Haematological Parameters among Sudanese Sickle Cell Anaemic Patients with Reference to their Tribal Affiliation, Khartoum Teaching Hospital, Khartoum State, Sudan

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B.Sc: in Medical Laboratory Science  Haematology & Blood Transfusion
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Department of Haematology
Faculty of Medical laboratory science
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Date: 18/2/2016
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قال تعالى:

(قل لو كان البحر مداداً لكلماتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ جَنَّا بِمِثْلِهِ مَدَداً)

صدق الله العظيم

(سورة الكهف الآية 109)
Dedication

To my Parents

To

My husband

my brothers and my sister  sow

To

my teachers

To

To everyone who support me in my study secure  and in my research…
Acknowledgment

First grateful thanks for Allah……

Then to my supervisor project prof. babkier Ahmed Mohamed for this guidance and constant supervision as well as providing necessary information regarding the project and also for his support in completing the project…….

All lab staff of medical laboratory and other department ……

Especially for all those have encouraged and supported me to finish this research…..

A lot of thanks to my member of my family and all My husband for their emotional and logistic support

……
Haematological Parameters among Sudanese Sickle Cell Anaemic Patients with Reference to their Tribal Affiliation, Khartoum Teaching Hospital, Khartoum State, Sudan

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Abstract
Sickle cell anaemia (SCA) is a major cause of morbidity and mortality in Africa where there is no readily available effective treatment. This study was aimed to determine the haematological parameter that can be used in monitoring the status and management of SCA patients and their relation to Sudanese tribes. This case control study of 50 patients suffering from SCA and 5 control free from SCA was included in the study, the information collected from Khartoum teaching hospital records. Blood samples were used to identify the SCA by complete blood count and sickling solubility test and electrophoresis, data was collected and analyzed by SPSS version 21. The fifty five patient distributed into case 50 and 5 control, out of 50 case the Hb SS was found (40) 80% and (10) 20% for Hb SA while in control group there is no Hb SS case but Hb SA (5) 100% among case group. Both genders had a general understanding of sickle cell disease from a medical standpoint. However, results showed that (21) 95.5% of males had Hb SS and (1) 4.5% of them their haemoglobin Hb SA. Females had also low number for Hb SS (19) 67.9% compared Hb SA (9) 32.1%. The large frequency number of participant tribes were Mesriria (19) 38.0% followed by Hawsa (7) 14%, Barno(5) 10% and Silahab(5) 10% three of the tribe had same frequency number of case were B.hasain, Mena and Zanda(2)4% for each one. The frequency found in Mahas, Noba, Rizagat and Tama which is (1) 2% for all of them. The result of red blood parameter was affected by SCA and male were the most a frequent for having SCA than female. Furthermore, the large number of participant belong to Mesriria tribe.
قياسات الدم بين مرضى الدم المنجلي السوداني مع الإشارة إلى انتقاءهم القبلي - مستشفى الخرطوم التعليمي

سماح أحمد محمد محمود

مستخلص الدراسة

الانيةما المنجلية هي واحدة من أكثر المشاكل الوراثية التي تؤدي إلى الأمراضية والموت في أفريقيا كما أنه لا يوجد علاج مناسب وفعال. يمنع المرض من هذا المنطلق هدف الدراسة القياس كل مكونات الدم عند الأشخاص المصابين بالمرض وعمر الخالق الذين ينتمون إليه، هذه الدراسة الرعوية ضمت خمسة من المرضى المشخيصين بمرض الأنيميا المنجلية وخمسة من الضحايا الذين لم تكن لديهم أصابات ك(expected) للجودة. جمعت المعلومات من سجلات مستشفى الخرطوم التعليمي حيث أن عينات الدم جمعها وإجراء لها الفحوص على جهاز العد الكامل لتحلى الدليل. ويضا أجري لها فحص من الجملة لتحديد الكريستات للهيموجلوبين وحجز المرحلة الكريستات. جمعت جميع المعلومات وحلت عن طريق الحاسب الآلي حيث وجد ان عدد عينات خمسة وخمسة عينة توزعها بين خمسون حالة دراسة وخمسة ضابط للجودة. في الخمسين عينة وجد هيوموغلوبين اس اس في (40) 80% وهيموجلوبين اس (0) 20% وفي ضابط للجودة لا يوجد حالة هيوموغلوبين اس (0) وهيموجلوبين اس اس (0) 100% أما بالنسبة إلى توزع المرض بين الجنسين وجد أن الرجال (21) 95% بالنسبة ل هيوموغلوبين س و (1) 4.5% و هيوموغلوبين اس. وهم أكثر اصابات من النساء. هيوموغلوبين اس (19) 67% وهيموجلوبين اس (9) 32% وهيموجلوبين اس (1) 4.5%. حصلت قبيلة المسيري (19) 38% على أكبر نسبة من المصابين بالأنيميا تليتها قبيلة الهوسا (14%), البرتول الصليح (5) ولي ثلاثة من القبائل نفس النسبة من الإصابات (2) 4% وهي بني حسين والماي والزائدي وأخيراً قبيلة قبيلة الهوسا الرتبة (2) هي المحس، والنروي، النابوي العرائذات. وقد سمعت في عدد الإصابات تأثر تعداد وحجم الخلايا الحمراء تأثيراً واضحاً بالنسبة للمصابين بالأنيميا المنجلية نوع و هيوموغلوبين اس. وهذا التأثير واضح في الرجال أكثر من النساء والأكثر من ذلك هو أن قبيلة المسيري هي القبيلة التي تضم إكتر المصابين. لذا نحن نوصي بضرورة عمل الفحص الحيني للأنيميا قبل الزواج لتجنب ولادة أطفال مصابين بالمرض.
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List of Abbreviation

