

Sickle Cell Disease in Sub-Saharan Africa: Molecular Mechanisms Underlying Episodic Crises, Current and Emerging Therapeutic Strategies in Treatment

REVIEW

Ajonuma Louis Chukwuemeka^{1,3}, Yusuf Azeez¹, Bamiro Adebisi Saka¹, Makonjuola Samira Lobo², Ajuonuma Udochukwu Joshua³, Ajuonuma Mary Uchechi³, Emmanuel Blessing Chioma^{3,4}, Ogbedeagu Virgilus Mahakwe⁵, Chuku Chika Lawson⁶, Adabale Khedijat Abosede⁷, Dosunmu Adedoyin Owolabi⁷

Abstract

Sickle cell disease (SCD) is a haematological disease that affects multiple organs, thus eliciting episodes of chronic pain, acute anaemia and infection, due to a single nucleotide mutation in the β -globin gene, which results in the substitution of a glutamic acid residue in place of valine on the β -globin chain of the resultant haemoglobin protein molecule, the sickle haemoglobin (HbS). SCD is a major cause of morbidity and mortality characterized by episodes of vaso-occlusive crises, pain syndromes and end organ dysfunctions. Its global prevalence is highest in Sub-Saharan Africa with 75% of global births living in this region, of which Nigeria has the highest number of SCD patients with about 100.000 births each year. The burden of SCD in the sub-Saharan region of Africa is enormous. Emotional, financial and total healthcare costs are monumental. An understanding of the mechanism underlying the vaso-occlusive crises, pain syndromes, inflammatory conditions and other sequelae of SCD appears to be essential in providing more rational treatments. The present review discusses the prevalence of SCD in Africa, molecular mechanisms underlying SCD episodic crises including vaso-occlusive syndrome, anaemia and infection. Current available treatments modalities in Sub-Saharan Africa and possible new treatment methods that cure SCD are re-examined in light of these mechanisms.

- 1 Department of Physiology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.
- 2 Department of Pharmacology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.
- 3 St. Marys' Hospital Amakohia, Ihitte – Uboma, Imo State, Nigeria.
- 4 School of Nursing, Imo State University, Owerri, Imo State, Nigeria.
- 5 University of Abuja Teaching Hospital, Gwagwalada, Abuja, Nigeria.
- 6 Department of Internal Medicine, Braitheweith Specialist Hospital, Port Harcourt, Rivers State, Nigeria.
- 7 Department of Hermatology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.

Contact information:

Ajonuma Louis Chukwuemeka.
Department of Physiology, Medical Research Complex.

Address: Lagos State University College of Medicine (LASUCOM), 1-5 Oba Akinjobi Way, Ikeja, Lagos, Nigeria.
Tel: +234 7012436468.

✉ Louisca@alumni.cuhk.net

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Introduction

Sickle cell disease (SCD) is a haematological disease that affects multiple organs through episodes of acute illnesses such as chronic pain, acute anaemia and infection, in both children and adults. The disease develops due to a single nucleotide mutation in the β -globin gene, which results in the substitution of a glutamic acid residue at the 6th position into a valine on the β -globin chain of the resultant haemoglobin protein molecule, the sickle haemoglobin (HbS). This causes the formation of a defective protein that affects the structure of the red blood cell (RBC), producing a sickle shaped cell [1]. As an autosomal recessive disorder, SCD is the one of the most common monogenic disorders known and it is characterized by phenotypic heterogeneity due to occurrence of various types of mutation in the β -globin gene [2]. The predominant clinical phenotype is the homozygote HbSS otherwise called the sickle cell anaemia (SCA), while other complex heterozygotes also exist due to different mutation in the β -globin gene. For instance the HbS- β thalassaemia, HbSC (with HbSS in Africa), HbSO and HbCO (Arab) and HbD (Punjab) all exist to constitute what is known as SCD [3-5]. The African HbS globin haplotypes are present in three geographical locations, Senegal, Benin and Bantu regions with a fourth Arab-Indian haplotype believed to have originated from Arab slave trades in Africa [6]. The HbS gene exists in high frequency in populations that experienced a selective pressure from *Plasmodium falciparum*, a protozoan agent for malaria because the resulting HbAS heterozygote is conferred with some form of protection against the deleterious consequences of malaria. As such, SCD exists in high prevalence in areas where malaria is endemic.

