

Chapter One

Introduction and Literature Review

Chapter One

1. Introduction and Literature Review

1.1. Introduction

Sickle cell anaemia (SCA), the most common inheritable disease in Africa, leading to public health problem in the region and elsewhere where descendants of Africans have settled (Roberts and Montalembert, 2007). Worldwide, it is recognized as a major cause of morbidity and mortality with tremendous social and economic impact mainly due to the recurrent acute episodic clinical events called “crises” and hospitalization (Lena *et al.*, 2012). In Africa, SCA is estimated to contribute to an equivalent of 5% of under-five deaths, and only half of the affected children live beyond their fifth birthday (Lena *et al.*, 2012).

The symptoms and complications of sickle cell disease (SCD) arise mainly from the crises (clinical and subclinical). Activation and damage of endothelial cells with activation of adhesion molecules lead to inflammation, release of C-reactive protein (CRP) and other inflammatory mediators and subsequent enhancement of ischemia. (Manwani and Frenette, 2013).

A study done by Raphael and Vichinsky, (2005) have shown that inflammatory markers such as C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF- α) are elevated in SCA patients. Also (Akinlade *et al.* 2013) reported elevated CRP level in SCA patients.

The above and other lines of evidence suggest that SCD is associated with a chronic inflammatory state, in which inflammation, oxidative stress and tissue oxidative damage occur, leading to various degrees of disease severity and end-organ dysfunction (Bandeira *et al.*, 2014).

1.2. Literature Review

1.2.1 .Sickle cell anaemia:

Sickle-cell anaemia (SCA), is a hereditary blood disorder, that results from a single point mutation in the haemoglobin B chain by substitution of a valine in state of glutamic acid at 6 position (Schnog *et al.*,2004).

1.2.1.1. History of sickle cell anemia:

In 1910, Herrick described an anemia characterized by bizarre, sickle-shaped cells. The role of deoxygenation was discovered in the 1920's by Hahn and Gillespie. The hereditary nature of the disease was suspected but not demonstrated until 1949 by Dr. James V. Neel. The association with hemoglobin was discovered by Linus Pauling and Harvey Itano in 1951 and the actual amino acid substitution by Vernon Ingram in 1956. Thus the 100th anniversary marks the discovery of this ancient disease from Africa by western medicine and naming of the disease for a simple agricultural implement to which a medical resident in 1910 likened the shape of the abnormal cells he saw under the microscope (Herrick, 2001).

1.2.1.2. Inheritance:

Sickle cell disease is inherited as an autosomal recessive condition whereas sickle cell trait is inherited as an autosomal dominant trait. This means that the gene can be passed on from a parent carrying it to male and female children. In order for sickle cell disease to occur, a sickle cell gene must be inherited from both the mother and the father, so that the child has two sickle cell genes (Samir, 2012).

The inheritance of just one sickle gene is called sickle cell trait or the "carrier" state. Sickle cell trait does not cause sickle cell anaemia. Patients with sickle cell trait

usually do not have many symptoms of disease and have normal hospitalization rates and life expectancies.

1.2.1.3. Distribution of sickle cell anaemia

Worldwide, it is recognized as a major cause of morbidity and mortality with tremendous social and economic impact mainly due to the recurrent acute episodic clinical events called “crises” and hospitalizations (Lena *et al.*, 2012).

1.2.1.3.1. In Africa:

SCA is estimated to contribute to an equivalent of 5% of under-five deaths, with up to 16% in some countries such as Nigeria and only half of the affected children live beyond their fifth birthday.

The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Saharan Africa, India and the Middle-East (Weatherall and Clegg, 2001). Migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades and in some European countries sickle-cell disease has now overtaken more familiar genetic conditions such as hemophilia and cystic fibrosis (Roberts and Montalembert, 2007).. In 2010, there were about 29,000 deaths attributed to sickle-cell disease globally (Weatherall and Clegg, 2001).

Sickle-cell disease occurs more commonly among people whose ancestors lived in tropical and sub-tropical sub-Saharan regions where malaria is or was common. Where malaria is common, carrying a single sickle-cell allele (trait) confers a selective advantage in other words, being a heterozygote is advantageous. Specifically, humans with one of the two alleles of sickle-cell disease show less severe symptoms when infected with malaria. (Wellems *et al.*, 2009)

1.2.1.3.2. Western Sudan:

The presence of HbS is already well documented among Kordofan and Darfur region inhabitants, especially Albaggara, an Afro-Arab constellation of tribes with a predominantly African descent (Adams, 2009). Some findings of a study conducted in Elobied hospital in North Kordofan State, showed that sickle cell trait in relatives of patients suffering from sickle cell disease (SCD) who were referred to this Hospital, was 54% of target samples, which concentrated mainly in two tribes, Bederia and Fulani. Sickle cell disease in Messeryia of Darfur and Messeryia Hummer of Kordofan showed a prevalence of 30.4% and 18% respectively. It is estimated that one in every 123 children born in Messeryia tribe is at risk of having SCD (Balgir, 2012). Many indigenous tribes that inhabit Darfur region and belong to the Negroid ethnic group and are a part of Nilo-Saharan language family such as the Berge, Fur and Masaleet had the highest frequencies of the S gene among them (Paul *et al.*, 2011).

1.2.2. The symptoms of sickle cell anaemia:

The following is symptoms and complications associated with sickle cell anaemia. Each child may experience symptoms differently. Symptoms and complications may include, but are not limited to, the following: The most common symptom of anemia is fatigue (feeling tired or weak). Other signs and symptoms of anemia may include: Shortness of breath, Dizziness, Headaches, Coldness in the hands and feet, Paler than normal skin or mucous membranes (the tissue that lines the nose, mouth, and other organs and body cavities) and Jaundice (Platt *et al.*, 1994).

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1.2.2.1. Acute anemia

Defined as a decline in hemoglobin concentration by 2.0 g/dL or more below the patient's baseline value, may have diverse etiologies. Therefore, splenic sequestration in a child or an aplastic episode at any age may require urgent evaluation and therapy. The reticulocyte count is an important index to assess diminished red blood cell production (low reticulocyte count, as can occur in parvovirus infected individuals, results in aplastic crisis, accelerated hemolysis, or sequestration in the lungs, spleen, or liver. An aplastic crisis is a common feature of SCD, especially in childhood. (Smith *et al.*, 2004).

1.2.2.2. Pain in sickle cell disease

Recurrent episodes of acute, severe pain are the hallmark of SCD. The pain is highly variable both within and among patients, and is the result of complex and poorly understood interactions between biological and psychosocial factors. Vaso-occlusion within the bone marrow vasculature leads to bone infarction, which in turn results in the release of inflammatory mediators that activate afferent nerve fibers and cause pain. Although the basic mechanism is simple, the precise details of the vaso-occlusion are poorly understood, involving complex interactions between red cells, endothelium, white cells and platelets. The unpredictability of the pain is a major factor in undermining the patient's ability to cope (Ballas, 2005). Acute pain frequently occurs spontaneously, but may be precipitated by infections, skin cooling, dehydration or stress. Acute pain in SCD is described as throbbing, sharp or gnawing, and patients can usually recognize whether it is typical of their SCD. If the patient thinks the pain is atypical, then other causes of pain should be sought. Acute painful episodes may occur on top of chronic pain, or be precipitated by other painful events, such as cholecystitis. Hospital admissions for acute pain in SCD typically last 4–10 days, but this varies widely. If persist more than 3 months it's considered

as chronic, chronic pain may be an extension of recurrent acute painful episodes or in a specific tissue or organ, such as avascular necrosis of the hips, or leg ulcers. Chronic pain is often associated with other conditions that enhance its chronicity. These include psychosocial factors such as depression, anxiety, feelings of despair, insomnia, loneliness, helplessness and dependence on pain medications, chronic pain can be hard and may lead in mental draining. (Serjeant, 2001).

