Cape Peninsula University of Technology

COMPARATIVE STUDY ON BIOLOGICAL FACTORS INFLUENCING THE DEVELOPMENT OF ALLOIMMUNIZATION IN SICKLE CELL DISEASE PATIENTS IN ILE-IFE, NIGERIA.

by

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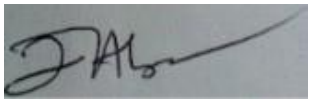
DECLARATION

I, Florence Ifechukwude Aboderin declare that the contents of this thesis represent my work solely, and that the thesis has not previously been submitted for any academic examination towards any qualification. Furthermore, it represents my ideas and not necessarily those of the Cape Peninsula University of Technology.



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ABSTRACT

Sickle cell disease (SCD) is a chronic haemolytic anaemia that remains a healthcare challenge in Africa. Sickle haemoglobinopathies occur due to a mutation resulting in the formation of an abnormal haemoglobin (Hb-S). In a reduced oxygen state (deoxygenation), HbS polymerises, and the red cells become rigid and deformed, assuming a sickle crescent shape. Blood transfusion remains the most common form of therapy despite the risks. Patients with SCD have high levels of alloimmunization, which may result in delayed haemolytic transfusion reactions. Research has demonstrated an association between alloimmunization and SCD patients who have received multiple blood transfusions. This study, therefore, aimed to investigate the biological factors, including inflammatory, oxidative stress markers and the red cell phenotype of SCD, in order to understand the mechanisms involved in the high rate of alloimmunization. This study also assessed the dietary intake and nutrient adequacy among young adults with SCD in Ile-Ife. To further establish that adequate nutrition improves red blood cells and overall health outcomes.

We determined the dietary intake level, nutritional status, and demographic characteristics among 50 young adults between 18 - 48 years and 50 age-matched non-SCD as controls. Dietary data were obtained by 24-hour dietary recall. A food frequency questionnaire was supplied to all participants, and body mass index (BMI) results show that only about 23.7% of the participants met the required daily calorie intake. In comparison, 76.3% of the participants did not meet the normal calorie needed. Carbohydrates and proteins are major micronutrients that measured the normal requirement by 93.8% and 54.6%, respectively. Minerals salts and vitamins showed inadequate intake, especially retinol, beta carotene, folate, and vitamin D. Fibre intake was insufficient, about 80.4%. The relationship between oxidative stress

and inflammatory profiles was investigated in sickle cell patients who took blood transfusions of two or more units. Results showed biochemical parameters such as SOD, AST, ALT, Creatinine and Urea, with inflammatory markers such as CRP and TNF being significantly increased (P = 0.003) in the test group compared to the control group. Additionally, the results demonstrated a significant positive correlation (P< 0.05 r=) between CRP and IL-6, TNF and Catalase. However, there was a negative correlation between AST, IL-β, SOD, Creatinine and Urea (P < 0.05 r=). Haematological parameters, inflammatory markers and the incidence of alloimmunization in both test and controls were determined. Red cell antibody typing was determined by saline and anti-human globin (AHG) methods and was interpreted using the ID panel profile. CRP, TNF, IL-1, IL-6, IL -1β were analysed using enzyme-linked immunosorbent assay (ELISA) technique. Full blood count (FBC) was processed on an auto-analyser. The test group consisted of patients with SCD confirmed with haemoglobin electrophoresis who had been transfused with at least two pints of blood, while those in the control group were not SCD patients but received the same units of blood. Results showed elevated alloimmunization in SCD and significantly increased platelet counts compared to the control group. In addition, the test group displayed evidence of inflammation with significantly increased levels (P = 0.0001) of C-reactive protein and the pro-inflammatory cytokine TNF. This was supported by a higher neutrophil count, which can also indicate elevated inflammation. Furthermore, the pattern of antibodies detected in SCD was anti-Kell, JKa and Fya, which was different from the control, which displayed anti-M and similarity with Kell antibodies. There was, however, no significant correlation between inflammatory markers and alloimmunization.

In conclusion, this study shows that undernutrition and inadequate nutrients, if not corrected, could result in high alloimmunization rates in SCD patients. High levels of oxidative stress and inflammation also contribute to multidimensional factors liable for elevated alloimmunization. In the management of SCD, it is imperative that nutritional monitoring be encouraged alongside medical care to ensure adequate levels of macro and micronutrients needed for maximum supply for body upkeep.

PREFACE

This thesis comprises of six chapters written in article format. The chapters are presented according to the templates of the journals where they were published or submitted.

Chapter one provides a concise introduction and background, outlining the study's interests, including the aims and objectives.

Chapter two: A literature review of sickle cell anaemia and alloimmunization.

This chapter offers a comprehensive review on the relationship between the immune response, inflammation, oxidative stress and the pathogenesis of sickle cell anaemia; to better understand the concept of the whole study. This has been published in Biomedicine 2023. (Aboderin et al., 2023) doi: 10.3390/biomedicines 11(09)2413. PMID:37760854; PMCID: PMC 10525295.

Chapter three deals with a research article titled “Dietary Intake and Nutrients Adequacy among Young Adults’ Sickle Cell Patients”. The manuscript has been published in the African Journal of Biomedical Research. ‘’Aboderin, F. I., Alagbo, P.K., Davison, G.M. and Oguntibeju O.O., 2024. Dietary intake and nutrient adequacy among young adults with sickle cell disease in Ile -Ife, Southwest Nigeria’’. African Journal of Biomedical Research, 27(1): 49-54. Doi: http://doi.org/10.4314/ajbr.v27i1.6.

Chapter four is also a research article titled: Inflammatory and oxidative stress markers in multi-transfused sickle cell disease patients,” which Has been accepted and is to be published in the September 2024 edition of Medicine Science / International Medical Journal. ‘’Manuscript MS -2024-06-054’’.

Chapter five, an original article titled: “Investigation of haematological, inflammatory parameters and the incidence of alloimmunization in multi-transfused sickle cell disease patients”, has been submitted to the Journal of “Haematology, Transfusion and Cell Therapy”. It is still under review.

Finally, chapter six includes a general discussion, conclusion and contribution to the entire research, as well as recommendations, limitations and suggestions for future research.

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- To all my Siblings, both at home and abroad, I thank you. I'm grateful.

To my dearest husband, Professor A.O Aboderin, thank you for your love and support.

DEDICATION

I dedicate this thesis to the Almighty God, the giver of wisdom, knowledge, and understanding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY

AAG	Lysine
AGE	Advanced glycation end-products
AHG	Anti-human globin
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CAT	Catalase
CBC	Complete blood count
CD4+, CD8+	Lymphocytes
CR	Creatinine
CRP	C- reactive protein
DHTR's	Delayed haemolytic transfusion reactions
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FBC	Full blood count
FDA	Food and Drug Administration
FRAP	Ferric antioxidant
Fya	Duffy antigens
GAG	Glutamic acid
GPX	Glutathione peroxidase
HB	Haemoglobin
HLA	Human leukocyte antigens

HRP	Avidin Horseradish peroxidase
IL -1	Interleukin 1
IL -6	Interleukin 6
IL-B	Interleukin 1β
Jka	Kidd antigens
K	Kell antigens
Lea	Lewis antgens
MBT	Multiple blood transfusion
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCP-1	Monocyte chemotactic protein-1
MCV	Mean cell volume
MDA	Melondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric Oxide
OD	Optical density
PMN'S	Polymorphonuclear neutrophils
ROS	Reactive oxygen species
SCD	Sickle cell disease
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TH1	T Helper type 1
TH2	T Helper type 2
TNF	Tumor necrosis factor

U	Urea
VEGF	Vascular endothelial growth factor
VOC	Vaso-oclusive crises

DEFINITION OF TERMS

Alloimmunization: The Body's reaction against foreign antigens from another person. E.g., blood transfusion or pregnancy.

Ameliorate: to make better or subside.

Antibody: an immunoglobulin which is produced in the system as a response to a stimulus by an antigen; which reacts specifically with it.

Antigen: this is a substance which when introduced into the system stimulates the production of an antibody and specifically reacts with it.

Antioxidant: a substance that protects the body from being attacked by substances that damage the cells e.g. Vit. C, vit. E, beta carotene, and vit A.

Delayed haemolytic transfusion reaction:

Haemolysis: lysing of blood or the breakdown of blood components.

Immune response: body's reaction against the foreign substance.

Inflammation: it is a reaction of the immune system in the body, with symptoms of swelling, pain, redness and heat.

Major histocompatibility complex: they assist the immune system to recognise foreign antigens or substances. They are also known as group of genes that codes proteins.

Multiple alloantibodies: immune antibodies produced only when exposed to strange antigens.

Multiple Transfusion: one receiving many units of blood transfusion.

Oxidative stress: an unevenness of free radicals and antioxidants that results in cell damage in the body.

Pathogenesis: the source and the progression of a disease condition.

Post transfusion: after transfusion.

Pretransfusion: before a blood transfusion.

Rheological Properties: the properties of materials that are controlled by a specific way in which deformation or flow behaviour occurs.

Sickle cell: red cell with sickle shape.

Transfusion: a process whereby blood is transfused into a patient’s bloodstream through the vein.

Vaso- Occlusive: the blockage of the microcirculatory vessels by sickled red blood cells. Thereby causing Ischemic injury to the body's organs.

CHAPTER ONE

INTRODUCTION

1.1 Statement of Research Problem

The risks involved in blood transfusion are enormous and should be critically examined, especially in sickle cell disease. Sickle cell disease results in a state of anaemia in which blood transfusion is a necessity. Blood transfusion remains a major therapy for the treatment of most anaemias, especially SCD. Red blood cell alloimmunization poses a serious negative complication for transfusion-dependent patients. There is an association between delayed haemolytic blood transfusion and alloimmunization, especially in patients who have had multiple blood transfusions like SCD compared to other disease conditions. It is therefore important to investigate the biological factors, markers of inflammation, oxidative markers and red cell phenotype of patients with sickle cell anaemia to understand better the mechanisms involved in the high alloimmunization rate.

1.2 Background

Sickle cell disease (SCD) is characterized by abnormal haemoglobin (HB) in which the sickle β-globin gene is inherited. The resulting abnormal sickle β-globin chain is due to the replacement of valine with glutamic acid in position 6 of the β-chain. Homozygous sickle cell anaemia (HB SS) is the most common of the inherited haemoglobinopathies (Ahmed and Ibrahim, 2017). When HB is exposed to reduced oxygen tension, it becomes insoluble and forms crystals. Polymerisation occurs, causing the elongation of red cells and the formation of a ‘sickle’ shape, which may block different microcirculations, causing infarcts of various organs. The red cell structure can be restored in the presence of an optimal oxygen supply, but continuous crisis or episodes enhances the destruction of the cell membrane, making the sickle shape irreversible (Thompson et al., 2019). SCD patients suffer from many complications such as pain, anaemia, and infection, and the abnormal sickle cell shape hinders the free flow of blood in the blood vessels, causing vaso-occlusion and leading to a shortage of oxygen (ischemia) and inflammation. SCD also presents with

jaundice of the eyes and skin, painful episodes, haemolytic anaemia, and organ damage which eventually results in death (Egesa et al., 2022). Various therapies have been successfully used in the management of sickle cell disease, such as transplantation, hydroxyurea, gene therapy, and stem cell transplantation. However, despite these treatments, blood transfusion remains the most common therapy in patients with sickle cell disease (Wang and Klein, 2010, Jersild and Hafner, 2017) , however, there are disadvantages and risks (Natukunda et al., 2010). These risks include blood-borne diseases, iron overload, and alloimmunization. This is likely due to a mismatch in antigenicity among recipients and donors, resulting in delayed transfusion reactions (Natukunda et al., 2010).

Oxidative stress is an additional pathophysiological phenomenon experienced by sickle cell patients, which is because of an imbalance between the production and accumulation of oxygen reactive species (ROS) in cells and organs and the inability of the biological system to neutralise the reactive products. This may lead to endothelial dysfunction and acute inflammation. The increase in ROS in SCD is as a result of increased intravascular haemolysis, chronic inflammation and ischemia-reperfusion injury (Keleku-Lukwete et al., 2015, Obeagu, 2018).

ROS and antioxidants work together in the body system as a regulatory check. Ordinarily, ROS are produced as intermediaries in healthy individuals (van Beers and van Wijk, 2018) which means that there is always a balance between ROS and antioxidants (e.g. glutathione peroxidase, superoxide dismutase, ascorbate pyruvate, catalase). These antioxidants assist in reducing or averting oxidative damage (Namazi et al., 2019). Increased oxidants and or decrease in antioxidants trigger oxidative reactions, thereby destroying proteins, lipids, and DNA, eventually leading to cell death. ROS and oxidative stress are major features of SCD (Kato et al., 2018). ROS and oxidative stress are common phenomenon which play a vital role in the inflammation process and are associated with numerous chronic inflammatory diseases such as sickle cell anaemia, diabetes, cancer, Alzheimer's disease, arthritis, asthma etc (Conran and Belcher, 2018). Besides being bactericidal, ROS also induces the expression of adhesion molecules, endothelial damage and the activation of inflammatory mediators (Conran and Belcher, 2018). Chronic proinflammatory states

are features of sickle cell patients. F_2-isoprostanes have been identified in SCD and are markers of oxidative stress (Reid, 2013). A likely origin of ROS emanating from SCD- related inflammation is revealed in the high number of activated neutrophils which generate ROS during an NADPH oxidase –mediated burst (Wang and Zennadi, 2021). It was reported that when transgenic mice were tested for hypoxia/reoxygenation-induced inflammatory response, a significant increase in the amount of platelets and white blood cells was observed (Bermudez-Gonzalez et al., 2022).

Other sources of oxidative stress in SCD include excessive free haemoglobin coupled with its breakdown products in oxidation reactions (Matters, 2015) and an increase in autoxidation of sickled haemoglobin-HbS (Strader et al., 2020; Conran and Belcher, 2018). Oxidative decomposition of polyunsaturated fatty acids gives rise to the production of F_2–isoprostanes and malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), respectively (Yadav et al., 2018, Zhang et al., 2019). This can be determined in blood or urine samples and can detect the level of oxidative stress. F_2- Isoprostanes and malondialdehyde hydroxynonenal (4-HNE) are increased in SCD (Akohoue *et al.,* 2007); Milne *et al.,* 2005) and it has been reported that AGE'S are elevated in oxidative conditions and are related with disease severity in inflammatory conditions (Nasif et al., 2016). Plasma levels of AGE'S are high in SCD and correlate positively to the presence of haemolysis-related organ complications (Ataga et al., 2015, Tantawy et al., 2014)

1.3 Rationale for the study

Alloimmunization is a global challenge which develops due to multiple transfusions. In some disease conditions, such as SCD, studies have shown that patients develop significantly higher rates of allogeneic antibodies, of which many are new and unidentified (Thompson et al., 2019). Despite the routine compatibility testing between a patient and a donor, alloimmunization remains increased and is often due to the fact that red cell antigens of the donor are phenotypically different from those of SCD patients, who are mainly of African descent. (Yazdanbakhsh, 2016).

As effective as blood transfusion is for the treatment of SCD, many risks remain, including alloimmunization, iron overload and transmission of blood-borne diseases.

The disparity between a patient and a donor in terms of blood group antigenicity can result in the development of alloantibodies in the recipient as well as cause a delayed type of transfusion reaction which can be fatal. (Adewoyin and Oyewale, 2015). There is a relationship between multiple blood transfusions in SCD patients and alloimmunization, inflammation and oxidative stress as well as delayed transfusion reactions. The proposed research will investigate the role of inflammation, particularly the increase of oxidants such as reactive oxidant species and other inflammatory markers in SCD patients. Furthermore, as there is currently no indigenous published work on this subject generally among Ife region in Nigerian, this study is designed to bridge that gap.

1.4 Aim

The research investigated the biological factors influencing the development of allogeneic antibodies in patients with sickle cell anaemia.

1.5 Objectives

1. Assess the dietary adequacy of the patients.
2. To determine the development of the following allogeneic antibodies (i.e., Anti Kell, Duffy, Kidd, Lewis, MNS, P1, Lutheran, etc) in patients receiving multiple transfusions for SCD and to compare them to non-sickle cell disease patients receiving a similar number of transfusions (control group).
3. To correlate markers of inflammation (CRP, Cytokines) with the development of allogeneic antibodies.
4. To compare and correlate oxidative stress markers (malondialdehyde (MDA)) with the development of allogeneic antibodies.
5. To investigate the relationship between alloimmunization, inflammation and oxidative stress. (The inflammatory and oxidative stress investigation will take place at CPUT).

1.6 Hypothesis

H_0: The development of allogeneic antibodies in SCD patients is influenced by high levels of oxidative stress and inflammation.
H_1: The development of allogeneic antibodies in SCD patients is not influenced by high levels of oxidative stress and inflammation.

1.7 Significance of study

Delayed allogeneic reactions and the development of antibodies have been described in SCD patients who have received multiple blood transfusions. This leads to potential risks such as delayed haemolytic reactions. This study is significant in that it will attempt to provide insight into the mechanisms involved in increased alloimmunization rates and strategies that could predict and prevent this. These strategies could benefit the long-term clinical outcome of SCD patients.

1.8 Ethical considerations

The study received ethical clearance from both Cape Peninsula University of Technology (CPUT/HWS-REC2021 renewal) Bellville, South Africa (the Human Research Ethics Committee, Faculty of Health and Wellness Sciences), and the Ethics Committee of Obafemi Awolowo University Health Centre, Ref: (D.MHS/2023) Ile-Ife. Informed written consent was obtained from all participants involved in the research.

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CHAPTER TWO

LITERATURE REVIEW

A REVIEW OF THE RELATIONSHIP BETWEEN THE IMMUNE RESPONSE, INFLAMMATION, OXIDATIVE STRESS, AND THE PATHOGENESIS OF SICKLE CELL ANAEMIA

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2.1 Abstract

Sickle cell anaemia (SCA) is a life-threatening haematological disorder that is predominant in sub-Saharan Africa. Sickle cell anaemia (SCA) is triggered by a genetic mutation in the β-chain of the haemoglobin gene, resulting in the substitution of glutamic acid with valine. This mutation leads to the production of an abnormal haemoglobin molecule called haemoglobin S (HbS). When deoxygenated, haemoglobin S (HbS) polymerizes, resulting in a sickle-shaped red blood cell that is rigid with a significantly shortened life span. Various reports have shown a strong link between oxidative stress, inflammation, the immune response, and the pathogenesis of sickle cell disease. Sickle cell anaemia (SCA) patients are constantly subjected to chronic inflammation and oxidative stress. The consequence of this leads to the development of vasculopathy (disease of the blood vessels) and several other complications. The role of the immune system, particularly the innate immune system, in the pathogenesis of SCA has become increasingly clear in recent years of research. However, little is known about the roles of the adaptive immune system in this disease. This review examines the interaction between the immune system, inflammation, oxidative stress, blood transfusion, and their effects on the pathogenesis of sickle cell anaemia.

Keywords: sickle cell anaemia; chronic inflammation; immune system; oxidative stress; haemolysis; blood transfusion

2.2 Introduction

SCD is a global health issue that affects millions of people globally [1, 2]. Africa has the highest sickle cell disease (SCD) prevalence, with Nigeria being the epicentre, having 4 to 6 million affected individuals. [3]. Fraiwan *et al.* [4] reported that every year, approximately 150,000 children in Nigeria are born with SCD, with 70-90 % dying before the age of five.

Sickle cell disease (SCD) is a genetic red blood cell disease that can be passed down to children from parents who are carriers [5]. Haemoglobin SS, also known as sickle cell anaemia (SCA), has been reported as the most prevalent and severe type of sickle cell disease [6-8]. Moreover, it is now established that SCD is not only a rheological

disease but is also characterized by chronic inflammation and oxidative stress, leading to the development of chronic vasculopathy and several chronic complications [9]. SCA is a genetic haemoglobin disorder caused by a single-point mutation in the β-chain of the haemoglobin gene, resulting in the replacement of glutamic acid with valine [10, 11]. The consequence of the mutation leads to the production of abnormal haemoglobin (HbS), which polymerizes in low oxygen concentrations [12, 13]. This leads to the dysfunction and deformation of the red blood cells to a sickle-shaped form [2, 9].

