MODIFICATIONS IN HAEMORHEOLOGICAL PARAMETERS IN SICKLE CELL ANAEMIA

BY

OBARISIAGBON SHADRACK
MAT NO: PG/BMS0609904

DEPARTMENT OF PHYSIOLOGY
SCHOOL OF BASIC MEDICAL SCIENCES
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN

SUPERVISOR: DR (MRS) R.O. AIKPITANYI-IDUITUA

DECEMBER, 2025.

MODIFICATIONS IN HAEMORHEOLOGICAL PARAMETERS IN SICKLE CELL ANAEMIA

BY

OBARISIAGBON SHADRACK
MAT NO: PG/BMS0609904

BEING A THESIS WRITTEN IN THE DEPARTMENT OF HUMAN PHYSIOLOGY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, IN PARTIAL FULFILMENT FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN HUMAN PHYSIOLOGY IN UNIVERSITY OF BENIN, EDO STATE

SUPERVISOR: DR (MRS) R.O. AIKPITANYI-IDUITUA

DECEMBER, 2025

CERTIFICATION

This is to certify that this study was carried out by **OBARISIAGBON SHADRACK,** with Matriculation Number **PG/BMS0609904**, titled, "**MODIFICATIONS IN HAEMORHEOLOGICAL PARAMETERS IN SICKLE CELL ANAEMIA"** in partial fulfillment of the requirement for the award of the Master of Science (M.Sc.) Degree in Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

_____ _____

Obarisiagbon Shadrack **DATE**
(STUDENT)

_____ _____

Dr. (Mrs.) R.O. Aikpitanyi-Iduitua **DATE**

(PROJECT SUPERVISOR)

_____ _____

Dr. (Mrs.) B.O. Eiya **DATE**

(PG – COORDINATOR)

_____ _____

Prof. O.K. Uche **DATE**

(HEAD OF DEPARTMENT)

DEDICATION

I dedicate this work to God Almighty, the creator of the universe, and to my family, friends and loved ones.

ACKNOWLEDGEMENT

Foremost, with humility and unreserved reverence, I give my heartfelt thanks to the Most High God, the beginning and the end of knowledge, wisdom, and understanding.

My deepest gratitude goes to my wife, Mrs. Jacinta Oghenevwaire Obarisiagbon (nee Ajemrena), for her immense contribution and unwavering support over the years. I also appreciate Prof. O.K. Uche as the Head of Department of Physiology.

I also sincerely appreciate the tireless efforts and motherly figure of my supervisor, Dr. (Mrs.) R.O. Aikpitanyi-Iduitua for her guidance, advice, patience, and encouragement throughout this work. To all my lecturers in the department, I remain deeply grateful for your invaluable contributions to my academic growth.

My sincere and warm gratitude also goes to the following persons: my elder brother, Mr. Nosa Ginua Obarisiagbon, Dr. Emmanuel Ogechukwu Aloamaka, Mr. Edeha Daniel, Mr. Silas Wisdom, Mr. Ayodele Abayomi, Mr. Paul Alufohai, Mr. and Mrs. Fidelis Ajemrena and family and others whom time will not permit me to mention, for their support, prayers and encouragement, which continually fueled my passion that keep adding fuel to my passion.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Sickle Cell Disease (SCD) is a hereditary blood disorder characterized by the production of abnormal haemoglobin (HbS), leading to the deformation of red blood cells (RBCs) into a sickle shape. This study aims to investigate modifications in haemorheological parameters in sickle cell anaemia. A comparative analysis was conducted involving 60 participants aged 16-40 years, categorized into three groups: sickle cell patients in crisis, sickle cell patients in steady state, and non-sickle cell controls. Parameters such as osmotic fragility, ESR, fibrinogen concentration, and red cell deformability were measured using standardized laboratory techniques. The study found a significant increase in osmotic fragility among sickle cell subjects during crises compared to steady state and controls ($p < 0.05$). Fibrinogen concentrations were markedly elevated in both steady-state and crisis groups, with the highest levels observed during crises ($p < 0.05$). Similarly, ESR values were significantly higher in sickle cell subjects compared to controls ($p < 0.05$). However, no significant difference in red cell deformability was observed between sickle cell and normal subjects ($p > 0.05$). The findings underscore the critical role of haemorheological parameters in the pathophysiology of SCD, highlighting osmotic fragility, fibrinogen elevation, and ESR as important markers of disease activity and crisis severity.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Sickle cell disease (SCD) is a hereditary blood disorder characterized by the production of abnormal haemoglobin, known as haemoglobin S (HbS) and vaso-occlusive crisis. This condition leads to the deformation of red blood cells (RBCs) into a sickle shape, particularly under low oxygen conditions. The deformation of RBCs can result in various complications, including vaso-occlusive crisis, haemolytic anaemia, and increased susceptibility to infections (Kato *et al*., 2018). This vaso-occlusive crisis is characterized by severe pain due to microvascular occlusion, leading to ischaemia and potential organ damage (Rees *et al*., 2010). These complications can lead to reduction in the quality of life and productivity of the affected individual. Understanding the differences in haemorheological parameters among subjects experiencing sickle cell crises, those in steady-state conditions, and non-sickle cell individuals is crucial for elucidating the pathophysiology of SCD and developing effective management strategies.

Haemorheological parameters such as osmotic fragility, erythrocyte sedimentation rate (ESR), fibrinogen concentration, and red cell deformability are critical in determining the clinical outcomes of subjects with SCD. Osmotic fragility indicates the susceptibility of RBCs to haemolysis when exposed to varying osmotic environments (Tzounakas *et al*., 2021). Studies such as Parrow *et al*. (2017) have shown that sickle cell subjects exhibit increased osmotic fragility, particularly during crises, which correlates with the extent of haemolysis and contributes to anaemia. Increased osmotic fragility in these subjects can lead to a higher rate of

RBC destruction, exacerbating the anaemia commonly seen in SCD. This parameter is therefore essential for understanding the stability of RBC membranes under stress and can provide insights into the severity of haemolytic events in SCD.

Red cell deformability refers to the ability of RBCs to change shape under shear stress (Sankaran *et al*., 2016). This property is crucial for the passage of RBCs through narrow capillaries, and impaired deformability can lead to vaso-occlusive crises. Studies such as Barodka *et al*. (2014) have demonstrated that sickle cells exhibit reduced deformability, which is exacerbated during crises, thereby contributing to the pathophysiology of SCD. Impaired deformability can result in increased blood viscosity and hindered microcirculation, leading to tissue ischaemia and pain.

The Erythrocyte Sedimentation Rate (ESR) reflects the degree of inflammation in the body, which can be significantly altered in subjects with SCD, particularly during crises (Piccin *et al*., 2022). Elevated ESR levels have been associated with increased inflammatory responses and can serve as an indirect marker of disease activity (Alharthi *et al*., 2024). The ESR can provide valuable information regarding the presence of underlying inflammation, which is often exacerbated during vaso-occlusive crises. Monitoring ESR levels can help clinicians assess the effectiveness of anti-inflammatory treatments and adjust therapeutic strategies accordingly.

Fibrinogen concentration, a key factor in coagulation, can indicate the risk of thrombotic events, which are often elevated in subjects with SCD (Nasimuzzaman and Malik, 2019). Increased fibrinogen levels have been linked to vaso-occlusive events, suggesting that monitoring this parameter could be vital for preventing complications associated with SCD (Nader *et al*., 2021). Fibrinogen concentration may serve as a biomarker for assessing the thrombotic risk in sickle cell subjects, particularly during crises.

This study therefore aims to explore the differences in these haemorheological parameters among three groups: subjects with sickle cell disease during vaso-occlusive crises, those in steady-state conditions, and non-sickle cell individuals. By comparing these groups, knowledge can be gained as it relates to the unique challenges faced by sickle cell subjects and how these challenges differ in acute and stable phases of the disease.

1.2 Statement of the Problem

Sickle Cell Disease (SCD) remains a significant public health challenge in sub-Saharan Africa, particularly in Nigeria, which accounts for the highest global burden of the disease. Despite advances in diagnosis and management, vaso-occlusive crises continue to be the leading cause of morbidity, hospitalization, and mortality among affected individuals. These crises result from the obstruction of blood flow due to sickled red blood cells, which leads to tissue ischaemia, severe pain, and organ damage. However, the underlying haemorheological alterations that distinguish the crisis phase from the steady state remain poorly characterized in many clinical settings across Nigeria.

Haemorheological parameters such as osmotic fragility, erythrocyte sedimentation rate (ESR), fibrinogen concentration, and red cell deformability play vital roles in determining blood flow properties and cellular interactions within the microcirculation. Alterations in these parameters can significantly influence the severity and frequency of vaso-occlusive episodes. Although previous studies have shown that sickle cell subjects generally exhibit abnormal haemorheological profiles, there is insufficient comparative data examining how these parameters differ between crisis and steady-state conditions in the same population group.

Furthermore, the relationships between these haemorheological changes and the clinical manifestations of sickle cell crises have not been adequately established in local contexts. Most existing studies have focused on haematological indices or biochaemical markers, with limited attention to the rheological properties of blood that directly affect oxygen delivery and vascular flow. Consequently, there remains a gap in understanding how modifications in these parameters contribute to disease severity and progression.

This knowledge gap poses a challenge to clinicians seeking to predict or prevent crises and hampers the development of targeted therapeutic strategies aimed at improving blood flow and reducing complications. Therefore, a systematic evaluation of haemorheological parameters particularly osmotic fragility, ESR, fibrinogen concentration, and red cell deformability in sickle cell subjects during crisis and steady-state phases is essential. Such an analysis would not only enhance the understanding of the pathophysiology of SCD but could also aid in identifying potential biomarkers for monitoring disease activity and guiding clinical management.

1.3 Justification of Study

Understanding the differences in haemorheological parameters among sickle cell subjects in crisis, those in steady state, and non-sickle cell individuals can elucidate the mechanisms underlying the complications associated with SCD. By examining parameters such as osmotic fragility, ESR, fibrinogen concentration, and red cell deformability, clinicians can gain insights into the pathophysiological processes that contribute to proper management of issues due to vaso-occlusive crises and other complications.

Additionally, if specific haemorheological parameters are found to be significantly altered during crises compared to steady-state conditions and non-sickle cell individuals, they could serve as potential biomarkers for predicting vaso-occlusive crises. This capability would enhance the ability of healthcare providers to anticipate and manage crises proactively, which is essential for reducing the incidence of acute complications associated with SCD.

1.4 Aim and Objectives

Aim

The aim of this study is to investigate modifications in haemorheological parameters in sickle cell anaemia.

Specific Objectives

1. To evaluate the osmotic fragility of red cells of subjects with sickle cell disease (HbSS) compared to steady-state conditions, and in non-sickle cell subjects (HbAA).
2. To measure the erythrocyte sedimentation rate in sickle cell subjects experiencing crises, those in steady state, and non-sickle cell subjects.
3. To evaluate the concentration of fibrinogen in the blood of sickle cell subjects during crises and steady state, and compare it with levels in non-sickle cell subjects.
4. To investigate the deformability of red blood cells in subjects with sickle cell disease during crises and in steady-state conditions compared to non-sickle cell subjects.

1.5 Research Questions

1. How does osmotic fragility differ among subjects with sickle cell disease (HbSS) during crises, in steady-state conditions compared with non-sickle cell individuals (HbAA)?
2. What variations exist in erythrocyte sedimentation rate between sickle cell subjects in crisis, those in steady state, and non-sickle cell subjects?
3. How does fibrinogen concentration change in sickle cell subjects during crises and steady state compared to non-sickle cell individuals?
4. What is the impact of sickle cell disease on red cell deformability in subjects during crises and in steady-state conditions compared to non-sickle cell individuals?

1.6 Significance of the study

Alteration in acid-base homeostasis may result in significant modifications in osmotic volume and rheology in sickle cell subjects during vaso-occlusive crisis. Evaluation of these modifications may help to improve therapeutic interventions, management and predict crisis episodes.

Furthermore, the significance of this study also lies in its potential contributions to both clinical practice and the broader understanding of sickle cell disease (SCD). By investigating the differences in haemorheological parameters among sickle cell subjects in crisis, those in steady state, and non-sickle cell individuals, this research aims to provide valuable insights into the pathophysiology of SCD. Understanding these haemorheological changes is crucial as it can help elucidate the mechanisms that lead to complications in sickle cell subjects, thereby enriching the scientific literature on the disease.

Moreover, the findings from this study may inform clinical practice by identifying specific haemorheological markers that correlate with the severity of sickle cell crises. Such identification could pave the way for the development of targeted interventions aimed at managing and mitigating crises more effectively, ultimately improving patient outcomes. By enhancing the precision of patient management strategies, healthcare providers may be better equipped to address the unique challenges faced by individuals with SCD.

The results of this study may also lay the groundwork for future research into the role of haemorheological parameters in sickle cell disease. Subsequent studies could build upon these findings to explore additional factors influencing haemorheology and their implications for treatment strategies. This could lead to a deeper understanding of the disease and inform the development of novel therapeutic approaches.

1.7 Scope of the Study

This study focused on subjects diagnosed with sickle cell disease, specifically examining those experiencing vaso-occlusive crises, those in steady-state conditions, and a control group of non-sickle cell individuals. The research will be conducted in a clinical setting, utilizing laboratory techniques to measure the specified haemorheological parameters. The findings will contribute to a deeper understanding of the relationship between haemorheological changes and the clinical manifestations of sickle cell disease. The study aims to provide valuable insights that may ultimately inform clinical practice and improve the management of subjects with SCD. Experimental subjects (HbSS) were recruited from Sickle Cell Centre, Benin-city. Control subjects were recruited from Benson Idahosa University, Benin City (Aged 16-21). The informed consent was obtained before participation in the research.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Sickle cell disease (SCD) is a complex hereditary disorder characterized by the presence of abnormal haemoglobin, leading to significant alterations in haemorheological parameters. This chapter reviews the existing literature on haemorheological changes in SCD, focusing on osmotic fragility, erythrocyte sedimentation rate (ESR), fibrinogen concentration, and red cell deformability.

2.2 Haemorheological Parameters in Sickle Cell Disease

2.2.1 Osmotic Fragility

Osmotic fragility is a critical haemorheological parameter that refers to the susceptibility of red blood cells (RBCs) to haemolysis when exposed to varying osmotic environments (Walski *et al*., 2014). This phenomenon is particularly significant in the context of sickle cell disease (SCD), a hereditary blood disorder characterized by the production of abnormal haemoglobin, known as haemoglobin S (HbS). Under conditions of low oxygen tension or dehydration, RBCs in SCD subjects undergo deformation, leading to a sickle shape that compromises their structural integrity and functionality.

Mechanisms of Osmotic Fragility in Sickle Cell Disease

The osmotic fragility of RBCs is influenced by several intrinsic and extrinsic factors. Intrinsically, the structural composition of the RBC membrane plays a vital role. In SCD, the presence of HbS causes polymerization of haemoglobin under low oxygen conditions, leading to

the distortion of the RBC shape (Mandal *et al*., 2020). This distortion increases the rigidity of the membrane, making it more susceptible to haemolysis when subjected to osmotic stress. Tzounakas *et al*. (2021) demonstrated that the osmotic fragility of sickle cells is markedly elevated compared to healthy individuals, correlating with the severity of haemolysis and anaemia in SCD subjects.

The osmotic fragility test typically involves exposing RBCs to hypotonic solutions, which causes the cells to swell and eventually lyse if they are unable to withstand the osmotic pressure (Ciepiela *et al*., 2018). In normal RBCs, the membrane is flexible and can accommodate changes in volume without rupturing. However, in sickle cells, the rigid structure and altered membrane properties lead to increased fragility (Narla and Mohandas, 2017). This fragility is not only a consequence of the abnormal haemoglobin but also of the biochaemical changes that occur in the RBCs due to chronic haemolysis and oxidative stress.

Clinical Implications of Increased Osmotic Fragility

Increased osmotic fragility has profound clinical implications for subjects with SCD. One of the most significant consequences is the exacerbation of anaemia (Beri *et al*., 2025). In SCD, haemolysis occurs at an accelerated rate due to the fragile nature of sickle RBCs. As these cells undergo haemolysis, the body attempts to compensate for the loss by increasing erythropoiesis; however, this compensatory mechanism often falls short, leading to chronic anaemia. The severity of anaemia in SCD subjects is closely linked to the degree of osmotic fragility, as higher fragility correlates with a greater rate of RBC destruction.

Moreover, the relationship between osmotic fragility and vaso-occlusive crises is critical. During these crises, the sickling of RBCs not only leads to increased haemolysis but also contributes to

the formation of microthrombi, which can obstruct blood flow and exacerbate tissue ischaemia (Nader *et al*., 2021; Paramo, 2021). The interplay between increased osmotic fragility and the occurrence of vaso-occlusive crises highlights the need for effective management strategies aimed at reducing haemolysis and improving the stability of RBC membranes.

Factors Influencing Osmotic Fragility

Several factors can influence osmotic fragility in sickle cell subjects. They include the following:

Hydration

Hydration plays a pivotal role in the overall health of sickle cell subjects. Dehydration can exacerbate the sickling phenomenon, characterized by the distortion of RBCs into a sickle shape (Brugnara, 2018). This morphological change not only impairs the cells' ability to function but also heightens their osmotic fragility, making them more susceptible to rupture when exposed to hypotonic solutions. When subjects are dehydrated, the blood becomes haemoconcentrated, leading to increased blood viscosity (Connes, 2024). This condition promotes further sickling, creating a vicious cycle that heightens the risk of vaso-occlusive crises. Therefore, maintaining adequate hydration is essential for managing SCD and mitigating the risks associated with increased osmotic fragility. Clinicians often emphasize the importance of hydration, especially during episodes of illness or stress when fluid loss may occur due to fever, vomiting, or diarrhea.

The Presence of Comorbidities

The presence of comorbidities can complicate osmotic fragility in sickle cell subjects. Infections are particularly problematic, as they are common in individuals with SCD due to splenic dysfunction (Ochocinski *et al*., 2020). The spleen plays a vital role in filtering pathogens and

managing the immune response; however, its dysfunction in SCD subjects can lead to increased susceptibility to infections (Sahu *et al*., 2024). When an infection occurs, the body mounts an inflammatory response, which can exacerbate haemolysis and increase osmotic fragility. Inflammatory cytokines released during this response can alter the properties of the RBC membrane, making thaem more prone to rupture under osmotic stress. This interplay between inflammation and osmotic fragility underscores the need for comprehensive management strategies that address both the haematological and non-haematological aspects of SCD.