SCD is a major cause of morbidity and mortality, and its global prevalence is highest in Africa, South America, India, Middle East and the Caribbean [7]. The highest global prevalence of SCD is in Sub-Saharan Africa with 75% of global births existing in this region and within this region, Nigeria has the

highest number of SCD patients with about 100,000 births each year amounting to 33% of global annual births [8, 9]. It has been reported that childhood mortality due to the disease in Africa ranges between 50-90%, although accurate information on this information is still lacking [10]. According to the World Health Organisation, 70% of deaths of SCD patients in Africa is preventable through low cost interventions such as new born screening for early identification of SCD patients and comprehensive care provision for disease prevention [11]. Although, increased incidence of mortality in children has been nearly eliminated in North American continent due to advancement in medical care and new born screening as well as provision of prophylactic antibiotics and pneumococcal vaccinations to prevent infection [12, 13], these still constitute a disease management that is not curative. The major forms of treatment available in this part of the world with significant effect include heamatopoietic stem cell transplant (HSCT), bone marrow transplant and red cell transfusion. Currently, only HSCT successfully carried out using stem cells or haematopoietic stem cells that have been genetically modified (gene therapy) can result in total cure by restoring normal erythropoiesis [14].

With the exception of South Africa where there are stem cell transplant centres [15], unfortunately in most parts of Africa, there is currently no curative treatment and the major form of treatments that have been in use in the region includes blood transfusion and prophylactic treatments. For instance, a new study reported that health care centres in Nigeria now prescribe patients with hydroxyurea (for those who can afford the drug when prescribed), folate supplements and penicillin prophylaxis [16]. Based on these facts, the treatment modalities currently available in sub Saharan Africa does not suffice for the burden of morbidity due to the disease in the continent harbouring more than 50% of the affected individuals with SCD. Here, we review the current treatment modalities in Africa and

suggest ways of better managing the disease as well as providing timely and effective treatments for patients with SCD.

Results

Signs and Symptoms of SCD

SCD is a haematological disease that stems from the production of defective RBCs. Nonetheless, patients suffering from the disease presents with a number of clinical signs and symptoms that represent multisystem failure/organ damage. The major symptoms are mainly categorized into three; haematological complications and associated syndromes, vaso-occlusive crises and pain syndromes, and end organ dysfunction.

Haematological complications and associated syndromes

SCD is a disease of the blood and it is mainly associated with complications arising from dysfunction of the RBCs. Chronic anaemia is a classic symptom in SCD patients occurring throughout their lives. Depending on genotype, the severity of anaemic crises in SCD varies and patients with homozygote HbSS experiencing the most severe anaemia due to the highest reduction in haemoglobin level, while those with β -thalasemia experience the least severe crises. Although SCD patients do not present with anaemia at birth due to protection from foetal haemoglobin (HbF), anaemia arises due to production of the adult Hb later in life resulting in acute episodes of haemoglobin reduction, hence anaemic crises. During the first five years of life, the Hb level remains virtually stable, although clinically significant lowering of Hb do occur at intervals resulting in such secondary symptoms as hyperhaemolysis, splenic complications, and aplasia.

Although severe anaemia is a hallmark of SCD, hyperhaemolysis is characterised with accelerated drop in steady state level of Hb with a corresponding

increased rate of haemolysis. It is also occasionally accompanied with increased erythropoiesis, increase in steady state reticulocyte count (about 25%) and exaggerated hyperbilirubnemia which may result in gall bladder disease [8, 17]. Hyperhaemolysis sometimes results during RBC transfusion where delayed haemolytic transfusion reaction causes destruction of both autologous and homologous RBCs by an innocent bystander mechanism. This coupled with transfusion induced suppression of erythropoiesis leads to the marked increase in haemolysis and is often followed with reticulocytopenia [18].

Splenic complications are one of the most observed crises in patients with SCD particularly those with SCA during the early stage of the disease and they are associated with increased morbidity and mortality. Acute splenic sequestration occurs in the first decade after birth and can occur as early as 2 months after birth due to excessive sequestration of both normal and sickled RBC into the spleen often resulting in severe anaemia and splenomegaly [19]. This thus leads to reduction in the level of RBC/Hb present in the blood by $> 2\text{g/dL}$, increased reticulocytosis and thrombocytopenia most times affecting packed cell volume [20]. These are the classical signs observed during aplastic crisis, although it is usually induced by infection such as that of parvovirus B19. Infection of erythroid progenitor cells in peripheral blood and bone marrow by parvovirus B19 results in cessation of erythropoiesis for as much as 10 days resulting in severe anaemia due to very short life span of RBC in SCD.

Priapism, a painful and prolonged penile erection occurs in about 30% of adolescents with SCD and it is as a result of increased arterial inflow of blood with reduced venous outflow causing the trapping of RBCs in erectile bodies. It has been determined to be associated with increased haemolysis, since haemolysis results in depletion of nitric oxide (NO), an important smooth muscle cell relaxant that plays important role regulating erectile functions [21].

Haemolysis remains a major life threatening event in SCD patients mainly because of the secondary complications that arises as a result. One of the most common symptoms of SCD is the acute chest syndrome (ACS) [22]. Adults suffering from SCD are at high risk of developing systemic and pulmonary hypertension, impaired vasodilatation due to changes in the intima and smooth muscles of blood vessels, and endothelial dysfunction [23]. With disease progression and age, there is increase in the burden of morbidity due to end organ failure such as avascular bone necrosis as a result of bone marrow infarction, chronic nephropathy due to haematuria, hyperfiltration, microalbuminuria and proteinuria, and haemorrhagic and non-haemorrhagic stroke [24-26].