1.2.3. Complications of Sickle Cell Disease

1.2.3.1. Sickle cell crisis

The term "sickle cell crisis" is used to describe several independent acute conditions occurring in patients with sickle cell disease, the most common complication of SCD is an acute episode of severe pain referred to as an acute vaso-occlusive crisis (VOC). AVOC is defined as pain resulting from tissue ischemia caused by vaso-occlusion most commonly in the bone(s) and bone marrow (Platt *et al.*, 1994).

1.2.3.2. Vaso-occlusive crisis (VOC)

A VOC is the hallmark acute complication for SCD and manifests as acute severe pain. The sickled erythrocytes block the flow of blood through the small blood vessels (capillaries) resulting in ischemia.

Sudden episodes of pain throughout the body are a common symptom of SCD. This sudden pain can range from mild to very severe form and usually lasts from hours to a few days. VOCs and their accompanying pain most commonly occur in the extremities, chest, and back. When they occur in other sites, they can be confused with, or can be the prodromal stage of other acute complications (e.g., head (stroke), flank (papillary necrosis), and abdomen (hepatic or splenic sequestration, constipation from opioid toxicity, or another Hepatobiliary complication). Patients

with more than three hospitalizations for a VOC in a year are considered to be at an increased risk of early death (Ballas *et al.*, 2005)

1.2.3.3. Hand-Foot Syndrome

When sickle cells block the small blood vessels at hands or feet, pain and swelling along with fever may occur. The first VOC may appear as early as at 6 months of age, often presenting as dactylitis, but there after VOCs occur with variable frequency. This may be the first sign of sickle cell anemia in infants (Pham, 2005)

1.2.3.4. Fever- Infections

Patients with SCD have an increased risk for severe bacterial infection, resulting primarily from reduced or absent splenic function (Booth *et al.*, 2010). The result is an extremely high risk of septicemia and meningitis, primarily due to *Streptococcus pneumoniae*. The risk of such infections continues throughout childhood and to a lesser extent in adults. Fever, as a presenting symptom, heralds many acute and sometimes life-threatening conditions, such as ACS and osteomyelitis. It is critical that fever alone is taken seriously in these patients and considered a potential emergency situation. Fever associated with pain should not be considered a VOC until infection is ruled out. Acute osteomyelitis, another complication associated with fever, may be unifocal or multifocal and may be caused by *Staphylococcus aureus*, salmonella, or other enteric pathogens (Booth *et al.*, 2010).

Pneumonia is the most common cause of death in young children with sickle cell disease. Meningitis, influenza, and hepatitis are other infections that are common in people with sickle cell disease (Olujohungbe *et al.*, 2011).

1.2.3.5. Acute Renal Failure

Acute renal failure is defined as a rapid reduction in renal function manifested by a rise in serum creatinine and reduction in glomerular filtration rate (GFR), with or without a decline in urine output. Acute renal failure may be due to pre-renal (e.g., dehydration) or post-renal (e.g., obstruction) insults, or result from intrinsic renal disease (e.g., glomerular injury). It may occur during an acute VOC, most often in association with ACS or acute multisystem organ failure. Renal papillary necrosis due to medullary infarction from obstruction of the blood supply in the vasa recta affects up to 15–30 percent of individuals with SCD (Pham *et al.*, 2005). Signs and symptoms include pain and hematuria. When present, fever suggests possible infection. The serum creatinine levels are generally low or low-normal in patients

With SCD and the values in acute renal function may still be within normal limits even if serum creatinine level increase two times from baseline (Pham *et al.*, 2005). Identification of early renal disease in people with SCD is important as these patients hyper secrete creatinine through the proximal tubules, thus making significant renal impairment before the serum creatinine rises (Ataga and Orringer , 2000). Micro albuminuria is most often the first manifestation of chronic kidney disease in SCD. Proteinuria due to glomerular injury is also common, but both micro albuminuria and macro albuminuria are typically asymptomatic. The most common renal complication in people with SCD is hyposthenuria, or the Inability to concentrate the urine, which is progressive with age (Francis and Worthen, 1968).

1.2.3.6. Hepatobiliary complications

Biliary tract abnormalities are common in SCD patients. These abnormalities include cholelithiasis, acute cholecystitis, and biliary sludge. Hemolysis of any etiology results in increased secreted unconjugated bilirubin that tends to precipitate

and leads to gallstones and sludge. Acute hepatic sequestration of red blood cells in the liver often develops over a few hours to a few days, and the resultant stretching of the hepatic capsule is usually painful. Acute intrahepatic cholestasis (also called sickle cell hepatopathy or “drepanocyte” liver) is also associated with SCD. It is characterized by the sudden onset of pain, increasing jaundice, a progressively enlarging and extremely tender liver, light-colored stools, and extreme hyperbilirubinemia (both conjugated and Unconjugated). This complication may prove fatal if not recognized and treated promptly (Shao and Orringer, 1995).

1.2.3.7. Splenic sequestration

Splenic sequestration is defined as sudden enlargement of the spleen and reduction in hemoglobin concentration by at least 2 g/dL below the baseline value. Splenic sequestration (pooling) - crises are a result of sickle cells pooling in the spleen. This can cause a sudden drop in hemoglobin and can be life threatening if not treated promptly. The spleen can also become enlarged and painful from the increase in blood volume. After repeated episodes of splenic sequestration, the spleen becomes scarred, and permanently damaged. Most children, by the age of 8 years old, do not have a functioning spleen from repeated episodes of splenic sequestration. The risk of infection is a major concern of children without a functioning spleen. Infection is the major cause of death in children under the age of 5 years in this population. It is a major cause of acute anemia and it may present acutely accompanied by severe anemia and hypovolemic shock. The reticulocyte count and circulating nucleated red blood cells are usually elevated (Platt *et al.*, 1994).

1.2.3.8. Acute chest syndrome (ACS)

Acute chest syndrome is a life-threatening condition for SCD patients. It is the second most frequent reason for hospitalization in children and adults with SCD and

the most common cause of death. It's similar to pneumonia and is caused by an infection or by sickle cells trapped in the lungs. Patients with this condition usually have chest pain, fever, and an abnormal chest x ray. Over time, lung damage may lead to pulmonary hypertension (Smith *et al.*, 2000).

1.2.3.9. Acute stroke

Stroke is one of the most common and devastating complications of SCD (Ohene-Frempong *et al.*, 1998) Sickle-shaped red blood cells may stick to the walls of the tiny blood vessels in the brain. This type of stroke occurs mainly in children. This complication presents as sudden onset of weakness, aphasia, and sometimes seizures or coma and results in adverse motor and cognitive sequelae. In the absence of secondary prevention measures such as a chronic transfusion program or hematopoietic stem cell transplantation, recurrence rates have been shown to range between 46 and 90 percent in children with SCD. Brain hemorrhage occurs more often in adult's patients (Verduzco and Nathan, 2005).

1.2.3.10. Priapism

Males with sickle cell disease may have painful and unwanted erections lasting about 4 hours, called priapism. This happens because the sickle cells stop blood flow out of an erect penis. Priapism is a common complication of SCD, affecting 35 percent of male patients, over time, priapism can damage the penis and lead to impotence (Olujohungbe *et al.*, 2011).

1.2.3.11. Multisystem organ failure

Multisystem organ failure is a severe, rare and life-threatening complication usually associated with a VOC and characterized by failure of the lungs, liver, and/or kidneys. Symptoms linked to this complication are fever and changes in mental

status such as sudden tiredness and loss of interest in their surroundings. The incidence of chronic complications appears to increase with age and understanding of the pathophysiology and the involved factors is necessary to prevent or reduce long-term morbidity (Hassell *et al.*, 1994).