Other Factors

The age of a patient can influence osmotic fragility (Walski *et al*., 2014). Older subjects may experience more pronounced complications due to cumulative damage to their RBCs over time. This age-related decline in RBC integrity can lead to increased fragility and a higher risk of haemolysis. Additionally, nutritional status plays a significant role. Deficiencies in essential vitamins, such as folate and vitamin B12, can impair RBC production and integrity, further contributing to increased fragility (Mikkelsen and Apostolopoulos, 2019).

Genetic background is another factor that can influence osmotic fragility in sickle cell subjects. Genetic variations may affect the severity of sickle cell disease and the resilience of RBCs under osmotic stress (Wang and Zennadi, 2021). Environmental stressors, such as high altitude, extreme temperatures, and physical exertion, can also impact hydration levels and increase the likelihood of sickling (Tewari *et al*., 2015; Obeagu and Obeagu, 2024). These factors can lead to dehydration and exacerbate the sickling process, further increasing osmotic fragility.

Medications can also play a role in influencing osmotic fragility. Certain drugs may impact RBC stability and membrane integrity, leading to increased susceptibility to haemolysis (Jeswani *et al*.,

2015). Additionally, blood pH levels can affect RBC properties; acidosis can alter membrane characteristics, potentially increasing osmotic fragility (Oyewale *et al*., 2011).

Therapeutic Implications

Understanding the concept of osmotic fragility has important therapeutic implications for managing SCD. Interventions aimed at reducing haemolysis and improving RBC stability can potentially mitigate the complications associated with increased osmotic fragility. For instance, hydroxyurea, a medication commonly used in SCD management, has been shown to increase fetal haemoglobin (HbF) levels, which can reduce the sickling tendency of RBCs and improve their overall stability (McGann and Ware, 2015). By increasing HbF levels, hydroxyurea can lead to decreased osmotic fragility and a reduced risk of haemolysis.

Moreover, the use of blood transfusions is another therapeutic approach that can impact osmotic fragility (Ojo *et al*., 2022). Transfusions can help dilute the sickled cells and increase the proportion of normal RBCs in circulation, thereby reducing the overall haemolytic burden. This approach is particularly beneficial during acute crises when the risk of severe anaemia and complications is heightened. However, the risks associated with blood transfusions, such as iron overload and alloimmunization, must be carefully managed.

Furthermore, supportive measures such as hydration and the management of infections can play a crucial role in minimizing osmotic fragility. Maintaining adequate hydration helps ensure that RBCs retain their shape and volume, thereby reducing the likelihood of haemolysis. Additionally, prompt treatment of infections can prevent the exacerbation of inflammation and its subsequent impact on osmotic fragility.

The Role of Nutrition

Nutrition also plays a significant role in influencing osmotic fragility and overall health in subjects with SCD. Certain nutrients, such as folic acid, vitamin B12, and iron, are essential for erythropoiesis and can impact the stability of RBCs (Bhadra and Deb, 2020). A well-balanced diet that addresses the specific nutritional needs of sickle cell subjects can contribute to improved haematological parameters and reduced haemolysis.

For example, folic acid is crucial for DNA synthesis and the production of new RBCs. In SCD subjects, where RBC turnover is increased due to haemolysis, ensuring adequate folic acid intake can help support erythropoiesis. Furthermore, antioxidants such as vitamins C and E may help mitigate oxidative stress, which can further compromise RBC integrity and increase osmotic fragility.

2.2.2 Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate (ESR) is a widely used laboratory test that measures the rate at which red blood cells (RBCs) settle in a column of blood over a specified period, typically one hour (Ramsay and Lerman, 2015). The ESR is a non-specific test that serves as an indirect marker of inflammation in the body.

An increased ESR generally indicates enhanced systemic inflammation, increased acute-phase protein production (particularly fibrinogen), and greater erythrocyte aggregation. Physiologically, elevated fibrinogen reduces the erythrocyte zeta potential, promoting cell aggregation and faster sedimentation, while also reflecting endothelial activation and altered blood rheology (Aytekin, 2018). In sickle cell disease, a high ESR suggests intensified inflammatory activity and

haemolytic stress, particularly during vaso-occlusive crisis, although it is influenced by the abnormal morphology of sickled erythrocytes (Rees et al., 2010).

Conversely, a decreased ESR reflects reduced inflammatory activity or impaired erythrocyte aggregation. In sickle cell disease, reduced ESR may result from the rigid, elongated shape and poor deformability of sickled erythrocytes, which interfere with rouleaux formation despite elevated inflammatory markers (Connes et al., 2016). Thus, a low ESR may indicate that abnormalities in erythrocyte morphology and membrane mechanics predominate over the pro-aggregatory effects of plasma proteins.

Factors Influencing Erythrocyte Sedimentation Rate

While ESR is a valuable indicator of inflammation, it is influenced by a variety of factors that can affect its accuracy and interpretation. These factors include:

Inflammatory Conditions

One of the primary factors influencing ESR is the presence of inflammatory conditions. Both acute and chronic inflammation can significantly elevate ESR levels (Alharthi *et al*., 2021). Acute inflammation, which may occur due to infections, trauma, or other inflammatory processes, leads to an increase in the production of acute-phase reactants like fibrinogen. This protein promotes the aggregation of RBCs, enhancing their sedimentation rate. Similarly, chronic inflammatory conditions, such as autoimmune diseases or chronic infections, can result in persistently elevated ESR levels due to ongoing inflammatory responses (Arida *et al*., 2018).

Infections

Infections also play a critical role in influencing ESR. Bacterial, viral, and fungal infections can lead to marked increases in ESR due to the body's inflammatory response. For instance, bacterial infections often result in higher ESR levels compared to viral infections (Li *et al*., 20224). This difference reflects the severity of inflammation and the body's immune response, highlighting the importance of considering infection status when interpreting ESR results.

Anaemia

Anaemia is another significant factor that can affect ESR readings. Different types of anaemia can have varying impacts on ESR. In general, the presence of anaemia, particularly normocytic or macrocytic anaemia, can lead to elevated ESR levels (Tefferi, 2003). This elevation is often attributed to changes in plasma protein levels and alterations in RBC morphology. Conversely, microcytic anaemia may not significantly affect ESR (Urrechaga *et al*., 2015). This underscores the importance of confirming the haematological status of subjects when interpreting ESR results.

Age and gender

Age and gender are demographic factors that also influence ESR. ESR tends to increase with age, and older adults often exhibit higher ESR levels (Siemons *et al*., 2014). This increase can be attributed to age-related changes in plasma proteins and the immune system. Gender differences are also notable; women typically have higher ESR levels than men, particularly during menstruation and pregnancy (Bray *et al*., 2016). Hormonal fluctuations throughout the menstrual cycle can lead to variations in plasma protein levels, thereby affecting sedimentation rates.

Pregnancy

Pregnancy induces physiological changes that can significantly impact ESR levels. During pregnancy, especially in the third trimester, ESR levels can rise considerably due to increased plasma volume and elevated levels of fibrinogen and other acute-phase reactants (Mba *et al*., 2018). This physiological increase should be taken into account when evaluating ESR in pregnant subjects, as it may not necessarily indicate pathological inflammation.

Obesity

Obesity is associated with chronic low-grade inflammation, which can lead to elevated ESR levels (Koca, 2017). Increased adipose tissue produces inflammatory cytokines that contribute to higher sedimentation rates. This connection underscores the importance of considering a patient's body mass index (BMI) and overall health status when interpreting ESR results.

Medications

Medications can also influence ESR readings. Certain drugs, such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), can reduce inflammation and lower ESR levels (Lam *et al*., 2021). The use of hormonal contraceptives may also affect ESR, potentially leading to higher levels due to changes in plasma proteins. Clinicians should be aware of a patient's medication history when interpreting ESR results, as these factors can significantly alter the expected outcomes.

Plasma protein levels

Plasma protein levels, particularly fibrinogen and globulins, play a crucial role in determining ESR. Elevated concentrations of these proteins increase the aggregation of RBCs, leading to faster sedimentation (Simeon *et al*., 2024). Conditions that alter plasma protein levels, such as

liver disease or nephrotic syndrome, can also affect ESR readings, making it essential for healthcare providers to consider these underlying conditions.

Characteristics of RBCs

The characteristics of RBCs themselves can influence ESR. The shape and size of RBCs affect their sedimentation rate. For instance, conditions that alter RBC shape, such as sickle cell disease, can significantly impact ESR measurements (Connes *et al*., 2016). Sickle cells tend to be more rigid and may aggregate, affecting their behavior in the bloodstream and leading to abnormal sedimentation patterns. Additionally, the deformability of RBCs plays a role; impaired RBC deformability can lead to increased ESR.

Hydration Status

Hydration status is another important factor that can influence ESR. Dehydration can concentrate blood components, potentially leading to higher ESR levels. Conversely, overhydration may dilute plasma proteins and lower ESR readings (Georgescu *et al*., 2017). Therefore, clinicians should assess a patient's hydration status when interpreting ESR results, particularly in acute care settings.

Chronic Diseases

Chronic diseases, including cancer and autoimmune disorders, often lead to elevated ESR levels due to persistent inflammation and the production of acute-phase reactants. For instance, in conditions like rheumatoid arthritis or systemic lupus erythaematosus, ongoing inflammation can result in consistently high ESR readings, complicating the interpretation of results and necessitating careful clinical correlation (Dima *et al*., 2016).

Smoking

Smoking is a lifestyle factor associated with increased ESR levels. Smoking has been linked to systemic inflammation, which can elevate ESR (Mohamed *et al*., 2023). The presence of nicotine and other harmful chaemicals in tobacco can contribute to inflammatory processes, further complicating the assessment of ESR in smokers.

Erythrocyte Sedimentation Rate in Sickle Cell Disease

Evidently, ESR is particularly useful in clinical settings to assess the presence and intensity of inflammatory processes, making it a valuable tool in the management of various diseases, including sickle cell disease (SCD).

In subjects with sickle cell disease, the ESR can be significantly altered, especially during vaso-occlusive crises and other acute events. During these crises, the sickling of RBCs leads to increased blood viscosity and subsequent alterations in haemodynamics, which can affect the ESR readings (Connes *et al*., 2016). Elevated ESR levels in sickle cell subjects are often associated with heightened inflammatory responses, serving as an important indicator of disease activity. Okoye *et al*. (2023) reported that increased ESR levels correlate with the severity of inflammation in sickle cell subjects, suggesting that monitoring ESR can provide insights into the disease's progression and the patient's overall inflammatory state.

Mechanisms Behind ESR Variations in SCD

The mechanisms underlying variations in ESR among sickle cell subjects are multifactorial. Inflammation plays a critical role in the pathophysiology of sickle cell disease (Conran and Belcher, 2018). When RBCs sickle, they can cause microvascular occlusion, leading to tissue

ischaemia and subsequent inflammation. Conran and Belcher (2018) assert that this inflammatory response involves the release of various cytokines and chaemokines, which further perpetuate the cycle of inflammation. The presence of these inflammatory mediators can increase the production of acute-phase reactants, such as fibrinogen, which contributes to the elevation of the ESR.

Moreover, the unique properties of sickle cells thaemselves can influence ESR measurements. Sickle cells tend to be more rigid and have a tendency to aggregate, which can affect their sedimentation rate (Perazzo *et al*., 2022). During a vaso-occlusive crisis, the increased number of sickled cells can lead to a higher ESR due to their altered behavior in the bloodstream. This phenomenon highlights the importance of understanding the specific context of ESR measurements in sickle cell subjects, as elevated levels may not solely reflect systemic inflammation but also the dynamics of sickle cell pathology.

Clinical Implications of Elevated ESR in SCD

The clinical implications of elevated ESR levels in sickle cell disease are significant. Alharthi *et al*. (2024) emphasized the role of ESR in monitoring the effectiveness of anti-inflammatory treatments in sickle cell subjects. Elevated ESR levels can indicate the need for therapeutic interventions aimed at reducing inflammation and preventing complications associated with SCD. For instance, the use of non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids may be warranted during acute pain episodes or when there is evidence of exacerbated inflammation.

Furthermore, ESR can serve as a valuable tool in assessing the response to treatment. A decrease in ESR following the initiation of anti-inflammatory therapy may indicate a positive response and a reduction in inflammatory activity (Brzustewicz *et al*., 2017). This information can guide

clinicians in adjusting treatment regimens and making informed decisions about patient management. Monitoring ESR levels can also help in identifying subjects who may require more aggressive interventions, such as transfusions or hydroxyurea therapy, to manage their disease effectively.

Variations in ESR Among Different Patient States

Understanding the variations in ESR among different patient states is crucial for interpreting test results accurately. In sickle cell disease, ESR levels can fluctuate based on several factors, including the presence of infections, the occurrence of pain crises, and the overall health status of the patient (Ochocinski *et al*., 2020). For instance, during acute infections, ESR levels may rise significantly due to the body's inflammatory response. This elevation may not necessarily indicate a worsening of sickle cell disease but rather reflect the concurrent infectious process.

Additionally, chronic inflammation associated with sickle cell disease can lead to persistently elevated ESR levels, complicating the interpretation of results. Clinicians must consider the patient's clinical context when evaluating ESR, as elevated levels may be indicative of ongoing disease activity or secondary processes, such as infections or other inflammatory conditions.

ESR as a Prognostic Indicator

Beyond its utility in monitoring inflammation, ESR may also serve as a prognostic indicator in sickle cell disease. Studies such as Daniels *et al*. (2017) have shown that elevated ESR levels can correlate with adverse outcomes, including increased hospitalization rates and complications related to SCD. By identifying subjects with persistently high ESR levels, healthcare providers can implement more proactive management strategies to mitigate potential complications.

For instance, subjects with elevated ESR may benefit from closer monitoring and more aggressive management of their disease. This could involve regular assessments for signs of infection, pain management strategies, and consideration of disease-modifying therapies, such as hydroxyurea, which has been shown to reduce the frequency of vaso-occlusive crises and improve overall health outcomes in sickle cell subjects.

Limitations of ESR Testing

Despite its usefulness, there are limitations to the ESR test that must be acknowledged. The ESR is a non-specific test, meaning that it can be influenced by a variety of factors unrelated to sickle cell disease, such as age, gender, and other medical conditions (Kahar, 2022). Consequently, elevated ESR levels may not always accurately reflect the degree of inflammation related to SCD (Van Beers *et al*., 2015). Additionally, certain factors, such as anaemia and the presence of sickle cells, can further complicate the interpretation of ESR results.

Moreover, the variability in ESR measurements can be influenced by laboratory techniques and patient-specific factors, leading to inconsistencies in results (Queiro *et al*., 2025). Therefore, while ESR can provide valuable information regarding inflammation and disease activity, it should be used in conjunction with other clinical assessments and laboratory tests to obtain a comprehensive understanding of a patient's condition.

2.2.3 Fibrinogen Concentration

Fibrinogen is a soluble plasma glycoprotein that plays a critical role in the coagulation cascade, ultimately leading to the formation of fibrin clots (Litvinov *et al*., 2020). Produced by the liver, fibrinogen is converted to fibrin by the action of thrombin during the coagulation process. This conversion is essential for haemostasis, preventing excessive bleeding following vascular injury.

Fibrinogen Concentration in Sickle Cell Disease

In certain medical conditions, including sickle cell disease (SCD), elevated fibrinogen levels can contribute to a hypercoagulable state, increasing the risk of thrombotic events (Faes *et al*., 2018). This section examines the significance of fibrinogen concentration in SCD.

Fibrinogen and its Role in Coagulation

Fibrinogen is a key component of the haemostatic system, serving not only as a precursor to fibrin but also playing a role in platelet aggregation and stabilization of the clot. When vascular injury occurs, fibrinogen is rapidly converted to fibrin, which forms a mesh that traps blood cells and platelets, effectively sealing the wound (Kearney *et al*., 2022). This process is essential for normal clot formation and wound healing. However, an increase in fibrinogen concentration can lead to excessive clot formation, contributing to thrombotic complications.

Fibrinogen in Sickle Cell Disease

Under low oxygen conditions, HbS polymerizes, causing red blood cells (RBCs) to assume a rigid, sickle shape. These deformed cells can obstruct blood flow in small vessels, leading to vaso-occlusive crises, pain, and other complications. The hypercoagulable state associated with SCD is multifactorial, involving changes in blood viscosity, endothelial dysfunction, and alterations in the coagulation cascade, including elevated levels of fibrinogen (Lizarralde-Iragorri and Shet, 2020).

Research has shown that fibrinogen levels are often elevated in subjects with SCD, particularly during vaso-occlusive crises. Nasimuzzaman and Malik (2019) conducted a study that demonstrated significantly higher fibrinogen concentrations in sickle cell subjects compared to

healthy controls. They also suggested that elevated fibrinogen levels may contribute to the pathophysiology of SCD by promoting thrombus formation and exacerbating vascular occlusion. This finding underscores the need for clinicians to monitor fibrinogen levels in SCD subjects to assess thrombotic risk accurately.

Thrombotic Risk and Hypercoagulable State

The relationship between elevated fibrinogen levels and thrombotic risk in SCD is critical for understanding the complications associated with the disease. Nader *et al*. (2021) highlighted the importance of monitoring fibrinogen concentration as a potential biomarker for assessing thrombotic risk in sickle cell subjects. Elevated fibrinogen levels can indicate a hypercoagulable state, characterized by an increased tendency to form clots. This hypercoagulability is compounded by other factors commonly seen in SCD, such as increased blood viscosity, platelet activation, and endothelial injury.

Thrombotic events in SCD can manifest as acute complications, including stroke, acute chest syndrome, and pulmonary embolism (Dessap *et al*., 2011). These events are often life-threatening and can lead to significant morbidity and mortality. The presence of elevated fibrinogen levels may serve as a warning sign, indicating that a patient is at increased risk for such complications. Therefore, regular monitoring of fibrinogen concentrations can facilitate early intervention and management strategies aimed at reducing thrombotic events.

Clinical Implications

Given the association between elevated fibrinogen levels and thrombotic risk in SCD, there is a pressing need for targeted interventions to prevent complications. Clinicians can consider implementing routine fibrinogen monitoring as part of the comprehensive care for subjects with

SCD. This approach would enable healthcare providers to identify subjects at higher risk of thrombotic events and tailor treatment plans accordingly.