CBC: complete blood counts.
Hb: haemoglobin.
MCH: mean cell haemoglobin.
MCHC: mean cell haemoglobin.
MCV: mean cell.
PCV: pack cell volume.
RDW: red cell distribution width.
SCA: Sickle cell anaemia.
SCD: Sickle cell disease.
VOC: vaso-occlusive crisis.

**Key word:** Sickle cell, Hemoglobin SS, Electrophoreses, complete blood counts
CHAPTER ONE

1. Introduction

1.1 Introduction:

Sickle-cell disease (SCD), or sickle-cell anaemia (SCA), is a hereditary blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape. Sickling decreases the cells' flexibility and results in a risk of various complications (Obeagu, et al 2014). The sickling occurs because of a mutation in the haemoglobin gene. Individuals with one copy of the defunct gene display both normal and abnormal haemoglobin. Life expectancy is shortened. In 1994, in the US, the average life expectancy of persons with this condition was estimated to be 42 years in males and 48 years in females (Platt, et al. 1994), but today, thanks to better management of the disease, patients can live into their 70s or beyond. 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 gene confers a fitness. Specifically, humans with one of the two alleles of sickle-cell disease show less severe symptoms when infected with malaria (Wellems et al., 2009).

Many patients with SCA are in reasonably good health most of the time and achieving a steady state level of fitness. This state of relative well-being is periodically interrupted by crisis of which the vaso-occlusive crisis (VOC) is the most common and hallmark of patients with SCD (Beutler E, 1990). The importance of early recognition and subsequent clinical and haematological assessment of the disease are greatly facilitated by familiarity with the patient’s steady state. A patient with SCA is said to be in steady state when there is absence of infection, acute complicating factors or acute clinical symptoms or crisis for at least three months. (Bookchin RM, 1996) Crisis refers to episodes of acute illness attributable to the sickling phenomenon in which there is a sudden deviation for the worse or a sudden exacerbation of symptoms and signs of patients with SCA who had hitherto been in stable condition. (Gustave KK, et al, 2003).

Many previous studies have lumped the haematological variables in steady state and crisis together. This study will determine if there is a difference in the haematological variables
between steady state period and VOC. Furthermore, many of the established values were determined over a decade ago. Various changes in therapeutic, economic, social, technological measures have taken place since then. This study will therefore determine if these changes have affected what we accept as the ‘normal’ values in these patients. Less commonly determined variables such as erythrocyte indices, red cell distribution width, haemoglobin A2 and haemoglobin F will also be determined along with the routine haematological tests.
CHAPTER TWO

2.1 Literature review

2.1 Disease definition:
Sickle cell haemoglobin (Hb S) results from a single-base mutation of adenine to thymine which produces a substitution of valine for glutamine at the sixth codon of the P-globin chain (a2p26glu^val). In the homozygous state \textit{(sickle cell anaemia)} both genes are abnormal (Hb SS), whereas in the heterozygous state \textit{(sickle cell trait, Hb AS)} only one chromosome carries the gene. As the synthesis of Hb F is normal, the disease usually does not manifest itself until the Hb F decreases to adult levels at about 6 months of age. The disease occurs mainly in Africans (25\% carry the gene) but is also found in India, the Middle East and southern Europe. (\textit{Weather, 2001})

For the purpose of this literature review, these phrases, frequently referenced throughout the text, are defined as follows:

\textbf{Genetic counseling:} Communication process between health care provider and client that emphasizes and provides accurate and up-to-date information about a genetic disorder in a sensitive and supportive, non-directive manner (\textit{SCDAA, 2005}).

