College of Applied Medical Sciences / University of Kerbala

21 / 9 / 2023

Approval Certification

We certify that the thesis entitled (**Evaluation the Level of Some Hemolysis Markers Visfatin, sVcam-1 in Steady and Crisis State of Sickle Cell Anemia Patients and Related with Vasculopathy)** fulfills partial requirements of the degree of Master in Clinical Laboratories.

Signature

Head of Clinical Laboratories Department

Assist. Prof. Dr. Linda Hameed Turki

College of Applied Medical Sciences

University of Kerbala

\ \ 2023

Signature

Vice Dean Scientific Affairs

Assist. Prof. Dr. Huda Abdalreda Abdullah

College of Applied Medical Sciences

University of Kerbala

21\ 9\ 2023

List of Contents

List of Tables

List of Figures

List or Appendices

List of Abbreviations

	Items
CAM	Cell adhesion molecules
CBC	complete blood count
CRP	C- reactive protein
D-D	D-Dimer
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immunosorbent assay
Fe (II)	Ferrous
HB	Hemoglobin
HbF	Hemoglobin Fetal
HbSB-thalassemia	Hetrozygous hemoglobin
HbSC	Hetrozygous hemoglobin
HbSS	Homozygous hemoglobin S
HCT	Hematocrit
HRP	Horseradish Peroxidase
HUVECs	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule-1
LDH	Lactate Dehydrogenase
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean-corpuscular volume
NO	Nitric Oxide
PLT	Platelets
RBCs	Red blood cells

SCA	Sickle cell Anemia
SCD	Sickle cell Disease
TNF	Tumor necrosis factor
VCAM-1	Vascular Cell Adhesion Molecule-1
VOC	vaso-occlusive crises
VSMC	Vascular smooth muscle cell
WBC	White blood cells

Summary

Sickle cell anemia (SCA) is a known hematological disorder that arises from a single point mutation in codon 6 of the β-globin gene that results in a glutamic acid to valine substitution.This mutation leads to the formation of abnormal hemoglobin called HbS. Homozygous hemoglobin S (HbSS disease) is the most common form of SCD, In addition to homozygous SCD (HbSS), other forms such as HbSC and HbSB thalassemia also exist.

This study has been conduct during 3 months between November/2022 and January /2023 in the hereditary blood disease center / Karbala Health Directorate. The total number of participants are (100) person including (35) patients with painful crisis, (35) patients with steady state, and (30) control group, all of the patients and control group were male, in addition, There ages ranged from (3-55).

Patients and healthy individuals provided venous blood samples totaling 5 ml. Each person also provided personal information, including their age, height, and weight. visfatin, and vcam-1 were done by manual kit with Enzyme-linked ImmunoSorbent Assay , while ferritin, Complete Blood Count, C-Reactive Protein, D-dimer, and Lactate dehydrogenase were done by auto chemistry analyzer.

In this study, we aimed to study the role of VCAM-1 as a marker of endothelial dysfunction in SCA patients. Investigation of the role of visfatin, as a marker of inflammation in SCA patients. Compared the expression of visfatin, sVCAM-1, Ferritin, and D dimer, in SCA patients that to control group. Comparing the severity of these markers D dimer, Ferritin, visfatin, and sVCAM-1 in children and adults. Evaluate the association between serum visfatin, sVCAM-1 level in SCA patients, and the frequency of vaso-occlusive crises. Possible usage of these markers as a

predictive index for VOC occurrence. Study correlations among visfatin, sVCAM-1, Ferritin, and D dimer markers with different hemolysis and inflammation markers. The results showed there has been significant differences in concentrations of D dimer, Ferritin, visfatin, CRP, HCT, sVCAM-1, LDH, HB, WBC, RBC, and Retic count, in SCA patient (painful crisis, steady state) in comparison with the control group for both adults and children. Also observed no significant differences in MCH levels between SCA patients and the control group for both adults and children.

The results of MCHC showed that there were significant differences between patients with sickle cell anemia and healthy people, in children only. While in adults there are no significant differences between patients and healthy.

The results also showed that there were significant differences in platelet, and MCV levels between patients with sickle cell anemia and healthy subjects, in adults only, but in children, there were no significant differences between patients with sickle cell anemia and healthy group.

In this study, SCA patients with the painful crisis have significant correlations between (visfatin, LDH), and (visfatin, Ferritin).

Chapter One
Introduction

Chapter One: Introduction

1.1. Introduction

Sickle cell anemia (SCA) is a known hematological disorder that arises from a single point mutation in codon 6 of the β-globin gene that results in a glutamic acid to valine substitution (Benjamin *et al*., 2023). this mutation leads to the formation of abnormal hemoglobin called HbS (Shantanam, 2018). When Hb S is deoxygenated, it becomes less soluble and precipitates due to intraerythrocytic polymerization of deoxyhemoglobin S, and displays morphological change to a crescent shape, Hemolytic anemia, and complications are brought on by these rigid sickle cells (Yousry *et al*., 2016). .

Homozygous hemoglobin S (HbSS disease) is the most common form of Sickle Cell Disease (Noronha *et al*., 2016), In addition to homozygous SCD (HbSS), other forms such as HbSC and HbSB-thalassemia also exist(Adly *et al*., 2016).

The pathophysiology of sickle cell anemia (SCA) arises from hemolytic anemia and acute vaso occlusion; organ damage develops from recurrent erythrocyte sickling, chronic hemolysis, and progressive endothelial Vasculopathy (Ware, 2010). Most of the time, many children with sickle cell anemia are in relatively good health. This is otherwise known as the steady state, This is interrupted occasionally by crises, especially the vaso occlusive type, which is the hallmark of SCA, Crises are characterized by episodes of acute illnesses that cause sickle cell anemia's clinical signs, such as pain, anemia, or jaundice (Nnajekwu *et al*., 2022).

Hemoglobin electrophoresis is the definitive diagnostic for SCA; Nonetheless, a rapid screening test known as the "sickled test" can detect levels of HbS of more than 10% in emergency conditions, but it cannot distinguish between SCD and sickle cell trait (M. Wilson *et al*., 2009).

visfatin is a protein mediator produced by fat cells (Having high levels of expression in visceral fat cells) (Desai & Somannavar, 2011), It appears that other organs and cells, like skeletal muscle, the liver, immune cells, cardiomyocytes, and the brain, are also involved in its production (Dahl *et al*., 2012). visfatin is widely known to be produced by hepatocytes, myocytes, and lymphocytes. Visfatin has several important roles, including promoting preadipocyte differentiation into adipocytes and promoting cytokine production such as tumor necrosis factor-alpha, interleukin-1beta, (Kolańska-Dams *et al*., 2021). Visfatin has been suggested as a marker of endothelial dysfunction, it can directly promote vascular inflammation by activating different cell types including endothelial cells and vascular smooth muscle cells (Romacho, Sánchez-Ferrer, *et al*., 2013), in sickle cell disease patients (Hamed, 2018).

Its broad spectrum of effects is mirrored by its potential involvement in a variety of diseases, such as human immuno- deficiency virus infection, septicemia, myocardial failure, atherosclerosis, metabolic disorders, inflammatory diseases, malignancies, and aging (Dahl *et al*., 2012).

Vascular Cell Adhesion Molecule-1 VCAM-1 is a 90-kDa glycoprotein that is inducible and predominantly expressed in endothelial cells. In 1989, VCAM-1 was first recognized as a cell adhesion molecule, that helps regulate inflammation-associated vascular adhesion and the trans endothelial migration of leukocytes, such as macrophages and T cells (Kong *et al*., 2018).

Several studies show that levels of soluble VCAM-1 increased in sickle cell anemia patients. VCAM-1 is among the most important inflammatory factors and plays a major role in sickle cell anemia. It is a useful marker for SCA management, as they are endothelial surface activation indicators can be connected with the disease's

severity (Al Mudhafar *et al*., 2019).

The higher levels of sVCAM observed in SCA patients confirm that these patients have elevated endothelial dysfunction. In addition, the high connection between sVCAM and markers of hemolysis suggests that endothelial dysfunction may, in part, be a result of decreased nitric oxide availability (Ataga *et al*., 2008). During hemolysis, free hemoglobin is decompartmentalized from the erythrocyte and is released into plasma, where it quickly interacts with and destroys NO, as a result, NO consumption is excessively high, and reactive oxygen species are produced, ultimately inhibiting vasodilation (Vilas-Boas *et al*., 2010).

D-dimer is a fundamental degradation fragment of fibrin and acts as an indicator of the syntheses and breakdowns of fibrin. D-dimer has proved instrumental in diagnosing, monitoring, and addressing the impacts of venous thrombosis and embolism (VTE) and has now become a part of routine healthcare precautions, Chronic inflammatory conditions like asthma, malignancies, sickle cell disease, and rheumatoid arthritis (Hashim *et al*., 2023).

The level of D-dimer was significantly higher in patients with SCD; and significant elevation was observe when comparing individuals with VOC to steady state, this is in keeping with many reports of elevated D-dimer in SCD. Elevated plasma D-dimer levels indicate increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation, Increasing thrombin activity and fibrin formation are features of steady-state sickle cell disease, and they further increase during painful crises

(Mohamed *et al*., 2020).

Ferritin: one of the key proteins regulating iron homeostasis, it is used as a biomarker to evaluate iron deficiency, insulin resistance, and metabolic syndrome and may serve to detoxify excess iron. Thus, serum ferritin frequently used as a first-line blood test when iron overload is suspect. It has been reported that serum ferritin levels were significantly increased in sickle cell anemia and β-thalassemia (Alsultan *et al*., 2010).

Ferritin is a protein found inside the cells of the body and very little of it is released into the serum (Mostafa *et al*., 2020) but is in high concentrations in marrow macrophages, the spleen, and the liver (Cullis *et al*., 2018).it is important to understand the physiology of iron uptake. The eventual destruction of aged erythrocytes (whether native or transfused) and hemoglobin degradation takes place in the macrophages of the liver, bone marrow, and spleen. Hemoglobin is digest and iron is released bound to transferrin. The remaining iron enters the storage pool as ferritin or hemosiderin(Smith *et al*., 2014).

Multiple etiologies can result in elevated serum ferritin levels, including iron overload, inflammation, liver or renal disease, malignancy, and the recently described metabolic syndrome, while low serum ferritin levels invariably indicate reduced iron stores (Cullis *et al*., 2018).

Aims of the study:

1. Aim to study the role of VCAM-1 as a marker of endothelial dysfunction in SCA patients.
2. Investigation of the role of visfatin, and C- Reactive Protein, as a marker of inflammation in SCA patients.
3. Comparing the expression of visfatin, sVCAM-1, Ferritin, and D dimer, in SCA patients that to healthy individuals.
4. Investigation of the role of CBC, LDH, and D-dimer, as a marker of hemolysis in SCA patients.
5. Comparing the severity of these markers D dimer, Ferritin, visfatin, and sVCAM-1 in children and adults.
6. Evaluating the association between serum visfatin, sVCAM-1 level in SCA patients, and the frequency of vaso-occlusive crises. Possible usage of these markers as a predictive index for VOC occurrence.
7. Studying correlations among visfatin, sVCAM-1, markers with different hemolysis and inflammation markers.

Chapter Two
Literature Review

Chapter Two: Literatures Review

2.1. Literatures Review

2.1.1. Sickle Cell Anemia: is a genetic disease resulting from the exchange of amino acids in the hemoglobin protein, which carries oxygen in the blood, transforming the natural disk shape of red blood cells into the sickle-like shape (Chillab *et al*., 2019),it was first described by Herrick in 1910 when JAMES B.HERRICK described a case of anemia in a dental student from Grenada that was associated with "peculiar elongated and sickle-shaped red blood corpuscles" on microscopy (Williams & Thein, 2018).

The inheritance of sickle cell anemia occurs via an autosomal recessive gene with both parents, in general, asymptomatic individuals who carry a single affected gene (heterozygous), transmitting the defective gene to their child, who therefore is homozygous (Hb SS) (Lervolino *et al*., 2011). The discovery of hemoglobin S (HbS) by Linus Pauling and colleagues in 1949 was the first demonstration that the production of an abnormal protein could be the cause of a genetic disorder (Odièvre *et al*., 2011).

This type of hemoglobin is produce by replacing glutamic acid instead of valine in position six of the β-chain on chromosome 11, which further produces deoxygenation-induced polymerization and finally results in the abnormal crescent shape of red blood cells (RBCs). Also, this sickled hemoglobin makes RBCs vulnerable to broken easily resulting in extravascular and intravascular hemolysis with a result of low hemoglobin levels (Ali Hazzazi *et al*., 2020).

Standard RBCs have a spherical form and are compact and resilient, which makes it easy for them to pass through narrow capillaries (Sen *et al*., 2021). Low oxygen tension promotes the sickling of red blood cells and repeated episodes of sickling damage the cell membrane and reduce its flexibility, in sickle cell disease. When

normal oxygen tension is restored, these cells fail to return to their original form, due to their rigidity, these blood cells cannot deform as they pass through narrow capillaries, which causes vascular blockage and ischemia (Obeagu *et al*., 2015).

2.1. Etiology of sickle cell anemia:

Genetic causes: A single-base mutation in the sixth codon of the beta-globin chain on chromosome 11 is what causes SCA. This leads to the formation of mutant sickle hemoglobin (HbS) (Hebbel *et al*., 2012), On chromosome 11, this mutation causes valine to replace glutamic acid at position six of the beta-globin chain gene (Elnaim *et al*., 2020), and HbS β-globin chains are substituted for normal HbA β-globin chains (Steinberg, 2008).

2.2. Type of Sickle Cell Disease (SCD):

There are at least five different sickle-gene mutation haplotypes, each with a unique HbF level and illness severity, The Arab-Asian and Senegal haplotypes are associated with relatively higher HbF levels and milder sickle cell disease (SCD), while the Bantu ,Benin, and Cameroon haplotypes are associated with relatively lower HbF levels and severer SCD (Ahmed & Ibrahim, 2023).

Homozygous HbSS (sickle cell anemia) is the most common and severe form of SCD with βS inheritance from both parents, Which permits the formation of the pathological sickle hemoglobin tetramer (α2 βS 2, HbS) (Ware *et al*., 2017), hemoglobin SC, resulting from a compound hetero- zygosity for two different mutations (S and C), is the second most common genotype representing about one-quarter the cases, Compared to homozygous patients, hemoglobin SC disease is characterized by a lesser degree of hemolysis and higher levels of hemoglobin (Hammoudi *et al*., 2020).

Sickle β thalassemia, a type of SCD that affects Hb concentration, is a hereditary autosomal recessive conditions, each copy of the patient's HbB gene carries a different mutation: one that results in crescent-shaped red blood cells (RBCs) and a second that is associated with β-thalassemia. People may have sickle B- zero thalassemia, which is the absence of normal Hb, or sickle B plus - thalassemia, which is a reduction in normal Hb based on the -thalassemia mutation (Benjamin *et al*., 2023).

HbSE disease (compound heterozygosity for the HbS and HbE genes), and HbSO Arab (compound heterozygosity for the HbS and HbO Arab genes) Other rare genotypes of SCD are also found (Steinberg, 2009).

HbSS disease and sickle β0 thalassemia often are referred to as sickle cell anemia because of their similar severity (Noronha *et al*., 2016).

2.3. Epidemiology:

SCA is the most common hemoglobinopathy globally, Recent estimates suggest that globally, more than 300,000 children are born with SCA every year (Williams & Thein, 2018), and its incidence is expected to increase to 400,000 neonates born per year by 2050 (Allali *et al*., 2020).

SCA is prevalent in areas of Africa, the Mediterranean, the Middle East, India, the peninsula, and Southeast Asia. (Atiyah *et al*., 2021), the United States, and South or Central America (Tanabe *et al*., 2020). The greatest burden of sickle cell anemia (SCA) is in sub-Saharan Africa (SSA), where 75% of the 300,000 global births of affected children live, and estimates suggest that 50–80% of these patients will die before adulthood (Makani *et al*., 2011).

In Iraq, Sickle cell disorders, are less uniformly distributed with carrier rates varying from 0 to 16.0%, and they cluster in the extreme north and south of the country

(Badr, 2022). the prevalence of sickle cell disease hemoglobin was low 0.56%, which is not representing the true figure of sickle cell carriers in the Karbala governorate because there is no definitive cure for these genetic conditions (Atiyah *et al*., 2021).

In Iraq Patients with SCA are clustered in two geographical areas, where they represent a major health problem. The highest frequency is among the Arabs in the extreme south where 6.48% of the how population of Basra are carriers for the sickle cell trait, and the Kurdish population in the north is the second-most prevalent area, where 1.2% of individuals of Dohuk are carriers (Uzsoy, 2020).

In Iran, one of the major health problems is sickle cell anemia. The native and main region of SCD is southwest of Iran (Jalali Far *et al*., 2021). In Oman, SCA is a major public concern, where 5.7% of the population carries the sickle cell gene and 0.3% of Omani people have SCA (Alkindi *et al*., 2021). According to estimates, there are 24 cases of SCA for every 10,000 people in Saudi Arabia. The regional distribution showed that the eastern and southern regions have the highest prevalence rates. (Kotb *et al*., 2019).

2.4. Steady-state sickle cell anemia:

The SCD clinical course is defined by stable, pain-free periods of comparatively good health known as a "steady state.", which is intermittently interrupted by painful and unstable periods referred to as ‘crisis’ (Ahmed & Ibrahim, 2023).

Ballas defined a steady state as a period during which the patient is not having any acute painful crisis or any changes due to therapy. SCA participants must fulfill the following criteria reported by Ballas to be in a steady state.

There should be no history of an acute painful episode that required treatment in the emergency department or the hospital for four consecutive weeks after a previous painful crisis. Second, there is no previous history of emergency department or hospital admissions 2–3 days after the point in time in question. Third, no history of blood transfusion during the previous four months. Fourth, no history of intercurrent illness such as infection, or inflammation during the previous 4 weeks, and last, no treatment with medications such as antibiotics that may affect the blood counts during the previous 3 weeks (I *et al*., 2022).

2.5. Pathogenicity of sickle cell Anemia (SCA):

Hemoglobin (Hb) is about 95% of the dry erythrocytes' weight; it is a necessary protein for erythrocytes to carry out their oxygen transport function. Hb transports around 23% of the total CO2 and waste product of metabolism (Saeed & Abolaban, 2021). Hemoglobin A, the standard form of adult hemoglobin, has two alpha-globin chains and two beta-globin chains (Lucarelli, 1997).

The primary event in the pathogenesis of SCA is HbS polymerization occurring in deoxygenated erythrocytes, the sickled erythrocytes shorten the lifespan of red blood cells and block blood vessels, leading to hemolysis, vasculopathy, and acute vaso,-occlusion (Disorders, 2012). The pathophysiology of SCD includes a chronic hemolytic anemia and red blood cells sickling because of acute hemoglobin polymerization with deoxygenation leading to repeated painful vaso-occlusive crises, endothelial cell dysfunction, and inflammation (Hammoudi *et al*., 2020).

2.5.1 Hemolysis: The substitution of one amino acid in the hemoglobin molecule results in sickle hemoglobin. As a result, RBCs sickle in low oxygen states causing occlusion of blood vessels, increased viscosity, and inflammation. These RBCs are

prematurely removed from the circulation, resulting in chronic hemolytic anemia (Kavanagh *et al*., 2022).

Hemolysis in SCA results in increased levels of free plasma hemoglobin, which has been shown to bind and inactivate nitric oxide (NO)(Rees & Gibson, 2012). Severe hemolysis, SCA is characterized by the release of cell-free hemoglobin and arginase into the plasma. arginase competes with eNOS for L-arginine, leading to reduced NO production, It is now recognized that hemolysis also plays a significant role in disease pathogenesis, possibly via decreased bioavailability of nitric oxide (NO) (Akinsheye & Klings, 2010),

Impaired NO bioavailability represents the primary characteristic of endothelial dysfunction, and is a common denominator in the pathogenesis of vasculopathy in sickle cell disease (SCD) (Melchionda et al., 2013). Endothelial dysfunction is associated specifically with anticoagulant properties, increased platelet aggregation, and increased expression of vascular adhesion molecules (VCAM-1, ICAM-1, and E-Selectin) (Antwi-Boasiako, Donkor, *et al*., 2018).