Several therapeutic strategies can be considered to manage elevated fibrinogen levels in SCD. Anticoagulant therapy, such as the use of low-molecular-weight heparin or direct oral anticoagulants, may be beneficial in preventing thrombotic events in high-risk subjects (Marcucc *et al*., 2022). Additionally, the use of hydroxyurea, a medication commonly prescribed for SCD, has been shown to reduce the frequency of vaso-occlusive crises and may also lower fibrinogen levels (McGann and Ware, 2015). Hydroxyurea works by increasing fetal haemoglobin (HbF) levels, which helps reduce sickling and improves overall blood flow, potentially mitigating the hypercoagulable state.

Moreover, lifestyle modifications, such as maintaining adequate hydration, regular exercise, and avoiding triggers that precipitate crises, can also play a role in reducing thrombotic risk (Samuel *et al*., 2024).

2.2.4 Red Cell Deformability

Red cell deformability is a critical physiological property that refers to the ability of red blood cells (RBCs) to change shape in response to shear stress (Kim *et al*., 2015). This characteristic is essential for the passage of RBCs through narrow capillaries, ensuring efficient microcirculation and oxygen delivery to tissues.

Red cell deformability is vital for maintaining proper blood flow and tissue perfusion (Barshtein *et al*., 2021). Under normal conditions, RBCs can easily deform as they navigate through the microvasculature, which is often narrower than the diameter of the cells themselves. This ability allows RBCs to traverse capillaries efficiently, facilitating the exchange of oxygen and carbon

dioxide between blood and tissues. Factors that influence red cell deformability include the mechanical properties of the cell membrane, the cytoskeletal structure of the RBCs, and the viscosity of the surrounding plasma (Kim *et al*., 2012).

In healthy individuals, RBCs exhibit high deformability, allowing for smooth passage through the capillary network. However, in SCD, the abnormal shape of sickle cells often rigid and less flexible impairs their ability to deform under stress (Perazzo etal., 2022). This deficiency can lead to increased blood viscosity and hindered microcirculation, resulting in tissue ischaemia and pain.

Red Cell Deformability in Sickle Cell Disease

In sickle cell disease, red cell deformability is significantly impaired. This impairment has profound implications for the pathophysiology of SCD, particularly during vaso-occlusive crises, which are marked by severe pain and tissue ischaemia.

Mechanisms of Impaired Deformability in Sickle Cell Disease

The impairment of red cell deformability in SCD is primarily attributed to the polymerization of haemoglobin S under low oxygen conditions (Williams and Wood, 2023). When deoxygenated, HbS molecules aggregate, forming long, rigid polymers that distort the shape of RBCs into a sickle form. This sickling process is exacerbated by various factors, including low pH, high levels of 2,3-bisphosphoglycerate (2,3-BPG), and dehydration (Bhatt *et al*., 2024). More specifically, the impairment of red cell deformability in SCD can be attributed to the following:

1. **Haemoglobin Polymerization**: The polymerization of HbS is the central mechanism leading to sickling. When RBCs are deoxygenated, the solubility of HbS decreases,

causing it to polymerize (Ferrone, 2018). This polymerization results in the formation of rigid structures within the RBCs, leading to their characteristic sickle shape. These sickle-shaped cells have a reduced surface area-to-volume ratio, which compromises their ability to deform and pass through narrow capillaries (Esperti, 2023).

2. **Membrane Properties**: The RBC membrane plays a crucial role in maintaining cell shape and deformability. In SCD, the sickling process leads to alterations in the lipid bilayer and cytoskeletal components of the RBC membrane (Wang and Zennadi, 2021). These changes can increase membrane rigidity and decrease the overall flexibility of the cell, further impairing deformability.

3. **Viscosity and Shear Stress**: In sickle cell disease, the increased viscosity of blood due to the presence of sickle cells can create a feedback loop that exacerbates deformability issues (Connes *et al*., 2016). Higher viscosity leads to increased shear stress on RBCs, which can further promote sickling and reduce the ability of cells to deform. This situation creates a vicious cycle where impaired deformability leads to increased viscosity, which in turn causes more sickling.

4. **Inflammation and Endothelial Dysfunction**: SCD is characterized by chronic inflammation and endothelial dysfunction, both of which can contribute to impaired red cell deformability (Nader *et al*., 2024). Inflammatory cytokines can alter the mechanical properties of RBCs and their interaction with the endothelium, leading to increased adhesion of sickle cells to the vessel wall and further compromising microcirculation (Kato *et al*., 2017).

Clinical Implications of Reduced Red Cell Deformability

The clinical manifestations of SCD are closely linked to the impairment of red cell deformability. Vaso-occlusive crises, which are hallmark features of SCD, occur when sickle cells obstruct blood flow in small vessels, leading to tissue ischaemia and severe pain (Darbar *et al*., 2020). More specifically, the following are the clinical the following are clinical manifestations of reduced red cell deformability in SCD:

1. **Vaso-Occlusive Crises**: Vaso-occlusive crises are acute episodes of pain caused by the obstruction of blood flow due to sickle cell aggregation (Darbar *et al*., 2020). Reduced deformability of sickle cells contributes to the likelihood of occlusion in small vessels. During a crisis, the sickling of RBCs is exacerbated, leading to further impairment of deformability and increased risk of blockage. This results in ischaemia and pain, which can be severe and debilitating.

2. **Acute Chest Syndrome**: Acute chest syndrome is another serious complication of SCD characterized by chest pain, fever, and respiratory distress (Sysol, J.R. and Machado, 2016). It can result from vaso-occlusion in the pulmonary vasculature or from infections. Impaired red cell deformability can contribute to the development of acute chest syndrome by promoting sickling in the lung circulation, leading to reduced oxygenation and respiratory complications.

3. **Stroke**: Subjects with SCD are at increased risk for cerebrovascular accidents (strokes), particularly in childhood (Hirtz and Kirkham, 2019). The impaired deformability of sickle cells can lead to occlusion of cerebral vessels, resulting in ischaemic stroke. Studies have shown that children with higher levels of sickle cell deformability have a lower incidence of stroke, highlighting the importance of this property in preventing cerebrovascular complications (Alakbarzade *et al*., 2023).

4. **Organ Damage**: Chronic haemolysis and recurrent vaso-occlusive events can lead to organ damage over time in SCD subjects (Jang *et al*., 2021). The impaired microcirculation resulting from reduced red cell deformability contributes to organ ischaemia, which can affect the spleen, kidneys, liver, and other vital organs. This damage can manifest as functional impairment and increased morbidity.

Measuring Red Cell Deformability

Assessing red cell deformability is essential for understanding the pathophysiology of SCD and evaluating potential therapeutic interventions. Several methods exist for measuring RBC deformability, including:

1. **Microfluidic Devices**: These devices use narrow channels to simulate the microvascular environment. By measuring the ability of RBCs to pass through these channels, researchers can quantify deformability under various conditions (Matthews *et al*., 2022).
2. **Osmotic Fragility Tests**: These tests assess the ability of RBCs to withstand osmotic stress, providing insights into membrane integrity and deformability (Orbach *et al*., 2017).
3. **Viscometry**: This technique measures blood viscosity under shear stress, helping to evaluate the overall flow properties of blood, including the contribution of red cell deformability (Pryzwan *et al*., 2024).
4. **Laser Diffraction Techniques**: Advanced imaging techniques, such as laser diffraction, can be used to assess the shape and deformability of RBCs under controlled shear stress conditions (Nikitin *et al*., 2011).

2.3 Empirical Review

This section synthesizes findings from several studies examining changes in haemorheological parameters during crises and steady states in SCD subjects, focusing on RBC deformability, Fibrinogen concentration, osmotic fragility, and erythrocyte sedimentation rate (ESR) organized into relevant subheadings.

2.3.1 Haemorheological Alterations in Sickle Cell Disease

RBC Deformability

RBC deformability is vital for the passage of cells through narrow capillaries. In SCD, the deformability of sickle cells is significantly impaired, particularly during vaso-occlusive crises. Caprari *et al*. (2019) demonstrated that sickle red blood cells exhibit reduced deformability due to increased intracellular haemoglobin concentration and viscosity, leading to frequent vaso-occlusive episodes and tissue ischaemia. Their study highlighted that haemorheological profiles in SCD subjects showed high blood viscosity, increased RBC aggregation, and decreased deformability, which were associated with oxidative damage to RBC membranes.

Parrow *et al*. (2017) further explored the relationship between RBC deformability and haemoglobin composition, finding that the presence of fetal haemoglobin (HbF) improved RBC deformability and hydration. The study utilized ektacytometry to measure deformability and found a correlation between the extent of diffraction pattern distortions and the percentage of HbF and HbA2. This suggests that higher levels of HbF may mitigate the severity of SCD by enhancing RBC flow properties.

Blood Viscosity

Blood viscosity is another critical haemorheological parameter affected in SCD. Caprari *et al*. (2019) reported elevated blood viscosity in SCD subjects, which contributes to the risk of vaso-occlusive crises. Increased viscosity is primarily due to the presence of sickle-shaped cells that aggregate more readily than normal RBCs, leading to impaired microcirculation.

The study by Antwi-Baffour *et al*. (2019) also indicated that the severity of anaemia in SCD subjects correlates with coagulation parameters, which can further influence blood viscosity. The findings suggest that managing anaemia could be a potential strategy to improve overall haemorheological properties and reduce the risk of complications.

Osmotic Fragility

Osmotic fragility refers to the susceptibility of RBCs to haemolysis when exposed to hypotonic solutions. Adegoke *et al*. (2014) assessed the effect of garlic extract on the osmotic fragility of RBCs in sickle cell subjects, finding that garlic administration reduced osmotic fragility in sickle cells. This suggests that dietary interventions may have a role in managing haemorheological parameters in SCD.

Dobrynina *et al*. (2020) highlighted the predictive value of osmotic fragility in the development of cerebral small vessel disease, indicating that increased osmotic fragility may also have implications for vascular health in SCD subjects. Their findings suggest that osmotic fragility can serve as a biomarker for assessing the risk of vascular complications in SCD.

Erythrocyte Sedimentation Rate (ESR)

ESR is a widely used laboratory test that measures the rate at which RBCs settle in a tube over a specified period. It serves as a marker of inflammation and can provide insights into disease

activity in SCD. Ahmed *et al*. (2000) reported that ESR values were significantly higher during painful crises compared to steady states in children with SCD. Their study found mean ESR values of 5 mm/hr during steady state, 25.8 mm/hr during painful crises, and even higher during infections, indicating that ESR can reflect the inflammatory status and haemolytic activity in SCD subjects.

Aytekin (2018) emphasized the importance of standardizing ESR measurement methods, as variations in techniques can affect the interpretation of results. The study highlighted that elevated ESR during crises correlates with increased haemolysis and inflammation, reinforcing its potential as a clinical indicator for monitoring disease progression and response to treatment.

Fibrinogen Concentration

Fibrinogen is a key plasma protein involved in coagulation and inflammation, and its concentration can significantly influence haemorheological properties in SCD. Elevated fibrinogen levels are associated with increased blood viscosity and enhanced RBC aggregation, both of which contribute to the pathophysiology of vaso-occlusive crises. Kucukal *et al*. (2020) demonstrated that fibrinogen levels were markedly higher during crises compared to steady states, suggesting that fibrinogen may play a role in promoting RBC adhesion to the endothelium.

The study by Nasimuzzaman *et al*. (2019) further supports this by showing that increased fibrinogen levels correlate with the severity of vaso-occlusive events and the risk of thrombotic complications.

2.3.2 Mechanisms Underlying Haemorheological Alterations

The mechanisms driving the observed haemorheological alterations in SCD are multifaceted. The polymerization of HbS under hypoxic conditions leads to the sickling of RBCs, which in turn affects their deformability and increases blood viscosity. Zhang *et al*. (2020) utilized boundary integral simulations to demonstrate how sickle cells segregate in flow, leading to increased shear stress on endothelial cells and contributing to vascular inflammation. This inflammation can exacerbate haemorheological alterations, creating a feedback loop that perpetuates the cycle of vaso-occlusive crises.

Additionally, the studies by Kucukal *et al*. (2020) and Nasimuzzaman *et al*. (2019) emphasize the role of fibrinogen in promoting RBC adhesion to the endothelium, which can further impair blood flow and increase the risk of thrombotic events. The interaction between sickle RBCs and activated endothelium is mediated by fibrinogen and is associated with haemolysis, indicating that inflammatory processes are closely intertwined with haemorheological changes in SCD.

2.4 Gap in Literature

Limited Understanding of Haemorheological Changes Across States

Current Knowledge: While previous studies have documented haemorheological alterations in SCD, there is insufficient comparative analysis between steady-state and crisis situations.

Gap: The present study seeks to provide a detailed examination of how haemorheological parameters, such as RBC deformability, blood viscosity, osmotic fragility, and ESR, differ significantly between these two states.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

This study adopted an experimental and comparative research design aimed at evaluating the modifications in haemorheological parameters among subjects with sickle cell disease (SCD) during vaso-occlusive crises, those in steady-state conditions, and non-sickle cell (control) individuals. The design enabled the measurement and comparison of fibrinogen concentration, erythrocyte sedimentation rate (ESR), osmotic fragility, and red blood cell (RBC) deformability across the three groups under controlled laboratory conditions.

3.2 Study Area

The study was conducted in Benin City, Edo State, Nigeria. Sickle cell patients were recruited from the Sickle Cell Centre, Benin City, while non-sickle cell control subjects were recruited from Benson Idahosa University, Benin City. All laboratory analyses were performed in the Haematology Laboratory using standardized procedures and calibrated equipment.

3.3 Study Population

The study population consisted of individuals aged 16–40 years, categorized into three groups:

1. Group I: Sickle cell patients (HbSS) in vaso-occlusive crisis.
2. Group II: Sickle cell patients (HbSS) in steady-state condition (no crisis within the last 4 weeks).
3. Group III: Non-sickle cell individuals (HbAS and HbAA) serving as control subjects.

3.4 Sample Size and Sampling Technique

A total of 60 participants were recruited for this study, comprising 20 normal controls (HbAA), 20 sickle cell patients in the steady state (HbSS), and 20 sickle cell patients in vaso-occlusive crisis (HbSS). Because this was a hospital-based comparative study, the sample size was determined by the number of eligible participants who met the inclusion criteria and consented to participate during the study period. A purposive (consecutive) sampling technique was employed to recruit participants until the required sample size of 60 was attained. Participants were selected based on confirmed haemoglobin phenotype determined by haemoglobin electrophoresis and fulfillment of the study inclusion and exclusion criteria.

3.5 Inclusion Criteria

1. Confirmed diagnosis of SCD (HbSS) by electrophoresis.
2. Subjects aged 16–21 years.
3. Individuals in crisis or steady state as defined by clinical presentation.
4. Non-sickle cell individuals (HbAA or HbAS) as controls.

3.6 Exclusion Criteria

1. Subjects with other haematological disorders or infections.
2. Individuals on anticoagulant or anti-inflammatory therapy.
3. Pregnant women.
4. Subjects unwilling to give consent.

3.7 Ethical Considerations

Ethical approval was obtained from the Health research and Ethics Committee of Ministry of Health Edo State with approval number: HA/737/26/C/04101113. Written informed consent was obtained from all participants prior to sample collection. All procedures were performed following the Helsinki Declaration (2013) on ethical principles for medical research involving human subjects.

3.8 Materials and Equipment

The following materials and instruments were used throughout the experiment:

1. Citrated containers
2. EDTA containers
3. Test tubes and racks
4. Water bath (37 °C)
5. Bucket centrifuge
6. Spectrophotometer
7. Applicator sticks
8. Retort stand and clamps
9. Syringes (1 ml and 2 ml)
10. Beakers and white filter cloth
11. Cotton wool, 70% alcohol, surgical gloves
12. Stopwatch

13. Sodium citrate (3.8%)
14. Calcium chloride solution (0.025 mol/L)
15. Normal saline (NaCl) solutions (0.1%–0.9%)

3.9 Sample Collection and Handling

Venous blood samples (2–5 ml) were collected aseptically from the antecubital vein using sterile disposable syringes and transferred into anticoagulated containers (EDTA or sodium citrate, as appropriate). Samples were properly labelled and processed immediately to avoid haemolysis or coagulation. Standard biosafety precautions were observed throughout the procedures.

3.10 Experimental Procedures

3.10.1 Determination of Fibrinogen Concentration (Gravimetric Method)

The fibrinogen concentration was determined using the gravimetric method as described by Higgins *et al*. (2016) and Dacie and Lewis (2011).

Procedure:

1. About 2.25 ml of venous blood was collected into a container with 0.25 ml of 3.8% sodium citrate (blood-to-anticoagulant ratio of 9:1).
2. The sample was centrifuged at 3,000 rpm for 15 minutes to obtain platelet-poor plasma.
3. About 1 ml of plasma was transferred into a test tube containing 1 ml of pre-warmed 0.025 mol/L calcium chloride.
4. Tubes were incubated in a water bath at 37 °C for 30 minutes, with intermittent stirring using an applicator stick.

5. The formed fibrin clot was removed, blotted with filter paper to remove moisture, and dried at room temperature.

6. The mass of the dried fibrin clot (mg) was recorded as the fibrinogen concentration.

3.10.2 Determination of Erythrocyte Sedimentation Rate (ESR)

The ESR was measured using the Westergren method following ICSH (2017) standards.

Procedure:

1. About 2 mL of venous blood was collected into an EDTA container.

2. The sample was mixed gently to prevent clotting.

3. A Westergren pipette was filled to the zero mark with anticoagulated blood.

4. The pipette was placed vertically in the stand at room temperature (18–25 °C), free from vibration.

5. After 1 hour (60 minutes), the distance from the top of the plasma column to the red cell sediment was recorded in mm/hour as the ESR.

3.10.3 Osmotic Fragility Test (OFT)

The osmotic fragility of erythrocytes was determined according to Parpart *et al*. (1947) and Bain (2017).

Procedure:

1. Serial dilutions of NaCl (0.1%–0.9%) were prepared from a 0.9% stock solution.

2. About 5 mL of venous blood was collected into an EDTA container and centrifuged at 3,000 rpm for 10 minutes.
3. The plasma was discarded and replaced with 0.9% saline; the washing process was repeated three times.
4. About 0.5 ml of washed RBC suspension was added to 3 ml of each NaCl concentration and allowed to stand for 20 minutes at room temperature.
5. Samples were centrifuged again at 3,000 rpm for 10 minutes.
6. The absorbance of the supernatant was measured at 540 nm using a spectrophotometer to determine the degree of haemolysis.

