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SICKLE CELL DISEASE AND ASSOCIATED COMPLICATIONS: A MOLECULAR VIEW

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ABSTRACT: Sickle cell disease (SCD) is an autosomal recessive hematological disorder, caused by a single mutation in the beta hemoglobin gene. In SCD the blood cells become crescent or sickle shaped and block the blood capillaries. The blockage of capillaries leads to two main pathophysiologic conditions; hemolysis and vaso-occlusion crisis leading to several acute and chronic clinical complications like pulmonary hypertension, retinopathy, inflammation, oxidative stress etc. These complications alter biological molecules like monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), endothelial nos-3 (eNOS3), toll like receptor (TLR), etc. which destabilize vital organs of our body resulting in organ damage and early mortality in SCD patients. These molecules also serve as a potential biomarker for the identification of level of manifestation of disease. In this review we have focused on some major complications associated with SCD and understanding of these biomarkers and their levels can also help in better assessment and management of complications associated with SCD.

KEYWORDS: Sickle cell disease, Pathophysiology, Complications, Biomarkers, Hematological diseases, Disease Management.

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1. INTRODUCTION

Sickle cell disease (SCD) is an inherited hematological disorder, affecting millions of people worldwide [1] caused by the single nucleotide mutation GAG to GTG of the β-globin gene in hemoglobin. The change in single base pair causes the codon to change from; which gives rise to a

hemoglobin that is designated as HbS or sickle hemoglobin. Sickle cell trait carriers are more than 200 million worldwide [2]. According to several reports it is estimated that over 300,000 children are born each year with a severe inherited hemoglobinopathy, in the low or middle-income countries 220,000 newborns are affected by this SCD [3]. The complications of SCD varies, but the most common acute events are vaso-occlusive pain crisis, caused by physical and adhesive entrapment of red cells containing hemoglobin S in the microcirculation, and the acute chest syndrome [4]**,** lung injury syndrome [5]. Affected adults are also at risk for a progressive vasculopathy [6] characterized by systemic and pulmonary hypertension, endothelial dysfunction, and proliferative changes in the smooth muscle of blood vessels [7], With increasing age, chronic end-organ complications begin to appear, including chronic renal failure [8], hemorrhagic and non-hemorrhagic stroke [9],avascular necrosis of bone, and pulmonary hypertension [10] the acute chest syndrome and pulmonary hypertension are the most common causes of death in patients with sickle cell disease [11]**.** Clinical manifestation of SCD varies from mild to severe forms that are associated with high mortality rates. Complications in SCD are varied and cannot be explained by a single mutation. There are a number of molecules involved in these complications associated with sickle cell disease. This review, gives an overview of the molecules involved in the complications associated with sickle cell disease. This article also describes the complications that are particularly characteristic of SCD and are due to the sequence of events that result from the pathophysiologic biology of the abnormal sickled red cell.

Complications associated with SCD

Renal abnormalities

Sickled blood cells in the small blood vessels decreases modularly blood flow which leads to impairment of urinary concentration ability, renal acidification and hematuria and causes sickle cell nephropathy. Sickle cell nephropathy also causes ischemia, microinfarct and papillary necrosis [12], in sickle cell nephropathy monocyte chemoattractant protein-1(MCP-1) secreted by a variety of cells that attracts blood monocytes and tissue macrophages through interaction with the cell surface receptor chemokine c-c motif receptor-2 (CCR2). MCP-1 is produced by kidney cells in response to proinflammatory response along with tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). MCP-1, TNF- α and IL can work as a potential biomarker of renal lesion as it secreted by the cells in response to damage (proinflammatory response), and it is also reflecting the damage caused by the oxidative stress in SCD [13]. There is a high level of creatinine based estimated glomerular hyper-filtration (GHF), renal insufficiency (RI) and renal failure (RF), age and body surface area are significantly more in SCD individuals with normal kidney function and GHF. A person with RF also shows a higher level of blood urea and fetal hemoglobin, GHF and RI are the potential indicators of kidney damage [14]. Monocyte chemoattractant protein-1(MCP-1), glomerular filtration rate, renal insufficiency estimation (creatinine based) can work as a biomarker to identify the severity of SCD and kidney damage in SCD patients.