1.2.3.12. Avascular necrosis

Avascular or aseptic necrosis can occur when capillaries are occluded by sickled erythrocytes at distal portions of a bone, near a joint, where hypoxia is maximal and collateral circulation is inadequate, the femoral neck is the most common site of aseptic necrosis. It causes chronic severe pain and disability (Diggs, 1967).

1.2.3.13. Leg ulcers

Leg ulcers are a common complication of SCD. Sickle cell ulcers usually begin as small sores on the lower third of the leg. Leg ulcers occur more often in males than in females and usually appear between the ages of 10 and 50. The cause of leg ulcers is not clear. Some heal rapidly, but others persist for years or recur. (Mason, 1922).

1.2.3.14. Pulmonary Hypertension

Pulmonary hypertension (PH) is defined as an elevation of the resting mean pulmonary arterial pressure (>25 mmHg) as determined by right heart catheterization (RHC). PH can occur in chronic hemolytic anemia and in the setting of chronic lung disease, chronic thromboembolic disease, or can be due to unclear and multiple mechanisms. Initial testing for PH has been done with an echocardiography assessment to estimate pulmonary artery pressure using tricuspid regurgitant jet velocity (TRV), but diagnosis requires right heart catheterization and direct measurement of the pulmonary arterial pressure and vaso-reactivity of the vessels. Excessive shortness of breath is an important symptom of PH (Badesch *et al.*, 2009).

1.2.3.15. Ophthalmologic complications

Chronic ophthalmological complications of SCD include proliferative sickle retinopathy and vitreous hemorrhage. They occur in up to 50 percent of patients with SCD and are associated with significant visual loss (Moriarty *et al.*, 1988).

The symptoms and complications of sickle cell disease (SCA) arise mainly from the crisis, activation and damage of endothelial cells with activation of adhesion molecules lead to inflammation, release of C-reactive protein (CRP) and other inflammatory mediators and subsequent enhancement of ischemia (Manwani and Frenette, 2013).

The above and other lines of evidence suggest that SCD is associated with a chronic inflammatory state, in which inflammation, oxidative stress and tissue oxidative damage occur, leading to various degrees of disease severity and end-organ dysfunction. Exploring the role of CRP in this chronic inflammatory state is very important as we search for therapeutic targets in this disease.

1.2.4. The C-reactive protein

The C-reactive protein (CRP) is an acute phase reactant, a protein made by the liver and released into the blood within a few hours after tissue injury, the start of an infection, or other cause of inflammation. Discovered in Oswald Avery's laboratory during the course of studies of patients with *Streptococcus pneumonia* infection (Tillet, and Francis, 1930) Sera obtained from these patients during the early, acute phase of the illness were found to contain a protein that could precipitate the “C” polysaccharide derived from the pneumococcal cell wall. Forty years later, Volakis and Kaplan identified the specific ligand for CRP in the pneumococcal C polysaccharide as phosphocholine, part of the teichoic acid of the pneumococcal cell wall, although phosphocholine was the first defined ligand for CRP, a number of

other ligands have since been identified. In addition to interacting with various ligands, CRP can activate the classical complement pathway, stimulate phagocytosis, and bind to immunoglobulin receptors (FcγR), (Volanakis and Kaplan, 1971). In humans, plasma levels of CRP may rise rapidly and markedly, as much as 1000-fold or more, after an acute inflammatory stimulus, largely reflecting increased synthesis by hepatocytes. CRP induction is part of a larger picture of reorchestration of liver gene expression during inflammatory states, the acute phase response, in which synthesis of many plasma proteins is increased, whereas that of a smaller number, notably albumin, is decreased. At least 40 plasma proteins are defined as acute phase proteins, based on changes in circulating concentration of at least 25% after an inflammatory stimulus. This group includes clotting proteins, complement factors, anti-proteases, and transport proteins. These changes presumably contribute to defensive or adaptive capabilities (Volanakis and Kaplan, 1971).

1.2.4.1. The structure of CRP

CRP consists of five identical, noncovalently associated ~23-kDa protomers arranged symmetrically around a central pore. The term “pentraxins” has been used to describe the family of related proteins with this structure. Each protomer has been found by x-ray crystallography to be folded into two antiparallel β sheets with a flattened jellyroll topology similar to that of lectins such as concanavalin A (Shrive *et al.*, 1996). Each protomer has a recognition face with a phosphocholine binding site consisting of two coordinated calcium ions adjacent to a hydrophobic pocket. The co-crystal structure of CRP with phosphocholine suggests that Phe-66 and Glu-81 are the two key residues mediating the binding of phosphocholine to CRP, Phe-66 provides hydrophobic interactions with the methyl groups of phosphocholine whereas Glu-81 is found on the opposite end of the pocket where it interacts with

the positively charged choline nitrogen. The importance of both residues has been confirmed by mutagenesis studies (Agrawal *et al.*, 2002).

The opposite face of the pentamer is the effector face, where complement C1q binds and Fcγ receptors are presumed to bind. A cleft extends from the center of the protomer to the central pore of the pentamer, and several residues along the boundaries of this cleft have been shown to be critical for the binding of CRP to C1q, including Asp-112 and Tyr-175. The crystal structure of the globular head domain of C1q was recently solved, and a model for C1q binding to CRP was proposed in which the top of the predominantly positively charged C1q head interacts with the predominantly negatively charged central pore of the CRP pentamer. The CRP test is not diagnostic of any condition, but it can be used together with signs and symptoms and other tests to evaluate an individual for an acute or chronic inflammatory condition. For example, CRP may be used to detect or monitor significant inflammation in an individual who is suspected of having an acute condition, such as: A serious bacterial infection like sepsis, fungal infection, pelvic inflammatory disease (Black *et al.*, 2003).

The CRP test is useful in monitoring people with chronic inflammatory conditions to detect flare-ups and/or to determine if treatment is effective. Some examples include: Inflammatory bowel disease, some forms of arthritis, autoimmune diseases, such as lupus or vacuities. CRP may sometimes be ordered along with an erythrocyte sedimentation rate (ESR), another test that detects inflammation. While the CRP test is not specific enough to diagnose a particular disease, it does serve as a general marker for infection and inflammation, thus alerting health practitioners that further testing and treatment may be necessary. Depending on the suspected cause, a number of other tests may be performed to identify the source of inflammation (Andreeva and Melbye, 2014). The CRP test may be ordered when an individual is suspected

of having a serious bacterial infection based on the person's medical history and signs and symptoms. It may be ordered, for example, when a newborn shows signs of infection or when an individual has symptoms of sepsis, such as fever, chills, and rapid breathing and heart rate. It may also be ordered on a regular basis to monitor conditions such as rheumatoid arthritis and lupus and is often repeated at intervals to determine whether treatment is effective. This is particularly useful for inflammation problems since CRP levels drop as inflammation subsides (Devkota, 2014).

1.2.4.2 Values of CRP

The level of CRP in the blood is normally low. A high or increasing amount of CRP in the blood suggests the presence of inflammation but will not identify its location or the cause. In individuals suspected of having a serious bacterial infection, a high CRP can be confirmatory. In people with chronic inflammatory conditions, high levels of CRP suggest a flare-up or that treatment has not been effective. If the CRP level is initially elevated and drops, it means that the inflammation or infection is subsiding and/or responding to treatment (Genzen, 2014).