3.10.4 Determination of Red Blood Cell Deformability (Relative Viscosity Method)

Red cell deformability was evaluated using a viscosity-based method based on Poiseuille's law (Guyton and Hall, 2021).

Procedure:

1. A 1 ml syringe was vertically clamped to a retort stand, needle tip pointing downward.
2. A piece of white cloth was inserted between the barrel and needle hub to act as a porous barrier.
3. Reference flow (Water): 1 ml of distilled water was allowed to drain under gravity, and the flow time was recorded three times.
4. Blood flow: 1 ml of freshly collected venous blood (EDTA sample) was allowed to drain similarly, and the mean flow time was recorded.

5. Relative viscosity (RV) was calculated as:

RV = Drain time of blood / Drain time of water

3.11 Data Analysis

The results obtained were expressed as mean ± standard error of the mean (SEM). Statistical analyses for comparisons among groups were performed using GraphPad Prism version 10.2.0. Differences among the study groups were analysed using one-way analysis of variance (ANOVA) and two-way ANOVA, followed by Tukey's post hoc test for multiple comparisons where appropriate. In addition, IBM Statistical Package for the Social Sciences (SPSS) version 26.0 was used to perform Pearson's correlation analysis to determine the relationships among osmotic fragility, erythrocyte sedimentation rate (ESR), blood viscosity, and fibrinogen concentration within the normal control, sickle cell steady-state, and sickle cell crisis groups. SPSS was selected for the correlation analysis because it provides comprehensive correlation matrices, corresponding significance (p) values, and efficient subgroup analyses, making it particularly suitable for evaluating relationships among multiple continuous variables. Statistical significance was accepted at $p < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION OF FINDINGS

4.1 Results

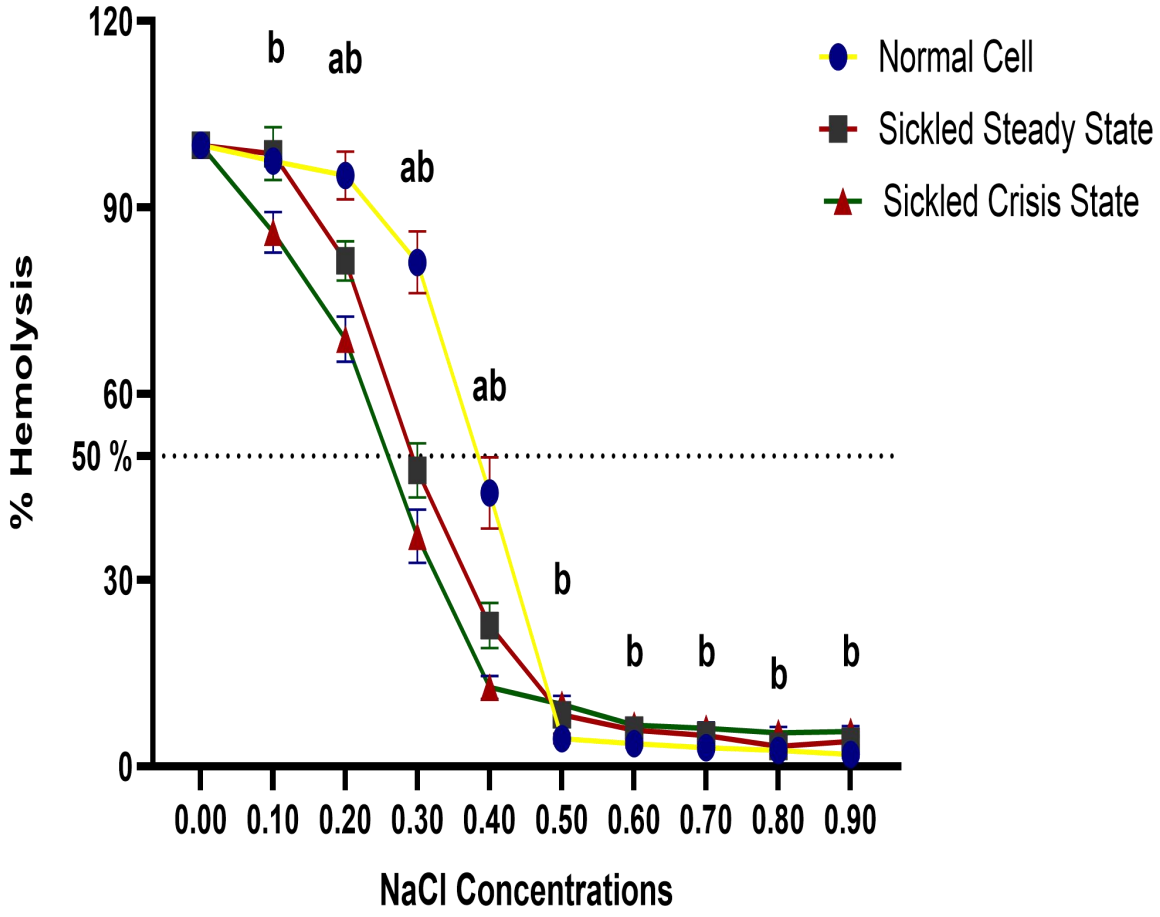

Figure 1: Osmotic fragility of normal red blood cells, sickled cells in crisis and steady state

a: $p<0.05$ normal cell compared to sickled steady state, and b: $p<0.05$ normal cell compared to sickled cell crisis state.

Results in figure 1 showed a shift to the left in sickled cells (SC) in crisis compared to the steady state and normal red blood cells when placed in hypotonic (0.1 to 0.4) sodium chloride solutions ($^{a}p<0.05$). The observed leftward shift in the osmotic fragility curve of erythrocytes from

subjects in sickle cell crisis indicates a statistically significant increase in osmotic fragility when compared with steady-state sickle cell subjects and normal controls ($p < 0.05$).

More specifically, in subjects with sickle cell disease who were sampled during crisis, the osmotic fragility pattern showed a distinct deviation from that of steady-state individuals and normal controls. Across the sodium chloride concentrations examined (0.1–0.4%), erythrocytes from the crisis group exhibited earlier and more pronounced haemolysis, indicating significant membrane instability. At relatively higher concentrations, where minimal haemolysis is expected in normal red blood cells, the crisis samples already showed substantial lysis. For instance, at concentrations around 0.40%–0.45% NaCl, where normal erythrocytes remain largely intact, red cells from the crisis group demonstrated notable haemolytic activity.

As the saline concentration decreased further towards the more hypotonic range (0.2–0.3%), haemolysis in the crisis samples became almost complete, reflecting their markedly increased fragility. In contrast, steady-state sickle cells required slightly more hypotonic conditions before comparable levels of haemolysis occurred, and normal cells maintained their structural integrity until the lowest concentrations (0.1–0.2%).

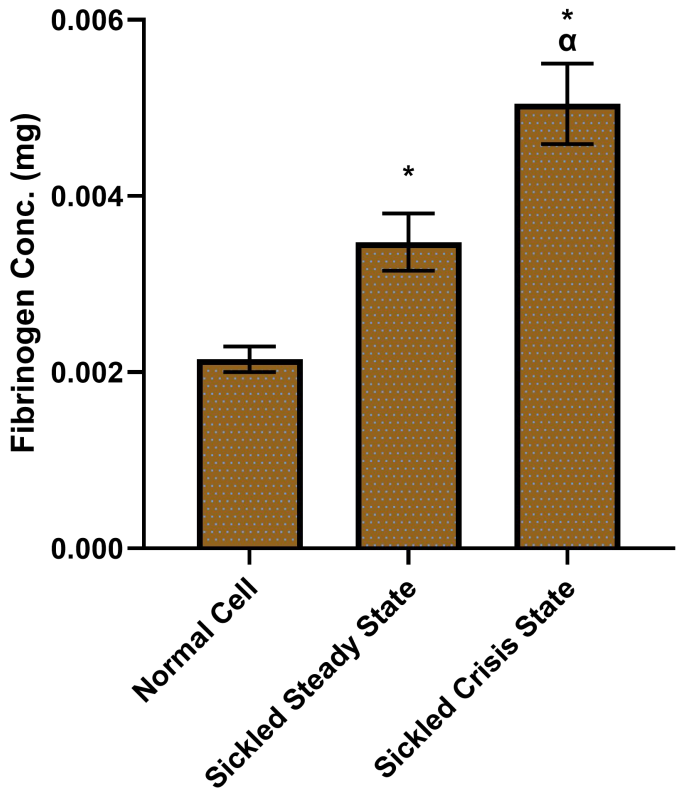

Figure 2: Fibrinogen concentration in normal, sickled (steady and crisis state)

The result in figure 2 shows a statistically significant increase in fibrinogen concentration in sickled cells (steady and crisis states) compared with normal cells (*$p<0.05$), and a statistically significant increase in fibrinogen concentration in sickled cells in the crisis state compared with the steady state ($^{\alpha}p<0.05$).

More specifically, the pattern presented in Figure 2 demonstrates clear and statistically significant differences in fibrinogen concentration among the study groups. Subjects with sickle cell disease, both in the steady state and during crisis, showed markedly higher fibrinogen concentrations when compared with normal (HbAA) individuals ($p < 0.05$). This indicates an overall elevation of fibrinogen levels in sickle cell disease, reflecting the chronic inflammatory and hypercoagulable state associated with the condition.

Furthermore, a comparison between the sickle cell subgroups revealed an additional statistically significant increase in fibrinogen concentration among individuals sampled during crisis compared with those in the steady state ($\alpha p < 0.05$). This suggests that fibrinogen levels rise even further during vaso-occlusive episodes, likely due to intensified inflammatory activity and enhanced acute-phase responses triggered by the crisis event.

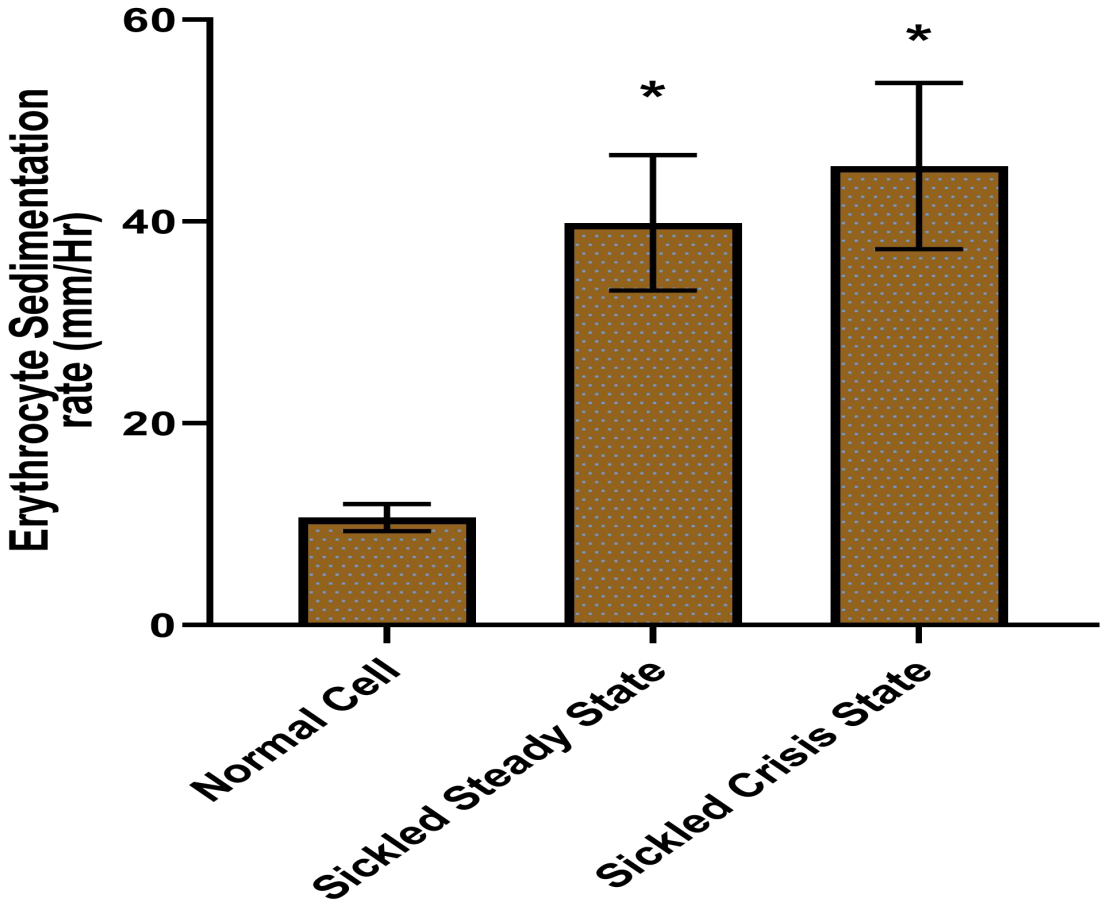

Figure 3: Erythrocyte sedimentation rate in normal, sickled (steady and crisis state)

The results in figure 3 show a statistically significant increase in erythrocyte sedimentation rate in sickled cells (steady and crisis states) compared with normal cells (*$p<0.05$). More

specifically, Figure 3 shows a clear and statistically significant difference in erythrocyte sedimentation rate across the study groups. Both sickle cell subjects in the steady state and those in crisis exhibited significantly elevated ESR values when compared with normal individuals ($p < 0.05$). This increase suggests heightened inflammatory activity and altered plasma protein composition characteristic of sickle cell disease. The presence of higher ESR in both sickle cell groups suggests that the disease, even in its stable phase, is associated with a persistent inflammatory state.

Although ESR values were elevated in both sickle cell groups relative to the normal controls, the crisis state demonstrated a further upward trend, consistent with the acute inflammatory response typically triggered during vaso-occlusive episodes. However, the primary statistically significant contrast noted in this figure lies between the sickle cell groups collectively and the normal group.

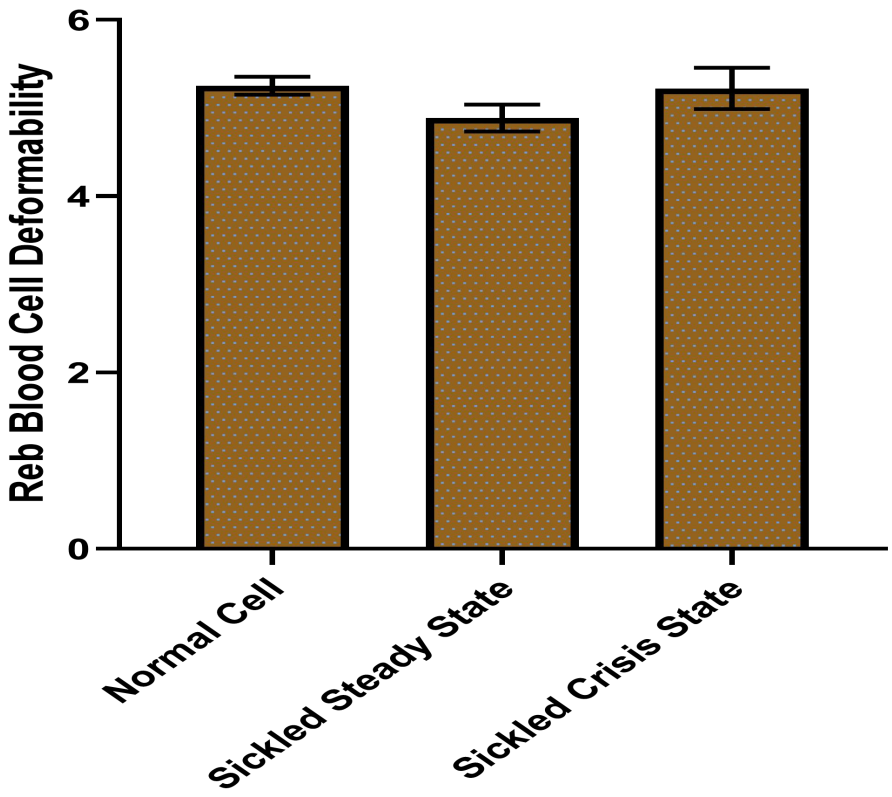

Figure 4: Blood red blood cell deformability in normal, sickled (steady and crisis state)

Figure 4 illustrates the comparative red blood cell deformability across normal individuals and subjects with sickle cell disease in both steady and crisis states. The results show no statistically significant difference in deformability values between the sickle cell groups (steady and crisis states) and the normal control group ($p > 0.05$). The absence of significant differences in the result suggests that the measured deformability parameters remained relatively similar across all groups.

This finding may indicate that, under the specific test conditions employed, the inherent rigidity of sickled cells did not manifest strongly enough to produce measurable differences when

compared with normal erythrocytes. It is also possible that compensatory mechanisms, such as varying hydration states or the presence of younger erythrocyte populations (reticulocytes), contributed to maintaining comparable deformability levels across the groups.

Pearson Correlation Analysis

Pearson's correlation analysis was performed separately for the normal control group, sickle cell patients in the steady state, and sickle cell patients in crisis to determine the relationships among osmotic fragility, erythrocyte sedimentation rate (ESR), blood viscosity, and fibrinogen concentration.

Table 4.1 Pearson Correlation Matrix for Normal Controls (HbAA) (n = 26)

Variables	Osmotic Fragility	ESR	Blood Viscosity	Fibrinogen
Osmotic Fragility	1	0.083	-0.262	-0.238
ESR	0.083	1	0.394*	-0.063
Blood Viscosity	-0.262	0.394*	1	-0.120
Fibrinogen	-0.238	-0.063	-0.120	1

*Correlation is significant at $p < 0.05$.

Among the normal control subjects, osmotic fragility showed weak negative correlations with blood viscosity ($r = -0.262$, $p = 0.195$) and fibrinogen concentration ($r = -0.238$, $p = 0.242$), and a weak positive correlation with ESR ($r = 0.083$, $p = 0.686$) as shown in table 4.1. None of these associations were statistically significant ($p > 0.05$). However, a moderate positive correlation was observed between ESR and blood viscosity ($r = 0.394$, $p = 0.046$), indicating that individuals with higher erythrocyte sedimentation rates tended to have slightly higher blood viscosity values. No significant relationship was found between fibrinogen concentration and any of the other measured variables.