Haemoglobin polymerization triggers a sequence of events that result in a variety of complications, including vascular-endothelial dysfunction, anti-inflammatory (nitric oxide) deficiency, inflammation, oxidative stress, hypercoagulability, and recurrent immune cell activation [2, 14, 15]. These pathological events result in an increase and excess of free radicals through the release of free haemoglobin, heme, and activation of pro-oxidant enzymes [2, 16]. Excessive amounts of free radicals contribute to increased oxidative stress, which induces chronic inflammation in SCA patients and a reduced life expectancy [17]. Patients with SCA require regular blood transfusions, which increases exposure to foreign antigens and elevates the risk of producing alloantibodies that may cause delayed haemolytic transfusion responses and make it difficult to find suitable blood [18-20]. Moreover, it has been reported that blood transfusion may lead to infectious disease and iron overload [21, 22].

Besides the effect of inflammation, oxidative stress, and blood transfusion on the pathogenesis of SCA, the effects of a dysregulated immune system have also been investigated [23, 24]. The innate and adaptive immune responses in SCD patients are impaired and cannot effectively protect against infection [25], with the dysfunction being linked to chronic inflammation [26]. Some researchers have reported that the persistent activation of the innate immune system results in the generation of excessive amounts of reactive oxygen species (13, 28-30). According to Ahmad and Ahsan [27], Engwa *et al.* [28] and Atiku *et al*. [10], elevated amounts of reactive species induce oxidative stress and tissue injury. Some studies have also revealed that the adaptive immune cells are dysfunctional in SCA patients, resulting in lower antibody levels than healthy individuals [24]. In addition, A few preliminary studies have

revealed that T and B cell numbers and functions are also impaired [24, 26, 29]. Based on these previous studies, this review aims to examine the current literature to understand the relationship between the immune response, inflammation, oxidative stress, blood transfusion, and the pathogenesis of SCA.

2.3 Immune Mechanisms Involved in the Pathogenesis of Sickle Cell Anaemia

Leukocytes such as neutrophils, eosinophils, basophils, monocytes, lymphocytes, and platelets have been implicated in the pathogenesis of SCD, as evidenced by several studies [23, 24, 30, 31]. These immune cells have all been reported to be responsible for promoting inflammation, adhesion, and painful crises in SCD [23, 32]. Even in the absence of infection, leucocytosis is a common phenomenon in SCD patients. A flow-cytometric method was adopted for the sensitive detection of active CD18 by monoclonal antibody 327C in whole blood samples. Findings from the study show that neutrophils present in SCD patients have a greater potential for response to an inflammatory stimulus, which results in rapid adhesion of CD18 to ligands including ICAM-1 on endothelium and ICAM-4 which is upregulated on SCD RBCs [33, 34]. Subsequently, SCD neutrophils adhere with higher affinity in vascular regions of chronic inflammation to propagate VOC by actively recruiting sickle RBCs from the flowing blood. [35]. Looking inward, this research affirms what the literature earlier reviewed.

Polymorphonuclear leukocytes (PMNs) are also activated with reduced L-selectin expression, enhanced CD64 expression and elevated levels of L-selectin, SCD16 and elastase, resulting in increased adhesiveness to the endothelium [36]. According to Antwi-boasiako *et al.* [37], male and female SCD patients who experienced complications had noticeably higher leukocyte counts than their healthy counterparts. Furthermore, the white blood cell count is frequently used by clinicians to accurately predict or detect stroke and acute chest syndrome [38]. Free haemoglobin and heme released during haemolysis have been reported to be one of the key players in the mechanisms leading to the activation of the innate and adaptive immune response [9,

23]. It has been reported that patients with SCD with high haemolysis rates are at greater risk of early mortality [2, 19]. The continual breakdown and destruction of red blood cells result in sustained activation of innate immune cells, resulting in a chronic inflammatory state [24, 39, 40].

Endothelial cells are one of the first cell types to be activated in the presence of heme in the bloodstream. Heme activates endothelial cells, inducing the expression of adhesion molecules (E-selectin, intercellular P-selectin, vascular cell adhesion molecule 1, adhesion molecule 1) and initiates the activation and recruitment of other immune cells, including macrophages, neutrophils, mast cells, and platelets. This process results in a vaso-occlusive crisis, which is commonly described in patients with SCD [15, 41]. The activated macrophages lead to an elevated production of pro-inflammatory cytokines, especially IL-1β, through activation of the NLRP3 inflammasome [23].

Heme has also been reported to have a direct link with the activation of neutrophils by acting as a prototypical pro-inflammatory molecule to recruit neutrophils to the site of injury via the stimulation of protein kinase C and ROS generation [2, 15]. Heme inhibits neutrophil apoptosis via modulation of phosphoinositide 3-kinase and NF-κB signalling, which further contributes to the development of chronic inflammation [42]. Neutrophils play a significant role in the pathogenesis of VOC with higher neutrophil counts being associated with clinical complications, such as earlier death and haemorrhagic stroke [41].

Besides neutrophils and macrophages, other innate immune cells, such as platelets, have been implicated in the pathogenesis of SCD. Malik [43], reported that platelet activation and a decrease in nitric oxide (NO) are triggered by the release of heme into circulation. Their findings are consistent with those of Nolfi et al. [44]. Patients with SCD experience thrombosis and pulmonary hypertension due to the release of soluble mediators from activated platelets, such as CD40 ligand and thrombospondin [23, 43]. These molecules bind to CD36, also known as glycoprotein IV, on sickle RBCs and endothelial cells. Platelets further associate with other immune cells, including neutrophils, macrophages, and monocytes [40]. It has been demonstrated that

activated platelets bind to neutrophils and monocytes in a P-selectin signalling pathway to form aggregates that promote VOC, inflammation, and thrombosis through various mechanisms [30]. In SCD mice, Allali *et al*. [23] reported that platelet-neutrophil aggregates may be an important factor in the development of pulmonary arteriole micro-emboli.

Although many studies have investigated the role of the innate immune system, the role of the adaptive immune response is still poorly understood. Studies performed on human and animal subjects have reported that both T and B lymphocytes are dysfunctional in SCD [45, 46]. To further analyse the variation of T cell counts among SCD patients, the relationship between splenic size and lymphocyte counts has been investigated by several researchers [24, 47-50]. Ojo *et al.* [51] used flow cytometry to analyse blood samples from 40 SCD patients at steady state for CD4+ T lymphocytes and ultrasonography to determine spleen size. They discovered that the mean CD4+ count in HbS patients with auto splenectomy (shrinkage and non-functionalization of the spleen) was slightly lower than in HbS patients with a normal-sized spleen. Several studies have further investigated the effect of hydroxyurea (HU) on lymphocyte subset counts [46, 52, 53]. These have shown that SCD patients receiving HU had lower total lymphocytes, T cells, CD4+ T cells, memory CD4+ T cells, and memory CD8+ T cells compared to those who were untreated [24]. Other studies showed that alloimmunization, which is an important complication related to chronic blood transfusion in SCD patients, also influences lymphocyte counts [24, 48, 54-57]. Furthermore, alloimmunized SCD patients demonstrated a significant decline in regulatory CD4+ T lymphocytes while showing an increase in regulatory CD8+ T lymphocytes [58]. This implies that a reduction in both CD4+T lymphocyte and CD8+T lymphocytes may result in an autoimmune state.

Furthermore, studies examining B cells have demonstrated that they are functionally abnormal in patients with SCD. These abnormalities include decreased antigen-specific B cell proliferation and IgM secretion. According to Ochocinski *et al.* [29], defects in B cell lymphocyte function in children affect the production of natural anti-polysaccharide antibodies, making children with SCA more susceptible to infection and disease.

2.4 Autoimmunity in Sickle Cell Disease

Autoimmunity results when the immune response of an organism is directed against its normal body constituents, such as cells and tissues. Any disease resulting from this immune response is termed an autoimmune disease. It is also the presence of antibodies produced by B lymphocytes and T lymphocytes directed against normal components of an individual. Patients with sickle cell disease (SCD) manifest an abnormal activation of the alternate complement pathway that increases the risk of infection and is thought to predispose them to autoimmune disease (AID) [59]. De Vlam *et al.* [60] and Hilario *et al.* [61] reported that the prevalence of antinuclear antibodies ranges from 12 to 30% in healthy individuals in total, whereas in Africa, it ranges from 7 to 39% [62]. However, high autoantibody titres have been reported in SCD without autoimmunity [63, 64]. The activity underlying the formation of autoantibodies in SCD is unidentified but may involve impairment of the spleen function. Significantly, autoantibody titres have been documented after splenectomy in the absence of AIDS [65]. Moreover, the suggested areas of focus involves chronic inflammation [28] and alloimmunization by multiple transfusions [27]. This has not been properly investigated in clinical studies [63, 64]). The coexistence of SCD and AIDS is difficult to treat, as patients receiving steroids experience repeated vaso-occlusive crises. Steroids are meant to ameliorate inflammatory conditions and calm the immune system's activity against illness and infection [66-68]. To emphasise this point, Bernini et al. [69] and others reported a case of 4 SCD patients who were subjected to increased doses of corticosteroids during SCD pain crises and chest pain syndrome. The result of steroid treatment seemed to shorten the period of complication, but inversely, the high dosage of corticosteroid also resulted in severe VOC episodes and haemorrhagic stroke in many patients. Their findings were similar to what the literature reported. Furthermore, recurrent blood transfusion or exchange transfusion, although assisting in the prevention of vaso-occlusive crises, leads to increased risks of producing antigenic alloimmunization [70]. Biological therapies, such as anti–TNF–related treatment, may be beneficial to SCD-associated AIDS/ inflammation, and haematopoietic stem cell transplantations remains a better therapeutic choice [71].

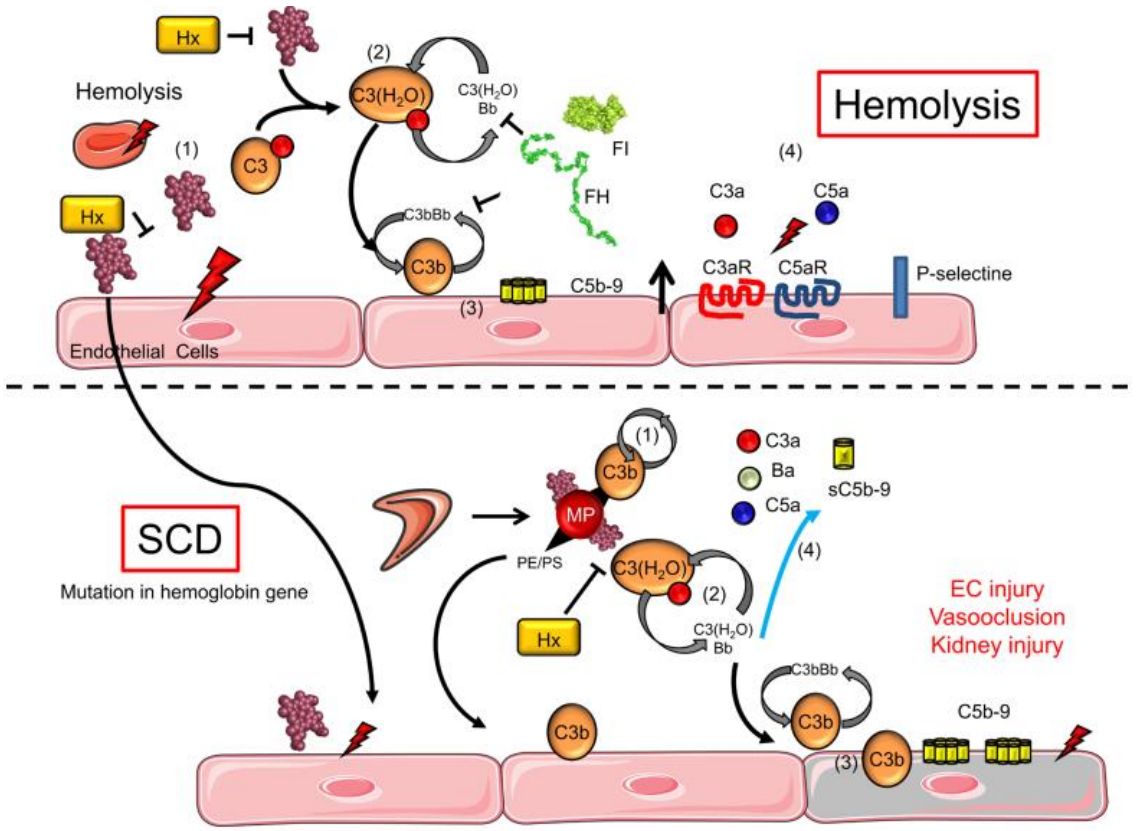

Figure 2.1: Complement activation pathway and haemolysis in SCD patients [61].

2.5 The role of oxidative stress in the pathogenesis of sickle cell anemia

Oxidative stress is an important contributor to the pathogenesis of sickle cell anaemia (SCA) and associated complications such as sickling, vaso-occlusion, and ischemia-reperfusion injury [2, 27, 37, 72, 73]. Oxidative stress occurs due to an imbalance between the production of reactive species such as oxygen species (ROS) and reactive nitrogen species (RNS) and the ability of antioxidant agents, including enzymes such as superoxide dismutases, catalase, and glutathione peroxidase, to neutralize them [28, 37, 74]. Patients with SCA are frequently exposed to oxidative stress, and studies have found higher levels of ROS in RBCs of SCA patients compared to healthy RBCs. The concentration of the reactive intermediates generated from the oxidative reactions has often been used as markers of disease severity [28, 75]. The mechanisms leading to oxidative stress in SCA patients' are well established. Some of these include haemoglobin (Hb) autoxidation. When SaQ1234567890 */haemoglobin is released into the bloodstream as a result of haemolysis, superoxide

(O^{-}_{2}) is produced, which can dismutate into hydrogen peroxide (H_2O_2) and serve as a starting point for additional oxidative reactions [9, 76]. Apart from haemoglobin (Hb) oxidation, other factors enhancing ROS production include ischemia-reperfusion injury caused by oxygen deprivation [12]. Ischemia-reperfusion injury has been reported to promote the activation of proinflammatory mediators such as xanthine oxidase, NADPH oxidase, nitric oxide synthase, and lipoxygenase [2, 28]. Another factor contributing to the excessive ROS in SCD patients is the release of iron and heme from unstable HbS, which may catalyse the Fenton reaction. Iron (II) will react with hydrogen peroxide ions, leading to the formation of ion (III) and hydroxyl radical [12]. Figure 2.1 illustrates each of the above mechanisms.

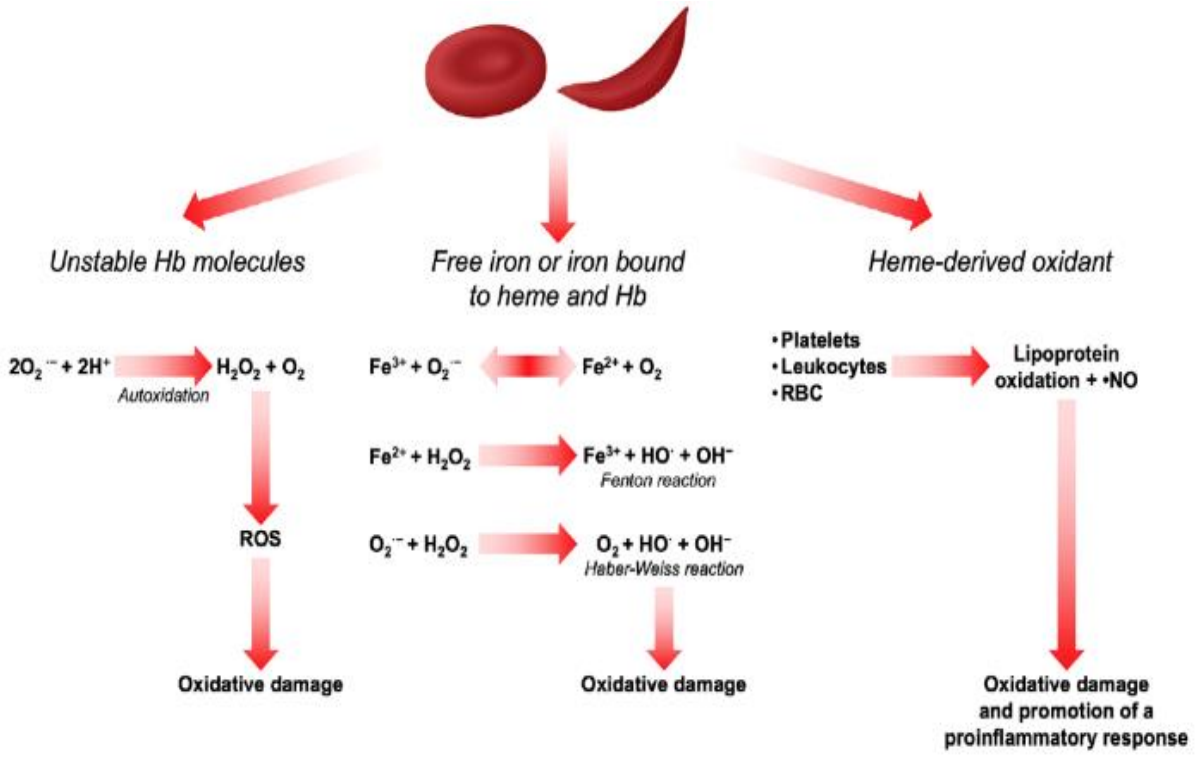

Figure 2.2: Sources of ROS in RBCs (adapted from [80].

To counteract radicals, the body produces antioxidants. [39, 78, 79]. These include non-enzymatic antioxidants such as microelements carotenoids and ascorbic acid [80], and enzymatic antioxidants including dismutase, catalase, glutathione peroxidase and heme oxygenase-1 [2, 28]. However, due to the high levels of oxidative stress in SCD patients, antioxidants are overwhelmed by the continual

source of ROS. Some unneutralised ROS have been reported to oxidize membrane lipids, proteins, and DNA, causing cell death and organ damage [81]. This damage leads to further ROS production, thereby aggravating the disease. The oxidative damage to lipids known as lipid peroxidation happens when membrane phospholipids are exposed to a hydroxyl radical ($HO^{\bullet}$) and hydroperoxyl ($HO^{\bullet}_2$), which have been reported as the two most prevalent ROS that can affect lipids [82, 83]. During lipid peroxidation, highly toxic molecule end products, including malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), can easily interact with proteins and DNA, causing damage [2, 80]. Malondialdehyde (MDA) is an important marker for evaluating oxidative stress in patients with SCD [28].

A study in Cameroon observed an increase in MDA in SCD patients compared to healthy individuals [84]. Similarly, in Ghana, Antwi-Boasiako *et al*. [37] reported that MDA levels were significantly higher in SCA patients with vaso-occlusive crises, followed by patients in steady-state. F_2-isoprostanes, as a marker of oxidative stress, has been reported to be higher in sickle cell patients compared to healthy controls [85, 86]. Nader et al., 2020 [87] assessed the contributions of NO and oxidative stress to cryptos (apoptosis of red cells) and the release of RBC-Mp and its contribution to vascular dysfunction in SCA. It was discovered that oxidative stress will affect eryptosis and the release of MPs generated during elevated eryptosis may be significant in macrovascular dysfunction in SCA patients.

RBC-MP's may have harmful effects on the microcirculation's endothelial cells in part by activating TLR4 and encouraging the expression of adhesion molecules and cytokines release, both of which may exacerbate vascular dysfunction. This discovery offers fresh insight into the underlying processes of vascular dysfunction in SCA. Still, more research is encouraged to determine the specificity of SCA RBC-MP'S at the source of TLR4 activation and to investigate new therapeutic targets that aim at preventing eryptosis and /or TLR4 activation in SCA [87].

It is well known that oxidative stress in SCA has clinical repercussions and is associated with worsening symptoms, including accelerated haemolysis [88],

endothelial damage [82], decreased NO bioavailability [2], and hypercoagulability [89]. Oxidative stress is inevitable in a patient with SCA; however, some antioxidant therapeutic strategies, including the use of L-glutamine, N-acetylcysteine, and manganese porphyrins, have been suggested to reduce the detrimental effects, though future investigation is still required [72].

