Hemostatic studies in Sudanese patients with sickle cell anemia.

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١٠٠ كَذَٰلِكَ قَالَ ﴿لَوْ كَانَ أَوْلِيَاءُ الْأَمْرِ أَنْ أُنْهِيَنَّكَ مِنْهُ﴾.

١٠١ لَكِنَّهُمُ الْحَرَّامُ ﴿لَا تَأْتِيهِمْ﴾.

١٠٢ ثُمَّ قَالَ ﴿لَمَّا كُلِّفَ سَنِيعٌ﴾.

١٠٣ لَهُمْ رَبُّهُمْ ﴿إِلَّا أَنْ يَأْتِيَهُمْ قَضَىٰ حُكْمٍ﴾.

١٠٤ كَذَٰلِكَ تَصَدَّىٰ لَهُمْ ﴿وَلَا تَأْتِيَهُمْ﴾.

١٠٥ الَّذِينَ ﴿أَفْحَّطُونَ﴾.

١٠٦ كَذَٰلِكَ ﴿ذَٰلِكَ صَدَقَةٌ مَّعِينٌ﴾.

١٠٧ كَذَٰلِكَ ﴿لَا يُقْسَمُ الْجَنُّ﴾.

١٠٨ لَكُمْ ﴿وَلَا تَأْتِيَهُمْ﴾.

١٠٩ كَذَٰلِكَ ﴿لَا يُقْسَمُ الْجَنُّ﴾.

١١٠ لَعِبْنَ ٍشِيْحَةٍ ﴿إِنَّ الْإِسْلاَمَ وَالْإِكْفَانَ﴾.
Dedication

To all the mothers who suffer a lot to raise their children healthy, lovely, comfortable, and indeed with good manners...

To my children whom I wish to grow up happy and well...

To my husband who helped me alot...

To tito...
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Abstract

Background: Sickle cell anemia is a common disease in Sudan. Homozygous patients suffer from hemolytic anemia and other serious complications. The underlying pathology of much of these complications is the occurrence of vaso-occlusion due to micro thrombi formation resulting in organ ischemia. Activation of coagulation system as well as increased fibrinolysis have been reported in SCA. This is the first study in Sudan in which hemostatic derangements in patients with sickle cell anemia are examined. Patients and controls: Fifty Sudanese patients (homozygous for hemoglobin S) of both sexes with ages ranging between 9 months and 17 years were included in this study. They were either in vaso-occlusive crisis or in steady state.11 apparently healthy controls were also studied. Laboratory methods: PT, APTT, Fibrinogen level, platelets counts and D-dimer levels were carried out in all patients and controls. Results: All controls had normal PT, APTT, Fibrinogen, and D-dimer level. While all patients had elevated D-dimer levels with normal fibrinogen concentration. All the patients had normal PT. 66% of patients had normal APTT, 22% had prolonged, and 12% had shortened APTT. Conclusion: Elevated levels of D-dimer occur in sickle cell anemia regardless of the clinical phase of the disease thus indicating a state of continuous fibrinolysis. The shortened APTT in some of the patients is an indication of a hypercoagulable state while the prolonged APTT in others is suggestive of a state of DIC.
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<tbody>
<tr>
<td>APTT</td>
<td>Activated Partial Thromboplastin time</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>DVT</td>
<td>Deep vein thrombosis</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra acetic acid</td>
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<tr>
<td>EIA</td>
<td>Enzyme immuno assay</td>
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<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbant assay</td>
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<tr>
<td>FEU</td>
<td>Fibrinogen equivalent unit</td>
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<tr>
<td>FPA</td>
<td>Fibrinopeptide A</td>
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<td>FPB</td>
<td>Fibrinopeptide B</td>
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<tr>
<td>FDP</td>
<td>Fibrin degradation products</td>
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<tr>
<td>Hb</td>
<td>Hemoglobin</td>
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<td>HbS</td>
<td>Hemoglobin S</td>
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<td>HbF</td>
<td>Hemoglobin F</td>
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<tr>
<td>HbSC</td>
<td>Hemoglobin SC</td>
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<tr>
<td>HbA</td>
<td>Hemoglobin A</td>
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<tr>
<td>KCEH</td>
<td>Khartoum Children Emergency Hospital</td>
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<tr>
<td>MoAb</td>
<td>Monoclonal antibodies</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>PLts</td>
<td>Platelets</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin time</td>
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<tr>
<td>PIH</td>
<td>Pregnancy Induced Hypertension</td>
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<td>WBCs</td>
<td>White blood cells</td>
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<td>SCD</td>
<td>Sickle Cell Disease</td>
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<td>SCA</td>
<td>Sickle Cell Anemia</td>
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<td>SS RBCs</td>
<td>Red cell homozygous for sickle hemoglobin</td>
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<tr>
<td>RBCs</td>
<td>Red blood cells</td>
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<td>vWF</td>
<td>von Willebrand Factor</td>
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CHAPTER ONE

1. INTRODUCTION & LITERATURE REVIEW

1.1. Normal structure of hemoglobin:

Most of the hemoglobin exists as a tetramer of two $\alpha$ chains, each with 141 amino acids, and two $\beta$ chains, each with 146 amino acids. About 75 percent of the amino acids in $\alpha$ or $\beta$ chains are in helical form as one heme molecule; oxygen is carried by the iron of the heme.\(^{(1,2)}\)

The four globin chain subunits fit together into a quaternary configuration, which is found in two structures, the (T) state (tense) and (R) state (relaxed) structures. Respective alternative terms are unliganded and liganded states or deoxy and oxy states.\(^{(2,3)}\)

Perurz in 1970 had proposed that the stereo chemical mechanism of Co-operative effects of hemoglobin arise from equilibrium between the two alternative quaternary structures just mentioned: the liganded structure whose oxygen affinity is high, and the deoxy structure whose oxygen affinity is low.\(^{(4)}\)

1.2. Definition of sickle cell disease:

The sickle cell diseases (SCD) are inherited disorders of hemoglobin (Hb) structure and synthesis, and include homozygous sickle cell anemia
(HbSS), sickle-C disease (HbSC), double-heterozygous combinations of
the HBS and B-thalassemia (HbS/thal) and other less common variants. (5)

The gene of sickle hemoglobin results in the substitution of valine for
the glutamic acid normally present at the sixth position of \( \beta \) chain of
hemoglobin. This changes the behavior of hemoglobin molecules, which
tend to polymerize on deoxygenation. (6) This is because the formed HB S
becomes an association of heme plus 2 normal (\( \alpha \)) alpha-chains and 2
abnormal (\( \beta \)) beta globin chains. It carries oxygen normally but begins to
form semi-solid aggregated structures once oxygen is unloaded to the
tissues. These hemoglobin S aggregates distort the red blood cells and
cause them to lose their normal elasticity. At first, HB S retains its ability
to return to its soluble form, and red cells, can regain their elasticity upon
re-oxygenation. However, this process damages the red
cell, and with repeated deoxygenation cycles, permanent red-cell damage
ensues causing rigid deformation of cell. (7) This deformation impairs the
ability of the cell to pass through small vascular channels. (8)

More causes of the sickle red blood cell damage is that it has increased
amount of denaturated hemoglobin. This high density enriched sickle cell,
especially that does not contain HbF, has very short in vivo survival. The
binding of the denaturated hemoglobin to the membrane forces a
redistribution of survival membrane compounds leading to membrane damage and enhanced cell dehydration. Membrane damage is also caused by an excessive accumulation of oxidant damage. The most important contributors to the dehydration of sickle red cells are potassium-chloride co-transport and calcium activated potassium efflux. The final stage of this process, membrane damage and cell dehydration, is the irreversibly sickled cells. (9, 10, 11, 12, 13, 14)

Another factor that affects sickling and the formation of irreversibly sickled erythrocytes is the heterogeneous distribution of hemoglobin types. It was found that the synthesis of hemoglobin F is least in erythroid cells destined to become irreversibly sickled. An irreversibly sickled cells has a high hemoglobin concentration and a high calcium and low potassium content, and it may be ATP depleted. Sickled cells have a short intravascular life span and the severity of the hemolytic process is directly related to the number of these cells in patient's circulation. (1, 15, 16, 17, 18)

Therefore, the pathophysiology of vaso-occlusive episodes is complex, involving not only polymerization of the mutant hemoglobin, but also interaction between SS red blood cells, endothelium, platelets, leucocytes and plasma constituents. (19)
That is why the hallmarks of sickle cell disease are chronic hemolytic anemia and vaso-occlusive painful crisis. \(^{(20)}\)

1.3. Historical background:

Sickle cell disease was first described by Herrick in the United States in 1910, when he described peculiar elongated and sickled-shaped red blood cell corpuscles in a case of severe anemia. \(^{(21)}\)

Emmel demonstrated that red cells sickled when blood from such patients was sealed under glass and allowed to stand at room temperature for several hours. But the fact that the transformation to sickle cells occurs in response to a fall in oxygen tension was not recognized until the classic studies of Harlan and Gillespie in 1927.