Vaso-occlusive crises and pain syndromes

Vaso-occlusion is the major symptom and complications observed in SCD patients resulting ultimately in painful episodes [27, 28]. Vaso-occlusion arises due to the obstruction of micro-vessels consequent of trapped sickled RBCs resulting in ischemia induced organ damage and painful episodes lasting from few hours to days or even weeks beginning from early childhood to adolescence, aggravating in adulthood when there may be persistent acute pain in the bones and joints. It results in body pains affecting different parts of the body and it is believed also to be the cause of priapism and splenomegaly due to acute splenic sequestration [29] as stated above. Recurrent acute pain episodes may result in neurological changes that may heighten sensitivity to pain. ACS is also a common occurrence in SCD characterised with chest pain, wheezing, cough, refractory hypoxemia and tachypnea, and it is as a results of the obstruction of micro-vessels by sickled RBCs [30].

Leg Ulcer

Leg ulcers are relatively common complications of SCD and can be debilitating. It is uncommon in the first decade of life. Pathogenesis of chronic leg ulcers

in SCD is complex and may be due to the following factors: 1) Mechanical obstruction of the microcirculation by dense sickled RBCs, 2) bacterial infections, 3) chronic anaemia due to hemolysis with decrease in RBCs oxygen carrying capacity, 4) decreased nitric oxide bioavailability leading to impaired endothelial function. Production of endothelin-1, a potent vasoconstrictor and subsequent increased expression of endothelial cells adhesion molecules, thrombosis and venous incompetence have all been proposed as potential contributing factors [31, 32].

Most of the time it is on the dependent part, especially the lower extremities that are involved, in areas with thin skin, less subcutaneous fat and with decreased blood flow (**Figure 1**). Trauma and inflammatory conditions may be the initiating factors.

Figure 1: A photomicrograph showing a leg ulcer that resulted from a trauma more than one year ago in a 25 years old female SCD patient. The ulcer is necrotic, with gangrenous borders, full thickness skin loss, destruction of other supporting structures and possible accompanying osteomyelitis. SCD = Sickle cell disease



End organ dysfunction

Advancement in treatment of SCD has increased the survival rate in SCD patients but due to frequent episodes of haemolytic anaemia, ischemia and reperfu-

sion injury in different organs, this group of people develop progressive organ dysfunction and eventual organ failure. SCD is associated with cardiovascular abnormalities due to increased cardiac output and resultant morphologic and physiologic changes to the cardiac system. Such changes include thickened ventricular septum, left ventricular hypertrophy and abnormal left ventricular diastolic function leading to overt cardiovascular dysfunction [33]. Pulmonary hypertension is a common end organ complication that is seen in about 30% of SCD adults, posing a major life threat and increasing mortality. Patients with pulmonary hypertension have reduced NO in the circulation due to chronic anaemia and as a consequent, there is development of vasculopathies characterised with endothelial dysfunction, increase in vascular tone, hypercoagulability and remodeling/destruction of the pulmonary vasculature [34]. Such patients present with ACS accompanied by severe pain that is ameliorated with NO inhalation. Patients with ACS present with cough, fever, chest pain, hypoxia, dyspnoea and wheezing due to pulmonary infection that arises from colonisation of the upper respiratory tract with bacteria or viruses such as *E. coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* [22, 35]. Bone marrow infarction and subsequent pulmonary infarction or fat embolism due to thromboembolism in micro-vessels have also been observed from autopsy reports of SCD patients and has also been associated with SCD and as such can be diagnosed using bronchioalveolar lavage to examine lipid laden macrophages [36].

Pathophysiology of SCD Molecular mechanisms of RBC membrane fragility and haemolysis in SCD

The normal Hb molecule is highly soluble and its solubility is the main property that allows RBCs to easily bind the molecule in high concentration to facilitate transport of oxygen molecule efficiently. The RBCs possess structural flexibility that aids

loading and unloading of oxygen molecules, thus, when the Hb molecule is deoxygenated and passed into the microcirculation, it undergoes a structural change to allow oxygenation traversing the pulmonary circulatory system. The replacement of glutamic acid in the β -globin molecule of HbS with valine results in hydrophobic interaction between adjacent deoxy-HbS molecules on the β -globin chain forming polymeric units. This event characterises the initiation, chain extension and stabilisation of a polymeric fibre of deoxy-HbS moieties which also groups together forming a fragile structure that disrupts the cytoskeletal framework and stiffens the structure of RBCs resulting in a sickled shape cells with very limited structural flexibility or reduced deformability [6]. The polymeric deoxy-HbS fibre in turn triggers the activation of signalling cascades that initiates the pathophysiological mechanism.