NO is a soluble gas produced by endothelial cells which is a known endogenous potent vasodilator and has a blood flow regulatory effect (Antwi-Boasiako & Campbell, 2018), In the normal physiology of the vasculature, it is a major endothelial-derived relaxing factor and plays a central role in vascular homeostasis (Antwi-Boasiako & Campbell, 2018), reduces endothelial activation, and limits platelet activation (Schnog *et al*., 2005), also regulates some vessel-platelet interactions. At the release site, it mediates local vasodilatation, antagonizes platelet aggregation, and inhibits vascular smooth muscle cell proliferation (Melchionda *et al*., 2013).

2.6. Clinical Manifestation of sickle cell Anemia (SCA):

The clinical manifestation of sickles corpuscles differ. Many people may have simple manifestations but other patients possess hard manifestations and should be set in a hospital for therapy (Bookchin & Lew, 1996). The clinical outcome of SCA ranges from mild to moderate to severe with acute to chronic clinical manifestations (L. V. Bhaskar, 2019).

The signs and symptoms of sickle cell anemia, which differ from person to person, and changed over time, include; anemic episodes, painful swelling of hands and feet, frequent infraction, delayed growth and visual problems Sickle cell anemia can lead to stroke: it can occur if sickle cells abstruct blood flow to area of the brain. Acute chest syndrome, hyper pulmonary tension, organ damage, blindness and leg ulcer with gallstones and another symptom of sickle cell crisis or sickling crisis may be used when describing several independent acute conditions that were occurring in people with sickle cell anemia or in patients with sickle cell diseases (Elnaim *et al*., 2020).

2.7. Sickle Cell Anemia complications:

Patients with sickle cell anemia usually need to stay in hospitals multiple times because of the related complications, additionally, many of the affected children suffer from premature death. About 50% of individuals who survive have documented irreversible organ damage. The risk of early death is highest among patients who have had severe complications, such as recurrent acute chest syndrome, and pulmonary hypertension, and renal failure (Kotb *et al*., 2019).

Acute complications are more likely to occur in sickle cell anemia (SCA) patients, such as a vaso-occlusive crisis, acute chest syndrome, acute bone marrow necrosis, stroke. Chronic organ damage from abnormal hemoglobin can also occur, especially

when in heart , kidneys, eyes (retinopathy), skin (ulcers), or bones (aseptic osteonecrosis) (Stankovic Stojanovic & Lionnet, 2016). Chronic complications range from chronic pain, cerebro-vascular disease/stroke, and hepatopathy, Chronic complications can be attributed to the vicious cycle of sickling, which causes endothelial injury, inflammation, leading to a vasculopathy (National Association of Resident Doctors of Nigeria, 2015). With the use of the vaccination program and the development of therapeutic care, the development of tissue and organ complications in childhood is delayed until older ages, The main clinical problem that causes these individuals' frequent hospitalizations is recurrent acute vaso-occlusive episodes (L. V. K. S. Bhaskar, 2021).

Acute Vaso-Occlusion crisis (painful crisis):

The acute sickle cell painful crisis (acute vaso-occlusion) is the hallmark of SCA and the first cause of hospitalization. clinical features of a typical painful crisis were described accurately by Diggs, that “patients experience a sudden onset of pain in the low back, in one or more joints (Ballas *et al*., 2012), bones, chest, or other parts of the body, and may last several hours to days (Aboursheid *et al*., 2022).

The proximate cause of vaso- occlusive pain crisis is occlusion of the microcirculation of the bone marrow by sickled red blood cells (Coates *et al*., 2011).

Sickle cell pain often becomes chronic, resulting in poorer quality of life , early and aggressive treatment of acute sickle cell pain may reduce the development of chronic pain , Chronic pain is often associated with other morbidities, including depression, anxiety, despair, insomnia, loneliness, helplessness, and dependence on pain medications (Yawn *et al*., 2014).

2.8. Diagnosis:

More than 900 hemoglobin (Hb) variants are currently known. An estimated 150 million people Worldwide, carry Hb variants. and hemoglobinopathies are the commonest inherited disorders, constituting a significant healthcare problem. Therefore, reliable detection and identification methods are essential (Jeddoa *et al*., 2011).

Sickle cell disease can be simply diagnosed, since there is an abundance of hemoglobin in the blood and numerous techniques can identify HbS and variant hemoglobins, Electrophoresis separating normal from abnormal hemoglobins is the most common technique, using standard alkaline gel, capillary electrophoresis, isoelectric focusing or high-performance liquid chromatography Chemical (Ware *et al*., 2017). DNA testing can help in clarify the diagnosis in cases when Hb electrophoresis is inconclusive (Mbayabo *et al*., 2022).

The essential laboratory tests for evaluating a patient with suspected sickle cell disease are a complete blood count (CBC), a reticulocyte count, and a peripheral blood smear. The peripheral smear is available for review more quickly than the results of the hemoglobin electrophoresis. Characteristic findings on the peripheral smear include sickle forms (HbSS), target cells and HbC crystals (HbSC or one of the HbSb thalassemias), and Howell– Jolly bodies(Pecker & Lanzkron, 2021).

2.9. Prevention and Treatment:

There are a few management strategies from vitamin intake to bone marrow or stem cell transplant to subdue pain and complications of sickle cell anemia, treatment is necessary to reduce mortality and morbidity in patients with sickle cell disease. (Joseph, 2022).

Pain may require hospitalization for more analgesics, and blood transfusion is often required. any infection that is suspected should be treated with broad-spectrum antibiotics after a culture of blood, sputum, and urine (M. Wilson *et al*., 2009). In cases of extreme pain or symptoms where at home care management is not working seeking a doctor is advised. During this hospital visit a provider may check for infection, fever, crisis triggers, or dehydration. For Pain patients may be prescribed more aggressive pain medication such as oxycodone, codeine or other nonsteroidal anti-inflammatory drugs depending on their level of pain. In severe cases, providers may prescribe meperidine, hydromorphone or morphine (Joseph, 2022).

Without adequate treatment, sickled RBCs and the associated hemolytic anemia results in innumerable acute and chronic clinical complications, including increased susceptibility to invasive bacterial infection due to splenic auto infarction, acute chest syndrome, stroke, chronic organ damage, and shortened lifespan (Shantanam & MUELLER, 2018b).

In Karbala governorate established hereditary blood disease center in 2004 for manage and follow up patients, and put premarital hemoglobinopathies screening program, it consider important tool for prevention and raising awareness diseases (Attiyah & Al-Najafi, 2020).

2.10. Markers:

2.10.1 Visfatin:

Adipose tissue is a known endocrine organ secreting several soluble factors, known as adipocytokines or adipokines, some of these being adiponectin, leptin, resistin, and, more recently, visfatin. They can partly explain the link between obesity, insulin resistance, beta-cell dysfunction, endothelial dysfunction, and atherosclerosis (Saddi-Rosa *et al*., 2010).

Visfatin is a multifaceted adipokine whose circulating levels are enhanced in different metabolic diseases, which is synthesized and released by Human endothelial cells (mainly released by visceral fat) (Romacho, Villalobos, *et al*., 2013), Visfatin is produced by a variety of sources such as macrophages, chondrocytes, and amniotic epithelial cells in addition to adipose tissue. The main effect of visfatin is to promote inflammatory processes. Visfatin also has important effects on the cardiovascular system such as endothelial dysfunction, atherosclerosis, myocardial damage, and fibrosis (Erten, 2021).

Visfatin is a pro-inflammatory cytokine that released by macrophages and monocytes that infiltrate tissue spaces in response to inflammation and can directly interact with vascular cells. Serum visfatin levels are elevated in SCD patients compared to healthy individuals. and is positively correlated with the frequency of VOC; It serves as a predictor of the occurrence and follow-up of VOC crises (Benjamin *et al*., 2023).

2.10.2. Vascular Cell Adhesion Molecule-1 (VCAM-1):

Cell adhesion molecules (CAM) are glycoproteins expressed on the surface of endothelial cells. CAM are responsible for leukocyte adhesion and transendothelial

migration to target tissues, which are essential in several biological events such as cell growth and differentiation, response to infection and inflammation, tissue healing, and immune cell response (Monteiro *et al*., 2023). They have been divid into four groups: selectins, cadherins, immunoglobulin-like adhesion molecules, and integrins. Cell adhesion is Important to maintain the structure and normal function of tissues. It is essential for providing an effective immune response to foreign infections. Vascular cell adhesion molecule-1 (VCAM-1) is expressed on activated endothelial cells and is an immunoglobulin-like adhesion molecule. It is able to bind to integrin 41, which is expressed constitutively on lymphocytes, eosinophils, and monocytes (Al Mudhafar *et al*., 2019).

The migration of activated T lymphocytes and macrophages from blood arteries is mediated by the interaction of selectins, such as E-selectin, with endothelial adhesion molecules, such as VCAM-1. During the inflammatory process, it also initiates the adhesion of neutrophils, monocytes, eosinophils, and T lymphocytes to the vascular endothelium. (Zdanowska *et al*., 2020).

VCAM-1 can also be cleaved from the surface of the endothelium or other cells to produce a soluble form (sVCAM-1), as such, serum sVCAM-1 levels had elevated in patients with immunologic disorders, such as rheumatoid arthritis, and autoimmune disorders, including multiple sclerosis, interestingly, The serum levels of sVCAM-1 is also increased in patients with various malignancies. Specifically, in many types of cancers, such as breast, gastric, ovarian and melanoma (Torre & Emert, 2019).

Soluble VCAM-1 serves as a promising biomarker of pain in VOC, higher levels of VCAM-1 might be very useful in predicting patients at high risk of endothelial dysfunction especially, in the case of SCA patients with VOC (Antwi-Boasiako, Donkor, *et al*., 2018).

2.10.3. D dimer:

D-dimer is a substance that is created when cross-linked fibrin is broken down with the aid of factor XIII. Its presence in the bloodstream is an indication that the hemostatic system is still being activated (Hashim *et al*., 2023). D-dimer is a biomarker that globally indicates the activation of hemostasis and fibrinolysis. it is a fibrin degradation product that is created when plasmin-induced fibrinolytic activity breaks down cross-linked fibrin (Ay *et al*., 2012).

D-dimers are products gotten from the degradation of cross-linked fibrin. Its assay commonly used to rule out venous thromboembolism for diagnostic and prognostic purposes. D-dimer is considered to be a particular product of the thrombosis process, In the process of thrombosis, D-dimer is considered a particular end product (AO *et al*., 2022).

D- dimer assays were first introduced in the 1970s, they were primarily used to check for evidence of disseminated intravascular coagulation, a condition that promotes rapid turnover of clot formation and breakdown within the microvasculature (Linkins & Takach Lapner, 2017). D-dimer can be elevated during pregnancy and puerperium, increasing age (>65 years), African American heritage, cigarette smoking, recent trauma, and the postoperative period, Thrombosis, Cardiovascular disease, Renal disease, Liver disease, Alzheimer's disease, sickle cell disease (Pulivarthi & Gurram, 2014). concentration of D-dimer higher in crisis than in the steady state (I *et al*., 2022).

D-dimer levels and other markers of coagulation activation are elevated in SCA and have been shown to correlate to stroke, retinopathy, and vasoocclusive crises (Naik *et al*., 2016). Chronic inflammatory conditions like asthma, malignancies, sickle cell disease, and rheumatoid arthritis have an inclination of raising D- dimer titer among

individuals (Hashim *et al*., 2023). The level of D-dimer increased when anemia was induced. Thromboembolism is one of the hemostatic consequences that typically accompanied sickle cell anemia, Increased D-dimer concentrations in the blood are an indication that thrombi have been formed and degraded in the body (AO *et al*., 2022).

2.10.4. C-Reactive Protein (CRP):

Sickle cell disease has been described by many scholars as a chronic inflammatory disease that is linked to several factors such as, increased synthesis of reactive oxygen species, hemolysis, endothelial destruction, and increased synthesis of pro-inflammatory cytokines among others. Inflammatory processes play a significant role in the activation of the acute painful vaso-occlusion crisis, which is the primary cause of sickle cell anemia patients' hospitalization (Obeagu & Muhimbura, 2022). Inflammation among sickle cell disease patients is a ceaseless process that occurs even during a steady state (Abdelmaged, n.d.).

Tillett and Francis first discovered C-reactive protein (CRP) in 1930. as precipitation was observed with the addition of pneumococcal C-polysaccharide to the serum of a patient with acute pneumonia (Ingle & Patel, 2011). CRP has become a non-specific but sensitive indicator of inflammation since its discovery (Mahmoud & Rivera, 2002).

The liver produces CRP as part of the acute phase reaction in response to a variety of pro-inflammatory cytokines. CRP has wide acceptance as a reliable indicator of systematic inflammation and tissue damage (Kaddam & Kaddam, 2020). During 50 hours, it quickly reaches its peak levels, falls once the inflammatory stimulus is removed, and has a half-life of 18 h (A. M. Wilson *et al*., 2006).

Circulating CRP levels are generally stable, are determined by their rate of synthesis, and fall within a characteristic range for each individual (Mohammed *et al., 2010)*. Increased CRP levels are thought to be an important risk indicator for peripheral vascular disease, ischemic stroke, atherosclerosis, and myocardial infarction. Weight loss, anorexia-cachexia syndrome, the severity of the illness, and recurrence in advanced cancer are all positively correlated with it (Mahmoud & Rivera, 2002), and it is consider the early predictor of vaso occlusive crisis in a patient with SCA (Al-Basheer *et al*., 2015). CRP has been broadly approved as reliable indicators of systemic inflammation and tissue damage, In sickle cell disease patients, high levels of CRP have been reported (Abdelmaged, n.d.).

2.10.5. Lactate Dehydroginas (LDH):

An intracellular enzyme called lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate during anaerobic glycolysis (Li *et al*., 2020), LDH is rapidly released into the cell culture supernatant when the plasma membrane is damaged, a key feature of cells undergoing apoptosis, necrosis, and other forms of cellular damage (Kumar *et al*., 2018). The LDH reaction is reversible and is required to use lactate, a major circulating carbohydrate in mammals, as a fuel to support cellular functions, LDH is thus a key node in carbohydrate metabolism (Hicks *et al*., 2023).

LDH has five different isoenzymes, as determined by their electrophoretic migration. Although LDH is present in all tissues, each isoenzyme dominates in a specific tissue (Stankovic Stojanovic & Lionnet, 2016): LDH-1 and LDH-2 are found in erythrocytes and cardiac muscle; LDH-3 in lymphatic tissue, platelets, lung, and the pancreas; and LDH-4 and LDH-five in the skeletal muscle and liver (Mecabo *et al*., 2015).

Blood LDH is the marker enzyme, which predicts mortality in many conditions such as ARDS, serious COVID-19, and cancer patients. Lactate plays a critical role in the normal physiology of humans including as an energy source, a signaling molecule, and a pH regulator (Gupta, 2022).

LDH is a tissue damage indicator that is elevated in a variety of clinical conditions, such as hemolysis, cancer, severe infections and sepsis, liver diseases, hematologic malignancies, and many others (Wu *et al*., 2020), cell death, hypoxia (in the course of respiratory failure), diseases of the hematopoietic and lymphatic systems, or inflammation of the lungs, pericardium, and pancreas. The highest concentrations are found in the heart, lungs, liver, skeletal muscle, and many others (Fialek *et al*., 2022). Serum LDH has been the most widely used marker of intravascular hemolysis (van Vuren *et al*., 2021). LDH elevation is thought to be a marker of SCA patients with the syndrome of hemolysis-associated nitric oxide (NO) resistance, endothelial dysfunction, and end-organ vasculopathy (Gurkan *et al*., 2010). LDH levels in SCA patients increase in severity of VOC (Meher *et al*., 2019).

2.10.6. Ferritin:

Ferritin was first identified in 1937 when French scientist Laufberger found a novel protein from horse spleen that contained up to 23% by dry weight of iron, and this protein was later named ferritin (W. Wang *et al.*, 2010).it is a water-soluble protein that naturally exists in most organisms. Ferritin is a 450 kDa spherical hollow nanocage, its internal diameter is around 8 nm, and its outside diameter is approximately 12 nm (X. Sun *et al*., 2021). initially, ferritin was considered predominantly a cytosolic protein with a general iron storage function. Ferritin can also be found in other cell compartments such as the nucleus, mitochondria, or lysosomes where it may play various roles depending on the cellular context (Plays *et al*., 2021).

Ferritin is a valuable tool for the doctor's evaluation, both for the evaluation of illness conditions, such as iron deficiency anemia, and for the evaluation of hereditary and acquired iron overloads conditions, such as hereditary hemochromatosis and chronic transfusion therapy. To identify and treat these conditions, doctors typically perform a panel of blood tests, including serum ferritin levels (W. Wang *et al*., 2010). Normal serum ferritin levels vary between laboratories, but generally, concentrations >300 µg/L in men and postmenopausal women and >200 µg/L in premenopausal women are considered to have elevated levels. Low ferritin values provide absolute evidence of iron deficiency, increased levels usually indicate an excess of iron, however, they are not specific, as ferritin is an acute phase protein and is also released from damaged hepatocytes; Therefore, levels are increased in inflammatory disorders, liver disease, excessive alcohol consumption, or cancer (Koperdanova & Cullis, 2015).

The iron absorbed in the small intestine is transported by transferrin to the bone marrow for the production of hemoglobin and excess iron is stored as ferritin intracellularly, A small part of ferritin is secreted into the blood (Sukla *et al*., 2022).

The rise in the serum ferritin in sickle cell anemia patients of this study may be due to the following reasons:

(a) Excess free iron, due to excessive Hb breakdown and abnormal Hb circulation mass in reticuloendothelial cells, exerts positive feedback on ferritin synthesis.

(b) patients did not donate blood transfusion, but continuous severe anemia may predispose them to excessive gastrointestinal absorption of iron from dietary iron intake, specifically haem iron from red meat (which is the normal diet in the eastern area of Saudi Arabia), which is known as one of the main determinants of body iron causing an increase in ferritin level (Alsultan *et al*., 2010).

2.10.7. Complete Blood Count (CBC):

2.10.7.1. Hemoglobin (HB):

In 1840, H. unfold accidentally found hemoglobin (Hb) in samples of earthworm blood held between two glass slides. He occasionally discovered tiny plate-like crystals crystals in desiccated swine or human blood samples. Later, in 1864, Hoppe-Seyler named these crystals the name "Haemoglobin". Around 1870, Claude Bernard discovered its role as an oxygen carrier. But Perutz, along with Sir John Kendrew, received the Nobel Prize in Chemistry (1962) for discovering the detailed three-dimensional structure of Hb by X-ray crystallography (Saha *et al*., 2014).

Hemoglobin (Hb) made up of two globin dimers, each with an associated heme moiety. In most adults, HbA (a2, b2) makes up 97% of the total Hb, A2 (a2, d2) makes up 1.5–3.5%, and fetal hemoglobin (HbF; a2, c2) makes up less than 2%.These percentages may vary with some hemoglobinopathies. For example, the presence of hereditary persistence of HbF, beta-thalassemia, sickle cell disease, pregnancy, anemia, and some leukemias causes an increase in HbF levels. levels may be elevated among individuals who are hospitalized (Gallagher *et al*., 2009).