In 1923 the sickling phenomena was shown to be inherited as an autosomal dominant trait. Much later, Need and Beet clarified the genetic basis of sickle cell anemia by demonstrating that heterozygosity for the sickle gene resulted in sickle cell trait without significant clinical symptoms, while homozygosity resulted in sickle cell anemia.

In 1949 Pauling and his colleagues found that all the hemoglobin in patients with sickle cell anemia showed an abnormally slow rate of migration on electrophoresis, while the parents of these patients had normal as well as abnormal hemoglobin. \(^{(22)}\) The biochemical nature of the
defect in sickle cell anemia was elucidated by Ingram who digested hemoglobin with trypsin and separated the resulting peptide on paper by electrophoresis in one direction and chromatography in the other. This technique (finger-printing) demonstrated that one of the digestion products of sickle hemoglobin migrates differently from that of normal hemoglobin.

Demonstration of the amino acid position of this peptide indicated that sickle cell anemia was the result of the replacement of glutamic acid residue by valine. This discovery established that the substitution of a single amino acid in a polypeptide chain can alter the function of the gene product sufficiently to produce widespread clinical defects.\(^{(23)}\)

1.4. Prevalence and geographical distribution:

- **Sex:**

  Hemoglobin S is transmitted as an autosomal co-dominant characteristic. Therefore, the disease is found with equal frequency in males and females.

- **Age:**

  Clinical characteristics generally are not seen in children younger than 6 months, when fetal hemoglobin levels decline sufficiently for abnormalities caused by hemoglobin S to be recognized.\(^{(7)}\)

Around the world the sickling disorders are found very frequently in the Afro-Caribbean population, and sporadically throughout Mediterranean
region, India and Middle East. The frequency of the gene occurs within particular ethnic groups because of the selective advantage against (plasmodium falciprum malaria) offered by the carrier state. The hemoglobinopathies are rarely found in the northern European population, but have come to Europe and North America with population migration. (24)

The frequency of sickle cell disease varies greatly among different populations. Among African Americans approximately 7.8% are carriers of the sickle mutation, and the predicted frequency of the homozygous state for sickle hemoglobin is approximately 1:650. The sickle cell trait occurs in 10-30% of people in Equatorial Africa but is infrequent in North and South Africa. In Saudi Arabia, there is a prevalence of 43% for sickle cell disease and 37% for Hb SS. In Northern Greece, the prevalence of sickle cell trait is as high as 30%. In many communities in the Indian States of Orissa, there is a prevalence of 20-30%. In Jamaica, the incidence of sickle cell disease, at birth is 3.2 per 1000 live births. (1, 5, 25, 26, 27)

In the Sudan sickle cell anemia was first reported in 1926 by Archibald. Three foci of the disease have been described: Western Sudan, with prevalence rate up to 30% among Baggara tribe by Vella, (28) Southern Sudan, where the prevalence rate up to 18% was found among the Southern Nilotes by Foy, et al, (29) and in the Blue Nile province, Central
Sudan, where prevalence ranging from 0.5% among indigenous population and up to 16% was found among the immigrating tribes in the area, by Ahmed and Baker.\textsuperscript{(30)} Ibrahim in 1970, found that sickle cell hemoglobin and β thalassemia are the major hemoglobin anomalies in Khartoum.\textsuperscript{(31)}

Generally, the mutation that results in hemoglobin S is felt to have originated in several locations in Africa and India. Its prevalence is variable, but very high in these countries. As a result of migration, both forced and voluntary, it is now found worldwide. Recent data indicated that 51% of children born with sickle cell anemia die by the age of 20 years.\textsuperscript{(32)} So, it carries high morbidity and mortality to the affected individuals.

1.5. Pathophysiology of sickle cell anemia:

The short survival of the sickle erythrocytes and the removal of the abnormal cells from circulation, at a rate exceeding the capacity of the marrow to replace them, result in hemolytic anemia.

The obstruction of small vessels by aggregates of sickle cells results in painful episodes and repeated infarctions which lead to gradual involvement of all organ systems, most notably: spleen, lungs kidneys, and bones.\textsuperscript{(1, 3)}
Symptoms vary from a mild asymptomatic disorder to a severe hemolytic anemia and recurrent severe painful crisis. The condition may present in childhood with anemia and mild Jaundice. The hand and foot syndrome due to infarcts of small bones is quite common in children and may result in digits of varying lengths.

Many patients achieved steady state of relatively well being interrupted by episodic events of crisis, types of crisis include: vaso-occlusive crisis, aplastic crisis, hemolytic crisis, and sequestration crisis.\(^{(33)}\)

Various types of vaso-occlusive crises occur, including:

- Bone: pain (commonest).
- Cerebral; hemiparesis, fits.
- Penis: priapism.
- Chest: pleuritic pain.
- Spleen: painful infarcts.
- Liver: pain with abnormal biochemistry.
- Kidney: papillary necrosis causing hematuria, renal tubular defect resulting in lack of concentration of urine.

Attacks of pain with low-grade fever last from few hours to a few days. In a given patient the degree of anemia is usually stable during a crisis, Hb does not fall unless there is one or more of following : a- plasia, auto-
sequestration, hemolysis, long-term problems e.g: infection, chronic leg ulcers, gall stones, aseptic necrosis of bone, blindness and chronic renal disease.\(^{33}\)

Individuals who are affected suffer a wide range of clinical problems that result from vascular obstruction and ischemia \(^{(7)}\). Therefore the vaso-occlusive crises are classified as the major complications of sickle cell anemia.

1.6. Vaso-occlusive crisis:

Factors contributing to vaso-occlusion include polymerization of abnormal hemoglobin molecule with impaired deformability of sickle red blood cell, abnormal interaction between sickled red blood cell and vascular endothelium, high granulocyte counts, and fat embolism among others. \(^{34}\)

The sickling of erythrocytes increases viscosity and reduces the rate of both local circulation and arterio-venous transit time. This causes occlusion of capillaries by “micro thrombi”. The occlusion is implicated in the multiplicity of vaso-occlusive complications of both acute and chronic nature. \(^{35}\) All that makes vaso-occlusive crisis the principal complication of sickle cell disease. Hand and foot syndrome, which is the earliest clinical manifestation of sickle cell disease, was reported by Watson in
Vaso-occlusive crisis may affect any tissue but the pain occurs especially in the bones, chest and abdomen. In young children, pain often involves the extremities. In older patients, head, chest, abdominal and back pain occur more commonly. Fever is common even in apparently uncomplicated painful crisis, suggesting that fever is characteristic of painful crisis itself and not necessarily indicative of infection.\(^{(36, 37, 38, 39, 40)}\)

The rate of painful crisis varies considerably, some patients never experience severe pain, and others require frequent hospital admission for painful crisis.\(^{(36, 38)}\)

The clinical manifestations of SCD include chronic hemolysis, frequent infections, and a variety of other acute and chronic complications that produce organ damage, disability, and death. As current thinking suggests that micro-vascular occlusion by poorly deformable sickle erythrocytes (SS RBCS) is a key pathophysiologic event in SCD, here we summarize and evaluate current concepts regarding the cause (s) and consequences of vascular occlusion in SCD.

1.6.1. Micro-vascular occlusion by RBCs in SCD:

I. In vivo studies in humans:

Direct evidence regarding the dynamic behavior of SS RBC in the human micro-vascular is essentially limited to a single study, in which
blood flow in nailfold capillary loops was investigated using intravital microscopy. In this study, Hb SS subjects were reported to exhibit increased frequency of intermittent flow and flow stasis compared with Hb AA subjects. Overall, an average of 8.2% of nailfolded capillaries were obstructed by RBCs in steady state Hb SS subjects and 1.0% in Hb AA subjects, these differences were not statically significant. These findings clearly indicate that SS RBCs can obstruct capillaries in human subjects, but (at least in the nailbed) such obstruction in human subjects appears to be relatively limited in extent, largely transient, and insufficient to reduce overall flow except under conditions of artificially induced reactive hyperemia. \(^{(41)}\)

The technique of intravital nailbed microscopy does not permit direct observation of arterioles or venules, two potentially important sites of micro-vascular occlusion by SS RBCs. Recently, however, capillary volumetric blood flow in the skin has been shown to exhibit an abnormal oscillatory flow in SS subjects, consistent with synchronization of flow at the arteriolar level.\(^{(42,43)}\) These could represent an exaggeration of normal tendency of pressure-sensitive terminal arterioles toward rhythmic vasomotion, which might function in SCD to clear transient micro-vascular obstructions by inducing periodic (bolus) flow.\(^{(42,43)}\)
The cause, significance, and frequency in other vascular beds of synchronized oscillatory capillary flow in SCD are not known. Because, these findings were not predicted by any existing model of micro-vascular dynamics in SCD, they emphasize the need of additional direct in vivo studies as a prerequisite to understanding the process of micro-vascular occlusion in SCD.