Because of the rigidity of the cell, dysregulation of ion transport across the membrane is affected. Most importantly, there is activation of ion channels such as the calcium dependent potassium channel (Gardos channel) and the potassium chloride co-transport system activation of which causes an influx of Ca^{2+} and a concomitant outflux of K^{+} resulting in RBC dehydration. This in turn increases the intracellular concentration of Hb and thus favours polymerisation of more deoxy-HbS [37]. Intracellular hemichromes from denatured Hb interacts with band 3 proteins, spectrin and actin leading to loss of Fe^{3+} from the heme creating an oxidising environment that disrupts the polarity of membrane phospholipids, particularly flipping of phosphatidylserine to the outer membrane surface. The mutated β -globin molecule also undergo auto-oxidation to form the superoxide (O_2^-) produced during reoxygenation of Hb to form methaemoglobin, which in the presence of hydrogen peroxide (H_2O_2) decomposes the Hb releasing free iron to create a highly oxidising microenvironment [38]. Ca^{2+} -calmodulin complex activates the erythrocyte adenosine monophosphate deaminase (AMPD) and increases the

intracellular level of inosine monophosphate (IMP) from deamination of AMP, rendering the cell energy deficient and in turn increasing the translocation of phosphatidyl-serine to the outer membrane surface [39]. These processes result in RBC membrane fragility and eventual haemolysis.

Pathophysiology of vaso-occlusion

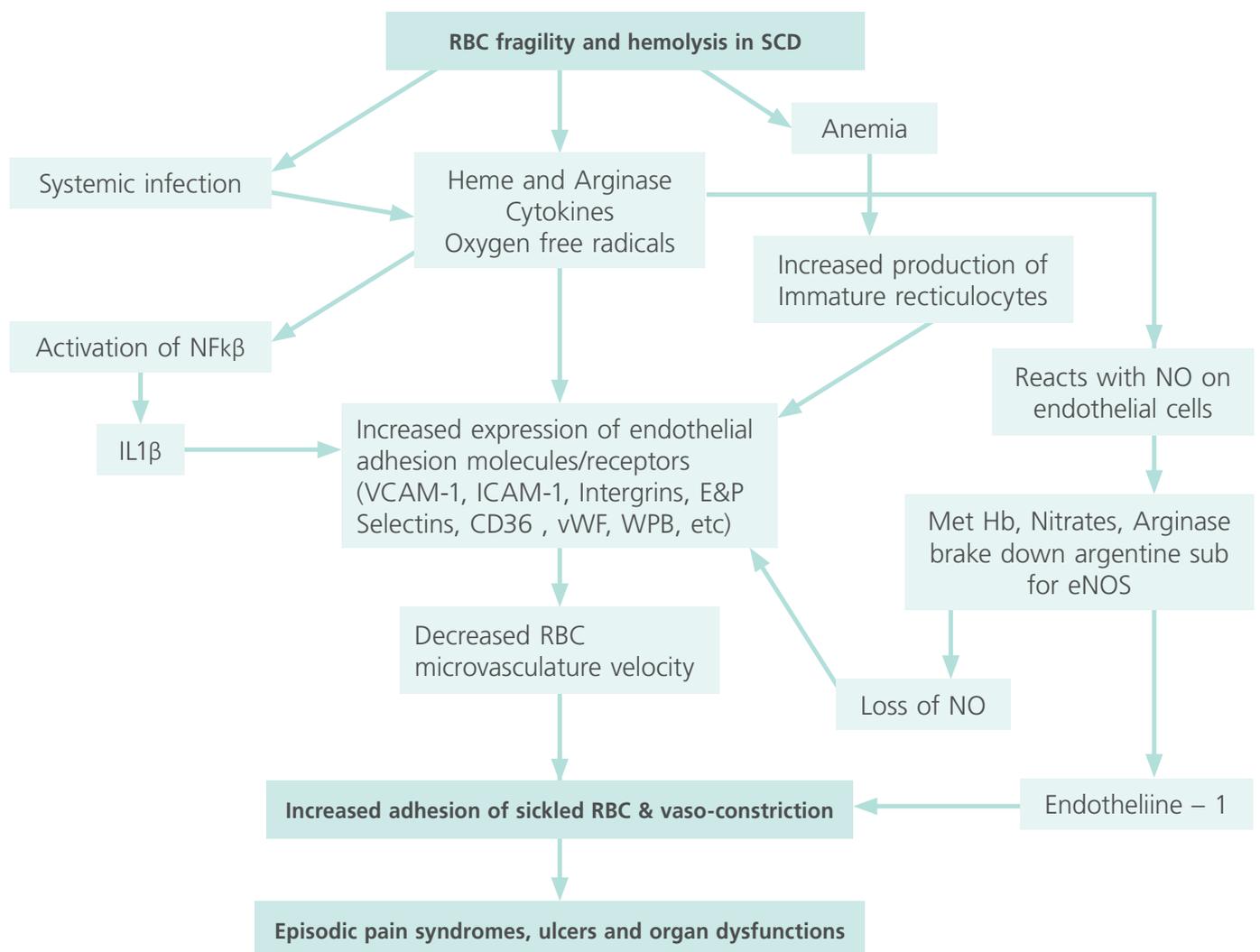
RBCs fragility and stiffness are both respectively responsible for the haemolysis and obstruction of microcirculation that results in vaso-occlusion, both of which characterise the pathophysiological bases of SCD. Vaso-occlusion arises due to adhesion of sickled RBCs to the endothelium of micro-vessels resulting in the obstruction of blood flow and subsequently ischemia in affected tissues. Vaso-occlusion is propagated in two phases. In the first, the reticulocytes produced prematurely in the bone marrow in an attempt to relieve anaemic stress adhere to blood vessels with the aid of various adhesion proteins expressed on their cell surfaces. This reduces the velocity of RBC in the microcirculation, maintaining the RBC in a hypoxic environment long enough for the formation of deoxy-HbS and Hb polymerization into fibres. In the second phase, sickled RBCs are entrapped in the micro vessels by binding to endothelial cells via different adhesion proteins [40]. Sickled RBC binding to the endothelium is facilitated by processes and factors such as the Von Willebrand factor (vWF) bridging integrin receptors with similar receptors on endothelial cells of large vessels, binding of $\alpha 4\beta 1$ on sickled RBC to vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells in micro-vessels, bridging of CD36 on sickled RBC with $\alpha \nu \beta 3$ integrin receptor on endothelial cells by plasma thrombospondin and binding of sickled RBCs to E- and P-selectins on cytokine activated endothelium [41].

