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# PROTEIN C: A MISSING LINK IN THE SUSCEPTIBILITY AND SEVERITY OF SICKLE CELL ANAEMIA

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## ABSTRACT

Sickle cell anemia belongs to a group of diseases called sickle cell disease. Sickle cell anemia is an inherited red blood cell disease in which there is not enough healthy red blood cells to transport oxygen throughout the body. Sickle cell anemia is caused by a mutation in a gene (hemoglobin), which is an iron-rich compound that allows red blood cells to carry oxygen from the lungs to the body. In sickle cell anemia, abnormal hemoglobin can cause red blood cells to become stiff, sticky, and deformed. Protein C is a vitamin K-dependent coagulation inhibitor produced in the liver. It works with its cofactor protein S (PS). The activated PC inhibits activated factors V and Viii, thereby reducing the production of thrombin. PC deficiency is associated with an increased risk of thrombotic complications. Protein C, activated by thrombin and in the presence of protein S, inactivates factors V and VIII. Thus, the protein C / S pathway is an effective source of natural anticoagulant activity. Protein C and protein S genetic defect status is associated with an increased risk of venous thromboembolism. In SS disease, both adults and children have been reported to have low levels of protein C and S, which can theoretically lead to a tendency for thrombosis.

***Keywords***: *protein c, a missing link, susceptibility, severity, sickle cell anaemia*

## INTRODUCTION

Sickle cell anemia belongs to a group of diseases called sickle cell anemia. Sickle cell anemia is an inherited red blood cell disease in which there are not enough healthy red blood cells to carry oxygen throughout the body. Under normal circumstances, round, flexible red blood cells can easily move through blood vessels. In sickle cell anemia, the red blood cells are shaped like a sickle or crescent. These tough, sticky cells can get stuck in small blood vessels, which can slow or impede the flow of blood and oxygen to certain parts of the body (Mayo Clinic, 2019). Sickle cell anemia is caused by mutations in a gene (hemoglobin), which is an iron-rich compound that allows red blood cells to carry oxygen from the lungs to all parts of the body. In sickle cell anemia, abnormal hemoglobin can cause red blood cells to become hard, sticky, and misshapen (CDC, 2019). Sickle cells divide and die easily, leaving very few red blood cells. Normal red blood cells can usually survive for about 120 days before they need to be replaced, but sickle cells usually die within 10 to 20 days, leading to a shortage of red blood cells (anemia) (GHR, 2019) . Sickle cell anemia (SCD) is one of the most common genetic diseases in the world, with the highest prevalence in the Middle East, the Mediterranean, Southeast Asia and sub-Saharan Africa, especially Nigeria (Pielet al., 2013).

SCD is a chronic hemolytic disease characterized by the tendency of hemoglobin molecules in red blood cells to aggregate and deform sickle-shaped (or crescent-shaped) red blood cells, leading to characteristic vascular occlusion events and accelerated hemolysis. It is inherited in an autosomal recessive manner, whether it is homozygous or double heterozygous. When it is inherited in a homozygous state, it is called sickle cell anemia (SCA). Other known SCD genotypes include SC hemoglobinopathy, sickle beta thalassemia and sickle beta zero thalassemia (the severity of which is similar to sickle cell anemia), Punjab SD hemoglobinopathy, SO Arab hemoglobinopathy, etc. (pielet al. , 2013).

Protein C, also known as autologous prothrombin IIA and coagulation factor XIX, is an activated form of proenzyme that plays an important role in the regulation of anticoagulation, inflammation and cell death, and in the maintenance of permeability of cells. blood vessels of humans and other animals (Huo and Morton, 2014). Activated protein C performs these operations primarily by proteolytically inactivating protein factors Va and Viiia. APC is classified as a serine protease because its active site contains serine residues (Kisielet al., 2010). In humans, protein C is encoded by the PROC gene, which is located on chromosome 2 (Marti et al., 2012).

Protein C is a vitamin K-dependent blood clotting inhibitor produced in the liver. It works with its protein S (PS) cofactor. Activated PC inhibits activated factors V and Viii, thereby downregulating thrombin production. Lack of CP is associated with an increased risk of thrombotic complications. Protein C deficiency can be hereditary or acquired; it can be a qualitative or quantitative defect. Genetic defects of PC are divided into type I and type II defects. Type II is due to reduced activity

despite the normal antigen (Reitsma et al., 2004). Some researchers report that patients with sickle cell anemia have reduced levels of natural coagulation inhibitors (including PC) and increased thrombin production (Westerman et al., 1999).