CRP levels can be elevated in the later stages of pregnancy as well as with use of birth control pills or hormone replacement therapy (i.e., estrogen). Higher levels of CRP have also been observed in people who are obese. The erythrocyte sedimentation rate (ESR) test will also be increased in the presence of inflammation; however, CRP increases sooner and then decreases more rapidly than the ESR.

1.2.5. Laboratory Diagnosis:

In HbSS, the complete blood count reveals hemoglobin levels in the range of 6–8 g/dl with a high reticulocyte count (as the bone marrow compensates for the destruction of sickled cells by producing more red blood cells). In other forms of

sickle-cell disease, Hb levels tend to be higher. A blood film may show features of hyposplenism (target cells and Howell-Jolly bodies).

Sickling of the red blood cells, on a blood film, can be induced by the addition of sodium metabisulfite. The presence of sickle hemoglobin can also be demonstrated with the "sickle solubility test". A mixture of hemoglobin S (Hb S) in a reducing solution (such as sodium dithionite) gives a turbid appearance, whereas normal Hb gives a clear solution (Lee *et al.*, 2000).

Sickle cell disease can be diagnosed in newborns, as well as older persons, by hemoglobin electrophoresis, isoelectric focusing, high-performance liquid chromatography or DNA analysis. In general, these tests have comparable accuracy.

Solubility testing methods and sickle cell preparations are inappropriate diagnostic techniques. Although these tests identify sickle hemoglobin, they miss hemoglobin C and other genetic variants. Furthermore, solubility testing is inaccurate in the newborn, in whom fetal hemoglobin is overwhelmingly predominant. Solubility testing methods also fail to detect sickle hemoglobin in persons with severe anemia.

DNA analysis provides the most accurate diagnosis in patients of any age, but it is still relatively expensive (Clarke and Higgins, 2000).

Abnormal hemoglobin forms can be detected on hemoglobin electrophoresis, a form of gel electrophoresis on which the various types of hemoglobin move at varying speeds. Sickle-cell hemoglobin (HgbS) and hemoglobin C with sickling (HgbSC)—the two most common forms—can be identified from there. The diagnosis can be confirmed with high-performance liquid chromatography. Genetic testing is rarely performed, as other investigations are highly specific for HbS and HbC (Clarke and Higgins, 2000).

An acute sickle-cell crisis is often precipitated by infection. Therefore, a urinalysis to detect an occult urinary tract infection, and chest X-ray to look for occult pneumonia, should be routinely performed (Kwiatkowski, 2005).

People who are known carriers of the disease often undergo genetic counseling before they have a child. A test to see if an unborn child has the disease takes either a blood sample from the fetus or a sample of amniotic fluid. Since taking a blood sample from a fetus has greater risks, the latter test is usually used. Neonatal screening provides not only a method of early detection for individuals with sickle-cell disease, but also allows for identification of the groups of people that carry the sickle cell trait (Lee *et al.*, 2000).

1.2.4. Previous studies

Suba Krishnan *et al.*, (2010). showed that Several lines of evidence suggest that sickle cell disease (SCD) is associated with a chronic inflammatory state, high sensitivity C-reactive protein (hs-CRP), a marker of low-grade, systemic inflammation, emerged as the most significant laboratory correlate of hospitalizations for pain or vaso-occlusive (VOC) events. While markers of increased hemolytic status, endothelial activation and coagulation activation all correlated positively with VOC events by univariate analysis, baseline hs-CRP levels provided the most significant contribution to the association in multiple regression models (22%), and, hs-CRP, along with age, provided the best fit in negative binomial models.

Fatima *et al.*, (2010). Done study on sickle cell anemia (SCA) with chronic inflammation, and given the role of high sensitivity C-reactive protein (hs-CRP) as inflammatory mediator, they hypothesized that SCA vasoocclusive crisis (VOC) is associated with heightened hs-CRP levels.

Elena M James *et al.*, (2014). On their study analyzed the utility of creatinine (Cr), C-reactive protein (CRP), and lactate dehydrogenase (LDH) as indicators of acute VOC and increased disease severity .they hypothesized that increased levels of these markers correlated with acute VOC and increased SCD severity because they are suggestive of renal injury, accelerated hemolysis, and inflammation with VOC onset.

CE Okocha1, *et al.*, (2014). They stated that CRP is significantly increased in crisis compared with the stable state in HbSS individuals, and in HbSS compared with HbAS and HbAA individuals. This due to, CRP production is part of a nonspecific acute phase response to inflammation and tissue necrosis. Evidence suggesting that sickle cell disease (SCD) is associated with a chronic inflammatory state. C-reactive protein (CRP) is known to modulate inflammation. Its role in the chronic inflammation of SCD may make it valuable as a therapeutic target

1.3. Rational

Sickle cell anemia (SCA) is widely distributed in Sudan with high rate of morbidity and mortality and associated with a chronic inflammatory state. C-reactive protein (CRP) is known to modulate inflammation. This concept can be strengthened by the study of (CE , and Manafa, 2014) who showed there is evidence suggests that sickle cell disease (SCD) is associated with a chronic inflammatory state, also Fatima *et al.*,(2010), on their study hypothesized that SCA vasoocclusive crisis (VOC) is associated with heightened CRP levels.

This is one of rare studies done in Sudan and regional countries, so that there is need to assessing the baseline values of inflammatory markers and susceptibility of patients with vasoocclusive crisis to chronic inflammatory state.

1.4. Objectives

1.4.1 General objectives:

The aim of this study is to determine the role of C- reactive protein level in vaso-occlusive crisis in patients with sickle cell anemia.

1.4.2 Specific objectives:

- 1- To measure C - reactive protein level in patients with sickle cell anemia.
- 2- To measure CBC in patient with sickle cell anemia.
- 3- To determine the difference of C- reactive protein level in steady state and in vaso-occlusive crisis in patients with sickle cell anemia.

Chapter two

Material and Method

Chapter Two

2. Material and Methods

2.1. Study design

This is a cross sectional study, conducted at Alkuwaity Pediatrics Hospital, Northern Kordofan State, Western Sudan, from February to June 2015.

2.2. Study area

Alkuwaity Pediatrics Hospital, in El Obied city, Northern Kordofan State Western Sudan.

2.3. Study population

Patients with sickle cell anaemia attended to Alkuwaity Pediatrics Hospital in the period of study, 74 with vaso-occlusive crises and 26 in steady state

2.4. Inclusion criteria

All patients with sickle cell anaemia of both sexes attending to the hospitals were included in this study.

2.5. Exclusion criteria

Patients use Hydroxyurea or any treatment which affect the result, and in cooperation patients were excluded from the study.

2.6. Data collection

Questionnaires were used to collect the information about demographic data, family history, and symptoms.

2.7. Method of Sample collection

A sample of 2.5 ml of blood was collected from each participant, dispensed into ethylene diamine tetra acetic acid (EDTA) for measurement of complete blood count (CBC) and then the plasma was separated in to plain tubes for determination of CRP.

2.8. Methodology

2.8.1. CBC

Evaluation of the blood cell count were performed by Sysmex automated hematological analyzer, which could perform 18 hematological parameters with high accuracy and precision. Principally Sysmex analyzer is based on the electronic resistance (impedance) detection method for counting and sizing recognition of the leukocytes, erythrocytes, and platelet. Through using three preliminary hydraulic systems for WBCs, RBCs, platelet and hemoglobin, and display the mode of the cells blood count results on the liquid crystal displayer (LCD) with histogram and printed out the results in thermal paper (Dacie and lewis, 2006).