The main protein found in red blood cells, hemoglobin (Hb), is a tetrameric metalloprotein made up of four iron-containing heme parts and a globular protein part. Many diseases, including anemia, thalassemia, heart disease, leukemia, sickle cell disease, and excessive blood loss, can be diagnosed by measuring the Hb concentration (Y. Sun *et al*., 2016). The function of hemoglobin (Hb) is to carry oxygen throughout the body and is the basis of life support; Therefore, the body needs correct management of Hb synthesis. Due to the fact that adult erythrocytes have a 120-day lifespan, mature erythrocyte Hb cannot sensitively reflect Hb synthesis. While this is happening, reticulocytes are released into the peripheral

blood from the bone marrow, and they later develop into mature erythrocytes in the following 1–2 days (Ogawa *et al*., 2020).

Several genes encode different types of globin proteins, and their various tetrameric combinations generate multiple types of Hb, which are normally expressed at different stages of life — embryonic, fetal, and adult (Kato *et al*., 2018). Hemoglobin (Hb), the primary blood oxygen (O2) carrier, is affected by globin gene mutations frequently; it is believed that 7% of the world's population is affected (Weatherall and Clegg 2001; Kohne 2011). These mutations can be widely separated into those that affect the production of globin protein subunits (thalassemias) and those that result in structurally abnormal globin proteins (Hb variations). The latter class is mainly made up of missense mutations that result in a single amino acid substitution in the globin protein, producing an abnormal or "variant" Hb tetramer. Naturally occurring Hb mutations cause a range of biochemical abnormalities, some of which produce clinically significant symptoms. The most common and medically important Hb variants include HbS, HbC, and HbE (Thom *et al*., 2013).

2.10.7. 2. White Blood Cell (WBC):

White Blood Cell (WBC) or leukocyte is one of the components of human blood, in addition to red blood cells and platelets. Every blood cell has its function.it is closely related to the human immune system which is useful to fight viruses and bacteria (Mohd Safuan *et al*., 2018).

immune system includes white blood cells, which perform a variety of functions related to organism defense. In order to reach the sites of action (such as sites of inflammation) WBCs must migrate to the vessel walls through a process known as margination, adhere efficiently to vascular endothelium (mediated by adhesion proteins, and then migrate into tissue surrounding it (Fedosov *et al*., 2012). In the

medical field, the analysis of white blood cells (WBC) is of vital importance for diagnosing diseases, usually, the proportion of WBC reveals important diagnostic information which can help clinicians make correct treatments for their patients and observe the curative effect. Therefore the detection of WBC is a meaningful task(X. Wang *et al*., 2018).

WBCs can be classify into two types, defined by the appearance of the cytoplasm. The first type is Granulocytes and includes Basophils, Eosinophils, and Neutrophils. The second group, called AGranulocytes, includes Lymphocytes and Monocytes (Sahlol *et al*., 2020).

2.10.7.3. Red Blood Cell (RBC):

Human red blood cells (RBCs) are highly differentiated cells that, during the course of maturation, have lost all organelles and most internal machinery. RBCs play a critical role in nearly all basic physiologic dynamics and are essential components of the body's respiratory system because they transport oxygen to all cells and tissues, and delivery of carbon dioxide to the lungs. RBCs can deform due to their flexible shape, allowing them to pass through all blood vessels and even small capillaries (Pretini *et al*., 2019).

Erythropoietin (EPO), a hormone produced by fibroblasts of peritubular capillaries in the kidney that regulates the production of new RBCs by stimulating proliferation and differentiation of erythroid precursor cells in the bone marrow (Arias & Arias, 2017).

Sickling of RBCs is the main pathologic event in SCD. Under hypoxic conditions, sickle RBCs undergo a dramatic change in morphology due to the polymerization of abnormal hemoglobin tetramers. These changes result in the formation of sickle-shaped RBCs with decreased flexibility (Faes *et al*., 2019).

2.10.7.4. Platelets:

The smallest blood cells are platelets, numbering 150 to 350 109/L in healthy individuals. The 19th century was the first description of activated platelets' ability to adhere to injured vessel walls and form aggregates. Platelets are increasingly recognized to play vital roles in numerous other pathophysiological processes, such as inflammation, atherogenesis, antimicrobial host defense, tumor growth, and metastasis, in addition to their long-established roles in thrombosis (Gremmel *et al*., 2016). In humans, platelets, anucleate blood cells with multiple functions and a short lifespan (2-4 m in diameter), circulate in the blood for 7–10 days before being removed by the spleen and liver. Platelet production, or thrombopoiesis, primarily occurs in the bone marrow and is preceded by the differentiation of hematopoietic stem cells into polyploid megakaryocytes (50-100 m in diameter) (van der Meijden & Heemskerk, 2019).

2.10.7.5. Hematocrit:

Hematocrit (HCT) is the volume fraction of red blood cells (RBC) in blood, which is an important indicator of a patient's blood condition. Measurement of the HCT is useful for the diagnosis of several diseases and conditions, such as anemia, polycythemia, dehydration, blood loss due to internal bleeding, and blood doping (Petersson *et al*., 2018).It is a parameter that reflects the fractional volume of a blood sample that is occupied by packed red blood cells (RBCs). The normal range of HCT is 40–54% for men and 37–47% for women (Kim *et al*., 2012).

2.10.7.6. Reticulocytes:

Reticulocytes in turn are generated in the bone marrow from mature erythroblasts by enucleation (Ney, 2011).and begin as large, multi-lobular, motile cells known as

R1 reticulocytes. To mature into RBCs, they must lose 20% of their plasma membrane, lose any residual organelles or internal membranes and selectively reduce or remove cytoplasmic and membrane proteins that are not required by RBC (Stevenz & Bruce, 1970). The bone marrow releases reticulocytes into the peripheral blood, and they subsequently differentiate into mature erythrocytes in the following 1–2 days (Ogawa *et al*., 2020). it comprises 0.6% - 2.9% of the RBCs in adult blood and 1.7% - 5.0% of those in umbilical cord blood (Yamada et al., 2020).

2.10.7.7. Mean Corpuscular Volume (MCV):

MCV is a measure of average RBC volume that is determined using the Wintrobe, Diem, and Center formula. MCV is equal to Hematocrit X 10/RBC. The MCV is measured in femtoliters. 80 to 100 fL is the normal range of MCV. Microcytic blood is described as being low in MCV, whereas macrocytic blood is described as being high in MCV. Blood with normal MCV is referred to as normocytic (Khan et al., 2013).

2.10.7.8. Mean Corpuscular Hemoglobin (MCH):

MCH is the amount of hemoglobin by weight in average Red blood cell count and is calculated by the Wintrobe, Diem, and Clenter formula, MCH is equal to hemoglobin multiplied by 10/R.B.C. A pictogram is used to express it. The normal range of MCH is 27–33 picograms. Blood is referred to as hypochromic when MCH is low and hyperchromic when it is high (Khan et al., 2013).

2.10.7.9. Mean Corpuscular Hemoglobin Concentration (MCHC):

MCHC is the concentration of hemoglobin in the average red blood cells or the ratio of the weight of hemoglobin to the volume in which it is contained an is calculated

by the Wintrobe, Diem, and Clenter formula ,Its normal values are 32-36 g/dL (Khan et al., 2013).

Chapter Three
Materials and Methods

Chapter Three: Materials and Methods

3.1. Materials

3.1.1. Chemical and kits

The chemical and kits used in the current study are list in Table (3-1).

Table (3-1): chemicals and kits of the study

No	kits	Company	country
1	Ethanol	Rihana Al-Warith	Iraq
2	Visfatin kit	Elabscience	China
3	Vcam-1 kit	Elabscience	China
4	D-Dimer kit	Biomerieux	France
5	CRP Kit	Hipro	China
6	LDH Kit	DIRUI	China
7	Ferritin kit	Biomerieux	France

3.1.2. Instrument and Equipment

The Instrument and Equipment used in this work are listed in Table (3-2).

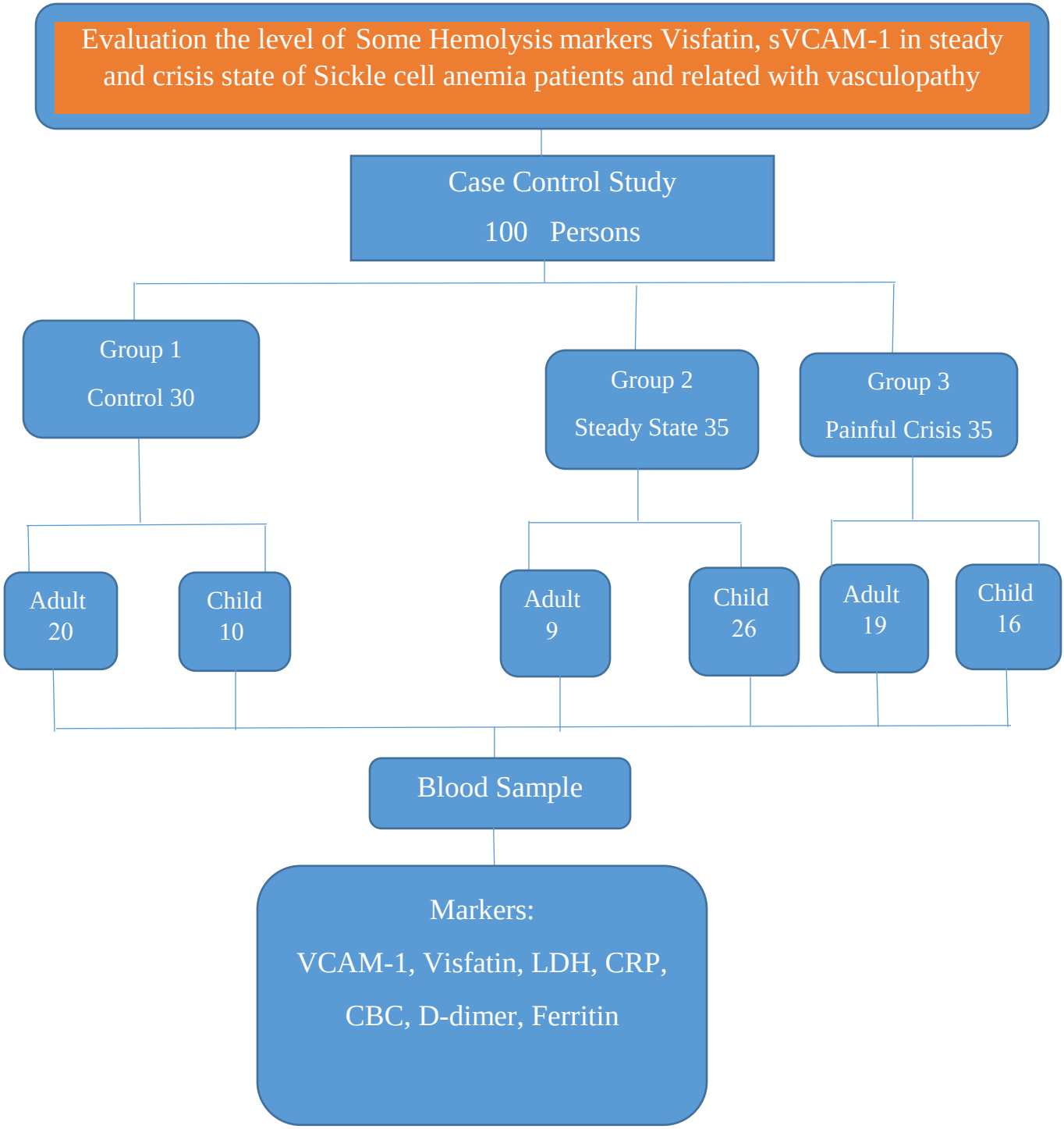
Table (3-2): Instrument and Equipment of the study

No	Instrument and Equipment	Company	Country
1	Gloves	HAN-CARE	Germany
2	Mask	Makena	China
3	Watte Cotton Pleats	Al Salama	Iraq
4	Disposable Syringe 5 ml	AL Shifa	KSA

5	Tourniquet	Voltaren	China
6	Gel Tube	Vacuum blood collection tube	China
7	EDTA Tube	Vacuum blood collection tube	China
8	Eppendorf Tube	TRUST LAB	China
9	Pasteur Pipette	TRUST LAB	China
10	Blue tips	TRUST LAB	China
11	Yellow tips	TRUST LAB	China
12	Wells centrifuge tubes racks	TRUST LAB	China
13	Micropipettes	Micropipettes	Germany
14	Swelab	Swelab Alfa Plus Standard	Sweden
15	Centrifuge	ROTOFIX 32 A (Hettich)	Germany
16	Washer Eliza	Biotek	USA
17	Reader Eliza	Biotek	USA
18	Printer Eliza	Biotek	USA
19	MINI Vidas	Biomerieux	France
20	Refrigerator	LG	Korea
21	Deep freeze	ALS	Italy
22	VORTEX MIXER	HIGH TOP	China
23	Microcentrifuge	HIGH TOP	China
24	HIPRO	Hipro Biotechnology Co	China
25	DIRUI	Auto Chemistry Analyzer (CS180)	China
26	Blue tube	Vacuum blood collection tube	China

3.2 Methods

3.2.1. Study Design



3.2.2. Patient

In the hereditary blood disease center/Karbala Health Directorate, a case-control study of patients with sickle cell anemia was conducted. Hematopathologist doctor diagnosed 70 patients, both new and old cases, with sickle cell anemia, according to data that patients provided to the center, Additionally, the patients' ages ranged from three to fifty-five, and they were all male.

3.2.2.1. Ethical Management of Studies

This study was approved by Ethical Committee at College of Applied Medical Science/ University of Kerbala. All subjects involved in this work were informed and agreement was obtaine verbally from each one before the collection of samples. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 3 (including the number and the date in 1/11/2022) to get this approval.

3.2.2.2. Collection of samples

Five mL of venous blood was drawn in an aseptic condition using a plastic syringe and the standard safety procedures used during venipunctures and divided into two parts:

1. One mL was added to (EDTA) tube for a complete blood count (CBC) and one ml blue tube for a D-dimer test.
2. Three mL were taken in a gel tube and left for 30 minutes to clot, then centrifugations were carried out for 20 minutes at 1500 xg, and serum was separated for analyzing parameters (Mostafa *et al*., 2020).

3.2.3. Control group

Healthy people were chosen from the general population in university, relatives, and population.

3.2.4. Exclusion criteria and Inclusion Criteria:

Exclusion criteria: Patients were exclude from the study if they had any of the following: Acute illness or fever, Malignancy, Hepatic or renal dysfunction, Autoimmune disease or cardiopulmonary disease, and Surgery or trauma within a month before the study.

Inclusion Criteria:

SCA patients who served as case group, Healthy subject, who served as control group.

3.2.5. Markers

3.2.5.1. Estimation of the level of serum Visfatin by ELISA Kit

3.2.5.1.1.Principle: This ELISA kit uses the Sandwich-ELISA principle. An antibody specific to Human VF has been pre-coated on the micro ELISA plate included in this kit. Samples are added with the specific antibody in the micro ELISA plate wells. Then a biotinylated detection antibody specific for Human VF and Avidin-Horseradish Peroxidase (HRP) conjugate is added successively to each microplate well and incubated. Washing removes free components. Each well is filled with the substrate solution. Specifically, just those wells that have Human VF, biotinylated detection antibody, and Avidin-HRP conjugate will appear blue in color. The addition of a stop solution and the transition to yellow signal the end of the enzyme-substrate reaction. At a wavelength of 450 nm + 2 nm, the optical density (OD) is determined spectrophotometrically. The Human VF concentration is directly

related to the OD value. You can calculate the concentration of Human VF in the samples by comparing the OD of the samples to the standard curve.

3.2.5.1.2. Reagents: Reagent preparation

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly
with a pipette. This reconstitution produces a working solution of 100 ng/mL(or add 1 mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles
generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 100, 50, 25, 12.500, 6.250, 3.130, 1.56, 0 ng/mL.
Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 100 ng/mL working solution to the first tube and mix up to produce a 50 ng/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube. Gradient diluted standard working solution should be prepared just before use.
4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent (Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).The working solution should be prepared just before use.
5. **Concentrated HRP Conjugate working solution:** HRP Conjugate is HRP conjugated avidin. Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate

Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).The working solution should be prepared just before use.

3.2.5.1.3. Procedure of Visfatin:

1- Add 100 microliters standard or sample to the wells.

Incubate for 90 min at 37 c

2- Discard the liquid, and immediately add 100 microliters of Biotinylated Detection Ab working solution to each well. Incubate for 60 min at 37c 3- Aspirate and wash the plate for 3times.

4- Add 100 microliters of HRP conjugate working solution. Incubate for 30 min at 37c. Aspirate and wash the plate 5 times.

5- Add 90 microliters of Substrate Reagent. Incubate for 15 min at 37c.

6- Add 50 microliters of Stop Solution.

7- Read the plate at 450nm immediately. Calculation of the results.

3.2.5.1.4. Calculation of results:

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log axis, with standard concentration on the x-axis and OD values on the yaxis. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

3.2.5.2. Estimation of the level of serum Vcam-1 by ELISA Kit

3.2.5.2.1. Principle: As above 3.2.5.1.

3.2.5.2.2. Reagent preparation: As above 3.2.5.1.2.

3.2.5.2.3. Procedure of vcam-1: As above 3.2.5.1.3.

3.2.5.2.4. Calculation of results: As above 3.2.5.1.4.

3.2.5.3. Estimation of the level of D-dimer

D-Dimer concentrations were measure automatically using the VIDAS® D-Dimer Exclusion IITM (DEX2).

3.2.5.3.1. Procedure of D-dimer:

Analysis of D-dimers by its means is a semi-automated procedure that re- quires manual sample pipetting in the appropriate test strip well. Results are available within 20 minutes, and the assay is linear from 0.05 to 10.0 mg/L FEU. Additionally, samples with D-dimer concentrations above the upper quantification limit can be manually diluted with the appropriate diluent in the ratio 1:5 and reanalyzed, achieving test re-porting up to 50.0 mg/L FEU (Prce & Zadro, 2020).

3.2.5.4. Estimation of the level of C - Reactive Protein (CRP)

C - Reactive Protein (CRP) was measured automatically by HIPRO Equipment.

3.2.5.5. Estimation of the level of Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH) was measure automatically by DIRUI (Auto Chemistry Analyzer CS180).

3.2.5.6. Estimation of the level of Ferritin

Ferritin was measure automatically by MINI Vidas Equipment.

3.2.5.7. Complete Blood Count (CBC)

CBC was measure automatically by SWELAB Equipment

3.2.6. Statistical analysis:

The version 24 of the computer program, Statistical Package For Social Sciences (SPSS), ANOVA table – one way, SD, R test and the results have been considered to have statistical significance at ($p \leq 0.05$).

Note 1:

G1 pointing to control group

G2 pointing to steady state group

G3 pointing to painful crisis group

Note 2:

Similar small letters indicate that there are no significant differences between groups.

Different small letters indicate significant differences between groups.

Chapter Four
Results and Discussion

Chapter Four: Results and Discussion

4. General parameters

4.1.1. Vcams-1 (ng/mL):

Referring to table (4-1)'s findings, both the adults and children patient groups in comparison to the control showed significant differences ($P \leq 0.05$). In the same table revealed Vcam-1 level higher in all patient groups (painful crisis and steady state) compared to control.

Table 4-1: Concentration of Vcam-1 (ng/mL) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	1.36 ± 0.36 [ab] (N=26)	2.32 ± 0.19 [a] (N=16)	0.41 ± 0.20[b] (N=10)	0.0000 *	1.458
Adults	1.73 ± 0.31 [b] (N=9)	2.79 ± 0.16 [a] (N=19)	0.95 ± 0.33 [c] (N=20)	0.0000 *	0.458
Total	1.46 ± 0.38	2.57 ± 0.29	0.83 ± 0.38		

* Means significant differences at $P \leq 0.05$

The data in table (4-1) showed that, as compared to the control group, the mean Vcam-1 values of SCA patients significantly increased. Numerous academics have characterized sickle cell disease as a chronic inflammatory condition that is associated with a number of factors, including an increase in the production of reactive oxygen species, hemolysis, endothelium damage, and pro-inflammatory cytokines all these facters lead to released VCAM-1 in high levels in patient with SCA (Obeagu & Muhimbura, 2022). The findings of the current analysis, which demonstrate that Vcam-1 levels were higher in SCA patients (painful crises and steady state) than in controls,

are addressed and supported by a number of studies (Antwi-Boasiako, Donkor, *et al*., 2018).

