Diplomarbeit

THE EFFECTS OF SICKLE CELL ANEMIA ON VASCULAR ENDOTHELIUM AND CURRENT THERAPEUTICAL OPTIONS: A LITERATURE REVIEW

eingereicht von

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Graz, 20.07.2017

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, 20.07.2017 *Linda-Ijeoma Jooda eh*

ACKNOWLEDGEMENTS

I would like to first of all thank the Almighty God for taking me through the medical school successfully. My sincere gratitude goes to my supervisor Ass. -Prof. Priv.-Doz. Dr. med. Nandu Goswami PhD. and my co-supervisor Dr. Oleksandr Bondarenko for the tremendious and continuous assistance rendered to me in the course of this review.

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ABBREVIATIONS

DA	Diplom Arbeit
HLA	Human leukocyte antigen
MCH	Mean corpuscular haemoglobin
RBC	Red blood cell
RNA	RiboNucleic Acid
SC	Sickle Cell
SCA	Sickle cell anemia
SCD	Sickle cell disease
VOC	Vaso-occlusive crisis
WBC	White blood cells

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ABSTRACT

Sickle cell anemia (SCA) is a hereditary disease. Pathologically, this sickle cell haemoglobin is known to be prone to polymerisation, producing deformable red blood cells, and resulting in sickling. It is mostly associated with vaso-occlusive crisis (VOC) and haemolytic anemia which can later result in chronic multi-organ system disease as well as very high morbidity and early mortality if not treated on time.

This diploma thesis explored sickle cell anemia, and then discussed current literature showing factors that might contribute to vaso-occlusion. This diploma thesis carried out **an extensive update of the current literature related to sickle cell anemia, paying particular attention to vascular endothelium.** Since vascular endothelium (dys) function changes could lead to arteriosclerosis, thrombosis, heart attack or even stroke, these aspects were examined in detail.

PubMed was used as the primary database where literature that were appropriate to this topic were retrieved. Articles written in English, published between 1979 and 2017 were explored. Abstracts as well as full texts were combined as primary literature. Web of science database and also list of References were also utilised in finding articles that were important to the area of search. Google was used for the searching of figures and specific tables related to the theme of this diploma thesis. Some textbooks, which covered sickle cell anemia, were used as secondary literature.

Taking into consideration refined criteria that were specific for this diploma thesis, **21 publications were found to be directly relevant.** These were then categorised into 3 sub-sections: Studies showing *factors that might contribute to vaso-occlusion via vascular interactions in adults*, in *children* and the *current therapeutical options needed to treat this disease.*

This work is important to me personally because apart from being a Nigerian, I also have relatives who are also victims of this disease. It is my hope that the findings of this diploma thesis will alleviate the sufferings of millions of people who are currently affected by this scourge.

ZUSAMMENFASSUNG

Sichelzellenanämie ist eine erbliche Erkrankung. Pathologisch, ist das Sichelzellenhämoglobin bekannt anfällig für Polymerisation und produziert verformbare Erythrozyten, resultierend in Sicheln. Es ist meistens mit einer vasookklusiven Krise und hämolytischer Anämie verbunden und kann nachfolgend in einem chronischen Multiorgan-Systemversagen resultieren, bzw. in sehr hohe Morbidität und frühe Mortalität, wenn nicht zeitgerecht behandelt wird.

Diese Diplomarbeit erforscht Sichelzellenanämie, diskutiert in Folge aktuelle Literatur und zeigt Faktoren auf, welche möglicherweise zu einer Vasookklusion beitragen können. Diese Arbeit führt **eine umfassende Aufdatierung der aktuellen Literatur bzgl. Sichelzellenanämie durch und schenkt dem Gefäßendothel besondere Aufmerksamkeit.** Da Funktionsveränderung des vaskulären Endotheliums (dys) zu Arteriosklerose, Thrombose, Herzinfarkt oder sogar Schlaganfall führen können, werden diese Aspekte im Detail examiniert.

PubMed wurde als primäre Datenbank verwendet und geeignete Literatur abgerufen. Artikel in englischer Sprache, publiziert zwischen 1979 und 2017 wurden erforscht. Abstrakte wie auch gesamte Texte wurden als primäre Literatur kombiniert. Web of science Datenbank und Referenzlisten wurden genutzt um wichtige Artikel dieser Thematik zu finden. Google wurde verwendet um Abbildungen und Tabellen zu diesem Thema in diese Diplomarbeit einfließen zu lassen. Lehrbücher, welche auf Sichelzellenanämie Bezug nehmen wurden als sekundäre Literatur hinzugezogen. Mit Rücksichtnahme auf die Präzisierung der Diplomarbeit wurden **21 Publikationen gefunden, welche von direkter Relevanz sind.** Diese wurden in 3 Unterkategorien aufgeteilt: Studien, welche Faktoren die möglicherweise zu Vasookklusion via Gefäß Interaktionen bei Erwachsenen, bei Kindern beitragen und die gegenwärtigen therapeutischen Möglichkeiten welche zur Behandlung dieser Krankheit benötigt werden.

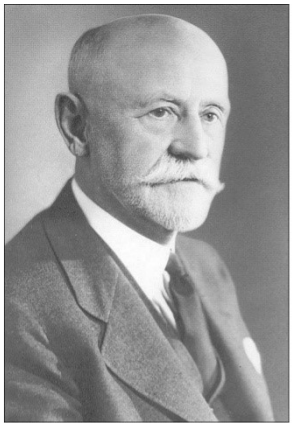
Diese Arbeit ist deshalb von großer Bedeutung für mich, abseits meiner Nigerianischen Nationalität, weil ich Verwandte habe, die Opfer dieser Krankheit sind. Es ist meine Hoffnung, dass die Forschungsergebnisse dieser Diplomarbeit die Leiden von Millionen Menschen welche derzeit von dieser Plage betroffen sind lindern.

1. INTRODUCTION

Sickle cell anemia (SCA) is a hereditary disease. This disease occurs mainly in the red blood cell (RBCs). It results in the deformation of the RBCs which in turn leads to hemolysis (the destruction of the RBCs) and finally to anemia (shortage of RBCs). It's also a vaso-occlusive and a pro-inflammatory disease accompanied by chronic multi-organ system disease as well as very high morbidity and early mortality. Before exploring SCA, let us first of all take a look at its history.

1.1 The history and discovery of Sickle Cell Anemia

In December 1904, in Chicago, a dental student who was apparently suffering from anemia, was admitted in the Chicago Presbyterian Hospital and Dr. Ernest Irons examined his blood and noticed that the shape of the RBC was not the same and called them "peculiar, elongated and sickled-shape" blood cells. However, its first clinical findings were recorded by an American cardiologist, Dr. James Bryan Herrick in 1910 (Fig. 1). (1)



James Bryan Herrick and his intern discovered sickle cell anemia.

Figure 1: Dr. James Herrick *(2)*

In 1949, two articles were published separately, one by a military doctor, Col. E.A. Beet and the other by Dr. James V. Neel, who worked at the University of Michigan

stating that SCA was a genetic disease and that people with the trait were heterozygous (carriers or AS) and those with the disease were homozygous (SS).

In the late 1950´s and early 1951´s, an American chemist, Dr. Linus Carl Pauling and his colleague Dr. Harvey Itano carried out a research on the SCA and the result showed that the hemoglobin molecule wasn´t looking normal- i.e. the haemoglobin (oxygen-carrying protein in the blood) of those suffering from SCA had a different chemical structure therefore giving it the name „ Sickle Cell Anemia, a Molecular Disease “. (1)

Since then till date many researches have been carried out to show how these abnormal structures affect the red blood cells and also better ways of detecting the disease have been improved.

1.2 The Physiology of Blood

Sickle cell anemia affects the blood, hence the subsequent topics in this chapter will provide a brief overview on the physiological features of the blood then take a closer look of the red blood cell.

1.2.1 What is Blood?

Blood is a special fluid found in the body which consists of blood cells and plasma. The blood cells comprise of: RBCs or erythrocytes, white blood cells (WBC) or Leukocytes and the platelets or Thrombocytes. See fig.2

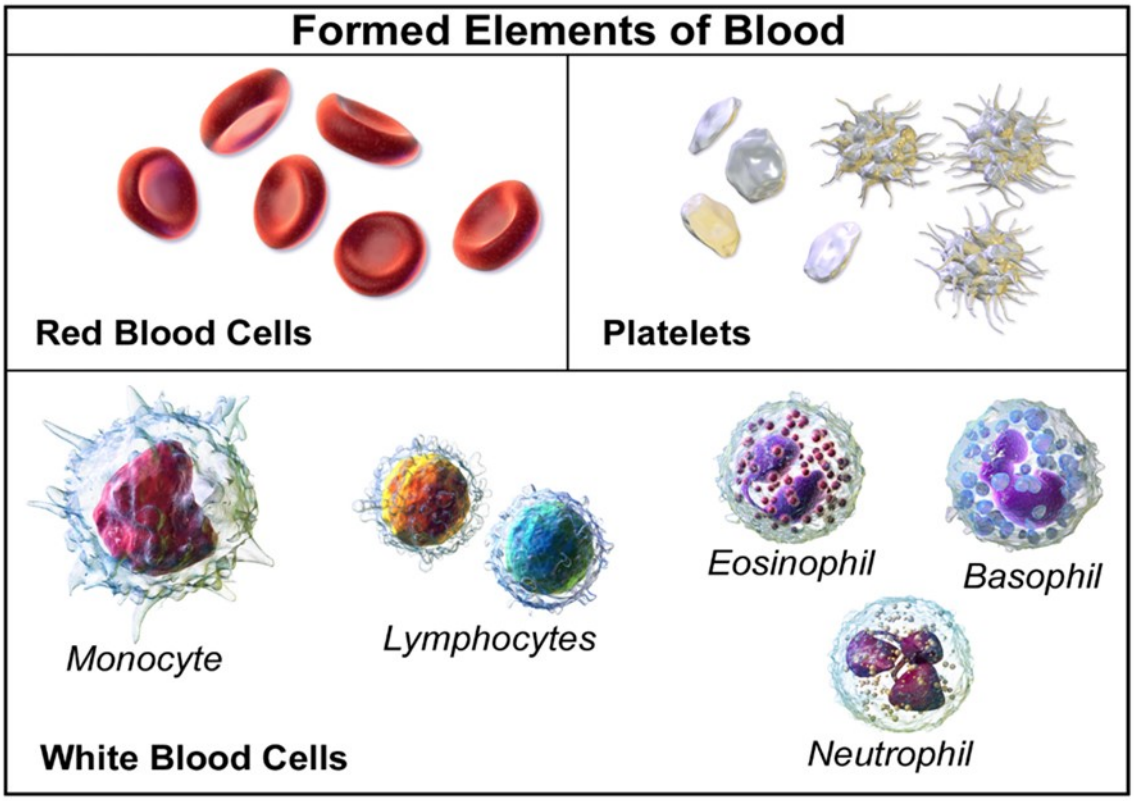


Figure 2: An illustration showing the blood cells*(3)*

1.2.2 Composition of Blood

Plasma

Blood consists of about 55% of blood plasma which happens to be blood´s liquid medium. The plasma is made up of mostly water 55%, proteins (Albumin, Globulin and Clotting proteins), glucose, mineral ions, hormones, enzymes, vitamins, etc. Albumin is the major protein found in the plasma, it helps in the maintainance of the colloidal osmotic pressure of blood.

White blood cells or leucocytes

WBCs are different from RBCs due to the fact that they lack hemoglobin therefore are transparent and are bigger in size (10-14 micro meters in diameter) than the RBCs. There are different types of WBCs (Fig. 2): **Granular Leucocytes** are: Neutrophils, Basophils, and Eosinophils. **Agranular Leucocytes** are: Monocytes

and Lymphocytes. These cells help the body to fight against pathogens (immune system), they also destroy and remove toxins, old, worned-out and abnormal cells. In other word, they are phagocytes i.e. they surround things or cells and swallow them.

Platelets (Thrombocytes)

They are membrane-bound cell fragments and they make up less than 1% of a whole blood. They are between 1-2 micro meters in diameter. They function in blood clotting (coagulation).

Red blood cells (erythrocytes)

RBCs contains hemoglobin which gives blood its bright red colour. This haemoglobin binds to oxygen therefore supplying oxygen to the rest of the body. The size of the red blood cell is 3.5 micro meter (minimum diameter), Despite the size of the red blood cell, oxygen must be able to pass through this cell. Though the red blood cell has a high concentration of haemoglobin, oxygen still helps in the maintainance of osmotic equilibrium. The red blood cell is a flexible biconcave disc which helps it to function well. It is able to generate energy as adenosine triphosphate (ATP) by the anaerobic glycolytic pathway.(4,5)

The volume percentage of the RBCs occupied in a proportion of blood is called hematocrit. The size of an erythrocyte is 7-8 micro meters in diameter and there are about 5 million erythrocytes in one microliter of blood.