2.6 The role of inflammation in the pathogenesis of sickle cell anaemia

Inflammation is the body's natural response to protect itself against harm (toxic chemicals, infection, and injury). Although it is difficult to determine the exact events that trigger the chronic inflammatory state in sickle cell disease (SCD), some pathophysiological mechanisms have been reported [31, 46]. The sources of inflammation in SCD include red cell alterations, haemolysis, vaso-occlusive processes, ischemia-reperfusion injury, infections, histamine, oxidative stress, thrombin generation and activation of complement [31, 86]. Many reported complications, such as acute chest syndrome, stroke, leg ulcers, nephropathy, and pulmonary hypertension, have been caused by inflammatory processes [90].

Haemolysis is the major inflammatory trigger that affects the bioavailability and function of anti-inflammatory molecules such as nitric oxide (NO) and heme oxygenase 1 (HO-1) [89, 91, 92]. Heme oxygenase 1 (HO-1) is an enzyme with numerous anti-inflammatory properties, including the breakdown of heme and the generation and release of various reaction products, including carbon monoxide, ferrous ions, and biliverdin [93-97]. However, continuous haemolysis leads to the overproduction of heme, which in turn accelerates the HO reaction, causing excessive reaction products to accumulate and, if not sufficiently sequestered, will have serious consequences [93]. During haemolysis, free haemoglobin and heme destroy nitric oxide (NO) produced by endothelial nitric oxide synthase, which plays an important role in leukocyte activation and emigration from blood vessels to tissue [2]. Researchers have demonstrated that free haemoglobin in the plasma destroys NO 1,000-fold faster than haemoglobin encapsulated within the red blood cells [9].

Neutrophils are one of the first lines of action of immune cells to infections. The movement of neutrophils to the site of injury or inflammation is usually triggered by

PAMPs from microbes or DAMPs derived from disrupted host cells. Neutrophils release ROS and proteases to combat foreign organisms at the site of infection [98]. The neutrophil upon activation and degradation releases enzymatic proteins such as myeloperoxidase, defensins, cathepsin G, and alastase which are major protein enzymes involved in many inflammatory responses [99] By processes of cell-to-cell contact, chemokines and cytokines are produced, They regulate dendritic cell regeneration, transform and represent antigen to memory $CD4^+$ T cell as well as to naïve CD8+ T cells, which consequently magnify the $CD8^+$ T cell response to antigen [100]. Stoppacciaro *et al*. [101] and Ma *et al*. [102] reported that there was a regression of cancer cells as a result of the interaction between neutrophils and T cells. Also, ROS, produced by the activated neutrophil, hinders the function of effector NK cells, while GM-CSF and IFN-γ produced from activated NK cells prolong the survival of neutrophils in an in vitro system [103]. In addition, the degradation of neutrophils impairs the recruitment of monocytes and lymphocytes to the inflammatory site. At the same time, the immune-suppressive capacity of neutrophils in T cell propagation during acute systematic inflammation has been reported [104].

Considering the numerous advantages of neutrophils in inflammation, it was perceived that neutrophils played an important role during a haemolytic condition. Therefore, the response of neutrophils with other blood cells to metHb and LTA was investigated. The report showed that met Hb is an endogenous DAMP ligand for TLR2 and that neutrophils are one of the most sensitive cell types responding to (metHb + LTA) – induced production of ROS. Interestingly, it was also observed that the effect diminishes by the presence of other white cells indicating that the white blood cells communicate with each other to modulate cellular responses during a haemolytic reaction [105]

Vaso-occlusive crises (VOC), commonly called sickle cell painful crises, happen when sickled red blood cells obstruct blood flow to the point that tissues are deprived of oxygen [24, 106, 107]. This, in turn, triggers an inflammatory reaction as the body attempts to correct the condition. In SCD, vaso-occlusive processes generate ischemia-reperfusion injury, known as tissue damage, caused by a disruption in blood

supply [2, 107, 108]. Ischemia-reperfusion damage increases oxidant generation and leukocyte adhesion, contributing to chronic inflammation.

Transforming growth factor (TGF-) and interleukin-17 (IL-17), as well as other inflammatory mediators, are considerably higher in patients with SCD in a steady state compared to controls; Tumour necrosis factor (TNF-), IL-6, and IL-8 have also been found to be elevated in patients with SCD and VOCs compared to controls [41, 108].

2.7 The relationship and interdependence between inflammation and oxidative stress in the pathogenesis of SCA

Comprehensive studies have demonstrated that oxidative stress and inflammation are closely linked and that both processes can easily induce one another [78, 83, 121]. Both mechanisms occur concurrently in many pathological conditions, including sickle cell disease (SCD), resulting in a vicious cycle that aggravates the disease [9, 122]. Several factors contribute to the overproduction of reactive oxygen species (ROS) in SCA, which, if not immediately sequestered, can create a chain reaction that results in chronic inflammation [12, 78]. Oxidative stress can activate a wide range of transcription factors and receptors, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) [77, 78]. These can control the expression of a wide variety of genes, including those responsible for producing pro-inflammatory and anti-inflammatory cytokines [77, 123].

Pattern recognition receptor toll receptor 4 (TLR4) triggers the innate and adaptive immune response by promoting the secretion of proinflammatory cytokines like TNF, IL-1, and IL-6, IL-12. This process can be activated by oxidative stress and thus leads to inflammation [73, 124]. Cell-free haemoglobin, derived from sickle RBCs, contributes to vascular dysfunction by promoting inflammation via the activation of TLR4 [9, 12]. Besides the direct activation of transcription factors and receptors via oxidative stress, several molecules with inflammatory potential known as damage-associated molecular patterns (DAMPs) released during haemolysis cause damage to some biomolecules. As a result, these damaged biomolecules promote inflammation through the NF-κB pathway [125]. Hydrogen peroxide, for example, can react with nitric oxide (NO) to form peroxynitrite, a highly reactive oxidizing and

nitrating agent capable of damaging lipids, DNA, and protein. These reactions promote cellular necrosis and apoptosis [31].

On the other hand, chronic inflammation can induce oxidative stress through the continuous activation of immune cells [73, 78, 126]. At the site of injury, immune cells such as phagocytic and non-phagocytic cells release reactive oxygen species (hydrogen radical, superoxide, hydrogen peroxide, etc.), chemical mediators (cytokines, nitric oxide, etc.), and enzymes (lipases, phosphatases, etc.) contributing to higher oxidative stress at the site of inflammation [127-129]. Nonphagocytic cells have been reported to generate reactive species in response to proinflammatory cytokines, leading to an imbalance between proinflammatory and anti-inflammatory cytokines and oxidative stress [27, 83]. Figure 2 depicts the relationship between oxidative stress and inflammation in SCA. If oxidative stress appears as the primary abnormality, it will stimulate inflammation, which will further induce oxidative stress and vice versa.

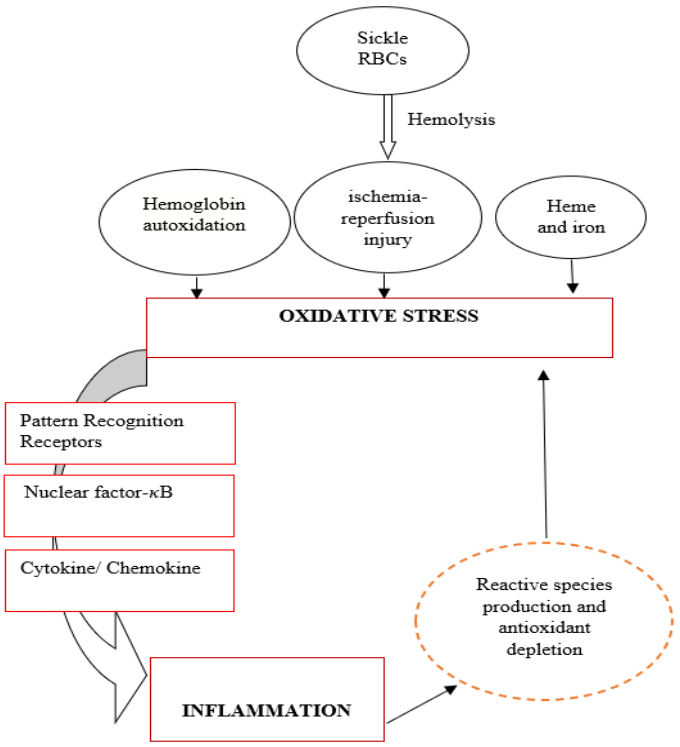

Figure 2.3: Relationship between oxidative stress and inflammation in the pathogenesis of SCA

Due to the interaction between oxidative stress and inflammation, some researchers have discovered that using antioxidants to treat only oxidative stress may not always be successful [2, 15, 72]. Inflammation and oxidative stress work together to amplify each other and cause progressive damage once the process begins. Finding antioxidants that can simultaneously prevent oxidative and inflammatory pathways has proven to be complicated. Hence, a comprehensive understanding of these pathological events occurring in SCA could contribute to the development of novel therapeutics.

2.8 Inflammation and Blood Transfusion in SCD

SCD patients suffer from many complications such as pain, anaemias, infection, and the abnormal sickle cell shape hinders the free flow of blood in the blood vessels, causing vaso-occlusion leading to a shortage of oxygen (ischemia) and inflammation in the system [41]. SCD also presents with jaundice of the eyes and skin, painful episodes, haemolytic anaemia, and organ damage, eventually resulting in death [130]. Continuous activation of leucocytes, platelets, and endothelial cells causing

haemolysis and VOC, premeditated by ischemia-reperfusion cycles [131]. Numerous therapies, such as hydroxyurea, gene therapy, and stem cell transplantation, have been used successfully in treating sickle cell disease. Despite all these treatments, blood transfusion remains the most effective therapy [90]. However, there are also some disadvantages and risks involved. These include alloimmunization, blood-borne diseases and iron overload. The build-up of alloantibodies [132] is most likely due to incompatibility in antigenicity between donors and recipients and may lead to delayed transfusion reactions [57].

2.9 The Cause of High Alloimmunization in SCD Patients

High alloimmunization is prevalent in SCD patients as traces of unidentified antibodies are present, which increases with the number of blood transfusions, age, genetics, sex, and requires extensive blood screening before transfusion [55]. The use of generic instruments and methods does not capture all the antibodies. Nebie *et al*. [133] stated that screening methods and instruments should be customized as their study showed that available instruments could not screen local patients' antibodies, hence the unidentified antibodies present in SCD patients after multiple transfusions. Thompson *et al*. [55] linked high alloimmunization in SCD patients to chronic inflammatory disorders which triggers the development of auto-antibodies and alloimmunization. Low expression of CD64 (FcyR1) in classical and intermediate monocytes and the inflammatory milieu found in SCD patients contribute to their high alloimmunization [134]. A miss-match between the donor and the recipient has also been linked to high alloimmunization in SCD patients. Reports from studies in related countries like Uganda, Burkina Faso, and Egypt showed that alloimmunization is lower since both donor and recipient belong to the same ethnic group [57, 90]. This finding was supported by the increase in alloimmunization found in Cape Town (South Africa), which was attributed to the increase in migration and genetic differences between donors and recipients [55]. There could be other factors such as iron overload and pregnancy.

2.10 Blood Types and blood transfusion in sickle cell Patients

Blood transfusion is a lifesaving, routine medical procedure. It entails several procedures, starting with pretransfusion screening before blood can be certified suitable for the patient needing it. Pretransfusion testing (antibodies screening) typically includes Rhesus and ABO grouping. However, other blood group systems,[including Kell, Kidd, Duffy, Lewis, Lutheran, P, and MNS, often regarded as minority or weak blood groups, have been linked to alloimmunization or antibody formation [136, 137]. Alloimmunization can lead to life-threatening events such as delayed haemolytic transfusion reaction [138], auto-immunization [21], and hyperhaemolysis syndrome [19]. Considering this, it has become imperative that routine blood grouping should include other blood group antigens for effective, complete, and accurate pretransfusion screening. This is crucial in the case of sickle cell disease patients who require multiple blood transfusions to increase their oxygen-carrying capacity and assist in the replacement of defective red blood cells with normal ones [37, 139]. Patients with sickle cell disease are more at risk of developing alloimmunization as a result of frequent blood transfusions, which makes cross-matching and suitable blood for transfusions problematic when the issues of minor antigens are not considered during the transfusion [134, 140]. According to Boateng *et al.* [141], the frequency of alloantibody development in patients with SCD is as high as 76% compared to the general population, which suggests that SCD patients are more vulnerable to the formation of alloantibodies [142]. The frequency of red blood cell alloimmunization in SCD patients may not be the same in every part of the world due to blood transfusion rates, racial mismatch sources, and the age of the initial transfusion [20, 143, 144].

2.11 Other Treatment Options

The US Food and Drug Administration has approved HU, L-glutamine, crizanlizumab, and voxelotor to reduce the acute complications of SCD [24, 31]. Hydroxyurea is the most commonly used of these, while other drugs, including L-glutamine and crizanlizumab, have not been widely adopted despite European approval [109]. Additionally, although HU is effective in reducing acute complications, improving quality of life and organ function and prolonging survival, it also remains underutilised primarily due to inexperience and unfounded safety concerns [110]. Although the

mechanism of action of HU is still unclear, previous studies have shown that after treatment, nitric oxide (NO) production is improved, and the concentration of foetal haemoglobin a(HbF) in erythrocytes is enhanced, thereby preventing HbS polymerisation[11, 111]. Several studies have investigated the effectiveness and safety of these drugs in reducing the frequency of VOC and inflammation in SCD patients [112-114, 135] and have reported that voxelotor increases haemoglobin levels, does not impair oxygen delivery, reduces hospitalisation for VOC and decreases sickle red blood cell levels. It has been reported that L- L-glutamine and crizanlizumab reduce VOC episodes and prolong the time between the first and second pain crises. In this study, it was reported that inflammatory molecules were reduced with HU therapy in children with sickle cell SCD [41]. Others have supported this and have observed that patients receiving HU had lower interleukin IL-6 levels [116, 117]. In contradiction, however, others have reported elevated levels of interleukin (IL)-6 compared to untreated patients [79, 106, 118-120].

2.12 Delayed haemolysis related to transfusion

Although transfusion seems effective treatment for most major anaemia cases, such as sickle cell disease can cause delayed transfusion reactions and haemolysis. Post-transfusion haemolysis is one of the most common immunological reactions that take place after transfusion. The rate of occurrence is underestimated because of its biological and clinical characteristics. The high incidence of alloimmunization in SS anaemia patients is the main reason for the delayed reactions. Studies have revealed that only 30% of cases have no detectable antibodies [144]. Delayed haemolytic transfusion reactions (DHTRs) occur in patients who previously received a blood transfusion with low antibody titres that were undetectable on pre-transfusion testing. After the transfusion of incompatible red blood cells (RBCs), the immune system is exposed to an antigen, which triggers and initiates sensitization and increases the synthesis of corresponding antibodies. The antibody titre becomes high enough to haemolyse transfused RBCs within a period. It is estimated that the frequency of DHTRs is 1 case per 5400 red cell units transfused [144], and it has been hypothesized that DHTRs could be an immune response that develops due to differences in compatibility of the donor red cells antigens of blood donors of European

descent and patients of African descent. Clinicians must be aware of DHTR so that it can be investigated if patients who have had a transfusion experience pain [145]. Patients with SCD require frequent blood transfusion therapy [146], to increase oxygen-carrying capacity or improve blood's rheological properties [147] and therefore, there is an increased chance of complications such as iron overload, infections, and delayed haemolytic transfusion reactions (DHTR) due to alloimmunization [148, 149].

2.13 Conclusions

The review presented here shows that SCD is a disease that affects millions of people worldwide. Extensive research has been conducted over many years to alleviate the complications endured by SCD patients, but the mechanisms involved in those complications need further investigation. The immune system is important when dealing with SCA patients, as its dysfunction results in oxidative stress and a pro-inflammatory environment. The preceding discussions have shed light and provided a better understanding of the roles of immune cells, oxidative stress, and inflammation in the pathogenesis of SCD. Although the role of innate immune cells in SCD pathogenesis has been broadly and extensively described, more research on the roles of the adaptive immune system in this disease is needed due to a scarcity of data. In addition to the complications caused by the constant activation of immune cells, which results in a chronic inflammatory state, it is important to note that haemolysis plays a significant role in inflammation by releasing toxic-free heme and haemoglobin, which affects the bioavailability of anti-inflammatory substances. Therefore, the pathogenesis of sickle cell anaemia is complex. Further research that would examine more specific factors in detail regarding the pathogenesis of sickle cell disease is recommended.

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injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

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CHAPTER THREE

DIETARY INTAKE AND NUTRIENTS ADEQUACY OF YOUNG ADULTS WITH SICKLE CELL DISEASE IN ILE-IFE, SOUTHWEST NIGERIA

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3.1 Abstract

3.1.1 Background

Sickle cell disease (SCD) is a chronic and genetically mutated disease seen among African descent, of which the need to study the nutritional status and the socio-demographic characteristics is a challenge. The study aims to determine the dietary intake, level of nutritional status, and sociodemographic characteristics of people with sickle cell disease in Ile-Ife, Osun State, Nigeria.

3.1.2 Methods

The study involved 100 participants, 50 in the test group (Hb SS patients) and 50 in the control group (other genotypes/non-Hb SS patients), recruited at the Obafemi Awolowo University's health centre in Ile-Ife. A questionnaire was issued to gather information on participants. Dietary data were obtained by using 24-hour dietary recall food frequency questionnaire which was distributed to the participants. Also, the socio-demographic characteristics, including their body mass index (BMI), height and weight, were measured.

3.1.3 Results

From the study, about 76.3% of the respondents did not meet the total calories needed, while 23.7% met the expectation of the total calories needed per day. Proteins and carbohydrates as major macronutrients met the requirement of 54.6% and 93.8% respectively. Fibre intake was grossly inadequate, 80.4% of mineral salts and vitamins showed inadequate intake, most especially folate, retinol, beta carotene and vitamin D.

3.1.4 Conclusion

Nutritional management should go with medical care in the management of patients with sickle cell disease. Nutritional management should also focus on a conscious selection of food to ensure the adequacy of both micronutrients and macronutrients necessary for the maximum functioning of the body and maintaining good nutritional status.

Keywords: Sickle cell disease, anaemia, Dietary intake, Nutritional status, Haemoglobin.

3.2 Introduction

Sickle cell disease (SCD) is a chronic genetically inherited disease, usually seen among those of African descent and is characterized by abnormal haemoglobin in which the sickled beta-globin is inherited (Nader et al., 2020). SCD remains a global problem affecting 20-25 million people worldwide with affected infants in Africa dying before the age of five years are 50- 80% (Stephen *et al.*, 2018). This blood disorder diminishes the ability of the red blood cells to carry oxygen. One major characteristic of this disease is the sickling of the blood cells, which results when the deoxygenated haemoglobin molecules affect the normal shape of the red blood cells (Nader et al., 2020). The resultant effect of the abnormality of the shape of the red blood cells is vaso-occlusive events and increased haemolysis. Vaso-occlusion (VOC) may cause damage to the tissue, bone, and organ, while haemolysis leads to anaemia (Darbari *et al.,* 2020). Vaso-occlusive results in system-level damage and other medical-related complications, including delayed growth**,** sexual maturation, poor health, pulmonary dysfunction, stroke, aseptic necrosis of the hip or shoulders, retinopathy associated with sickle cell, cognitive impairments related to hypoxia, ulcers of the skin, severe pain, and low body weight (Linton et al., 2021). Pharmacologic and psychotherapeutic management is the treatment for sickle cell complications, this manages painful crises (Ojo *et al.,* 2023). This management improves caloric intake, which counters the negative effects of poor nutrition and low body weight. Reducing pain during a painful crisis results in reduced disability, increased activity, and better nutrition (Sharma, 2021). Also, **c**omorbidities with sickle cell disease have been shown to affect dietary intake negatively (Sagi *et al.*, 2021) and a report has shown that SCD could cause death and disability (Pietl et al, 2014).