4.1.2. Visfatin (ng/mL):

According to the findings of table (4-2), SCA patients had higher concentrations of Visfatin than controls. In the same table it is also indicated significant differences (P≤ 0.05) between adults, children in both patient groups compared with control.

Table 4-2: Concentration of Visfatin (ng/mL) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	1.36 ± 0.25 ^{ab} (N=26)	2.28 ± 0.31 ^{a} (N=16)	0.29 ± 0.17 ^{b} (N=10)	0.0000 *	1.398
Adults	1.81 ± 0.38 ^{b} (N=9)	2.85 ± 0.15 ^{a} (N=19)	0.97 ± 0.36 ^{c} (N=20)	0.0000 *	0.458
Total	1.48 ± 0.34	2.59 ± 0.37	0.82 ± 0.43		

* Means significant differences at P ≤ 0.05

Depending on the findings of the current investigation, patients' levels of visfatin elevated in response to the inflammatory process compared to control subjects. or may be due to the increased synthesis of reactive oxygen species, hemolysis, endothelial destruction, and increased synthesis of pro-inflammatory cytokines among others. Inflammatory processes play a significant role in the activation of the acute painful vaso-occlusion crisis, which is the primary cause of sickle cell anemia patients' hospitalization, these factors lead to released Visfatin in high levels in patient with SCA (Obeagu & Muhimbura, 2022). Numerous studies have addressed and supported the findings of this investigation, which demonstrates that the Serum visfatin is

increased in SCD patients compared with that in healthy children and is associated with the frequency of VOC; it can be used as a predictive index for VOC occurrence and follow-up in those patients (D. M. Habashy & Shams, 2017).

4.1.3. C-Reactive Protein (CRP) (mg/L):

Table (4-3) showing that CRP is higher in SCA patient (painful crisis and steady state) compared with control group in both child and adult .There are significant differences between adults, child in both patient groups compared with control ($P \leq 0.05$).

Table 4-3:Concentration of CRP (mg/L) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	1.64 ± 1.30 [b] (N=26)	25.95 ± 42.15 [a] (N=16)	1.05 ±0.47 [b] (N=10)	0.008 *	8.067
Adults	3.01 ± 3.97 [b] (N=9)	43.29 ± 37.78 [a] (N=19)	0.95 ± 0.46 [b] (N=20)	0.0000 *	30.915
Total	2.00 ± 2.30	35.36 ± 40.20	0.98 ± 0.45		

* Means significant differences at $P \leq 0.05$

The data presented in table (4-3) showed that the patient groups' CRP concentrations were significantly higher than those of the control group. Sickle cell diseases has been described by many scholars as a chronic inflammatory disease that is linked to several factors such as, increased synthesis of reactive oxygen species, hemolysis, endothelial destruction, and increased synthesis of pro-inflammatory cytokines among others. Inflammatory processes play a significant role in the activation of the acute painful vaso-occlusion crisis, which is the primary cause of sickle cell anemia patients' hospitalization (Obeagu & Muhimbura, 2022). Inflammation among sickle cell disease patients is a ceaseless process that occurs even during a steady state (Abdelmaged,

n.d.). These results are in agreement with certain studies that reported an increase in CRP levels. Studies have assessed its value in SCD. high levels of SCA in the steady state have been correlated with increased frequency of acute pain in children with SCD Most studies find that levels increase during acute vaso-occlusion (Rees & Gibson, 2012).other study showing that C-reactive protein (CRP) levels, was significantly different during VOC compared with the previous steady state values in both adults and child (Karahan *et al*., 2022).

4.1.4. Lactate dehydrogenase (LDH) (U/L):

According to table (4-4)'s findings, there are significant differences (P≤ 0.05) between adult, child in both patient groups compared with control. In the same table revealed LDH level higher in all patient groups (painful crisis and steady state) compared to control.

Table 4-4: Concentration of LDH (U/L) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	929.34 ± 173.03[b] (N=26)	1964.93 ± 546.1[a] (N=16)	633.16 ± 91.92 [c] (N=10)	0.0000 *	7.982
Adults	569.33 ± 69.08 [b] (N=9)	959.57 ± 93.40 [a] (N=19)	282.80 ± 72.72 [c] (N=20)	0.0000 *	0.596
Total	836.77 ± 220.51	1419.17 ± 551	363.65 ± 168.44		

* Means significant differences at P ≤ 0.05

The concentration of LDH in SCA patients was significantly higher in both crisis and steady state compared to control groups in this work; hemolysis always present is supported scientifically with high values of Lactate Dehydrogenases, in some study that supports current results the Serum LDH is increased in SCA patients (crisis,

steady) compared with that in healthy (Kreka et al., 2022). In this study also Lactate dehydrogenase levels were significantly increased in VOC state compared to steady-state in both children and adults (Kingsley *et al*., 2022).

4.1.5. D-Dimer (mg/mL):

In table (4-5) Mean concentrations of D-dimer, is higher in the SCA patient (painful crisis and steady state) if compared with control group. There are significant differences between (child and adult) in all patient group compared with control ($P \leq 0.05$).

Table 4- 5: Concentration of D.dimer (mg/mL) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	446.0 ± 109.27 [b] (N=26)	1040.1 ± 145.87[a] (N=16)	73.0 ± 17.77 [c] (N=10)	0.0000 *	4.957
Adults	811.6 ± 150.80 [b] (N=9)	1930.2 ± 549.5 [a] (N=19)	199.9 ± 64.32 [c] (N=20)	0.0000 *	9.687
Total	540.02 ± 201.05	1523.34 ± 609.6	170.61 ± 78.61		

* Means significant differences at $P \leq 0.05$

In this study's SCA patients, the serum D-dimer level was considerably greater than in healthy controls. Elevated plasma D-dimer levels indicate increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation. Increasing of thrombin activity and fibrin formation these increasing are features of steady-state sickle cell disease, and that they further increase during painful crisis. The results of the agreement study (Mohamed et al., 2020) are consistent with recent findings that showed greater serum D. dimer levels

in both crisis and steady state conditions. The level of D-dimer was significantly higher in of the patients; and significant elevation was observe in patients with VOC when compared with steady state.

4.1.6. Retic count (%):

In table (4-6) Mean percentage of Retic count, is higher in the SCA patient (painful crisis and steady state) if compared with control group. There are significant differences between (children and adults) in all patient groups compared with control ($P \leq 0.05$).

Table 4-6: Percentage of Retic count (%) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	3.69 ± 4.11 [b] (N=26)	7.39 ± 4.26 [a] (N=16)	2.63 ± 2.41 [c] (N=10)	0.009 *	0.770
Adults	2.99 ± 4.44 [ab] (N=9)	5.48 ± 4.64 [a] (N=19)	1.69 ± 1.01 [c] (N=20)	0.006 *	3.412
Total	3.51 ± 4.14	6.35 ± 4.51	1.91 ± 1.45		

* Means significant differences at $P \leq 0.05$

These findings were consistent with other previousiy published data in which authors reported that Retic count higher in painful crisis, steady state compared with control groups. Due to the fact that adult erythrocytes have a 120-day lifespan, mature erythrocyte Hb cannot sensitively reflect Hb synthesis. While this is happening, reticulocytes are released into the peripheral blood from the bone marrow, and they later develop into mature erythrocytes in the following 1–2 days (Ogawa *et al*., 2020) this leading to increase in Retic count. The difference in mean of Retic count was statistically significant ($p \leq 0.05$) in both children and adults (Ishau, 2022).

4.1.7. Red Blood Cell (RBC) (10^12/1):

According to the results of table (4-7), there have been significant differences ($P \leq 0.05$) between adult, child in both patient groups compared with control. In the same table revealed Vcam-1 level higher in all patient groups (painful crisis and steady state) compared to control.

Table 4-7: a count of RBC (10^12/1) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	3.51 ± 0.57 [b] (N=26)	3.22 ± 0.74 [b] (N=16)	4.73 ± 0.57 [a] (N=10)	0.0000 *	1.196
Adults	3.39 ± 1.24 [b] (N=9)	3.74 ± 0.92 [b] (N=19)	5.03 ± 0.47 [a] (N=20)	0.0000 *	1.104
Total	3.48 ± 0.77	3.50 ± 0.87	4.96 ± 0.50		

* Means significant differences at $P \leq 0.05$

SCD patients with HbSS VOC have significantly lower levels of HCT and RBC counts compared to those in the steady state due to the chronic haemolysis associated with SCD. The rate of RBC destruction is also very high in SCD and hence contributes to the lower levels of RBC and other RBC indices. The sickle cell anemia patients are continually hemolysing their red cells with a short survival rate of the erythrocytes between 12-14 days and as a result, the hemoglobin, hematocrit, and red blood cells are usually lower than normal healthy individuals. The result of the current investigation is consistent with previous recent study which found that the differences in their means were statistically significant ($p \leq 0.05$) between SCA patient group compared with control. Red blood cell in VOC were significantly lower than the mean steady state and the controls in both children and adults (C Onyekwelu *et al*., 2019).

4.1.8. White Blood Cell (WBC) (10^9/1):

In table (4-8) Mean a count of WBC, is higher in the SCA patient (painful crisis and steady state) if compared with control group. There are significant differences between (child and adult) in all patient group compared with control ($P \leq 0.05$).

Table 4-8: a count of WBC (10^9/1) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	11.84 ± 5.47 [a] (N=26)	11.85 ± 5.67 [a] (N=16)	5.73 ± 0.48 [b] (N=10)	0.037 *	5.662
Adults	10.36 ± 4.45 [b] (N=9)	12.57 ± 6.78 [a] (N=19)	7.43 ± 1.50 [c] (N=20)	0.007 *	1.577
Total	11.46 ± 5.20	12.24 ± 6.22	7.04 ± 1.59		

* Means significant differences at $P \leq 0.05$

Significant difference in white blood count reported in vaso-occlusive crisis in this study compared with lower values in a steady state may be due to redistribution of white cells from margination to the circulating pool as aresult of pain, nausea, vomiting, and anxiety associated with the vaso-occlusive crisis. Sickle cell anemia is associated with an abnormal chronic inflammatory reaction that results from recurrent episodes of ischemia/hypoxia and reoxygenation, which are more pronounced during crises and are often associated with hyperleukocytosis and increased neutrophil activation. Leukocytes play an important role in the pathophysiology of SCA; neutrophils have roles in the initiation and propagation of VOCs by slowing the blood flow in the microvasculature, prolonging the transit time of sickle RBCs and therefore increasing HbS polymerization (Abdul-Hussein *et al*., 2021).The result of the current investigation is consistent with previous recent study which found that The differences

in their means were statistically significant (p≤ 0.05) between SCA patient group compared with control (Ishau, 2022).

4.1.9. Platelet (10^9/1):

Table (4–9) displays the findings of the platelet levels in both the patient and control groups. was found significantly higher concentration of platelets in SCA patients (painful crisis and steady state) as compared to the control group (P≤0.05).

Table 4-9: Concentration of Platelet (10^9/1) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	329.8 ± 237.6 (N=26)	315.5 ± 200.8 (N=16)	289.5 ± 161.4 (N=10)	0.916	NS
Adults	390.0±155.8 [a] (N=9)	260.36±150.4 [b] (N=19)	230.25±48.5 [b] (N=20)	0.006 *	119.68
Total	345.28±219.01	285.6 ± 174.82	243.92 ± 87.5		

* Means significant differences at P ≤ 0.05 NS: no significance

Only in adults did the results of table (4-9) show statistically significant variations in platelet concentrations between SCA patients and controls, this result agreement with other study that found the mean of platelet values both in VOC and steady state were significantly lower than the controls values in adults and children. The higher PLT count seen in patients with SCD could be attribute to a possible splenic sequestration, reduction or absence of spleen resulting from hyposplenism in SCD autosplenectomy, as well as the underlying chronic inflammation (Antwi-Boasiako, Ekem, *et al*., 2018). or due to hypercoagulability and thromboembolic complications. Autosplenectomy associated with sickle cell diseases which causes reduced or absent splenic

sequestration could account for thrombocytosis seen in the disease (I *et al*., 2022). These results are in agreement with certain studies that reported an increase in PLA levels in SCA patient in both adults and children (Antwi-Boasiako, Ekem, *et al*., 2018).

4.1.10. Hematocrit (HCT) (%):

According to Table (4-10)'s findings, there are significant differences (P≤ 0.05) between adults, children in both patient groups compared with control. In the same table it is reveal that HCT level higher in all patient groups (painful crisis and steady state) compared to control.

Table 4-10: Concentration of HCT (%) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	26.53 ± 4.28 [b] (N=26)	23.75 ± 4.93 [c] (N=16)	37.45 ± 7.69 [a] (N=10)	0.0000 *	1.344
Adults	24.02 ± 6.29 [c] (N=9)	28.47 ± 5.44 [b] (N=19)	42.77 ± 1.03 [a] (N=20)	0.0000 *	0.550
Total	25.88 ± 4.90	26.31 ± 5.66	41.54 ± 5.77		

* Means significant differences at P ≤ 0.05

HCT levels for SCA patients significantly decreased as compared to healthy individuals, according to the results in the table (4–10), The sickle cell anemia patients are continually hemolysing their red cells with a short survival rate of the erythrocytes between 12-14 days and as a result, the hemoglobin, hematocrit, and red blood cells are usually lower than normal healthy individuals. The results are similar and relate to some previous research. Compared to SCA patients, HCT has been observed to have significantly increase (C Onyekwelu *et al*., 2019) .

4.1.11. Hemoglobin (HB) (g/dL):

When compared to the control group, the results of HB in table (4–11) showed a significant rise ($P \leq 0.05$) in Hb concentration in SCA patients. The level of hemoglobin in SCA patients (painful crisis, steady state) is higher if compared with and the control group.

Table 4-11: Concentration of HB (g/dL) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	9.59 ± 1.50 [b] (N=26)	8.65 ± 1.69 [c] (N=16)	12.11 ± 2.18 [a] (N=10)	0.0000 *	0.459
Adults	8.53 ± 2.11 [c] (N=9)	10.20 ± 1.77 [b] (N=19)	14.68 ± 1.88 [a] (N=20)	0.0000 *	0.556
Total	9.32 ± 1.71	9.49 ± 1.88	14.09 ± 2.20		

* Means significant differences at $P \leq 0.05$

The data in table (4-11) showed a significant rise in HB concentration in the control groups when compared to patient groups in the case when compared with control groups. Sickled hemoglobin makes RBCs vulnerable to broken easily resulting in extravascular and intravascular hemolysis with a result of low hemoglobin levels in patient groups comparing with control group, These results are in line with several studies, the hematological indices during crisis and steady state showed that the mean hemoglobin in VOC were significantly lower than the mean steady state and the controls (C Onyekwelu et al., 2019).

4.1.12. Ferritin (ng/mL):

Results of table (4-12) demonstrated that there were significant changes ($P \leq 0.05$) in the ferritin concentration in both children and adults with SCA when compared to the control group. The ferritin concentration increased in patients with SCA (painful crisis, steady state) when compared to the control group.

Table 4-12: Concentration of Ferritin (ng/mL) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	730.76 ± 252.1 [b] (N=26)	1178.37 ± 493.1 [a] (N=16)	181.0 ± 314.1 [c] (N=10)	0.0000 *	5.313
Adults	998.0 ± 162.3 [b] (N=9)	2625.6 ± 687.6 [a] (N=19)	404.05±575.8 [c] (N=20)	0.0000 *	2.880
Total	799.48 ± 258.82	1964.05 ± 944.85	352.57 ± 530.0		

* Means significant differences at $P \leq 0.05$

The patients group with SCA patient group (painful crisis and steady state), according to the most recent findings in table (4-12), had considerably higher ferritin concentrations than the control group. The rise in the serum ferritin in sickle cell anemia patients due to the excess free iron, due to the excess breakdown of Hb and the abnormal circulating mass of Hb in reticuloendothelial cells, exert a positive feedback on ferritin synthesis, ferritin was significantly higher in patients with SCA during both steady state and acute VOC compared to control (Alsultan *et al*., 2010).

4.1.13. MCV (fL):

Table (4-13) showing that MCV is higher in SCA patient (painful crisis and steady) compared with control group in both child and adult. There are no significant differences between children in both patient groups and control but there are significant differences between adults in both patient groups and control ($P \leq 0.05$).

Table 4-13: Concentration of MCV (fL) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	74.11 ± 10.86 (N=26)	74.67 ± 11.28 (N=16)	78.81 ± 10.97 (N=10)	0.641	NS
Adults	73.29±13.33 [b] (N=9)	75.57 ± 16.18 [b] (N=19)	85.02 ± 5.76 [a] (N=20)	0.023 *	9.145
Total	73.90 ± 11.35	75.16 ± 13.96	83.59 ± 7.51		

* Means significant differences at $P \leq 0.05$ NS: no significance

A significant increase in MCV concentration was seen in the SCA patient group (painful crisis and steady state) according to the most recent findings in table (4-13) when compared to controls in adults, Due to the rapid rate of RBC destruction in SCD, RBC levels and other RBC indices like MCV are lower in SCA patient compared to control group, These results agreement with other studies that found the value of MCH increase in SCA patient (painful crisis, steady state) compared to control group with significant differences in adults (Antwi-Boasiako *et al*., 2019). But our results disagreement with these study in children because we found no significant differences between SCA patient and control in children due to the change in geographical area,

environmental factors, sample size, and age differences (Antwi-Boasiako, Ekem, *et al*., 2018).

4.1.14. MCH (pg):

Table (4-14) showing that MCH is lower in SCA painful crisis and steady state compared with control group in both children and adults .but there are no significant differences between adults , children in both patient groups compared with control.

Table 4-14: Concentration of MCH (pg) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	27.54 ± 3.32 (N=26)	26.98 ± 4.22 (N=16)	25.59 ± 2.93 (N=10)	0.490	NS
Adults	26.78 ± 6.47 (N=9)	28.31 ± 5.39 (N=19)	29.22 ± 3.32 (N=20)	0.463	NS
Total	27.34 ± 4.25	27.70 ± 4.87	28.38 ± 3.54		

* Means significant differences at $P \leq 0.05$ NS: no significance

The results of MCH are present in Table (4-14). When compared to adults with SCA, healthy persons had a significantly higher level of MCH, but in children level of MCH for healthy individuals decrease compared with the SCA patients. The rate of RBC destruction is also very high in SCD and hence contributes to the lower levels of RBC and other RBC indices like MCH, these results are similar and deals with some previous research, one of these study had showed there was no significant difference in the MCH values between the Steady-state and the VOC state compared with control in adults and children (Kingsley *et al*., 2022).

4.1.15. MCHC (g/dL):

Table (4-15) showing that MCHC is higher in SCA patient (painful crisis and steady) compared with control group in both children and adults. There are no significant differences between adults in both patient groups and control but there are significant differences between children in both patient groups and control ($P \leq 0.05$).

Table 4-15: Concentration of MCHC (g/dL) in patients with SCA and compare with control group

Categories	Mean ± SD			*P value*	LSD
	Patients (70)		Healthy (30)		
	Steady (35)	Painful (35)			
Children	36.15 ± 1.25 [a] (N=26)	36.6 ± 1.08 [a] (N=16)	32.6 ± 2.40 [b] (N=10)	0.0000 *	0.4525
Adults	36.25 ± 2.85 (N=9)	36.00 ± 1.25 (N=19)	34.46 ± 2.58 (N=20)	0.051	NS
Total	36.17 ± 1.75	36.27 ± 1.19	34.03 ± 2.62		

* Means significant differences at $P \leq 0.05$ NS: no significance

Current research shows that patients with SCA (painful crisis and steady state) have significantly higher MCHC concentrations than controls in adults, as shown in table (4-15). The rate of RBC destruction is also very high in SCD and hence contributes to the lower levels of RBC and other RBC indices like MCHC, This results agree with other studies that found the value of MCHC increase in SCA patient (painful crisis, steady state) compared to control group with significant differences in children (Antwi-Boasiako et al., 2019). But our results disagree with these study in adults because we found no significant differences between SCA patient and control in adults due to the change in geographical area, environmental factors, sample size, and age differences (Antwi-Boasiako, Ekem, et al., 2018).