1.2.3 Red Blood Cell

RBC´s Production

The production of RBC´s is controlled by erythropoietin, a hormone produced by the kidneys and liver. RBCs commence as an immature cell, (Erythroblasts) in the bone marrow (Fig.3). The earlier cells are larger, with more basophilic cytoplasm. The cytoplasm of the cells which develop later is more eosinophilic as a result of

hemoglobin formation and accumulation. They also contain progressively more hemoglobin (which stains pink) in the cytoplasm (Fig.3). The nucleus is finally ejected from the normoblast and a reticulocyte is formed, which still retains some ribosomal RiboNucleic Acid (RNA) and can synthesise hemoglobin. Before the red blood cell matures to an erythrocyte, it circulates in the peripheral blood for 1-2 days. Approximately 10-15% of developing erythroblasts die within the bone marrow without producing mature RBCs. This ineffective erythropoiesis is increased and becomes an important cause of reduced hemoglobin concentration (anemia) in various pathological states, e.g. thalassaemia major, myelofibrosis, megaloblastic anemia. Erythropoietin stimulates erythropoiesis in the bone marrow by low O_2 supply and so increases O_2 delivery. Conversely, increased O_2 supply to the tissues reduces the erythropoietin drive (Fig.4).(4–6)

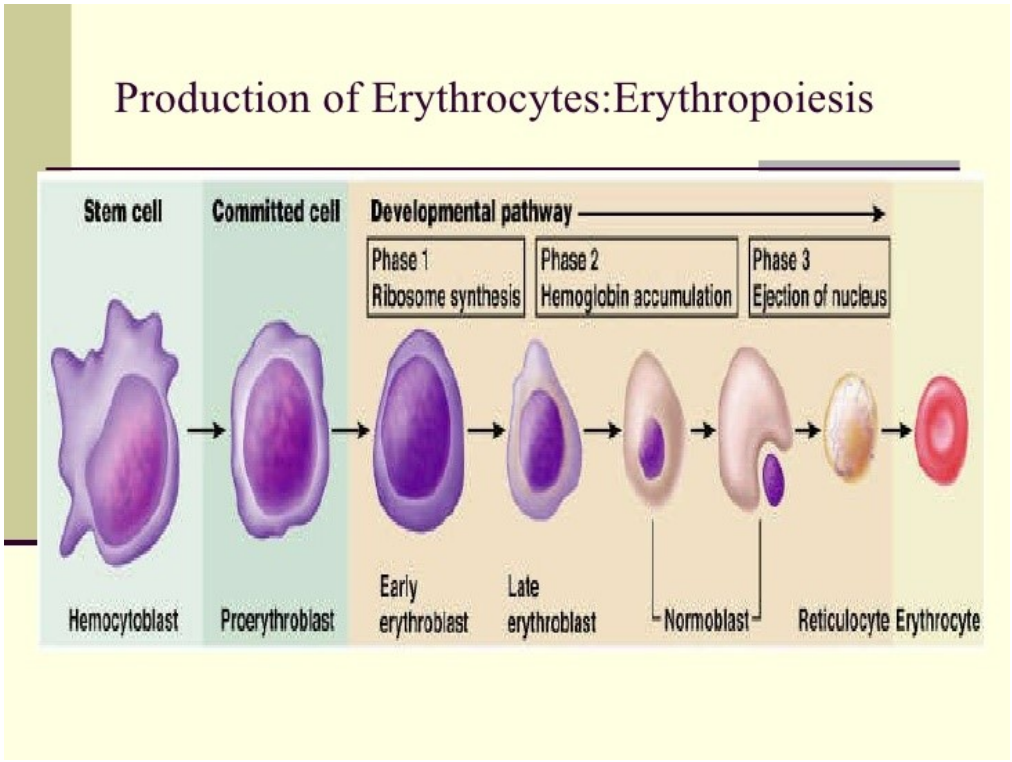


Figure 3: Erythroblasts at varying phases of development*(7)*

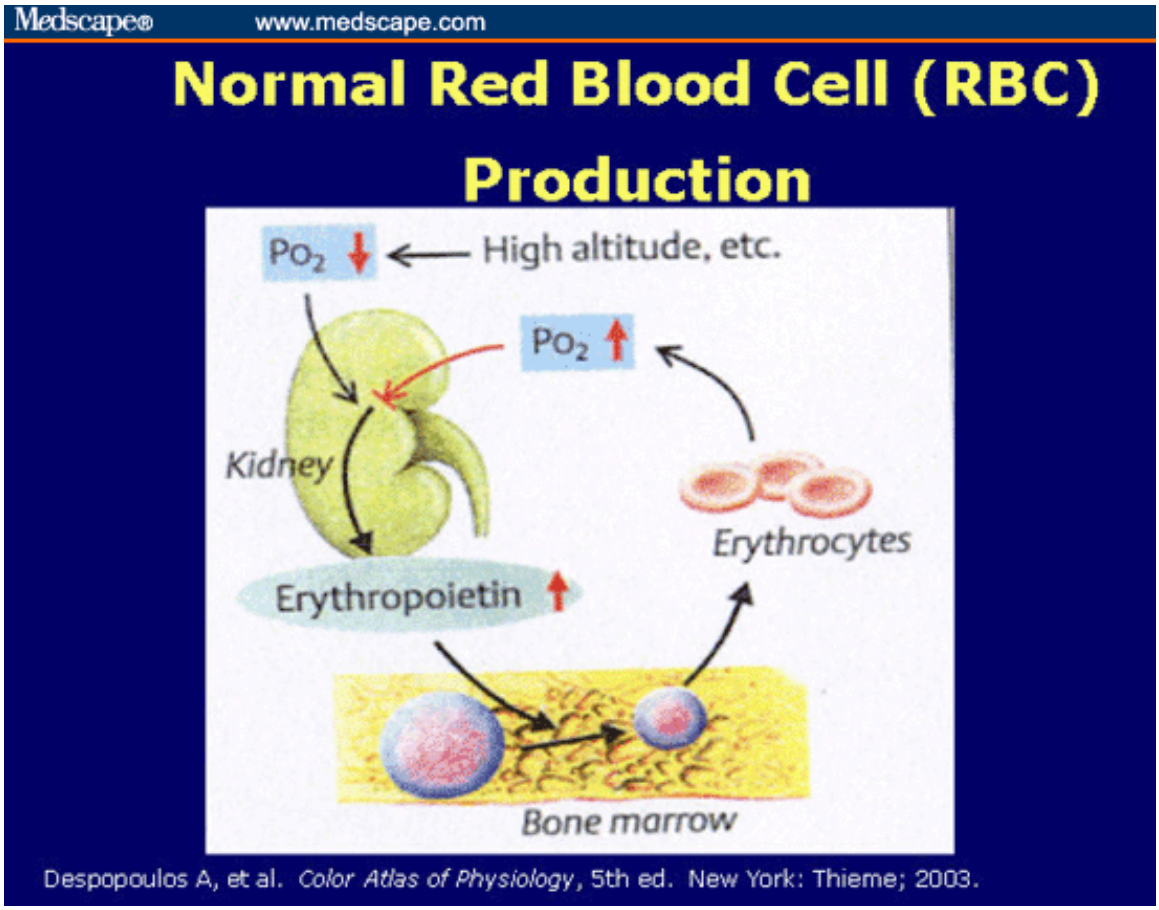


Figure 4: The production of erythropoietin by the kidney *(8)*

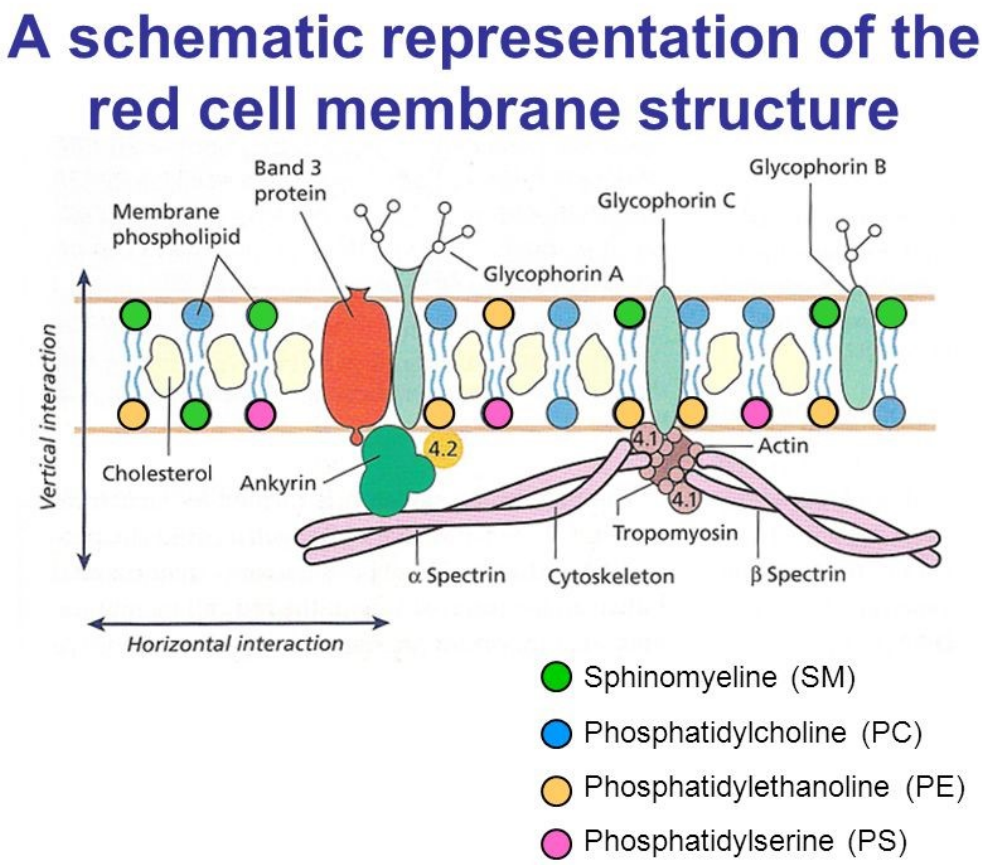
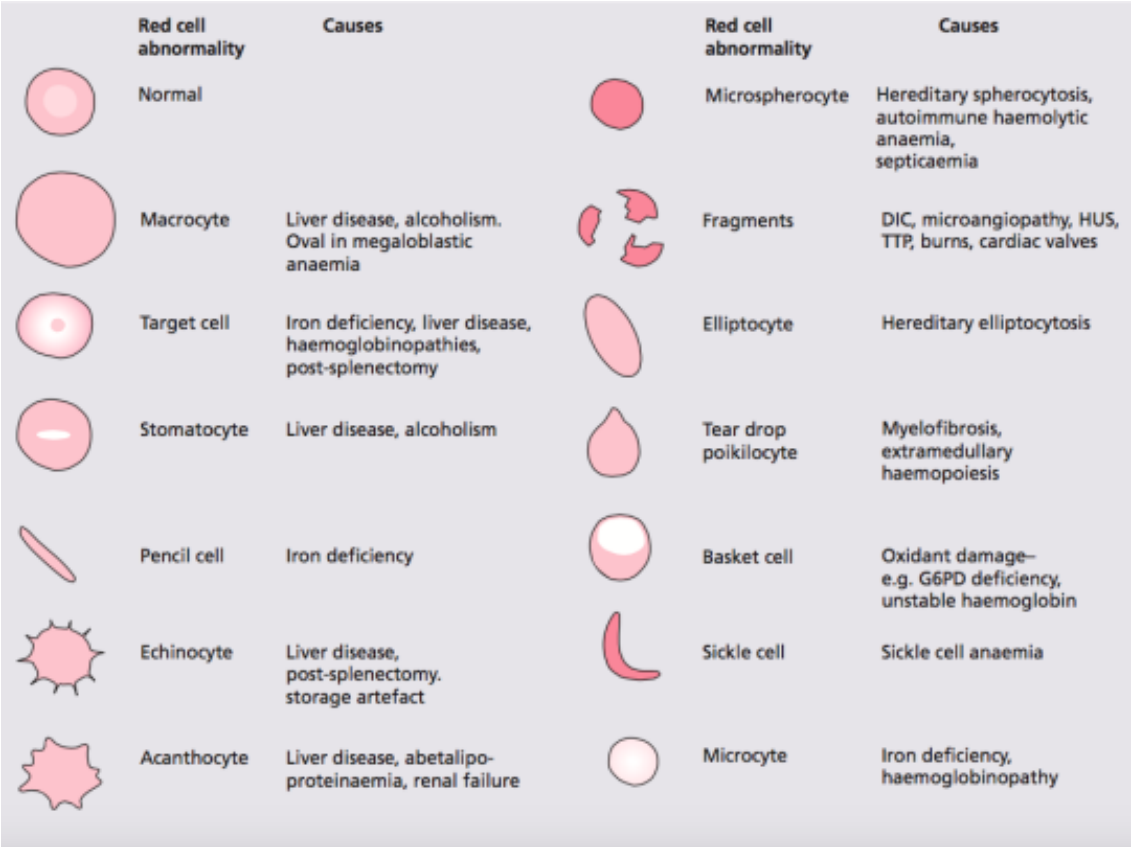


Figure 5: The structure of the RBC membrane*(9)*

RBC´s Membrane

The RBC´s membrane comprises of a bipolar lipid layer that anchors integral membrane proteins, surface antigens, and a membrane skeleton (Ankyrin, protein 4.1, spectrin and actin) (fig. 5). This membrane helps to hold the shape of the red cell thereby preventing it from being deformed. These proteins contain many sulphydryl (-SH) groups which are necessary for the structure of the cell. A defect on the membrane proteins leads to abnormalities of the shape of the red cell (e.g. elliptocytosis, hereditary spherocytosis and sickle cell anemia).(5)



Red cell abnormality	Causes	Red cell abnormality	Causes
Normal		Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy	Elliptocyte	Hereditary elliptocytosis
Stomatocyte	Liver disease, alcoholism	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
Pencil cell	Iron deficiency	Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
Echinocyte	Liver disease, post-splenectomy. storage artefact	Sickle cell	Sickle cell anaemia
Acanthocyte	Liver disease, abetalipo-proteinaemia, renal failure	Microcyte	Iron deficiency, haemoglobinopathy

Figure 6: More frequent variations in size and shape of the RBC*(10)*

Hemoglobin (Hb) Structure and function

Hemoglobin is a specialised protein which gives the RBC´s its bright red colour thereby enabling it to carry out its function as mentioned above. The hemoglobin gene is made up of four polypeptide chains, two subunits known as alpha-globin (Hb A) and another two subunits called beta-globin (Hb B), (Fig. 7). The Hb B gene gives out the command for the production of the beta-globin which can be found in the red blood cells. The function of this hemoglobin is to carry the iron-containing molecule known as heme on each of its subunits (i.e. each of the four protein Subunits carries a molecule making four molecules) which is important for the supply of oxygen in the tissues. A complete hemoglobin protein is able to carry four heme molecules at the same time.(4,5)

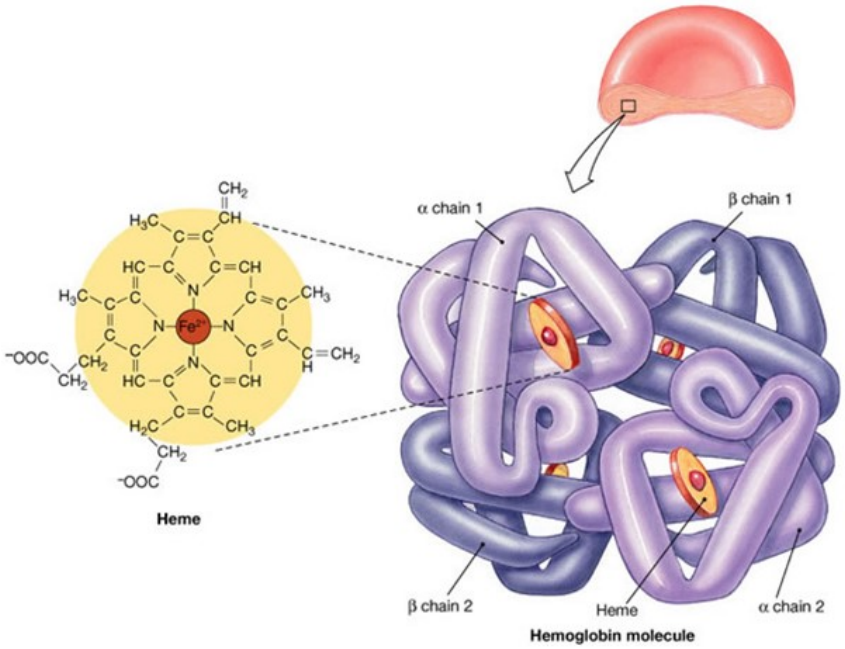


Figure 7: Structure of hemoglobin*(11)*

In the systemic circulation, the red blood cell carry oxygenated blood (blood rich in oxygen) from the lungs to the tissues and return to the lungs with deoxygenated blood (blood rich in carbondioxide). This only takes place with the help of external chemical factors. The haemoglobin molecule exhibits a greater bonding ability towards oxygen molecule in cases where there are higher levels of oxygen (in the lungs). When the oxyhemoglobin binds to the maximum capacity, it becomes saturated but its affinity towards oxygen increases whereas when its binding loose O_2 the affinity reduces. This regulation activity is also known as cooperativity and is measured as the hemoglobin- O_2 dissociation curve (Fig. 10).(4,5)

The normal position of the curve depends on the concentration of 2, 3-di phosphoglycerate (DPG), pH and CO_2, and the presence of sickled Hemoglobin (Hb S), shift the curve to the right

(Oxygen is being release more easily= decreased affinity). The different types of Hb is described in table 1 below.