Retinopathy and ocular manifestations

A complication of proliferative sickle cell retinopathy (PSCR) is a major contributor for vision loss and visual impairment in 10-20% of affected eyes in SCD patients. Micro-vascular occlusions being the most common cause of visual loss, it is logical to assume that sickle cell disease patients to suffer repeated episodes of vasocclusion are vulnerable to visual loss causing blindness. Overexpression of sICAM-1(Soluble cell adhesion molecules -1) in transgenic mice shows a reduced neutrophil and monocyte recruitment resulting in diminished leukocyte transmigration [15] suggesting that more sICAM-1might in fact protect against retinopathy and may suggest leukocyte extravasation is an important step in the development of proliferative sickle retinopathy, which leads to significant increase of Pigment epithelium-derived factor *(*PEDF) in SCD patients. Circulating sICAM-1 and PEDF are associated with sickle cell retinopathy [16].

Some other ophthalmological complications of SCD include retinal changes, refractive errors, vitreous hemorrhage and abnormalities of the cornea. The 63.5% of patients have myopia followed by 19.8% hyperopia in SCD patients. Genotyping in SCD patients for NOS3 (nitric oxide synthase 3) 27bp, VNTR (variable number tandem repeats) and IL4 (interleukin 4) intron 3, relieve several VNTR polymorphisms in the NOS3 of SCD patients; which attributes to the ophthalmological complications like retinopathy. Suggesting that NOS3 VNTR contributes to the susceptibility to the development of myopia in SCD patients [17]. Overexpression of ICAM also reported in SCD patients. Thus, studying the effect of ICAM and NOS3 in SCD patient help us to understand better about the ocular manifestation like myopia and retinopathy in SCD patients.

Inflammation

Inflammation is a known to be key component in SCD pathophysiology [15]**,** When the innate immune system detects the damaging agents the start inflammatory response by activation of neutrophils. Neutrophils migrate towards the inflamed tissue, recruit inflammatory monocytes and makes a potential proinflammatory environment [18]**.** In SCD, there is an increased leukocyte count and activation of granulocytes, monocytes, and platelets can be seen [19]**.** A sickled red blood cell (SS-RBC) also induces Toll-like receptors (TLR) and Nod-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome expression in peripheral blood mononuclear cells (PBMC). Sickle RBC's also acts as Damage-associated molecular patterns (eDAMPs) by stimulating TLR and NLR expression and IL-1β cytokine and LTB4 (leukotriene B4) production in PBMC cultures, thus contributing to inflammation. Sickled RBC also interferes with the gene expression of inflammatory molecules and may trigger a complex network in the pathogenesis of SCD [20]**.** TGF-β1 also has an important anti-inflammatory role and can also be used as a potential target for the development of new therapeutic strategies in SCD [21]**.** There is also a correlation of telomere length with disease severity and inflammation in SCD. Telomere length correlated with IL-8 level [15] and total lymphocyte count. Telomere lengths are greatly influenced by inflammation and reactive

oxygen species (ROS) in SCD, thus telomere length is associated with disease severity and chronic inflammation in SCD [22]. A number of molecules are involved in inflammation response; these responses could be anti-inflammatory or proinflammatory, TLR activation in SCD patients activates the innate immune system of the patient [20] Likewise, eDAMP also activates the immune system of patient by producing the cytokines, interleukins and up regulating certain adhesion proteins present in cells. Activation of these factors also responsible to generates the reactive oxygen species in SCD patients. A better understanding of activation and expression of these factors could help us to control the inflammation associated in SCD**.**