4.2. Correlations:

4.2.1. Correlation between Vcam-1 and Visfatin in painful patients group:

In our study,we first determined the differences in two adipocytokines,vasfatin and vcam-1,between adults and children in SCA patient during painful crisis in our study the results showed positive significant Correlation serum visfatin and vcam-1 levels (R2 Linear = 0.555). This indicates that the rise in Vcam-1 is associated with the rise in visfatin in painful crisis.may be due to the increased synthesis of reactive oxygen species, hemolysis, endothelial destruction, and increased synthesis of pro-inflammatory cytokines among others. Inflammatory processes play a significant role in the activation of the acute painful vaso-occlusion crisis, which is the primary cause of sickle cell anemia patients' hospitalization, these factors lead to released Visfatin and VCAM-1 in high levels in patient with SCA (Obeagu & Muhimbura, 2022).

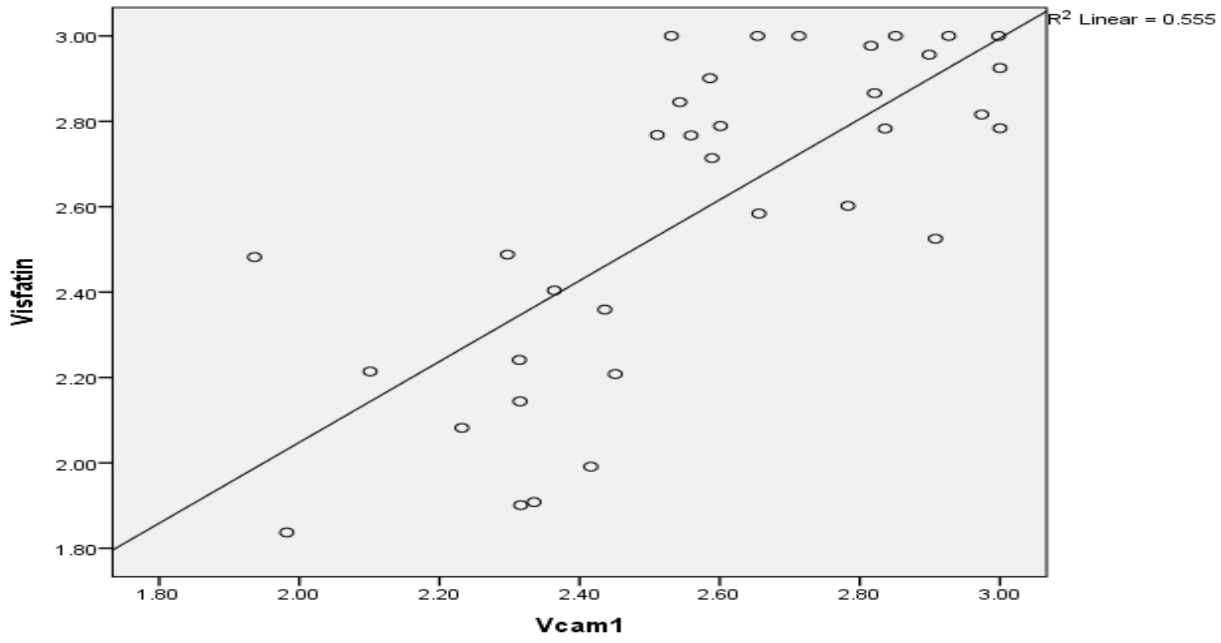


Figure 1: Correlation between Vcam-1 and Visfatin in painful patients group.

4.2.2. Correlation between Vcam-1 and LDH in painful patients group:

Based on the results in Figure (2) there was negative significant correlation between Vcam-1 and LDH in painful patients crisis group in both children and adults (R2 = 0.432). When LDH levels increase VCAM-1 levels will be decrease. The correlation between LDH levels with serum sVCAM-1 levels could indicate a link of sVCAM- 1 to the stress erythropoiesis characteristic of HbSS patients. The correlation between lactate dehydrogenase levels, and serum VCAM-1 might also be explained by the direct immune activating effect of free hemoglobin, The results of the current analysis, which demonstrate a negative relationship between sVCAM-1 and serum lactate dehydrogenase levels, had been addressed by and supported by several papers (Schnog *et al*., 2003).

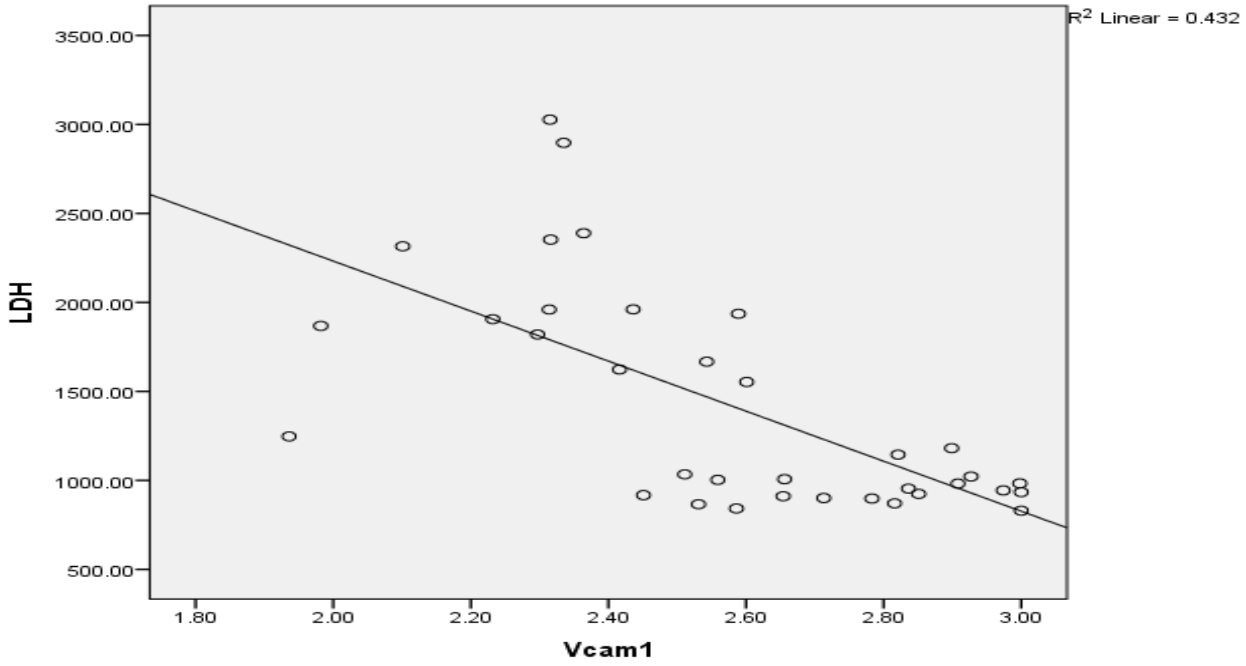


Figure 2: Correlation between Vcam-1 and LDH in painful patients group.

4.2.3. Correlation between Vcam-1 and D-dimer in steady patients group:

According to the results in Figure (3) there were positive significant correlation between Vcam-1 and D-dimer in SCA steady patients group in both children and adults (R2 = 0.407). This indicates that the rise in D-dimer is associated with the rise in Vcam-1. According to the results of this study (Mohamed et al., 2020) D-dimer level Elevated due to the increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation. Increasing of thrombin activity and fibrin formation these increasing are features of steady-state sickle cell disease that lead to endothelial dysfunction ending with released VCAM-1 in high level.

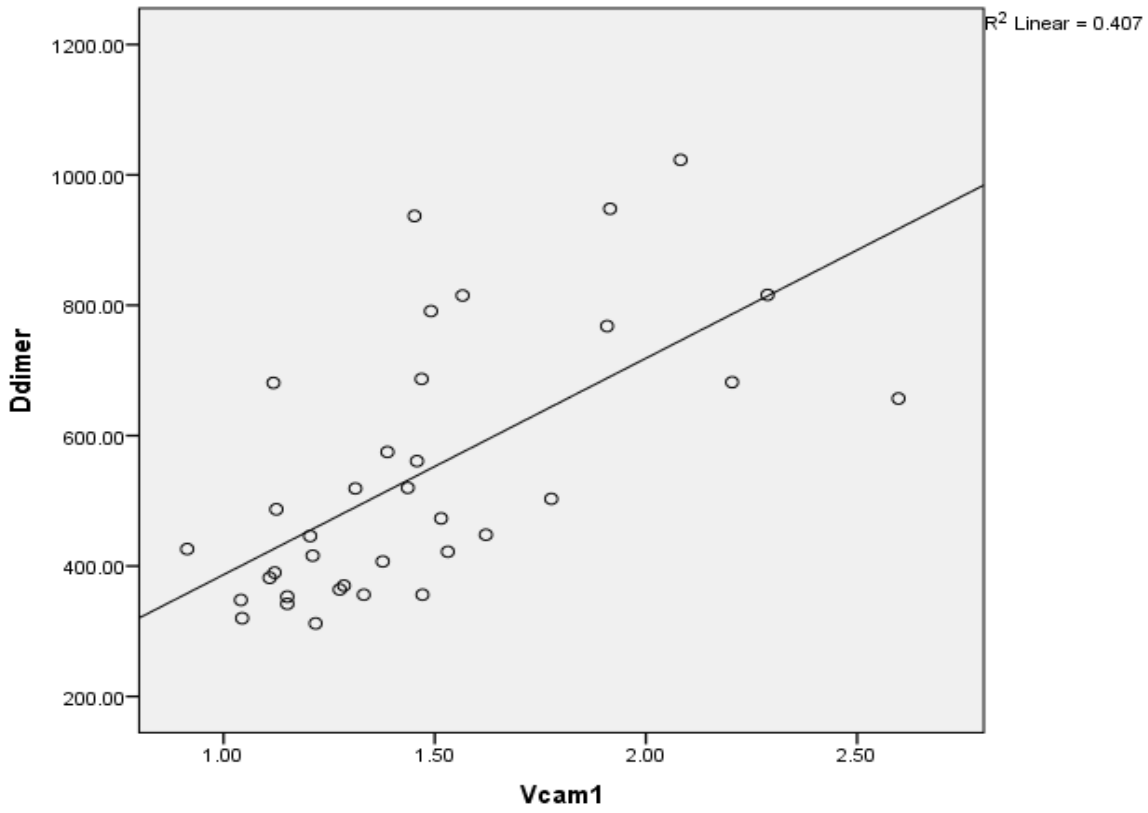


Figure 3: Correlation between Vcam-1 and D-dimer in steady patients group.

4.2.4. Correlation between Vcam-1 and D-dimer in painful patients group:

Based on the results in Figure (4) there were positive significant correlation between Vcam-1 and D-dimer in SCA painful patients group in both children and adults (R2 = 0.511). When D-dimer levels increase VCAM-1 levels will be increase. Depending on the results of this study (Mohamed et al., 2020) D-dimer level Elevated due to the increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation. Increasing of thrombin activity and fibrin formation these increasing during painful crisis sickle cell disease that lead to endothelial dysfunction ending with released VCAM-1 in high level. These results are in agreement with several studies' suggestions that there are present significant correlations between soluble vascular cell adhesion molecule-1 and D-dimer (Ataga *et al*., 2012).

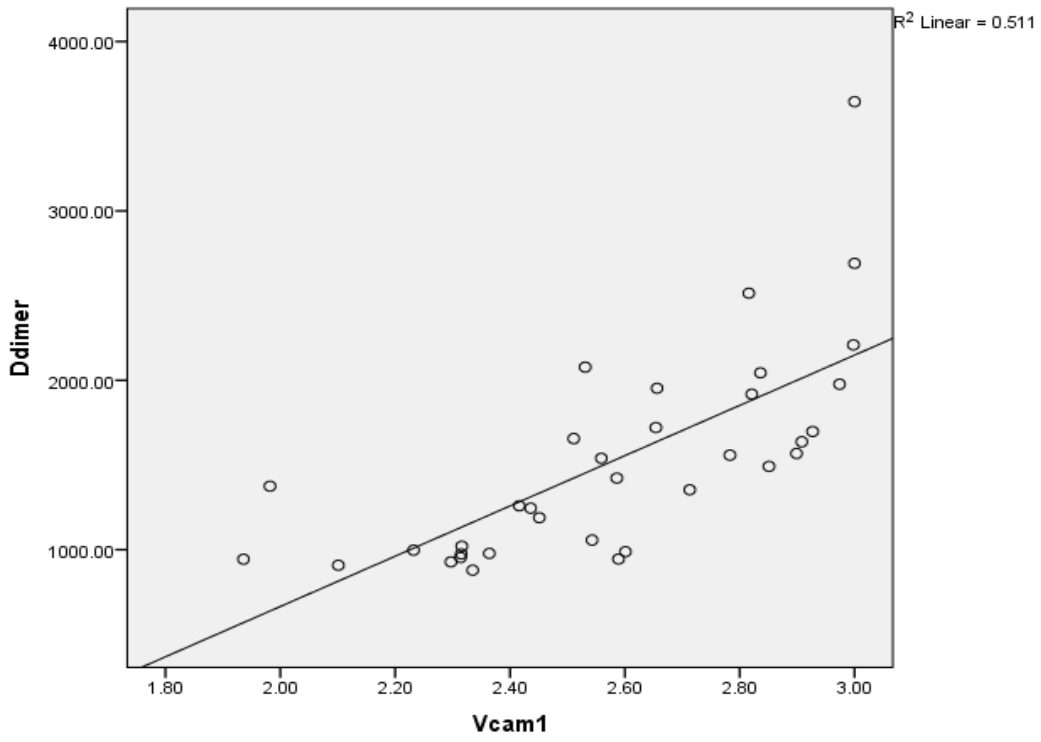


Figure 4: Correlation between Vcam-1 and D-dimer in painful patients group.

4.2.5. Correlation between Vcam-1 and Ferritin in painful patients group:

According to the results in Figure (5) there was positive significant correlation between Vcam-1 and Ferritin in painful patients group in both children and adults (R2 = 0.515). This indicates that the rise in ferritin is associated with the rise in Vcam-1. In sickle cell anemia patients serum ferritin increase due to the excess free iron, the excess breakdown of Hb and the abnormal circulating mass of Hb in reticuloendothelial cells exert a positive feedback on ferritin synthesis, HB will bind with NO and lead to endothelial dysfunction ending with released VCAM-1 in high level. Correlation between Vcam-1 and Ferritin in painful patients group was not study before in SCA.

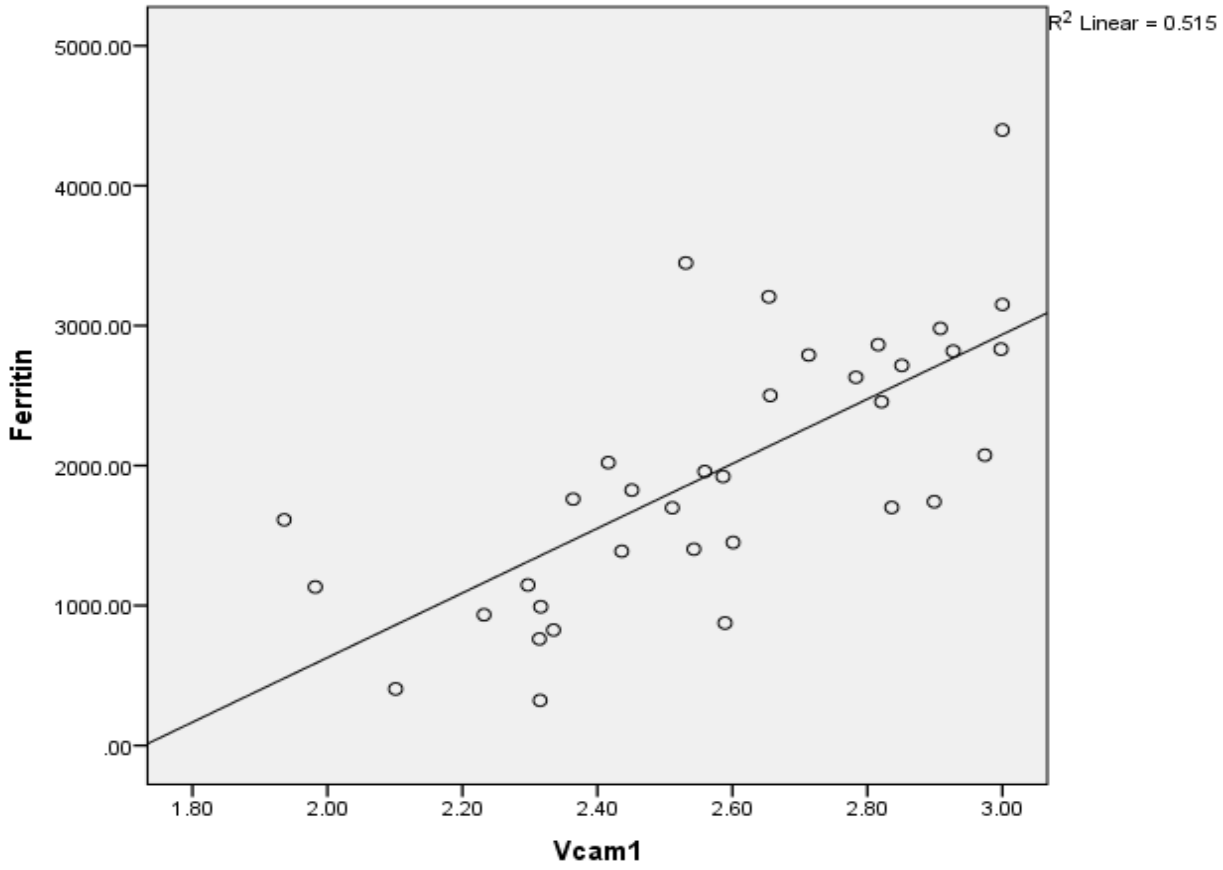


Figure 5: Correlation between Vcam-1 and Ferritin in painful patients group.

4.2.6. Correlation between Visfatin and LDH in painful patients group:

According to the results in Figure (6) there were negative significant correlation between Visfatin and LDH in painful crisis patients group in both children and adults (R2 = 0.553). This indicates that the rise in LDH is associated with the decrease in visfatin levels. LDH elevation believed to be a marker of SCA patients with a syndrome of hemolysis associated nitric oxide (NO) resistance, endothelial dysfunction, and end-organ vasculopathy (Gurkan *et al*., 2010). While due to Hyper inflammation, excessive release of free radicals, and the occurrence of high oxidative stress in SCA patients, the secretion and synthesis of Visfatin is downregulated, so its concentration decreases (Romacho, Villalobos, *et al*., 2013).