Table 4.2 Pearson Correlation Matrix for Sickle Cell Patients in Steady State (n = 26)

Variables	Osmotic Fragility	ESR	Blood Viscosity	Fibrinogen
Osmotic Fragility	1	-0.191	-0.090	-0.120
ESR	-0.191	1	0.225	-0.271
Blood Viscosity	-0.090	0.225	1	-0.257
Fibrinogen	-0.120	-0.271	-0.257	1

No significant correlations were observed ($p > 0.05$).

Among sickle cell patients in the steady state, all observed correlations were weak and statistically non-significant ($p > 0.05$) as shown in table 4.2. Osmotic fragility demonstrated weak negative relationships with ESR ($r = -0.191$), blood viscosity ($r = -0.090$), and fibrinogen concentration ($r = -0.120$) as shown in table 4.2. Likewise, ESR showed weak positive correlation with blood viscosity ($r = 0.225$) and weak negative correlation with fibrinogen concentration ($r = -0.271$) as shown in table 4.2. These findings indicate that under steady-state conditions, changes in one parameter were not significantly associated with changes in the others.

Table 4.3 Pearson Correlation Matrix for Sickle Cell Patients in Crisis (n = 26)

Variables	Osmotic Fragility	ESR	Blood Viscosity	Fibrinogen
Osmotic Fragility	1	-0.030	-0.448*	0.510**
ESR	-0.030	1	-0.081	0.083
Blood Viscosity	-0.448*	-0.081	1	-0.061
Fibrinogen	0.510**	0.083	-0.061	1

*Correlation is significant at $p < 0.05$.
**Correlation is significant at $p < 0.01$

In contrast, the crisis group demonstrated two statistically significant relationships. Osmotic fragility showed a moderate negative correlation with blood viscosity ($r = -0.448$, $p = 0.022$), indicating that increased erythrocyte fragility was associated with lower blood viscosity measurements as shown in table 4.3. Furthermore, osmotic fragility exhibited a moderate positive correlation with fibrinogen concentration ($r = 0.510$, $p = 0.008$), suggesting that higher

fibrinogen levels were associated with increased osmotic fragility during vaso-occlusive crisis. No significant relationship was observed between ESR and osmotic fragility ($r = -0.030$, $p = 0.885$), blood viscosity ($r = -0.081$, $p = 0.696$), or fibrinogen concentration ($r = 0.083$, $p = 0.688$).

Hence, the correlation analysis indicates that significant interrelationships among the measured haematological parameters were evident primarily during sickle cell crisis, whereas the normal control and steady-state groups exhibited predominantly weak and non-significant associations. These findings suggest that vaso-occlusive crisis is accompanied by stronger interactions between erythrocyte membrane fragility, blood rheology, and inflammatory biomarkers, consistent with the increased pathological activity observed during acute sickle cell episodes.

4.2 Discussion of Findings

The present study evaluated the haemorrheological parameters among sickle cell disease (SCD) subjects in steady-state conditions, individuals experiencing vaso-occlusive crisis, and non-sickle cell controls. The parameters assessed included osmotic fragility, red cell deformability, erythrocyte sedimentation rate (ESR), fibrinogen concentration, and blood viscosity.

4.2.1 Osmotic Fragility

The present study demonstrated clear and statistically significant differences in osmotic fragility among the normal control group, sickle cell patients in the steady state, and those experiencing vaso-occlusive crisis, indicating that erythrocyte membrane behaviour and volume regulation are profoundly influenced by the pathophysiological state of sickle cell disease (SCD). Osmotic fragility assesses the susceptibility of erythrocytes to haemolysis following exposure to progressively hypotonic saline solutions and provides an indirect measure of membrane integrity, intracellular hydration, surface area-to-volume ratio, and the capacity of red blood cells (RBCs)

to accommodate osmotic swelling before membrane rupture. Unlike tests that evaluate only membrane structure, osmotic fragility reflects the combined effects of membrane elasticity, cytoskeletal stability, ion transport, and cellular hydration.

In the present study, normal erythrocytes demonstrated a rightward shift of the osmotic fragility curve in markedly hypotonic saline concentrations (0.1–0.4% NaCl) compared with erythrocytes from both steady-state and crisis SCD patients. Physiologically, this finding indicates that HbAA erythrocytes tolerated greater osmotic stress before haemolysis occurred. The high deformability of normal RBCs is attributed to their biconcave disc shape and an intact membrane cytoskeleton composed primarily of spectrin, ankyrin, actin, protein 4.1, and band 3 proteins, which maintain membrane flexibility while allowing considerable expansion during water influx. As extracellular osmolarity decreases, water enters normal erythrocytes through aquaporin channels, causing progressive cell swelling. Because normal erythrocytes possess a favourable surface area-to-volume ratio, they are capable of accommodating substantial increases in cell volume before reaching the critical membrane tension required for lysis (Paradkar and Gambhire, 2021). Consequently, haemolysis occurs only in more severely hypotonic solutions, accounting for the observed rightward displacement of the osmotic fragility curve.

In contrast, erythrocytes obtained from sickle cell patients, particularly during vaso-occlusive crisis, exhibited a significant leftward shift of the osmotic fragility curve compared with the steady-state group (**$p < 0.05$**), indicating reduced osmotic fragility or increased osmotic resistance. Physiologically, this phenomenon is primarily a consequence of chronic erythrocyte dehydration rather than improved membrane integrity. Repeated cycles of deoxygenation promote polymerization of haemoglobin S (HbS), resulting in the formation of long intracellular

polymers that distort erythrocytes into the characteristic sickle shape. Polymerization also activates membrane ion transport pathways, including the calcium-activated Gardos channels and potassium-chloride cotransport system, leading to potassium efflux followed by osmotic water loss. The resulting dehydration increases intracellular haemoglobin concentration (MCHC), reduces cell volume, and lowers the intracellular water available for osmotic expansion. Consequently, dehydrated sickled erythrocytes require greater hypotonic stress before swelling sufficiently to rupture, producing the reduced osmotic fragility observed in the crisis group (Brugnara, 2018).

Although crisis erythrocytes exhibit reduced osmotic fragility, this should not be interpreted as evidence of healthier or structurally superior membranes. On the contrary, vaso-occlusive crisis is characterized by repeated HbS polymerization and depolymerization, oxidative stress, calcium overload, lipid peroxidation, protein oxidation, and progressive disruption of the membrane cytoskeleton. Recurrent sickling damages membrane phospholipids and cytoskeletal proteins, promotes phosphatidylserine externalization, and stimulates microvesicle shedding, leading to progressive membrane stiffening and irreversible sickling. These changes substantially reduce erythrocyte deformability and impair microvascular transit despite the apparent increase in osmotic resistance (Alexy *et al*., 2022). Therefore, the reduced osmotic fragility observed during crisis primarily reflects profound cellular dehydration and altered cell geometry rather than preservation of membrane function.

The behaviour of erythrocytes in near-isotonic saline concentrations (0.5–0.9% NaCl) further supports this physiological interpretation. Within this concentration range, normal erythrocytes demonstrated relatively less haemolysis than sickled erythrocytes because they remained within

their physiological osmotic tolerance limits. Conversely, sickled erythrocytes, despite being osmotically resistant in more hypotonic solutions, possess rigid membranes with markedly reduced deformability and diminished capacity to respond to subtle osmotic fluctuations. Chronic activation of Gardos channels and increased membrane permeability maintain a dehydrated intracellular environment, while repeated oxidative injury further limits membrane elasticity (Brugnara, 2018). Consequently, osmotic fragility in SCD reflects not only membrane integrity but also erythrocyte hydration status, intracellular haemoglobin concentration, membrane surface area, and the functional integrity of ion transport mechanisms responsible for maintaining cell volume.

The present findings differ from those reported by Adegoke *et al*. (2014), who observed increased osmotic fragility in patients with sickle cell disease and attributed this to oxidative membrane injury resulting from repeated sickling and unsickling cycles. Their study further demonstrated that treatment with garlic extract, an antioxidant, significantly reduced erythrocyte fragility, emphasizing the contribution of oxidative stress to membrane instability. While oxidative membrane damage undoubtedly contributes to erythrocyte dysfunction in SCD, the discrepancy between their findings and those of the present study may reflect differences in the predominant physiological mechanisms influencing osmotic behaviour. In the present study, crisis erythrocytes appeared to exhibit greater osmotic resistance because cellular dehydration, increased intracellular haemoglobin concentration, and reduced cell water content outweighed the membrane-destabilizing effects of oxidative injury. Thus, although oxidative stress is markedly intensified during vaso-occlusive crisis, the concurrent activation of ion transport pathways and progressive erythrocyte dehydration may shift the osmotic fragility curve toward increased resistance rather than increased susceptibility to haemolysis. This observation

highlights the complex interplay between membrane injury and cellular hydration in determining the osmotic behaviour of sickled erythrocytes and suggests that osmotic fragility is governed by multiple physiological mechanisms acting simultaneously rather than membrane damage alone.

Hence, the present findings demonstrate that osmotic fragility in sickle cell disease is determined by the interaction between HbS polymerization, erythrocyte dehydration, membrane biomechanics, oxidative injury, and ion transport. While normal erythrocytes exhibit high deformability and a large osmotic reserve due to intact membrane architecture, sickled erythrocytes undergo profound alterations in cell hydration and membrane mechanics that modify their osmotic response according to disease state. The significantly reduced osmotic fragility observed during vaso-occlusive crisis therefore reflects the physiological consequences of chronic cellular dehydration and altered membrane rheology rather than improved erythrocyte integrity.

4.2.2 Fibrinogen Concentration

The present study demonstrated significantly elevated fibrinogen concentrations in individuals with sickle cell disease (SCD) compared with the normal control group ($p < 0.05$**),** with patients experiencing vaso-occlusive crisis exhibiting the highest concentrations. Furthermore, fibrinogen levels were significantly higher in the crisis group than in the steady-state group ($p < 0.05$), indicating that fibrinogen production is progressively enhanced as the disease transitions from chronic physiological compensation to acute vaso-occlusion. These findings emphasize the fundamental physiological role of fibrinogen in regulating haemostasis, inflammation, blood rheology, and vascular homeostasis, all of which become profoundly altered in SCD.

Fibrinogen is a 340-kDa soluble glycoprotein synthesized primarily by hepatocytes under the regulation of inflammatory cytokines, particularly interleukin-6 (IL-6), interleukin-1β, and tumour necrosis factor-α (TNF-α). Under normal physiological conditions, circulating fibrinogen participates in haemostasis by serving as the precursor of fibrin following thrombin-mediated cleavage and by facilitating platelet aggregation through glycoprotein IIb/IIIa receptors. Beyond coagulation, fibrinogen also contributes to vascular homeostasis by influencing plasma viscosity, erythrocyte aggregation, endothelial function, and cellular signaling (Lizarralde-Iragorri & Shet, 2020). In healthy individuals, fibrinogen concentrations are tightly regulated to balance efficient coagulation with maintenance of normal blood fluidity and microvascular perfusion.

In sickle cell disease, however, chronic haemolysis, recurrent tissue hypoxia, oxidative stress, and endothelial activation establish a persistent inflammatory milieu that continuously stimulates hepatic fibrinogen synthesis. Even during the clinically stable or steady state, polymerization of haemoglobin S (HbS) during intermittent deoxygenation results in repeated sickling and unsickling of erythrocytes, leading to membrane injury and intravascular haemolysis. Free haemoglobin and erythrocyte-derived microparticles released during haemolysis scavenge nitric oxide, impair endothelial function, and promote the release of pro-inflammatory cytokines that stimulate acute-phase protein production, including fibrinogen. Consequently, the significantly elevated fibrinogen concentrations observed in the steady-state subjects in the present study indicate that chronic inflammation persists despite the absence of overt vaso-occlusive symptoms.

The significantly higher fibrinogen concentrations recorded during vaso-occlusive crisis reflect the amplification of these physiological processes during acute disease exacerbation. Vaso-occlusion produces widespread tissue ischemia followed by reperfusion injury, generating large

quantities of reactive oxygen species that further activate endothelial cells, leukocytes, monocytes, and platelets. Activated endothelial cells release cytokines, chemokines, and adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and E-selectin, all of which amplify inflammatory signaling and stimulate further hepatic synthesis of acute-phase proteins. Maciel *et al*. (2024) demonstrated that inflammatory activation increases substantially during vaso-occlusive crisis, explaining the marked rise in circulating fibrinogen concentrations. Therefore, the significantly higher fibrinogen levels observed in the crisis group in the present study are consistent with the physiological progression from chronic low-grade inflammation during steady state to an intense acute-phase inflammatory response during vaso-occlusion.

The present findings closely agree with those of Kucukal *et al*. (2020), who demonstrated that elevated fibrinogen concentrations directly enhance interactions between sickled erythrocytes and the vascular endothelium. Physiologically, fibrinogen functions not only as a coagulation protein but also as an adhesive ligand capable of binding integrins expressed on erythrocytes, leukocytes, platelets, and endothelial cells. These molecular interactions strengthen the adhesion of sickled erythrocytes to activated endothelial surfaces through adhesion molecules such as VCAM-1 and other endothelial receptors, thereby reducing erythrocyte transit through the microcirculation. Reduced cellular movement prolongs erythrocyte residence time within capillaries, increasing the likelihood of HbS polymerization during deoxygenation and promoting further sickling. Thus, elevated fibrinogen contributes directly to the self-perpetuating cycle of erythrocyte adhesion, vascular obstruction, tissue hypoxia, and inflammatory activation that characterizes vaso-occlusive crisis.

The findings of the present study are also consistent with those of Ajayi *et al*. (2007), who reported significantly elevated fibrinogen concentrations in several chronic inflammatory and cardiovascular disorders, including sickle cell disease. Their findings suggest that fibrinogen represents a common physiological response to sustained inflammatory stimulation irrespective of the underlying disease process. In SCD, continuous haemolysis and endothelial dysfunction maintain persistent cytokine production, thereby sustaining elevated fibrinogen concentrations even outside acute crises. This supports the present observation that fibrinogen is elevated in both steady-state and crisis patients, although the magnitude of elevation becomes significantly greater during vaso-occlusive episodes.

Similarly, Nasimuzzaman *et al*. (2019) demonstrated that increasing fibrinogen concentrations are associated with greater frequency and severity of vaso-occlusive and thrombotic complications in SCD. Physiologically, elevated fibrinogen modifies blood rheology through several complementary mechanisms. First, fibrinogen molecules adsorb onto erythrocyte membranes and form reversible molecular bridges between adjacent erythrocytes, promoting erythrocyte aggregation under low-shear conditions. This aggregation, commonly described as rouleaux formation in normal erythrocytes, increases blood flow resistance and reduces microvascular perfusion (Rampling, 2019). Although classical rouleaux formation is less pronounced in sickled erythrocytes because of their abnormal morphology, elevated fibrinogen nevertheless promotes abnormal cellular interactions among sickled erythrocytes, leukocytes, platelets, and endothelial cells, thereby increasing vascular resistance.

Although the present study did not demonstrate significant differences in whole-blood viscosity among the study groups, the markedly elevated fibrinogen concentrations observed in sickle cell

patients remain physiologically important. Whole-blood viscosity measured under laboratory conditions reflects bulk rheological behaviour, whereas the microcirculation operates predominantly under very low shear conditions where erythrocyte aggregation, plasma viscosity, endothelial interactions, and cellular deformability exert much greater influence on blood flow. Consequently, substantial rheological abnormalities may exist within capillaries despite relatively modest changes in bulk viscosity measurements. This physiological explanation agrees with the observations of Rampling (2019), who emphasized that fibrinogen exerts its greatest rheological effects under low-shear conditions characteristic of post-capillary venules and capillary networks. Similarly, Alexy *et al*. (2022) demonstrated that elevated fibrinogen increases plasma viscosity and further impairs microvascular blood flow by acting synergistically with the reduced deformability of sickled erythrocytes.

From a physiological perspective, fibrinogen contributes to SCD pathogenesis through multiple interconnected mechanisms. In addition to promoting erythrocyte aggregation, elevated fibrinogen increases plasma viscosity, thereby increasing vascular resistance and reducing tissue perfusion. Furthermore, fibrinogen interacts directly with endothelial cells through integrin receptors, activating intracellular signaling pathways that stimulate the production of cytokines, chemokines, and adhesion molecules (Hsieh *et al*., 2017). Endothelial activation subsequently enhances leukocyte recruitment and promotes firm adhesion of erythrocytes and platelets to the vascular wall, thereby perpetuating vaso-occlusion. Simultaneously, increased circulating fibrinogen provides abundant substrate for thrombin-mediated fibrin generation, enhancing clot formation and contributing to the hypercoagulable state characteristic of sickle cell disease (Hsieh *et al*., 2017). Thus, fibrinogen functions not merely as a passive biomarker of

inflammation but as an active physiological regulator of vascular function, haemostasis, inflammatory signaling, and blood rheology.

The significantly elevated fibrinogen concentrations observed in the present study therefore indicate both increased inflammatory activity and progressive disruption of normal haemorheological homeostasis. Duvoix *et al*. (2013) described fibrinogen as a sensitive biomarker of systemic inflammatory burden because its synthesis closely reflects cytokine activity during inflammatory stress. However, fibrinogen also functions as an active mediator of disease progression. Davalos and Akassoglou (2012) demonstrated that fibrinogen participates directly in inflammatory signaling by activating leukocytes, endothelial cells, and macrophages, thereby linking coagulation with innate immune responses. Consequently, the significantly higher fibrinogen concentrations observed in crisis patients compared with steady-state patients in the present study reflect not only greater inflammatory stimulation but also increased physiological disturbance of vascular homeostasis, endothelial function, and blood rheology.

Hence, the findings of the present study demonstrate that fibrinogen occupies a central physiological position in the pathogenesis of sickle cell disease. Its progressive elevation from normal controls to steady-state patients and subsequently to vaso-occlusive crisis reflects increasing activation of inflammatory, coagulation, and rheological pathways. Rather than functioning solely as an acute-phase protein, fibrinogen actively modulates erythrocyte aggregation, plasma viscosity, endothelial activation, coagulation, and microvascular blood flow. These physiological interactions explain why fibrinogen concentrations increase significantly during vaso-occlusive crisis and underscore its importance as both a biomarker of inflammatory

activity and a key contributor to the haemodynamic and microvascular disturbances that characterize sickle cell disease.