2.8.1.1. Principle of Sysmex model 21 hematology analyzer

Measurement of blood cells (RBCs, WBCs, and platelet). And hemoglobin concentration obtained by aspiration of small volume of well mixed (k2EDTA) blood by sample probe and mixed with isotonic diluents in nebulizer. Diluted aspiration delivered to RBCs aperture bath for providing information about RBCs and platelet. Other portion of aspirated sample induced in to WBCs bath in which hemolytic reagent (stromatolyzer) added to break down (RBCs) and release of hemoglobin which measured in build colorimeter, based in cyanomethemoglobin method (HICN). The through three sensing apertures for each cell type, cell counted and size information generated in triplicate pulses acting to electronic conductivity. Mentioned pulses converts in to digital number using in build calculator

programmed and designed for RBCs, WBCs counts. Some portion of diluted sample delivered to in build hemoglobin meter at the same time, hence three values directly measured (RBCs, WBCs, Hb) and displayed on (LCD). Other values of red cell indices, leukocyte differential and absolute count calculated from given information, the result printed out according to the setting mode.

On the other hand, platelet count and histogram determined from pulses acting to size of the platelet (Dacie and lewis, 2006).

2.8.2. CRP Estimation

The level of CRP was assayed using commercially available CRP kits by use icromaTM CRP.

2.8.2.1 Principle of CRP:

The test uses a sandwich immunodetection method, such that the detector antibody in buffer binds to CRP in sample and antigen – antibody complex are captured to another CRP antibody that has been immobilized on test strip as sample mixture migrates nitrocellulose matrix. Thus the more CRP antigen in sample, the more antigen -antibody complex accumulated on the test strip. Signal intensity of fluorescence on detector antibody reflects the amount of antigen captured and is processed by ichromaTM reader to show CRP concentration in specimen.

2.8.2.2. Components and reagents:

ichroma^{T M} CRP consist of a test cartridge, an ID chip, a blood collecting capillary, and a detection buffer tube.

- the test cartridge contain a test strip on the membrane of which, murine antibodies against CRP and rabbit IgG have been immobilized at the test line respectively.

- Each test cartridge is individually sealed in an aluminum foil pouch containing adesiccant. 25 sealed test cartridges are packed in a box which also contains an ID chip.
- The detection buffer pre-dispensed in a tube contains fluorochrome –labeled anti-CRP antibodies, fluorescent-labeled anti-rabbit IgG, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is dispensed in each detection buffer tube. 25 detection buffer tubes are packed in a separate box which is further packed in a Styrofoam box provided with ice packs for the purpose of shipment.
- Blood collection capillary is used for picking up 10 micro liter of whole blood, serum, plasma, or control solution.

2.8.2.3. Material supplied

- Test cartridge box:

-sealed test cartridge	25
-ID chip	1
-Package insert	1
-Blood collecting capillary	25
- Box containing detection buffer tube

-detection buffer tubes	25
-------------------------	----

2.8.2.4. Test procedure

1. Prepare the plasma, make puncture on the top of the detector tube by inserting an empty blood collection capillary.

2. Draw the sample with the blood collection capillary, assemble the capillary and tube in to one, and shake the assembled tube 10 times by inversion to take the blood out of capillary.
3. Remove the cap off the top of tube, discard two drops of reagent, then apply two drop onto the sample well of a cartridge.
4. Insert into the test cartridge holder of the ichromaTM reader.
5. Press select button on the ichromaTM reader to start the scanning process (read within 3 minutes) read the test result on display screen of the ichromaTM reader.

IchromaTM reader calculate the result automatically and display CRP concentration in terms of mg/l.

Working range of ichromaTM CRP is 2.5-300 mg/l.

2.8.2. 5. Reference range

CRP concentration >5 mg/l may reflect an acute-phase response to infectious diseases or disorder characterized by acute inflammation.

2.9. Ethical consideration:

Ethical clearance Obtained from the ethical committee from Alkuwaity Pediatrics Hospital.

Verbal consents were obtained from all patients involve in the research.

2.10. Data analysis

Data obtained was analyzed using Statistical Package for Social Sciences (SPSS) software package version 13. (By use T tests)

Chapter three

Results

Chapter Three

3. Results

3.1. Demographic Data

The age range between 8 months and 15-year, with a high frequency 65/100 (65%) seen in the age group of patients ranged between 1- 5years (Figure 3.1).

According to the sex, the results showed increased frequency of male patients 58/100 (58%) compared with female patients 42/100 (42%). Most of the patients had known family history 80/100(80%).

The majority of the patients belong to Afro-Asiatic tribes 52/100 (52%), followed by Niger-Congo 48/100 (48%) [Figure 3.3].

3.2. Clinical Data of the Study patients

The patients were presented with different clinical feature yet dominated by paler which found in 76/100 (76%) patients [Table 3.1].

3.2.1 Sickle Cell Crises

Of the 100 patients with sickle cell anemia, 74/100 (74%) showed vaso occlusive crises while the remaining 26/100(26%) were in steady state [Figure3.4].

3.3 Hematological Data

Statistical analysis of the patients sample in vaso occlusive crisis and steady state showed that Hb concentration ($6.55\text{g/dl} \pm 1.3$) and ($9.8\text{g/dl} \pm 2.4$) respectively with $P < 0.000$.

PCV ($20.4\% \pm 3.9$) and ($30.7\% \pm 7.1$) respectively with $P < 0.000$, RBCs ($2.5 \times 10^6/\mu\text{l} \pm 0.57$) and ($3.9 \times 10^6/\mu\text{l} \pm 1.1$) respectively with $P < .000$

Red cell indices in vaso occlusive crisis and steady state showed MCV ($78.7\text{fl} \pm 9.6$) and ($75.5\text{fl} \pm 7.7$) respectively with $P < 0.135$, MCH ($26.1\text{pg} \pm 3.8$) and ($25.5\text{pg} \pm$

4.6) respectively with $P < 0.48$, MCHC ($32.2\text{g/dl} \pm 3.3$) and ($32.6\text{g/dl} \pm 3.3$) respectively with P.value 0.66.

While the WBC showed ($19.36 \times 10^9/\text{l} \pm 10.645$) and ($76.76 \times 10^9/\text{l} \pm 20.19$) respectively with $P < 0.000$, Platelets showed ($357.108 \times 10^9/\text{l} \pm 162.838$) and ($290.461 \times 10^9/\text{l} \pm 124.40$) with $P < 0.045$ (Table 3.2)

Statistical analysis of C. reactive protein showed significant differences between vaso occlusive crises and steady state patients CRP ($16.43\text{mg/l} \pm 12.35$) and ($4.56\text{mg/l} \pm 1.45$) respectively, with $P < .000$.