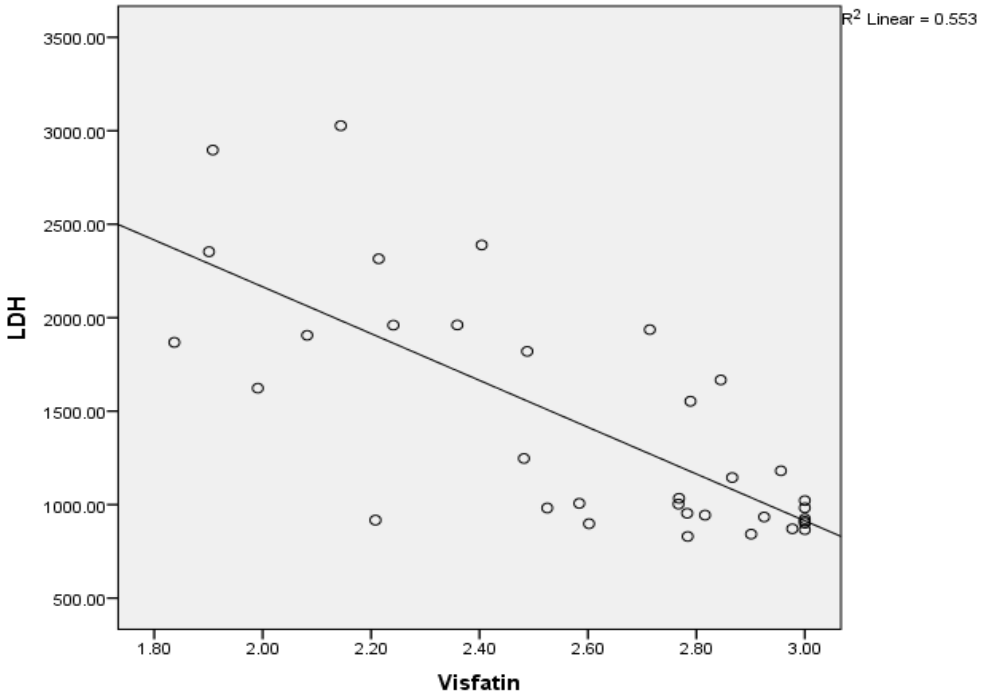


Figure 6: Correlation between Visfatin and LDH in painful patients group.

4.2.7. Correlation between Visfatin and D-dimer in steady patients group:

Based on the results in Figure (7) there were positive significant correlation between Visfatin and D-dimer in steady patients group in both children and adults (R2 = 0.355). This indicates that the rise in D-dimer is associated with the rise in visfatin. According to the results of this study (Mohamed et al., 2020) D-dimer level Elevated due to the increased plasmin degradation of cross-linked fibrin, and are therefore an indirect indication of increased thrombin activity and fibrin formation. Increasing of thrombin activity and fibrin formation these increasing are features of steady-state sickle cell disease that lead to endothelial dysfunction ending with released Visfatin in high level. Correlation between Visfatin and D-dimer in steady patients group was not study before in SCA.

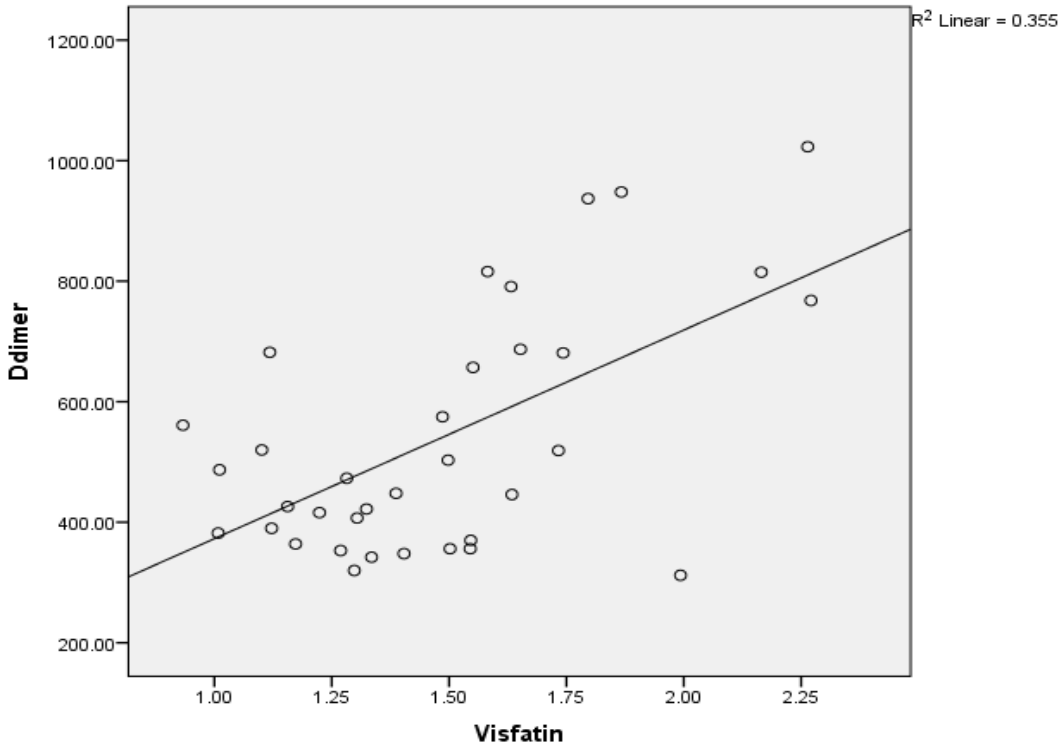


Figure 7: Correlation between Visfatin and D-dimer in steady patients group.

4.2.8. Correlation between Visfatin and D-dimer in painful patients group:

According to the results in Figure (8) there was positive significant correlation between Visfatin and D-dimer in painful crisis patients group in both children and adults (R2 = 0.294). This indicates that the rise in D-dimer is associated with the rise in visfatin. Depending on the results of this study (Mohamed et al., 2020) D-dimer level Elevated due to the increased plasmin degradation of cross-linked fibrin, and therefore an indirect indication of increased thrombin activity and fibrin formation. Increasing of thrombin activity and fibrin formation are features in painful crisis sickle cell disease that lead to endothelial dysfunction ending with released Visfatin in high level. Correlation between Visfatin and D-dimer in painful patients group was not study before in SCA.

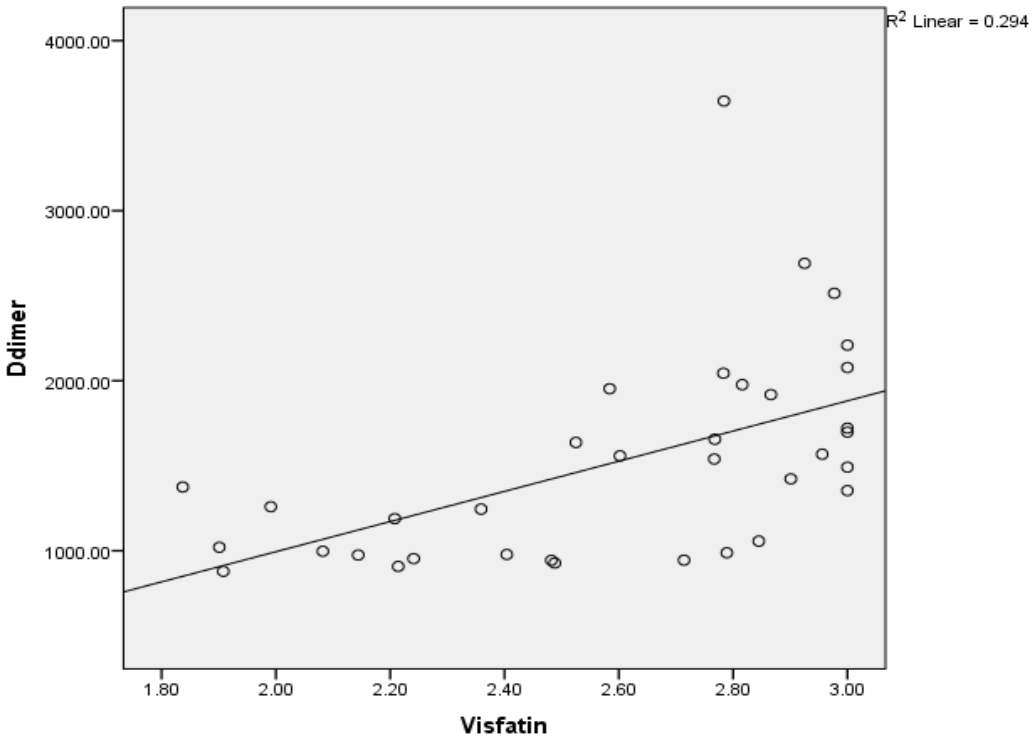


Figure 8: Correlation between Visfatin and D-dimer in painful patients group.

4.2.9. Correlation between Visfatin and Ferritin in painful patients group:

According to the results in Figure (9) there was positive significant correlation between Visfatin and Ferritin in painful crisis patients group in both children and adults (R2 = 0.450). The rise in the serum ferritin in sickle cell anemia patients due to the excess free iron. The excess breakdown of Hb and the abnormal circulating mass of Hb in reticuloendothelial cells, exert a positive feedback on ferritin synthesis (Alsultan *et al*., 2010), HB will bind with NO and lead to endothelial dysfunction ending with released Visfatin in high level. This indicates that the rise in Ferritin is associated with the rise in Visfatin in painful crisis. These findings were in agreement with other previous published data in which authors reported that Serum visfatin level was positively correlated with serum ferritin level (D. M. M. Habashy & Shams, n.d.).

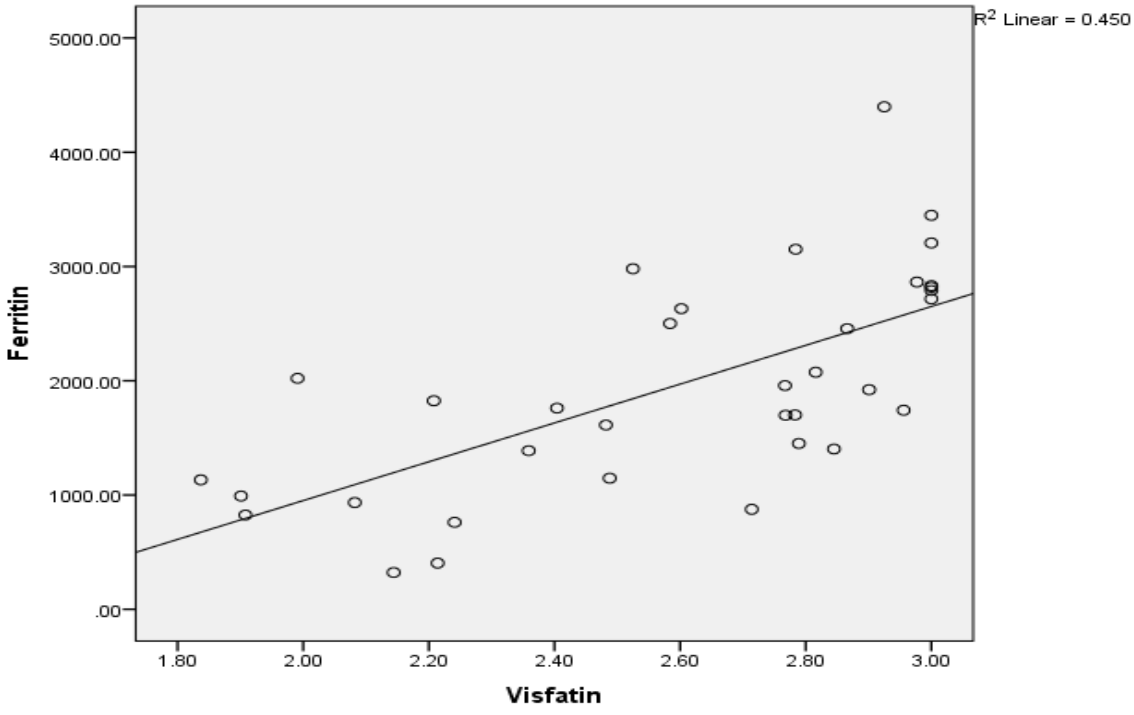


Figure 9: Correlation between Visfatin and Ferritin in painful patients group.

Conclusions and Recommendations

Conclusions:

1- The study concluded that the level of Visvatin in the blood is significantly higher among patients with sickle cell anemia in both (steady state and painful crisis) compared to control, with a positive relationship between the level of Visfatin in the blood and the frequency of vascular occlusion crises.
2- The level of Vcam-1 is higher in patients compared to healthy subjects, with a positive relationship between Vcam-1 level and endothelial dysfunction

Recommendations:

1- Larger sample size should be tested in the future to increase the accuracy of results.
2- Studying the effect of these markers on females.
3- Studying the effect of other markers such as (Total bilirubin, Nitric Oxide Synthase, Peroxidase, and Malondialdehyde) on patients with sickle cell anemia in (painful crisis, steady state) and comparing them with healthy subjects.

The References

References:

Abdelmaged, D. (n.d.). *Efficacy of Gum Arabic as Anti-inflammatory Agent in Sickle Cell Anemia Pediatric Patients : A randomized , double-blind , placebo-controlled Phase III trial Background :*

Abdul-Hussein, H. K., Al-Mammori, H. S., & Hassan, M. K. (2021). Evaluation of the expression of red blood cell CD36, interleukin-6 and interleukin-8 in sickle cell anemia pediatric patients. *Cytokine*, *143*(January), 1–6. https://doi.org/10.1016/j.cyto.2021.155534

Abboursheid, T., Albaroudi, O., & Alahdab, F. (2022). Inhaled nitric oxide for treating pain crises in people with sickle cell disease. *Cochrane Database of Systematic Reviews*, *2022*(7). https://doi.org/10.1002/14651858.CD011808.pub3

Adly, A. A., Ismail, E. A., Andrawes, N. G., Mahmoud, M. M., & Eladawy, R. (2016). Soluble Fas/FasL ratio as a marker of vasculopathy in children and adolescents with sickle cell disease. *Cytokine*, *79*, 52–58. https://doi.org/10.1016/j.cyto.2015.12.022

Ahmed, S. G., & Ibrahim, U. A. (2023). *Pathophysiologic Basis of Haemolysis in Patients with Sickle Cell Disease in Steady State and in Hyperhaemolytic States : Aetiopathogenesis , Management , and Mitigation*. https://doi.org/10.4103/njbcs.njbcs

Akinsheye, I., & Klings, E. S. (2010). Sickle cell anemia and vascular dysfunction: The nitric oxide connection. *Journal of Cellular Physiology*, *224*(3), 620–625. https://doi.org/10.1002/jcp.22195

Al-basheer, J. G., Abdelgadir, O., Eldin, A., & Mustafa, M. (2015). C - reactive Protein Level and WBC Count as Biomarkers for Vaso-Occlusive Crisis among Patients with Sickle Cell Disease. *American Journal of Medicine and Medical Sciences*, *5*(6), 283–286. https://doi.org/10.5923/j.ajmms.20150506.03

Al Mudhafar, A. M. H., Hadi, N. R., Salman, R. A., Jasim, T. A., Hasoon, H. K., Jamil, D. A., & Al-Aubaidy, H. A. (2019). Effects of hydroxyurea on cerebral blood flow in sickle cell anemia patients. *World Heart Journal*, *11*(1), 25–32. https://doi.org/10.14456/ITJEMAST.2021.166

Ali Hazzazi, A., Ageeli, M. H., Alfaqih, A. M., Ali Jaafari, A., Malhan, H. M., & Bakkar, M. M. (2020). Epidemiology and characteristics of sickle cell patients admitted to hospitals in Jazan region, Saudi Arabia. *Journal of Applied Hematology*, *11*(1), 10–14. https://doi.org/10.4103/joah.joah_67_19

Alkindi, S., Al-Ghadani, A. R., Al-Zeheimi, S. R., Alkindi, S. Y., Fawaz, N., Ballas,

S. K., & Pathare, A. V. (2021). Predicting risk factors for thromboembolic complications in patients with sickle cell anaemia – lessons learned for prophylaxis. *Journal of International Medical Research*, *49*(12). https://doi.org/10.1177/03000605211055385

Allali, S., Maciel, T. T., Hermine, O., & De Montalembert, M. (2020). Innate immune cells, major protagonists of sickle cell disease pathophysiology. *Haematologica*, *105*(2), 273–283. https://doi.org/10.3324/haematol.2019.229989

Alsultan, A. I., Seif, M. A., Amin, T. T., Naboli, M., & Alsuliman, A. M. (2010). Relationship between oxidative stress, ferritin and insulin resistance in sickle cell disease. *European Review for Medical and Pharmacological Sciences*, *14*(6), 527–538.

Antwi-Boasiako, C., & Campbell, A. D. (2018). Low nitric oxide level is implicated in sickle cell disease and its complications in Ghana. *Vascular Health and Risk Management*, *14*, 199–204. https://doi.org/10.2147/VHRM.S163228

Antwi-Boasiako, C., Dankwah, G. B., Aryee, R., Hayfron-Benjamin, C., Donkor, E. S., & Campbell, A. D. (2019). Oxidative Profile of Patients with Sickle Cell Disease. *Medical Sciences (Basel, Switzerland)*, *7*(2), 1–8. https://doi.org/10.3390/medsci7020017

Antwi-Boasiako, C., Donkor, E. S., Sey, F., Dzudzor, B., Dankwah, G. B., Otu, K. H., Doku, A., Dale, C. A., & Ekem, I. (2018). Levels of Soluble Endothelium Adhesion Molecules and Complications among Sickle Cell Disease Patients in Ghana. *Diseases*, *6*(2), 29. https://doi.org/10.3390/diseases6020029

Antwi-Boasiako, C., Ekem, I., Abdul-Rahman, M., Sey, F., Doku, A., Dzudzor, B., Dankwah, G. B., Otu, K. H., Ahenkorah, J., & Aryee, R. (2018). Hematological parameters in Ghanaian sickle cell disease patients. *Journal of Blood Medicine*, *9*, 203–209. https://doi.org/10.2147/JBM.S169872

AO, O., EO, A., ED, E., BO, O., & RB, J. (2022). Haemostatic Parameters in Normal and Anemia-Induced Rabbits Treated With Anti-Sickling Polyhedral Mixture. *J Hematol Thrombo Dis.*, *10*(4), 1000487. https://doi.org/10.35248/2

Arias, C. F., & Arias, C. F. (2017). How do red blood cells know when to die? *Royal Society Open Science*, *4*(4). https://doi.org/10.1098/rsos.160850

Ataga, K. I., Brittain, J. E., Desai, P., May, R., Jones, S., Delaney, J., Hinderliter, A., & Key, N. S. (2012). *Association of Coagulation Activation with Clinical Complications in Sickle Cell Disease*. *7*(1). https://doi.org/10.1371/journal.pone.0029786

Ataga, K. I., Moore, C. G., Hillery, C. A., Jones, S., Whinna, H. C., Strayhorn, D., Sohier, C., Hinderliter, A., Parise, L. V., & Orringer, E. P. (2008). Coagulation activation and inflammation in sickle cell disease-associated pulmonary hypertension. *Haematologica*, *93*(1), 20–26. https://doi.org/10.3324/haematol.11763

Atiyah, M. N., Akeel, B., Ali, A., & Abd, H. M. (2021). *Two years of premarital screening program for hemoglobinopathies in Karbala , Iraq ; Outcome and options for improving the program* . *25*(6), 623–630.

Attiyah, M. N., & Al-najafi, W. K. (2020). *Original Paper Premarital Screening Program for Hemoglobinopathies in*. *13*(1), 2293–2300.

Ay, C., Dunkler, D., Pirker, R., Thaler, J., Quehenberger, P., Wagner, O., Zielinski, C., & Pabinger, I. (2012). High D-dimer levels are associated with poor prognosis in cancer patients. *Haematologica*, *97*(8), 1158–1164. https://doi.org/10.3324/haematol.2011.054718

Badr, S. H. (2022). *Quality of life among adolescents with hemoglobinopathies*. *6*(April), 2960–2967.