**II. Animal studies:**

A number of potentially important findings have come from animal studies. Studies of the behavior of unfractionated SS RBCs in animal microvascular beds have demonstrated that whereas oxygenated non-irreversibly sickled SS RBCs are generally quite deformable and readily pass through the microvasculature without causing obstruction, deoxygenated and irreversibly sickled SS RBC can cause significant obstruction of arterioles, capillaries, and venules, in part via increased adherence to micro-vascular endothelium. Studies using various density-defined sub-populations of SS RBC indicate that the least-dense deformable (reversible) SS RBC are the most adherent to endothelium in vivo, and appear to play a key role in initiating micro-vascular occlusion by adhering to the post venular capillary endothelium, thereby creating a partial obstruction that selectively traps the
densest and least-deformable (irreversible sickled) RBC, resulting in complete obstruction. (44, 45, 46, 47)

This model of micro-vascular occlusion in SCD is consistent with in vitro evidence that less-dense SS RBC are more adherent. (48,49) It is also consistent with clinical evidence that RBCs adhesiveness correlates directly with the overall severity of vascular occlusive disease in SS subjects (50), and that the incidence of painful crisis is higher in SS subjects with better hydrated, more deformable RBC (51,52), which appear to be more adherent to endothelium (48,49), and therefore presumably more likely to initiate vascular occlusion. Finally, it is consistent with a report that the percentage of circulating dense cells decrease during painful crisis, possibly reflecting selective entrapment of these cells in the concluded microvasculature.

1.6.2. RBC -endothelial adherence and micro-vascular occlusion:

SS RBCs are abnormally adherent to vascular endothelium in vitro, under both static and dynamic flow conditions, (48, 50, 53, 54) and in vivo. (41,46) Although direct observations of SS RBC endothelial adhesion in vivo have thus far been confined to the micro-vasculature, it is possible that SS RBC endothelial adhesion also occurs in the large arteries and veins, and could contribute to large-vessel endothelial injury, vascular intimal hyperplasia,
and thrombosis. Many intrinsic abnormalities of SS RBC have been implicated as causes of increased adhesiveness, including membrane sialic acid abnormalities (55), oxidative membrane damage (56,57), loss of membrane phospholipid asymmetry (58,59) and binding to various plasma adhesive proteins, including fibrinogen, fibrinectin, and von Willebrand Factor (vWF). (60,61)

1.6.3 White blood cells (WBC) and micro-vascular occlusion:

Although less numerous than RBC, WBCs are considerably less deformable (62), and therefore potentially more occlusive in the micro-vasculature. Many subjects with SCD have moderately elevated WBC counts in the steady state due to absent splenic function, and marked leucocytosis is often seen during painful crisis. (63) Neutrophil adhesiveness has been reported to increase significantly during painful crisis. (64) Thus, the contribution of WBCs to micro-vascular occlusion in SCD deserves further investigation.

1.7. Factors that affect severity of SCD:

1.7.1. Fetal hemoglobin:

Fetal hemoglobin protects the red cell from sickling. It is a potent inhibiter of the polymerization of deoxyhemoglobin S, the gamma-chain lacks the valine which interacts hydrophobically with hemoglobin S
molecules, thus reducing sickling and vascular occlusions. Patients with high Hb F have lower number of irreversibly sickled cell and have a concomitant decrease in number of adhesion markers, and so less adherent erythrocytes. (65, 66, 67, 68, 69)

Therefore, it was found that Hb F decreased incidence of chest syndrome at Hb F > 20%; decreased incidence of aseptic necrosis and stroke at Hb > 10% (70), in addition to its inverse correlation with stroke, leg ulcers, retinopathy, aseptic necrosis of femoral head and splenic sequestration crisis. (71, 72, 73, 74, 75)

1.7.2. Other factors include:

- Alpha thalassemia.
- Beta thalassemia.
- G6PD deficiency.
- Other beta globin haplotypes.
- Environmental factors. (37)

1.8. Diagnosis:

Sickle cell anemia is diagnosed by the findings of a variable degree of anemia (Hb 6-8 g/dl), an elevated reticulocyte count of (10-20%) sickled erythrocytes on the peripheral blood film, a positive sickling test, and hemoglobin electrophoretic pattern characterized by absence of Hb A and a
preponderance of Hb S with a variable amount of Hb F. The diagnosis is confirmed by finding the sickle cell trait in both parents.\textsuperscript{(76)}

1.9. Blood coagulation and sickle cell disease:

1.9.1: Normal coagulation:

The central event in the coagulation pathway is the production of thrombin, which acts upon fibrinogen to produce fibrin and thus fibrin clot. There are at least five components involved: blood vessels, platelets, plasma coagulation factors, their inhibitors and the fibrinolytic system. The two commonly used coagulation tests, the activated partial thromboplastin time (APTT) and the prothrombin time (PT), have been used historically to define two pathways of coagulation; the intrinsic and the extrinsic pathway.\textsuperscript{(77)}

1.9.2: Fibrinogen:

Fibrinogen is a large dimeric protein, each half consisting of three poly peptides named $A\alpha$, $B\beta$ and $G$ gamma held together by 12 disulphide bonds. The two monomers are joined together by further three disulphide bonds. Fibrin is formed from fibrinogen by thrombin cleavage of the A and B peptides from fibrinogen. This results in fibrin monomers that associate to form a polymer, which is the visible clot. The central E domain exposed by thrombin cleavage, then binds with a complementary region on the
outer or D domain of another monomer. The monomers thus assemble into a staggered overlapping two-stranded fibril. More complex interaction subsequently leads to branched and thickened fiber formation. (78, 79)

1.9.3. Structure of fibrin:

The peptides that are cleaved from fibrinogen by thrombin, create two forms of fibrin: {fibrin I} which is a fibrinogen molecule devoid of fibrinopeptide A (FPA), the peptide from the amino terminal of the two A\(\alpha\) chain, and {fibrin II}; the fibrinogen without (FPA) and fibrinopeptide B (FPB), the peptide that is released from the amino terminal of the fibrinogen B chains. (80, 81, 82)

1.9.4 Formation of fibrin degradation products:

Plasmin digests the different forms of fibrin, fibrin I and fibrin II, which consist of very long polymers of fibrin I and II in which the sub-units are cross-linked by isopeptide between alpha (\(\alpha\)) and gamma chains. Plasmin will attack the sub-units in these polymeric structures in a random order. This will result in soluble fragments of the original polymer, with a range of molecular weights. (81, 83) These are collectively designated as x-oligomers. (83, 84, 85) Extensive digestion of these x-oligomers will yield, the so called fragments D-dimer and fragments E_I or E_{II}. (81, 83)

1.9.5. Fragment D-dimer:
Fragment D-dimer can be described as two fragments D covalently kept together by two isopeptide bonds (cross-links) between the gamma chain remnants of the two D-fragments.

Methods of D-dimer estimation include enzyme immuno assays (EIA) and latex agglutination assays which are based on monoclonal antibodies (MoAb) with specificity which is compatible with the use of plasma. (81, 83)

1.9.6. Abnormalities of coagulation in SCD:

Sickle cell disease is a thrombophilic state with evidence for in vivo thrombin generation, endothelial activation and clinically evident thrombotic predisposition. (86, 87)

Coagulation activation has been documented with in vivo demonstration of thrombin generation both in steady state and during vaso-occlusive crisis. (88, 89) In some studies this coagulation activation was shown to be more accentuated during painful crisis (90), and thrombosis probably does play an important role in several other recognized complications including stroke, acute chest syndrome, leg ulceration and placental infraction. (91, 92)

This thrombophilic state is associated with complex perturbations of plasma and cellular hemostatic mechanism. (93) These changes include evidence for thrombin generation, (94) depletion of natural anticoagulants,
Moreover, a number of observations suggest that activation of coagulation with formation of fibrin occurs in sickle cell disease and may contribute to the pathogenesis of micro vascular occlusion. Fibrin is present in vessels occluded by sickle cell erythrocytes. Fibrinogen survival is shorter than normal in the steady state and further shortens during painful crisis. Levels of factor V are reduced in the steady state, consistent with ongoing thrombin activity, and fibrin-fibrinogen fragment E is elevated. The latter reflects plasmin proteolysis of fibrinogen and/or fibrin, however, and thus is not specific for either thrombin activity or fibrin formation. More recently, assays for specific markers of thrombin action on fibrinogen have been applied to the study of coagulation in sickle cell disease. Fibrinopeptide A (fpa) is produced by the action of thrombin on the alpha chain of fibrinogen and is the first fragment produced in the transformation of fibrinogen to fibrin. Fpa has been reported to be elevated during painful crisis, but to be normal in uncomplicated sickle cell disease during the steady state.