Table 1: Types of normal hemoglobin

Hb A or Hb A1 (Adult Hb)	• Normal Hb in adults • Consists of about 97% of total Hb. • Comprises of 2α and 2β chains.
Hb A2	• Minor adult Hb, • Consists of 3% of normal adult Hb. • Comprises of 2α and 2δ chains
Hb F (fetal Hb)	• Main Hb during fetal life and in new-borns • Disappear gradually where it is replaced by Hb A shortly after birth. • Comprises of 2α and 2γ chains. • Has greater bonding ability towards O_2 than Hb A • Ensures O_2 transfer from maternal circulation to fetus through placenta.

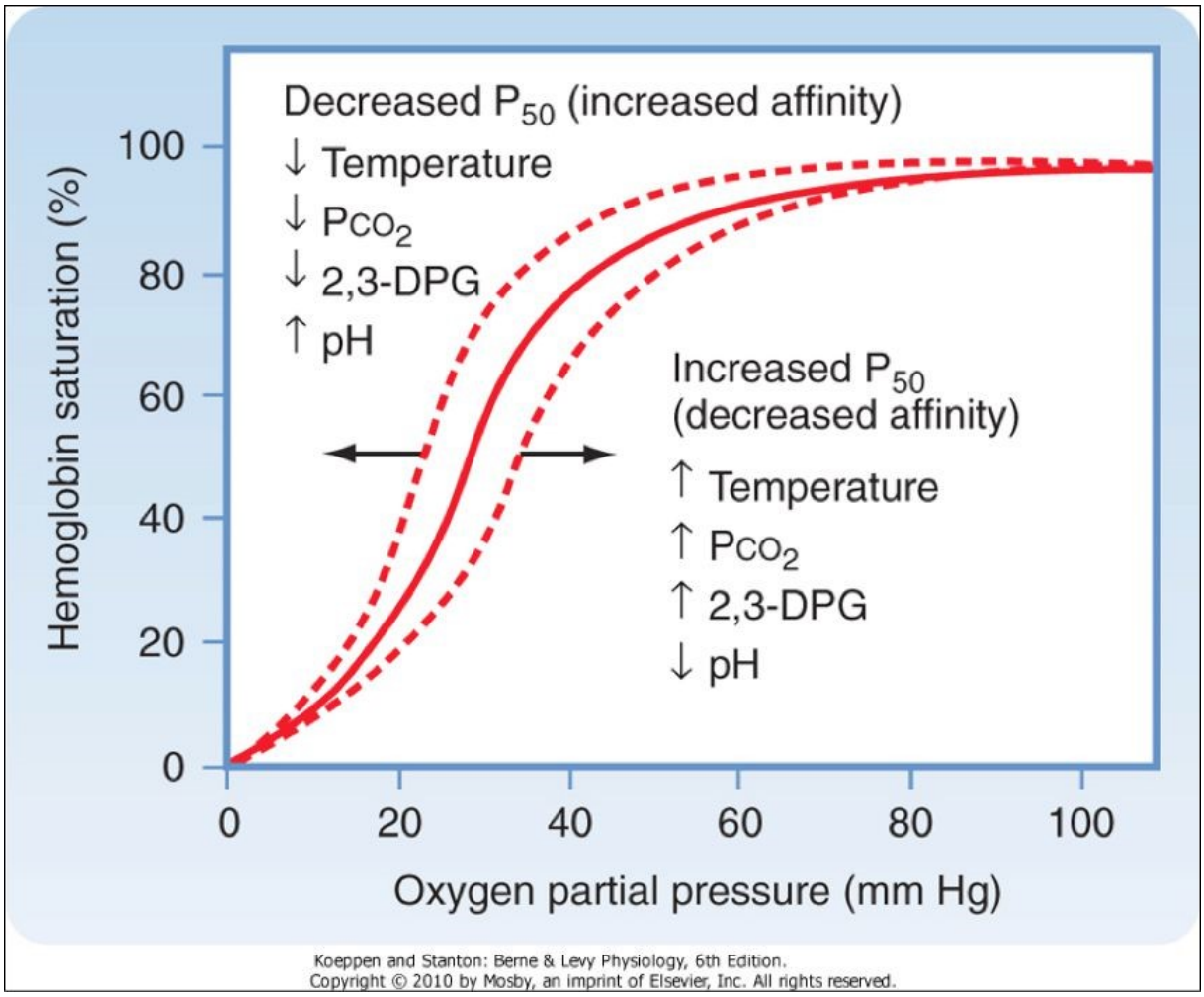


Figure 8: The partial pressure of oxygen at which Hb is 50% saturated.*(12)*

Decreased oxygen affinity, with increasing p50 (right-shift) occurs with an increase in Temp., P_{CO_2}, 2,3-DPG level and a decrease in pH. Increased oxygen affinity occurs during the opposite circumstances resulting to a decreasing p50 (left-shift).

Hemoglobin synthesis

Hem synthesis takes place mainly in the mitochondria and in the cytosol (Fig. 9). This synthesis undergoes different biochemical reactions commencing with the condensation of glycine and succinyl coenzyme A by 5-Aminolavulinic acid (ALA) synthase to form ALA. This molecule is then transferred to the cytosol, were chains of reactions take place resulting to a ring structure called Coproporphyrinogen. This molecule is then taken back to the mitochondria where another reaction takes place

and produces protoporphyrin. Ultimately, protoporphyrin combines with iron in the ferrous (Fe^{2+}) state to form heme (Fig.9).(4,13)

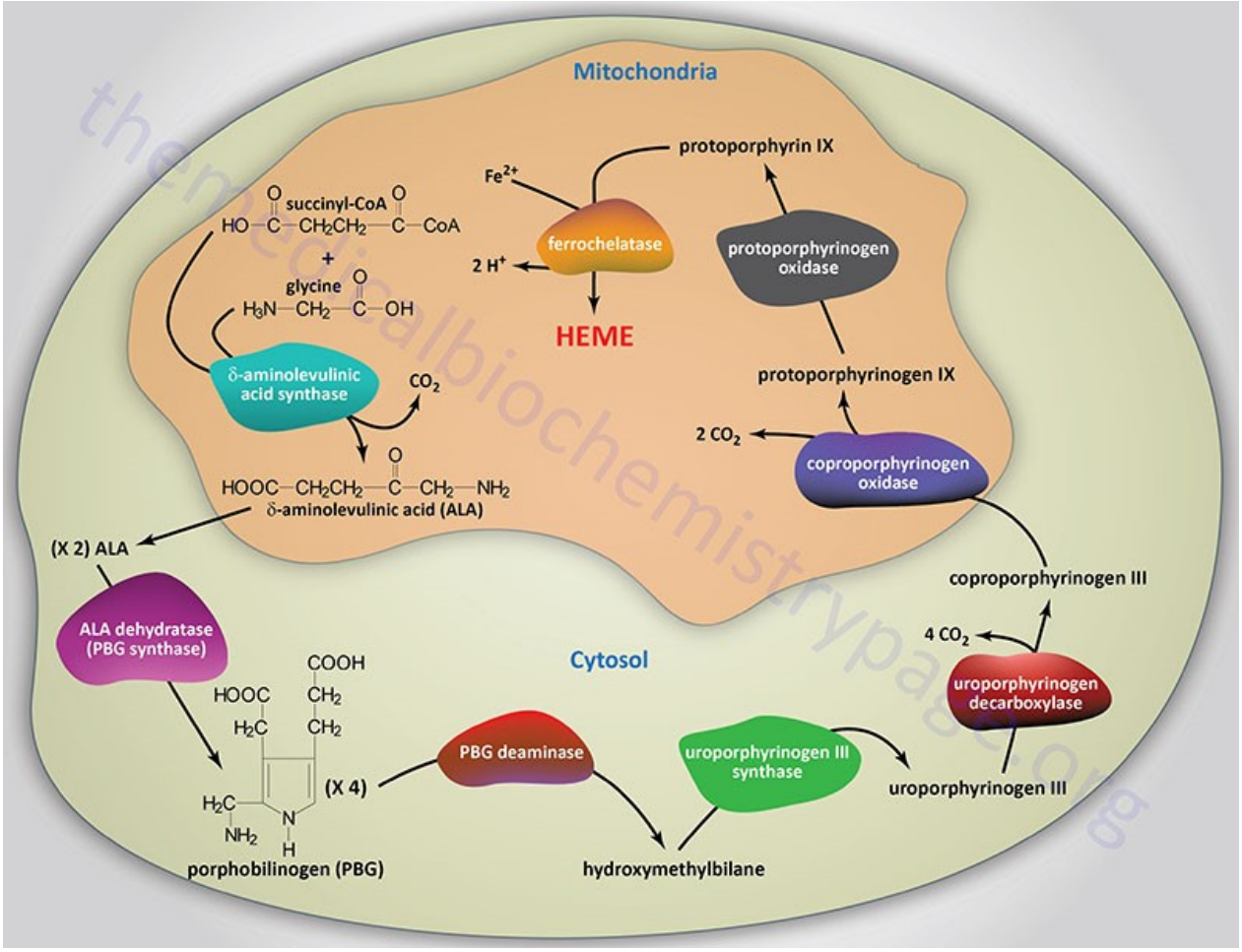


Figure 9: Hemoglobin synthesis in the developing red blood cell(14)

1.2.4 Functions of Blood

The blood plays many important roles within the body such as:

- They deliver oxygen to tissues via the RBCs.
- Supply nutrients e.g. glucose, amino acids and fatty acids via the plasma proteins
- They get rid of waste such as carbon dioxide, urea and lactic acid

- ❖ Immune system, by mobilising the WBCs
- ❖ Coagulation (blood clotting)
- ❖ Regulating the body pH and body temperature (thermoregulation)

1.3 Pathophysiology of Sickle cell anemia

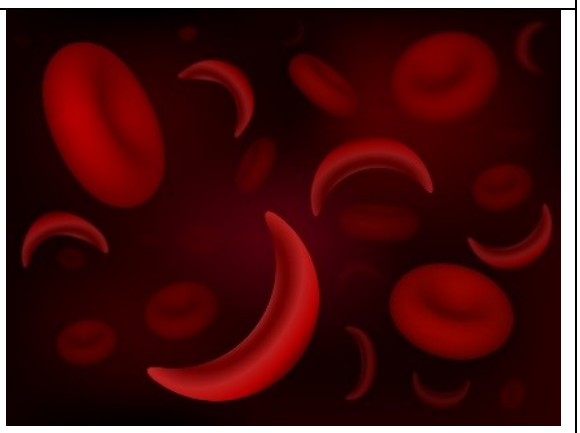
Deoxygenation seems to be the most important variable when it has to do with the occurrence of sickling (fig. 11). Genetics also plays a key role in the formation of SCA. First of all, the differences between the normal and sickled RBC are listed in (Table 2) below then more light will be thrown on the pathophysiology of the disease.

As earlier stated, SCA is a group of hemoglobin disorders which is caused as a result of mutation of the β-globin gene and is inherited as an autosomal recessive trait. This gene is referred to as “sickle β-globin gene”. The abnormality of this gene is caused by the substitution of valine for glutamic acid in position 6 in the β chain (Fig. 10 & 12). (4)

This change causes the Hb S (sickle cell hemoglobin) to be insoluble in its deoxygenated state, thereby forming crystals in the RBCs. Deoxygenated Hb S polymerises into long fibres (Fig. 13) causing sickling, vascular occlusion, also hemolytic anemia (shortening of RBC lifespan from 120 days to 20 days or less) which takes place in the spleen (Fig.12).

Table 2: Comparision of normal red blood cell and sickle red blood cell

NORMAL	**SICKEL**
Disc-shaped	Sickle-shaped
Soft (like a bag of jelly)	Hard (like a piece of wood)

Easily flow through small blood vessels	Often get stucked in small blood vessels
Lives for 120 days	Lives for 20 days or less
 (*http://cadawest.org/category/resources/self-employed-dancer*)	 (http://blog.targethealth.com/step-toward-gene-therapy-for-sickle-cell-disease/)

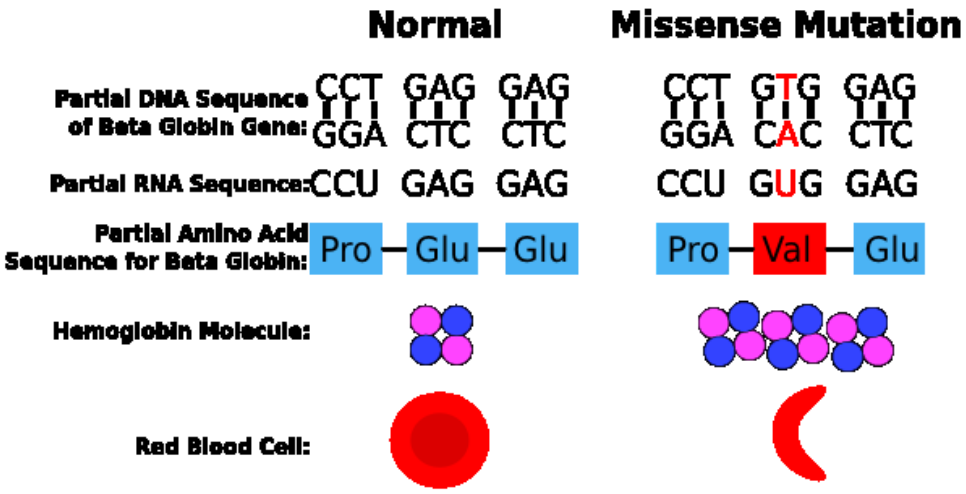


Figure 10: Molecular pathology of sickle cell anemia(15)