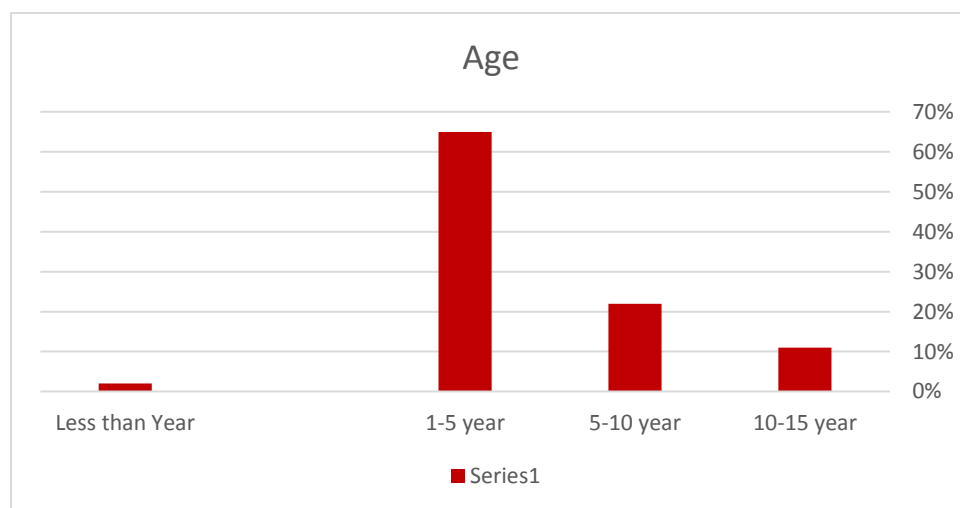


Figure 3.1 Distribution of the study population according to the age

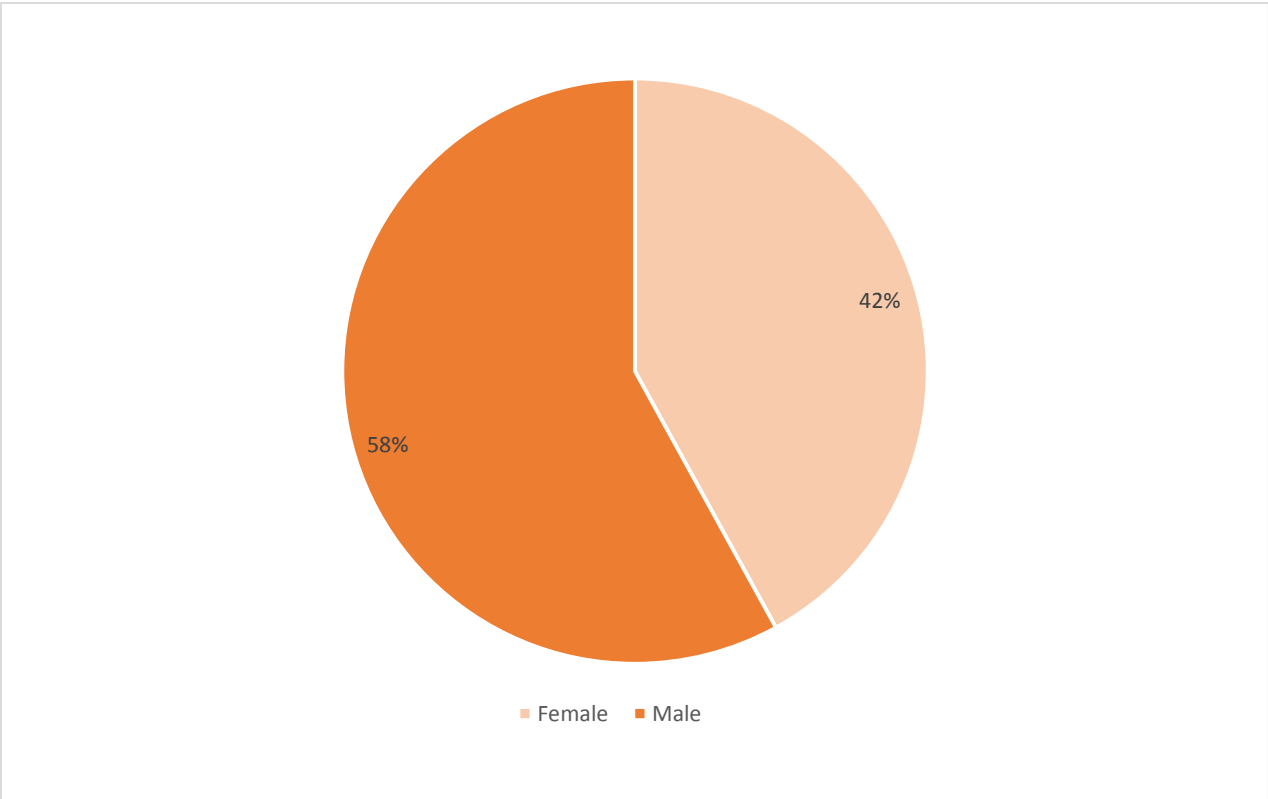


Table: 3.2 Distribution of the study population according to sex

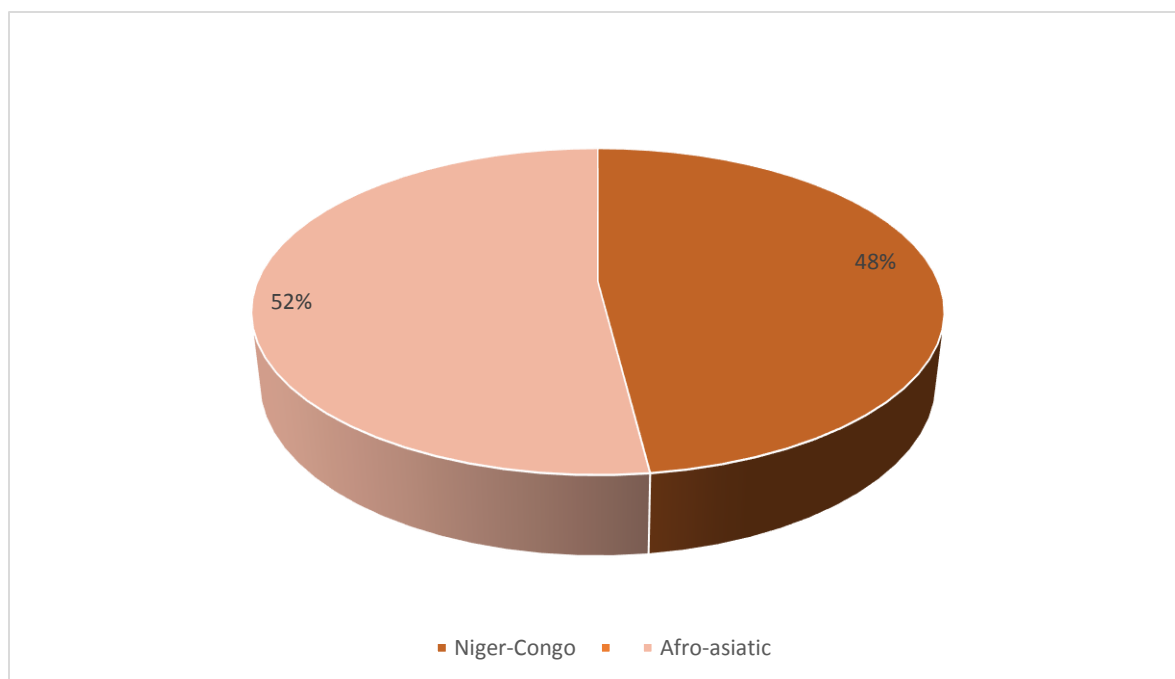


Figure: 3.3 Tribal origins in the patients with sickle cell anemia

Table 3.1. Clinical Feature of the Study patients

Clinical Feature	Frequency	Percentage
Pale	76	76%
Abdominal Pain	21	21%
Jaundice	51	51%
Swollen	46	46%