Undernutrition has been considered a complication of sickle cell disease and should be considered in clinical care. However, awareness in past decades has not been addressed adequately at an empirical level (Singer *et al.,* 2020). There are several approaches used in the management of SCD, including natural products, hydroxyurea,

blood transfusion, and nutrient supplements; despite all of these, adequate dietary intake, protein-energy calorie deficiency still exists. This implies a shortage of nutrients needed for growth and development despite the adequacy of nutrients. Hence, there is a need to proffer solutions to the inadequacy of nutrients in SCD, which could be through supplementation of nutrients or providing adequacy of nutrient**s** (Martyres *et al.*, 2016)**.**

Studies suggest that nutrient deficiencies in sickle cell patients are more likely due to increased nutrient requirements due to the burden of the disease (Charlotte *et al.,* 2022). Evidence to review the role of macronutrient deficiencies causing nutritional deficiencies was scanty in the last two decades. However, more recent findings established lower than normal anthropometric measurements in adults and adolescent sickle cell patients, especially in males (Martyres et al., 2016).

A supplementation trial carried out by Buchmann *et al.* (2022), which was the first of its kind, undoubtedly revealed that insufficient macronutrient intake was ameliorated via macronutrient supplementation with five growth retarded HBSS patients. The result from the research showed that protein energy supplements could improve clinical status and growth, as shown in the 2 HBSS patients who were fed supplements of proteins and calories via nasogastric tubes in addition to their regular diets. Although the findings of this research are limited in interpretation due to the small number of patients, the results establish a role for malnutrition as one of the complications of HBSS patients and the benefits of routine supplements.

Presently, there is no special recommended dietary allowance for HBSS patients with the burden of the disease, unlike in pregnancy or certain growth spurts (Zemel et al., 2002). Micronutrient deficiencies in HBSS patients have been associated mostly with iron, folic acid, zinc, copper, and pyridoxine, and the role of these deficiencies has mediated with immunity, as in the case of zinc, the imbalance between TH1 and TH2 functions leads to decreased cell-mediated immune functions (Prasad *et al.,* 1988) and growth (Zemel *et al.,* 2002). Evidence shows that the pathophysiology of SCD has significant nutritional implications which encompasses increased nutrient requirements, nutrient deficiencies and abnormalities in growth. (Al-Saqladi *et al.*, 2008, Bello-Manga *et al.*, 2016, Platt *et al.*, 1984).

Nutritional inadequacies in HBSS patients may be due to decreased nutrient intake, high catabolism due to the disease, and malabsorption along the intestinal tract. Recent data from previous studies revealed normal food intake in HBSS patients. However, as the age of the patients advances, there is a decline in the adequacy of dietary intake. Report shows that IL-6 pro-inflammatory cytokine is elevated in HBSS patients, and this protein is associated with decreased appetite and wasting, suppressed appetite, leading to a reduction in food intake (Desal et al (2020).

Bbosa (2019) and Desai et al. (2020) in their research on supplementation in managing sickle cell anaemia complications, inferred that there is improved weight gain, a decrease in the level of inflammation, decreased oxidative stress, and improved muscle strength and endurance with protein and arginine supplementation, respectively. Similarly, Parveen *et al*. (2017), Rodrigues *et al*. (2021), and Bhagat and Singh (2022) observed that there was improved sexual maturation and reproductive capacity, in linear growth, and decreased number of painful days as a result of zinc and magnesium supplementation. It is believed that a cogent driver of disease complication in SCD is increased rates of metabolic expenditure in individuals (Akohoue *et al.,* 2007, Hyacinth *et al.*, 2010).

Nutritional care should be focused on an aspect of supportive management for patients with SCD since nutritional intervention can be used to address increased energy expenditure and nutritional requirements (CHA 2014; NHS 2010). It was hypothesized that in Africa, undernutrition is a major factor that worsens the prognosis of SCD as a result of inadequate nutrition intake among a significant proportion of the population (Piel *et al.,* 2014). Children in middle- and lower-income countries with SCD may have a higher risk of developing malnutrition. Delayed maturity, stunted growth and poor immunologic functions seen in SCD are mainly attributed to undernutrition associated with the disease (Behera *et al*., 2012).

A major explanation for nutritional deficiency in HBSS patients is hypermetabolism; increased metabolic requirements with reduced nutrient intake, thus lead to nutrient inadequacy. Nutrient supplementation is an area to be investigated in ensuring nutritional adequacy in HBSS patients. One small study revealed improved bone mineral density and normalized vitamin D status with supplements of oral vitamin D and calcium (Grover *et al.,* 2021). It is getting more obvious that emphasis should be

placed on adequate intake of macronutrients in the recommendations for SCD than the traditional supplementation with micronutrients which studies have addressed in comparison with SCD (Hyacinth *et al*., 2010). Erythropoiesis, protein catabolism, myocardial energy expenditure and proinflammatory cytokines contribute to the higher energy requirement (Hibbert *et al.*, 1992, Hibbert et al., 2005, Hibbert *et al.,* 2006). Nutrients from diet and amino acids from protein catabolism are channelled to the replacement of red blood cells which are constantly removed because of haemolysis. These irregularities in the metabolism elevate the energy requirement of the body and impair the availability of nutrients necessary for growth, development and maintaining adequate muscle mass in adults. This leads to severe undernutrition which is clinically manifested.

Singhal *et al*. (2002) studied a group of SCD children and a control group. It was discovered that with similar energy intake, the ratio of energy intake to resting metabolic rate was significantly lower for the SCD children than the control group. It was concluded that the observation indicates a relative energy deficiency in SCD. This agrees with the hypothesis for the increased need for energy from macronutrients. Although this study is on children, it is likely to be the case for adults who inherited the SCA genotype.

The approach to the management of SCD is complex and multifactorial. Nutritional risks are high in SCD, and the use of nutrition as adjuvant therapy in combating several diet-related chronic disorders that are found with SCD is still not made a priority for providing sufficient treatment. The focus had been on increasing red cell count through various means without paying attention to the changes in the forms and function of the sickled red cell which may correlate with developing a nutrient deficiency. Red cell production requires many substrates, most importantly protein. Protein synthesis is associated with a high energy cost and limited nutrient availability for growth and maintenance of body mass. It is important to say that a sufficient diet for a health age, gender and body mass for an individual will not cover the nutritional needs of the person grappling with SCD. Therefore, this study assessed the dietary adequacy and sociodemographic of patients with sickle cell disease.

3.3 Methodology

This is a cross-sectional study conducted at the Department of Haematology and Immunology, Obafemi Awolowo University Teaching Hospital, Ile-Ife Osun State, Nigeria. Ethical approval was sought and obtained from the research and ethics committee of Obafemi Awolowo University Health Centre, Ref: (D.MHS/2023) Ile-Ife, for the study. Informed written consent was obtained from all participants. Ethical approval was also sought and obtained at the Human Research Ethics Committee, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, (CPUT/HWS-REC2021 renewal) Bellville, South Africa.

A total of hundred (100) participants were recruited for the study. Fifty (50) young adults ages 18 to 48 years with sickle cell disease attending the haematology Day Clinic of the Obafemi Awolowo University Health Centre and 50 age-match SCD participants as controls.

Dietary data were obtained through a single 24-hour dietary recall food frequency questionnaire. The patients were made to recount all food and beverages they had consumed in the past 24 hours. An estimate of the quantity of food consumed was derived using food models and serving sizes. Dietary intakes were converted into energy and nutrients using the Nigerian Food Composition Tables, which cover major food concerns in Nigeria and mostly in the Locality. Height in metres and weight in kg were measured using a Stadiometer and a body weighing scale. The height and body weight were recorded for each participant. The height in metres and weight in kg were used to derive the patient's Body Mass Index (BMI).

3.3.1 Data analysis

Data entry and analysis were conducted using SPSS version 21. Body mass index was calculated and graded into thin, normal, overweight, and obese. Food and beverages consumed were converted via the information on serving sizes and provided food models. An estimate of food and beverages consumed were converted to grams. Nigerian food composition tables were used to recall all nutrients and their quantities available in the estimate of food and beverages consumed by respondents.

3.4 Results

The study involved 100 participants, 50 in the test group (Hb SS patients) and 50 (other genotypes/non-Hb SS patients) in the control group. Among the participants, 51% were females, 49% were males of which 77% were under 30 years of age. In the control group,58% were males and 42% females, while males were 40% and females 60% in the test group. Most (96%) of the respondents in the control group and 58% in the test group were under 30 years. The mean age of the respondents was 26.3± 8.61 years. The mean age in the control and test group was 22.5± 4.46 years and 30.2± 9.98 years, respectively.

3.4.1 Nutritional status

The mean weight of the respondents is 57.8 ± 9.50 kg, height 1.7± 0.14 M and BMI 21.3± 3.02 Kg/M^2. The control and test group nutritional status are quite similar; the mean height of the control and test group is 1.70±0.16M and 1.6± 0.08M, respectively. Similarly, the mean weight of the control and test is 63.7± 8.85Kg and 52.0± 5.87Kg, respectively. Also, the mean BMI from the control and test groups are 22.1± 3.04Kg/M^2 and 20.6± 2.85Kg/$M^{2,}$ respectively.

The result of the nutritional status of respondents as measured by the BMI shows that 18% of the respondents were underweight, indicated by a BMI < 18.5 Kg/m2, 73% were within the optimal/ normal range, indicated by a BMI 18.5-24.9 Kg/M^2 and 9% of the respondents were overweight which was indicated by a BMI ranging between 25.0-29.9Kg/M^2.

In the test group, 26% were underweight, while the majority (68%) were within the normal BMI range and a few 6% were overweight. The BMI distribution according to sex in both the control and test groups shows that 6.9% of males and 14.3% of females were underweight in the control group. 79.3% male and 76.2 % female were within the normal range, 13.8% male and 9.5% females were overweight.

In the test group, 30% males and female 23.3% of female were underweight. 13% of males and 21% of females were within the normal BMI range, and 5% of males and 6.7% of females were overweight.

3.4.2 Nutrient adequacy

When the intake of nutrients was compared to the recommended daily allowance, to determine adequacy or inadequacy, it was discovered that most of the respondents (76.3%) did not meet the total calorie expected and 23.7% met the expectation of the total calorie needed daily. More than half (54.6%) met the recommended allowance for protein intake. Similarly, the requirement for carbohydrates as a major macronutrient was observed to be met adequately by 93.8% of the respondents.

However, 80.4% of the respondents had inadequate fibre intake. Additionally, zinc (56.7%), copper (93.8%) and Iron (55.6%) met the recommendation and found adequate in the diet.

In contrast, calcium (99%), magnesium (83.5%), phosphorus (76.3%), potassium (83.5%), sodium (87.6%), vitamin A (85.6%), vitamin E (97.9%), thiamine (70.1%), riboflavin (55.7%), Niacin (81.4%), vitamin B 12 (80.9%) and vitamin C (92.6%) recommendations were not met and found deficient or inadequate in the diet. Also, no respondent met the recommendation for folate, retinol, beta carotene and vitamin D.

Comparing the results between the control and test groups, there were similarities and few contrasts in the adequacy of some nutrients. We observed that 34% of the respondents in the control group had adequate calorie intake and a few (12.8%) of the test group had adequate calorie intake. There was dissimilarity in the protein adequacy in the groups. 78% of the respondents in the control group were reported to have adequate protein intake while just 29.8% in the test group reported having protein adequacy.

Carbohydrate adequacy was found to be similar across the two groups, 92% and 95.7% of the respondents were reported to consume adequate carbohydrates in the control and test group respectively. Fibre consumption was recorded to be generally low compared to the daily recommendation among the respondents, 18.8% and 20.4% had adequate consumption in the control and test group respectively.

There was no record of any respondents in the test group with calcium adequacy and only 2% of respondents in the control group recorded calcium adequacy. Findings from the result show that iron intake was more pronounced among the test group with 79.6% of the respondents having Iron intake adequacy, also, 68% of the respondents in the control group had iron intake adequate.

Some micronutrients such as magnesium, phosphorus, potassium and sodium had similar findings across the groups. The results of the findings on the adequacy of these nutrients across the groups show that most of the respondents had inadequate intake of these nutrients in their diet. For Zinc consumption there were dissimilarities in the findings across the groups, 77% of the respondents had adequate consumption in the control group while more than half (63.3%) in the test group had inadequate consumption.

Copper intake was excellent across the groups, 95.8% and 91.8% of the respondent's intake was adequate in the control and test groups respectively. Results of other vitamins and micronutrients were similar across the group as no respondent was shown to have adequacy of any in their diet.

3.4.3 Mean of Nutrient Intake

The mean of all nutrients is shown in Table 1. The values for major micronutrients and nutrients of importance are stated below.

Mean energy intake in the control and test are 1916 ± 112.8 and 1461 ± 74.93 respectively. The mean values for carbohydrate intake in the control and test are 309.5 ± 18.69 and 264.7 ± 11.77, respectively. The mean values for protein intake in the control and test are 220.2 ± 48.91 and 60.27 ± 9.420, respectively. The mean values for Fat intake in the control and test are 40.78 ± 4.318 and 20.27 ± 2.761 respectively. The mean values for Iron intake in the control and test are 15.85 ± 0.969 and 11.17 ± 0.95 respectively. The differences across the groups are of significance. The test group have lower intakes in comparison to the control group.

Table 3.1: Dietary Intakes of Rsespondents Based on a Single 24-Hr Recall

Nutrient	Daily Mean Intake n = 100			Proportions of Respondents Who Met Recommended Values			
	Control (n = 50)	Test (n = 50)	P value	Control (n = 50) (%)	Test (n = 50) (%)	P value	Chi-square
Energy (kcal)	1.916 ± 112.8	1461 ± 74.93	0.0011	17 (34.0)	6 (12.8)	0.014	6.039
Protein (g)	220.2 ± 48.91	60.27 ± 9.420	0.0018	39 (78)	14 (29.8)	0.001	22.721
Carbohydrate (g)	309.5 ± 18.69	264.7 ± 11.77	0.0453	46 (92)	45(95.7)	0.444	0.585
Fat (g)	40.78 ± 4.318	20.27 ± 2.761	0.0001	-	-	-	-
Fibre (g)	12.29 ± 2.705	27.84 ± 6.225	0.0242	9 (18.8)	10 (20.4)	0.837	0.042
Calcium (g)	260.3 ± 35.37	196.4 ± 29.50	0.1687	1 (2)	0 (0)	0.320	0.990
Iron (mg)	15.85 ± 0.969	11.17 ± 0.95	0.0008	34 (68)	10 (20.4)	0.001	22.701
Magnesium (mg)	187.0 ± 35.59	214.3 ± 36.27	0.5922	9 (18.8)	8 (16.3)	0.754	0.099
Phosphorus (mg)	1001 ± 168.3	657.8 ± 125.1	0.1053	16 (33.3)	7 (14.3)	0.027	4.863
Potassium (mg)	1353 ± 342.3	15830 ± 1463	0.3249	9 (18.8)	7 (14.3)	0.554	0.351
Sodium (mg)	543.4 ± 145.9	676.8 ± 184.9	0.5721	9 (18.8)	3 (6.1)	0.059	0.351
Zinc (mg)	15.98 ± 1.166	11.06 ± 0.94	0.0014	37 (77.1)	18 (36.7)	0.001	16.079
Copper (mg)	18.49 ± 1.932	18.97 ± 4.45	0.9213	46 (95.8)	45 (91.8)	0.414	0.667
Manganese (mg)	30.34 ± 10.15	26.80 ± 9.152	0.7958	21 (43.8)	25 (51.0)	0.473	0.514
Vitamin A (mg)	30.34 ± 10.15	174.6 ± 50.48	0.6938	8 (16.7)	6 (12.2)	0.536	0.384
Retinol (mg)	87.80 ± 18.43	21.78 ± 7.909	0.0013	-	-	-	-
β Carotene (mcg)	4336 ± 1462	3417 ± 1100	0.6154	-	-	-	-
Vitamin D (mcg)	25.30 ± 11.58	1.599 ± 0.462	0.0414	-	-	-	-
Vitamin D (mcg)	1.754 ± 0.516	1.743 ± 0.471	0.9875	-	-	-	-

3.4.4 Age of the individual and body mass index

The result shows that the higher the age of the respondents, the more the BMI values. This implies that underweight abound more in the younger respondents.

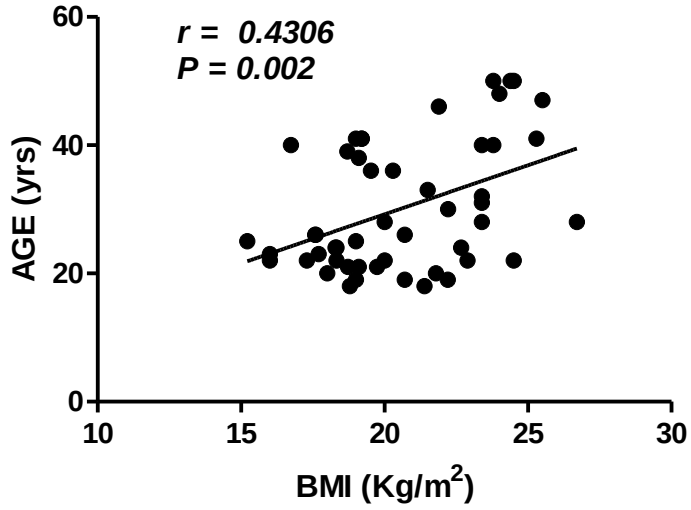

Figure 3.1: Positive correlation between age and body mass index.

3.5 Discussion

The majority of the participants (60%) in the test group were female, this finding was inconsistent with the findings of Osei-Yeboah and Rodrigues (2011), whose male participants were (57%) and Al-Saqladi et al. 2010 whose participants constituted 54.9% male.

According to Aderibigbe *et al.* (1999), research carried out in Ilorin, Nigeria, the findings show that there was significantly lower weight in individuals with SCD. This is similar to the findings of the current research. VanderJagt *et al*. (2000) have similar findings to Aderibigbe *et al.* (1999), as there was significantly lower weight in males with SCD aged 10-18 years. Also, significantly lower BMI was noted for males in the same category. VanderJagt *et al*. (2002) also reported that there was significantly lower weight and BMI in females and males with SCD. Al-Saqladi *et al*. (2008) reported significantly lower weight in individuals with SCD aged 18 years. Glew *et al.* (2003) reported significantly lower weight and height in individuals with SCD when

compared with a control group. Okolosi (2020) reported that SCD is significantly associated with underweight and stunting with males more likely than females. Chinawa *et al*. (1969), report significantly lower weight in individuals with SCD and 48% with SCD were underweight, 13% of controls were underweight. Esezobor *et al*. (2016) reported that 2% of individuals with SCD were overweight or obese. Toly-Ndour *et al*. (2011) reported that 4.3% were overweight or obese and significantly lower BMI in individuals with SCD in Nigeria. Onukwuli *et al.* (2018) observed significantly lower BMI in females individuals with SCD.

3.5.1 BMI

The findings in this study show that overweight exist in sickle cell patients and this agrees with the findings of (Chawla *et al.*, 2013) who reported 13% being overweight and obese as a young adult with SCD. A study by Akodu *et al*. (2012) showed that 2.5% of his subjects were obese, this is quite different from the findings of our study, as no respondent was categorized as obese. The findings of the nutritional status of the test and control groups are different from the findings of Ukoha *et al*. (2020) as a significant proportion of the sickle cell patients are within the normal BMI range.

The general belief with sickle cell patients has been that most patients are stunted from their childhood. Hence, the findings from this study are of interest as we observed that 6% were overweight. This is similar to the findings by Ukoha *et al*. (2020). In the reports from Chawla *et al*., 22.4% and Halpern 25% are quite higher than that of this study. Also, findings from Akodu *et al.* (2012) research were lower as it was reported that 2.5% of the respondents were obese.

3.5.2 Nutrient adequacy

Vitamin D is essential for calcium homeostasis and bone mineralization. Its deficiency is common in sickle cell disease which results from dark skin pigmentation, limited sun exposure, and increased catabolism of nutrient and energy intake. This deficiency affects up to 8% of SCD patients and it contributes to the occurrence of osteopenia and osteoporosis (Umeakunne and Hibbert, 2019). It was discovered that dietary

intake of vitamin D among the SCD patients was inadequate, this is consistent with the findings of Osei-Yeboah and Rodrigues (2011).