Ballas, S. K., Gupta, K., & Adams-Graves, P. (2012). Sickle cell pain: A critical reappraisal. *Blood*, *120*(18), 3647–3656. https://doi.org/10.1182/blood-2012-04-383430

Benjamin, A., Adeshola, A., Fatima, A. M., John, J., Akintunde, A. A., Mohammed, I., Benedict, A., Jatau, A. A., & Haruna, M. M. (2023). *Chronic myeloid leukemia coexisting with non-Hodgkin ' s lymphoma : an uncommon presentation of bilineage hematological malignancy*. 227–231. https://doi.org/10.4103/ejh.ejh

Bhaskar, L. V. (2019). Factor V Leiden and prothrombin G20210A mutations and risk of vaso-occlusive complications in sickle cell disease: A meta-analysis through the lens of nephrology. *Journal of Nephropharmacology*, *8*(2), 16–16. https://doi.org/10.15171/npj.2019.16

Bhaskar, L. V. K. S. (2021). Susceptibility to vascular complications in sickle cell anemia patients is associated with intron 4a/b polymorphism of the NOS3 gene: A meta-analysis. *Meta Gene*, *28*(October 2020), 100870. https://doi.org/10.1016/j.mgene.2021.100870

Bookchin, R. M., & Lew, V. L. (1996). Pathophysiology of sickle cell anemia. *Hematology/Oncology Clinics of North America*, *10*(6), 1241–1253. https://doi.org/10.1016/S0889-8588(05)70397-X

C Onyekwelu, K., A Ufelle, S., E Ikekpeazu, J., C Ezeh, R., Z Uche, C., & Philip Udoh,

I. (2019). Changes during vaso-occlusive crisis (VOC) and normal state in sickle cell disease patients. *Medical Case Reports and Reviews*, *2*(1), 1–4. https://doi.org/10.15761/mcrr.1000122

Chillab, E. D., Talib, R. A., & Al-Awsi, G. R. L. (2019). Genetics of sickle cell anemia disorders in Baghdad City, Iraq. *Indian Journal of Public Health Research and Development*, *10*(2), 817–822. https://doi.org/10.5958/0976-5506.2019.00396.6

Coates, D. R., Chin, J. M., & Chung, S. T. L. (2011). 基因的改变NIH Public Access. *Bone*, *23*(1), 1–7. https://doi.org/10.1016/j.jpeds.2011.07.018.Markers

Cullis, J. O., Fitzsimons, E. J., Griffiths, W. J. H., Tsochatzis, E., & Thomas, D. W. (2018). Investigation and management of a raised serum ferritin. *British Journal of Haematology*, *181*(3), 331–340. https://doi.org/10.1111/bjh.15166

Dahl, T. B., Holm, S., Aukrust, P., & Halvorsen, B. (2012). Visfatin/NAMPT: A multifaceted molecule with diverse roles in physiology and pathophysiology. *Annual Review of Nutrition*, *32*, 229–243. https://doi.org/10.1146/annurev-nutr-071811-150746

Desai, B., & Somannavar, M. S. (2011). *Visfatin – a review*. 9–14.

Disorders, R. C. (2012). *A rticles and B rief Reports Hemoglobin sickle cell disease complications : a clinical study of 179 cases St or ti Fo un da tio n Fe rra ta St or ti Fo un da tio n*. *97*(8). https://doi.org/10.3324/haematol.2011.055202

Elnaim, E. G., Ibrahim, S., Ahmed, D., Aldaw, R., Salih, N., Musa, M., Yosuif, E., Ali, S. A., Hamed, H. A. A., Mohammed, E. H., & Bilal, N. E. (2020). Investigate of Haemostatic and Fibrinolytic System Parameters among Sickle Cell Anaemia Patients in the Khartoum State. *Indian Journal of Pharmaceutical and Biological Research*, *8*(02), 01–05. https://doi.org/10.30750/ijpbr.8.2.1

Erten, M. (2021). Visfatin as a promising marker of cardiometabolic risk. *Acta Cardiologica Sinica*, *37*(5), 464–472. https://doi.org/10.6515/ACS.202109_37(5).20210323B

Faes, C., Ilich, A., Sotiaux, A., Sparkenbaugh, E. M., Henderson, M. W., Buczek, L., Beckman, J. D., Ellsworth, P., Noubouossie, D. F., Bhoopat, L., Piegore, M., Renoux, C., Bergmeier, W., Park, Y., Ataga, K. I., Cooley, B., Wolberg, A. S., Key, N. S., & Pawlinski, R. (2019). Red blood cells modulate structure and dynamics of venous clot formation in sickle cell disease. *Blood*, *133*(23), 2529–2541. https://doi.org/10.1182/blood.2019000424

Fedosov, D. A., Fornleitner, J., & Gompper, G. (2012). Margination of White blood

cells in microcapillary flow. *Physical Review Letters*, *108*(2), 1–5. https://doi.org/10.1103/PhysRevLett.108.028104

Fialek, B., Pruc, M., Smereka, J., Jas, R., Rahnama-Hezavah, M., Denegri, A., Szarpak, A., Jaguszewski, M. J., Peacock, F. W., & Szarpak, L. (2022). Diagnostic value of lactate dehydrogenase in COVID-19: A systematic review and meta-analysis. *Cardiology Journal*, *29*(5), 751–758. https://doi.org/10.5603/CJ.a2022.0056

Gallagher, E. J., Le Roith, D., & Bloomgarden, Z. (2009). Review of hemoglobin A(1c) in the management of diabetes. *Journal of Diabetes*, *1*(1), 9–17. https://doi.org/10.1111/j.1753-0407.2009.00009.x

Gremmel, T., Frelinger, A. L., & Michelson, A. D. (2016). Platelet physiology. *Seminars in Thrombosis and Hemostasis*, *42*(3), 191–204. https://doi.org/10.1055/s-0035-1564835

Gupta, G. S. (2022). The Lactate and the Lactate Dehydrogenase in Inflammatory Diseases and Major Risk Factors in COVID-19 Patients. *Inflammation*, *45*(6), 2091–2123. https://doi.org/10.1007/s10753-022-01680-7

Gurkan, S., Scarponi, K. J., Hotchkiss, H., Savage, B., & Drachtman, R. (2010). Lactate dehydrogenase as a predictor of kidney involvement in patients with sickle cell anemia. *Pediatric Nephrology*, *25*(10), 2123–2127. https://doi.org/10.1007/s00467-010-1560-8

Habashy, D. M. M., & Shams, M. A. (n.d.). *Serum visfatin in sickle cell disease : association with frequency of vaso-occlusive crises*. 52–57. https://doi.org/10.4103/ejh.ejh

Habashy, D. M., & Shams, M. (2017). Serum visfatin in sickle cell disease: association with frequency of vaso-occlusive crises. *The Egyptian Journal of Haematology*, *42*(2), 52. https://doi.org/10.4103/ejh.ejh_21_17

Hamed, M. H. A. (2018). *Downloaded from https://academic.oup.com/qjmed/article/111/suppl_1/hcy200.155/5244509 by guest on 24 April 2023 Downloaded from https://academic.oup.com/qjmed/article/111/suppl_1/hcy200.155/5244509 by guest on 24 April 2023*. *111*, 69–70.

Hammoudi, N., Lionnet, F., Redheuil, A., & Montalescot, G. (2020). Cardiovascular manifestations of sickle cell disease. *European Heart Journal*, *41*(13), 1365–1373. https://doi.org/10.1093/eurheartj/ehz217

Hashim, Z., Alsharafa, Y., Malik, A., Al, I., & Al-sabbagh, W. R. M. (2023). *EVALUATION OF D-DIMER VALUES IN COVID-19 IRAQI PATIENTS AND ITS*

CORRELATION WITH THE UNDERLYING CO-MORBID DISEASES. *April*, 209–215.

Hebbel, R., Vercellotti, G., & Nath, K. (2012). A Systems Biology Consideration of the Vasculopathy of Sickle Cell Anemia: The Need for Multi-Modality Chemo-Prophylaxis. *Cardiovascular & Hematological Disorders-Drug Targets*, *9*(4), 271–292. https://doi.org/10.2174/1871529x10909040271

Hicks, K. G., Cluntun, A. A., Schubert, H. L., Hackett, S. R., Berg, J. A., Leonard, P. G., Ajalla Aleixo, M. A., Zhou, Y., Bott, A. J., Salvatore, S. R., Chang, F., Blevins, A., Barta, P., Tilley, S., Leifer, A., Guzman, A., Arok, A., Fogarty, S., Winter, J. M., … Rutter, J. (2023). Protein-metabolite interactomics of carbohydrate metabolism reveal regulation of lactate dehydrogenase. *Science (New York, N.Y.)*, *379*(6636), 996–1003. https://doi.org/10.1126/science.abm3452

I, I. N., Augustine, B., M, S. A., O, A. I., & D, A. C. (2022). *Uche Ebele I a , Hassan Aderonke O b , Akinbami Akinsegun A b , Bamiro Fafatu A b , Assessment of serpin A5 levels and coagulation parameters of sickle cell anemia patients during bone pain vaso-occlusive crisis and steady state*. 152–157. https://doi.org/10.4103/ejh.ejh

Ingle, P. V., & Patel, D. M. (2011). C- reactive protein in various disease condition - an overview. *Asian Journal of Pharmaceutical and Clinical Research*, *4*(1), 9–13.

Ishau, M. (2022). *Evaluation Of Reduced Glutathione As A Marker Of Oxidative Stress In Sickle Cell Disease Patients In A Tertiary Hospital In Nigeria*. *8*(4).

Jalali Far, M. A., Oodi, A., Amirizadeh, N., Mohammadipour, M., & Keikhaei Dehdezi, B. (2021). The Rh blood group system and its role in alloimmunization rate among sickle cell disease and sickle thalassemia patients in Iran. *Molecular Genetics and Genomic Medicine*, *9*(3), 1–9. https://doi.org/10.1002/mgg3.1614

Jeddoa, Z. M., Mohammad, Z., & Jeddoa, A. (2011). Evaluation of (HPLC) Patterns of Sickle Cell Anaemia Patients in Comparison with Apparently Healthy Individuals. *Karbala J. Med*, *4*(1), 980–987.

Joseph, R. (2022). *Sickle Cell Anemia & Associated Neurological Complications : A Literature Review Sickle Cell Anemia*.

Kaddam, L. A., & Kaddam, A. S. (2020). Effect of Gum Arabic (Acacia senegal) on C-reactive protein level among sickle cell anemia patients. *BMC Research Notes*, *13*(1), 1–5. https://doi.org/10.1186/s13104-020-05016-2

Karahan, F., Ünal, S., Topçu, D. B., Öztaş, Y., & Bozlu, G. (2022). The role of immature granulocyte percentage in predicting acute chest syndrome and the

severity of the vaso-occlusive crisis in sickle cell disease. *Turkish Journal of Pediatrics*, *64*(1), 92–97. https://doi.org/10.24953/turkjped.2021.1385

Kato, G. J., Piel, F. B., Reid, C. D., Gaston, M. H., Ohene-Frempong, K., Krishnamurti, L., Smith, W. R., Panepinto, J. A., Weatherall, D. J., Costa, F. F., & Vichinsky, E. P. (2018). Sickle cell disease. *Nature Reviews Disease Primers*, *4*, 1–22. https://doi.org/10.1038/nrdp.2018.10

Kavanagh, P. L., Fasipe, T. A., & Wun, T. (2022). Sickle Cell Disease: A Review. *Jama*, *328*(1), 57–68. https://doi.org/10.1001/jama.2022.10233

Khan, Z., Nawaz, M., Khan, A., & Bacha, U. (2013). Hemoglobin, red blood cell count, hematocrit and derived parameters for diagnosing anemia in elderly males. *Proceedings of the Pakistan Academy of Sciences*, *50*(3), 217–226.

Kim, M., Kim, A., Kim, S., & Yang, S. (2012). Improvement of electrical blood hematocrit measurements under various plasma conditions using a novel hematocrit estimation parameter. *Biosensors and Bioelectronics*, *35*(1), 416–420. https://doi.org/10.1016/j.bios.2012.02.010

Kingsley, A., Ofonime, E., & Edakabasi, A. (2022). *ADAMTS13 levels in sickle cell anaemia : is there a change between steady-state and vaso-occlusive crisis ?* 26–34. https://doi.org/10.4103/ejh.ejh

Kolańska-Dams, E., Boinska, J., & Socha, M. W. (2021). Adipokine levels and carbohydrate metabolism in patients diagnosed de novo with polycystic ovary syndrome. *Qatar Medical Journal*, *2021*(2), 1–8. https://doi.org/10.5339/QMJ.2021.34

Kong, D. H., Kim, Y. K., Kim, M. R., Jang, J. H., & Lee, S. (2018). Emerging roles of vascular cell adhesion molecule-1 (VCAM-1) in immunological disorders and cancer. *International Journal of Molecular Sciences*, *19*(4), 13–17. https://doi.org/10.3390/ijms19041057

Koperdanova, M., & Cullis, J. O. (2015). Interpreting raised serum ferritin levels. *BMJ (Online)*, *351*(August), 9–11. https://doi.org/10.1136/bmj.h3692

Kotb, M. M., Almalki, M. J., Hassan, Y., Al Sharif, A., Khan, M., & Sheikh, K. (2019). Effect of Health Education Programme on the Knowledge of and Attitude about Sickle Cell Anaemia among Male Secondary School Students in the Jazan Region of Saudi Arabia: Health Policy Implications. *BioMed Research International*, *2019*. https://doi.org/10.1155/2019/9653092

Kreka, M., Nastas, E., Refatllari, E., Idrizi, A., Shpata, V., Kreka, B., & Godo, A. (2022). Laboratory Indicators and Some Considerations in Albanian Patients with

Beta Thalassemia Major and Sickle Cell Disease. *Journal of Biosciences and Medicines*, *10*(01), 141–149. https://doi.org/10.4236/jbm.2022.101011

Kumar, P., Nagarajan, A., & Uchil, P. D. (2018). Analysis of cell viability by the lactate dehydrogenase assay. *Cold Spring Harbor Protocols*, *2018*(6), 465–468. https://doi.org/10.1101/pdb.prot095497

Lervolino, L. G., Baldin, P. E. A., Picado, S. M., Calil, K. B., Viel, A. A., & Campos, L. A. F. (2011). Prevalence of sickle cell disease and sickle cell trait in national neonatal screening studies. *Revista Brasileira de Hematologia e Hemoterapia*, *33*(1), 49–54. https://doi.org/10.5581/1516-8484.20110015

Li, C., Ye, J., Chen, Q., Hu, W., Wang, L., Fan, Y., Lu, Z., Chen, J., Chen, Z., Chen, S., Tong, J., Xiao, W., Mei, J., & Lu, H. (2020). Elevated Lactate Dehydrogenase (LDH) level as an independent risk factor for the severity and mortality of COVID-19. *Aging*, *12*(15), 15670–15681. https://doi.org/10.18632/AGING.103770

Linkins, L. A., & Takach Lapner, S. (2017). Review of D-dimer testing: Good, Bad, and Ugly. *International Journal of Laboratory Hematology*, *39*(February), 98–103. https://doi.org/10.1111/ijlh.12665

Lucarelli, M. E. (1997). Hydroxyurea for the treatment of severe sickle cell anemia. *American Family Physician*, *55*(2), 658–660.

Mahmoud, F. A., & Rivera, N. I. (2002). The role of C-reactive protein as a prognostic indicator in advanced cancer. *Current Oncology Reports*, *4*(3), 250–255. https://doi.org/10.1007/s11912-002-0023-1

Makani, J., Cox, S. E., Soka, D., Komba, A. N., Oruo, J., Mwamtemi, H., Magesa, P., Rwezaula, S., Meda, E., Mgaya, J., Lowe, B., Muturi, D., Roberts, D. J., Williams, T. N., Pallangyo, K., Kitundu, J., Fegan, G., Kirkham, F. J., Marsh, K., & Newton, C. R. (2011). Mortality in sickle cell anemia in africa: A prospective cohort study in Tanzania. *PLoS ONE*, *6*(2). https://doi.org/10.1371/journal.pone.0014699

Mbayabo, G., Lumbala Kabuyi, P., Ngole, M., Lumaka, A., Race, V., Maisin, D., Gruson, D., Matthijs, G., Minga, T. M., Devriendt, K., Van Geet, C., & Tshilobo, P. L. (2022). Value of DNA testing in the diagnosis of sickle-cell anemia in childhood in an environment with a high prevalence of other causes of anemia. *Journal of Clinical Laboratory Analysis*, *36*(8), 1–6. https://doi.org/10.1002/jcla.24593

Mecabo, G., Yamamoto, M., Biassi, T. P., & Figueiredo, M. S. (2015). Lactate dehydrogenase isoenzyme 3 and hemolysis in sickle cell anemia: A possible correlation? *Blood*, *125*(24), 3821–3822. https://doi.org/10.1182/blood-2015-04-

641522

Meher, S., Patel, S., Das, K., Dehury, S., Jit, B. P., Maske, M. M., Das, P., Dash, B. P., & Mohanty, P. K. (2019). Association of plasma homocysteine level with vaso-occlusive crisis in sickle cell anemia patients of Odisha, India. *Annals of Hematology*, *98*(10), 2257–2265. https://doi.org/10.1007/s00277-019-03776-x

Melchionda, F., Oncology, P., Spreafico, F., Unit, P. O., Hemato-oncology, P., Ciceri, S., Unit, G. T., Medicine, P., Lima, M., Unit, P. S., Collini, P., Pession, A., Massimino, M., Radice, P., & Perotti, D. (2013). LETTER TO THE EDITOR A Novel WT1 Mutation in Familial Wilms Tumor. *Pediatric Blood & Cancer*, *February*, 1388–1389. https://doi.org/10.1002/pbc

Mohamed, E. A., Elgari, M. M., Babker, A. M., & Waggiallah, H. A. (2020). Comparative study of hypercoagulability change in steady state and during vaso-occlusive crisis among sudanese patients living with sickle cell disease. *African Health Sciences*, *20*(1), 392–396. https://doi.org/10.4314/ahs.v20i1.45

Mohammed, F. A., Mahdi, N., Sater, M. A., Al-Ola, K., & Almawi, W. Y. (2010). The relation of C-reactive protein to vasoocclusive crisis in children with sickle cell disease. *Blood Cells, Molecules, and Diseases*, *45*(4), 293–296. https://doi.org/10.1016/j.bcmd.2010.08.003

Mohd Safuan, S. N., Md Tomari, M. R., & Wan Zakaria, W. N. (2018). White blood cell (WBC) counting analysis in blood smear images using various color segmentation methods. *Measurement: Journal of the International Measurement Confederation*, *116*(November 2017), 543–555. https://doi.org/10.1016/j.measurement.2017.11.002

Monteiro, J., Cople-rodrigues, C., & Citelli, M. (2023). *Vitamin D and its effects on cell adhesion molecules : A systematic review*. 1–18.

Mostafa, G. G., Zahran, F. E., Omer, S. A., Ibrahim, A., & Elhakeem, H. (2020). The effect of serum ferritin level on gonadal, prolactin, thyroid hormones, and thyroid stimulating hormone in adult males with sickle cell anemia. *Journal of Blood Medicine*, *11*, 27–32. https://doi.org/10.2147/JBM.S232562

Naik, R. P., Wilson, J. G., Ekunwe, L., Mwasongwe, S., Duan, Q., Li, Y., Correa, A., & Reiner, A. P. (2016). Elevated D-dimer levels in African Americans with sickle cell trait. *Blood*, *127*(18), 2261–2263. https://doi.org/10.1182/blood-2016-01-694422

National Association of Resident Doctors of Nigeria. (2015). Nigerian Journal of Medicine. *Medical Journal*, *24*(1), 17–27. https://doi.org/10.4103/NJM.NJM

Ney, P. A. (2011). Normal and disordered reticulocyte maturation. *Current Opinion in Hematology*, *18*(3), 152–157. https://doi.org/10.1097/MOH.0b013e328345213e

Nnajekwu, U. C., Nnajekwu, C. O., Onukwuli, V. O., Chukwu, B. F., Ikefuna, A. N., & Emodi, I. J. (2022). Folate levels in children with sickle cell anaemia on folic acid supplementation in steady state and crises at a tertiary hospital in Enugu, Nigeria: a prospective, comparative study. *Malawi Medical Journal*, *34*(2), 132–137. https://doi.org/10.4314/mmj.v34i2.9

Noronha, S. A., Sadreameli, S. C., & Strouse, J. J. (2016). Management of sickle cell disease in children. *Southern Medical Journal*, *109*(9), 495–502. https://doi.org/10.14423/SMJ.0000000000000523

Obeagu, E. I., & Muhimbura, E. (2022). *An Update on Interferon Gamma and C Reactive Proteins in Sickle Cell Anaemia Crisis Abstract*. https://doi.org/10.36648/2254-609X.11.10.84

Obeagu, E. I., Ochei, K., & Okoro, O. (2015). *Sickle Cell Anaemia : A Review Sickle Cell Anaemia : A Review*. *3*(April 2016), 2244–2252. https://www.researchgate.net/profile/Emmanuel_Obeagu/publication/301327927_Sickle_Cell_Anaemia_A_Review/links/571214f008ae39beb87a2f11/Sickle-Cell-Anaemia-A-Review.pdf%0Ahttps://www.researchgate.net/publication/301327927_Sickle_Cell_Anaemia_A_Review

Odièvre, M., Verger, E., Silva-pinto, A. C., Elion, J., & Indies, F. W. (2011). *Ijmr-134-532*. *October*, 532–537.