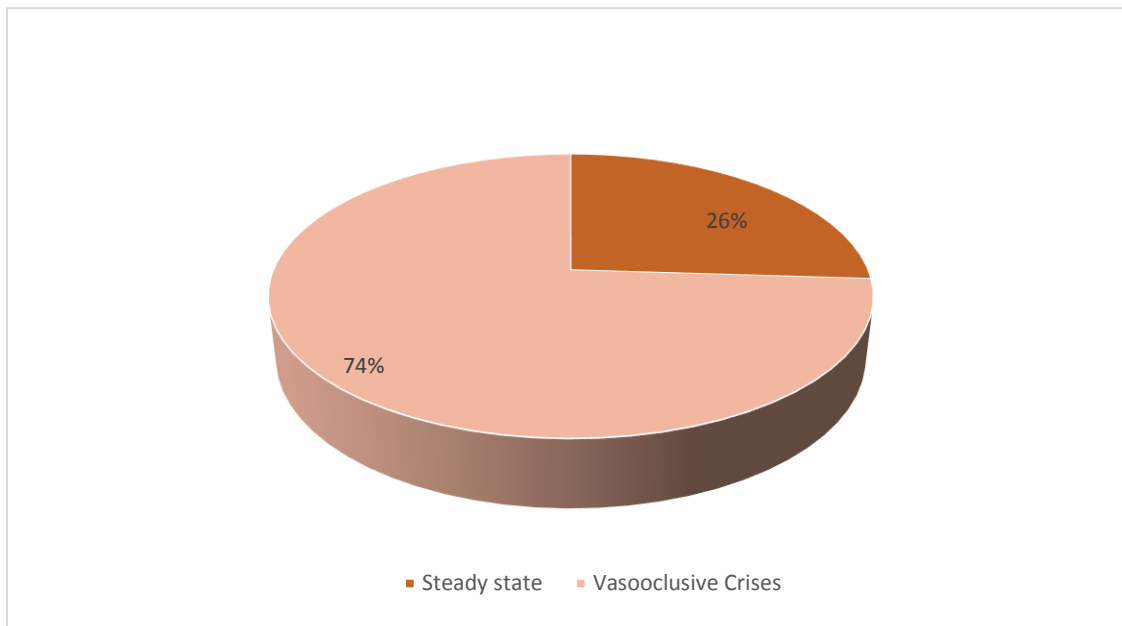


Figure: 3.4 Tribal origins in the patients with sickle cell anemia

Table 3.2 Hematological picture of the study patients

Hematological variable	Patient state	Mean	±SD	P.value
Hb g/dl	Vasooclusive Steady state	6.55 9.80	1.30 2.40	.000
PCV%	Vasooclusive Steady state	20.40 30.69	3.96 7.14	.000
RBCs×10 ⁶ /μl	Vasooclusive Steady state	2.48 3.88	.57 1.13	.000
MCV fl	Vasooclusive Steady state	78.68 FL 75.53 fl	9.60 7.70	.135
MCH pg	Vasooclusive Steady state	26.16 pg. 25.50 pg.	3.85 4.63	.477
MCHC g/dl	Vasooclusive Steady state	32.21 g/dl 32.56 g/dl	3.35 3.34	.659
TWBC×10 ⁹ /l	Vasooclusive Steady state	19.36 76.76	10.64 20.19	.000
Platelts×10 ⁹ /l	Vasooclusive Steady state	357.10 290.46	162.83 124.40	.045

Table: 3.3 Comparison of C. Reactive protein between Vaso occlusive crisis and steady state

Patients state	Mean of CRP	±SD	P.value
Vaso occlusive crises	16.43 mg/l	12.35	.000
Steady state	4.56 mg/l	1.45	

Chapter Four

Discussion, Conclusion, Recommendations

Chapter Four

4. Discussion, Conclusion, Recommendations

4.1. Discussion

Sickle cell disease (SCD) is one of the most common inherited disorders of hemoglobin in Africa. Rates of SCA and trait varied in different areas in Sudan with the highest rates reported from Western and Eastern Sudan (Majdi and Hanan, 2014)

A total of 100 patients with Sickle cell anemia attended to the Sickle cell center during the period of study were enrolled in this study. Most of them showed vaso occlusive crises while the remaining were in steady state, all study patients are children and adolescent, with high frequency seen in the children.

This study showed that CRP is significantly increased in crisis compared with the steady state this result is agreement with CE Okocha *et al.*, (2012) they stated that CRP is significantly increased in crisis compared with the stable state in HbSS individuals, and in HbSS compared with HbAS and HbAA individuals. This is due to, CRP production is part of a nonspecific acute phase response to inflammation and tissue necrosis. The hypothesis here is that repeated obstruction of blood vessels and eventual reperfusion of necrosed tissue lead to the formation of oxygen radicals, which damage the tissues and set up a low level state of ongoing inflammation (Bandeira *et al.*, 2014).

In this study the males had the highest frequency than the female but this were not found in literature review, and most of study population have family history. This study shows that the majority of the patients belong to Afro-Asiatic tribes followed by Niger-Congo, the study done by Majdi and Hanan, (2014), they showed that the presence of HbS is already well documented among Kordofan and Darfur region inhabitants, especially Albaggara, an Afro-Arab constellation of tribes with a

predominantly African descent. Sickle cell disease is most common in Messeryia of Darfur followed by Messeryia of Hummer in Kordofan.

The mean hemoglobin, RBCs counts and Hct values in VOC are lower values compare to steady state this agrees with Omoti, (2005) who showed that The Hb, RBCs, and PCV obtained in steady state and VOC are decreased. This is due to the SCA patients are continually haemolysing their red cells with a short survival rate of the erythrocytes between 12-14 days hence the hemoglobin and PCV values are usually lower than normal individuals. This is shown in this study where the hemoglobin and PCV values in VOC were significantly less than in control.

The total white blood cell count (WBCs) in this study was increased in vaso occlusive crisis than in steady state, this is similar to report of Suba Krishnan *et al.*, (2010) they showed in the HbSS group, inflammatory markers CRP and WBC, were strongly associated with clinical outcome of hospitalization for pain events, This is expected because of the basic mechanisms which cause an increase concentration of neutrophils in venous blood of SCA patients which include demargination of intravascular neutrophils, accelerated release from the bone marrow and reduction in the rate at which neutrophils leave the blood (Akinlade *et al.*, 2013).

The total platelets count is also significantly increased in vaso occlusive crisis than in steady state this agreed with study done by Berney *et at.*, (2006), who the haemoglobin levels were significantly lower and platelet counts significantly higher in subjects with SCA compared with controls. Haemostasis and coagulation were significantly enhanced in SCA due to Constant intravascular and extravascular distruction of sickle red blood cell lead to chronic and acute haemolytic anemia associated with a high prevalence of clinical thrombotic events (Stuart *and* Setty, 2001).

4.2. Conclusion

- CRP level was increased in vasoocclusive crisis compared with steady state.
- According to the sex, the results showed increased male patients more than female patients. Most of the patients had family history of sickle cell anemia.
- The majority of the patients belong to Afro-Asiatic tribes, followed by Niger-Congo.
- Clinical feature in the study patients were paler, jaundice, swollen and abdominal pain.
- Hemoglobin level, PCV and RBCs are more decreased in crisis than in steady state.
- Mean cell volume (MCV), Mean cell hemoglobin (MCH), and Mean cell hemoglobin concentration (MCHC) showed low level in all patients with no significant differences between vaso occlusive crises patients and steady state patients.
- The white blood cell (WBC) and Platelets showed significantly higher level in vaso occlusive crises than in steady state patients.

4.3. Recommendation

1. Routine CRP measurement should be done for all patients as follow up visits.
2. Adequately controlled studies using more inflammatory markers are warranted to define the role of chronic inflammatory state with vaso-occlusive crises and specific complications of the disease.
3. Active community medical education about the sickle cell disease through medical personnel and public societies
4. Counseling before marriage should be implemented for prevention of sickle cell diseases.

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Appendices

Appendix I
بسم الله الرحمن الرحيم
Questionnaire
Sudan University of Science and Technology
College of Graduate Studies

**C-reactive protein level as marker for vaso-occlusive crisis in patient with sickle
cell anemia At North Kordofan State- Sudan Feb 2015**

1. Demographic data

Name.....

Age () sex () Tribes () family history ()
)

2. Clinical presentations

Paler () Abdominal pain ()
Swollen () Jaundice ()

Crisis.....