Cardiovascular complications

Sickle cell disorders are associated with multiple clinically significant cardiac abnormalities, primarily but not exclusively in adults. The anemia of SCD is associated with a chronic high cardiac output state. Morphologic and physiologic changes include a thickened interventricular septum, increased left ventricular (LV) mass, abnormal left ventricular diastolic filling, and left ventricular diastolic dysfunction, among others. Cardiac dysfunction also potentially has implications for other end-organ functions [23]. Multiple pathologic and clinical studies have shown that patients with pulmonary hypertension (PH) and diastolic LV dysfunction represent a particularly high-risk subgroup. These cardiopulmonary complications contribute to a markedly low functional capacity and associated high risk of both sudden death and severe multi-organ dysfunction**.**

Bacterial infection

Infection in SCD occurs due to impaired splenic function. Infection contributes to morbidity and mortality in SCD. The sickle gene confers an increased susceptibility to infection, especially to certain bacterial pathogens, and at the same time infection provokes a cascade of SCD specific pathophysiological changes. Respiratory tract infections are the most common infections in SCD. Gram negative organism *Klebsiella pneumoniae* are the most common organism isolated followed by *E. coli, Salmonella* and *Acinetobacter.* Among Gram positive organism, *Staphylococcus aureus, enterococcus, Streptococcus pneumoniae* are common bacterial isolates [24]. In SCD the spleen is damaged by the sickled blood cells and it is not able to remove bacteria from the blood thus making the patient susceptible for infections apart from the splenic dysfunction, CD209-336 A>G polymorphism also leads to a number of bacterial infections. Genotypic frequency of CD209-336 A>G polymorphism also varies among different ethnic population of SCD patients and CD209-336 A>G polymorphism and bacteria responsible for infection can be used as a predicator of susceptibility to infections in patients with SCD [25].

Oxidative stress

Erythrocytes have an environment of continuous pro-oxidant generation due to the presence of hemoglobin (Hb), which represents an additional and quantitatively significant source of superoxide (O_2^-) generation in biological systems. Erythrocytes have a self-sustaining antioxidant defense

system to counteract oxidative stress [26], Red blood cells uniquely function to protect hemoglobin via a selective barrier allowing gaseous and other ligand transport as well as providing antioxidant protection not only to themselves but also to other tissues and organs in the body. Sickle hemoglobin molecules suffer repeated polymerization/depolymerization generating greater amounts of reactive oxygen species, which can lead to a cyclic cascade characterized by blood cell adhesion, hemolysis, vaso-occlusion, and ischemia–reperfusion injury. In other words, SCD is intimately linked to a pathophysiologic condition of multiple sources of pro-oxidant processes with consequent chronic and systemic oxidative stress [27]. Thus, SCD patients are at high risk of oxidative damage. This can be identified by the use of biomarkers for oxidative stress [28]. Reduced glutathione (GSH) is a major tissue antioxidant. In the event of cells exposed to increased oxidative stress, GSH changes to oxidized glutathione (GSSH) and, hence, decreases the ratio of GSH/GSSG. In SCD patients, GSH levels as well as GSH/GSSG ratio are decreased [29]. Therefore, measurement of GSH levels may serve as a useful biomarker of *in vivo* oxidative stress [30]**.** In addition, GSH and glutamine concentrations are among the indirect markers to assess the oxidative stress on tissues and cells in disorders such as SCD [31]. As the oxidative stress contributes to pathophysiology of SCD, identification of oxidation causing markers like GSH are needed for better assessment of sickle cell disease.

Vaso-occlusive crisis (VOC)