\textbf{Hemoglobin:} Chemical substance (an iron containing protein) of the red blood cell, which carries oxygen to the tissues, and gives the cell its red color (\textit{SCDAA, 2005}).

\textbf{Hemoglobin A (HbA):} Hemoglobin is composed of two alpha globins and two beta globins, normally produced by children and adults (\textit{Jones, 2008, p. 119}).

\textbf{(AC):} Inheritance of one gene for the usual hemoglobin (A) and one gene for \textbf{Hemoglobin C trait} hemoglobin (C). A person who has the hemoglobin C Trait (AC) is a carrier of the hemoglobin C gene, and is not affected by the gene (\textit{SCDAA, 2005}).

\textbf{Hemoglobin C disease:} A person has both HbS and HbC and is often referred to as \textquotedblright\textipa{HbSC}\textquotedblright. Hemoglobin C causes red blood cells to develop. Having just some hemoglobin C and normal hemoglobin, a person will not have any symptoms of anemia. However, if the sickle hemoglobin S is combined with the target cell, some mild to moderate anemia may occur (\textit{UMMC, 2010}).
**Hemoglobin E disease:** Similar to sickle cell-C disease except that an element has been replaced in the hemoglobin molecule under certain conditions, such as exhaustion, hypoxia, severe infection, and/or iron deficiency (*UMMC, 2010*).

**Hemoglobin S-beta-thalassemia:** An inheritance of both the thalassemia and sickle cell genes. The disorder produces symptoms of moderate anemia and many of the same conditions associated with sickle cell disease, but to a milder degree (*UMMC, 2010*).

**Sickle cell anemia (SS):** An inherited disorder of the red blood cells in which the hemoglobin is different from the normal hemoglobin. This unusual hemoglobin results in the production of unusually shaped cells and is referred to as “HbSS.” It is the most common and severe form of the sickle cell variations (*SCDAA, 2005*).

**Sickle cell disease (SCD):** An inherited disorder of the red blood cells in which one gene is for sickle hemoglobin (S) and the other gene is for unusual hemoglobin such as S, C, Thal (*SCDAA, 2005; UMMC, 2010*).

**Sickle cell trait:** A person carrying the defective gene, HbS, but also has some normal hemoglobin HbA. Persons with the sickle cell trait are usually without symptoms of the disease, but mild anemia may occur under intense, stressful conditions, exhaustion, hypoxia (low oxygen), and/or severe infection. The sickling of the defective hemoglobin may occur and result in some complications associated with sickle cell disease (*UMMC, 2010*).

### 2.2 Pathogenesis:

Deoxygenated Hb S molecules are insoluble and polymerize. The flexibility of the cells is decreased and they become rigid and take up their characteristic sickle appearance. This process is initially reversible but, with repeated sickling, the cells eventually lose their membrane flexibility and become irreversibly sickled. This is due to dehydration, partly caused by potassium leaving the red cells via calcium activated potassium channels called the Gados channel. These irreversibly sickled cells are dehydrated and dense and will not return to normal when oxygenated. (Weather, 2001)

Sickling can produce:

A shortened red cell survival and impaired passage of cells through the microcirculation, leading to obstruction of small vessels and tissue infarction. Sickling is precipitated by infection, dehydration, cold, acidosis or hypoxia. In many cases the cause is unknown, but adhesion proteins on activated endothelial cells (VCAM-1) may play a causal role, particularly in
vasoocclusion when rigid cells are trapped, facilitating polymerization. Hb S releases its oxygen to the tissues more easily than does normal Hb, and patients therefore feel well despite being anemic (except of course during crises or complications). Depending on the type of hemoglobin chain combinations, three clinical syndromes occur:

- Homozygous Hb SS have the most severe disease.
- Combined heterozygocity (Hb SC) for Hb S and C who suffer intermediate symptoms.
- Heterozygous Hb AS (sickle cell trait) usually have no symptoms. (Weather, 2001)