It is known that the endothelium plays a crucial role in development of vaso-occlusion by mediating RBC adhesion through the expression of several adhesion molecules/receptors such as VCAM-1,

Lu/BCAM, c-type lectins (selectins), and CD36 on the surfaces of endothelial cells, and the molecular mechanisms that triggers endothelial activation till date are not fully understood [42]. In SCD, the activation of endothelial cells result in endothelial dysfunction and aberrant adhesion molecule expression often characterising a deviation from basal quiescent state of endothelium to a more active state [42]. This activation is believed to be triggered in part by processes due to sickled RBCs haemolysis such as heme liberation and release of inflammatory cytokines from cytolysed sickled RBCs. In a murine sickle cell model, heme activated nuclear factor kappa- β (NF κ β) of endothelial cells causes degranulation of Weibel-Palade body (WPB) by mobilising WPB, vWF and P-selectin to the surface of the cells and endothelial walls through the action of toll-like receptor 4 (TLR4) [43]. Oxygen radicals produced from the interactions of sickled RBCs with the endothelium and subsequent activation of endothelial NF κ β also up-regulate the transcription of adhesion molecules such as VCAM1, E-selectin and ICAM1 giving the circulating endothelial cells an activated phenotype through the expression of these adhesion molecules (**Figure 2**) [44, 45]. P- and E-selectin have been suggested to be potential factors in the development of vaso-occlusion but the implication of P-selectin in the pathogenesis of vaso-occlusion and as a potential therapeutic target is further emphasised by reduced sickled RBC and leukocyte adhesion to endothelium and increased microvascular blood flow in SCD mice treated with anti-P-selectin antibody [46]. We are also of the opinion that inflammatory cytokines produced during infection and during RBC haemolysis play significant roles in the expression endothelial cell adhesion molecules/receptors.

An in vitro study also demonstrated the role of platelets in endothelial activation in SCD patients since platelets circulate in an activated form in these patients. Steady state SCA platelets co-cultured with human umbilical endothelial cells (HUVEC) ac-

Figure 2: Schematic illustration of proposed mechanisms underlying increased adhesion of sickled RBCs to vascular endothelium and vaso-constriction leading to episodic pain syndromes, leg ulcers and organ dysfunctions in SCD: Rupture of RBCs could result in systemic infection anaemia and release of cellular molecules such as heme, arginase, superoxides and inflammatory cytokines. This could lead to activation of NF κ B and reduced NO production through release of the cellular molecules or increased production of immature reticulocytes consequent of anaemia. These factors thus could result in increased expression of adhesion molecules that will mediate binding of sickled RBCs to endothelial surface of the vasculature causing vaso-occlusive episodes.



eNOS = endothelial nitric oxide synthase, IL1 β = Interleukin -1 beta, RBC = Red blood cell, SCD = Sickle cell disease, NF κ B = nuclear factor kappa beta, VCAM-1 = vascular cellular adhesion molecule 1, ICAM-1 = intracellular adhesion molecule -1, E&P Selectins, vWF = Von Willebrand factor, WPB = Weibel-Palade body, NO = nitric oxide, E-Selectins = endothelial-leukocyte adhesion molecule 1.

tivated the endothelial cells (EC) through activation of EC NF κ B and increased IL1 β expression and a resulting high expression of adhesion molecules such as ICAM1 and E-selectin [47].