VOC is thought to be the underlying cause of painful crises, acute splenic sequestration, and priapism (painful and prolonged penile erection). The sickle-shaped red blood cells can't flow freely through blood vessels and cause vascular occlusion, if lasting for 2 or more hours that is attributable to SCD. VOC is considered as the hallmark of SCD [32]. VOC, the most common complication of SCD, results from tissue ischemia**.** Sudden onsets of pain throughout the body are a common symptom of SCD. This ranges from mild to very severe form. VOCs and their accompanying pain most commonly occur in the extremities, chest, and back. the sites which are normally affected includes the arms, legs, back, abdomen, chest, and head can be confused with, or can be the prodromal stage of, other acute complications (e.g., head (stroke), flank (papillary necrosis), and abdomen (hepatic or splenic sequestration, constipation from opioid toxicity, or another hepatobiliary complication). Patients with SCD are at high risk for acute and chronic complications that may result in disability or death. In VOC the white blood cell count (WBC) and C-reactive protein (CRP) level increases; this is a sign of inflammatory response. There is also an association between WBC count and CRP level in SCD in VOC events [33]**.** Elevated WBC count could be predictors of VOC and determination of WBC and CRP amount can help in prevention and management of VOC crisis in SCD patient [33]**.** An interaction between sickle erythrocytes and vascular endothelial cells also plays a significant role in the initiation of VOC crisis, with an inverse relation between selectins, TSP (thrombospondin) and vWF (von Willebrand factor) levels, in SCD

patients with elevated TSP levels during active VOC [34]. The activities of purinergic system ecto-enzymes present on the platelet surface as well as CD39 (cluster of differentiation) and CD73 expression on platelets of SCD patients. Revealed that the activities of ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) and ectonucleoside adenosine deaminase (E-ADA) are increased in platelet of SCD patients, which could be attributed to the thrombo regulatory processes and VOC crisis associated with SCD and low expression of CD39 observed in SCD patients could be a compensatory mechanism that could help in activating high energy phosphate for several physiological processes in SCD patients [35]. Patients with SCD have increased expression of E-selectin and P-selectin in the serum plays an important role in the pathogenesis of VOC [10]. A number of proteins like cytokines, adhesion protein, cluster of differentiations, MCP, CRP are involved in VOC crisis associated with SCD. Studying the relationship between sickle red blood cells and different molecules which are associated with VOC crisis like MCP, CRP, and endothelial cell proteins could help us to combat the consequences resulting from the SCD.

Pulmonary hypertension

Pulmonary hypertension (PH) is characterized by high blood pressure in the lungs occurred due to damage to the pulmonary arteries. Pulmonary arteries are the ones used to transport blood from the right heart ventricle to the lungs. In SCD patients' arteries become narrowed and blocked [36]. Thus, the heart needs to work under stress to properly pump the blood in body. This stress of pumping the blood arteries enlarges and weakens the organ which can lead to right heart failure. Hemolytic anemia also reported [37] as an important mechanism leading to pulmonary vasculopathy by inhibiting all NO signaling and produce vasoconstriction [38]. Hemolysis also releases other red cell enzymes that have the potential to inhibit NO signaling. Other biomarkers such as brain natriuretic peptide (BNP) [39] and endothelin-1 show a significant correlation with uric acid in SCD patients. An increase in uric acid (UA) level was reported to be associated with increase pulmonary hypertension. Uric acid [40] level can be used as a reproducible biological marker to assess the severity of PH in SCD. Elevated pulmonary artery pressures in patients with SCD also been associated with low hemoglobin concentration, and high levels of serum lactate dehydrogenase (LDH) [41] With PH in SCD WBC [42] count and phospholipase A2 (PLA2) levels [43] also increase. Studying the levels of these biomarkers; BNP, LDH, NOs, UA and PLA2 and their correlation with each other can help us to manage the severity of pulmonary hypertension.

Stroke

In SCD, stroke is caused by the clumping of blood cells and blockage of small blood vessels which provide blood to brain. Larger arteries going to the brain are the primary site of strokes in SCD patients, due to rigidity of red blood cells they clump along the walls of larger arteries and damages the vessel walls and exposing tissue that gathers a greater number of sickle cells and further narrowing the vessels leading to brain. Clumping of red blood cells along with the endothelium