3.6 Conclusion

The findings from this study showed that the dietary intake of SCD patients' needs improvement to ensure nutrient adequacy. From the literature reviewed, it was observed that SCD patients may need to consume more than their non-SCD counterparts to maintain a healthy body due to the burden the disease places on their nutrient store. The findings from this study reveal that the diet of SCD patients examined was less adequate. Carbohydrate intake examined was met, however, protein intake was found inadequate. Although, iron intake was found to be excellent in SCD patients all other micronutrients were not adequately consumed. The nutrient adequacy measured reflects the nutritional status of SCD patients as a BMI < 18.5Kg/M^2 was found. There is a need for nutritional management to go with the medical care in the management of SCD patients. Nutritional management should focus on the conscious selection of food to ensure the adequacy of macro and micronutrients necessary for optimal functioning of the body and maintaining good nutritional status.

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CHAPTER FOUR

INFLAMMATORY AND OXIDATIVE STRESS MARKERS IN MULTI-TRANSFUSED SICKLE CELL DISEASE PATIENTS

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4.1 Abstract

4.1.1 Background

Sickle cell anaemia (SCA) is a form of haemolytic anaemia caused by an abnormal composition of the globin chains of haemoglobin. When the mutated haemoglobin is exposed to low-oxygen concentration, it polymerises into long crystals of red blood cells giving the cell an abnormal sickled shape with extreme fragility, and less flexible erythrocytes with a reduced lifespan. These changes lead to various complications such as stress, trauma, dehydration, vascular occlusion, and haemolysis including inflammation and oxidative stress which has been implicated in SCD patients. This study investigated the relationship between markers of inflammatory and oxidative stress in alloimmunized sickle cell patients.

4.1.2 Methods

A hundred (100) participants were involved in the study; fifty (50) young adults aged 18 to 48 years, diagnosed with SCD and receiving care at the Haematology Day Clinic within the Obafemi Awolowo University Health Centre, and a healthy group of fifty (50) individuals, matched for age but without SCD disease, served as the control group. This was a cross-sectional study and samples were analysed for inflammatory and oxidative biomarkers as well as liver and kidney function tests following the manufacturer's instructions.

4.1.3 Results

From the analysis, both biochemical parameters and inflammatory markers show a statistically significant increase ($p = 0.003$) in the means of test groups compared to the means of the control groups for the following: SOD (pg/mL) = 284.2, AST (μ/L) = 46.94, ALT (μ/L) = 37.0, CREAT (μmol/L) = 56.71, and Urea (μmol/L) = 3.4. Additionally, there was a significant difference ($p = 0.003$) between the means of CRP (ng/mL) = 3.9 and TNF (pg/mL) = 8.1 in the test groups compared to the control groups.

4.1.4 Conclusion

Frequent transfusions in SCD serve to prevent and alleviate microcirculatory complications and the breakdown of red cells that incite inflammation and oxidative stress, potentially mimicking alloimmunization. It also serves to reduce the risk of organ damage by maintaining low serum creatinine and urea levels. From this study, it is important that oxidative and Inflammatory markers should be included in the proper monitoring of SCD patients.

4.2 Introduction

Sickle cell disease (SCD) is a worldwide health concern ravaging the health of the African population, especially in Sub-Saharan Africa, where its prevalence is highest [1]. This hereditary genetic condition, linked to chromosome 11a, stems from alterations in the deoxyribonucleic acid (DNA) structure, causing the replacement of glutamic acid with valine at the sixth position of the beta-globin chain. About 20 million people are affected globally, with more than 11.5 million prevalence in Africa alone [2]. Children under the age of 5 years with SCD record a mortality rate of 85 % in Africa [3]. In Ghana, the prevalence rate is 2% of the annual births [4]. Nigeria shares 2-3% SCD of the total populace, with a 24% prevalence of sickle cell trait and about 100,000 annual SCD infant deaths [5].

Sickle cell patients often suffer from the complications of oxidative stress and inflammation [6, 7]. The complexity of SCD extends beyond the genetic mutation, encompassing the intricate interplay of inflammation and oxidative stress. These complications, working synergistically, manifest either as oxidative stress due to the gradual increase of inflammatory processes or inflammation resulting from the aftermath of oxidative reactions. Oxidative stress, an additional pathophysiological phenomenon in SCD, results from an imbalance between the release of reactive oxygen species (ROS) and the body's defence capacity [7, 8]. These molecules are generated during regular metabolic processes and play essential roles in signalling, receptor activation, nuclear transduction, and gene expression. However, when there's an imbalance between ROS production (including ROS and reactive nitrogen species, RNS) and the body's antioxidant defences, oxidative stress occurs, leading to cellular

damage [6]. It is intimately linked to inflammation and endothelial destruction, exacerbated by chronic inflammation and intravascular haemolysis [6].

The origin of the chronic inflammatory state in SCD remains unpredictable, although certain factors could contribute to it such as red cell deformability, haemolysis, histamine release, thrombin generation, and complement activation collectively resulting in inflammation in SCD patients, with haemolysis playing a pivotal role]9]. Haemolysis, particularly its impact on anti-inflammatory molecules like nitric oxide (NO) and heme oxygenase 1 (HO-1), obstructs their functionality, leading to excessive development of reaction products [7-12].

Blood transfusion is important as it is a lifesaving therapy for SCD patients, but often results in severe iron overload [8], increased biomarkers of oxidative damage [7], immune antibodies, and haemoglobin binding to the cell membrane acting as a Fenton reagent, thereby elevating the production of oxidants such as superoxide and hydroxyl radical [7, 9]. The increase in the components of haemolysis such as intravascular haemoglobin and oxidants may contribute to the consumption of nitric oxide in SCD [11]. In contrast, a reduced level of NO, may lead to haemodynamic instability [12, 13], and a reduction in antioxidant capacity [14]. Efforts to monitor oxidative damage in SCD involve measuring biomarkers such as F2-isoprostanes, 4-hydroxynonenal (4-HNE), SOD, and malondialdehyde (MDA). These stable end products of oxidative reactions provide valuable insights into oxidative stress levels and disease severity, with increased levels noted in SCD [15, 16]. Advanced glycation end-products (AGE) also serve as indicators, correlating positively with haemolysis-related tissue complications in patients with SCD [17].

Besides blood transfusion, other therapies used in the management of SCD complications include hydroxyurea, voxelotor, crizanlizumab and L-glutamine therapy, of which voxelotor 900mg seems to be more effective, in that the efficacy yielded elevated haemoglobin levels and reduced markers of haemolysis in SCD [18]. In addition, voxelotor reduces the risk of stroke, albuminuria, pulmonary arterial hypertension and mortality [18].

Inflammation and oxidative stress pose significant challenges for individuals with SCD who undergo frequent transfusions. The repetitive administration of blood transfusions can induce the production of allogeneic antibodies among other complications, thereby exacerbating oxidative stress levels. Moreover, chronic inflammation is a characteristic of SCD, as the presence of damaged red cells triggers immune responses. Vaso-occlusive crises (VOCs) and iron overload resulting from recurrent transfusions can inflict damage on various organs, such as kidneys, lungs, pancreas and the brain [14]. Consequently, the focus of this study is to unravel the relationship between markers of inflammation and oxidative stress and organ damage in patients with SCD.

4.3 Aim

This study assessed inflammatory and oxidative stress markers in multi-transfused patients with sickle cell disease to better understand their roles in the pathophysiology of SCD.

4.4 Research Design and Methodology

This is a cross-sectional study conducted at the Haematology and Immunology Department of Obafemi Awolowo University Teaching Hospital, Nigeria. We investigated the relationship between oxidative stress and inflammatory profiles in sickle cell patients who received two or more pints of blood transfusion with a group of individuals without the disease (the control group) but received equivalent blood. A questionnaire was served to compile some information from participants.

4.5 Inclusion criteria

Inclusion criteria for this study involved SCD patients with multiple blood transfusions. The diagnosis of SCD was confirmed with the haemoglobin electrophoretic method.

4.6 Exclusion

Patients who were not diagnosed with SCD and were not between 18 and 48 years of age were excluded. Additionally, individuals experiencing crisis were also excluded.

4.7 Ethical Consideration

The study received ethical clearance from both Cape Peninsula University of Technology, (CPUT/HWS-REC2021 renewal) Bellville, South Africa (the Human Research Ethics Committee, Faculty of Health and Wellness Sciences), and the ethics committee of Obafemi Awolowo University Health Centre, Ref: (D.MHS/2023) Ile-Ife. Informed written consent was obtained from all participants involved in the research.

4.8 Analysis

Enzyme-linked immunosorbent assay (ELISA) KITS for Tumour Necrotic Factor (TNF), C-Reactive Protein (CRP), Interleukin 6 (IL-6), and Interleukin 1 beta (IL-1β) manufactured by Elabscience Biotechnology. USA. were used for the inflammatory analysis.

Using the micro-ELISA plate, samples were added to the wells and incubated with specific antibodies after which antibody specific for Human IL-1β, TNF- α, IL-6, and CRP. Avidin-horseradish peroxidase (HRP) conjugate was included in all the microplate wells for incubation, after which washing took place and substrate solution was added. Those containing Human IL-1β, TNF- α, IL-6, and CRP and Avidim -HRP conjugate will appear blue, the reaction is stopped by adding a stop solution and it turns yellow. The concentration of the samples is proportional to the optical density values read at a wavelength of 450 ± 2mm. The analytes' levels in the quality control reagents of the kits were within the expected ranges [19].

4.8.1 Urea (Kidney Function Tests)

Urea is hydrolysed to produce ammonia and carbon dioxide in the presence of water and urease. The ammonia released combines with -oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH) to yield glutamate and oxidised nicotinamide (NAD+) in the presence of glutamate-dehydrogenase. Levels were determined according to previously described methods ([20, 21]

4.8.2 Creatinine

The muscle tissues are responsible for the production of creatine and phosphate in the body. Creatinine is a Nitrogenous byproduct. Creatinine is not stored in the body; therefore it cannot be reutilised but is excreted as waste proportional to the body's muscle mass. Creatinine measurement determines kidney efficiency. It has some merits over the assessment of urea because it does not depend on any substances like protein ingestion, or water intake and is a constant. An increased amount is indicative of kidney dysfunction. Decreased levels of plasma creatinine seem rare and not clinically significant. It was determined according to previously described method [22]

4.8.3 AST (Aspartate aminotransferase or glutamate oxaloacetate) Liver Function Test

Aminotransferases are a variety of enzymes that speed up the conversions of amino acids and α- oxoacids by the transfer of amino groups. Some cells have been studied and AST is seen in cytoplasmic and mitochondria of cells. In cases of mild tissue damage (e.g. Liver) AST is more domicile in the cytoplasm than mitochondria, therefore severe organ destruction results in increased mitochondria enzymes being released. Increased levels of AST indicate hepatic disease, muscular dystrophy, myocardial infarction, and organ damage. This was determined according to previously described methods [23, 24].

4.8.4 ALT (Alanine Aminotransferase)

This is a maximum standard method according to the concentrations recommended by the International Federation of Clinical Chemistry (IFCC) [25].

4.9 Statistical Analysis

Data were to undergo statistical analysis using SPSS version 24.0 statistical package and relevant statistical values were obtained. Student *t-test* was carried out and data were presented as mean ± standard deviation (SD), A Chi-square test was also carried out where appropriate. Values of $P<0.05$ were considered statistically significant.

4.10 Results

4.10.1 Demographics profile of the participants

This study comprised of two different groups: the control and the test group. The control group consisted of 26 (52.00%) females and 24 (48.00%) males, while the test group consists of 30 (60.00%) females and 20 (40.00%) males. The subjects were educated young African adults, aged 18 to 48 years. The body mass index (BMI), weight, and height were measured. There was no significant difference between gender, age, and haemoglobin genotypic variation. as shown in **Table 4.1**

Oxidative stress markers are represented in **Table 4.2**. Superoxide dismutase (SOD) showed a significant difference with P = 0.008 in the test group compared to the control group, whereas catalase showed no significant difference (P= 0.079) in both the test and the control groups.

Table 4.3 Represents markers of inflammation. CRP and TNF were significantly higher in the test group compared to the means of the control group. Whereas IL-6 and IL-1β showed no significant difference between the means of the test and control groups. Biomarkers such as creatinine (CR) and urea (U) showed a significant increase in the means of the control groups compared with the test groups, as represented in **Table 4.4.** Additionally, the enzymes AST and ALP showed a significant increase (P = 0.036 and P = 0.001 respectively) in the test group compared with the control groups. **Table 4.5** shows a notable positive correlation between CRP and IL-6, TNF and catalase. However, a significant negative correlation (P< 0.05 r=) with AST and ALT, IL-1β, SOD, creatinine and Urea.

Table 4.1: **Demographics of sickle cell disease and control subjects**

Age (years)	Control n=50(%)	SCD n= 50(%)	Total n=100(%)	Statistical indices
<= 20	08 (16.00)	08 (16.00)	16 (16.00)	X^2 = 0.1926, df =3 P-value = 0.979
21 – 30	19 (38.00)	21 (42.00)	21 (11.73)	
31 – 40	13 (26.00)	12 (24.00)	54 (30.17)	
41+	10 (20.00)	09 (18.00)	27 (9.50)	
Gender				
Female	26 (52.00)	30 (60.00)	56 (28.00)	X^2 = 0.6494, df =1 P-value = 0.4203
Male	24 (48.00)	20 (40.00)	44 (22.00)	
Diagnosis				
ANAEMIA	21 (42.00)	0 (0.00)	21 (21.00)	X^2 = 100.00, df =7 P-value < 0.0001*+
CML	04 (8.00)	0 (0.00)	04 (4.00)	
HB SS	0 (0.00)	50 (100.00)	50 (50.00)	
MM	01 (2.00)	0 (0.00)	01 (0.50)	
SEPSIS	04 (8.00)	0 (0.00)	04 (4.00)	
STOMACH PAIN	01 (2.00)	0 (0.00)	01 (0.50)	
SURGERY	15 (30.00)	0 (0.00)	15 (15.00)	
TRANSFUSION	04 (08.00)	0 (0.00)	04 (2.00)	

+ Significant p value, * chi square test

Sickle cell disease patients are less likely to be diagnosed with different diagnoses than control subjects. However, there was no significant association between gender, age and haemoglobin genotypic variation.

Table 4.2: Mean and standard deviation of biochemical enzymes of all participants

	M	SD	P-value
AST (µ/L)	46.9	38.70	0.036*
ALT (µ/L)	37.0	37.44	0.001*
CREATININE (µmol/L)	56.7	23.98	0.005
UREA (µmol/L)	3.4	1.68	0.004
CATALASE (µ/mL)	139.6	73.82	0.079
SOD (ng/dL)	271.8	47.52	0.008

M= mean, **SD**= standard deviation
AST: Aspartate transaminase; ALT: Alanine transaminase; SOD: Superoxide dismutase

Table 4.3: **Inflammatory parameters of participants by study group**

	GROUP						
	Control		Test				
	M	SD	M	SD	t	Df	p
CRP	1.2	2.78	3.9	5.62	-3.099	97	0.003 *
IL-6	84.3	101.96	58.1	86.10	1.380	97	0.171
TNF	2.8	5.76	8.1	14.31	-2.449	97	0.016 *
IL-1β	2.5	.38	4.1	6.73	-1.710	97	0.090

CRP: C-reactive protein; IL-6: Interleukin 6; TNF: Tumour necrosis factor; IL-1: Interleukin 1

Table 4.3: Shows the independent-samples t-test for the inflammatory parameters of participants by study group. The result showed that there was a significant (p = 0.003) difference between the mean of the control and test groups for CRP(ng/mL)= 3.9 and TNF(pg/mL) = 8.1; (p = 0.016). The result also showed no significant difference between the mean of the control and test groups for IL-6 pg/mL = 84.3 vs 58.1; p = 0.171 and IL-1βpg/mL = 2.5 vs 4.1; p = 0.090 respectively.

Table 4.4: Mean and standard deviation of biochemical enzymes of participants by study group

	GROUP						
	Control		Test				
	M	SD	M	SD	T	Df	p
AST (µ/L)	29.94	40.51	46.94	38.90	2.130	97	0.036*
ALT (µ/L)	14.90	13.65	37.00	37.63	3.866	97	0.001*
CREATININE (µmol/L)	140.82	203.72	56.71	24.98	2.899	97	0.005
UREA (µmol/L)	6.886	8.31	3.4	1.68	2.918	97	0.004
CATALASE (µ/mL)	152.7	77.92	126.7	67.87	1.778	97	0.079
SOD (ng/dL)	259.1	41.91	284.2	49.77	-2.716	97	0.008*

P<0.05 indicates a significant difference.
AST: Aspartate transaminase; ALT: Alanine transaminase; SOD: Superoxide dismutase

Table 4.5: Correlation between CRP and other study parameters.

CRP.	AST (µ/L)	ALT (µ/L)	IL-6	TNF	IL-1β	CATALASE (µ/mL)	SOD (ng/dL)	CREATININE (µmol/L)	UREA (mmol/L)
--	-.094	-.096	-.210*	.210*	.018	-.222*	-.079	-.109	-.112

* indicates a significant correlation between CRP and study parameters

4.11 Discussion

Findings from this research revealed that the inflammatory parameters of participants were significantly increased in the test group for C-reactive protein (CRP) (ng/mL) = 3.9; p = 0.003 and tumor necrosis factor (TNF) (pg/mL) = 8.1; p = 0.016 compared to the control group. Using T-test with SPSS statistical package version 24, these results are consistent with earlier studies by [26] which reported higher CRP and TNF levels in SCD compared to controls. Clinically, increased CRP in SCD patients denotes the presence of a severe health condition that causes acute inflammation. Repeated CRP levels are useful in monitoring patients with inflammatory challenges like SCD, especially in the first week of follow-up of antibiotic treatment. A prolonged delay in the reduction of CRP levels could be associated with an increased risk of inappropriate treatment with antibiotics [27]. The increase in CRP is indicative of inflammation, infection, or injury in the body. It is associated with vaso-occlusion [28] but evidence suggests that patients with SCD have moderately increased CRP during steady state and that this increases significantly during painful vaso-occlusive crisis and haemolysis.

TNF was significantly increased in SCD patients compared to controls and this concurs with previous reports [29]. TNF-alpha is a pro-inflammatory cytokine and an early indicator of inflammation secreted by macrophages/ monocytes during acute inflammation and is accountable for a huge range of signaling events that lead to

apoptosis and necrosis. This protein is also necessary for the inhibition of infection and cancers[30].

Additionally, as observed in this study, SOD showed significantly increased activity in the test group compared to the control which supports studies by Gizi, Papassotiriou [31], and Younus [32]. SOD is an essential antioxidant defense against oxidative stress in the body. It acts both as an enzyme and curative agent ROS. Antwi- Boasiako et al. [33] reported low SOD, and Repka and Hebbel [9] recorded an increase in biomarkers of oxidative damage both in SCD and thalassaemia. Some other authors, like Lazzaretti et al. [34] and, Schater et al. [35], also reported low activity. In this study, SOD was significantly increased. This might be because our subjects had received a series of blood transfusions [35].

The cause of inadequate oxygen in SCD patients is the sickled shape of the erythrocytes, which deprives and short-changes the normal amount of oxygen to the organs of the body (Ischaemia). This is one of the challenges that regular blood transfusion tends to solve although some risks are involved such as increasing chances of foreign antigens and the risks of producing alloantibodies which cause DHTRs [36]. Conrath [37] and Khatun [15] reported that multiple blood transfusions (MBT) can lead to transfusion-transmitted infections, iron overload, and inflammation [16, 17]. The end products of haemolysis (haem and free haemoglobin) have been implicated in the activation of the innate and adaptive immune responses. Allalli et al. [38] suggested that patients with high haemolysis rates are at greater risk of early mortality [37]. The continual breakdown and destruction of erythrocytes result in sustained activation of innate immune cells, resulting in a chronic inflammatory state [39]; [40]; [41].