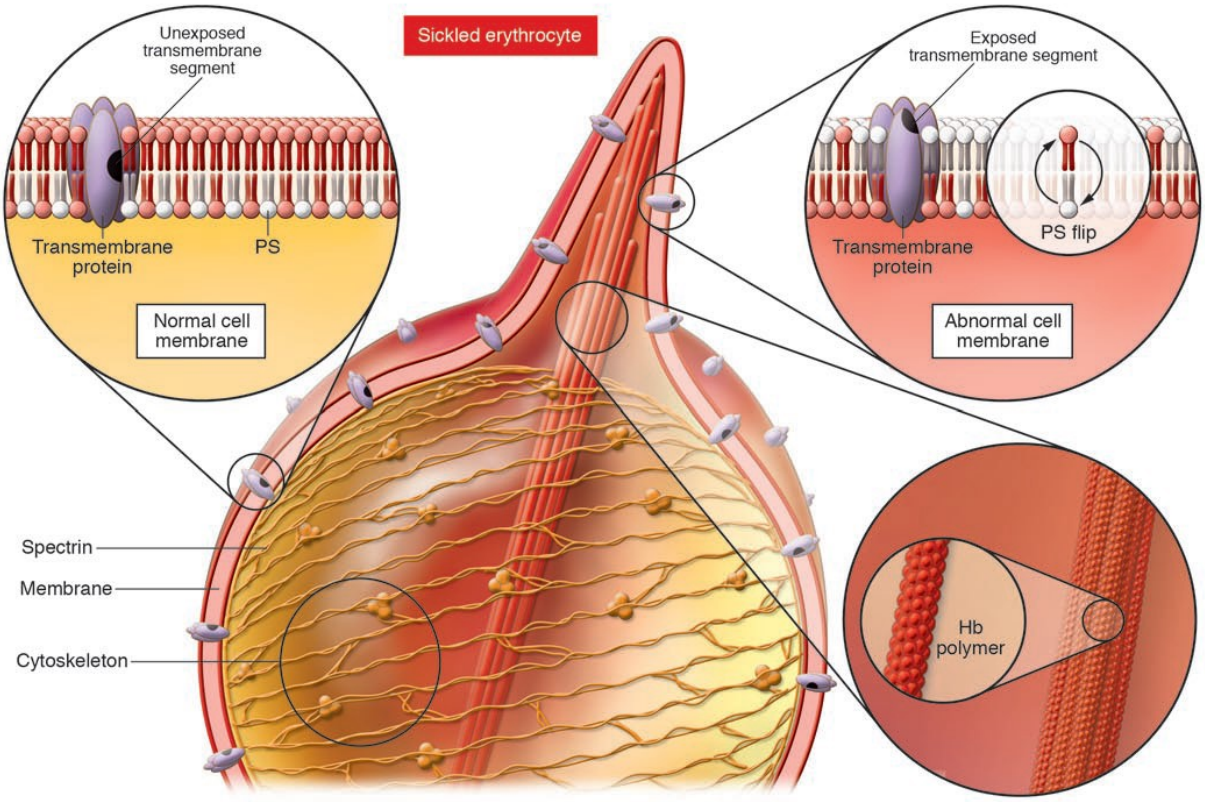


Figure 11*:* Alteration of the RBC membrane by polymers (16)

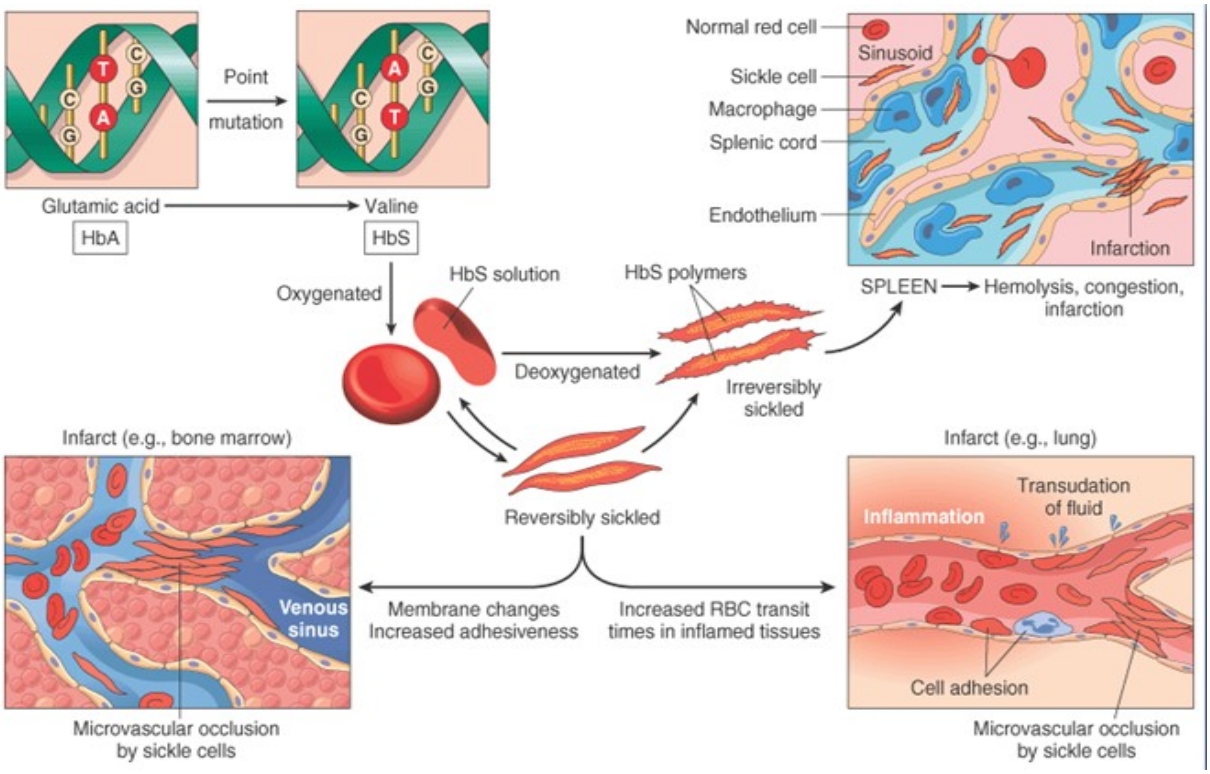


Figure 12: Pathophysiology of Sickle Cell Anemia*(17)*

As a result of the exchange of valine for glutamic acid, there is a structural change in the haemoglobin which leads to the formation of a hemoglobin Polymer. The hemoglobin polymers interfer with the RBC cytoskeleton thereby creating projections which give the RBC the sickle-like shape. The prothrombotic and proinflammatory state of sickle cell blood comes about when it is exposed to negatively charged glycolipids.(16)

Most sickled erythrocytes get back their original shape after rehydration and reoxygenation. Sickling can lead to irreversible plasma membrane damage. Such that the plasma membrane reduces in its performance in the area of active transport, thereby permitting an influx of calcium ions, which activate an ion channel that permits the efflux of potassium ion and water. Occasionally, irreversible sickling occurs in SCA but not in children with sickle cell trait.

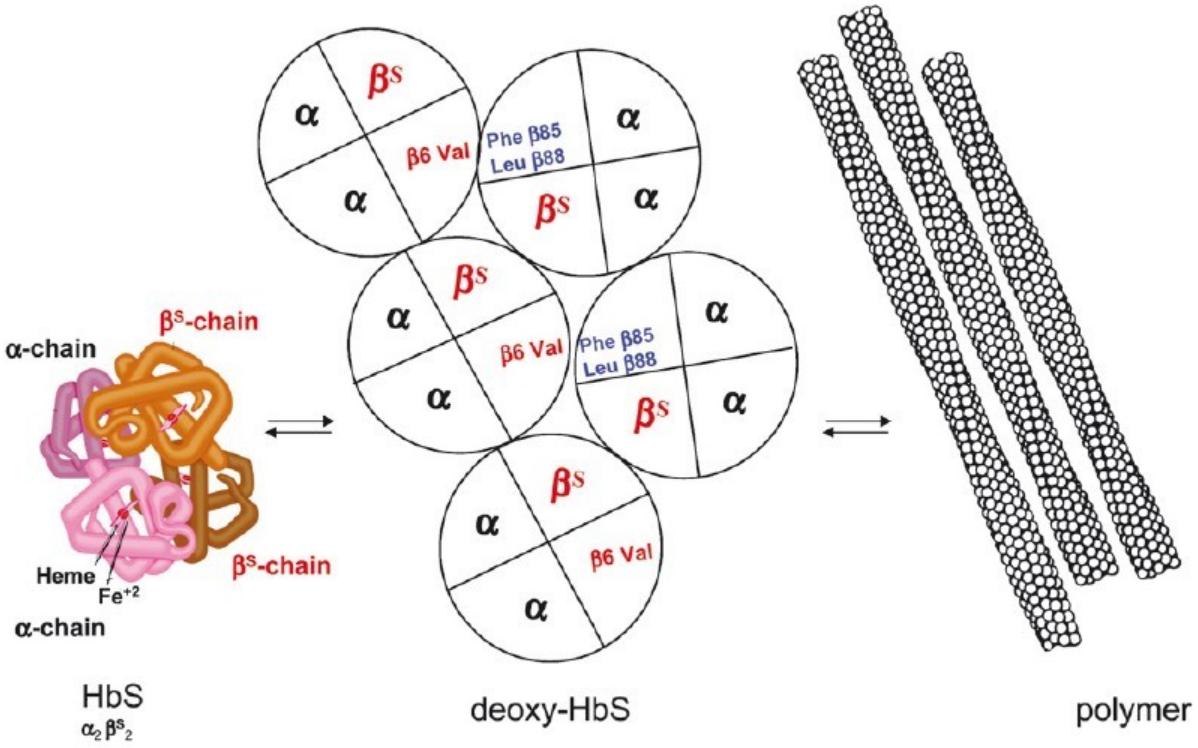


Figure 13: Polymerisation of hemoglobin in sickle cell anemia*(18)*

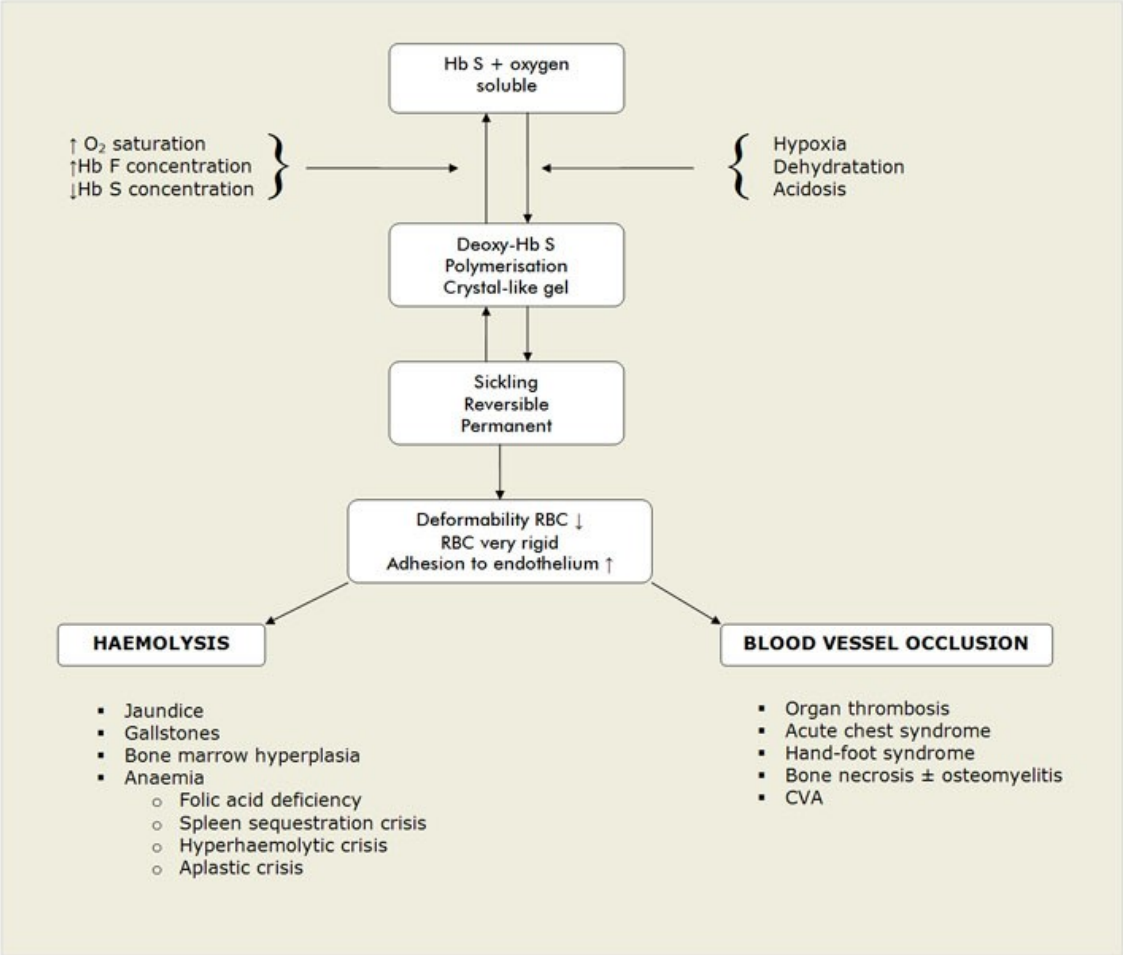


Figure 14: Precipitating factors that can trigger sickling(19)

(Top right): oxygen saturation (Hypoxia) of the blood, ↓ pH in the blood (acidosis) reduces the oxygen affinity of hemoglobin see (fig.4), (dehydration) leads to the reduction in volume of RBC cytoplasm, also ↑ plasma osmolality, ↓ plasma volume, and low temperature.

Factors that prevents Hb S polymerisation → top left.(20)

1.4 Genetics of SCA

1.4.1 Mutation of Hb B gene

The location of Hb B gene can be on the short (p) arm, especially from base pair 5,225,465 to base pair 5,227,070 on chromosome 11 (Fig.15). When there is a mutation (a change in structure) in the gene, through point mutation, it leads to the production of abnormal form of beta-globin called hemoglobin S or HbS. Both beta-

globin subunits are being exchanged for HbS resulting to a change in a single protein building block (amino acid). Due to the mutation, the amino acid glutamic acid which was originally located on the beta-globin will now be exchanged for amino acid valin at position 6 in beta-globin (Glu6Val or E6V). As a result of this change, the HbS molecules become stiff and long, thereby having the sickle-shape. Also, the cells have a very short lifespan leading to a reduction of the red blood cells (anemia). Due to the shape of these cells, closure of the small blood vessels also known as vaso-occlusion can occur resulting to pain and organ damage as shown above (Fig. 12). There can be other abnormalities in the beta-globin giving rises to other types of SCA (table 3). For example, only one beta-globin subunit is exchanged with hemoglobin S and the other subunit with hemoglobin C (HbSC) disease, the amino acid is also altered (in this case the amino acid glutamic acid is been replaced with amino acid lysine at position 6) written as Glu6Lys or E6K.

Hemoglobin C (HbC) occurs when the two subunits of the normal beta-globin are substituted with two hemoglobin C and the amino acid lysine takes over (Glu6Lys). This disease is usually associated with people from the Western part of Africa.

Hemoglobin E (HbE), as a result of Glu26Lys mutation in beta-globin (amino acid change takes place at position 26). This disease is mostly found in Southeast Asia.