4.2.3 Erythrocyte Sedimentation Rate (ESR)

The present study demonstrated that erythrocyte sedimentation rate (ESR) was significantly elevated in both steady-state and crisis sickle cell disease (SCD) subjects compared with the normal control group ($p < 0.05$), with the highest values occurring during vaso-occlusive crisis. These findings indicate progressive activation of inflammatory and haemostatic pathways as disease severity increases and support the concept that SCD is characterized by chronic basal inflammation that becomes markedly intensified during acute vaso-occlusive episodes. Physiologically, ESR reflects the rate at which erythrocytes sediment under gravity within anticoagulated blood and is determined by the interaction between plasma protein composition, erythrocyte morphology, erythrocyte surface charge, haematocrit, and the rheological properties of blood rather than inflammation alone (Simeon *et al*., 2024; Alharthi *et al*., 2024).

Under normal physiological conditions, erythrocytes remain suspended within plasma because their membranes possess a net negative surface charge, known as the **zeta potential**, which generates electrostatic repulsion between adjacent cells and limits spontaneous aggregation. Consequently, normal erythrocytes sediment slowly, producing relatively low ESR values. The principal physiological determinants of ESR include plasma concentrations of fibrinogen and immunoglobulins, erythrocyte size and shape, plasma viscosity, and haematocrit (Kahar, 2022). Acute-phase proteins, particularly fibrinogen, partially neutralize the zeta potential, allowing erythrocytes to aggregate into rouleaux. These larger aggregates possess greater mass and sediment more rapidly than individual erythrocytes, thereby increasing ESR.

In sickle cell disease, however, the physiological regulation of ESR becomes considerably more complex because the disease simultaneously alters both plasma composition and erythrocyte structure. Chronic polymerization of haemoglobin S (HbS) damages the erythrocyte membrane through repeated cycles of sickling and unsickling, producing rigid, elongated, and poorly deformable erythrocytes. These abnormal cells have a reduced capacity to form classical rouleaux because their distorted morphology interferes with the close membrane-to-membrane interactions required for aggregation. Under physiological conditions, this morphological abnormality would be expected to reduce sedimentation. Conversely, chronic haemolysis, endothelial activation, oxidative stress, and persistent cytokine production stimulate hepatic synthesis of acute-phase proteins, particularly fibrinogen, which enhance erythrocyte aggregation and accelerate sedimentation. The significantly elevated ESR observed in the steady-state subjects in the present study therefore suggests that the inflammatory increase in plasma proteins outweighed the inhibitory effects of abnormal erythrocyte morphology, resulting in an overall increase in sedimentation rate.

The significantly higher ESR values observed during vaso-occlusive crisis indicate further amplification of these physiological processes. Acute vaso-occlusion produces tissue hypoxia followed by ischemia-reperfusion injury, leading to extensive generation of reactive oxygen species, activation of endothelial cells, leukocytes, monocytes, and platelets, and increased release of inflammatory cytokines such as interleukin-6 and tumour necrosis factor-α. These cytokines stimulate hepatic synthesis of fibrinogen and other acute-phase proteins, increasing plasma protein concentration and promoting erythrocyte aggregation despite the abnormal morphology of sickled erythrocytes. Simultaneously, accelerated haemolysis releases free haemoglobin, haem, and erythrocyte-derived microparticles that further stimulate endothelial

dysfunction and inflammatory signaling, creating a self-amplifying inflammatory response. The markedly elevated ESR observed during crisis in the present study therefore reflects the cumulative effects of increased acute-phase protein production, enhanced inflammatory activity, and altered plasma rheology rather than changes in erythrocyte sedimentation alone.

The present findings agree with those reported by Ahmed *et al*. (2000), who demonstrated significant increases in ESR during painful vaso-occlusive crises in children with SCD. Their study showed that mean ESR increased from approximately 5 mm/hr during the steady state to approximately 25.8 mm/hr during vaso-occlusive crisis, with even greater elevations in patients who developed concurrent infections. These findings indicate that ESR responds dynamically to increasing inflammatory activity and haemolytic stress. Physiologically, infections further augment cytokine production and hepatic acute-phase protein synthesis, thereby accelerating erythrocyte sedimentation beyond the levels observed during uncomplicated vaso-occlusive crises. The similarity between the findings of Ahmed *et al*. (2000) and those of the present study suggests that progressive inflammatory activation is a major determinant of ESR elevation during sickle cell crisis.

The present findings are also consistent with Aytekin (2018), who emphasized that although ESR remains one of the oldest laboratory indices of inflammation, its physiological interpretation requires consideration of multiple factors influencing erythrocyte sedimentation. Aytekin (2018) noted that erythrocyte morphology, plasma protein concentration, haematocrit, temperature, and pre-analytical handling all influence ESR measurements. In sickle cell disease, these physiological variables are profoundly altered, making ESR a composite indicator of both inflammatory activity and changes in erythrocyte rheology rather than an isolated marker of

inflammation. Consequently, despite the abnormal morphology of sickled erythrocytes, the significant increase in ESR observed in the present study demonstrates that inflammatory alterations in plasma composition predominated over the inhibitory effects of reduced erythrocyte deformability.

The elevated ESR observed in the present study is further supported by the corresponding increase in fibrinogen concentration. As demonstrated in Section 4.2.2, fibrinogen concentrations increased significantly in both steady-state and crisis SCD patients ($p < 0.05$). Physiologically, fibrinogen promotes erythrocyte aggregation by adsorbing onto erythrocyte membranes and partially neutralizing their surface charge, thereby reducing electrostatic repulsion between adjacent cells. Although sickled erythrocytes do not form classical rouleaux as efficiently as normal erythrocytes, elevated fibrinogen enhances abnormal cellular aggregation involving erythrocytes, leukocytes, platelets, and endothelial cells, contributing to increased sedimentation and altered blood rheology. Thus, the parallel elevation of fibrinogen and ESR observed in the present study reflects activation of common inflammatory pathways and supports the role of fibrinogen as one of the principal physiological determinants of ESR during sickle cell disease.

The findings relating to osmotic fragility also provide additional physiological insight into the observed ESR changes. The reduced osmotic fragility demonstrated by crisis erythrocytes in the present study indicates chronic erythrocyte dehydration resulting from activation of potassium transport pathways, increased intracellular haemoglobin concentration, and altered membrane biomechanics. These physiological changes reduce erythrocyte deformability and impair normal microcirculatory flow. Although decreased deformability alone would be expected to reduce sedimentation because rigid erythrocytes aggregate less efficiently, the accompanying

inflammatory increase in plasma fibrinogen and other acute-phase proteins appears to overcome this inhibitory effect, resulting in the overall elevation in ESR observed during crisis. This illustrates that ESR in sickle cell disease reflects the combined influence of plasma protein composition and erythrocyte mechanical properties rather than either factor independently.

Hence, the present findings demonstrate that ESR is fundamentally a physiological indicator of the interaction between inflammation, plasma protein composition, erythrocyte membrane properties, and blood rheology. The progressive increase in ESR from normal controls to steady-state patients and subsequently to crisis patients reflects increasing activation of inflammatory pathways, enhanced hepatic synthesis of acute-phase proteins, persistent haemolysis, endothelial dysfunction, and progressive disruption of normal erythrocyte physiology. The agreement between the present findings and those reported by Ahmed *et al*. (2000) and Aytekin (2018) further supports the view that ESR remains a valuable indicator of the inflammatory and haemorheological alterations that characterize sickle cell disease. Rather than serving solely as a nonspecific inflammatory marker, ESR reflects the integrated physiological consequences of altered plasma composition, erythrocyte membrane dysfunction, and vascular inflammation, making it an important adjunctive parameter in understanding the pathophysiology of sickle cell disease.

4.2.4 Red Cell Deformability

The present study demonstrated no statistically significant difference in red blood cell (RBC) deformability between normal controls and individuals with sickle cell disease (SCD), whether in the steady state or during vaso-occlusive crisis ($p > 0.05$). This finding suggests that, under the experimental conditions employed in the present study, the overall mechanical behaviour of

erythrocytes from sickle cell subjects was comparable to that of normal (HbAA) erythrocytes. Physiologically, red cell deformability refers to the ability of erythrocytes to undergo reversible changes in shape while traversing the microcirculation, particularly capillaries whose diameters are frequently smaller than the resting diameter of the erythrocyte. This remarkable property depends on the coordinated interaction of membrane elasticity, cytoskeletal integrity, intracellular viscosity, cell geometry, and cellular hydration. Preservation of deformability is essential for maintaining efficient tissue perfusion, minimizing vascular resistance, and facilitating oxygen delivery to metabolically active tissues.

Under normal physiological conditions, erythrocytes possess exceptional deformability because of their biconcave disc shape, high surface area-to-volume ratio, and highly organized membrane cytoskeleton composed principally of spectrin, ankyrin, actin, protein 4.1, protein 4.2, and band 3 proteins. These structural components provide sufficient mechanical strength to preserve membrane integrity while simultaneously allowing extensive reversible deformation during passage through narrow capillaries and splenic sinusoids. In addition, the relatively low intracellular viscosity of haemoglobin A (HbA), together with tightly regulated membrane ion transport mechanisms that maintain optimal cellular hydration, enables normal erythrocytes to recover their original morphology after repeated mechanical deformation. Consequently, healthy erythrocytes exhibit excellent flexibility and minimal resistance to microvascular blood flow.

In sickle cell disease, however, erythrocyte deformability is expected to decline because of multiple physiological alterations affecting both the membrane and intracellular compartment. During deoxygenation, haemoglobin S (HbS) undergoes polymerization, producing rigid intracellular fibres that distort erythrocytes into the characteristic sickle shape. Repeated cycles

of polymerization and depolymerization generate cumulative membrane injury through oxidative stress, lipid peroxidation, calcium influx, and disruption of cytoskeletal proteins. Simultaneously, activation of membrane ion transport pathways, including the calcium-activated Gardos channel and potassium-chloride cotransport system, promotes potassium and water loss, leading to erythrocyte dehydration, increased mean corpuscular haemoglobin concentration (MCHC), and elevated intracellular viscosity. Collectively, these physiological changes reduce membrane flexibility, impair erythrocyte deformability, and increase mechanical resistance during microvascular transit.

Despite these well-established mechanisms, the present study did not demonstrate a statistically significant reduction in deformability among sickle cell subjects. This finding differs from that reported by Caprari *et al*. (2019), who observed significantly reduced erythrocyte deformability in patients with SCD together with increased blood viscosity and erythrocyte aggregation. Their study attributed these rheological abnormalities to oxidative damage involving membrane proteins, including spectrin degradation and increased membrane-bound globin, which altered membrane elasticity and impaired the ability of erythrocytes to undergo reversible deformation. Oxidative modification of membrane proteins weakens interactions between the lipid bilayer and cytoskeleton, thereby increasing membrane rigidity and reducing the capacity of erythrocytes to negotiate the narrow dimensions of the microcirculation. The findings of Caprari *et al*. (2019) therefore support the concept that progressive oxidative injury contributes significantly to the impaired rheological behaviour characteristic of sickle cell disease.

Similarly, Alapan *et al*. (2016), using a high-resolution microfluidic single-cell assay, demonstrated that erythrocytes containing HbS possessed significantly lower dynamic

deformability than normal HbAA erythrocytes. Importantly, their study also showed that sickle cell populations are mechanically heterogeneous, consisting of both highly deformable erythrocytes and markedly rigid, irreversibly sickled cells. Physiologically, this heterogeneity reflects differences in erythrocyte age, intracellular haemoglobin concentration, hydration status, cumulative oxidative damage, and the frequency of previous sickling episodes. Younger erythrocytes and reticulocytes generally retain greater membrane flexibility because they have undergone fewer cycles of oxidative injury and possess relatively intact cytoskeletal architecture, whereas older dense erythrocytes exhibit progressive dehydration, increased intracellular viscosity, membrane stiffening, and reduced deformability. This coexistence of mechanically distinct erythrocyte subpopulations contributes substantially to the complex rheological behaviour observed in sickle cell disease and explains why vaso-occlusion is often initiated by only a subset of rigid erythrocytes despite the presence of many relatively deformable circulating cells.

The absence of significant differences in deformability observed in the present study may therefore reflect the physiological heterogeneity of the erythrocyte population rather than true preservation of membrane mechanics. Bulk deformability assays determine the average mechanical behaviour of the entire erythrocyte population and consequently may underestimate the contribution of relatively small populations of rigid cells. If the study cohort contained a substantial proportion of reticulocytes, erythrocytes with relatively preserved membrane integrity, or cells containing higher concentrations of fetal haemoglobin (HbF) or residual haemoglobin A (HbA), the average deformability measurement would be expected to approximate that of normal erythrocytes. This interpretation agrees with the observations of Alapan *et al*. (2016), whose

single-cell analyses demonstrated that averaging the mechanical properties of heterogeneous erythrocyte populations can obscure clinically important differences between individual cells.

Methodological factors may also have contributed to the absence of significant differences in the present study. Erythrocyte deformability is highly dependent on the magnitude of mechanical stress, oxygen tension, osmotic conditions, and shear rate applied during measurement. Under physiological conditions, erythrocytes experience varying levels of shear stress as they move through arteries, arterioles, capillaries, and venules, and sickled erythrocytes often exhibit the greatest impairment under conditions of low oxygen tension or high mechanical stress that promote HbS polymerization. Consequently, deformability assays performed under relatively static or normoxic laboratory conditions may fail to reproduce the physiological environment in which sickled erythrocytes become maximally rigid. Caprari *et al*. (2019) similarly observed that reductions in deformability became more pronounced under conditions associated with increased oxidative stress and membrane injury, indicating that experimental conditions substantially influence the detection of mechanical abnormalities.

Intrinsic erythrocyte characteristics may have further contributed to the present findings. Haemoglobin composition is a major physiological determinant of erythrocyte deformability because HbF interferes with HbS polymerization, thereby reducing intracellular fibre formation and preserving membrane flexibility. Likewise, adequate cellular hydration reduces intracellular haemoglobin concentration and viscosity, enabling erythrocytes to deform more readily during passage through the microcirculation. Parrow *et al.* (2017) demonstrated that erythrocytes containing higher proportions of HbF or HbA_2 exhibit improved deformability under both osmotic and shear stress conditions because these haemoglobin variants reduce polymer

formation and preserve membrane mechanics. Therefore, if participants in the present study possessed relatively elevated HbF concentrations, whether through genetic modifiers or hydroxyurea therapy, or if their erythrocytes maintained relatively normal hydration status, deformability may have remained largely preserved despite the presence of HbS. Furthermore, a predominance of younger erythrocytes resulting from accelerated erythropoiesis could increase the overall deformability of the erythrocyte population because reticulocytes generally possess greater membrane elasticity than senescent erythrocytes.

Hence, the findings of the present study suggest that erythrocyte deformability in sickle cell disease is determined by the complex interaction between HbS polymerization, membrane cytoskeletal integrity, intracellular viscosity, erythrocyte hydration, oxidative injury, and cellular age. Although these physiological processes generally reduce erythrocyte flexibility in sickle cell disease, the absence of a statistically significant difference in the present study indicates that the combined influence of erythrocyte heterogeneity, haemoglobin composition, hydration status, and methodological factors may have preserved average deformability measurements. These observations reinforce the concept that erythrocyte deformability represents a dynamic physiological property governed by multiple interacting determinants rather than by haemoglobin genotype alone, highlighting the complexity of the haemorheological adaptations that occur in sickle cell disease.

4.2.5 Relationship between Haematological Parameters among Normal Controls, Sickle Cell Patients in the Steady State, and Sickle Cell Patients in Crisis

The present study evaluated the relationships among osmotic fragility, erythrocyte sedimentation rate (ESR), blood viscosity, and fibrinogen concentration in normal individuals (HbAA), sickle cell patients in the steady state, and sickle cell patients during vaso-occlusive crisis. The findings

demonstrate that the interactions among these haematological parameters differ according to disease status, with significant correlations becoming evident predominantly during vaso-occlusive crisis. These observations support the concept that acute sickle cell crisis is characterized by intensified interactions between erythrocyte membrane injury, inflammatory activation, and altered blood rheology.

Among the normal control group, osmotic fragility showed weak negative correlations with blood viscosity ($r = -0.262$, $p = 0.195$) and fibrinogen concentration ($r = -0.238$, $p = 0.242$), and a weak positive correlation with ESR ($r = 0.083$, $p = 0.686$), although none of these relationships were statistically significant. However, a moderate positive correlation was observed between ESR and blood viscosity ($r = 0.394$, $p = 0.046$). Physiologically, this finding is expected because healthy erythrocytes possess intact membranes, normal deformability, and maintain their biconcave shape, allowing efficient passage through the microcirculation with minimal resistance. Under normal conditions, blood viscosity is determined mainly by haematocrit, plasma proteins, and erythrocyte aggregation, while ESR reflects the tendency of erythrocytes to form rouleaux under the influence of plasma proteins, particularly fibrinogen. Consequently, individuals with relatively higher ESR values may also exhibit modestly higher blood viscosity because both variables are influenced by similar rheological factors. Connes *et al*. (2016) explained that erythrocyte aggregation and plasma protein composition are among the principal physiological determinants of blood viscosity, while Lawrence and Fabry (1986) reported that ESR under normal physiological conditions is largely influenced by plasma proteins, especially fibrinogen, although this influence becomes more pronounced during inflammatory states. The absence of significant correlations involving fibrinogen in the present study likely reflects the narrow physiological variation in fibrinogen concentrations among healthy individuals, where

homeostatic regulation minimizes fluctuations in plasma proteins and erythrocyte membrane stability.

Among sickle cell patients in the steady state, all observed relationships were weak and statistically non-significant ($p > 0.05$). Osmotic fragility demonstrated weak negative correlations with ESR ($r = -0.191$), blood viscosity ($r = -0.090$), and fibrinogen concentration ($r = -0.120$). Likewise, ESR showed a weak positive correlation with blood viscosity ($r = 0.225$) but weak negative correlations with fibrinogen ($r = -0.271$) and osmotic fragility ($r = -0.191$). These findings indicate that, despite the persistence of chronic haemolysis, oxidative stress, and membrane abnormalities, the haematological disturbances present during the steady state remain relatively compensated, preventing strong linear relationships among the measured variables. During the steady state, patients are free from acute vaso-occlusive episodes, although low-grade inflammation, endothelial activation, and chronic erythrocyte membrane damage persist. Consequently, alterations in osmotic fragility, ESR, blood viscosity, and fibrinogen do not necessarily occur simultaneously or proportionately. This finding differs somewhat from the observations of Richardson *et al*. (1976), who reported that plasma fibrinogen and whole blood viscosity increased significantly during vaso-occlusive crisis, suggesting that stronger rheological interactions occur primarily during acute inflammatory episodes rather than during clinical stability. Similarly, Phillips *et al*. (1991) demonstrated that blood viscosity correlated strongly with disease severity and chronic organ damage in sickle cell anaemia, reporting correlation coefficients ranging from $r = 0.73$ to $r = 0.83$. The weaker relationships observed in the present study therefore suggest that the physiological disturbances characterizing the steady state may not be sufficiently severe to produce measurable associations among these haematological variables.