A significant difference between the two groups was recorded for aspartate aminotransferase (AST) and alanine aminotransferase (ALT). This concurs with the findings of Taiwo et al., [42], who reported that there is dysfunction of the liver in the steady state SCD. Also, according to Kotila et al., [42] extreme haemolysis causes hyperbilirubinaemia, leading to increased alkaline phosphatase (ALP) in SCD patients. In addition, haemolysis raises AST and ALT levels in SCD. Emokpae and Umeadi [43]

reported a high levels of both AST and ALT in SCD patients for AST and ALT which is similar to this current study.

Creatinine (Cr) and urea (U) are markers of renal function. Cr showed a significant increase in the control group compared with the test group, which affirms report of Fowler et al [44] report. In our study, serum creatinine was reportedly low in SCD patients, which contradicts that of [44]. This discrepancy might be due to the series of precautionary measures prescribed for their patients before the test was carried out. They were not allowed to take medications, block the renal pathway to protein or creatinine load and alter renal haemodynamics. However, in our study, there were no restrictions related to renal function pathways and most of our subjects were multi-transfused.

Serum creatinine is a biochemical substance that usually is filtered out of the body through a healthy kidney. However, accumulation and increased levels of creatinine jeopardize and render the kidney dysfunctional and put the patient at risk. Furthermore, when sickled red blood cells lack sufficient oxygen (hypoxia), it results in dysfunction of the heme and triggers haemolysis (due to antigen-antibody incompatibility), accompanied by a sickle cell pain crisis Vaso-occlusion (VOC), microvascular occlusion and tissue Ischaemia brings about the pathophysiology of SCD which eventually prompt renal damage or failure. Consequently, creatinine and urea were significantly different to the control which support previous results [45, 46].

4.12 Conclusion

In summary, our findings revealed a significant increase in inflammatory markers, such as CRP and TNF, among sickle cell patients. Moreover, there was a notable increase in superoxide dismutase (SOD) levels alongside enzymes such as AST and ALT. Furthermore, sickle cell patients demonstrated lower urea levels than the control group. These results underscore the fact that haemolysis could trigger inflammation and pro-inflammatory cytokines and induce oxidative stress in sickle cell disease patients, ultimately posing challenges to the efficacy of the immune system in the long term. It is therefore recommended to include markers of inflammation and oxidative

stress in routine examinations to gain a better understanding of their roles in the pathophysiology and appropriate monitoring and management of SCD patients.

4.13 Recommendation

The primary strength of this study is that it is the first to be conducted in southwest Nigeria. It provides a better understanding of the relationship between inflammation and oxidative stress biomarkers in multiple transfused patients with sickle cell disease. The management of SCD should be supported with blood transfusions on the condition that the patients need it and it would improve the quality of life and longevity of the patients. Future research should explore specific immunological mechanisms to provide deeper insights.

4.14 Limitation

This study faced certain limitations. Initially, we intended to work with a larger sample size; however, logistical challenges and some individuals' unwillingness to participate prevented us from achieving this goal. A larger sample size could have provided more robust data.

4.15 Authors contribution

O.O Oguntibeju read, reviewed and edited the manuscript. He supervised the research work acted as resource administration and secured funding.

G.M Davison read, reviewed, and edited the manuscript. She co-supervised the research work.

T. Oduola, read, reviewed and co-supervised the research work.

F.I Aboderin: Conceived the idea, conception designed, data collection, sample analysis and writing manuscripts.

Conflict of interest: The authors do not have any conflict of interest.

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CHAPTER FIVE

INVESTIGATION OF HAEMATOLOGICAL, INFLAMMATORY PARAMETERS AND THE INCIDENCE OF ALLOIMMUNIZATION IN MULTI-TRANSFUSED SICKLE CELL DISEASED PATIENTS

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5.1 Abstract

Sickle cell disease (SCD) is a haemoglobinopathy caused by an aberrant mutation of the β globin chain resulting in the amino acid valine replacing glutamic acid at the 6th position. Patients with sickle cell disease suffer from complications including chronic inflammation and the development of allogeneic antibodies due to multiple blood transfusions. This study investigated the association between haematological, inflammatory markers and alloimmunization in multi-transfused patients with SCD.

5.1.1 Methods

This was a cross-sectional study, that enrolled 100 participants, including 50 young adults (18-48 years) with homozygous SCD from the Obafemi University Health Centre in Nigeria, and compared the results to a group of age and sex-matched individuals who did not have the disease (the control group) but who had also received blood transfusions.

Full blood counts (FBC) and differentials were processed on an auto-analyser (SFRI H18 Light, France). Red cell antigen Identification was by saline and anti-human globin (AHG) method while the abnormal haemoglobinopathy was evaluated using the electrophoretic method. ABO and Rhesus blood groups were analysed using a direct method on tile, and the determination of inflammatory markers, including C-reactive protein (CRP), Tumour necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-1β (IL-1β) was by the enzyme-linked immunosorbent assay (ELISA) technique. The data were statistically analysed using SPSS version 24.0 and GraphPad Prism version 8. Additionally, student t-tests and Chi-square tests were performed as appropriate. Data were presented as mean ± standard deviation, with $P<0.05$ considered statistically significant**.**

5.1.2 Results

As expected, those with SCD had an increased rate of alloimmunization and significantly reduced haemoglobin and red cell parameters except for the Mean Cell Volume (MCV) which was similar to those without SCD. Although both groups had platelet counts within the reference range, those with sickle cell disease had significantly elevated counts compared to those in the control group. Those with SCD

displayed evidence of inflammation with significantly increased levels ($p = 0.001$) of C-reactive protein and the pro-inflammatory cytokine TNF. This was supported by higher white cell counts and neutrophilia. Most of the antibodies detected in SCD were anti-Kell, Jka and Fya, while the controls displayed anti-M and Kell antibodies. Despite the elevated inflammatory markers, no significant correlation was observed between these and the rate of alloimmunization.

5.1.3 Conclusion

In this study of 100 participants, those with SCD had an elevated rate of alloimmunization with higher levels of anti-kell, Jka and Fya as well as the inflammatory markers, TNF and CRP when compared to the control group. However, despite these findings, no significant correlation between inflammatory markers and alloimmunization could be detected. This suggests that elevated alloimmunization rates are multifactorial and involve other processes which require further investigation.

5.2 Introduction

Sickle cell disease (SCD) is a genetic disorder characterized by haemoglobin S resulting from the inheritance of an abnormal β-globin chain gene from one or both parents. Globally, 50 million people are affected, with Africa experiencing approximately 50 to 90% childhood mortality [1]. In Ghana, screening of newborns between 1995 to 2004 recruited 177,283 newborns, and 3,346 were found to have SCD. SCD makes up about 2% of 5 million total births annually [2]. Meanwhile, in Nigeria, about 24% of the population has SCT, with an estimated 150,000 babies born with SCD annually. SCD makes up 3% of the total births, while the annual infant mortality is approximately 100,000 [3].

In Africa, blood transfusion remains the most common form of therapy for SCD and has effectively reduced complications, including vaso-occlusive crisis (VOC), acute pain, and chest pain syndrome, by increasing the oxygen-carrying capacity of blood [4]. However, there are risks associated with blood transfusions, such as blood-borne diseases and allergic and haemolytic reactions. Chronically transfused patients suffer

from iron overload and alloimmunization, which is particularly prominent in those suffering from SCD. The production of alloantibodies can affect up to one-third of the SCD population, potentially resulting in delayed haemolytic transfusion reactions (DHTR) [5]. Identifying suitable and compatible blood for a patient with multiple alloantibodies has, therefore, become a challenge [6].

The presence of delayed reactions and the development of antibodies in multi-transfused patients has been implicated in various pathological conditions. It has been proposed that one of the causes of the increased alloimmunization rate in patients with SCD is chronic inflammation [7].

Inflammation arises from an abnormal activation of innate immune responses, which can be initiated by the process of haemolysis. This commences chronically in SCD when red blood cells are damaged, releasing various molecules into the peripheral blood, including sickle haemoglobin (HbS) and heme (iron compound). It was recently discovered that free heme increases in both SCD and beta thalassaemia, however, the inflammation in SCD is triggered by the circulating abnormal haemoglobin [8]. The abnormal haemoglobin S binds to Toll-like receptor 4 (TLR4: also known as CD284), a key activator of the innate immune response expressed on monocytes. [9, 10]. The resulting inflammation has been correlated with mortality; therefore, it has been hypothesized that elevated levels of pro-inflammatory markers could predict the development of allogeneic antibodies.

In a murine model, it has been observed that inflammation plays an important role in RBC alloimmunization [11]. This work supported the theory that SCD is characterized by chronic inflammation [12] and has led to the hypothesis that inflammation plays a role in the increased rate of alloimmunization observed in these patients despite little published data. This hypothesis appears reasonable because inflammatory signals activate the immune response and advance the recognition of foreign antigens. The release of cytokines, such as interleukin -6 (IL-6), interleukin-1 (IL-1), and tumour necrosis factor-alpha (TNF-α), as well as the activation of antigen-presenting cells and tissue damage, may lead to the initiation of alloimmunization when foreign antigens are introduced during transplantation and transfusion [12-14].

Alloimmunization poses a complex challenge, with the risk increasing after every additional blood transfusion [13]. A study carried out in the United States reported that 50% of all immunized subjects had multiple antibodies [14] [15, 16]. Over time, many of these became undetectable, potentially challenging future transfusion and putting the patient at risk of a DHTR [17]. The most common red cell antigens involved are the Rh, Kell, Kid, Duffy, Lewis, and MNS blood group systems. [18], [19] Other factors include the recipient's age and sex, number and frequency of transfusions, history of pregnancy, recipient clinical diagnosis and treatment, ethnic differences between recipient and donors and genetic factors related to antigenic response [16].

The overall incidence of post-transfusion alloimmunization in Nigeria varies from 18.7% [20] to lower rates of 8.8% [21]. These depend on the region where the study took place. For instance, Kangiwa et al.'s study was carried out in Northern Nigeria while Obi et al.'s in the Eastern part of the country,

With this background, it has been hypothesized that those with elevated levels of inflammatory markers have a higher risk of alloimmunization and delayed transfusion reactions. Therefore, this study investigated the association between the increased incidence of alloimmunization in multi-transfused patients with SCD and chronic inflammatory markers. The study focused on a cohort of patients with SCD diagnosed at the haematology department of an academic hospital in Nigeria.

5.3 Research Design and Methodology

This was a cross-sectional study conducted at the Department of Haematology and Immunology, Obafemi Awolowo University Teaching Hospital, Nigeria. The full blood count, differential count, inflammatory markers, and blood group antigen profiles of sickle cell patients who received two or more pints of blood were compared to a group of individuals without the disease (the control group) but who also received blood transfusions.

5.3.1 Inclusion criteria

All patients who were diagnosed with SCD and had received at least two pints of blood were included in the test groups. The control group were made up of individuals who

did not have SCD but were age and sex-matched. This control cohort who had received at least two pints of blood for reasons which included anaemia, surgery, bleeding, leukaemia, and loss of blood through trauma.

5.3.2 Exclusion

All patients who did not have sickle cell disease (SCD) and were not between 18 and 48 years of age were excluded. Additionally, individuals who were experiencing a sickle cell crisis were also excluded.

5.3.3 Ethical Consideration

The study received ethical clearance from both the Human Research Ethics Committee, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology (CPUT/HWS-REC2021 renewal) Bellville, South Africa and the research and ethics committee of Obafemi Awolowo University Health Centre, Ref: (D.MHS/2023) Ile-Ife. Informed written consent was obtained from all participants involved in the research.

5.4 Diagnosis of sickle cell disease

All SCD patients were diagnosed according to established criteria using traditional haematological parameters, including blood smear morphology. Thereafter, the diagnosis was confirmed using haemoglobin electrophoresis, which was performed on the Helena manual electrophoresis instrument (Helena Biosciences UK) according to a method previously described ([22]

5.5 Analysis of samples

Six millilitres of blood were collected into ethylene diamine tetra acetic acid (EDTA) and serum separator tubes. A Complete Blood Count (CBC), Haemoglobin electrophoresis, ABO and Rhesus blood group and red cell antibody typing of Rh (D, C, E,c,e), Kell (K,k), Duffy (Fya, Fyb) and Kidd (Jka, Jkb) M, N, S,s, PI, Lu^a, Kp^a, Le^a, Le^b were examined in both the test group and controls. The red cell antibodies were interpreted using the ID panel profile. Tumour necrosis factor alpha (TNF-α), C-reactive protein (CRP), Interleukin 6 (IL-6), and Interleukin 1 beta (IL-1β) were also analysed using an ELISA.

5.5.1 Full blood count and blood smear analysis

Complete blood counts were performed using an H18Light auto analyser (SFRI, France which uses the impedance technique to enumerate the number of blood cells and spectrophotometry to determine haemoglobin levels. Red cell indices were calculated using the red cell count and haemoglobin values. Before analysis, 3 levels of control were used to ensure the accuracy of the autoanalyzer.

A routine blood smear was stained with Leishman stain for 3-5 minutes. After washing, the slides were allowed to dry before examining under x100 Objectives. A manual differential was performed, and the red cells were examined for sickling and other red cell abnormalities.

5.5.2 Haemoglobin Electrophoresis

Blood samples were haemolysed using haemolysate and an appropriate volume of Tris buffer (P^H 8.4) was added to the electrophoresis chamber. The cellulose acetate paper was soaked for 20 -30 minutes in the buffer, after which the excess was blotted and, 0.5-0.6 ml of the specimen was applied. The cellulose acetate paper was placed in the electrophoresis chamber and covered to run at 450 volts for 20 minutes. The cellulose paper was then removed and stained with Ponceau S for three minutes. The results of the abnormal haemoglobins were compared with the relative mobility of the control samples [23].

5.5.3 Determination of ABO and Rhesus Blood Group

Biotech's blood grouping reagents for ABO and Rhesus were used to determine the blood groups [24]. This was achieved by tile grouping and confirmed by tube grouping methods utilizing anti-sera A, B and D for Rhesus, while tube grouping employed pooled cells A, B and O. Equal volumes of each cell and antisera were added and mixed and agglutination was observed and interpreted appropriately. Red cell antibody analysis was carried out to determine the presence of clinically significant red cell antibodies such as Kell, Kidd, and Lewis which could cause alloimmunization. This was achieved using the ID panel cells for red cell antibodies (ID Panel cells, product

code PR144, NHSBT Reagents) which were processed according to the manufacturer's instructions (NHS Blood Transplant PR 1444 (2020).

5.5.4 Inflammatory markers:

ELISA KITS for TNF, CRP, IL-6, and IL-1β manufactured by Elabscience Biotechnology, USA. were used for the measurement of inflammatory cytokines. The micro-Elisa plates were pre-coated with antibodies specific to Human TNF-α, CRP, IL-6, and IL-1β. Samples (or standards) were added to the micro-ELISA plate wells and incubated with each specific antibody. Thereafter, a biotinylated detection antibody and Avidin- Horseradish peroxidase (HRP) conjugate was added to all the microplate wells. Free components were washed away, and a substrate solution was added. Only those wells that contained Human TNF-α, CRP, IL-6, and IL-1β, biotinylated detection antibody and Avidin-HRP conjugate appeared blue and the reaction was terminated by the addition of a stop solution resulting in the colour turning yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 ± 2mm with the OD values being proportional to the concentrations of the relevant analyte. The concentrations of each analyte were calculated by comparing the OD of all the respective samples to their standard curves.

5.6 Statistical Analysis

Data were subjected to statistical analysis using SPSS version 24.0 and GraphPad Prism version 8 statistical package and relevant statistical values were obtained. Student *t-test* was carried out and data were presented as mean ± standard deviation (SD). The Chi-square test was also carried out where appropriate. Values of $P<0.05$ were considered statistically significant.

5.7 Results

5.7.1 Demographics and clinical characteristics

All participants were of African descent and between the ages of 18 and 48 with no significant difference between the mean age of those with (30.66±9.25) and without (31.46±9.87) SCD. Thirty of those suffering from SCD were females compared to 26 in the control group. As expected, those suffering from SCD received significantly more

transfusions (165 vs 108) compared to the controls (p<0.001). On average, each person in the SCD group received 3.4 transfusions while the control group 2.2 (p<0.0001). 19(38%) of the test group experienced a transfusion reaction compared to 10(20%) of the control group. Although the number of reactions was higher in those with SCD, this was not significantly different (p=0.2038). (Table 5.1). The type of transfusion reactions observed were mostly haemolytic reactions and included allergy, coldness, shivering, rash, and itching.

Table 5.1: General characteristics of participants

	Control n=50(%)	SCD n= 50(%)	Total n=100(%)	p-value
Age (years)	31.46±9.877	30.66±9.255		0.6769
Gender				
Female	26 (52.00)	30 (60.00)	56 (56.00)	X^2 = 0.6494, df =1 P-value = 0.4203
Male	24 (48.00)	20 (40.00)	44 (44.00)	
Haemoglobin electrophoresis				
AA	36(72.00)		36(36.00)	X^2 = 100.00, df =3 P-value < 0.0001*+
AC	5(10.00)		5(5.00)	
AS	9(18.00)		9(9.00)	
SS	-	50(100.00)	50(50.00)	
The mean number of blood units transfused	2.160±0.5095	3.360±1.045		<0.0001
Mean number of blood transfusion reactions	0.400±0.8571	0.6327±0.9507		0.2038

Abbreviations: SCD: Sickle Cell Disease

5.7.2 Full blood count and haematology

As expected, the red cells, packed cell volume (PCV, haemoglobin and all red cell indices apart from the mean cell volume were significantly different between those with SCD and the control group. In addition, those with SCD had significantly higher neutrophil counts (p<0.0001) and lower lymphocyte numbers (P<0.0001). Although

both groups had platelet counts within the reference range, those with SCD had significantly higher platelet counts (p<0.0001) (Table 5.2).

Table 5.2: Comparing the haematological parameters in sickle cell anaemia disease and non-sickle cell anaemia patients.

Parameter	Control		Test		p-value
	Mean ± S.D	Median	Mean ± S.D	Median	
WBC (mm3)	15702 ±40587	5550	14803 ± 6453	13450	0.0001*
Platelets (mm3)	182300±87196	177500	289063±128976	267500	0.0001*
PCV (%)	31.20±23.13	32.50	23.13±4.741	23.00	0.0001*
Hb (g/dl)	10.41±2.852	11.45	7.642±1.482	7.650	0.0001*
MCV(FL)	79.72±4.468	80.00	80.75±4.468	80.00	0.3603
MCH (pg)	31.70±11.84	30.00	27.62±6.469	26.00	0.0001*
MCHC(g/dL)	31.76±1.364	32.00	31.45±0.9237	31.10	0.0763
Neutrophil (%)	57.74±12.58	59.00	67.60±9.498	70.00	0.0001*
Neutrophil (Absolute)	9883±25636	3051	10206±5029	9694	0.0001*
Lymphocyte (%)	39.74±12.62	40.00	30.00±9.042	29.00	0.0001*
Lymphocyte (Absolute)	4640±10137	2388	4275±2025	3585	0.0001*
Monocyte (%)	0.4800±0.8142	0.0000	0.5000±0.8864	0.0000	0.9225
Monocyte (Absolute)	62.80±114.0	0.0000	141.0±710.6	0.0000	0.5315
Eosinophil (%)	1.600±1.917	0.0000	1.600±1.953	1.0000	0.0065*
Eosinophil (Absolute)	737.1±3494	0.0000	217.4±265.1	148.5	0.0002*
Basophil (%)	0.5000±1.111	0.2500	0.4200±0.7025	1.0000	0.6397
Basophil (Absolute)	245.5±1135	0.0000	58.26±101.5	0.0000	0.3778

The test used Mann Mann-Whitney test. * Signifies a significant difference between the test and control group. P<0.05 indicates a significant difference. WBC: White blood cell; Hb: Haemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration.

5.7.3 Development of alloantibodies

Those with SCD had an increase of alloantibodies with a mean of 0.6 alloantibody reactions compared to 0.4 in the controls (p=0.2038). The majority of the antibodies in

those with SCD were anti-Kell, Jka and Fya whereas the control group was mostly anti-M, as well as Kell as shown in Table 5.3.