Aside endothelial activation, endothelial dysfunction which is characterised by the reduction in NO produced by endothelial cells is another contributor to development of vaso-occlusion. NO is a major vasodilator produced by nitric oxide synthase (NOS) through conversion of arginine to citrulline. NO, after production traverses the endothelial cell membranes to bind guanylate cyclase converting GTP to cGMP in smooth muscle cells, subsequently activating cGMP-dependent protein kinases that mediates vasodilation. During haemolysis, there is release of heme and arginase into the circulatory system. Heme reacts with NO forming nitrates and methHb while arginase breaks down arginine which is a substrate for NOS to replenish the scavenged NO (**Figure 2**) [48, 49]. This in turns leads to increased expression of endothelin-1, a vasoconstrictor, resulting in vasoconstriction and vaso-occlusion. Interestingly, it has been previously demonstrated that NOS gene therapy down-regulates the expression of ICAM1, VCAM1, E- and P-selectin, and inhibits the activation of platelets [50, 51]. This then suggests a role for NO in endothelial inactivation and further strengthens the fact that reduced bioavailability of NO in SCD may contribute to platelet activation and increased expression of adhesion molecules.

Current and emerging therapeutic strategies in the treatment of SCD

Fundamentally, apart from the prophylactic treatment such as Pneumococcal vaccination and penicillin injection given to SCD infant during early diagnosis, the treatment of SCD is based on either disease management procedure or a curative stem cell therapy (all discussed below), both of which exhibit a level of complications that are currently being investigated.

Prophylactic therapy

There are currently three prophylactic treatments that are widely accepted and predominantly in use; penicillin prophylaxis, pneumococcal vaccination and folate supplement. Penicillin prophylaxis is provided to infants shortly after birth and diagnosis of SCD. This is because within the first 5 years of life, SCD patients are prone to life threatening pneumonia infection due to vaso-occlusion in the spleen preventing antibody production against encapsulated micro-organisms such as *Streptococcal pneumonia* and *Haemophilus influenza*, and inability of the spleen to also produce complement factors and C-reactive proteins for bacteria sensing, recognition and phagocytosis. This thus result in chronic infection that can lead to death in the first 24 hrs from onset of the infection [52-54]. Immediately after diagnosis or at least by 2 months of age, SCD patients under the age of 5 years are treated with 125 mg of penicillin VK twice daily. This dosage is increased to 250 mg twice daily for patients over the age of 5 [54]. Because of the decline in episodes of infection in older SCD patients and ability to develop immune response, penicillin prophylaxis is advised to be discontinued to prevent development of resistant strains.

Pneumococcal infection is a common occurrence in SCD patients and the use of pneumococcal vaccines widely is to prevent this in SCD patients under 5 years of age. The vaccination involves immunisation with a heptavalent protein conjugate vaccine (PCV7) at ages 2, 4, 6 and 12 months. This is followed with immunisation with a tricosavalent pneumococcal polysaccharide vaccine at 2 and 5 years [55]. Because of the increased rate of folate turn over due to megaloblastic anaemia and bone marrow aplasia associated with haemolytic anaemia, SCD patients are treated with 1 mg of folate daily [56].

In addition to the above therapeutic combination, because of the prevalence of malaria amongst SCD patient in Nigeria and other parts of Sub Sa-

hara Africa, treatment with anti-malaria prophylaxis is often recommended to reduce episodes of crisis [57]. The most commonly used treatment ranges from combination of arthemisin based drugs to the use of insecticide treated nets. Chemoprophylaxis with proguanil or pyrimethamine is also widely used and has been found to reduce sickle cell crises [57, 58]. Daily dosage of 100 or 200 mg of proguanil or weekly dosage of 125 or 250mg mefloquine have also been shown to reduce sickle cell crises with at least 80% success rate and both regimen having comparable outcomes [59].

SCD treatments aimed at disease management

Hydroxyurea for HbF induction

In the first few months to couple of years of life, new born with SCD are prevented from vaso-occlusive and anaemic crises that characterise SCD due to the expression of HbF. HbF interferes with HbS polymerisation preventing the formation of deoxy-HbF polymeric fibre at a concentration that is enough to have toxic effect on the RBC. It has been reported that in compound heterozygotes of SCD trait having hereditary persistence of HbF expression is advantage. Twenty percent HbF is enough to prevent SCD crises even when there is about 80% HbS in the RBC [60]. Erythroid progenitors have high HbF levels and due to stringent differentiation and slow clonal expansion, only about 3% of circulating RBCs of adults contain a detectable concentration of HbF. Thus with rapid expansion of progenitor cells, the retention of γ -globin gene expression of HbF in RBC is maintained. Therefore, drugs such as 5-azacytidine and its analogues have been developed to induce expression of HbF through destruction of late erythroid progenitors that favour expression of β -globin gene of adult Hb. This results in accelerated expansion of erythroid progenitors into RBCs containing high HbF levels. Due to gross gene hypomethylation and cytotoxicity of these class of drug, their use

in the treatment of SCD is currently not approved. Hydroxyurea is the only drug approved for HbF induction to prevent vaso-occlusive crises and perhaps the most successful drug in the treatment of SCD [58]. Hydroxyurea acts by inhibiting ribonucleotide reductase, an enzyme that converts ribonucleotides to deoxyribonucleotides, thus, halting DNA synthesis [58].