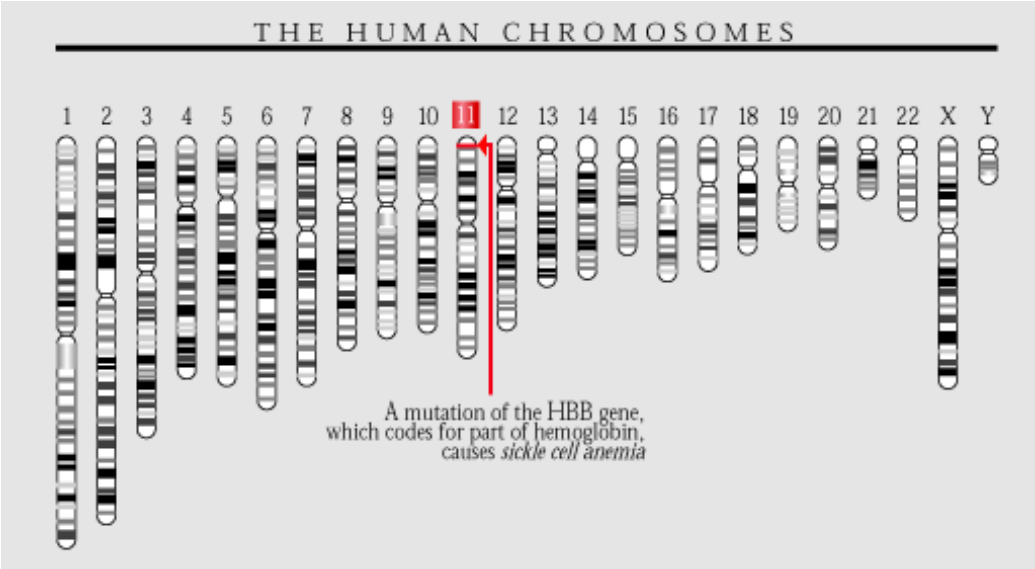


Figure 15: Location of Hemoglobin B gene*(21)*

Table 3: Genotypes of sickle cell anemia *(22)*

Abnormal haemoglobin type	Population affected	Trait (One mutated gene, one normal gene)	Outcome with two abnormal genes
Haemoglobin S	African American, Hispanic, East Indian, Mediterranean and Middle Eastern	Sickle trait - no symptoms	SCA
Haemoglobin C	West African descent	HbC trait - no symptoms	HbC disease - mild hemolytic anemia
Haemoglobin E	Southeast Asian descent. Common in Cambodia, Indonesia, Laos, Malaysia, Tailand and Vietnam	HbE trait - no symptoms	HbE disease - mild hemolytic anemia, microcytosis

1.4.2 SCA Inheritance

As earlier stated, SCA is a hereditary disease (can be passed on from one generation to the next generation or from parents to children), and we have also learned how the changes occur in the red blood cell, next we´ll be looking into how people inherit this disease.

It is an autosomal recessive pattern, i.e. the two copies of the genes are mutated (altered or changed). Usually parents of those affected have only one mutated gene (carriers or also called heterozygous) but do not exhibit signs and symptoms of this disease. The short arm of the Chromosome 11 of Hb B gene when mutated gives rise to sickle-cell anemia, however, a child can be a recipient of just one defective gene from either the mother or the father while the other one is healthy (heterozygous or Carrier) thereby producing just few sickled red blood cells, not enough to cause symptoms. On the other hand, when a child happens to receive two mutated genes (one from each parent) the child is said to have the disease (homozygous). If two parents who are carriers, the possibility of them having a child with the disease is 1-in-4 chance and the possibility of their child´s being just a carrier is 1-in-2 chance. See Fig. 16.

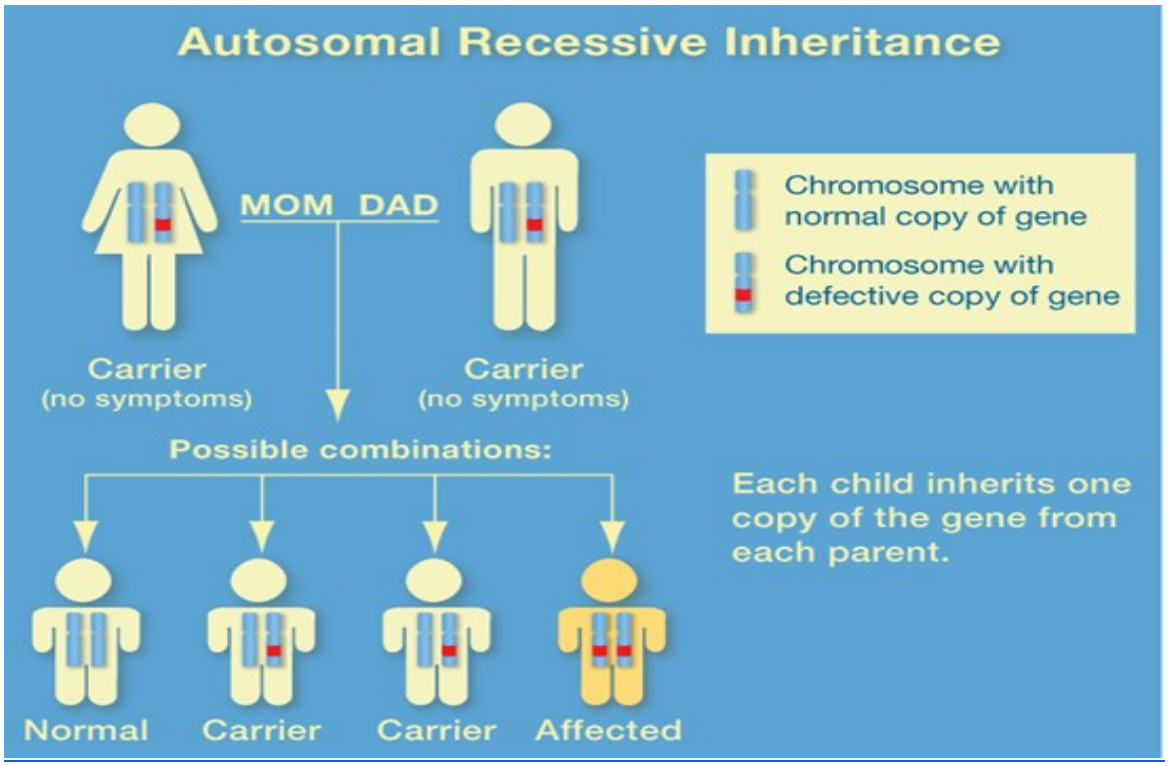


Figure 16: Sickle cell anemia inheritance pattern(23)

1.4.3 SCA Epidemiology

SCA is commonly found among people from Africa, India, Mediterranean countries South and Central America (Fig.18). (24)

About 2% of all children of SCA occurs in Africa. In Saudi Arabia about 4.2% of the population are heterozygote Sickle Cell (SC carrier) and 0.26% are homozygote (SCA). About 200 000 babies with SCA are given birth to annually in africa. In Nigeria for example, about 24% of the population are carriers of this gene. Implying that, about 150 000 children with SCA are been given birth to annually in Nigeria. (24)

The sickle-cell trait permits some resistance to malaria during the crucial, early childhood, thereby supporting the survival of the host and succeeding transmission of the abnormal hemoglobin gene. As a result of the complicated lifecycle of the malaria parasite, it lives part of its life in the red blood cell. (fig. 17)

In a SC carrier, the abnormal haemoglobin bursts before its time due to the presence

of the malaria parasite. As a result of this, the Plasmodium parasite is no longer capable of reproducing. This protection only takes place in heterozygous gen. Those with homozygous gen don´t receive such protection. (24)

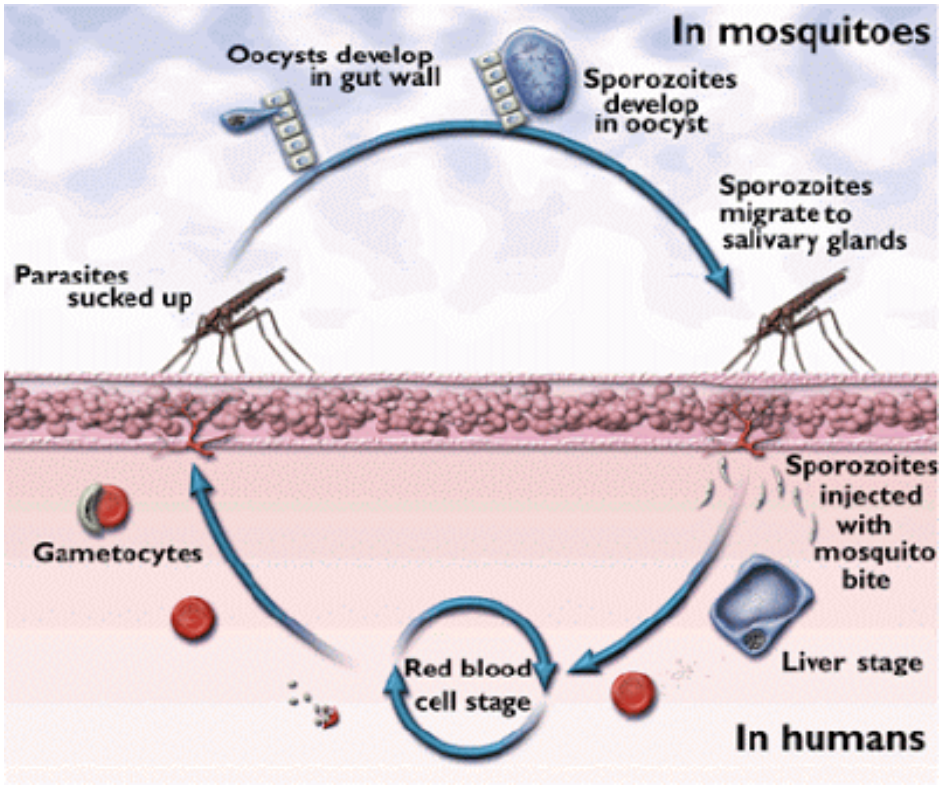


Figure 17: The life cycle of plasmodium falciparum *(*25*)*

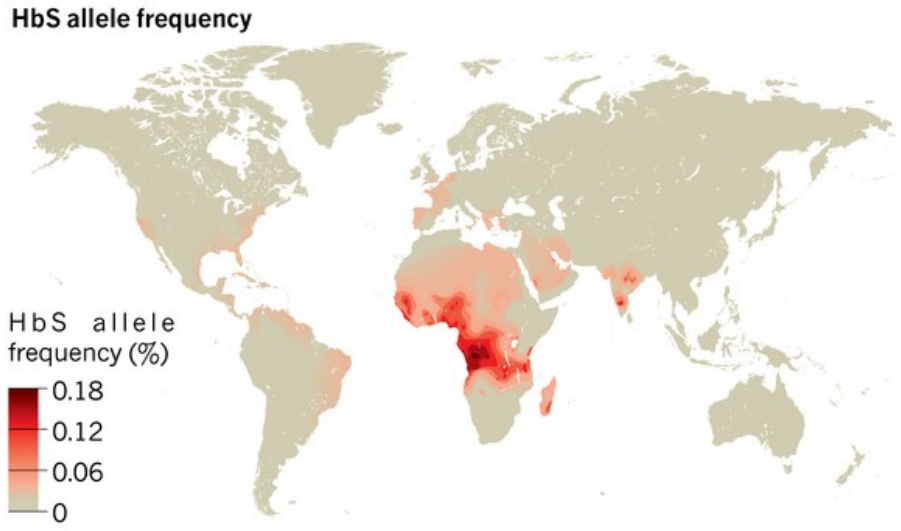


Figure 18*: Sickled Haemoglobin (*HbS) allele frequency*(*26*)*

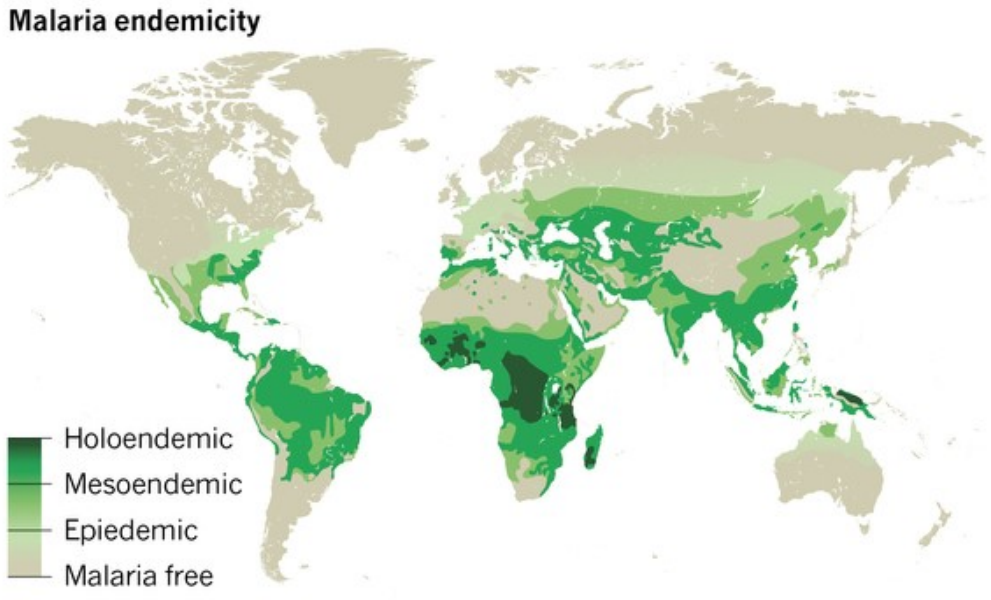


Figure 19: Malaria endemicity(26)

1.5 Clinical manifestations of SCA

The symptoms of SCA could start occurring from 6-12mths of age because of the replacement of fetal hemoglobin by HbS. The symptoms have two characteristics: a chronic disease with intensifying condition; the second is at it is a condition affecting RBCs that supply oxygen to all cells of the body. Therefore, any part of the body can be endangered by sickle cell disease.(20)

Vaso-occlusion and haemolytic anemia are to be related to the main signs and symptoms of this disease. This can later result in tissue ischemia, acute and chronic pain. Functional hyposplenism is an end result of splenic infarction which occurs during childhood and may later increase the infection risk. These complications have a major impact on mortality and morbidity.(27)

The major acute and chronic complications of SCD are listed below (table 4).

Table 4: Acute and Chronic complications of sickle cell anemia

	Acute	**Chronic**
Pain	Acute vaso-occlusive pain episodes, acute chest syndrome	Pain from tissue infarction, osteonecrosis, ulcers
Infection	Sepsis, pneumonia, Meningitis	Leg ulcers, osteomyelitis
Anemia	Aplastic crisis, splenic sequestration crisis	Compensated haemolytic anemia, chronic hypersplenism
Renal	Renal infarction, hematuria, acute renal failure	Hypertension, chronic renal failure, nephrogenic diabetes insipidus
CNS	Ischemic stroke, hemorrhagic stroke	Silent cerebral infarctions
Skeletal	Bone infarction	Oteoporosis, osteomyelitis
Pulmonary	Acute chest syndrome, asthma, pulmonary thromboembolism	Chronic restrictive lung syndrome, pulmonary hypertension
Cardiac	Myocardial infarction, autonomic dysfunction, sudden death	Diastolic dysfunction, heart failure
Other	Fetal and maternal complications, venous thromboembolism	Functional asplenia

The symptoms of hemolytic anemia are: jaundice, irritability, fatigue and pallor, sometimes they are accompanied by "*crises"* or pain.