In another study, coagulation activity and whole blood viscosity were measured in the steady state and serially during painful crisis and it was
demonstrated that platelets and coagulation were activated in steady state and this becomes more pronounced early in crisis. Whole-blood viscosity increased during crisis in parallel with plasma fibrinogen. Plasma heparin-neutralizing activity was also significantly raised and was followed over the next 2 days by a fall in platelets count. A significant elevation of factor VIII R: Ag/ F VIII: C ratio also occurred by the second day. Coagulation activation of this type may contribute to vascular sledging, and also promote further sickling by causing an increase in whole blood viscosity. Indeed elevated levels of factor VIII have been correlated to thrombus formation and vascular sledging in many clinical studied, which demonstrated correlation between elevated levels of F VIII and F IX and the risk of coronary heart disease, myocardial infraction, and ischemic stroke.

In addition to its effects on the cleavage of fibrinogen and its ability to activate platelets, the increase in circulating thrombin levels, with its wide-ranging effects on endothelial cells and blood vessel, may be important in the pathophysiology of sickle cell disease.

Thrombocytosis, defective thromboplastin generation, and elevation in Factor VIII levels, have also been reported in SCD. In the painful crisis, the platelets count may be further increased, and a
sequential activation of the coagulation system occurs, \textsuperscript{(111)}, accompanied by impairment of fibrinolysis. \textsuperscript{(116,117,111)}

Lesie J et al examined the coagulation changes during the steady state in homozygous sickle cell disease in Jamaica. Coagulation studies were carried out in 117 Jamaicans, and 40 local controls. The patients had significantly higher factor VIII levels, higher platelet counts, lower factor V and plasminogen levels, shorter thrombin times and higher serum fibrinogen degradation products (FDP) than the control group. The low factor V and plasminogen and high (FDP) levels might be explained by activation of the coagulation system and continuous clot lysis even in the absence of painful crisis. \textsuperscript{(101)}

Hager D et al, measured seventeen parameters of coagulation and fibrinolysis in 33 patients with sickle cell disease, 30 were tested in steady state (SS) and 19 in crisis. The same parameters were measured in 16 controls all with (Hb A) only. The results showed highly significant increase in D-dimer in both steady state and crisis patients compared with controls, and moreover no significant increase in fibrinogen level in both also. \textsuperscript{(118)}

Nsiri B, et al, studied the abnormalities of coagulation and fibrinolysis in a group of (28) children and young adults with homozygous sickle cell
disease either in steady state (n: 12), or during painful crisis (n: 16) significant elevation of D-dimer fragment was noted during sickle cell crisis. The results provide evidence for the presence of circulating activated clotting factors in SCD and for an imbalance of the profibrinolytic and anti-fibrinolytic systems. (119)

Famodu et al, made serial measurements of plasma fibrinogen concentrations in (9) homozygous (SCD) patients during vaso occlusive crisis. Fibrinogen concentration was shown to be rising sharply and significantly to a maximum on approximately the second day of the onset of pain crisis .This study suggests that serial measurement of fibrinogen could be used a sensitive parameter to monitor the progression of sickle cell pain crisis. (120)

Elevated levels of D-dimer indicate increased plasmin degradation of cross linked fibrin, which in turn indicates increased thrombin activity, since the formation of cross-linked fibrin requires the action of thrombin on both fibrinogen and factor XIII. (121) The fibrin D-dimer fragment is not produced by proteolysis of fibrinogen by plasmin or other non thrombin proteases. D-dimer levels in blood are quite insensitive to artifact, since the sequential actions of thrombin, factor XIII, and plasmin are not likely to
occur during sample collection and processing. Even clotted blood samples don’t exhibit artefactually increased levels of D-dimer. \(^{(121)}\)

Moreover, Ataga et al have found evidence of activation of both blood coagulation and platelets in sickle cell disease in samples obtained in the steady state and during painful crisis. They demonstrated high levels of thrombin generation, depletion of anticoagulant proteins, and abnormal activation of the fibrinolytic system. \(^{(110)}\)

1.10. D-dimer in disease:

1.10.1. Disseminated Intravascular Disease (DIC):

All patients with DIC appear to have elevated levels of D-dimer as assessed by (Elms and Wild), \(^{(122,123)}\), using latex agglutination assay for D-dimer.

Moreover, for diagnosis of DIC in new-borns done by (Ockelford), \(^{(124)}\), also D-dimers were found elevated with low level of fibrinogen.

1.10.2. Deep Vein Thrombosis (DVT):

Using EIA (Enzyme Immuno Assay) for D-dimer estimation, high levels occurred in hospitalized patients with DVT (Whitaker et al and Hochstein et al). \(^{(122,125)}\)
1.10.3. Pregnancy and (Pre) eclampsia:

In a study done by (Trofatter et al) D-dimer levels were increased in pre-eclamptic women due to hemostatic abnormalities that usually occur in these patients. 39% of these studied patients showed high levels of D-dimer. (126)

1.10.4. Malignancies:

D-dimer levels both in tissues and in plasma of patients with ovarian cancer were increased as compared to normal (Kroneman et al). (127) It has been suggested that in malignancies fibrinolysis is accompanied by fibrinogenolysis as assessed by (McCulloch et al). (128)

1.10.5. Sickle cell anemia and D-dimer:

Francis RB Jr. with an ELISA assay sensitive to plasma D-dimer levels of less than 50 g/ml, found an elevated level of D-dimer in (23) of (25) subjects with (SCD) tested during the steady state, and in all (21) subjects with (SCD) tested serially while hospitalized for painful crisis. D-dimer was elevated in all (7) patients tested more than (6) months after their last painful crisis. It was concluded that increased thrombin activity and fibrin formation are features of steady-state sickle cell disease, and that they further increase during painful crisis. (129)
DV Devin et al, examined the relationship between fibrin D-dimer levels and the occurrence of complications in patients with (SCD), using a commercially available latex bead agglutination assay. (57%) of (187) samples on (96) patients showed elevated levels of fibrin D-dimer. (90%) of (75) samples from asymptomatic patients were negative for fibrin D-dimer, but (97%) of (29) samples from vaso-occlusive crisis patients, and (85%) of (83) samples from patients with other complications of sickle cell diseases were positive.

They concluded that the complications of (SCD), including vaso-occlusive crisis, result in production of fibrin D-dimer, and its detection may be used as a marker for the presence of complications.\textsuperscript{(130)}

Shahid Ahmed et al, measured plasma D-dimer in (65) blood samples of 37 adult patients with (SCD) who were hospitalized for vaso-occlusive painful crisis. D-dimer levels of patients who were on low dose warfarin were compared with those of patients who were not on any anti-coagulant.

Overall median D-dimer level in (65) samples was 2.7µg fibrinogen equivalent units (FEU) / ml. Patients who were on low dose warfarin had a median level of 0.81µg FEU/ml compared with 1.3µg EFU / ml in the patients who were not on warfarin.
They concluded that patients with SCD during vaso-occlusive crisis have an elevated D-dimer level and low dose anti-coagulation treatment is associated with a significant reduction in the D-dimer levels. (131)

No study has been done in Sudan to assess activation of coagulation and fibrinolytic system in patients with (SCD).
2. RATIONALE

Sickle cell anemia (SCA) is very common in Sudan, and its complications cause a lot of morbidity and mortality. The most serious complication in this condition is vaso-occlusion resulting in severe pain and organ dysfunction. Activation of coagulation and fibrinolysis are thought to play a major role in vaso-occlusive crisis. It has been suggested in the literature that low dose anticoagulation can reduce such complications in sickle cell disease patients who have evidences of coagulation and fibrinolysis activation. It is the aim of this study to look for evidence of such changes in Sudanese patients with (SCA). This will provide evidence for clinicians to manage their patients properly and thus reduce serious complications.
3. OBJECTIVES

3.1. General objectives:
To assess coagulation and fibrinolysis in patients with sickle cell anemia.

3.2. Specific objectives:

3.2.1. To look for evidences of activation of coagulation during the steady and crisis state in homozygous (SCA) by measuring prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen level.