2.3. Epidemiology:
The highest frequency of sickle disease is found in tropical regions, particularly in tropical regions of India and the Middle-East. 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. Specifically, humans with one of the two alleles of sickle cell disease show less severe symptoms when infected with malaria. (Wellems, Hayton, Fairhurst 2009)

Three quarters of sickle cell cases occur in Africa. A recent WHO report estimated that around 2% of newborns in Nigeria were affected by sickle cell anemia. The carrier frequency ranges between 10% and 40% across equatorial Africa, decreasing to 1-2% on the north Africa coast and <1% in south Africa. (report WHO, 2010)

2.4 Signs and Symptoms:
Sickle cell disease may lead to various acute and chronic complications, several of which have a high mortality rate. (Yawn, et al, 2014)

- Painful swelling of the hands and feet, known as dactylitis.
- Fatigue or dizziness from anemia.
- A yellowish discoloration of eyes, known as jaundice that occurs due to hemolysis of red blood cells.

Complications:
Most episodes of sickle cell crisis last between five and seven days. (BestBets 2010) Although infection, dehydration and acidosis can act as triggers, in most instances, no predisposing cause is identified. (Kumar, et al, 2009)
• Vaso-occlusive crisis
Is caused by sickle shaped red blood cells that obstruct capillaries and restrict blood flow to an organ resulting in ischemia, pain, necrosis and often organ damage.

*Splenic sequestration crisis:
Are acute, painful enlargement of the spleen, caused by intrasplenic trapping of red cells and resulting in a precipitatus fall in hemoglobin levels with potential for hypovolemic shock. The spleen usually infarcted before the end of childhood in individuals suffering from sickle cell anemia. (Anie KA, Green J, 2012).

*Acute chest syndrome:
Is defined by at least two of the following signs and symptoms: chest pain, fever, pulmonary infiltrate or focal abnormality, respiratory symptoms, or hypoxemia.
It’s the second most common complications and its account for about 25% of deaths in patients with sickle cell disease, majority of cases present with vaso-occlusive crisis then they develop acute chest syndrome. (Paul RN et al, 2011)

*Aplastic crisis:
Are acute worsening of the patient’s baseline anemia, producing pale appearance fast heart rate, and fatigue. This crisis is usually triggered by parvovirus B19, which directly affects production of red cell precursors and multiplying in and destroying them. (Kumar et al, 2009)
Reticulocytes counts drop dramatically during the disease (causing reticulocytopenia), and the rapid turnover of red cells leads to drop in hemoglobin. This crisis takes 4 days to one week to disappear.

*Haemolytic crisis
Are acute accelerated drops in hemoglobin level. The red cells break down at faster rate.

Others complication:
• Increased risk of bacterial infections due to loss of functioning spleen tissue.
• Stroke, which result from progressive narrowing of blood vessels, prevents oxygen from reaching brain.
• Cholelithiasis and cholecystitis may result from excessive bilirubin production and precipitations due to prolong haemolysis.
• Avascular necrosis of the hip and other major joints may occur result to ischemia. (Marti-Carvajal et al, 2004)
- Acute papillary necrosis in the kidneys. This may lead to chronic kidney failure and may progress to end stage renal disease. (*Powars, et al 1991*)
- Leg ulcers. (*Rudge FW 1991*)
- Retinopathy.
- Chronic pain, even in the absence of acute vaso-occlusive crisis.
- Osteomyelitis, the most common organism is salmonella, followed by staphylococcus aureus and gram negative enteric bacilli perhaps because intravascular sickling of the bowel leads to patchy ischaemic infarction. (*Almeida A, Roberts I 2005*)

### 2.5 Laboratory diagnosis

These are some of the most common tests used to diagnose sickle cell syndrome:

1. **Hemoglobin Electrophoresis:**
   This is the test which is used to find out a person’s hemoglobin type. It is this test which tells you what type of sickle cell disease your child has. This test is also done when it is important to know how much sickle hemoglobin is in your child’s blood. Before a blood transfusion, doctors use the test to help decide how much blood should be given. After a transfusion, the test is used to see if enough blood was given to lower the amount of sickle hemoglobin and prevent complications from sickling.
   Always needed to confirm the diagnosis. There is no Hb A, 80-95%HbSS, and 2-20%Hb F.