Treatment with hydroxyurea is applicable to SCD patients of all ages [61] and it is recommended subsequent to presentation of symptoms such as priapism, persistent anaemia, vaso-occlusion related crises (more than 3 hospital admissions per year) and ACS. Starting dose of hydroxyurea is 15 mg/Kg/day which is increased by 5 mg/Kg/day every 3 months up to a maximum of 35 mg/Kg/day. Because of the effect of the drug on hematopoiesis, patients are closely monitored at 2 week intervals for complete blood counts to detect bone marrow aplasia. In adults that cannot tolerate the starting dosage, 500 mg/day dosage can be applied and the blood count checked fortnightly in many Sub Saharan centres. In the event of myelotoxicity, the drug is withheld for 2 weeks until stable blood count is achieved. When recurrent or persistent myelotoxicity occur, dosage may be reduced gradually by 2.5 mg/Kg/day until stable conditions are observed. Afterwards complete blood count may be monitored monthly for changes in platelet and leukocyte counts.

Hydroxyurea is a well-tolerated drug and treatment with hydroxyurea is associated with significant reduction in hospital admissions due to vaso-occlusive crises, ACS, priapism and hepatic complications [56]. Its effectiveness in preventing stroke is controversial anyway [62], but a clinical trial investigating this is currently underway [63]. On the contrary, there is increasing evidence that long term use of hydroxyurea has suggested by some studies, results in leukaemia in patients with essential thrombocytemia and polycythemia vera [64, 65], although a study involving 299 adult SCD patients found no association

between 9 years treatment with hydroxyurea and development of leukaemia of any sort [66]. Thus, there is need for a well-structured long term study that will further investigate association between treatment with hydroxyurea and leukomogenic risk in SCD patients.

Replenishment of circulating NO for vaso-occlusion treatment

NO is a known major vasodilator produced by endothelial cells and it plays a key role in vaso-occlusive crises of SCD [67, 68]. Although there is increased expression of NOS in SCD, the disease is characterised with inactivation of NOS, resistance to NO and impaired NO bioavailability resulting in vaso-occlusive related crises such as pulmonary hypertension and ACS [34, 69, 70]. As such, different studies have been designed to target increasing the bioavailability of circulating NO to compensate for the deficiency thus observed. A study showed that inhalation of NO significantly reduced the dependence of patient on morphine for pain relief after 6 hours of NO inhalation (0.26 mg/kg vs 0.44 mg/kg, case vs placebo group respectively, $p = 0.03$) and a 1 cm/h reduction in pain score compared to placebo group ($p = 0.02$) [71]. Because of the important role played by arginine in the biosynthesis of NO and pathogenesis of vaso-occlusion in SCD, several studies now look at the use of arginine supplements in treating vaso-occlusion. However, no standard dosage exists as yet. Patients treated with oral 100 mg/kg of L-arginine HCl thrice daily for 5 days had 15.2% reduction in arterial systolic pressure (63.9 ± 13 mmHg to 54.2 ± 12 mmHg, $p = 0.002$) (72). A recent randomised control trial reported that 100 mg/Kg L-arginine supplementation reduces opioid use for pain relief by 54% (1.9 ± 2.0 mg/kg vs 4.1 ± 4.1 mg/kg, admission vs discharge respectively, $p = 0.02$) and 50% reduction in pain score (1.9 ± 2.4 vs. 3.9 ± 2.9 , admission vs discharge respectively, $p = 0.01$) [73].

RBC transfusion

RBC transfusion in treating SCD is a major disease management procedure in adult patients with disease complications and vaso-occlusive crises and about 90% of adults suffering from SCD will receive this treatment at some point in their lives [74]. However, there is need to thoroughly consider the risks and benefits of the procedure to maintain a risk-benefit balance and avoid further disease complications and waste of resources. The major risks associated with transfusion include iron overload, increased blood viscosity, alloimmunisation against donor blood cells and delayed haemolytic transfusion reactions. Nonetheless, the requirement and recommendations for the use of RBC in treating SCD is increasing due to increased sophistication in the procedure with the availability of iron chelators and erythrocytapheresis to treat iron overload as well as extended phenotype matching for alleviating alloimmunisation.