3.2.2. To evaluate the activation of fibrinolysis in patients with Sickle Cell Anemia (SCA) by measuring the plasma D-dimer level in both steady and crisis state.

3.2.3. To correlate the degree of such activation of coagulation and fibrinolysis, if present, with the clinical state and severity of SCA.
CHAPTER TWO

2. MATERIALS AND METHODS

2.1. Study area and duration:

This is a cross-sectional study conducted at Khartoum Children Emergency Hospital (KCEH) which is part of Khartoum Teaching Hospital and is located at the center of Khartoum to the north of the main hospital. (KCEH) accommodates a referred clinic department in which different units follow up their patients. The study was conducted in the sickle cell anemia clinic in this hospital. The period of the study was from (June to December) 2006.

2.2. Study population:

Patients with sickle cell anemia who attend the referred clinic. All diagnosed by Hb electrophoresis.

2.2.1. Inclusion criteria: Patient with homozygous Hb S.

2.2.2. Exclusion criteria: Patients with other β chain variants eg: HbSC, HbS\thal.

2.3. Sampling:

2.3.1. Sample unit:

Patients with sickle cell anemia attending the referred clinic and who accepted to be included in the study.
2.3.2. Sample design:

A total coverage for all patients attending the referred clinic during the study period was the design which was chosen for sampling.

2.3.3. Sample size:

Fifty patients with sickle cell anemia and (11) controls were investigated in this study, and this sample size was determined according to availability of resources.

2.3.4. Clinical status on presentation:

According to the presenting symptoms, patients were grouped into:

1. Patients presenting during vaso-occlusive crisis (came with painful episodes).

2. Patients presenting during steady state, coming for follow up or with other insignificant complications.

2.3.5. Blood samples:

From each patient and control, 5 ml of venous blood were collected (with minimum torinquation) into a syringe, and then divided into two volumes:

2.3.5.1. 2.5ml of blood were immediately delivered into a container containing 0.25ml of 13.3g/l aqueous tri-sodium citrate (9 parts of freshly collected blood with 1 part of tri-sodium citrate). These were gently mixed and then platelet poor plasma was obtained from it by centrifugation at 2.500g for
15 min (within 1 hour of collection). Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) were done in this sample. The rest of the plasma were delivered into cryo-tubes and stored at -20 degrees centigrade till used later in batches for the determination of fibrinogen level and fragment D-dimer level.

2.3.5.2. The remaining 2.5ml of blood were delivered into a container containing K2EDTA and mixed gently. These were used to obtain basic hematological values by an automated cell counter (Sysmex KX-21).

2.3.6. Control Samples:

Control blood samples were collected from healthy subjects and platelet poor plasma was prepared as described above. The plasma samples were pooled in plastic containers and treated as the test sample in each batch.

2.4. Tests performed:

2.4.1. Prothrombin time (PT).

2.4.2. Activate Partial Thromboplastin Time (APTT).

2.4.3. Fibrinogen level.

2.4.4. Fragment D-dimer level.

2.4.5. Full Blood Count (FBC).

All the reagents for coagulation were from DiaMed Company.
2.5. Principles of Methods:

2.5.1. Prothrombin time (PT):

2.5.1.1. Sample preparation:

The platelet poor plasma was obtained as described above and the test was performed within 1 hour of collection for both patients and controls.

2.5.1.2. Procedure:

0.1 ml of pooled normal control and patient plasma were pipetted in test tubes containing 0.2 ml of PT reagent (DiaPlastin) pre-warmed at 37°C in a water bath. A stop-watch was started and clot formation observed. The stop-watch was stopped at the appearance of fibrin web. Then the time was recorded in seconds. (Appendix I)

2.5.2. Activated Partial Thromboplastin Time (APTT):

2.5.2.1. Sample preparation:

The platelet poor plasma was obtained as described above and the test was performed within one hour of collection for both patients and controls.

2.5.2.2. Procedure:

0.1 ml of control and patient's plasma were added (each) to 0.1 ml pre-warmed (APTT) reagent (DiaCelin). The mixture was incubated for 3 minutes at 37°C (in water bath), and then 0.1 ml of pre-warmed calcium chloride was added. The stop watch was started on the addition of calcium chloride, and
then stopped on the first appearance of the fibrin web. The result was recorded in seconds. (Appendix II)

2.5.3. Fibrinogen level:

2.5.3.1. Sample preparation:

The platelet poor plasma of both patients and controls were prepared and frozen at (–20) degrees centigrade as mentioned above.

2.5.3.2. Procedure:

Citrated plasma was diluted (1:10) with Owren’s Veronal Buffer [0,1ml plasma + 0,9ml buffer] and used immediately. Then into clean tubes:

0,2 ml of diluted plasma were pipetted, and left to warm 2-5 mins at 37°C, then 0.1 ml pre-warmed Diafibrinogen was added. Simultaneously a stop watch was started and clot formation was observed. At the appearance of the first visible fibrin web the stop watch was stopped.

Clotting time was obtained in seconds and the fibrinogen concentration was read in mg\dl from the calibration curve. (Appendix IV)

2.5.4. Fragment D-dimer level:

It was estimated by an instrument called DiaMed-LX2.

2.5.4.1. Sample preparation:

The platelet poor plasma of both patients and controls were already prepared and frozen at (–20) degrees centigrade as mentioned above.
2.5.4.2. Theory of the machine:

2.5.4.2.1. Definition:

The DiaMed-LX2 is a manual two channel photo-optical instrument. It is designed for the quantitative determination of D-dimer (DD) concentration in patient plasma.

2.5.4.2.2. Operating instructions:

Instrument is operated when the temperature indicator light is on. It takes approximately 10-15 minutes for the instrument to equilibrate to 37 degrees centigrade.

The default values for the system parameters are:

LANGUAGE: ENGLISH
D-DIMER: µg\L
TEMP.CONTROL: ON
SIGNAL: ON
CONTRAST OF LCD: VALUE: 25
SPEED OF MIXER: VALUE: 215
PATIENT IDENTIFICATION (PID): NUMBER

Each one of these items is chosen optionally by a direct order.

2.5.4.2.3. Calibration of D-dimer:
Dilutions of minimum three points are required, with a maximum of five points. Calibration data is obtained by testing reference plasma (D-dimer calibrator "Dia Cal DD") in duplicate in the "Analysis" mode.

2.5.4.2.4. Key Functions:

There are Optic keys (1&2). During analysis they are pressed once for patient identification (PID), twice to activate the channel and a third time to manually initiate the reaction.

2.5.4.2.5. Principle:

The DiaMed-l-LX2 is a highly sensitive 2-channel-photometer. The light source is provided by a laser LED-Optic at 660 nm. This intensive light is able to penetrate turbid solutions, such as latex suspension used for the determination of D-Dimer concentration. Latex particles designed specifically for automated DiaMed-LX2 testing, are coated with a monoclonal antibody specific for D-dimer. If D-dimer antigen is present in the sample, an antigen-antibody reaction occurs, with a simultaneous change in light transmission at 660 nm. The concentration of D-dimer in the sample is directly proportional to the rate of the antigen-antibody reaction.

2.5.4.2.6. Procedure:

Reaction happened when the start reagent is pipetted (Latex suspension) into the first cuvette position while simultaneously pressing the (Optic 1) key "a
beeping noise indicated the start of the reaction". Repeating for channel 2, and always pipetting from left to right (1 to 2). After the noise a blinking screen \{MIX NOW\} showed up. This was changed to \{RUNNING\} message-7 seconds after test start. During that time, the liquid in reagent was mixed. We avoided contact with the cuvette while "running" was shown. Beeping noise was sound when the reaction was complete and the result was displayed on the screen. Results were automatically printed.

**Steps:**

1. We pipetted 25\(\mu\)L plasma into cuvette.
2. Then we added 50 \(\mu\)L Reaction Buffer.
3. Incubation > 1 min.
4. We transferred cuvette to measuring position.
5. While incubating, we pressed "optic1". If selected, entered PID with numeric keys or up\/down keys. We confirmed by pressing "optic1" again.

   The message "ACTIVE" was displayed and channel one was ready to start reaction.
6. Then we added 50 \(\mu\)L pre-warmed latex and simultaneously pressed the "optic1" key. (immediately after start, we pipetted the fluid in the cuvette up and down three times to achieve best mixing). The test was automatically started.
7. The results were displayed in \(\mu\)g\(\cdot\)mL or \(\mu\)g\(\cdot\)L. \(^{(132)}\), (Appendix V)
2.5.5. Full blood count (FBC):

- The blood counter Sysmex kx-21 was used.
- The Sysmex kx-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by WBC detector block using the DC detection method.
- The RBCs count and Platelets are taken by the RBCs detector block, also using the DC detection method.
- The Hb detector block measures the hemoglobin concentration using non-cyanide hemoglobin method.