Ogawa, C., Tsuchiya, K., & Maeda, K. (2020). Reticulocyte hemoglobin content. *Clinica Chimica Acta*, *504*(October 2019), 138–145. https://doi.org/10.1016/j.cca.2020.01.032

Pecker, L. H., & Lanzkron, S. (2021). Sickle cell disease. *Annals of Internal Medicine*, *174*(1), ITC1–ITC16. https://doi.org/10.7326/AITC202101190

Petersson, K., Jakobsson, O., Ohlsson, P., Augustsson, P., Scheding, S., Malm, J., & Laurell, T. (2018). Acoustofluidic hematocrit determination. *Analytica Chimica Acta*, *1000*, 199–204. https://doi.org/10.1016/j.aca.2017.11.037

Plays, M., Müller, S., & Rodriguez, R. (2021). Chemistry and biology of ferritin. *Metallomics*, *13*(5). https://doi.org/10.1093/mtomcs/mfab021

Prce, A., & Zadro, R. (2020). *Original articles results*. *30*(3), 1–9.

Pretini, V., Koenen, M. H., Kaestner, L., Fens, M. H. A. M., Schiffelers, R. M., Bartels,

M., & Van Wijk, R. (2019). Red blood cells: Chasing interactions. *Frontiers in Physiology*, *10*(JUL), 1–17. https://doi.org/10.3389/fphys.2019.00945

Pulivarthi, S., & Gurram, M. K. (2014). Effectiveness of D-dimer as a screening test for venous thromboembolism: An update. *North American Journal of Medical Sciences*, *6*(10), 491–499. https://doi.org/10.4103/1947-2714.143278

Rees, D. C., & Gibson, J. S. (2012). Biomarkers in sickle cell disease. *British Journal of Haematology*, *156*(4), 433–445. https://doi.org/10.1111/j.1365-2141.2011.08961.x

Romacho, T., Sánchez-Ferrer, C. F., & Peiró, C. (2013). Visfatin/Nampt: An adipokine with cardiovascular impact. *Mediators of Inflammation*, *2013*(Cv). https://doi.org/10.1155/2013/946427

Romacho, T., Villalobos, L. A., Cercas, E., Carraro, R., Sánchez-Ferrer, C. F., & Peiró, C. (2013). Visfatin as a Novel Mediator Released by Inflamed Human Endothelial Cells. *PLoS ONE*, *8*(10), 1–7. https://doi.org/10.1371/journal.pone.0078283

Saddi-Rosa, P., Oliveira, C. S., Giuffrida, F. M., & Reis, A. F. (2010). Visfatin, glucose metabolism and vascular disease: A review of evidence. *Diabetology and Metabolic Syndrome*, *2*(1), 1–6. https://doi.org/10.1186/1758-5996-2-21

Saeed, A., & Abolaban, F. (2021). Spectroscopic study of the effect of low dose fast neutrons on the hemoglobin structure. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *261*, 120082. https://doi.org/10.1016/j.saa.2021.120082

Saha, D., Patgaonkar, M., Shroff, A., Ayyar, K., Bashir, T., & Reddy, K. V. R. (2014). Hemoglobin Expression in Nonerythroid Cells: Novel or Ubiquitous? *International Journal of Inflammation*, *2014*. https://doi.org/10.1155/2014/803237

Sahlol, A. T., Kollmannsberger, P., & Ewees, A. A. (2020). Efficient Classification of White Blood Cell Leukemia with Improved Swarm Optimization of Deep Features. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-59215-9

Schnog, J. B., Rojer, R. A., Gillavry, M. R., Cate, H., Brandjes, D. P. M., & Duits, A. J. (2003). Steady-state sVCAM-1 serum levels in adults with sickle cell disease. *Annals of Hematology*, *82*(2), 109–113. https://doi.org/10.1007/s00277-003-0609-1

Schnog, J. B., Teerlink, T., van der Dijs, F. P. L., Duits, A. J., & Muskiet, F. A. J. (2005). Plasma levels of asymmetric dimethylarginine (ADMA), an endogenous

nitric oxide synthase inhibitor, are elevated in sickle cell disease. *Annals of Hematology*, *84*(5), 282–286. https://doi.org/10.1007/s00277-004-0983-3

Sen, B., Ganesh, A., Bhan, A., & Dixit, S. (2021). "Deep Learning based diagnosis of sickle cell anemia in human RBC." *Proceedings of 2021 2nd International Conference on Intelligent Engineering and Management, ICIEM 2021*, 526–529. https://doi.org/10.1109/ICIEM51511.2021.9445293

Shantanam, S., & MUELLER. (2018a). **乳鼠心肌提取** HHS Public Access. *Physiology & Behavior*, *176*(1), 139–148. https://doi.org/10.1517/14740338.2015.1088827.Hydroxyurea

Shantanam, S., & MUELLER. (2018b). **乳鼠心肌提取** HHS Public Access. *Physiology & Behavior*, *176*(1), 139–148. https://doi.org/10.1002/cpt.2028.Changing

Smith, E., Lebensburger, J., Hilliard, L., Kelly, D., Fineberg, N., Bai, S., & Howard, T. (2014). Ferritin and LIC: Predicting liver injury in children with sickle cell. *Journal of Pediatric Gastroenterology and Nutrition*, *58*(3), 387–390. https://doi.org/10.1097/MPG.0000000000000235

Stankovic Stojanovic, K., & Lionnet, F. (2016). Lactate dehydrogenase in sickle cell disease. *Clinica Chimica Acta*, *458*, 99–102. https://doi.org/10.1016/j.cca.2016.04.035

Steinberg, M. H. (2008). Sickle cell anemia, the first molecular disease: Overview of molecular etiology, pathophysiology, and therapeutic approaches. *TheScientificWorldJournal*, *8*, 1295–1324. https://doi.org/10.1100/tsw.2008.157

Steinberg, M. H. (2009). Genetic etiologies for phenotypic diversity in sickle cell anemia. *TheScientificWorldJournal*, *9*, 46–67. https://doi.org/10.1100/tsw.2009.10

Stevens-hernandez, C. J., & Bruce, L. J. (1970). Reticulocyte Maturation. *Anesthesiology*, *32*(5), 485–485. https://doi.org/10.1097/00000542-197005000-00063

Sukla, S. K., Mohanty, P. K., Patel, S., Das, K., Hiregoudar, M., Soren, U. K., & Meher, S. (2022). Iron profile of pregnant sickle cell anemia patients in Odisha, India. *Hematology, Transfusion and Cell Therapy*, *xx*. https://doi.org/10.1016/j.htct.2021.06.012

Sun, X., Hong, Y., Gong, Y., Zheng, S., & Xie, D. (2021). Bioengineered ferritin nanocarriers for cancer therapy. *International Journal of Molecular Sciences*, *22*(13). https://doi.org/10.3390/ijms22137023

Sun, Y., Du, H., Lan, Y., Wang, W., Liang, Y., Feng, C., & Yang, M. (2016). Preparation of hemoglobin (Hb) imprinted polymer by Hb catalyzed eATRP and its application in biosensor. *Biosensors and Bioelectronics*, *77*, 894–900. https://doi.org/10.1016/j.bios.2015.10.067

Tanabe, P., Spratling, R., Professions, H., Smith, D., Grissom, P., Hulihan, M., & Disabilities, D. (2020). *HHS Public Access*. *119*(6), 26–35. https://doi.org/10.1097/01.NAJ.0000559779.40570.2c.Understanding

Thom, C. S., Dickson, C. F., Gell, D. A., & Weiss, M. J. (2013). Hemoglobin variants: Biochemical properties and clinical correlates. *Cold Spring Harbor Perspectives in Medicine*, *3*(3). https://doi.org/10.1101/cshperspect.a011858

Torre, E. A., & Emert, B. L. (2019). *Therapy Resistance*. 1–11.

Uzsoy, N. K. (2020). Findings in Patients with Sickle Cell Anemia *. *Heart Disease*, 23–29. https://doi.org/10.4103/ijh.ijh

van der Meijden, P. E. J., & Heemskerk, J. W. M. (2019). Platelet biology and functions: new concepts and clinical perspectives. *Nature Reviews Cardiology*, *16*(3), 166–179. https://doi.org/10.1038/s41569-018-0110-0

van Vuren, A. J., Minniti, C. P., Mendelsohn, L., Baird, J. H., Kato, G. J., & van Beers, E. J. (2021). Lactate dehydrogenase to carboxyhemoglobin ratio as a biomarker of heme release to heme processing is associated with higher tricuspid regurgitant jet velocity and early death in sickle cell disease. *American Journal of Hematology*, *96*(9), E315–E318. https://doi.org/10.1002/ajh.26243

Vilas-Boas, W., Cerqueira, B. A. V., Zanette, A. M. D., Reis, M. G., Barral-Netto, M., & Goncalves, M. S. (2010). Arginase levels and their association with Th17-related cytokines, soluble adhesion molecules (sICAM-1 and sVCAM-1) and hemolysis markers among steady-state sickle cell anemia patients. *Annals of Hematology*, *89*(9), 877–882. https://doi.org/10.1007/s00277-010-0954-9

Wang, W., Knovich, M. A., Coffman, L. G., Torti, F. M., & Torti, S. V. (2010). Serum ferritin: Past, present and future. *Biochimica et Biophysica Acta - General Subjects*, *1800*(8), 760–769. https://doi.org/10.1016/j.bbagen.2010.03.011

Wang, X., Xu, T., Zhang, J., Chen, S., & Zhang, Y. (2018). SO-YOLO Based WBC Detection with Fourier Ptychographic Microscopy. *IEEE Access*, *6*, 51566–51576. https://doi.org/10.1109/ACCESS.2018.2865541

Ware, R. E. (2010). How I use hydroxyurea to treat young patients with sickle cell anemia. *Blood*, *115*(26), 5300–5311. https://doi.org/10.1182/blood-2009-04-146852

Ware, R. E., de Montalembert, M., Tshilolo, L., & Abboud, M. R. (2017). Sickle cell disease. *The Lancet*, *390*(10091), 311–323. https://doi.org/10.1016/S0140-6736(17)30193-9

Williams, T. N., & Thein, S. L. (2018). Sickle cell anemia and its phenotypes. *Annual Review of Genomics and Human Genetics*, *19*, 113–147. https://doi.org/10.1146/annurev-genom-083117-021320

Wilson, A. M., Ryan, M. C., & Boyle, A. J. (2006). The novel role of C-reactive protein in cardiovascular disease: Risk marker or pathogen. *International Journal of Cardiology*, *106*(3), 291–297. https://doi.org/10.1016/j.ijcard.2005.01.068

Wilson, M., Forsyth, P., & Whiteside, J. (2009). Haemoglobinopathy and sickle cell disease. *Continuing Education in Anaesthesia, Critical Care and Pain*, *10*(1), 24–28. https://doi.org/10.1093/bjaceaccp/mkp038

Wu, M. Y., Yao, L., Wang, Y., Zhu, X. Y., Wang, X. F., Tang, P. J., & Chen, C. (2020). Clinical evaluation of potential usefulness of serum lactate dehydrogenase (LDH) in 2019 novel coronavirus (COVID-19) pneumonia. *Respiratory Research*, *21*(1), 1–6. https://doi.org/10.1186/s12931-020-01427-8

Yamada, M., Chishiki, M., Kanai, Y., Goto, A., & Imamura, T. (2020). Neonatal reticulocyte count during the early postnatal period. *Pediatrics and Neonatology*, *61*(5), 490–497. https://doi.org/10.1016/j.pedneo.2020.04.004

Yawn, B. P., Buchanan, G. R., Afenyi-Annan, A. N., Ballas, S. K., Hassell, K. L., James, A. H., Jordan, L., Lanzkron, S. M., Lottenberg, R., Savage, W. J., Tanabe, P. J., Ware, R. E., Murad, M. H., Goldsmith, J. C., Ortiz, E., Fulwood, R., Horton, A., & John-Sowah, J. (2014). Management of sickle cell disease: Summary of the 2014 evidence-based report by expert panel members. *JAMA - Journal of the American Medical Association*, *312*(10), 1033–1048. https://doi.org/10.1001/jama.2014.10517

Yousry, S. M., Ellithy, H. N., & Shahin, G. H. (2016). Endothelial nitric oxide synthase gene polymorphisms and the risk of vasculopathy in sickle cell disease. *Hematology*, *21*(6), 359–367. https://doi.org/10.1080/10245332.2016.1142710

Zdanowska, N., Owczarczyk-Saczonek, A., Czerwińska, J., Nowakowski, J. J., Kozera-żywczyk, A., Owczarek, W., Zdanowski, W., & Placek, W. (2020). Methotrexate and adalimumab decrease the serum levels of cardiovascular disease biomarkers (Vcam-1 and e-selectin) in plaque psoriasis. *Medicina (Lithuania)*, *56*(9), 1–9. https://doi.org/10.3390/medicina56090473

Appendices

Apendix 1: Questionnaire of patients

Questionnaire

Name:

Age:

Length:

Weight:

Steady state or Crisis state?

Complication	VOC	Painful crises	anemia	ACS

Hematologic and biochemical data of SCA patients	
CRP (mg/L)	
D-DIMER (mg/mL)	
FERRITIN (ng/mL)	
Hb (g/dL)	
HCT (%)	
LDH (U/L)	
MCH (pg)	
MCHC (g/dL)	
MCV (fL)	
Platelet (10^9/1)	
RBC (10^12/1)	
Reticulocytes Count (%)	
VCAM-1 (ng/mL)	
VISFATIN (ng/mL)	
WBC (10^9/1)	

الخلاصة

فقر الدم المنجلي هو اضطراب دموي معروف ينشأ من طفرة واحدة في الكودون 6 من الجين β-globin الذي ينتج عنه استبدال حمض الجلوتاميك ب حمض الفالين ، تؤدي هذه الطفرة إلى تكوين هيموجلوبين غير طبيعي يسمى HbS. الهيموجلوبين متماثل اللواقح (مرض HbSS) هو الشكل الأكثر شيوعًا لمرضى SCD. بالإضافة إلى SCD متماثل (HbSS)، توجد أيضًا أشكال أخرى مثل HbSC وHbSB-thalassemia.

اجريت الدراسة على مدى 3 اشهربين تشرين ثاني / 2022 وكانون ثاني / 2023 في مركز امراض الدم الوراثية / دائرة صحة كربلاء. عدد العينات المستخدمة في الدراسة (100) من الذكور يشمل (35) مريضاً يعانون من نوبة الألم و (35) مريضاً بحالة مستقرة، و (30) شخصا يتمتعون بصحة جيدة، ، بالإضافة إلى أعمارهم تراوحت بين (3-55).

تم الحصول على 5 مل من الدم الوريدي من المرضى و الأصحاء. بالإضافة إلى ذلك ، تم أخذ بعض المعلومات من كل شخص والتي تشمل (العمر والجنس ومدة تاريخ المرض والطول والوزن). تم قياس مستويات vcam-1 , visfatin يدويا بواسطة استخدام جهاز ELISA ، بينما تم قياس مستويات D-dimer , CRP , CBC , ferritin و LDH بواسطة محلل الكيمياء التلقائي.

في هذه الدراسة، نهدف إلى دراسة دور VCAM-1 كعلامة على الخلل البطاني في مرضى فقر الدم المنجلي. تقصي دور visfatinكعلامة للالتهاب في مرضى فقر الدم المنجلي. المقارنة بين تعبير visfatin و sVCAM-1 و Ferritin و D dimer في مرضى SCA مقارنة بالأفراد الأصحاء. مقارنة شدة هذه العلامات D dimer وFerritin وvisfatin وsVCAM-1 في الأطفال والبالغين. تقييم الارتباط بين مستوى visfatin، ومستوى sVCAM-1 في مرضى SCA،وتكرار أزمات انسداد الأوعية. الاستخدام المحتمل لهذه العلامات كمؤشر لحدوث نوبات الألم. دراسة الارتباطات بين علامات Visfatin وsVCAM-1 وFerritin وD dimer مع مختلف علامات التحلل والالتهاب.

أظهرت النتائج وجود فروقات معنوية في تركيزات D dimer و Ferritin و visfatin و CRP و HCT و sVCAM-1 و LDH و HB و WBC و RBC و Retic في مرضى SCA (حالة الأزمة ، حالة

مستقرة) بالمقارنة مع مجموعة الاصحاء لكل من البالغين والأطفال. كما لوحظ عدم وجود فروق معنوية في مستويات MCH بين مرضى SCA ومجموعة الاصحاء لكل من البالغين والأطفال.

أظهرت نتائج MCHC وجود فروق معنوية بين مرضـــى فقر الدم المنجلي والأشـــخاص الأصـــحاء عند الأطفال فقط. بينما في البالغين لا توجد فروق معنوية بين المرضـى والأصـحاء. أظهرت النتائج أيضًـا وجود فروق معنوية في مســتويات الصــفائح الدموية، MCV، بين مرضـــى فقر الدم المنجلي والأصـــحاء، عند البالغين فقط، ولكن عند الأطفال، لم تكن هناك فروق معنوية بين المرضـــى. مع فقر الدم المنجلي ومجموعة الاصحاء.

في هذه الدراسة، مرضى SCA الذين يعانون من أزمات الم لديهم ارتباط كبير بين

(visfatin ،VCAM-1)، (visfatin ،LDH)، (vcam-1 ،D-dimer)،

(LDH، vcam-1)، (Visfatin D -dimer)و (vcam-1، Ferritin).

و (visfatin, ferritin).

بينما يكون مرضـــى SCA في حالة مســـتقرة لديهم ارتباطات معنوية بين (visfatin، D-dimer) و (vcam-1، D-dimer) فقط.

خلصت الدراسة إلى أن مستوى الفسفاتين في الدم أعلى بشكل ملحوظ بين مرضى فقر الدم المنجلي في كل من (الحالة المستقرة وحالة نوبة الالم) مقارنة بالأفراد الأصحاء، مع وجود علاقة إيجابية بين مستوى الفسفاتين في الدم وتكرارحدوث أزمات انسداد الاوعية الدموية .

كما ان مستوى فيكام مرتفع بالمرضى مقارنة بالاصحاء مع وجود علاقة إيجابية بين مستوى فيكام والخلل البطاني

جامعة كربلاء

تقييم مستويات بعض علامات انحلال الدم Visfatin، sVcam-1 في الحالة المستقرة والأزمات لمرضى فقر الدم المنجلي وعلاقتها بأعتلالات الاوعية الدموية

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

كتبت بواسطة

نور يحيى عبد زيد محمد

بكالوريوس تحليلات مرضية/ 2015 كلية العلوم الطبية التطبيقية – جامعة كربلاء

بأشراف

أ.د. عبير جياد يوسف

(صفر) 1445 **(آب) 2023**