Indications for the recommendation of RBC transfusion include transient RBC aplasia, ACS, splenic sequestration induced hypovolemia, acute multi-organ failure and stroke, acute exacerbation of anaemia and pre-surgery blood transfusion for SCD patients [74-76]. Irrespective of the cause or requirement for transfusion, the need for the procedure should be based on the inability of the patient to compensate for the loss of Hb due to anaemia. As asymptomatic patients with moderate or even severe anaemia have a steady state reduction in Hb level, undergoing transfusion can lead to unwarranted and avoidable complications. Simple RBC transfusion are frequently used in event of acute splenic sequestration and transient RBC aplasia but patients with the latter are transfused with adequate monitoring of the Hb level which will increase as the disease alleviates. Transfusion with aliquots of 5 ml/Kg spaced over some hours up till Hb level rises to 10 mg/dl so as to avoid hyperhemoglobinemia post-transfusion due to disease alleviation [77]. In patients with acute ischemic stroke, chronic

transfusion can be used to prevent future episodes of the disease and can also prevent the disease development in high risk patients diagnosed early with transcranial Doppler ultrasonography [78] but recent studies have shown that patients that undergo chronic transfusion for prevention of recurrent stroke often develop cerebral vasculopathy and cerebral infarction [79-82].

Emerging treatment strategies

Use of African traditional medicines

There are some African traditional medicines that have shown great potentials in the stimulation of hematopoietic cells to rapidly produce blood and correct anaemia. It has also been suggested that they may be acting by increasing the population of circulating HbF. They may also be acting on any of the molecular pathways described above. However, these traditional medicines need purification to isolate active ingredient(s) and subjected to clinical trials before they can be widely used in SCD treatment.

Hematopoietic stem cell transplantation (HSCT) and gene therapy

HSCT is the only curative measure available for SCD treatment and considering the prevalence of the disease, it is still an underutilised form of treatment. Recommendation of HSCT is often limited because of rare existence of HLA-matched sibling donor (MSD). Apart from this, there are some associated complications such as graft versus host disease (GVHD) and post-HSCT toxicity. Children below 16 years stand a high chance of having successful HSCT due to absence of age-related comorbidities and organ failure. Advancement in research could help push the application of the procedure to older age groups with the use of reduced intensity treatment (RIT), a multistep strategy where immunosuppressive agents are used in low doses to prevent organ toxicities [83]. RIT procedures vary for each patient and the procedure is not to be considered the same

across population. In adult patients with advanced disease comorbidities, HSCT often results in high morbidity and mortality rate. However, presentation with recurrent stroke, hypertension and renal dysfunction, or patients at high risk of pulmonary complications and sudden death, qualifies for consideration for the procedure as benefits of performing HSCT can outweigh the complications. In this case, a matched unrelated donor (MUD) can be considered with the implementation of RIT if there is no MSD, showing the potential of using this treatment modality to increase donor pool irrespective of HLA status. A RIT trial in 14 patients treated with fludarabine, alemtuzumab and melphalan using MSD or MUD marrow produced a 95% overall and 79% event free survival rate in these patients [14]. RIT HSCT trials are currently underway at the National Institutes of Health and Blood and Marrow Transplant-Related Clinical Trials Network (BMT CTN) Sickle Cell Unrelated Transplant (SCURT) and it is currently in practice at the John Hopkins School of Medicine [84].

Apart from RIT-HSCT, a recent study showed that genetically modifying β -globin (autologous hematopoietic stem cell gene therapy) and subsequently transferring this gene into bone marrow stem cell to be transplanted could help reduce post-HSCT complications and other associated morbidities [85]. A lentiviral vector encoding wild type human Hb gene modified to impair HbS polymerisation was transferred into bone marrow CD34 to transduce the patients' HbS and thus prevent formation of sickled RBC. This study showed efficient transfection of the lentiviral vector and effective transduction of SCD bone marrows' CD34 progenitor to produce normal Hb and this could be moving into clinical trial soon.

Conclusions

In summary, the burden of SCD in sub-Saharan region of Africa is enormous. Emotional, financial

and total healthcare costs are monumental. Unfortunately, despite the burden of SCD in sub-Saharan Africa, there are no centres offering these new treatments methods or currently using molecular approaches in the treatment of SCD. Although more awareness about SCD and its complications is increasing in the region, research focusing on modern treatment methods is almost non existence. Targeting adhesion molecules known to circulate in the blood of SCD patients and or expressed on endothelial cells may lead to the development of treatments that will see a reduction in adhesion of sickled RBCs to the endothelium hence preventing vaso-occlusive episodes. Periodic monitoring of their plasma levels using molecular methods and subsequent administration of neutralizing antibodies may be a potent preventive measure. New research focusing on the molecular mechanism of vaso-occlusive syndromes may lead to further identification of molecular targets that can improve SCD treatments in the sub-Saharan region of Africa. SCD being a single gene defect will be adequately treated using the newly developed stem cell gene therapy and HSCT in sub-Saharan region of Africa.

Conflict of interest

We the authors declare that we have no conflict of interest.

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