This instrument works in two analysis modes: Whole blood mode and prediluted mode. Whole blood mode is the mode of analyzing collected blood sample in the whole blood status. The pre-diluted mode is used in analyzing a minute amount of blood collected from the ear lobe or finger tip. The mode, which was chosen for this study, was the whole blood mode in which the RBCs are counted in 1 µl of whole blood by a DC detection method and the Hb is measured as a volume in gram in 1 dl of whole blood using a non-cyanide hemoglobin analysis method.

**DC Detection Method:**

**Principle:**

- Blood sample is aspirated measured to predetermined volume diluted
at the specific ratio then fed into each transducer.

• The transducer chamber has a minute aperture. On both sides of this aperture there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture causing electric changes. The blood cell size is detected as electric pulse. Blood cell count is calculated by counting the pulses.

2.5.5.1. DC detection method for RBCs and PLT counts:

**Principle:** As mentioned above.

**Procedure:**

• Blood is aspirated from its container by the machine probe.

• 4.0 µl of blood is diluted in 1: 500 by adding 1.996 ml of the diluent, and brought to the mixing chamber as diluted sample.

• Out of the diluted samples, 40 µl is measured and rediluted by 1.960 ml of diluent, then transferred to the RBCs\ PLT transducer chamber.

• 250 µl of the sample in the RBCs \ PLT transducer chamber is aspirated through the aperture, and then the RBCs and PLT are counted by the DC detection method.
2.5.5.2. Non Cyanide Hemoglobin Analysis Method:

**Principle:**

Non cyanide Hemoglobin analysis method rapidly converts blood hemoglobin to methemoglobin which contains no poisonous substance.

**Procedure:**

- Blood is aspirated from the sample tube into the machine.
- 6µl of blood is transferred to the WBC transducer chamber along with 1.994 ml of diluent. At the same time 1.0 ml of WBC/Hb lyse reagent is added to prepare 1:500 dilution sample, RBCs is hemolysed and platelets shrink with WBCs membrane held together as they are, Hb is converted into red colored methemoglobin.
- Then, about 1.0 ml of the solution is transferred to the Hb flow cell, and 500 µl in the WBCs transducer is aspirated through the aperture, WBCs are then counted by the DC detection method.
- In Hb flow cell 555-nm wavelength beam irradiated from the light emitting diode (LED) is applied to sample.
- Concentration of this sample is measured as absorbance.
- This absorbance is compared with that of the diluent (The Hb lyse reagent) which was measured before addition of the sample, thereby calculating the Hb value.
CHAPTER THREE

3. RESULTS

3.1. Sex and Age Distribution:

61 Sudanese subjects were included in this study, 50 of them were patients with known SCA and 11 were controls. Twenty five patients (50%) were males and 25 (50%) were females (Figure 1). The ages of the patients ranged between (9) months and (17) years. (Figure 2)

The control groups were 45% males (5 members) and 55% females (6 members). (Figure 3). Their ages ranged from 8 to 18 years.

**Figure 1**: Distribution of sex in the patients group.
**Figure 2:** Distribution of the age groups in the patients’ population.

![Age Distribution Chart]

**Figure 3:** Distribution of sex in control group.

![Sex Distribution Chart]

**3.2. Sociodemographic data:**

3.2.1. Residence:

Most of the patients were resident in peripheral areas of Khartoum state or outside Khartoum in Mayo, Hag Yosif and Ombada being the most frequent areas. (Figure 4). Most of these areas are inhabited by people who migrate from different parts of Sudan.
3.2.2 Tribes:

The geographical distribution of the patients’ tribes showed that most of them were from western Sudan, (Figure 5), the Messeria tribe being the biggest source of patients' fathers and mothers. Table (1&2)

The crossing between fathers and mothers tribes showed that 54% were from the same tribe. (Figure 6)
**Figure 5:** Geographical distribution of the patients according to their tribes.
**Table 1:** Distribution of fathers’ tribes among patients group.

<table>
<thead>
<tr>
<th>Fathers' Tribes</th>
<th>Frequency</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Messeria</td>
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<td>4</td>
</tr>
<tr>
<td>Tama</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fallata</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Berno</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hamar</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Niema</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Taga</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Bar</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Fur</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Khizam</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Helbawy</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ashraf</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>maamla</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mahas</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Table 2: Distribution of mothers' tribes among patients group.

<table>
<thead>
<tr>
<th>Mothers' Tribe</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Messeria</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Bagara</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Selhab</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Hausa</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Rizegat</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Salamat</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Taaiisha</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Habanya</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tama</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dago</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Berno</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Hamar</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Niema</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Bar</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Fur</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mararit</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Helbawy</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ashraf</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>maamla</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>
3.3. Distribution of Clinical findings:

3.3.1. Presenting symptoms and clinical signs:

Different symptoms and clinical signs were seen in the studied patients, with painful episodes, Pallor, and Jaundice being the most frequent findings. No patient came with gallstones or leg ulcers. (Table 3&4).

Table 3: Distribution of presenting symptoms.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>6</td>
<td>12 %</td>
</tr>
<tr>
<td>Painful episodes</td>
<td>24</td>
<td>48 %</td>
</tr>
<tr>
<td>Others</td>
<td>7</td>
<td>14 %</td>
</tr>
<tr>
<td>Symptom less</td>
<td>13</td>
<td>16 %</td>
</tr>
</tbody>
</table>

Table 4: Distribution of clinical findings.

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pallor</td>
<td>41</td>
<td>82 %</td>
</tr>
<tr>
<td>Jaundice</td>
<td>38</td>
<td>76 %</td>
</tr>
<tr>
<td>Palpable liver</td>
<td>30</td>
<td>60 %</td>
</tr>
<tr>
<td>Palpable spleen</td>
<td>7</td>
<td>14 %</td>
</tr>
</tbody>
</table>
3.3.2. Clinical status:

According to the presenting symptoms 24 patients (48 %) were in vaso-occlusive crises as recognized by their complaining of pain while 26 patients (52 %) were in steady state either coming for follow up or presenting with minor complications. (Figure 7)

A severity score was calculated for each patient using accepted international criteria where the disease is considered as severe if patients experiences three or more painful episodes per year \(^{38,134,135}\). Nineteen (38%) of our patients had severe disease. (Table 5)

**Figure 7:** Distribution of patients according to clinical status.
Table 5: Percentages of pain episodes among patients population.

<table>
<thead>
<tr>
<th>No. of pain episodes per year</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>1</td>
<td>2 %</td>
</tr>
<tr>
<td>once</td>
<td>21</td>
<td>42 %</td>
</tr>
<tr>
<td>twice</td>
<td>9</td>
<td>18 %</td>
</tr>
<tr>
<td>three times</td>
<td>10</td>
<td>20 %</td>
</tr>
<tr>
<td>four times</td>
<td>4</td>
<td>8 %</td>
</tr>
<tr>
<td>five times</td>
<td>5</td>
<td>10 %</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100 %</td>
</tr>
</tbody>
</table>

3.4. Laboratory data:

3.4.1. Basic hematological data:

(Table 6) shows the mean values for Hb, WBCs and platelets in patients.

Table 6: Description of the basic hematological measurements of the patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>6.9</td>
<td>4.5</td>
<td>9</td>
</tr>
<tr>
<td>TWBCsX10^6/L</td>
<td>17.5</td>
<td>4.4</td>
<td>45.4</td>
</tr>
<tr>
<td>PlateletX10^9/L</td>
<td>382.04</td>
<td>94</td>
<td>756</td>
</tr>
</tbody>
</table>
3.4.1.1. Hemoglobin:

All were anemic with some showing very high WBCs and platelets counts.

All except one patient had Hb of (5-10) g/dl. That patient was severely anemic Hb (4.5 g/dl). (Table 7)

Hb values were slightly higher though statistically not significant in those who were not in crisis (P=0.235). (Figure 8)

Table 7: Hemoglobin level (g / dl) distribution among the patients.

<table>
<thead>
<tr>
<th>Hb (g/dl)</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>5-10</td>
<td>49</td>
<td>98</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 8: Comparison between the mean of Hb level in the study population.