Protein C deficiency is a rare genetic feature that is usually prone to thrombotic diseases. It was first described in 1981 (Griffin et al., 1981). The disease belongs to a group of inherited diseases called thrombosis. Protein C deficiency is associated with a higher incidence of venous thromboembolism (relative risk 8-10), but not with arterial thrombotic disease (Khan and Dickerman, 2006). The main function of protein C is its anticoagulant properties as inhibitors of coagulation factors V and VII. A deficiency of

A results in the loss of normal lysis of factors Va and VIIIa. There are two main types of protein C mutations that can cause protein C deficiency: ((Khan and Dickerman, 2006)

sickle cell disease

sickle cell disease is a multi-system disease caused by a single gene mutation. Almost every organ in the body will be affected. It is characterized by the presence of abnormal red blood cells destroyed by HbS. This variant of normal adult hemoglobin (HbA) is inherited from both parents (homozygous for the HbS gene) or from one parent, and another variant of hemoglobin, such as hemoglobin C (HbC) or beta thalassemia (compound heterozygosity) . When deoxygenated, HbS polymerizes, destroying red blood cells and causing them to lose cations and water. The rheological characteristics of these damaged cells and the abnormal expression of adhesion molecules lead to the possibility of hemolytic anemia and small blood vessel obstruction, which in turn leads to vascular occlusion. Vascular occlusion often leads to acute complications, including ischemic tissue damage, leading to severe pain or organ failure. Acute chest syndrome is a typical example of organ failure in sickle cell anemia and one of the main causes of hospitalization and death (Vishinski et al., 2010).

Patients with sickle cell anemia may have any of several hemoglobin genotypes. Almost all genetic studies of sickle cell anemia have focused on sickle cell genotypes (ie HBB Glu6Val, rs334). Other genotypes of sickle cell anemia are due to the compound heterozygosity of the HbS gene and other hemoglobin (Hb) variants such as HbC, HbE, and HbD, or are caused by multiple HbS-β-thalassemias. Except for HbS-β0-thalassemia (β0 means no HbA), the compound heterozygous genotype of sickle cell anemia is usually clinically less severe than the sickle cell genotype. However, there is significant phenotypic heterogeneity in every sickle cell disease genotype (Rees et al., 2010).

Sickle cell anemia

This is a type of sickle cell disease, also known as sickle cell disease, an inherited blood disease characterized by a genetic mutation in the gene encoding hemoglobin in human red blood cells. Hemoglobin is an iron-based metalloprotein that binds to the oxygen molecules in the lung capillaries, and then carries them through the bloodstream, releasing the oxygen molecules to the body cells and combining with carbon dioxide, and then releasing them back to the lungs

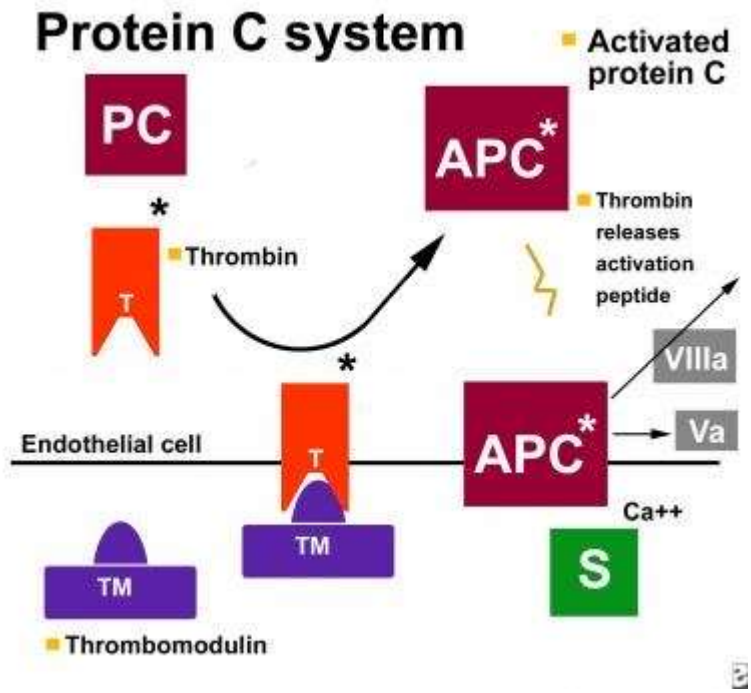
together with each body cell. Exhale (Maton et al., 1993).