3. Treatment

Folic acid () Hydroxyl urea ()
Osteocare () Omega 3 ()
Multi vitamins ()

4. Investigations

CBC

Hb: PCV:
RBCs count: MCV:

MCH:

MCHC:

TWBCS Count:

PLTS Count:

Differential leucocyte counts

Neutrophil:

lymphocyte:

Monocyte:

Eosinophil:

Basophil:

C reactive protein level:

By

Jowaireia Geber Al basheer AL Hassan

Appendix II

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا- برنامج الماجستير- مختبرات طبيه

براءة اخلاقيه

الاسم:.....

سوف يتم أخذ عينه من الدم من الوريد بواسطة حقنة طعن وذلك بعد مسح منطقة العينه بواسطة المطهر
وكل الأدوات المستخدمة لأخذ العينه معقمة ومتبع فيها وسائل السلامة المعملية, وسوف يتم تعداد الدم
وقياس مستوى بروتين سي التفاعلي في مرضى انيميا الخلايا المنجلية

وأنا أقر بأن هذه العينات سوف يتم تحليلها فقط لطلب البحث

أوافق أنا المذكور أعلاه أخذ عينه لأجراء الدراسة

الأمضاء:.....

التاريخ:.....

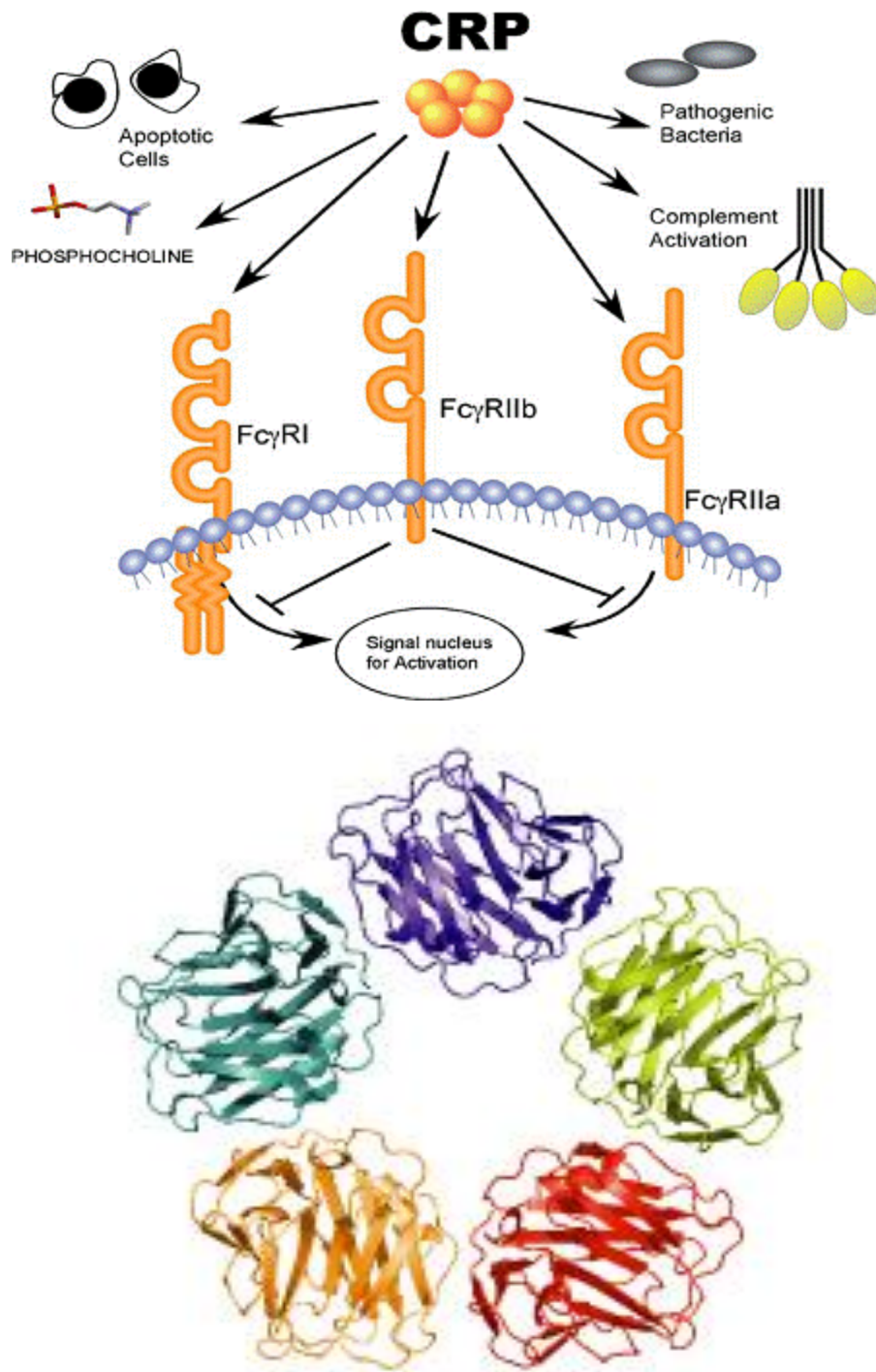


Figure 1.1. Structure of CRP

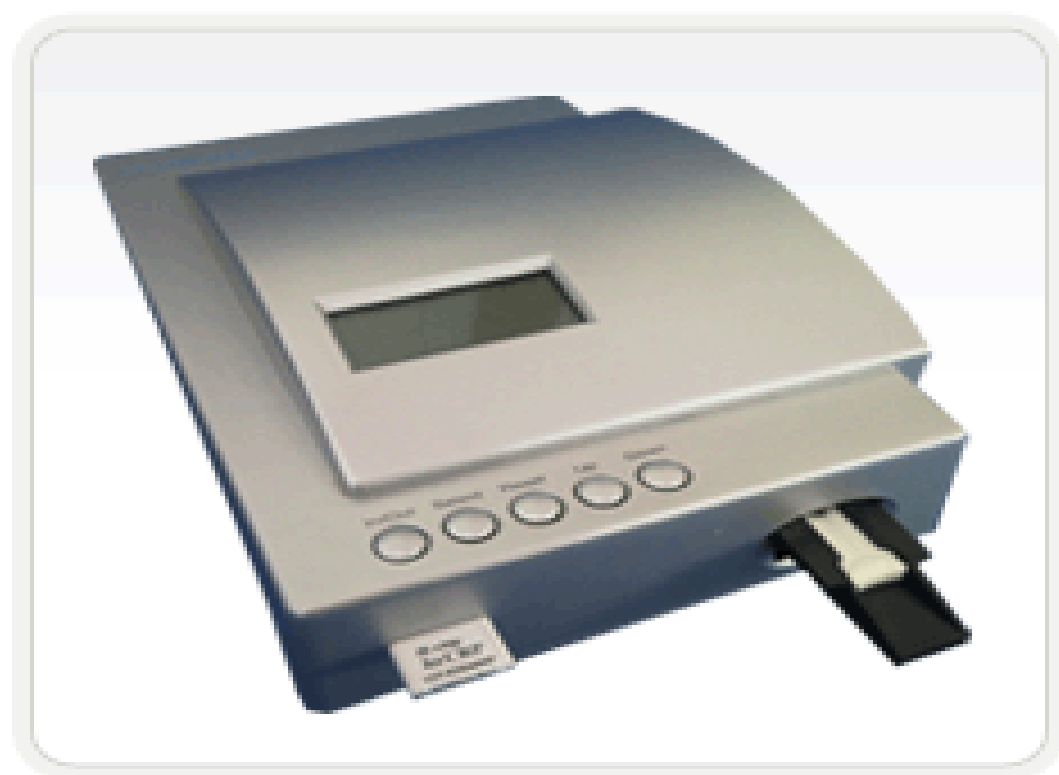


Figure 2.1. ichroma instrument