(Patients, Controls) P value = (0.000) Significant
(Crisis, Steady state) P value = (0.235) Not significant
3.4.1.2. Platelets Counts:

62% of the patients had normal platelet counts, 34% showed thrombocytosis while only 4% had thrombocytopenia. (Figure 9)(Table 8)

**Figure 9:** Distribution of platelets groups in patients’ population.

![Distribution of platelets groups](image)

**Table 8:** Distribution of the platelets groups in the patients' population.

<table>
<thead>
<tr>
<th>Platelets groups</th>
<th>Vaso-occlusive crisis</th>
<th>Steady state</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percent</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2</td>
<td>8.3</td>
</tr>
<tr>
<td>Normal</td>
<td>14</td>
<td>58.4</td>
</tr>
<tr>
<td>Thrombocytosis</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>100</td>
</tr>
</tbody>
</table>
Platelets counts of patients were significantly higher than the control group, (P=0.006). (Figure 10)

No significant variation appeared in platelets counts between patients with vaso-occlusive crisis and those who were in steady state (P=0.729), (Figure 10).

**Figure 10:** Comparison between the mean of platelets counts in the study population.
3.4.1.3. White blood cells count (WBCs):

Taking the normal range of (WBCs) count as (3-9) \( \times 10^6 \)/L, (96%) of the patients had (WBCs) count above the normal range and only (4%) were in the normal range. (Figure 11)

**Figure 11:** Distribution of WBCs groups among patients' population.

Significant differences were shown WBCs values when patients and control groups were compared, (\( P=0.000 \)). WBCs counts of patients were higher than the control group. (Figure 12)

WBCs counts were slightly higher in those who were in crisis but the difference was not significant (\( P=0.41 \)). (Figure 12)
**Figure 12**: Comparison between the mean of WBCs counts in the study population.

(Patients, Controls) P value = (0.000) Significant

(Crisis, Steady state) P value = (0.41) Not significant
3.4.2. Coagulation Tests:

(Table 9) summarizes the results of PT, APTT, fibrinogen and D-dimer in patients and controls.

The mean of values of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) in both patients and controls were within normal range, with no significant differences between patients and controls P value (0.6) and (0.6) for both respectively.

However mean of value of Prothrombin Time (PT) was significantly Shorter in crisis patients while mean (APTT) was significantly longer (P=0.03), (P=0.005) respectively.

Mean fibrinogen concentrations were within normal in patients and controls but were significantly lower in patients. No significant difference was noted between crisis and steady state patients, P value (0.03) and (0.8) for both respectively.

D-dimer levels were very much higher in patient’s population than the control group with crisis patients having significantly higher values, P value (0.000) and (0.5) for both respectively.
Table 9: Comparison between means of D-dimer, fibrinogen, PT, and APTT among the study population.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients mean</th>
<th>Controls mean</th>
<th>P Value</th>
<th>Crisis patients' mean</th>
<th>Steady state patients' mean</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>14.3</td>
<td>14.5</td>
<td>0.6</td>
<td>13.9</td>
<td>14.6</td>
<td>0.03</td>
</tr>
<tr>
<td>APTT</td>
<td>32.7</td>
<td>31.5</td>
<td>0.6</td>
<td>36.8</td>
<td>29.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>344.7</td>
<td>341.8</td>
<td>0.03</td>
<td>348.5</td>
<td>341.1</td>
<td>0.8</td>
</tr>
<tr>
<td>D-dimer</td>
<td>778.3</td>
<td>75.7</td>
<td>0.000</td>
<td>938.3</td>
<td>630.6</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*P > 0.05 (Not Significant)*

*P ≤ 0.05 (Significant)*
3.4.2.1. Prothrombin Time (PT) distribution and comparisons:

PT values were within normal range (12-16 seconds) in patients and controls. (Figure 13)

**Figure 13:** Distribution of the mean of PT values in the study population.

(Patients, Controls) P value = (0.6) Not significant
(Crisis, Steady state) P value = (0.03) Significant
3.4.2.2. *Activated Partial Thromboplastin Time (APTT)* distribution and comparisons:

66% of the patients were within the normal range for APTT level (26-36 seconds). Whereas the rest had either prolonged or shortened times. (Figure 14)

(Table 10) show us the distribution of APTT in patients. 45.9% of the patients in crisis had prolonged results and 20.8% had short APTT times. Only 4% of the steady state patients had short APTT times and the rest were normal.

**Figure 14:** Distribution of APTT Groups in patient’s population.

**Table 10:** Distribution of APTT groups among patients.

<table>
<thead>
<tr>
<th>APTT groups</th>
<th>Vaso-occlusive crisis patients</th>
<th>Steady state patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>Percentage</td>
</tr>
<tr>
<td>Prolonged</td>
<td>11</td>
<td>45.9%</td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
<td>33.3%</td>
</tr>
<tr>
<td>Short</td>
<td>5</td>
<td>20.8%</td>
</tr>
</tbody>
</table>
Patients had slightly longer APTT than the controls, but the difference was not significant, (P=0.6). The mean of APTT values was significantly higher in patients in crisis than in those in steady state. (P=0.005)(Figure 15)

**Figure 15:** Distribution of the mean of APTT values in the study population.

(Patients, Controls) P value = (0.6) Not significant  
(Crisis, Steady state) P value = (0.005) Significant
4.2.3. *Fibrinogen level distribution and comparisons:*

88% of our patients showed a concentration of fibrinogen within the normal range (150-450mg/dl). (Table 11)

The values were significantly higher in controls than the patients (P=0.03). Values were almost equal in crisis and steady state patients (P=0.8). (Figure 16).

The lowest levels of fibrinogen were associated with the severe cases in our population of patients (who had four and five pain episodes per year). (Figure 17)

**Table 11:** Fibrinogen distribution among patients.

<table>
<thead>
<tr>
<th>Fibrinogen concentration</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 150mg/dl</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>150-450 mg/dl</td>
<td>44</td>
<td>88%</td>
</tr>
<tr>
<td>&gt; 450 mg/dl</td>
<td>5</td>
<td>10%</td>
</tr>
</tbody>
</table>
**Figure 16:** Distribution of the mean of Fibrinogen values in the study population.

(Patients, Controls) P value = (0.03) Significant
(Crisis, Steady state) P value = (0.8) Not significant

**Figure 17:** Distribution of the mean of fibrinogen concentration among pain episodes groups.
3.4.2.4. **D-dimer distribution and comparisons:**

The entire patient's population had D-dimer levels higher than the controls.

The difference was highly significant between patients and controls, (P=0.000), with the patients having markedly elevated levels of D-dimer. (Figure 18). Significant variations appeared when comparing D-dimer values of vaso-occlusive crisis patients and steady state patients, (P=0.05), values being higher in those patients who were in crisis. (Figure 18)

**Figure 18:** Distribution of the mean of D-dimer levels in the study population.

(Patients, Controls) P value = (0.000) Significant

(Crisis, Steady state) P value = (0.05) Significant
3.4.2.4.1. D-dimer and Platelets (Plts) count:

No significant correlation was found between D-dimer and platelets values, (P=0.5). (Figure 19)

**Figure 19:** Correlation between D-dimer level and platelets counts in patients group.

Pearson correlation
P. value: 0.5 (not significant)
3.4.2.4.2. D-dimer and white blood cells (WBCs):

Among the study patients high D-dimer levels were associated with leucocytosis patients, with no significance variation in D-dimer between different groups of WBCs count (P=0.07). (Figure 20)

**Figure 20:** Distribution of the mean of D-dimer among WBCs groups.

T test: P=0.07 (Not significant)
3.4.2.4.3. D-dimer and Activated Partial Thromboplastin Time (APTT):

Patients who had prolonged APTT showed the highest levels of D-dimer, followed by those with short APTT. Patients who had normal APTT had the lowest D-dimer values. Significant variation appeared when comparing D-dimer among the prolonged APTT group and that detected in the other groups (P=0.01) (Figure 21).

Correlation between D-dimer values and APTT levels in the whole group of patients give negative significance (P=0.5) (Figure 22).

**Figure 21:** Comparison in D-dimer level between different APTT groups among patients' population.

One way analysis of variance

P= 0.01

(Significant)
Figure 22: Correlation between D-dimer level and APTT in patients group.

Pearson correlation
P. value: 0.5 (not significant)
3.4.2.4.4. D-dimer and Fibrinogen:

Correlation between D-dimer values and Fibrinogen levels give negative significance (P=0.7) (Figure 23).

**Figure 23: Correlation between D-dimer and fibrinogen levels in patients group.**

Pearson correlation
P.value 0.7 (not significant)
3.4.2.4.5. D-dimer and severity score:

Significant increase in D-dimer was seen in patients with three and more painful crisis (P=0.04) (Figure 24).