The occurrence of sickle cell anemia is due to a single nucleotide (A to T) mutation in the β-globin gene, which causes glutamic acid to be replaced by valine at position 7 (position 6 in the historical nomenclature). Hydrophobic amino acid) substitution. This causes the protein to form hemoglobin S (HbS) in its final conformation instead of hemoglobin A (HbA), which is normal adult hemoglobin. Under normal circumstances, mutations are usually benign, and will not have a significant impact on the second, third or fourth level. Under normal oxygen concentration conditions, the phase hemoglobin structure is not affected. However, under conditions of low oxygen concentration, this mutation allows HbS to polymerize on its own. When HbS is under conditions of low oxygen saturation, the hydrophobic residue of valine (formerly called glutamic acid) at position 7 of the β chain of hemoglobin can bind to hydrophobic plaques, causing the hemoglobin molecules to S aggregate and form fibrous precipitates. These precipitates form long intertwined chains in blood cells, which elongate and distort their shape, causing them to acquire a unique deformed "sickle-like" shape, hence the name. These "sickle-shaped" cells transport oxygen much less efficiently than ordinary round cells. Also, after sickle cells, cells lose a lot of elasticity and become stiffer. This stiffness makes cells more likely to get trapped in small openings in capillaries and narrow blood vessels. As cells accumulate in blocked blood vessels, they can cause ischemia (hypoxia) and cell death in the affected area, a complication called sickle cell crisis. Sickle cell crisis is usually very painful and can be fatal if the blockage occurs around vital organs and causes organ damage or failure. Furthermore, the deformation of the cell by the hemoglobin fibers reduces the integrity of the cell membrane, which makes the cell easier to lyse. The damage is so severe that healthy red blood cells can generally function for 90-120 days, while sickle cells can only last 10-20 days before lysis (Maakaron, 2014). Chapter

Although compared to healthy people, the number of red blood cells produced by the bone marrow increases, but it cannot compensate for the rate of cell destruction. Patients' red blood cell levels are usually lower than normal and may have symptoms common in patients with anemia, such as feeling tired, weak, short of breath or poor exercise ability (Stedman, 2006).

**PROTEIN C**

Protein C is a 62-kD, vitamin K–dependent glycoprotein synthesized in the liver. It circulates in the blood as an inactive zymogen at a concentration of 4 µg/mL. Its activation into the serine-protease-like enzyme, activated protein C (aPC), is catalyzed by thrombin when it is bound to the endothelial proteoglycan thrombomodulin (Pepler *et al*., 2017).The protein C pathway is illustrated in the image below.




(Danese *et al*., 2010).

The protein C pathway. APC = activated protein C; PC = protein C; S= protein S; T = thrombin; TM = thrombomodulin; Va = factor Va; VIII = factor VIIIa (Pepler *et al*., 2017).

APC exerts its anticoagulant activity primarily through inactivation of coagulation factors Va and VIIIa, which are required for factor X activation and thrombin generation. The catalytic activity of APC is greatly enhanced by the vitamin K–dependent cofactor protein S.

Aside from its role in coagulation, APC subserves anti-inflammatory and cytoprotective functions, which are mediated through the endothelial protein C receptor and the protease-activated receptor–1 (PAR-1) (Danese *et al*., 2010).

A deficiency of aPC disturbs the delicate balance between procoagulant and anticoagulant proteins and engenders a prothrombotic environment. The role of APC and other anticoagulant proteins in this balance appears to be especially important in the slow-flowing venous circulation, in which procoagulant proteins and platelet phospholipids have prolonged exposure to the vessel wall. This may explain, in part, why protein C deficiency appears to be associated primarily with venous thrombosis.

**Genetics of protein C deficiency**

Heterozygous protein C deficiency is inherited in an autosomal dominant fashion. The gene for protein C is located on the long arm of chromosome 2 and nearly 200 pathogenic mutations of this gene have been described (D'Ursi *et al*., 2007). These mutations are divided into 2 type:

1. type I and
2. type II

On the basis of whether they cause a quantitative (type I) or functional (type II) deficiency of protein C.