Table 5.3: Distribution of alloantibodies in SCD and the Control group

Alloantibodies	Control n=50(%)	SCD n= 50(%)	Total n=(%)
c	2(4.00)	2(4.00)	4(4.00)
E	5(5.00)	2(4.00)	7(7.00)
Fya	3(6.00)	6(12.002)	9(9.00)
Fyb	1(2.00)	2(4.00)	3(3.00)
Jka	0(0.00)	8(16.00)	8(8.00)
K	9(18.00)	9(18.00)	18(18.00)
KPa	3((6.00)	4(8.00)	7(7.00)
Lea	5(10.00)	2(4.00)	7(7.00)
M	12(24.00)	5(10.00)	17(17.00)
N	1(2.00)	2(4.00)	3(3.00)
s	3(6.00)	2(4.00)	5(5.00)
C	5(5.00)	2(4.00)	7(7.00)
Cw	1(2.00)	2(4.00)	3(3.00)
e	0(0.00)	2(4.00)	2(2.00)

5.7.4 Markers of Inflammation

Patients with sickle cell disease had significantly elevated levels of c-reactive protein and the pro-inflammatory cytokine TNF. In contrast, there was no significant difference in the measurements for IL-6 and IL-1β. Table 5.4 demonstrates the independent-samples t-test for the inflammatory parameters of participants by study group. The result showed that there was a significant difference between the mean of the control and test groups for CRP [t(97) = -3.099; p = 0.003] and TNF [t(97) = -2.449; p = 0.016]. The result also showed that there was no significant difference between the mean of the control and test groups for IL-6 [t(97) = 1.380; p = 0.171] and IL-1β [t(97) = -1.710; p = 0.090].

Table 5.4: Inflammatory markers in those with SCD and the control group

	GROUP						
	Control		Test				
	M	SD	M	SD	T	df	p
CRP	1.2	2.78	3.9	5.62	- 3.099	97	0.003*
IL-6	84.3	101.96	58.1	86.10	1.380	97	0.171
TNF	2.8	5.76	8.1	14.31	-2.449	97	0.016*
IL-1β	2.5	.38	4.1	6.73	-1.710	97	0.090

*Signifies significant differences between study groups

M: Mean; SD Standard deviation; CRP: C-reactive protein; IL-6: Interleukin 6; TNF: Tumour necrosis factor; IL_1: Interleukin 1

5.7.5 Correlation between markers of inflammation and alloimmunization

Correlation analysis showed a weak positive correlation between alloimmunization and two of the pro-inflammatory markers, CRP, and TNF. In contrast, a very weak negative correlation between alloimmunization and two other inflammatory markers, IL-1β and IL6 was observed. These however were not statistically significant (Table 5.5)

Table 5.5: Correlation between inflammatory markers and alloimmunization

Inflammatory markers	**Pearson's Correlation**	**p-value**
CRP	**0.1127**	**0.2665**
IL6	**-0.08318**	**0.4130**
IL-1β	**-0.02402**	**0.8135**
TNF	**0.1179**	**0.2452**
Neutrophil	**-0.02975**	**0.7700**
WBC	**-0.03534**	**0.7284**

CRP; C-reactive protein, IL-6; interleukin- 6, IL-1 β; interleukin 1beta, TNF; Tumor necrosis factor.

5.8 Discussion

This cross-sectional study aimed to explain the complex relationship between inflammation and alloimmunization in patients with Sickle Cell Disease (SCD). It highlights the increased transfusion frequency and transfusion reactions in SCD

patients Those with SCD developed allogeneic antibodies which were mostly anti-Kell, Jka and Fya while the non-SCD group developed anti-Kell and antibodies to M. Despite those with SCD having significantly elevated levels of the pro-inflammatory markers (TNF and CRP), no significant correlation between any of these and the rate of alloimmunization could be detected. This suggests that while inflammation is prevalent in SCD patients, it does not directly predict alloimmunization [25].

The findings align with previous research by Kangiwa et al. (2015), which reported similar alloimmunization patterns but at higher rates. Differences in sample size, subject age, regional practices, and transfusion protocols likely contributed to the variance in alloimmunization rates. This study focused on young adults in the Western part of Nigeria, while Kangiwa et al. included both adults and children in the Eastern region, indicating possible regional differences in antigen prevalence and healthcare practices.

Other studies, such as those by Adewoyin, Lee [27]; Buhari, Sagir [28] and Erhabor, Erhabor [29] corroborate these findings, highlighting the clinically significant increase in Rhesus and Kell antibodies. The higher alloimmunization compared to other African countries like Ghana [30] Uganda [31] and Tanzania [17] show a steady increase in the rate of alloimmunization in Nigeria.

These differences could be explained by variations in the genetic backgrounds between populations, which can influence immune responses. For instance, the prevalence of specific antigens like Kell, Jka, and Fya might vary across different ethnic groups, affecting the likelihood of developing corresponding antibodies. Likewise, variations in blood transfusion protocols and donor screening processes between different regions and hospitals can impact alloimmunization rates and the types of antibodies formed.

At our institution, blood units are prophylactically phenotype-matched between the recipient and the donor using ABO and Rhesus grouping and once it is compatible, it is given to the recipient without necessarily carrying out antigen testing. However, an extended antigen testing panel was performed in this current study.

The immunogenicity of antigens may also significantly influence the likelihood of antibody development. Antigens like Kell, Jka, and Fya, which were elevated in our SCD group, are known to be highly immunogenic, leading to a higher prevalence of corresponding antibodies in transfused patients. The Duffy antigen, specifically Fya, is particularly immunogenic and prevalent in African populations, making it a common target for alloimmunization in SCD patients [32, 33].

In this current study, those with SCD had significantly higher levels of the proinflammatory proteins TNF and CRP, while IL-6 and IL1 were similar to the control group**.** The increase in inflammatory proteins has been reported in previous studies, such as Pascale [34]. It has been proposed that these proteins could be used as clinical biomarkers. For example, CRP has been associated with acute chest pain (ACP) and vaso-occlusive crisis (VOC) [35]. This has been confirmed by others whose findings were consistent with ours (Conran and Belcher [36] and Silva-Junior, Garcia [37].

TNF-α is a cytokine with several properties, including the activation of endothelial cells and leucocytes. The action of macrophages and the chemotaxis of inflammatory cells has been implicated in the pathogenesis of various acute and chronic states such as sepsis, chronic infections, and inflammatory conditions. TNF plays an essential role in the synthesis of protein and the expression of adhesion molecules in vascular endothelial cells [38, 39]. In SCD, this cytokine has been proposed as a risk factor for the occurrence of painful crises, as well as being involved in the occlusion of the microcirculation [40, 41].

High white cell counts and neutrophils are also associated with inflammation and during infections, neutrophils are the first cells to respond. Activated neutrophils release enzymes such as reactive oxygen species, proteases and myeloperoxidase, which combat foreign organisms at the site of infection [42]. These enzymes are also involved in several inflammatory processes [42]. When adhesion takes place, chemokines and cytokines are produced, which go on to stimulate dendritic cells, resulting in the presentation of antigen to memory CD4+ T cells as well as to naïve CD8+ T cells, which consequently leads to activation of the adaptive immune response [43].

Several other studies have reported elevated platelet counts in SCD patients [44-46]. Increased platelets in this study could contribute to the chronic and acute complications of SCD by promoting molecular and diverse cellular events within the microcirculation that eventually lead to vaso-occlusive and vascular injury in SCD.

Despite the increase in inflammatory markers, no significant correlation between the rate of alloimmunization and any of the inflammatory markers could be detected, which was similar to a previous study by Tatari-Calderone, Fasano [25]. Their study, conducted in Washington, DC, USA, involved 83 children with SCD who received multiple red blood cell transfusions for both the prevention and treatment of disease-related complications. The levels of cytokines were correlated with the development of anti-RBC antibodies within the seven years post-recruitment and demonstrated that twelve subjects had significantly elevated levels of all cytokines, both pro-inflammatory and anti-inflammatory. Interestingly, higher levels of cytokines were also found in the patients without anti-RBC allo- and/or auto-antibodies. Therefore, it was concluded that high cytokine levels were not indicative of alloantibody development and that the increased concentration of multiple cytokines is not a biomarker of either the presence of or susceptibility to the development of RBC alloimmunization.

Several other studies have reported increased inflammation and innate [47] immune activation in SCD patients [48, 49] however the pattern of cytokine expression varies [50] [51]. High plasma levels of TNFα have been reported while others have suggested that reduced production of IFNγ was the first evidence of the onset of DHTR in SCD patients [52].

Researchers have shown that various factors, aside from inflammation can influence the development of alloimmunization in SCD. These factors include iron overload, haemolysis, delayed haemolytic transfusion reactions, pregnancy, haemolytic disease of the newborn (HDN), infection, genetic factors, the antigenic immunogenicity of RBCs, recipient exposure to foreign donor antigens, the immunological status of the recipient, age at first transfusion, and the duration of transfusions. A further factor which could play a role is differences in the human leukocyte antigen (HLA) alleles. These findings all warrant future research.[53, 54]

5.9 Limitations

This study was conducted in only one region of Nigeria and therefore the results cannot be applied to the general Nigerian population or other countries within Africa. Regional genetic variations, environmental factors, and healthcare practices could influence the results, thereby limiting the broader applicability of the study's conclusions. A further limitation was the relatively low number of participants. A larger sample size would have provided more robust data and enhanced the statistical power of the study, allowing for more definitive conclusions. A further limitation was that only four inflammatory markers were analysed which may have overlooked other relevant biomarkers which could provide additional insights into the conditions being studied.

Despite these limitations, the results have indicated that SCD individuals in this region of Nigeria have high rates of alloimmunization and elevated levels of anti-Kell, Jka, and Fya antibodies, along with inflammatory markers TNF and CRF, compared to those without SCD. However, no significant correlation was found between the inflammatory markers and alloimmunization. The study underscores the multifactorial nature of alloimmunization in SCD and the importance of considering genetic, regional, and procedural factors to optimize transfusion practices. Further research is needed to explore these differences and develop strategies to reduce alloimmunization risks in SCD patients.

Authors contribution

O.O. Oguntibeju read, reviewed and edited the manuscript. He supervised the research work acted as resource administration and secured funding.

G.M. Davison read, reviewed, and edited the manuscript. She co-supervised the research work.

T. Oduola, read, reviewed and co-supervised the research work.

F.I. Aboderin: Conceived the idea, conception designed, data collection, sample analysis and writing manuscripts.

Conflict of interest: The authors do not have any conflict of interest.

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CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

Sickle cell disease is a chronic condition that constitutes a significant public health concern worldwide. The disease is majorly distributed among Africans, whose ancestors migrated from sub-Saharan Africa. The disease also domiciles in regions like South and Central America, Saudi Arabia, India and Mediterranean countries among others. Literature indicates that in Western Africa, Nigeria has the highest frequency of SCD cases (Fernandes et al., 2017). This study is a cross sectional one that compared the biological factors influencing the development of alloimmunization in SCD patients. From this study, our findings showed that SCD has a diet that is lower in nutritional intake, and they have elevated levels of oxidative stress and inflammation. However, this does not seem to be the sole basis for the higher alloimmunization rate.

Nutrition as part of this study is essential and cannot be trivialised or overlooked in solving the problem of alloimmunization rate in SCD. Most Clinicians focus on ensuring that packed cell volume (PCV) and blood cell counts are high and maintained but neglect the effect of nutrition in improving red blood cell and overall health outcomes. To further establish the usefulness of dietary intake and adequacy in nutrients, about 50 young SCD-confirmed persons and 50 controls were recruited to determine their nutritional status. Our findings showed only 23.7% met the calorie intake while the remaining 76.3% did not meet the standard requirement. Fibre intake was grossly low, too; about 80.4% of participants did not measure up to average values. Nutritional intake and amino acids from protein catabolism are seen substituting red cells because of haemolysis in SCD patients. This phenomenon elevates the energy and hinders the accessibility of nutrients needed for growth, muscle maintenance and development in the body, which eventually leads to serious undernutrition (Baranauskas et al., 2023, Weiler et al., 2023). It can also cause an imbalance in the immune system, thereby complicating the health of SCD patients.

Treatment of SCD is complex and includes blood transfusion, natural products, hydroxyurea, and nutrient supplements, which exist despite the persistent issues of adequate dietary intake and nutrient availability (Bell et al., 2024). Micronutrient deficiencies in SCD individuals are often associated with iron, folic acid, copper, pyridoxine, and zinc which affect immunity and growth (Gombart et al., 2020, Brittenham et al., 2023). Nutritional support has not been a fundamental focus. It is essential to state that an adequate diet fashioned to one's gender, age, and dietary intake will not adequately address the nutritional requirements of SCD unless nutritional strategies are mapped out for careful selection of food to ensure an adequate supply of macronutrients and micronutrients essentially needed for body maintenance and upkeep (Gombart et al., 2020).

Literature indicates that SCD has notable nutritional implications involving increased nutrient requirements, nutrient deficiencies, and growth abnormalities (Soliman et al., 2021; Obeagu and Obeagu, 2024). However, this study was necessary as it investigated dietary intake and nutritional adequacy to better understand how they affect the immune response among young adult sickle cell patients (Aboderin et al., 2023).

The second study examined the relationship between oxidative stress and inflammatory markers in multi-transfused SCD patients. This involved SCD patients who received two or more pints of blood transfusions, with a control group of individuals without the disease who received an equivalent number of blood transfusions. A questionnaire was issued to compile data. The following tests: AST, ALT, creatinine, urea, catalase, SOD, IL-1, IL-6, IL-1β, TNF, and CRP were determined using appropriate methods such as ELISA technique, Ultra-Violet and colourimetric methods were applied. Statistical analysis was performed using SPSS version 24.0, with data presented as mean ± standard deviation (SD). A chi-square test was also carried out where appropriate. Values of $p < 0.05$ were considered statistically significant.

Our findings revealed a significant increase in inflammatory markers which includes CRP and TNF, among sickle cell patients. moreover, there was a notable increase in superoxide dismutase (SOD) levels, alongside enzymes like AST and ALT. Furthermore, sickle cell patients displayed lower urea levels than the control group. These results underscore the fact that haemolysis could trigger inflammation and pro-inflammatory cytokines as well as induce oxidative stress in sickle cell disease patients, basically posing challenges to the efficacy of the immune system in the long term. The evidence of this is prominently displayed in our results. It is advisable to include markers of inflammation and oxidative stress in routine examinations to gain a better understanding of their roles in the pathophysiology and appropriate monitoring and management of SCD patients.

The management of SCD should be supported with minimal transfusion given only when needed to reduce alloimmunization rates and to improve the quality of life and longevity in the SCD patients. This cross-sectional study sheds light on the connection between inflammation, oxidation and alloimmunization, especially in patients with Sickle Cell Disease (SCD).

The third study highlights the link between inflammation and alloimmunization in (SCD) patients receiving multiple transfusions and comparing them with non-SCD patients receiving a similar number of transfusions. Maintaining the same number of participants, 50 SCD as test group and 50 control, questionnaires were used to gather information and the blood transfusion laboratory services records was consulted to confirm the number of blood transfusions received and the number of post -transfusion reactions. The study showed that individuals with SCD had significantly ($P < 0.0001$) more blood transfusions than the control group. The SCD received an average number of 3.4 while the control group received an average number of 2.2. Also, the SCD recorded more transfusion reactions compared to the control group. However, the difference was not statistically significant $p = 0.2038$). The pattern of antibodies identified in the SCD group were anti Kell, Jka, and Fya while the control displayed anti M, and Kell antibodies. Furthermore, in both test and control groups, the Rhesus antibodies were similar and next by Kell antibodies but differs in Jka Fya when

compared with control. This result implies a strong association between the antibody group and the test group.

In addition to the alloimmunization report, haematological parameters were significantly different in the SCD, especially with neutrophils and platelets counts. Inflammatory markers such as CRP, TNF, IL-1, IL-6, and IL-1β were determined, and those with SCD displayed evidence of inflammation with significantly increased levels (P= 0.0001) of the pro-inflammatory cytokines TNF and C-reactive protein. It has been hypothesized that there is an association between HLA types and alloimmunization in SCD, this was supported by a current study establishing a positive relationship between alloimmunization and HLA-DRB11503 (Meinderts et al., 2019). However, certain HLA types increase the risk of alloimmunization; others, like HLA-DRB10901, appear to confer protection (Hendrickson, 2020). Despite elevated inflammatory markers, (CRP and TNF), no significant correlations were noted between these markers and immunization rate.

Conclusion

The study on alloimmunization in sickle cell disease SCD individuals spotlights the complex relationship between inflammation, transfusion, oxidative stress, haematological factors and nutrition. It unveils that although not statistically significant, SCD patients experience an increase rate of alloimmunization compared to non- SCD patients with a distinct alloantibody pattern. The interconnection of chronic inflammation, oxidative stress and nutrients adequacy complicates immune function in SCD. Despite identifying major factors, the actual mechanisms remain unclear, necessitating further research for targeted therapies and better management strategies for SCD.

Recommendations

1. Dietary interventions to address the significant deficiencies in caloric intake, minerals, and vitamins observed among SCD patients are strongly advocated. Focusing on ensuring adequate intake of folate retinol, beta-carotene, and vitamin

D is essential to improving overall health and potentially mitigate some complications associated with SCD.

2. It is necessary to include regular assessments of inflammatory markers (such as CRP and TNF) and oxidative stress indicators (like SOD) in routine examinations of SCD patients. This will help monitor and understand these markers' role in disease progression and in tailoring individualized treatment plans.
3. Further research is suggested to elucidate the unclear pathways leading to chronic inflammation and alloimmunization in SCD. Developing therapies that specifically target these pathways could prevent or reduce the frequency of complications such as alloimmunization and stroke.
4. Develop and enforce stringent transfusion procedures to minimize the risk of alloimmunization. This includes pretransfusion matching for minor antigens and close monitoring for the development of new antibodies. Educating healthcare providers and patients about the importance of these procedures is also important.

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Addendum

Addendum 1: Research Outputs

Published Articles

- Florence I. Aboderin, Taofeeq Oduola, Glenda M. Davison, Oluwafemi O. Oguntibeju (2023). A review of the relationship between the immune response, inflammation oxidative stress, and the pathogenesis of sickle cell anaemiaS; Biomedicines 2023, 11(9), 2413; https://doi.org/10.3390/biomedicines 11092413
- Florence I. Aboderin, Patience K. Alagbo Glenda M. Davison, Oluwafemi O. Oguntibeju. (2024). Dietary Intake and Nutrients Adequacy among Young Adults with Sickle Cell Disease in Ile -Ife, Southwest Nigeria; African journal of biomedical research volume 27 January 2024; 49 -54; https://ojshostng.com/index.php/ajbr

Articles Under Review

- Florence Ifechukwude Aboderin, Taofeeq Oduola, Glenda Mary Davison, and Oluwafemi Omoniyi Oguntibeju. Inflammatory and oxidative stress markers in multi-transfused sickle cell disease patients (accepted).
- Florence Ifechukwude Aboderin, Taofeeq Oduola, Glenda Mary Davison, and Oluwafemi Omoniyi Oguntibeju. Investigation of haematological, inflammatory parameters and the incidence of alloimmunization in multi-transfused sickle cell disease patients (underreview).

Workshops Attended

- Hands-on training workshop on sickle cell disease diagnosis and management. Organised by the Institute for advanced medical research and training (IAMRAT), College of medicine university of Ibadan. Title: ‘’RECENT ADVANCES AND INNOVATIONS IN THE DIAGNOSIS AND TREATMENT OF SICKLE CELL DISEASE ‘’ with sub theme: Current trend in basic molecular Techniques. 6th -8th May2024
- Revolutionizing Healthcare with Artificial intelligence: Perspectives on Ethics, challenges, and Learnings. Lecture organised by IAMRAT 30TH May ,2024

Addendum 2: Ethics Certificates

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (CPUT HWS-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

31 March 2023
REC Approval Reference No:
CPUT/HWS-REC 2021/H32 (renewal)

Faculty of Health and Wellness Sciences

Dear Prof. Oguntibeju

Re: APPLICATION TO THE CPUT HWS-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to **Ms. F Aboderin** for ethical clearance. This approval is for research activities related to research for **Ms. F Aboderin** at Cape Peninsula University of Technology.