walls is mediated by von Willebrand's protein. This is an important marker of arterial occlusive stroke in SCD. Von Willebrand's protein enhances adherence of sickled red cells to the endothelial cells and endothelium walls [44]. The membranes of sickle red cells are strikingly abnormal, thus disturbing the normal ratio of membrane phospholipids and cholesterol [45] Which results in a *pro-coagulant* activity of sickled cells [46]. Therefore, a cascade could occur in which von Willebrand's protein promotes sickle red cell/endothelial cell adhesion, which leads to thrombus formation. Removing these abnormal sickled red cells would interrupt this cascade of events in thrombus formation and decrease in Von Willebrand's protein as well. In SCD there is an increased white blood cell counts which is related with increased frequency of pain, increased hemorrhagic stroke risk [9], and earlier death [47]. Increased IL-1 β levels also associated with high risk of stroke in SCD patients. Understanding the role of von Willebrand's protein, IL-1 β and WBC count can help us to assess their relation with stroke and to manage the stroked associated with SCD.

Acute chest syndrome

Acute chest syndrome (ACS) occurred often by the precipitation of a lung infection, which results in inflammation and loss of oxygen saturation leading to further sickling of red cells, thus causing pulmonary and systemic hypoxemia, sickling, and VOC. Although there is not a single condition that causes ACS, other complications in SCD can trigger it. That could be a lung infection or asthma; Asthma is a top cause of ACS in SCD children [48]. CRP is the most widely used marker of acute and chronic inflammation in ACS. High levels of SCD are correlated with increased frequency of acute pain in ACS [49]. In inflammatory response of ACS, Secretory phospholipase A2 (sPLA2) cleaves phospholipids and produce arachidonic acid leading to a cascade mechanism of inflammatory response [50]. sPLA2 levels can be used as a potential biomarker of ACS sensitivity and specificity [51]. Cardiac troponin I also increased in patients with ACS, increasing mortality rate in SCD patients [52]. CRP, sPLA2 and cardiac troponin I levels are needed to be assessed the ACS severity in SCD as the increase in these, three-protein level is directly related with the acute pain in ACS.

Priapism

Priapism is a common penile erection complication of SCD in men, occurred without any sexual activity or desire. The majority of priapism cases are ischemic, in which increased penile pressure compromises the vascular circulation, with time repeated episodes cause permanent damage and erectile dysfunction, due to all these manifestations priapism is considered a medical emergency. Timely diagnosis and appropriate management are required in preserving normal function of penis. This is a challenging management area in SCD patients. Severity of priapism is also associated with hemolysis. Thus, hemolysis can work as a marker for priapism severity. Lactate dehydrogenase (LDH), bilirubin and reticulocyte count also increases with an increase of priapism [53]. LDH levels also associated with the free-hemoglobin level in plasma, increased NO consumption and impaired vasodilation [41]. Chronic scavenging of NO results in a decreased expression and degradation of

cyclic guanine monophosphate (cGMP), the secondary messenger in NO signaling responsible for vasodilation in smooth muscles and affecting the nitric oxide synthase (eNOS), which also develops priapism severity [54]. A molecule phosphodiesterase type 5 inhibitor *(*PDE5 *inhibitor)* also gets impaired in SCD and affects cGMP and its effector, cGMP-dependent protein kinase (PKG) [55]. A low-dose PDE5 inhibitor treatment could help to reduce priapism in SCD patients [56]. Adenosine signaling also gets decreased in the SCD patients [57]. Additional reports are needed to further clarify the role of these molecules like PDE5, eNOS, cGMP and adenosine signaling pathway in priapism as well as appropriate targets for therapy.

Leg ulcers

The pathogenesis of chronic ulcers in SCD is quite complex to understand it may be due to mechanical obstruction by dense sickled red cells, venous incompetence or bacterial infections. An increase in vasoconstriction, *in situ* thrombosis, anemia and decrease in oxygen carrying capacity of blood, and decreased nitric oxide bioavailability leads to impaired endothelial function all are contributing factors of leg ulcers in SCD patients [58] Furthermore, inflammation, infection and trauma have all been cited as potential cause of lower extremity ulcerations in SCD patients, combination of all these factors, along with the interrupted microcirculation by sickle cells contributed to the pathophysiology of ulcerations in patients with SCD. Leg ulcers occur in areas which have lower amount of subcutaneous fat, thin skin, and with decreased level of blood flow. Patients with SCD and leg ulcers are also at a risk to develop other, more serious disease complications like PH, priapism and, possibly renal disease. Arginylglycyl aspartic acid (RGD) peptide matrix shows a significant reduction in ulcer size. Inflammatory and adhesion markers may be associated with leg ulceration in SCD as they are responsible for inflammation-mediated vasoocclusion/vasoconstriction [59].