**Type I deficiency:** Type I protein C deficiency refers to a quantitative lack of plasma protein C concentration. Heterozygous individuals usually exhibit protein C antigen and activity levels that are about half of normal patients'

plasma. Various causal genetic changes in the protein C promoter region and splice site, as well as the coding sequence of the protein C gene itself, have been reported (D`Ursi et al., 2007). Type I hybrid protein C deficiency families have obvious phenotypic variation, some families have serious thrombosis tendency, and some families are asymptomatic. Interestingly, this variability was observed even between different lineages with the same protein C mutation, indicating that the mutation itself does not fully explain the phenotypic variability. In some families lacking protein C, the presence of a second thrombotic mutation, such as factor V Leiden, is associated with a more severe phenotype (Limperger et al., 2014).

**Type II deficiency:** Type II protein C deficiency is less common than type I disease and is associated with decreased functional activity and normal immunologic levels of protein C. A number of point mutations within the protein C gene giving rise to this disorder have been described (D'Ursi *et al*., 2007). Individuals who are homozygous or compound heterozygous for a mutation or other genetic defect affecting the protein C, typically due to the inheritance of abnormal alleles from both parents, can experience neonatal purpura fulminans, intracranial thromboembolism, and thrombosis (Inoue *et al*., 2017).

**PROTEIN C IN SICKLE CELL ANAEMIA PATIENT**

Vascular obstruction is a countless clinical sequela of accumulated organ damage, reduced quality of life, and shortened life expectancy in patients with sickle cell disease (SCD). The pathophysiological mechanism of vascular occlusion in SCD is complex and involves the adhesion process between sickle red blood cells, white blood cells, and activated endothelial cells (Frenette, 2002). The characteristic of patients with sickle cell anemia is a state of hypercoagulability, which defines an imbalance in the hemostasis system, leading to an inadequate or excessive deposition of fibrin and / or platelets, leading to the clinical consequences of thrombosis. Many laboratory studies consistently report increased thrombin production and fibrinolytic activity, as well as decreased levels of natural anticoagulants (NOACs), such as protein C and S (Tomer et al., 2001). The hypercoagulable state observed in SCD has been identified as a variety of etiologies, from exposure to phosphatidylserine due to continuous red blood cell sickle cell formation and endothelial tissue factor expression to activation of induced clotting. cytokines (Bezeaud et al., 2002). Large vessel thrombosis causes and/or contributes to SCD-related brain and lung complications. Some studies have shown that decreased SCD protein levels are associated with an increased risk of stroke in SCD patients (Bayazit and Kilinc, 2001). Activated membrane-bound protein C and cofactor protein S together inactivate activated factors V and VIII, thereby preventing the production of thrombin (Bezeaud et al., 2002). HbSS patients generally have a more severe phenotype than HbSC patients, and NOAC disease (and other measured markers) in HbSS patients is consistently more severe, confirming that more severe phenotypes are associated with lower levels of HbSS.

Proteins C and S are vitamin K-dependent plasma glycoproteins that

contain carboxyglutamate and have important anticoagulant functions (Epstein et al., 2004). Protein S is necessary for protein C to function. It acts as a cofactor for protein C and forms a stoichiometric complex (Walker, 2001). In the presence of calcium, the complex regulates the coagulation process by inhibiting factors V and VIII stimulated by thrombin (Dahlback, 2004). Cases of congenital and acquired protein C and S deficiencies have been identified, and due to the reduced anticoagulant potential of the blood, they have an increased risk of thromboembolism and recurrent thrombotic events (Kamiya et al., 2006). Because activated protein C (a serine protease) plays an important regulatory role in blood coagulation through its ability to degrade coagulation factors V and VIII, lower protein C levels can lead to thromboembolic diseases. Congenital protein deficiency occurs but rarely occurs, and leads to fatal neonatal thrombosis, characterized by fulminant purpura (Seligsohn et al., 2004).