TITLE: Comparitive study on biological factors influencing the development of alloimmunization in sickle cell disease patients in IIfe-Ife, Nigeria

Supervisors: Prof.O Oguntibeju, Prof .G Davison and Prof. T Oduola

Comment:

Approval will not extend beyond 1 April 2024. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the CPUT HWS-REC in December of that particular year, for the CPUT HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Ms. Carolynn Lackay
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HWS-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

29 November 2021
REC Approval Reference No:
CPUT/HW-REC 2021/H32

Faculty of Health and Wellness Sciences

Dear Dr Prof Oguntibeju

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC to **Ms F Aboderin** for ethical clearance. This approval is for research activities related to research for **Ms F Aboderin** at Cape Peninsula University of Technology.

TITLE: Comparitive study on biological factors influencing the development of alloimmunization in sickle cell disease patients in IIfe-Ife, Nigeria

Supervisors: Prof O Oguntibeju, Prof G Davison and Prof T Oduola

Comment:

Approval will not extend beyond 30 November 2022. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Carolynn Lackay
Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, NIGERIA
HEALTH CENTRE

INTERNAL MEMORANDUM

FROM: Research and Human Resource Committee, Medical and Health Services, Health Centre	TO: Head of Units, Health Centre.
REF: D.MHS/ DATE: 2023	

The Committee has approved that ~~Mr.~~, ~~Miss.~~, Mrs., ~~Dr.~~, ~~Prof.~~ FLORENCE I. ABODERIN should carry on his/her research work titled: COMPARATIVE STUDY ON BIOLOGICAL FACTORS INFLUENCING THE DEVELOPMENT OF ALLOIMMUNIZATION IN SCD PATIENTS IN ILE-IFE within the Health Centre having fulfilled the requirement stipulated by the Committee.

PRO

Chairman

MEDICAL & HEALTH SERVICES
Obafemi Awolowo University
ILE-IFE

OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, NIGERIA
HEALTH CENTRE

INTERNAL MEMORANDUM

FROM: Research and Human Resource Committee, Medical and Health Services Health Centre	TO: Head of Units, Health Centre
REF: D.MHS/ DATE: 14/01/2021	

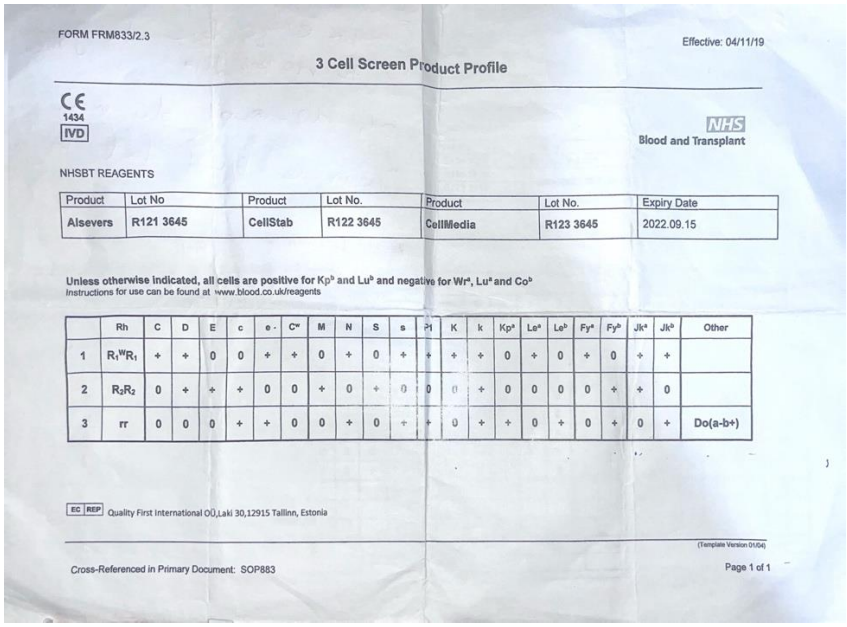
The Committee has approved that Mr., Miss., Mrs., Dr., Prof. F. I. ABODERIN should carry on his/her research work titled: COMPARATIVE STUDY ON BIOLOGICAL FACTORS INFLUENCING THE DEVELOPMENT OF ALLOIMMUNIZATION IN SCD PATIENTS IN ILE-IFE within the Health Centre having fulfilled the requirement stipulated by the committee.

PRO

MEDICAL & HEALTH SERVICES
MEDICAL RECORDS UNIT
Obafemi Awolowo University, Ile-Ife

Chairman

Addendum 3: Results and Questionnaires



FORM FRM833/2.3

Effective: 04/11/19

3 Cell Screen Product Profile

CE 1434 IVD

NHS Blood and Transplant

NHSBT REAGENTS

Product	Lot No	Product	Lot No.	Product	Lot No.	Expiry Date
Alsevers	R121 3645	CellStab	R122 3645	CellMedia	R123 3645	2022.09.15

Unless otherwise indicated, all cells are positive for Kp^b and Lu^b and negative for Wr^a, Lu^a and Co^b
Instructions for use can be found at www.blood.co.uk/reagents

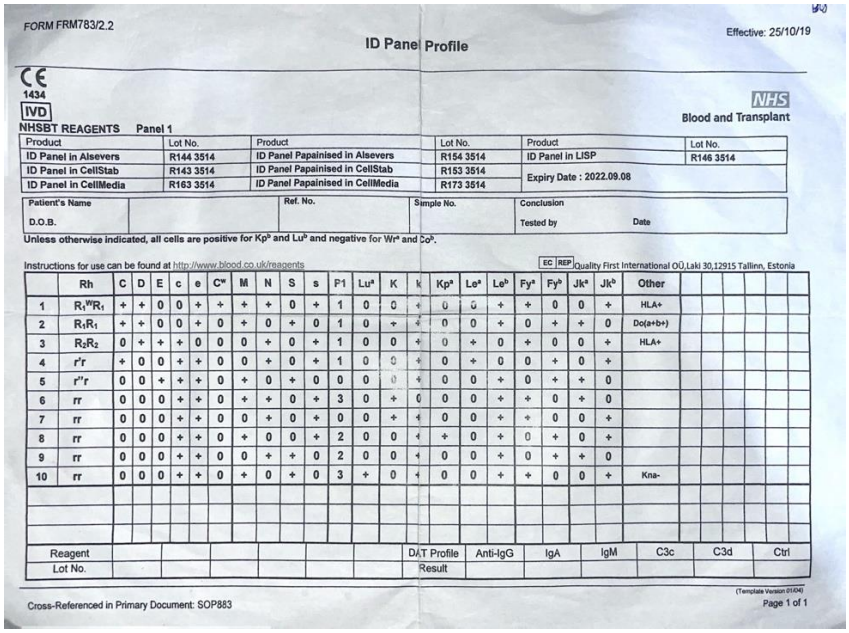
	Rh	C	D	E	c	e	C^w	M	N	S	s	P1	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other
1	$R_1^wR_1$	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	0	+	0	+	+	
2	R_2R_2	0	+	+	+	0	0	+	0	+	0	0	+	+	0	0	0	0	+	+	0	
3	rr	0	0	0	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	0	+	Do(a-b+)

EC REP Quality First International OÜ,Laki 30,12915 Tallinn, Estonia

(Template Version 01/04)

Cross-Referenced in Primary Document: SOP883

Page 1 of 1



FORM FRM783/2.2

Effective: 25/10/19

ID Panel Profile

CE 1434 IVD

NHS Blood and Transplant

NHSBT REAGENTS Panel 1

Product	Lot No.	Product	Lot No.	Product	Lot No.
ID Panel in Alsevers	R144 3514	ID Panel Papainised in Alsevers	R154 3514	ID Panel in LISP	R146 3514
ID Panel in CellStab	R143 3514	ID Panel Papainised in CellStab	R153 3514	Expiry Date : 2022.09.08	
ID Panel in CellMedia	R163 3514	ID Panel Papainised in CellMedia	R173 3514		

Patient's Name		Ref. No.		Sample No.		Conclusion	
D.O.B.						Tested by	Date

Unless otherwise indicated, all cells are positive for Kp^b and Lu^b and negative for Wr^a and Co^b.

Instructions for use can be found at http://www.blood.co.uk/reagents

EC REP Quality First International OÜ,Laki 30,12915 Tallinn, Estonia

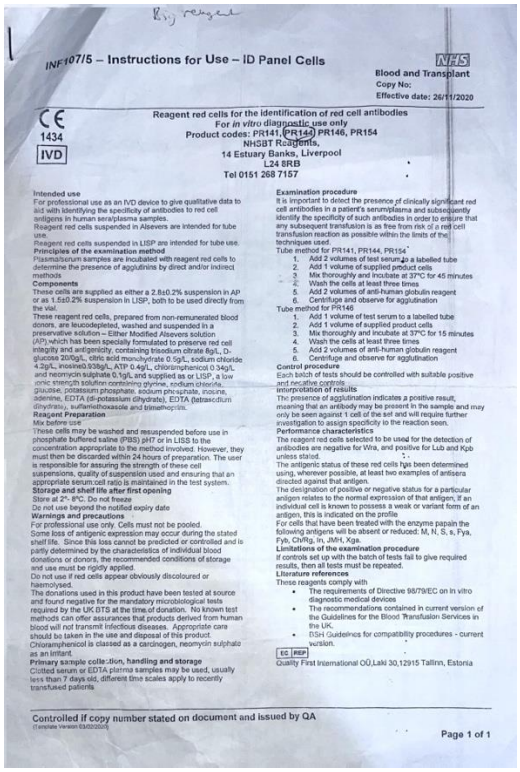
	Rh	C	D	E	c	e	C^w	M	N	S	s	P1	Lu^a	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other
1	$R_1^wR_1$	+	+	0	0	+	+	+	+	0	+	1	0	0	+	0	0	+	+	0	0	+	HLA+
2	R_1R_1	+	+	0	0	+	0	+	0	+	0	1	0	+	+	0	0	+	0	+	+	0	Do(a+b+)
3	R_2R_2	0	+	+	+	0	0	0	+	0	+	1	0	0	+	0	+	0	+	0	0	+	HLA+
4	r'r	+	0	0	+	+	0	0	+	0	+	1	0	0	+	0	+	0	0	+	0	+	
5	r''r	0	0	+	+	+	0	+	0	+	0	0	0	0	+	0	0	+	0	+	+	0	
6	rr	0	0	0	+	+	0	+	+	0	+	3	0	+	0	0	0	+	+	0	+	0	
7	rr	0	0	0	+	+	0	0	+	0	+	0	0	+	+	0	0	+	+	0	0	+	
8	rr	0	0	0	+	+	0	+	0	0	+	2	0	0	+	0	0	+	0	+	0	+	
9	rr	0	0	0	+	+	0	0	+	+	0	2	0	0	+	0	0	+	0	+	+	0	
10	rr	0	0	0	+	+	0	+	0	+	0	3	+	0	+	0	0	+	+	0	0	+	Kna-

Reagent								DAT Profile	Anti-IgG	IgA	IgM	C3c	C3d	Ctrl
Lot No.								Result						

(Template Version 01/04)

Cross-Referenced in Primary Document: SOP883

Page 1 of 1



R.j. reagent

INF107/5 – Instructions for Use – ID Panel Cells

NHS
Blood and Transplant
Copy No:
Effective date: 26/11/2020

CE 1434
IVD

Reagent red cells for the identification of red cell antibodies
For *in vitro* diagnostic use only
Product codes: PR141, PR144, PR146, PR154
NHSBT Reagents,
14 Estuary Banks, Liverpool
L24 8RB
Tel 0151 268 7157

Intended use
For professional use as an IVD device to give qualitative data to aid with identifying the specificity of antibodies to red cell antigens in human serum/plasma samples.
Reagent red cells suspended in Alsevers are intended for tube use.
Reagent red cells suspended in LISP are intended for tube use.

Principles of the examination method
Plasma/serum samples are incubated with reagent red cells to determine the presence of agglutinins by direct and/or indirect methods.

Components
These cells are supplied as either a 2.8±0.2% suspension in AP or as 1.5±0.2% suspension in LISP, both to be used directly from the vial.
These reagent red cells, prepared from non-remunerated blood donors, are leucodepleted, washed and suspended in a preservative solution – Either Modified Alsevers solution (AP) which has been specially formulated to preserve red cell integrity and antigenicity, containing trisodium citrate 8g/L, D-glucose 20/8g/L, citric acid monohydrate 0.5g/L, sodium chloride 4.2g/L, inosine0.938g/L, ATP 0.4g/L, chloramphenicol 0.34g/L and neomycin sulphate 0.1g/L and supplied as or LISP, a low ionic strength solution containing glycine, sodium chloride, glucose, potassium phosphate, sodium phosphate, inosine, adenine, EDTA (di-potassium dihydrate), EDTA (tetrasodium dihydrate), sulfamethoxasole and trimethoprim.

Reagent Preparation
Mix before use
These cells may be washed and resuspended before use in phosphate buffered saline (PBS) pH7 or in LISS to the concentration appropriate to the method involved. However, they must then be discarded within 24 hours of preparation. The user is responsible for assuring the strength of these cell suspensions, quality of suspension used and ensuring that an appropriate serum:cell ratio is maintained in the test system.

Storage and shelf life after first opening
Store at 2°- 8°C. Do not freeze
Do not use beyond the notified expiry date

Warnings and precautions
For professional use only. Cells must not be pooled.
Some loss of antigenic expression may occur during the stated shelf life. Since this loss cannot be predicted or controlled and is partly determined by the characteristics of individual blood donations or donors, the recommended conditions of storage and use must be rigidly applied.
Do not use if red cells appear obviously discoloured or haemolysed.
The donations used in this product have been tested at source and found negative for the mandatory microbiological tests required by the UK BTS at the time of donation. No known test methods can offer assurances that products derived from human blood will not transmit infectious diseases. Appropriate care should be taken in the use and disposal of this product.
Chloramphenicol is classed as a carcinogen, neomycin sulphate as an irritant.

Primary sample collection, handling and storage
Clotted serum or EDTA plasma samples may be used, usually less than 7 days old, different time scales apply to recently transfused patients

Examination procedure
It is important to detect the presence of clinically significant red cell antibodies in a patient's serum/plasma and subsequently identify the specificity of such antibodies in order to ensure that any subsequent transfusion is as free from risk of a red cell transfusion reaction as possible within the limits of the techniques used.
Tube method for PR141, PR144, PR154
1. Add 2 volumes of test serum to a labelled tube
2. Add 1 volume of supplied product cells
3. Mix thoroughly and incubate at 37°C for 45 minutes
4. Wash the cells at least three times
5. Add 2 volumes of anti-human globulin reagent
6. Centrifuge and observe for agglutination

Tube method for PR146
1. Add 1 volume of test serum to a labelled tube
2. Add 1 volume of supplied product cells
3. Mix thoroughly and incubate at 37°C for 15 minutes
4. Wash the cells at least three times
5. Add 2 volumes of anti-human globulin reagent
6. Centrifuge and observe for agglutination

Control procedure
Each batch of tests should be controlled with suitable positive and negative controls

Interpretation of results
The presence of agglutination indicates a positive result, meaning that an antibody may be present in the sample and may only be seen against 1 cell of the set and will require further investigation to assign specificity to the reaction seen.

Performance characteristics
The reagent red cells selected to be used for the detection of antibodies are negative for Wra, and positive for Lub and Kpb unless stated.
The antigenic status of these red cells has been determined using, wherever possible, at least two examples of antisera directed against that antigen.
The designation of positive or negative status for a particular antigen relates to the normal expression of that antigen. If an individual cell is known to possess a weak or variant form of an antigen, this is indicated on the profile.
For cells that have been treated with the enzyme papain the following antigens will be absent or reduced: M, N, S, s, Fya, Fyb, Chrlg, In, JMH, Xga.

Limitations of the examination procedure
If controls set up with the batch of tests fail to give required results, then all tests must be repeated.

Literature references
These reagents comply with
- The requirements of Directive 98/79/EC on in vitro diagnostic medical devices
- The recommendations contained in current version of the Guidelines for the Blood Transfusion Services in the UK.
- BSH Guidelines for compatibility procedures - current version.

EC REP
Quality First International OÜ,Laki 30,12915 Tallinn, Estonia

Controlled if copy number stated on document and issued by QA
(Template Version 03/02/2020)

Page 1 of 1

Site-ID:□□□-□□□□ RCID:□□□ CRF DATE:□□/□□□/□□□□ VISIT _____

Study ID Number _____ Date and Time at start of recall _____ through _____

Day of Week _____ Dietary Recall Number 1 2 3 4 5 6

Time	Meal type	Place consumed	Description of food or drink or snack	Brand/ Homemade	Amount/Quantity	Leftover Y/N	How was it prepared?	What were you doing when you were eating? Were you watching TV, using computer or mobile phone or tablet or none of above	Did you eat with anyone else Y/N	If Yes Who? Family member /others

1

QUESTIONARE FOR DATA COLLECTION

Date:

1). Names: First name Surname

2). Sex: Female ☐ Male ☐

3). Date of birth: .....

4). What is your blood group?: .....

5). Do you know your genotype?: .....

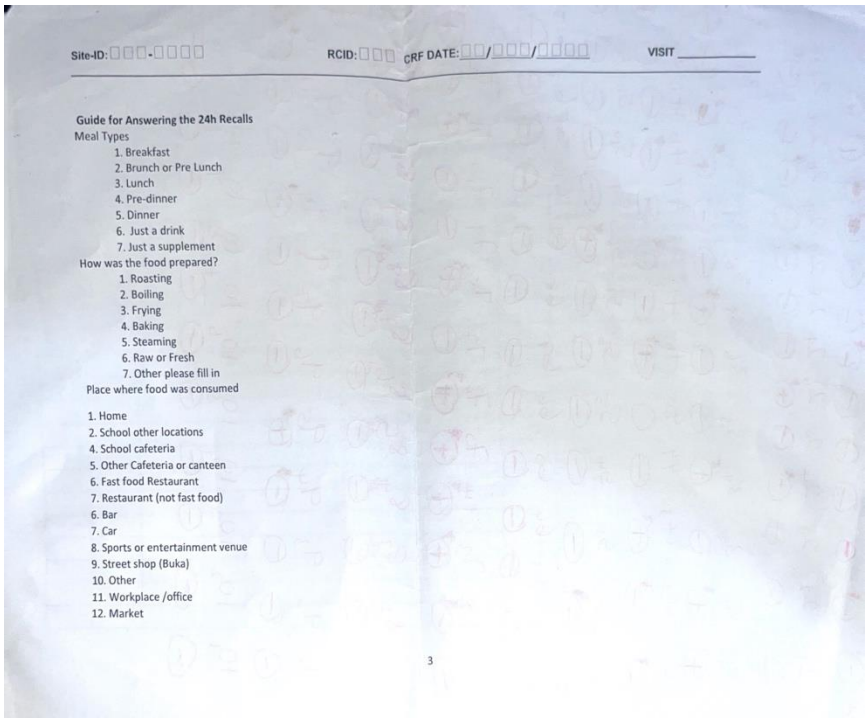
6). Any history of blood transfusion?: .....

7). If no 6 is yes, when were you transfused?: .....

8). How many units of blood were you transfused with?: .....

9). What is the nature of blood you received?: Whole blood ☐ packed cells ☐

10). After the blood transfusion was there any reaction?: like fever, head ache, shivering or pimples on your skin?



Site-ID:□□□-□□□□ RCID:□□□ CRF DATE:□□/□□□/□□□□ VISIT _____

Guide for Answering the 24h Recalls

Meal Types

1. Breakfast
2. Brunch or Pre Lunch
3. Lunch
4. Pre-dinner
5. Dinner
6. Just a drink
7. Just a supplement

How was the food prepared?

1. Roasting
2. Boiling
3. Frying
4. Baking
5. Steaming
6. Raw or Fresh
7. Other please fill in

Place where food was consumed

1. Home
2. School other locations
4. School cafeteria
5. Other Cafeteria or canteen
6. Fast food Restaurant
7. Restaurant (not fast food)
6. Bar
7. Car
8. Sports or entertainment venue
9. Street shop (Buka)
10. Other
11. Workplace /office
12. Market

3