Discussion

This article aims to discuss major complications associated with SCD and their role in high mortality and morbidity. Twelve specific complications have been selected for discussion due to their relative frequency or potential severity and are indicated by subheadings. This review is based on the recently published literature; we have selected some major complications and picked up the molecules involved in these complications. After selection of the complications we have gone through the literature to study the relation between the levels of these molecules level in normal person and a SCD person. In literature we have found a number of bio-molecules whose level decrease and increase in the SCD person, in comparison with normal person and these bio-molecules level varies with the complications caused by SCD, such as in renal dysfunction alteration in GFR and several protein concentrations found to be the major cause of renal failure in SCD. In renal dysfunction a MCP-1 protein also reported to be altered, which can act as a predictive biomarker of renal lesion. Likewise, in vaso-occlusive crisis (VOC) several proteins expression like cytokines,

endothelial surface proteins, (C-reactive proteins) CRP, adhesion proteins found to be altered with SCD. An extensive study of these proteins helps us to understand SCD associated VOC and tackle these complications in a better way. As the SCD also responsible for retinopathy in the patients some of the molecules like PEDF and sICAM1 are found to be associated with sickle cell retinopathy. Innate immunity system acts as first line of defense against pathogen, by recognizing PAMPs (pathogen associated molecular pattern) but also sense DAMPs (damage associated molecular patterns) from tissue injury. Some of the molecules like TGFβ1, annexin protein, adhesion protein also responsible for inflammatory response. Immune system activates Toll and NOD receptors to initiate inflammatory response. Study of these Toll and NOD receptors like TLRs and NLPs can help us to understand the actual mechanism to cure SCD**.** In the cardiac complications associated with SCD no cardiac failure mechanism of sudden death has been identified till now, several reports suggest that increase in the pulmonary pressure could be the main cause of these cardiac complications, as in the SCD patients the pulmonary pressure reported higher than the normal one. So pulmonary hypertension could be a sign of cardiac complications associated with SCD**.** As the SCD also responsible for splenic dysfunction which makes the person susceptibility for several bacterial diseases. An improved understanding of the mechanisms leading to increased susceptibility to bacterial infections in SCD may result in future in the development of logical interventions which should help further to reduce morbidity and mortality. Chemokine polymorphisms also provide some new possibilities for developing targeted drug therapy for many diseases. MCP1-2518A/G and V64I of C-C Chemokine receptor-2 (CCR2) also associated with clinical complication of SCD. A future treatment offering protection by inducing production or expression of the MCP1 and CCR2 protein may be beneficial for patients with SCD. Likewise, in priapism eNOS polymorphism also found which increase the splenic dysfunction. In acute chest syndrome CRP, sPLA2 and cardiac troponin I are found to be increased. In VOC and stroke an increased level of CRP and WBC also reported. In oxidative stress formation of reactive oxygen species and reduced level of reduced glutathione (GSH) also plays a major role. By doing an in-depth study about these molecules and their role in SCD severity and association with complication could help us to manage the disease by identifying the major molecules and their correlation with the mortality in SCD patients.

2. CONCLUSION

Sickle cell disease is characterized by a variety of complications with abnormal level of molecules (biomarkers) that have been identified and associated with different complications. A wide-ranging study and comparative analysis of the mechanism of action of these molecules can give an insight into the severity of complications associated with SCD like acute chest syndrome, pulmonary hypertension, cardiovascular disease, renal dysfunction, pain crisis and infection. To sum up, this review opens up new avenue into the comprehensive study of these molecules and their association with manifestations of SCD to understand different complications associated with SCD.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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