The most important findings of the present study were observed in the sickle cell crisis group, where two statistically significant relationships were identified. Osmotic fragility showed a moderate negative correlation with blood viscosity ($r = -0.448$, $p = 0.022$) and a moderate positive correlation with fibrinogen concentration ($r = 0.510$, $p = 0.008$), whereas ESR showed no significant relationship with osmotic fragility ($r = -0.030$, $p = 0.885$), blood viscosity ($r = -0.081$, $p = 0.696$), or fibrinogen concentration ($r = 0.083$, $p = 0.688$). These findings indicate that acute vaso-occlusive crisis strengthens the interactions between erythrocyte membrane integrity, inflammation, and blood rheology.

The moderate negative correlation between osmotic fragility and blood viscosity ($r = -0.448$) suggests that patients with greater erythrocyte membrane fragility tended to have lower measured blood viscosity during crisis. Although this finding appears contrary to the expectation that rigid sickled erythrocytes increase blood viscosity, it can be explained by the complex pathophysiological processes occurring during vaso-occlusive crisis. Increased osmotic fragility reflects greater membrane instability arising from repeated sickling and unsickling cycles, oxidative membrane damage, lipid peroxidation, calcium influx, and cytoskeletal disruption. Severely damaged erythrocytes become increasingly susceptible to intravascular haemolysis and are rapidly removed from circulation by the reticuloendothelial system. Consequently, although surviving sickled erythrocytes remain rigid and poorly deformable, accelerated destruction of fragile erythrocytes reduces the effective circulating red cell mass, partially offsetting the viscosity-enhancing effects of rigid erythrocytes. Furthermore, intravenous hydration, which is routinely administered during vaso-occlusive crisis, may reduce whole-blood viscosity through haemodilution despite persistent membrane injury. Connes *et al*. (2016) emphasized that blood viscosity in sickle cell disease is influenced not only by erythrocyte rigidity but also by

haematocrit, plasma viscosity, erythrocyte aggregation, and the proportion of dense circulating cells. Similarly, Akinola (1992) demonstrated that repeated sickling markedly impairs erythrocyte deformability and alters blood rheology throughout vaso-occlusive crisis.

The positive correlation observed between osmotic fragility and fibrinogen concentration ($r = 0.510$, $p = 0.008$) suggests that increasing inflammatory activity is associated with worsening erythrocyte membrane injury during vaso-occlusive crisis. Fibrinogen is a major acute-phase reactant synthesized by the liver in response to inflammatory cytokines, particularly interleukin-6, released during tissue ischaemia and endothelial injury. Elevated fibrinogen increases plasma viscosity, promotes erythrocyte aggregation, enhances adhesion of sickled erythrocytes to the vascular endothelium, and contributes to microvascular obstruction. Simultaneously, inflammatory mediators and oxidative stress damage erythrocyte membrane lipids and cytoskeletal proteins, increasing susceptibility to osmotic lysis. Therefore, the positive association observed in this study indicates that patients with greater inflammatory responses also experience more severe erythrocyte membrane instability. This finding agrees with Richardson *et al*. (1976), who reported significantly elevated plasma fibrinogen concentrations and increased whole-blood viscosity during sickle cell crisis, concluding that hyperfibrinogenaemia contributes directly to the rheological abnormalities associated with vaso-occlusion. Likewise, Awodu *et al*. (2009) demonstrated highly significant increases in plasma fibrinogen ($p < 0.0001$) and whole-blood viscosity ($p < 0.01$) during vaso-occlusive crisis, indicating that inflammatory activation substantially contributes to abnormal blood rheology. Although these studies primarily examined the relationship between fibrinogen and blood viscosity, the present findings extend their observations by demonstrating that increased fibrinogen is also associated with increased erythrocyte membrane fragility during crisis.

However, ESR showed no significant relationships with osmotic fragility ($r = -0.030$), blood viscosity ($r = -0.081$), or fibrinogen concentration ($r = 0.083$) during vaso-occlusive crisis. Physiologically, this finding reflects the unique behaviour of sickled erythrocytes. ESR depends largely on the formation of rouleaux, which requires normal biconcave erythrocytes capable of orderly aggregation. However, sickled erythrocytes are elongated, rigid, dehydrated, and structurally abnormal, making rouleaux formation difficult even in the presence of elevated fibrinogen concentrations. Consequently, ESR becomes an insensitive marker of inflammatory activity in sickle cell disease because erythrocyte morphology overrides the normal influence of plasma proteins on sedimentation. This finding agrees with Awodu *et al*. (2009), who reported that ESR showed no significant day-to-day variation during vaso-occlusive crisis despite marked increases in plasma fibrinogen and blood viscosity. Similarly, Lawrence and Fabry (1986) observed that although ESR increased during painful crisis compared with the steady state, the sedimentation process remained strongly influenced by abnormal erythrocyte morphology rather than plasma fibrinogen concentration alone.

Hence, the present findings demonstrate that the physiological relationships among osmotic fragility, blood viscosity, fibrinogen concentration, and ESR vary according to disease status. In healthy individuals, these parameters remain largely independent because of intact erythrocyte membrane integrity and tightly regulated plasma protein concentrations, although ESR and blood viscosity exhibit a modest physiological association ($r = 0.394$, $p = 0.046$). During the steady state of sickle cell disease, chronic haemolysis and low-grade inflammation are relatively compensated, resulting in weak and non-significant correlations among the measured variables. In contrast, vaso-occlusive crisis is characterized by intensified inflammation, oxidative stress, endothelial dysfunction, membrane injury, and altered blood rheology, producing significant

relationships between osmotic fragility, blood viscosity, and fibrinogen concentration. These findings reinforce the central role of erythrocyte membrane damage, inflammatory activation, and haemorheological disturbances in the pathogenesis of vaso-occlusive crisis and suggest that these parameters may serve as complementary indicators of disease activity during acute sickle cell episodes.

Clinical Implications

The findings of the present study have important clinical implications for the management and monitoring of patients with sickle cell disease (SCD). First, the observed alterations in osmotic fragility, particularly the significantly increased fragility of erythrocytes during vaso-occlusive crisis, underscore the vulnerability of red blood cells to haemolysis under stress. Clinically, this highlights the need for interventions aimed at stabilizing erythrocyte membranes and mitigating oxidative stress during acute crises. Therapeutic strategies that enhance red cell membrane resilience, including antioxidant therapy, hydration management, and pharmacological agents such as hydroxyurea, may reduce haemolytic episodes and consequent complications such as anaemia, jaundice, and end-organ ischemia (Adegoke *et al*., 2014; Brugnara, 2018).

Second, the significant elevation of fibrinogen in both steady-state and crisis conditions has direct relevance for thrombotic risk and microvascular obstruction in SCD. Elevated fibrinogen levels increase blood viscosity and promote red cell aggregation, particularly under low shear conditions in the microcirculation, thereby exacerbating vaso-occlusion. Clinically, this supports the use of fibrinogen or other coagulation markers as part of routine risk assessment for thrombosis in SCD patients. It also underscores the potential benefit of therapies targeting hypercoagulability, such as antiplatelet agents, anticoagulants, or fibrinogen-lowering strategies, particularly during acute crises (Kucukal *et al*., 2020; Nasimuzzaman *et al*., 2019).

Third, the significantly elevated erythrocyte sedimentation rate (ESR) in both steady-state and crisis SCD subjects reinforces its utility as a non-specific but informative marker of inflammation and disease activity. Monitoring ESR in clinical practice can aid in the early detection of inflammatory exacerbations, track the severity of vaso-occlusive episodes, and guide timely therapeutic interventions, including anti-inflammatory or cytoprotective therapies (Ahmed *et al*., 2000; Aytekin, 2018). Persistently elevated ESR even in clinically stable patients highlights the chronic inflammatory state inherent in SCD and may indicate the need for preventive interventions.

Finally, although red cell deformability was not significantly different between sickle cell and normal subjects under the assay conditions used in this study, clinicians should interpret this finding cautiously. Functional impairments in RBC deformability may manifest under physiological flow conditions, hypoxia, or oxidative stress, which were not fully simulated in the assay. Thus, normal deformability in vitro does not preclude microvascular complications in vivo. Clinicians should consider comprehensive assessments of RBC function, including markers of rigidity, aggregation, and adhesion, to better predict and manage vaso-occlusive risk (Alapan *et al*., 2016; Caprari *et al*., 2019).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study investigated key haemorheological parameters such as osmotic fragility, fibrinogen concentration, erythrocyte sedimentation rate (ESR), and red blood cell (RBC) deformability in individuals with sickle cell disease (SCD) during vaso-occlusive crisis and steady-state conditions, and compared them with non-sickle cell (HbAA) controls. The findings provide important insights into the pathophysiological alterations associated with SCD and the mechanisms underlying disease severity.

The results demonstrated a significant increase in osmotic fragility among sickle cell subjects, particularly during crisis. The marked leftward shift of the fragility curve indicates that erythrocytes in crisis are more susceptible to lysis in hypotonic environments, reflecting greater membrane instability and dehydration. This underscores the enhanced vulnerability of HbSS cells to haemolysis during acute vaso-occlusive episodes.

Fibrinogen concentration was significantly elevated in both steady-state and crisis SCD subjects, with the highest levels observed during crisis. This pattern confirms the chronic inflammatory

and hypercoagulable state characteristic of SCD and highlights fibrinogen's important contribution to increased blood viscosity, red cell aggregation, and microvascular obstruction.

Similarly, ESR was significantly raised among sickle cell subjects compared with normal controls, reflecting ongoing inflammatory activity even during periods of clinical stability. Elevated ESR during crisis further supports the role of inflammation in triggering or exacerbating vaso-occlusive events.

In contrast, RBC deformability did not differ significantly between sickle cell and normal subjects under the conditions of our assay. While this may reflect methodological limitations or compensatory mechanisms (such as younger erythrocyte populations or higher HbF levels), the finding does not negate the well-documented impairment of deformability under physiological stress. Rather, it suggests that deformability alterations in SCD may require dynamic or microfluidic testing to be accurately captured.

Hence, the study confirms that haemorheological abnormalities particularly osmotic fragility, fibrinogen elevation, and increased ESR remain central to the pathophysiology of SCD and can serve as important markers of disease activity and crisis severity.

5.2 Recommendations

Based on the findings of this study, the following recommendations are proposed for clinical practice, patient management, and future research:

1. **Routine monitoring of haemorheological biomarkers:** Healthcare providers should incorporate osmotic fragility, fibrinogen levels, and ESR into routine evaluation of SCD

patients as these parameters can help identify early signs of impending crisis, monitor treatment response, and assess inflammatory and haemolytic burden.

2. **Targeted therapies to stabilize erythrocytes**: Interventions that improve membrane stability such as adequate hydration, antioxidant supplementation and hydroxyurea should be encouraged, particularly for patients with frequent crises or high osmotic fragility.
3. **Holistic crisis prevention strategies**: Because crisis episodes significantly exacerbate haemorheological abnormalities, emphasis should be placed on preventive care such as regular clinic visits, vaccination, prompt treatment of infections, hydration, pain management plans, and avoidance of known crisis triggers.
4. **Advanced assessment of RBC deformability**: Future clinical evaluations should incorporate more sensitive or dynamic methods of assessing RBC deformability, such as microfluidic flow assays or oxygen-gradient ektacytometry, to better detect functional deficits associated with vaso-occlusion.
5. **Strengthening patient education**: Patients and caregivers should be educated on the importance of hydration, nutrition, medication adherence, and early recognition of crisis symptoms, as these factors influence haemorheology and overall disease outcomes.
6. **Further research**: Larger multicenter studies using advanced biophysical tools are recommended to clarify the relationship between RBC deformability, crisis severity, and treatment status.

REFERENCES

Adegoke, O. A., Akinsulie, A. O., Temiye, E. O. (2014). Garlic extract and osmotic fragility in sickle cell anaemia. *Nigerian Journal of Clinical Practice*. **17**(1): 1–5.

Ahmed, S. G., Ibrahim, U. A., Hassan, A. W. (2000). Hematological parameters in sickle cell anaemia patients during steady state and vaso-occlusive crisis. *Nigerian Journal of Medicine*. **9**(1): 6–9.

Ajayi, O. I., Famodu, A. A., Oviasu, E. (2007). Fibrinogen concentration: A marker of cardiovascular disorders in Nigerians. *Turk. J. Hematol*. **24**: 18-22.

Akinola, N. O., Stevens, S. M., Franklin, I. M., Nash, G. B. and Stuart, J. (1992). Rheological changes in the prodromal and established phases of sickle cell vaso-occlusive crisis. *British journal of haematology*, **81**(4), 598–602.

Alakbarzade, V., Maduakor, C., Khan, U., Khandanpour, N., Rhodes, E., Pereira, A.C. (2023). Cerebrovascular disease in sickle cell disease. *Practical Neurology*. **23**(2): 131-138.

Alapan, Y., Matsuyama, Y., Little, J. A., Gurkan, U. A. (2016). Dynamic deformability of sickle red blood cells in microphysiological flow. *Technology*. **4**(02): 71-79.

Alexy, T., Detterich, J., Connes, P., Toth, K., Nader, E., Kenyeres, P., Simmonds, M. J. (2022). Physical properties of blood and their relationship to clinical conditions. *Frontiers in Physiology*. **13**: 906768.

Alharthi, A. H., Al-Shehri, S. H. A., Albarqi, M. A. A., Alshehri, M. S., Alshehri, A. M., Amer, A. M., Alassiry, A. M. A. (2024). Laboratory Markers of Inflammation: CRP and ESR in Clinical Practice. *Journal of International Crisis and Risk Communication Research*. **7**(S8): 2376.

Arida, A., Protogerou, A.D., Kitas, G. D., Sfikakis, P.P. (2018). Systemic inflammatory response and atherosclerosis: the paradigm of chronic inflammatory rheumatic diseases. *International journal of molecular sciences*. **19**(7): 1890.

Awodu, O. A., Famodu, A. A., Ajayi, O. I., Enosolease, M. E., Olufemi, O. Y., & Olayemi, E. (2009). Using serial haemorheological parameters to assess clinical status in sickle cell anaemia patients in vaso-occlussive crisis. *Clinical hemorheology and microcirculation*, **41**(2), 143–148.

Aytekin, M. (2018). Standardization of erythrocyte sedimentation rate measurements and its diagnostic significance. *Clinical Laboratory*. **64**(5): 693–700.

Ballas, S.K., Gupta, K., Adams-Graves, P. (2012). Sickle cell pain: a critical reappraisal. *Blood, The Journal of the American Society of Hematology*. **120**(18): 3647-3656.

Barodka, V.M., Nagababu, E., Mohanty, J.G., Nyhan, D., Berkowitz, D.E., Rifkind, J.M. Strouse, J.J. (2014). New insights provided by a comparison of impaired deformability with erythrocyte oxidative stress for sickle cell disease. *Blood Cells, Molecules, and Diseases*. **52**(4): .230-235.

Barshtein, G., Pajic-Lijakovic, I., Gural, A. (2021). Deformability of stored red blood cells. *Frontiers in Physiology*. **12**: 722896.

Beri, D., Rodriguez, M., Singh, M., McLaughlin, D., Liu, Y., Zhong, H., Mendelson, A., An, X., Manwani, D., Yazdanbakhsh, K., Lobo, C.A. (2025). Babesiosis and sickle red blood cells: loss of deformability, altered osmotic fragility, and hypervesiculation. *Blood*. **145**(19): 2202-2213.

Bhadra, P., Deb, A. (2020). A review on nutritional anaemia. *Indian Journal of Natural Sciences*. **10**(59): 18466-18474.

Bhatt, S., Argueta, D.A., Gupta, K., Kundu, S. (2024). Red blood cells as therapeutic target to treat sickle cell disease. *Antioxidants & Redox Signaling*. **40**(16-18): 1025-1049.

Bray, C., Bell, L.N., Liang, H., Haykal, R., Kaiksow, F., Mazza, J.J., Yale, S.H. (2016). Erythrocyte sedimentation rate and C-reactive protein measurements and their relevance in clinical medicine. *Wmj*. **115**(6): 317-21.

Brugnara, C. (2018). Sickle cell dehydration: Pathophysiology & therapeutic applications. *Clinical hemorheology and microcirculation*. **68**(2-3): 187-204.

Brzustewicz, E., Henc, I., Daca, A., Szarecka, M., Sochocka-Bykowska, M., Witkowski, J., Bryl, E. (2017). Autoantibodies, C-reactive protein, erythrocyte sedimentation rate and serum

cytokine profiling in monitoring of early treatment. *Central European Journal of Immunology*. **42**(3): 259-268.

Caprari, P., Ciampittiello, G., Arcieri, S., Rocchi, M. (2019). Hemorheology in sickle cell disease: The role of red cell deformability. *Clinical Hemorheology and Microcirculation*. **72**(1): 1–10.

Ciepiela, O., Adamowicz-Salach, A., Zgodzińska, A., Łazowska, M., Kotuła, I. (2018). Flow cytometric osmotic fragility test: Increased assay sensitivity for clinical application in pediatric hematology. *Cytometry Part B: Clinical Cytometry*. **94**(1): 189-195.

Clavería, V., Wagner, C., Connes, P., Villat, A., Abkarian, M. (2019). Aggregating and blood flow in health and disease. In *Dynamics of Blood Cell Suspensions in Microflows.* CRC Press. pp. 183-213.

Connes, P. (2024). Blood rheology and vascular function in sickle cell trait and sickle cell disease: From pathophysiological mechanisms to clinical usefulness. *Clinical Hemorheology and Microcirculation*. **86**(1-2): 9-27.

Connes, P., Alexy, T., Detterich, J., Romana, M., Hardy-Dessources, M.D., Ballas, S.K. (2016). The role of blood rheology in sickle cell disease. *Blood reviews*. **30**(2): 111-118.

Conran, N., Belcher, J.D., 2018. Inflammation in sickle cell disease. *Clinical hemorheology and microcirculation*. **68**(2-3): 263-299.