**Figure 24:** D-dimer distribution among pain episodes groups in the patients' population.

One way ANOVA: (P=0.04) (Significant)
3.4.2.4.6. D-dimer distribution according to presenting symptoms:

Patients who came to the clinic with pain episodes had the highest levels of D-dimer mean, followed by the patients who were not having symptoms (came for follow up), and lastly the patients who came complaining from fever and other complications of sickle cell anemia. (Figure 25)

**Figure 25:** Distribution of D-dimer level among presenting symptoms.
3.4.2.4.7. D-dimer and vaso-occlusive crisis state:

The Odd Ratio statistical formula can be used to see if we can predict the occurrence of vaso-occlusive crisis by measuring the D-dimer concentration.

An odds ratio of (1.0) means the two variables are independent. The greater the odds ratio are (above 1.0), the more effect the independent variable (vaso-occlusive crisis) has on the dependent variable (high D-dimer level). The further (below 1.0) the odds ratio are, the more is the negative association between the two variables.

In our patients the relationship between D-dimer level and vaso-occlusive crisis gave (odds ratio = 0.95), which is classified as a negative result, considering that 96% of the crisis patients have high D-dimer levels, and all (100%) of the steady state patients have also high D-dimer levels. (Table 12)

**Table 12:** The distribution of the high D-dimer levels among the patients group (both crisis and steady state).

<table>
<thead>
<tr>
<th>Vaso-occlusive occurrence</th>
<th>crisis</th>
<th>D-dimer group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>normal values</td>
<td>increased values</td>
</tr>
<tr>
<td></td>
<td>number</td>
<td>%</td>
<td>number</td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>4%</td>
<td>23</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>0%</td>
<td>26</td>
</tr>
</tbody>
</table>

Odds Ratio = 0.95 (negative result)
CHAPTER FOUR

1. DISCUSSION

Sickle cell anemia is very common in Sudan. It is a cause of morbidity and mortality and this is mainly due to the severe complications that occur in this disease. The worst of these is vasoocclusion which leads to very severe painful crisis and organ dysfunction due to repeated ischemic injury. Prevention of such crises can lead to better quality of life in these patients. A lot of workers in this field have tried to find ways of doing this by trying to predict the occurrence of such crisis and thus giving prophylactic measures. In this study we have tried to look for evidence of predicting the occurrence of vaso-occlusive crisis or at least predicting the severity of the disease.

Most of our patients were from western Sudanese tribes where the prevalence of the sickle gene is very high (Figure 5). Add to that the incidence of consanguinous and intratribal marriages is quite high amongst these tribes. Thus 54% of our patients resulted from such marriages. Nineteen of our patients (38%) had severe disease according to internationally published criteria, and this might reflect the fact that severe disease is very common in Sudanese sicklers (Table 5).
The studied group consisted of 24 patients (48%) presenting with painful crisis and 26 patients (52%) in steady state either suffering from complications other than crisis or coming for follow up. (Figure 7)

All patients were anemic and almost all (96%) had high WBC counts, both findings being known features of their disease (Figure 8, 11).

Haemostatic studies in the patients gave variable results. As far as coagulation tests were concerned, the PT was normal in all of them with however significantly shorter times in the crisis patients when compared to steady state patients. APTT on the other hand gave variable results as it was normal in 33 patients (66%), shortened in 6 patients (12%) and prolonged in 11 (22%), (Figure 14). Short APTT and PT are indicators of a hypercoagulable state which leads to elevation in the coagulation factors, with factor VIII being the first affected and this will explain the shortening of APTT in some of our patients. Such findings have been reported before (101,106,107,108,109,115). The prolongation of APTT seen in some of our patients is highly suggestive of a state of DIC which is expected in SCA and this is supported by the findings of Nisri B et al (119). It is worth mentioning that one of the patients in steady state had a shortened APTT. It can be speculated that this patient was experiencing a silent process of hypercoagulibility.
Fibrinogen levels were normal in 44 patients (88%), high in 5 patients (10%) and low in one patient (2%), (Table 11). However mean value in patients was significantly lower than controls. This again may indicate DIC. There is no agreement in literature in this matter; some studies reported low levels while others reported high or normal levels. \(^{(100,120)}\)

Fibrinolytic activity was assessed through D-Dimer assay. D-dimers are specific cleavage products of fibrin. High levels are known to occur in DIC \(^{(122,123,124)}\), thromboembolic disease \(^{(121)}\), deep vein thrombosis \(^{(122,125)}\) and PIH \(^{(126)}\). All of our patients had significantly higher levels than normal. In addition to that crisis patients had significantly higher levels than those in the steady state. In fact most of them had levels above \((579 \text{ ug}\text{L})\). These results are highly suggestive of an ongoing state of thrombosis and fibrinolysis in SCA regardless of the clinical phase of the disease. This state seems to worsen during vaso-occlusive crisis and we therefore think that a rising level is an indication of an impending crisis. All available literature reports confirm elevation of D-Dimer levels in SCD. There is however some controversy as to whether this elevation worsens in crisis or not. Our findings are in agreement with Devin D et al, \(^{(130)}\), who found 97% of their crisis samples and 85% of samples obtained from SCD patients with complications other than crisis having elevated D-dimer levels. They concluded that the complications of
SCD, including vaso-occlusive crisis, result in production of D-dimer. Although they did not specify that patients who were in crisis had higher D-dimers than those in steady state, yet their figures indicate that more patients in crisis had high D-dimer levels. Also Shahid Ahmed et al.\(^{(131)}\), found elevated D-dimer in all patients hospitalized for vaso-occlusive crisis. They concluded that patients with SCD during vaso-occlusive crisis have an elevated D-dimer level and that low dose anti-coagulation treatment is associated with a significant reduction in the D-dimer levels. Another support to our results is the report of Nisri B et al.\(^{(119)}\), who found significant increase in D-dimer during sickle cell crisis and that of Wild JT et al.\(^{(122)}\) who also found elevated levels of D-dimer. They attributed this to increased plasmin degradation of cross linked fibrin thus indicating increased thrombin activity which is common in SCD patients.

On the other hand Hager et al.\(^{(118)}\) found highly significant increases in D-dimers in both steady and crisis patients.

Platelet counts were normal or high except for two crisis patients who showed thrombocytopenia. They were both in crisis and so their low platelets can be part of a DIC process. Similar findings were reported by Alkjearsic N\(^{(91)}\), Richardson G\(^{(106)}\), and Gordon PA\(^{(137)}\).
Our present data are thus in agreement with most literature findings that report evidence of hypercoagulability, DIC and excessive fibrinolysis in patients with SCA. D-Dimer results in our patients have suggested that it might be useful in determining the severity of disease but its value in predicting crisis has yet to be looked into by repeated measurements on patients while they are in the steady state.

The fact that sicklers are in a continuous state of activation of their coagulation system should encourage treating doctors to introduce anticoagulant therapy especially for those with severe disease.
2. CONCLUSIONS

1. There is a state of hyper-coagulability and excessive fibrinolysis in patients with SCA, and this is not related to the severity of the disease.

2. This state of hypercoagulability and excessive fibrinolysis worsens during crisis.

3. D-dimer levels may not predict crisis, but they are good indicaters of the severity of the disease.

4. APTT is a better test for the assessment of a DIC state than the PT, since factor VIII is the first factor to become affected in such a state.
3. RECOMMENDATIONS

1. A study on a larger number of patients and including a larger number of tests should be done.

2. It will be more useful if a base line for D-dimer in SCA is done with regular follow up, to try to predict changes in the disease.

3. In sicklers with severe disease and evidence of hypercoagulable state, anti-coagulation therapy should be considered, because it will reduce the incidence of vaso-occlusive crisis.
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QUESTIONAIR
(0-18 yrs)

1. General Information:

1.1. Serial Number: ……. 
1.2. Date

1.3. Group:

1.3.1. Study group
1.3.2. Control group

1.4. Personal Information:

1.4.1. Name:………………………………………………

1.4.2. Age:………………………………………………

1.4.3. Sex:………………………………………………

1.4.4. Present residence:………………………………

1.4.5. Original home:

- North
- South
- West
- East
- Central

1.4.6. Tribe:
- Father tribe:………………………………
- Mother tribe:……………………………

2. Symptoms:

2.1. Thrombotic tendency, eg:
- Blindness
- Deafness
• Paralysis

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3. Past history:

3.1. Age at the first presentation: 

3.2. Cause of the first presentation: 

3.3. Number of hospital admissions: 

3.4. Number of blood transfusions: 

3.5. Date of last blood transfusion: 

3.6. Complications:

• Vaso occlusive crisis

• Other

Specify: 

• Follow up

3.7. Number of pain episodes per year: 

4. Investigations:

4.1. CBC:

• Hb:
• Platelets

• TWBCs

4.2. Coagulation:

4.2.1. PT:

4.2.2. APTT:

4.2.3. Fibrinogen level:

4.2.4. D-dimer level:
Appendix II
Appendix III