1.5.1 Infections

The main reason for high morbidity and mortality in patients with sickle cell disease (SCD) is infection. The mechanisms are: presence of catheter in the body,

functional hyposplenism or asplenism, reduced tissue perfusion, splinting, and hypoventilation. The development of functional hyposplenism starts from childhood (between 4 to 6 months), hence children have high infection risk. SCD patients have high viral infections (e.g., H1N1 Influenza, Zika Virus, parvovirus), probably due to enhanced inflammatory response and high sickling. (28) Malaria known to be a common cause of morbidity and mortality in children with homozygous SCA. Infections may present with fever and leucocytosis, and in some cases with headache, chest pain, wheezing cough; meningismus, seizures in meningitis; hypoxemia in acute chest syndrome.

1.5.2 Anemia

Sickle cells have a very short lifespan thereby going through a process known as hemolysis (fig.20). Contributing factors which helps in this process are: iron deficiency, low Serum erythropoietin concentrate and/or folate. The main causes of a reduction in hemoglobin level are: splenic sequestration crisis, hyperhemolytic crisis and aplastic crisis.(27)

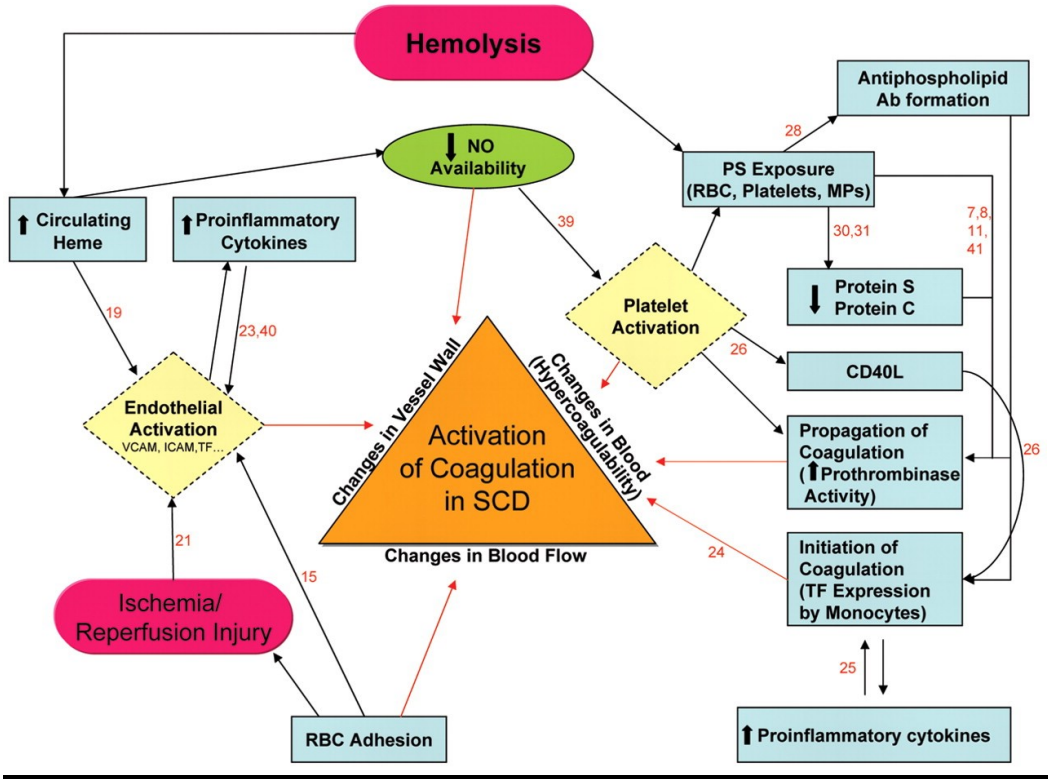


Figure 20: Mechanism of hemolysis(29)

A representation of pathophysiological mechanisms that leads to other hemolytic anemias and coagulation activation in SCA. Based on Virchow's triad, the pathways illustrated above helps in the activation of coagulation. It uses one of these three mechanisms: (i) Alteration in blood composition; (ii) Alteration in the endothelial wall; and/or (iii) Alteration in blood flow *Legend:* Ab: antibody; NO:nitric oxide; PS: phosphatidylserine; RBC: red blood cell; MPs: microparticles; TF: tissue factor.

Circulating microparticles from the endothelium, thrombocytes, red blood cells and monocytes, may also help during the hypercoagulable state in hemolytic anemia. The main instigator of coagulation is tissue factor and is abnormally expressed on circulating endothelium in SCD patients. (11)

2. AIMS AND OBJECTIVES

Sickle cell anemia is a vaso-occlusive and a pro-inflammatory disease, which can affect any part of the body. The major manifestations of SCD are related to hemolytic anemia and vaso-occlusion, which can, in turn, lead to acute and chronic pain and tissue ischemia or infarction. Although this disease could be very dangerous, SCA also has a positive effect in a SC carrier because the abnormal haemoglobin bursts before its time due to the presence of the malaria parasite. As a result of this, the Plasmodium parasite is no longer capable of reproducing. Sickle cell anemia is a genetically inherited disease, which not only affects the blood but also the vascular endothelium. The aim of this Diplom Arbeit (DA) is to **carry out an extensive update of the current literature related to SCA**, paying particular attention to vascular endothelium. Since vascular endothelium (dys-) function changes could lead to arteriosclerosis, thrombosis, heart attack or even stroke, this DA will also explore these aspects in detail. In addition, examined also will be literature related to current therapeutic options which are available to treat this disease.

This DA examines an important area of research, which is often under-studied. Despite 75% of SCA occurring in Africa, and in Nigeria alone approx. 200 000 babies being born every year with SCA - it is not exclusively an African disease anymore. Due to current migration and increased globalization, SCA is present among many African-Americans, Africans in Europe, as well as Africans in Austria. Moreover, being a Nigerian, SCA is a very interesting, deeply personal and motivating theme. Finally, it is my hope that the findings of this DA will alleviate the sufferings of millions of people who are currently affected by this scourge.

3. METHODOLOGY

Since this DA is about SCA, relevant publications related to this work were collected systematically. PubMed was used to carry out the primary literature review. This search strategy is divided into three groups:

First of all, keywords that were related to sickle cell anemia were used: sickle cell anemia, vascular endothelium, vaso-occlusive crisis, malaria and gender. Possible synonyms of these terms were then searched for: “sickle cell anemia” synonym “sickle cell disease” or “hemolytic anemia”, “vaso-occlusive crisis” synonym “pain”. These terms were then searched for in pubmed in order to acquire most of the relevant publications for this work.

Limiting the research criteria- The use of “AND” or “OR” helped in focusing on the field of interest and also to get appropriate papers for this research. The words that were combined are: sickle cell anemia AND vascular endothelium, sickle cell anemia AND vascular endothelium in children. These keywords used were in quote (“).

Sources of research- As stated earlier, **Pubmed** was the primary database used in this DA. It is an easy to access database that provides current and past research literature. Another database that was also used was **Web of Science**. From this database, one can know how many times a paper has been cited which in turn helps one find how relevant these articles are to the peers in the research area.

The list of References provided in every publication was then used to search for other publications. **Google** was used for searching of figures and specific tables related to the theme of this DA.

Some **textbooks** which covered sickle cell anemia were used in the as secondary literature. This information is largely described in the introduction section, where some background and general information related to sickle cell anemia is provided.

Choice of the literature- The literature selection to be used in the DA was based on the following criteria:

a) Was it relevant to the DA topic?
b) Were the articles published between 1979 and 2017?
c) Only articles with full texts were included as primary literature
d) Only publications written in English were used.

Organising the references used for this research- Zotero was used to save the references that were imported from Pubmed and Web of science. Zotero also formatted and created the Bibliography.

4. UPDATE ON CURRENT LITERATURE

After combining sickle cell anemia and vascular endothelium, there were 399 publications. For these publications, the search criteria were then refined to include only articles that were written in English language, and that were published from the period 1979-2017. This process reduced the number of publications to 30. Similarly, using another combination of "sickle cell anemia and vascular endothelium in children", led to the findings of 56 publications in this area. Taking into consideration both of the refined criteria that were specific for this DA, approximately **21 publications were found to be directly relevant.** These were then categorised into 3 sub-sections:

A) Studies showing factors that might contribute **to vaso-occlusion via vascular endothelium interactions in adults.**

B) Studies showing factors that might contribute to **vaso-occlusion via vascular endothelium interactions in children.**

C) Studies which examined the **current therapeutical options of SCA** and their side effects as well as potential nutritional supplements, which could be taken with minimal or no side effects.

A) Studies showing factors that might contribute to vaso-occlusion via vascular endothelium interactions in adults (n= 5 studies).

Author	Population Study	Aim	Results
Hebbel et al (1979)	• Preparation of endothelial cell: Human umbilical cord veins • Preparation of red blood cell: n=17 men and women	Could sickle red cell be less repulsive of the vascular endothelium?	• Approx.10 sickle red blood cell remained adherent to endothelium (after 12 washes) • Sickle red blood cell scatter themselves around the endothelial cell like rosette clusters.

Mohandas et al (1984)	n=2	Study to show the attachment of sickle erythrocytes to the endothelium	• 89%of Sickle cell adherence • $3x10^{-6}$dyne (strength of Adhesion), • >85% of Sickle cell attached themselves to the endothelium in plasma, • High density fractioned cells: attached themselves on large area.
Ballas et al (1988)	n=65 (men and women; 64 African-Americans and 1 Mediterranean origin)	Relationship between clinical severity and viscoelastic properties of sickle erythrocytes	• Negatively related to: - α-gene number with the HbA2, Hb, Hct - % of dense cells and painful crisis severity • Positively related with serum level and reticulocyte count • Positive relationship with frequency, red cell deformability and painful crisis severity.
Garrido et al (2013)	n=90 (men and women, 18-53 yrs.)	Effects of platelets on endothelial activation in vitro	• ↑Interleukin-1β, ↑P-selectin • ↑Interleukin-8 • ↑Intercellular adhesion molecule1 and nuclear factor kappa-light-chain-enhancer of activated B cells1
Connes et al (2014)	n=97 (men and women, all were in steady-state)	Study showing the relationship between parameters reflecting the hemolytic rate and the viscoelastic properties of red blood cell in sickle cell patients.	• No difference in the proportion of patients with↓ or↑ hemolytic component in the ↓ and↑ blood viscosity.

Hebbel and his colleagues (1979) (**30**) investigated the abnormal adherence of sickle erythrocytes to cultured vascular endothelium using the washing procedure. They found out that sickle red blood cell scattered themselves in rosette-like clusters around endothelial cell (Fig.21).

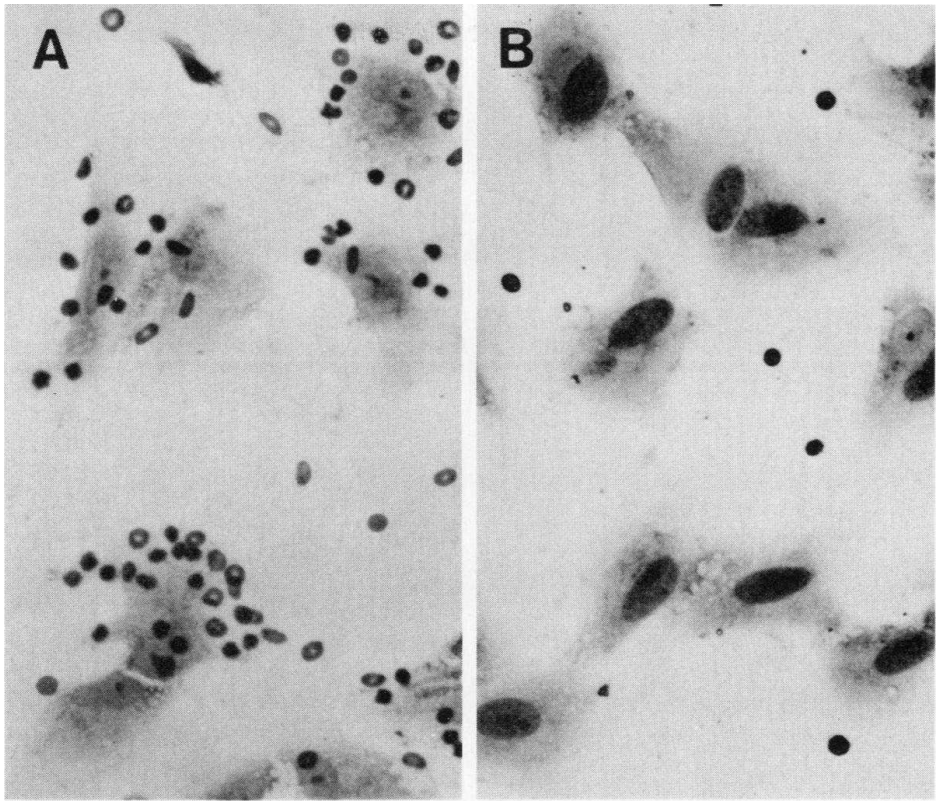


Figure 21: Distribution of red blood cell on sub confluent culture plates
Stained with Giemsa after the fifth plate wash. Sickle red blood cell distribute themselves in rosette-like clusters around endothelial cells (A), whereas normal red blood cell is present in fewer numbers and are randomly distributed (B). (30)

Another finding was that approx. 1-10 sickle red blood cell remained adherent to each endothelial cell after 12 washes. According to their finding, the reason behind this adherence is unknown but the sickle red blood cell membrane has been shown to have different abnormal features. (31)

Mohandas et al (1984)(32) unlike **Hebbel and his colleagues (1983)** used a different technique (micromechanical tech.) to get direct, quantitative measure of the adherence of individual red cells to vascular endothelial cell and also the strength of

adhesion. Although, they both obtained similar result (i.e. sickle cell adhering strongly to endothelial cell than normal cells), **Mohandas et al (1984)** used quite a few numbers of patients for this study than **Hebbel and his colleagues. Mohandas et al** major finding was that temporal changes in plasma factors and change in the cell membrane could enhance the adhesion of sickle cells on endothelial cell, which results to vaso-occlusive crisis.

Ballas et al. (1988)(**33**) explored the characteristics of the red blood cell that correlate with the frequency and severity of the sickle cell disease vaso-occlusive crisis. Before their haematological and rheological data were taken, they were in a steady state i.e. at least one week free of pain crisis. Their findings are:

1. The α-globin gene number had no predictive value of the frequency and severity of the painful crisis.
2. The red cell deformability shows a positive relationship with the frequency and severity of the crisis.
3. The next result showed negative relationship between the percentage of dense cells and the severity of the painful crisis. These results suggest that patients who have high percentage of dense cells and low red cell deformability may experience milder crisis than those who has higher RBC deformability.