Daniels, L. M., Tosh, P. K., Fiala, J. A., Schleck, C. D., Mandrekar, J. N., Beckman, T. J. (2017, November). Extremely elevated erythrocyte sedimentation rates: associations with Patients' diagnoses, demographic characteristics, and comorbidities. In *Mayo Clinic Proceedings.* **92**(11): 1636-1643.

Darbari, D.S., Sheehan, V.A., Ballas, S.K. (2020). The vaso-occlusive pain crisis in sickle cell disease: definition, pathophysiology, and management. *European journal of haematology*. **105**(3): 237-246.

Davalos, D., Akassoglou, K. (2012,). Fibrinogen as a key regulator of inflammation in disease. In *Seminars in immunopathology*. **34**(1): 43-62.

Dessap, A.M., Deux, J.F., Abidi, N., Lavenu-Bombled, C., Melica, G., Renaud, B., Godeau, B., Adnot, S., Brochard, L., Brun-Buisson, C., Galacteros, F. (2011). Pulmonary artery thrombosis during acute chest syndrome in sickle cell disease. *American journal of respiratory and critical care medicine*. **184**(9): 1022-1029.

Dima, A., Opris, D., Jurcut, C., Baicus, C. (2016). Is there still a place for erythrocyte sedimentation rate and C-reactive protein in systemic lupus erythematosus? *Lupus*. **25**(11): 1173-1179.

Duvoix, A., Dickens, J., Haq, I., Mannino, D., Miller, B., Tal-Singer, R., & Lomas, D. A. (2013). Blood fibrinogen as a biomarker of chronic obstructive pulmonary disease. *Thorax*. **68**(7): 670-676.

Esperti, S. (2023). *New mechanisms involved in the modulation of sickle red blood cells rheology and senescence* (Doctoral dissertation, Université Claude Bernard-Lyon I).

Faes, C., Sparkenbaugh, E.M., Pawlinski, R. (2018). Hypercoagulable state in sickle cell disease. *Clinical hemorheology and microcirculation*. **68**(2-3): 301-318.

Ferrone, F.A. (2018), April. Targeting HbS polymerization. In *Seminars in hematology*. **55**(2): 53-59. WB Saunders.

Georgescu, V.P., de Souza Junior, T.P., Behrens, C., Barros, M.P., Bueno, C.A., Utter, A.C., McAnulty, L.S., McAnulty, S.R. (2017). Effect of exercise-induced dehydration on circulatory markers of oxidative damage and antioxidant capacity. *Applied Physiology, Nutrition, and Metabolism*. **42**(7): 694-699.

Hirtz, D., Kirkham, F. J. (2019). Sickle cell disease and stroke. *Pediatric Neurology*. **95**: 34-41.

Hsieh, J. Y., Smith, T. D., Meli, V. S., Tran, T. N., Botvinick, E. L., Liu, W. F. (2017). Differential regulation of macrophage inflammatory activation by fibrin and fibrinogen. *Acta biomaterialia*. **47**: 14-24.

Jang, T., Poplawska, M., Cimpeanu, E., Mo, G., Dutta, D. & Lim, S.H. (2021). Vaso-occlusive crisis in sickle cell disease: a vicious cycle of secondary events. *Journal of Translational Medicine*. **19**(1): 397.

Jeswani, G., Alexander, A., Saraf, S., Saraf, S., Qureshi, A. (2015). Recent approaches for reducing hemolytic activity of chemotherapeutic agents. *Journal of Controlled Release*. **211**: 10-21.

Kahar, M. A. (2022). Erythrocyte sedimentation rate (with its inherent limitations) remains a useful investigation in contemporary clinical practice. *Ann. Pathol. Lab. Med*. **9**: R9-R17.

Kato, G. J., Piel, F. B., Reid, C. D., Gaston, M. H., Ohene-Frempong, K., Krishnamurti, L., Vichinsky, E. P. (2018). Sickle cell disease. *Nature reviews Disease primers*. **4**(1): 1-22

Kearney, K.J., Ariëns, R.A., Macrae, F.L. (2022). The role of fibrin (ogen) in wound healing and infection control. In *Seminars in Thrombosis and Hemostasis*. **48**(02): 174-187.

Kim, J., Lee, H., Shin, S. (2015). Advances in the measurement of red blood cell deformability: A brief review. *Journal of Cellular Biotechnolog.* **1**(1): 63-79.

Kim, Y., Kim, K., Park, Y. (2012). Measurement techniques for red blood cell deformability: recent advances. *Blood Cell—An Overview of Studies in Hematology*. **10**: 167-194.

Koca, T.T. (2017). Does obesity cause chronic inflammation? The association between complete blood parameters with body mass index and fasting glucose. *Pakistan journal of medical sciences*. **33**(1): 65.

Kucukal, E., Man, Y., Hill, A., Gurkan, U. A. (2020). Fibrinogen and its role in sickle cell vaso-occlusion. *Blood Advances*. **4**(5): 994–1003.

Lam, S.H., So, H., Cheng, I.T., Li, E.K., Wong, P., Li, T.K., Lee, A.P.W., Tam, L.S. (2021). Association of C-reactive protein and non-steroidal anti-inflammatory drugs with cardiovascular events in patients with psoriatic arthritis: a time-dependent Cox regression analysis. *Therapeutic Advances in Musculoskeletal Disease*. **13**: 1759720X211027712.

Lawrence, C., and Fabry, M. E. (1986). Erythrocyte sedimentation rate during steady state and painful crisis in sickle cell anemia. *The American journal of medicine*, **81**(5), 801–808.

Li, N., Jia, Y., Feng, J., Chang, H., Li, S. (2024). Changes in the levels of WBC count, PCT, CRP and ESR in Patients with acute Community-acquired Lower Respiratory tract infections and their diagnostic value. *Pakistan journal of medical sciences*. **40**(3Part-II): 405.

Litvinov, R.I., Pieters, M., de Lange-Loots, Z., Weisel, J.W. (2020). Fibrinogen and fibrin. *Macromolecular Protein Complexes III: Structure and Function*. 471-501.

Lizarralde-Iragorri, M. A., Shet, A. S. (2020). Sickle cell disease: a paradigm for venous thrombosis pathophysiology. *International journal of molecular sciences*. **21**(15): 5279.

Maciel, T. T., Rignault, R., Allali, S. (2024). Hemolysis and Innate Immunity. *Current Practices in Sickle Cell Disease*, **1**.

Mandal, A.K., Mitra, A. and Das, R. (2020). Sickle cell hemoglobin. *Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and other Body Fluid Proteins*. 297-322.

Marcucci, M., Etxeandia-Ikobaltzeta, I., Yang, S., Germini, F., Gupta, S., Agarwal, A., Ventresca, M., Tang, S., Morgano, G.P., Wang, M., Ahmed, M.M. (2022). Benefits and harms of direct oral anticoagulation and low molecular weight heparin for thromboprophylaxis in patients undergoing non-cardiac surgery: systematic review and network meta-analysis of randomised trials. *Bmj*. 376.

Matthews, K., Lamoureux, E.S., Myrand-Lapierre, M.E., Duffy, S.P., Ma, H. (2022). Technologies for measuring red blood cell deformability. *Lab on a Chip*. **22**(7): 1254-1274.

Mba, C.O., Adias, T.C., Eze, E.M. (2018). Acute phase reactant correlates among pregnant women in Port Harcourt, Nigeria. *European Journal of Biomedical and Pharmaceutical Sciences*. **5**(8): 87-94.

McGann, P.T., Ware, R.E. (2015). Hydroxyurea therapy for sickle cell anaemia. *Expert opinion on drug safety*. **14**(11): 1749-1758.

Mikkelsen, K., Apostolopoulos, V. (2019). Vitamin B12, folic acid, and the immune system. In *Nutrition and immunity*. 103-114.

Mohamed, S.E., Eid, H.A., Moazen, E.M., Abd El-Fattah, D.A. (2023). Impact of chronic cigarette smoking on blood count indices, erythrocyte sedimentation rate and C-reactive protein as inflammatory markers in healthy individuals. *Journal of Recent Advances in Medicine*. **4**(1): 74-85.

Nader, E., Conran, N., Romana, M., Connes, P. (2021). Vasculopathy in sickle cell disease: from red blood cell sickling to vascular dysfunction. *Comprehensive Physiology*. **11**(2): 1785-1803.

Nader, E., Romana, M., Connes, P. (2020). The red blood cell—inflammation vicious circle in sickle cell disease. *Frontiers in immunology*. **11**: 454.

Narla, J., Mohandas, N. (2017). Red cell membrane disorders. *International journal of laboratory hematology*. **39**: 47-52.

Nasimuzzaman, M. D., Malik, P. (2019). Role of the coagulation system in the pathogenesis of sickle cell disease. *Blood Advances*. **3**(20): 3170-3180.

Nikitin, S.Y., Priezzhev, A.V., Lugovtsov, A.E. (2011). Laser diffraction by the erythrocytes and deformability measurements. *Advanced optical flow cytometry: methods and disease diagnoses*. 133-154.

Obeagu, E.I., Obeagu, G.U., 2024. Implications of climatic change on sickle cell anaemia: A review. *Medicine*. **103**(6): e37127.

Ochocinski, D., Dalal, M., Black, L.V., Carr, S., Lew, J., Sullivan, K., Kissoon, N. (2020). Life-threatening infectious complications in sickle cell disease: a concise narrative review. *Frontiers in Pediatrics*. **8**: 38.

Ojo, A.S., Ojukwu, S., Asmare, W., Odipe, O., Larbi, D. (2022). Intravenous fluid administration and the risk of adverse outcomes in sickle cell disease patients hospitalized for vaso-occlusive crisis. *Journal of Hematology*. **11**(5): 159.

Okoye, H.C., Nonyelu, C.E., Akpa, C.O., Nwogoh, B., Muoghalu, E.A., Duru, A.N., Omunakwe, H., Korubo, K., Nnachi, O.C., Okorie, P.O. (2023). Correlation of Haematological Markers of Systemic Inflammation with Sickle Cell Disease Severity. *East African Medical Journal.* **100**(4).

Orbach, A., Zelig, O., Yedgar, S., Barshtein, G. (2017). Biophysical and biochemical markers of red blood cell fragility. *Transfusion Medicine and Hemotherapy*. **44**(3): 183-187.

Oyewale, J., Dzenda, T., Yaqub, L., Akanbi, D., Ayo, J., Owoyele, O., Minka, N., Dare, T. (2011). Alterations in the osmotic fragility of camel and donkey erythrocytes caused by temperature, pH and blood storage. *Veterinarski arhiv*. **81**(4): 459-470.

Paradkar, S., Gambhire, P. (2021). The Role of Cytoskeleton of a Red Blood Cell in Its Deformability. *Journal of the Indian Institute of Science*. **101**(1): 39-46.

Paramo, J.A. (2021). Microvascular thrombosis and clinical implications. *Medicina Clínica (English Edition)*. **156**(12): 609-614.

Parrow, N. L., Tu, H., Nichols, J., Violet, P. C., Pittman, C. A., Fitzhugh, C., Fleming, R.E., Mohandas, N., Tisdale, J.F., Levine, M. (2017). Measurements of red cell deformability and hydration reflect HbF and HbA2 in blood from patients with sickle cell anaemia. *Blood Cells, Molecules, and Diseases*. **65**: 41-50.

Perazzo, A., Peng, Z., Young, Y.N., Feng, Z., Wood, D.K., Higgins, J.M., Stone, H.A. (2022). The effect of rigid cells on blood viscosity: linking rheology and sickle cell anaemia. *Soft Matter*. **18**(3): 554-565.

Phillips, G., Jr, Coffey, B., Tran-Son-Tay, R., Kinney, T. R., Orringer, E. P. and Hochmuth, R. M. (1991). Relationship of clinical severity to packed cell rheology in sickle cell anemia. *Blood*, **78**(10), 2735–2739.

Piccin, A., O'Connor-Byrne, N., Daves, M., Lynch, K., Farshbaf, A. D., Martin-Loeches, I. (2022). Autoimmune disease and sickle cell anaemia:'Intersecting pathways and differential diagnosis'. *British Journal of Haematology*. **197**(5): 518-528.

Pryzwan, T., Dolibog, P., Kierszniok, K., Pietrzyk, B. (2024). Blood rheological properties and methods of their measurement. *Annales Academiae Medicae Silesiensis*. **8**: 1-10.

Queiro, R., Alonso, S., Burger, S., Pardo, E., Braña, I., Loredo, M., Alperi, M. (2025). Tailoring Inflammatory Biomarker Assessment in Axial Spondyloarthritis: A Comparative Study of Erythrocyte Sedimentation Rate and C-Reactive Protein Across Disease Profiles. *Journal of Personalized Medicine*. **15**(8): 329.

Rampling, M. W. (2019). Red cell aggregation and yield stress. In *Clinical blood rheology*. pp. 45-64.

Ramsay, E.S., Lerman, M.A. (2015). How to use the erythrocyte sedimentation rate in paediatrics. *Archives of Disease in Childhood-Education and Practice*. **100**(1): 30-36.

Rees, D. C., Williams, T. N., Gladwin, M. T. (2010). Sickle-cell disease. *The Lancet*. **376**(9757): 2018-2031.

Richardson, S. G., Breeze, G. R. and Stuart, J. (1976). Hyperfibrinogenaemia and hyperviscosity in sickle-cell crisis. *Journal of Clinical Pathology*, **29**(10), 890–893.

Sahu, T., Pande, B., Verma, H.K., Bhaskar, L.V.K.S., Sinha, M., Sinha, R., Rao, P.V. (2023). Infection and potential challenge of childhood mortality in sickle cell disease: a comprehensive review of the literature from a global perspective. *Thalassemia Reports*. **13**(3): 206-229.

Samuel, P.O., Edo, G.I., Emakpor, O.L., Oloni, G.O., Ezekiel, G.O., Essaghah, A.E.A., Agoh, E., Agbo, J.J. (2024). Lifestyle modifications for preventing and managing cardiovascular diseases. *Sport Sciences for Health*. **20**(1): 23-36.

Siemons, L., Ten Klooster, P.M., Vonkeman, H.E., van Riel, P.L., Glas, C.A., van de Laar, M.A. (2014). How age and sex affect the erythrocyte sedimentation rate and C-reactive protein in early rheumatoid arthritis. *BMC musculoskeletal disorders*. **15**(1): 368.

Simeon, C. A., Beega, G. F., Abiola, S. A., Wofuru, C. D., Eze, C. C. (2024). Significance of inflammatory biomarkers in clinical diagnostics: Erythrocyte sedimentation rate versus other inflammatory biomarkers—A review. *Int. J. Sci. Res. Arch*. **12**: 1980-1995.

Sysol, J.R., Machado, R. (2016). Sickle cell disease and acute chest syndrome: epidemiology, diagnosis, management, outcomes. *Hematologic abnormalities and acute lung syndromes*. 67-87.

Tefferi, A. (2003), October. Anaemia in adults: a contemporary approach to diagnosis. In *Mayo Clinic Proceedings.* **78**(10): 1274-1280.

Tewari, S., Brousse, V., Piel, F.B., Menzel, S., Rees, D.C. (2015). Environmental determinants of severity in sickle cell disease. *Haematologica*. **100**(9): 1108.

Tzounakas, V. L., Anastasiadi, A. T., Valsami, S. I., Stamoulis, K. E., Papageorgiou, E. G., Politou, M., Antonelou, M. H. (2021). Osmotic hemolysis is a donor-specific feature of red blood cells under various storage conditions and genetic backgrounds. *Transfusion*. **61**(9): 2538-2544.

Urrechaga, E., Hoffmann, J.J.M.L., Izquierdo, S., Escanero, J.F. (2015). Differential diagnosis of microcytic anaemia: the role of microcytic and hypochromic erythrocytes. *International journal of laboratory hematology*. **37**(3): 334-340.

Van Beers, E.J., Yang, Y., Raghavachari, N., Tian, X., Allen, D.T., Nichols, J.S., Mendelsohn, L., Nekhai, S., Gordeuk, V.R., Taylor, J.G., Kato, G.J. (2015). Iron, inflammation, and early death in adults with sickle cell disease. *Circulation research*. **116**(2): 298-306.

Walski, T., Chludzińska, L., Komorowska, M., Witkiewicz, W. (2014). Individual osmotic fragility distribution: a new parameter for determination of the osmotic properties of human red blood cells. *BioMed Research International*. **2014**(1): 162102.

Wang, Q., Zennadi, R. (2021). The role of RBC oxidative stress in sickle cell disease: from the molecular basis to pathologic implications. *Antioxidants*. **10**(10): 1608.

Williams, D.C., Wood, D.K. (2023). High-throughput quantification of red blood cell deformability and oxygen saturation to probe mechanisms of sickle cell disease. *Proceedings of the National Academy of Sciences*. **120**(48): e2313755120.

APPENDIX

LETTER OF ETHICAL APPROVAL

EDO STATE MINISTRY OF HEALTH
HEALTH RESEARCH ETHICS COMMITTEE

PROTOCOL NUMBER	**HA/737/26/C/04011113 (PLEASE QUOTE IN ALL ENQUIRIES)**
APPROVAL NUMBER	**HA/737/26/C/04101113**
TITLE OF RESEARCH PROPOSAL	**STUDY ON MODIFICATIONS IN HAEMORHEOLOGICAL PARAMETERS AS WELL AS THEIR RELATIONSHIP WITH HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN SICKLE CELL ANAEMIA**
PRINCIPAL INVESTIGATOR (S)	**DR. ROSEMARY OSAMEDE AIKPITANYI-IDUITUA, OBARISIAGBON SHADRACK**
DATE CONSIDERED	**10TH APRIL, 2026.**
DECISION OF THE COMMITTEE	**APPROVED**

THIS APPROVAL DATES 10/04/2026 TO 10/04/2027. IF THERE IS A DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC EDO SMoH SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK: Please kindly note that the HREC Edo SMoH seal authenticates this approval

DR (MRS) Omonyemen B. BELLO (MBBS, MPH, FPHCM) *(CHAIRMAN)* **SIGNATURE & DATE**.....

SUPERVISOR(S) .....

.....

ATTESTATION BY INVESTIGATOR(S)

No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the Edo SMoH HREC except in circumstances outlined in the Code. The Edo SMoH HREC reserves the right to conduct compliance visits to your research site without previous notification.

Signature & Date.....

EDO STATE MINISTRY OF HEALTH SECRETARIAT RESEARCH & ETHICS COMMITTEE