SCD patients had elevated levels of thrombin generation markers and thrombin antithrombin (AT) complex (F 1.2, TAT complex) without seizures. A reduction in natural anticoagulant protein levels can also be seen in SCD. Therefore, protein C and S levels will decrease in a steady state, seizures will not occur, and even decrease during acute pain episodes (Westerman et al., 1999). These may be the result of the following conditions: Due to the expression of tissue factor (TF) and red blood cell prothrombinase (RBC) activity in blood vessels, increased thrombin production leads to chronic depletion of proteins C and S. Due to exposure to the PS membrane, the binding of sickle red blood cells to protein S increases (Allan et al., 2002). Anti-phospholipid antibodies inhibit the binding of protein S to β2 glycoprotein 1 (Stuart and Setty, 2001), resulting in the inactivation of protein S due to circulating C4 binding protein. The mechanism of low protein C levels is unclear, but this may be due to wasting coagulopathy. However, Ibijola et al. conducted a similar study in Nigeria. (2016) Although the sample size is small, it shows that the protein C level of adult PCS patients is significantly reduced. This may be the result of mild inflammation in ACS patients (Stuart & Setty, 2001). It may also be due to continuous tissue destruction associated with continuous hemolysis or vascular occlusion, or the liver is often affected in SCD, and the tissue is the site of protein C production. Therefore, chronic liver disease with impaired liver function caused by sickle cell disease affects its production (it is also a vitamin K-dependent protein). All the protein C studies discussed above were conducted in adult PCS patients using different types of coagulation analyzers, and the sample size was similar to the sample size of this study. Microvascular occlusion in

SS disease is a multifactorial process. The homeostatic activation of coagulation has been reported (Hagger et al., 2005), and coagulopathy may play a role in the pathogenesis of vascular occlusion in sickle cell disease. Thrombin production is increased, and natural anticoagulant proteins C and S are reported to be lower in SS disease (Hagger et al., 2005). Protein C, activated by thrombin and in the presence of protein S, inactivates factors V and VIII. Thus, the protein C / S pathway is an effective source of natural anticoagulant activity. Protein C and

protein S genetic defect status is associated with an increased risk of venous thromboembolism. In SS disease, both adults and children have been reported to have low levels of protein C and S, which can theoretically lead to a tendency for thrombosis. The presence of resistance to activated protein C may further exacerbate this trend (Wright et al., 2007). Approximately 60% of circulating protein S binds to the C4B-binding protein. C4B-binding protein is an acute phase reactant involved in the complement pathway. An increase in the level of C4B-binding protein can reduce the efficiency of the protein C pathway by reducing the amount of free protein S (Walker, 2004).

Protein C and Protein S are produced in the liver and are vitamin K-dependent proteins. Impaired liver function due to decreased protein C and protein S synthesis may be another possible explanation. The liver is often affected in SS; red blood cells that pass through areas of hypoxic sinusitis are easily blocked by swollen Kupffer cells (Mills et al., 2008). The strongest evidence for liver dysfunction comes from the close correlation between prolonged prothrombin time and the low factor V, factor VII, and C4B binding proteins synthesized by the liver. Compared with biliary obstruction or diet-induced vitamin K deficiency, the lack of detectable PIVKA and low factor V (a protein independent of vitamin K) contributes to liver cell involvement.

**CONCLUSION**

Sickle cell anemia is a multi-system disease caused by a single gene mutation. Almost every organ in the body will be affected. It is characterized by the presence of abnormal red blood cells destroyed by HbS. Sickle cell anemia is a type of sickle cell disease, also known as sickle cell disease.

is an inherited blood disease characterized by a genetic mutation in the gene encoding hemoglobin in human red blood cells. Protein C, also known as autologous prothrombin IIA and coagulation factor XIX, is a proenzyme, an activated form that plays an important role in regulating anticoagulation, inflammation and cell death, and maintaining the permeability of blood vessel walls. It has been determined in different studies that the protein C level of patients with sickle cell anemia is reduced. It is said that these decrease in protein C levels are caused by different mechanisms, such as increased thrombin production due to the expression of tissue factor (TF) in blood vessels and the activity of red blood cells, leading to chronic depletion of protein C and S prothrombinase (RBC), Due to the exposure of the PS membrane, the binding of sickle erythrocytes to protein S increases. Antiphospholipid antibodies inhibit the binding of protein S to β2 glycoprotein 1, causing protein S to be inactivated by circulating C4 binding proteins, which in turn leads to protein S inactivation affecting protein C Because protein S is an important cofactor for activating protein C. Protein C and protein S are produced in the liver and are vitamin K-dependent proteins. The liver is often affected in SS disease, leading to or not producing protein C and S.

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