Years later, **Connes and her colleagues (2014) (34),** investigated the relationships between haemolytic component and rheological characteristics of the RBC in SCA patients. This study shows that (i) patients having an increased haemolytic component have decreased red blood cell deformability; (ii) both dense red blood cells (irreversible) and reversible red blood cells are vulnerable to fragmentation when they experience high shear stress. There was no difference in proportion of patients with low or high haemolytic component in the low or high blood viscosity groups. Their other findings match with that of **Ballas et al (1988)** although **Ballas et al** used Afro-Americans for their study population**.**

In conclusion, both **Ballas** and **Connes** studies show that, low deformed red blood cells may cause mechanical obstruction at the entrance of narrow capillaries resulting to decreased tissue perfusion. It also causes increased mechanical stress

when applied on endothelial cells enhancing malfunction and over-expression of vascular cell adhesion molecules.

Garrido et al (2013)(**35**) explored the effects of platelets on endothelial activation. The following methods were used for this study: Microfluidic platelet adhesion assay, In vitro co-cultured assay and Flow cytometry, High-sensitivity ELISAs and qPCR were used for the quantification of platelets and the adhesion molecules respectively. From the results, IL-1β (platelets) and αIIbβ3 (adhesion molecule) were more significantly increased than the rest. They also found out that the platelets had an increased adhesion to fibrinogen under flow conditions and are also have the potentials of provoking endothelial cell activation by the induction of the increased expression of endothelial inflammatory cell activation and adhesive proteins. Even though few patients were used for this study and some of these patients were in use of hydroxyurea during the study, the results derived backs up the hypothesis that the adhesion of inflammatory sickle cell disease platelets to components of the endothelial wall may be of great importance in sickle cell disease as it can lead to vaso-occlusive process. Could it be that the number of those that were on hydroxyurea therapy was very small that there was no significant effect on the endothelial activation or the hydroxyurea therapy has no effect on some aspects of platelet activation or inflammatory status? More investigations could be carried out on this area.

B) Studies showing factors that might contribute to vaso-occlusion via vascular endothelium interactions in children. (n=7 studies)

Author	Population Study	Aim	Results
Mehta et al. (1982)	n=10 (1-22yrs.)	Using plasma prostaglandin ($6kPGF_1\alpha$) level to study the mechanism of decreased platelet aggregation.	➢ Platelet aggregation ↓, ➢ $6kPGF_1\alpha$ steady state: ↑, ➢ Vaso-occlusive crisis: ↓ than in steady state

Longenecker et al. (1983)	n=25 (16 HbSS, 9 HbSC)	Study showing prostacyclin levels in SCD.	➢ Differences between sickle cell disease, sickle cell trait and normal control are significant, ➢ **Level in crisis**: intermediate between sickle cell disease and sickle cell trait
Setty et al (1995)	n=42 (mix gender, 2-22 yrs.)	Assessment of plasma prostaglandin and thromboxane (both in steady state and in crisis) in children with sickle cell diease	➢ **6kPGF$_1$α** steady state: significantly lower; ➢ Crisis state: ↑ than steady state but below normal range. ➢ **TxB$_2$:** similar to 6kPGF$_1$α.
Inwald et al (2000)	n=24 (13 males, 11 females, 8-18 yrs.) n=12 (No neurological sequelae of sickle cell disease) n=12 (Neurological sequelae)	Study exploring the Differences in inflammatory cell activation between sickle cell patients with and without neurological sequelae	➢ **Platelet:** CD62P and CD40L ↑, ➢ **Granulocyte:**CD11b ↑, Neutrophils and monocytes ↑, circulating platelet-granulocyte complex↓ , circulating platelet-erythrocyte complex↑, ➢ **No difference** in any inflammatory cell parameter between sickle cell patients and sickle cell patients with neurological sequelae**.**

Setty et al (2012)	n=30 (Mixed gender), (2-10 yrs.)	Identifying the presence of Tissue factor-positive monocytes in sickle cell patients and their relationship with the other coagulation markers.	➢ **Sickle cell**: white blood cells↑, Lactate dehydrogenase↑, Type-2 Phosphatidylserine (PS)-Positive Erythrocytes ↑, High-sensitivity C-reactive Protein↑, Tumor necrosis factor α↑, soluble vascular cell adhesion protein 1↑, soluble E-selectin↑, soluble P-selectin↑white blood tissue factor↑, thrombin-antithrombin complexes↑, D-dimer↑, ➢ **Sickle cell carrier**: Tumor necrosis factor α↑, soluble vascular cell adhesion protein 1↑
Krishnan et al (2009)	n=70 (Males= 37, Females= 33, 2-20 yrs.)	Association between the inflammatory biomarker High-sensitivity C-reactive Protein and hospitalisation for vaso-occusive events in paediatric sickle cell disease.	➢ F1.2↑, D-dimer↑↑, ➢ Lactate dehydrogenase↑↑, soluble vascular cell adhesion protein 1↑, ➢ sP-Selectin↑↑, High-sensitivity C-reactive Protein ↑↑
Hatzipantelis et al (2013)	n=41(22 Males, 19 Females, 2-24 yrs)	Determination and comparison of three panels of plasma circulating biomarkers both during vaso-occlusive crisis and at "steady state".	➢ **Comparison of sex:** no differences between patient and control group. ➢ **Comparison of VOC and "steady state":** erythrocyte sedimentation rate↑, C-reactive Protein↑, endothelin-1↑, soluble P-selectin↑, platelet-derived growth factor, nitric oxide↑ (vaso-occlusive crisis.) ➢ **VOC frequency:** endothelin-1↑, soluble P-selectin↑, soluble vascular cell adhesion protein 1↑

Mehta et al. (1982) (**36**) investigated the mechanism of decreased platelet aggregation by measuring the level of plasma 6kPGF$_1$α, prostacyclin in the class of prostaglandin, a stable metabolite from endothelial wall. Prostacyclin is a very potent anti-aggregate of platelets and can also be derived from arachidonic acid. Due to its half-life (approx.30 sec.), it cannot be measured directly hence measurements are done for its precursor (6kPGF$_1$α). After using cyclic endoperoxide analog, U 46619, a potent pro-aggregate to examine the platelet aggregation, platelet aggregation was still found to be reduced in sickle cell disease patients. These researchers also later discovered that the activity of the pro-aggregates prostaglandins is normally balanced by prostacyclin (anti-aggregate). The imbalance between these prostaglandins is believed to be a major factor in the maintenance of homeostasis. (37) The plasma was prepared in the presence of 1mM aspirin and radioimmunoassay was used to measure the Plasma 6kPGF$_1$α levels in the ten patients. One of the patients had acute vaso-occlusive crisis while the other had an infection. Both patients had low levels of prostacyclin compared with other patients in stable state. Compared to controls, however, there was an increase in prostacyclin level.

The above study was followed by another study by **Longenecker et al.1983** (**38**), who explored the prostacyclin level in children with sickle cell disease using serum prepared in the presence of indomethacin and radioimmunoassay was used for the measurement. In contrast to the results of **Mehta et al.1982** the Longenecker study showed a decreased prostacyclin level in stable state; the levels during vaso-occlusive crisis were higher. Probably the differences could be due to different methods (Mehta et al used aspirin and Longnecker study used indomethacin) or different patient groups there were used in the two studies.

Assessment of plasma levels of 6-keto-prostaglandin F$_1$α and thromboxane B$_2$ (TxB$_2$) in children with sickle cell disease was carried out by **Setty et al. (1995)** (**39**)**.** This investigation was done using radioimmunoassay after extraction of eicosanoids and separation by High-performance liquid chromatography. Their findings however showed that the levels of both 6kPGF$_1$α and TxB$_2$ (pro-aggregate prostaglandin) are

significantly lower when compared to the control group. But during vaso-occlusive crisis, though the levels of 6kPGF$_1$α and TxB$_2$ tend to rise above the steady state, but they were still below the normal range (Fig. 22). **Longenecker and colleagues (1983)** have previously carried out similar studies and they also arrived at similar results in serum 6kPGF$_1$α levels between patients and control group. Although more patients were used for this present study compared to **Longenecker** study, they both arrived at the same result. The high plasma levels of both 6kPGF$_1$α and TxB$_2$ during vaso-occlusive crisis observed in the Setty study (1995) could be as a result of platelet activation compared to steady state. The imbalance of these eicosanoids could have an impact in the potentiation of vaso-occlusive crisis in patients with sickle cell disease.

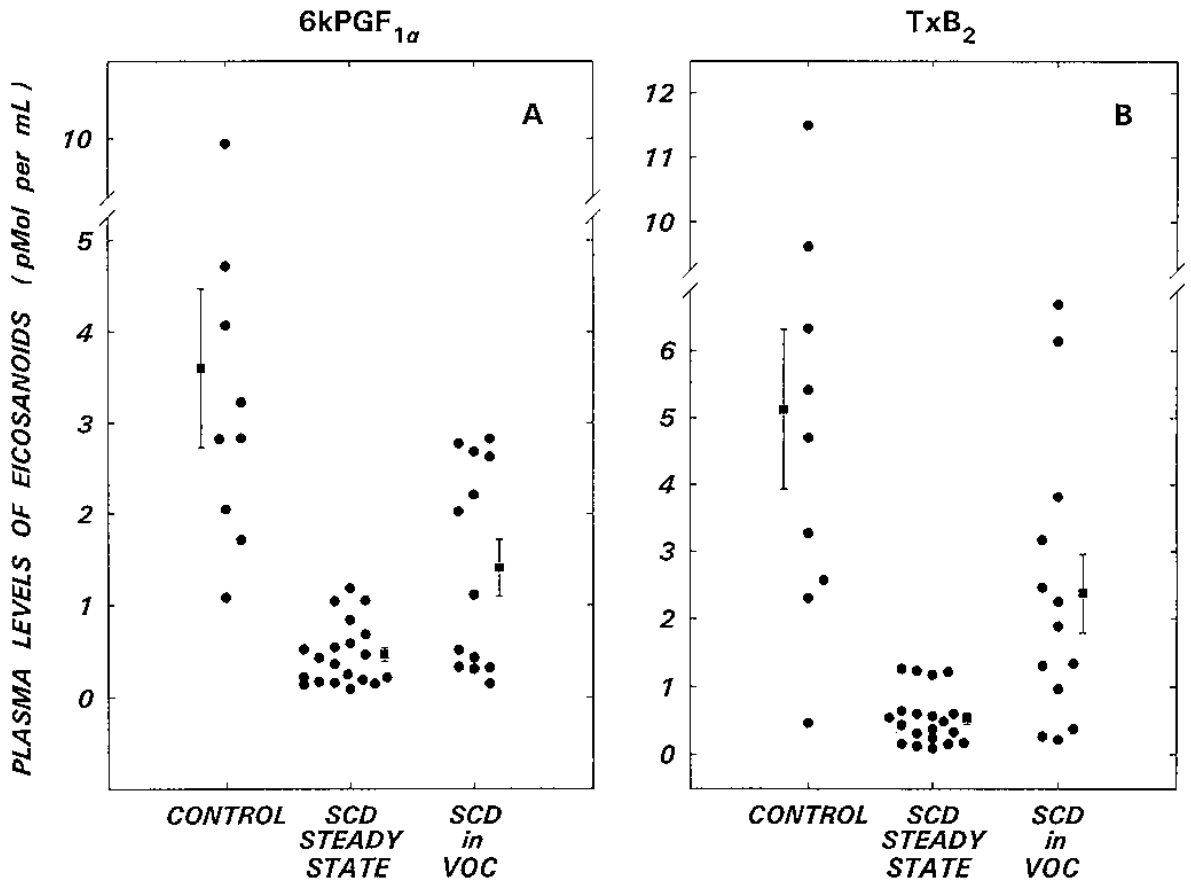


Figure 22: *Plasma levels of 6kPGF1 and TxB2 (39)*

Plasma levels of 6kPGF$_1$α ***(panel A),*** and **TxB$_2$ *(panel* B**) from control donors ***(n*** = 9) are compared with those from patients with SCD during steady state ***(n*** = 20) and in VOC ***(n*** = ***13).*** **(SCD: sickle cell disease, VOC: vaso-occlusive crisis)**

Inwald et al (2000) (**40**) aimed to test the differences in response of inflammatory cells in children with sickle cell and the sickle cell patients with neurological sequelae (stroke, seizures, and transient ischemic attacks) or frequent pain compared with healthy controls. They discovered that the children with sickle cell disease had high levels of granulocyte and platelet but the amount of circulating platelet –granulocyte complexes was low. The reasons behind this are not clear. In this study, they found out that there were no differences between sickle cell disease patients group and sickle cell patients with neurological sequelae. Probably, therapeutic interventions like transfusion could have concealed the differences in the group with neurological sequelae. The mechanism through which it took place is however, currently not known. Probably it could be as a result of oxygenation, hence reducing numbers of erythrocytes capable of sickling thereby reducing vaso-occlusion.

Setty et al. (2012) (**41**) explored the presence of tissue factor-positive monocytes in sickle cell disease and their relationship with the other coagulation markers (whole blood tissue factor, micro particle-associated tissue factor, thrombin-antithrombin complexes and D-dimer). In this study, the findings were: elevated circulating tissue factor-positive monocytes and significant correlations between tissue factor-positive circulating monocytes and markers of both early (e.g. Whole blood tissue factor) and late stages (e.g. Thrombin-antithrombin complex and D-dimer) of coagulation activation. These abnormalities in the coagulation activation are seen earlier in children with homozygous sickle cell disease and they lend further support to the notion that one of the cellular sources of the abnormality is the activated circulating monocyte. Another finding was that micro particle- associated tissue factor procoagulant activity was not elevated in the plasma of young children with sickle cell disease and it did not as well correlate with markers of coagulation activation. From these results, it shows that the circulating tissue factor-positive monocytes, not the micro particle-associated tissue factor, plays an important role in hemostatic activation in sickle cell disease.

Krishnan and colleagues (2009) (**42**) examined a broad panel of biomarkers associated with childhood sickle cell vaso-occlusive crisis. They reported that the inflammatory markers high sensitivity-C-reactive protein and white blood cell were

most significantly correlated with hospitalisations for vaso occlusive crisis, while markers of coagulation activation (F1.2, D-dimer) and endothelial activation (soluble vascular cell adhesion protein 1 and soluble P-selectin) were not associated with the clinical outcome of hospitalisation for pain events. This study highlights the complications related to clinical relevance of inflammation in micro vessel occlusion especially in sickle cell disease.

Hatzipantelis et al (2013) (**43**) determined and compared three panels of plasma of plasma circulating biomarkers both during vaso-occlusive crisis and at "steady state". In contrary to **Krishnan and colleagues** whose study took place in Philadelphia, USA, this current study was carried out in Thessaloni´ki, Greece. The biomarkers that were examined are as follows: **adhesion molecules** (vascular cell adhesion molecule, soluble P-selectin, and β2-intergrins) **endothelial factors** (Nitritoxide, endothelin-1, von Willebrand factor, platelet-derived growth factor, thrombomodulin and D-dimers) **acute phase reactants** (C-reactive protein erythrocyte sedimentation rate and Interleukin-6). In this present study, comparison of age showed that patients aged <14 yrs. exhibited increased levels of Endothelin-1, soluble P-selectin, Thrombomodulin and HbF and decreased levels of fibrinogen, D-dimers and Hb than older children. In the comparison of sex, there was no significant statistical difference. In both studies, there were significant higher values of acute phase reactants (Erythrocyte sedimentation rate and C-reactive protein) during crisis than in steady state. These results show that in the sickle cell disease, there is an induction in the circulating inflammatory cytokines expression via the endothelial cell activation. The chronic inflammatory state generated by this plays important roles in pathogenesis and progression in this disease. (44)

C) Studies which examined the current therapeutical options of SCA and their side effects as well as potential nutritional supplements, which could be taken with minimal or no side effects. (n= 9 Studies)

Author	Population Study	Aim	Results
Shome et al. (2015)	n=51 sickle cell disease (27males, 24 females)	Study showing the outcome of hydroxyurea therapy in sickle cell disease patients	➢ 4 patients= non-anemic post-treatment ➢ 77% of patients had ↑ in fetal hemoglobin fractions post-treatment ➢ ↑ In Hct, MCV, MCH and RDW. ↓ in the numbers of hospitalisation, pain crisis episodes and the number of transfused red blood cells
Aygun et al. (2011)	n=340 children with abnormal transcranial Doppler velocities	Determination of chronic transfusion practices for primary stroke prophylaxis in children with sickle cell disease	➢ 92% of patients: mean pretransfusion sickled hemoglobin percentage was 33.2 ± 14.0%
Alharbi et al. (2016)	n=2 (1 male, 1 female) (5years and 8 years)	Study on neurological side-effects after Blood Transfusion in sickle cell patients	➢ ↑ blood pressure, ↑ haemoglobin and neurological events
Paciaroni et al. (2015)	n= 31(2-17years.)	Study showing the effect of hematopoietic stem cell transplantation in children with sickle cell disease.	➢ All patients sustained engraftment ➢ 7 patients: grade 2, 5 patients: grade 3-4 acute graft versus host disease ➢ 28 survival without sickle cell disease thereafter.

Isgro et al. (2017)	n=37 (2-17 years)	Analysing pulmonary function in sickle cell disease patients that underwent bone marrow transplant.	➢ All patients sustained engraftment ➢ 48% of the patients: restrictive pulmonary patterns, 3 patients: acute graft versus host disease, ➢ No changes in spirometry value.
Morin et al. (2016)	n=11 (8 males, 3 females) (2-13 years)	A study determining the results (analytical and clinical) of sickle cell patients who had allogenic hematopoietic stem cell transplantation.	➢ 10 patients: A stable graft ➢ 7 patients: arterial hypertension, ➢ 3 patients: acute renal failure, ➢ 9 patients: cytomegalovirus reactivation, ➢ 4 patients: neurological complications, ➢ 6 patients: acute graft versus host disease, one of whom developed grade IV intestinal aGVHD causing his death. ➢ 90.9% overall survival, 81.9% event-free survival.
Ohnishi et al. (2000)	n=20 (12 males, 8 females), (18-26 years)	A study targeting the protection of the red blood cell membrane from oxidative injury using nutritional antioxidants supplements.	➢ ↑ in hematocrit level by 20%, ➢ ↓ in average number of painful crisis by 36%, ➢ Most of the patients were more energetic.
Arruda et al. (2012)	n=83 (18-68 years)	Exploration of the outcome using antioxidant vitamins C and E supplements	➢ No effect in reducing the symptoms and acute side-effects of sickle cell disease, ➢ Two patients experienced ischemic stroke, ➢ It is associated with the worsening of hemolysis and markers of inflammation in sickle cell patients.

Daak et al. (2013)	n=140 (2-24 years)	Investigation on the efficacy of vitamin E and omega-3 fatty acids in sickle cell patients.	➢ ↓ in vaso-occlusive crisis, ➢ Severe anemia and blood transfusion, ➢ ↑ in hemoglobin concentration, ↑ in percentage of fetal hemoglobin, ↑ blood flow and cell resistance to lysis, ➢ No effect on sequestration crisis, stroke, and vascular necrosis.

Shome et al. (2015) (**45**) explored the effect of hydroxyurea therapy in 51 sickle cell disease patients. As a result of the administration of hydroxyurea therapy, remarkable improvement in the clinical status of almost all of the patients was noticed. Only one patient had no reduction in the number of pain crisis or hospital visitations. There is a relationship between changes in fetal haemoglobin levels and the changes in mean corpuscular haemoglobin (MCH) and Hb. From the findings, hydroxyurea therapy could shorten the number of hemolysis thereby reducing disease morbidity.

Aygun et al. (2011) (**46**) investigated the current provision of chronic transfusion therapy in other to aid in the reduction of the risk of primary stroke in children with sickle cell disease. 32 clinical sites collected data on 340 children with sickle cell disease and abnormal transcranial Doppler velocities receiving chronic transfusions. The target of this study was to maintain sickle cell haemoglobin <30% but it was difficult to achieve. Possible hindrances from accomplishing this task include: (1) family failing to understand the need for prompt transfusions; (2) physician and staff goals in the aspect of transcranial doppler screening and chronic transfusion therapy (3) development of allo- and auto-antibodies; (4) hypersplenism; (5) problems associated with venous access. Receiving transfusions on time was seen to be the most important factor in the maintainance of HbS at ≤ 30%. Deviations from the standard mentioned above may change clinical outcomes such as transcranial doppler velocity reductions, change in cerebral vasculopathy, or stroke prevention.

Alharbi et al. (2016) (**47**) explored the neurological side-effects (Reversible posterior Leukoencephalopathy Syndrome, RPLS) after the administration of blood transfusions in two sickle cell disease patients. RPLS was first described in 15

patients who developed clinical features and had confirmatory radiological findings. (48) In this present study, both patients had elevated blood pressure (Case 1: 171/97, Case 2: 150/100), increase in hemoglobin after blood transfusion followed by neurological events (headache, unresponsive to painful stimuli). An MRI of one of the patients showed symmetrical T2 signal hyperintensity in the occipital and posterior lobes consistent with RPLS. Blood transfusions are administered to prevent stroke. However, much attention should be paid to the rate of administration and the volume. An increase in haemoglobin level can increase blood viscosity which can further lead to neurological complications like RPLS.

Paciaroni et al. (2015) (**49**) investigated the effect of hematopoietic stem cell transplantation in children between 2010 and 2014. These patients had bone marrow transplantation from human leukocyte antigen (HLA)- identical sibling donors following a myeloablative-conditioning regimen. The results show an overall survival of 90% and an event-free survival of >80%. Due to this result, this therapy could be considered for patients that are diagnosed with SCA and with an HLA-identical donor as soon as possible in order to avoid development of disease and treatment-related irreversible organ damage.

Another perspective of hematopoietic stem cell transplantation in children between 2010 and 2015 was provided by **Isgro et al. (2017)** (**50**) who analysed the pulmonary function in sickle cell patients that went through bone marrow transplantation using high-resolution computed tomography scan and spirometry, before and after transplant. The indications for this therapy are: repeated and severe vaso-occlusive crisis, stroke, acute chest syndrome in association with minimal or no access to hydroxyurea and/or transfusion therapy in their country. 3-6 months' post-transplant, there was no significant change in the spirometry value. The results indicate that restrictive pulmonary patterns are a common finding. Probably this might be from episodes of vaso-occlusion in the lung which can result in pulmonary infarction, necrosis of the alveolar wall, pulmonary fibrosis and progressive loss of lung function or it might also be a result of fibrosis after many pulmonary infection diseases.

Morin et al. (2016) (**51**) explored the clinical and analytical outcomes of patients with sickle cell disease that underwent hematopoietic stem cell transplantation in their hospital from 2010 to 2014. The table below shows their outcome.

Table 5: Outcomes and complications of post transplantation

Patient	**Acute neurologic complications (day of onset) Seizures**	**Acute neurologic complications (day of onset) SAH**	**Hypertension (day of onset)**	**Acute kidney failure**	**aGVHD-localisation and grade**	**Other complications post-HSCT**
Pt 1	Yes, +1 +27	Yes, +1	Yes, +1	No	Yes, Cutaneous II	Day +27: brain MRI: ciclosporin toxicity, Mild cognitive impairment, Mucositis grade 3
Pt 2	No	No	Yes (day 0)	No	No	
Pt 3	Yes, +29	No	Yes, +17	No	Yes	
Pt 4	No	No	No	No	No	Mucositis grade 4
Pt 5	No	No	Yes, +13	Yes	Yes, Cuteneous II	Possible Aspergillus Infection
Pt 6	No	No	No	No	No	Catheter-related infection and left gluteus myositis due to MRSA
Pt 7	Yes, +1	Yes, +1	Yes, +1	Yes	Yes Cutaneous IV	Virus-associated hemorrhagic

					Gastrointestinal IV Hepatic III	cystitis, Cholestasis and development of bilary sludge and cholelithiasis, fever without microbial isolation
Pt 8	No	No	No	No	Yes cutaneous I	Mucositis grade 4
Pt 9	No	Yes, +2	Yes (-1)	No	No	Mucositis grade 1, Virus-associated hemorrhagic cysistis
Pt 10	No	No	Yes, +23	Yes	Yes, cutaneous III	Mucositis grade 3 Fever and neutropaemia
Pt 11	No	No	No	No	No	Mixed chimerism: intensified immunosuppression for 1 year.

aGVHD: acute graft versus host disease, SAH: subarachnoid haemorrhage, HSCT: hematopoietic stem cell transplantation

The study of Morin and colleagues and that of **Paciaroni et al. (2015**) showed same percentage of overall survival and event-free survival. Though **hematopoietic stem cell transplantation** therapy may be the only curative treatment available to patients with SCD, caution must be observed during this therapy, as it is associated with a

high risk of death (9%), attributed largely to graft-versus-host disease (9%) and neurologic complications (36%).

Ohnishi et al. (2000) (**52**) explored the usage of nutritional antioxidants supplements inother to protect the red blood cell membrane from oxidative injury such as: 6g of vitamin C, 1200 IU of vitamin E and 6g of aged garlic extract,. In a pilot trial of limited sample size and a short duration (6 months), there was an increase in the hematocrit level by 20%. The goal of this pilot study was to show that the blending of different antioxidants would have more therapeutical impact by using relatively high doses of the supplements mentioned above. Using nutritional supplements for therapy is beneficial as it would not be associated with serious side effects (e.g. as occurs with chemotherapeutic agents). In the future, the blending of hydroxyurea therapy with nutritional supplementation for sickle cell anemia could be worth investigating.

Arruda et al. (2012) (**53**) evaluated the impact of vitamins C and E supplementation in sickle cell patients. The daily doses were: 1400mg of vitamin C and 800mg of vitamin E. The anticipated result was: reduction in hemolytic markers. Due to the fact that hemolysis, anemia and vaso-occlusion are, at least caused - or aggravated by - oxidative stress, antioxidants such as vitamins C and E were used. However, the treatment led to the worsening of anemia by significantly increasing all the markers of hemolysis as well as some inflammatory markers. In contrast to **Ohnishi et al. (2000)** this present study used very low dose of vitamins C and E and did not use aged garlic extract. Garlic is known to have additional beneficial pharmacologic effects such as increasing cellular levels of superoxide dismutase, glutathione peroxidase and glutathione S-transferase (54–56) (an important cellular defense component) and it also inhibits platelet aggregation thus helping in the prevention of painful crisis). (57–59)However, the mechanisms of this vitamin-induced worsening of hemolysis remain to be determined.

Daak et al. (2013)(**60**) explored the efficacy of vitamin E and omega-3 fatty acids in sickle cell patients. Omega-3 fatty acid consists of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Sickle cell patients have a low level of this acid. The results of this study show that this supplementation hinders hemolytic- and vaso-occlusive crises in sickle cell patients. It carries out this functions by: decreasing blood cell aggregation, decreasing inflammation, ↓ in adhesion, ↓ in oxidative stress and by increasing vasodilation - and consequently blood flow (61,62) - thereby health

and related quality of life are improved. From these findings, it appears that omega-3 fatty acids could be effective, safe, easily available and cheap options in sickle cell disease treatment.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Sickle cell anemia is a hereditary disease which mostly affects the red blood cells and also the vascular endothelium. It is mostly associated with haemolytic anemia and acute painful crisis as a result of vaso-occlusion. The studies described in this DA suggests that red blood cell adhesion to endothelium, elevated inflammation biomarkers and endothelial cell activation may contribute to vaso-occlusive process in sickle cell disease. However, some studies did not find any significance. Temporal changes in plasma factors and change in the cell membrane could enhance the adhesion of sickle cells on endothelial cell which in turn leads to vaso-occlusive crisis. A study discovered that the children with sickle cell disease had high levels of granulocyte and platelet but the amount of circulating platelet – granulocyte complexes was low. However, the reason is not known.

There was no significant difference between sickle cell patients group and the group of sickle cell with neurological sequelae. Could it be that the differences were concealed by the therapeutic intervention (blood transfusion) in the sickle cell group with neurological sequelae? The answer needs further explorations.

Similarly, inflammatory markers such as high sensitivity-C-reactive protein and white blood cell were reported to have the most correlation with hospitalisation for pains. However, endothelial activation (soluble vascular cell adhesion protein 1 and soluble P-selectin) and coagulation activation markers (F1.2, D-dimer) were not associated with the clinical outcome of hospitalisation for pain events.

Hydroxyurea therapy led to an increase in fetal haemoglobin levels with changes also in (Hb) and (MCH). This implies that an administration of hydroxyurea could result to a reduction of hemolysis thereby leading to reductions in disease morbidity.

Hematopoietic stem cell transplantation could be a useful therapeutic option for sickle cell disease as the results of some trials showed an overall survival rate of 90% in patients and an event-free survival of >80%. However, caution must be observed during this therapy because of its association with a high risk of death and neurological complications. In addition, the use of nutritional supplements like aged garlic extracts in combination with vitamin C and E were shown to increase the haematocrit level by 20%. Another nutritional supplementation was omega 3-fatty

acid. The results showed that omega 3-fatty acids could play important roles in the prevention of vaso-occlusive- and haemolytic crisis in sickle cell patients.

In summary, the explored studies in this DA suggest the effects of sickle cell disease on the vascular endothelium. The current therapeutic interventions could help in reducing disease morbidity, and consequently reducing hospitalisation. However, using novel approaches such as stem cell transplantation and nutritional approaches could be beneficial. More studies that investigate the use of these emerging therapeutic options need to be explored to improve the quality of millions affected by this debilitating disease.

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