2. **Complete Blood Count (CBC):**
   The most common blood test is the complete blood count (CBC). It is mainly done to find out the number, shape and size of the red blood cells and the hemoglobin level. This information is used to tell if any treatment is needed.
   The normal level of Hb in the adult range 6-8 g/dL with a high reticulocyte count (10-20%), while in children without sickle cell disease is cell disease usually have a lower hemoglobin level of 6-10 g/dl. This varies with the type of sickle cell disease. If your child’s hemoglobin level is less than 5 or 6 g/dl, she may need to be given blood or go to the hospital to be observed.

3. **Reticulocyte (Retic) Count:**
   Reticulocytes are young red blood cells. The number of these cells shows whether the bone marrow is doing its job well, making and releasing young red cells into the blood.
1. **Kidney and liver function tests**

These tests show if these organs have been damaged by sickle cell disease. In young children, damage is rare. However, over time, the sickle cells can plug up the small blood vessels of these organs so that they can become damaged.

2. **Urine test (urinalysis)**

In sickle cell disease, children can get kidney or urinary tract infections.

Urine is checked under a microscope for signs of infection. With a bacterial infection, urine is cloudy, smells bad and tiny swimming bacteria and white blood cells can be seen. Red blood cells in the urine can be a sign of slight bleeding from the kidney. A lot of protein in the urine can mean kidney damage, but a small amount doesn’t usually matter.

3. **Blood Chemistry Tests**

These tests measure substances in the blood which are important for health and growth, such as iron, glucose (sugar), and minerals.

1. **X-Rays**

X-Rays are used to see if there is an infection in the lungs and to look at bones which may be damaged by sickle cell disease

2. **Blood films**

Can show features of microcytic hypochromic cells.

3. **Sickling test**

Red cells on a blood film can be induced in the presence of sodium metabisulphite.

**Sickle Solubility Test.**

A mixture of HbS in a reducing solution such as sodium dithionite gives a turbid appearance because of precipitation of Hb S, whereas normal Hb gives a clear solution. A number of commercial kits such as Sickledex are available for rapid screening for the presence of Hb S, for example before surgery in appropriate ethnic groups and in the Accidents & Emergency department.

**Genetic study:**

The parents of the affected child will show features of sickle cell trait (*Kumar, 2004*).
2.6 Previous Studies

In study done in Nigeria (Obeagu, et al 2014) A great percentage of sickle cell anaemic patients in this University are in steady state because of increased level of fetal haemoglobin (HbF) in most of them but very few have minimal level of HbF which easily undergo crisis. Most of the patients were detected in the course of medical examination. Because of the delicate nature of these patients, the researchers of this study saw the necessity to carry out the study. 20 confirmed sickle cell patients were used as the patients aged 4-34 year, 14 males and 6 females and 40 subjects with haemoglobin genotypes AA were used as the controls. The study showed significant increase in WBC, Neutrophil and Lymphocytes (P<0.05), significant decrease in PCV (P<0.05) and no significant change in monocyte (P>0.05) when the mean values of the SCA patients were compared relative to HbAA subjects. SCA patients should be monitored closely and prevented from triggering factors to crisis.

Other study also done in Nigeria in the University of Benin Teaching Hospital, Benin City, A prospective study of 200 patients (81.3%) in steady state, 46 patients (18.7%) during vaso-occlusive crisis (VOC) and 84 control subjects seen between August 2001 and July 2002 in 3 centers in Benin City, Nigeria had their blood samples analyzed within two hours of collection. Automated Coulter Counter was used to determine the complete blood counts while the foetalhaemoglobin (HbF) was estimated by the modified Betke method and haemoglobin A2 by HbS-free microcolumn chromatography.

The mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in steady state were 79.38fl ± 22.41, 28.31pg ± 3.58 and 32.56g/dl ± 2.27 while in VOC they were 85.50fl ± 8.14, 28.79pg ± 2.78 and 33.76g/dl±3.44 respectively. The red cell distribution width (RDW), haemoglobin A2 and F in steady state were 23.76% ± 6.49, 4.52% ±1.16 and 2.17% ±1.81 while during VOC they were 21.62% ±5.11, 3.82%±1.27 and 2.05%±1.19 respectively. The neutrophil count (P<0.01), MCV (P<0.01) and MCHC (P<0.05) were significantly higher during VOC than steady state while the RDW (P<0.05) and haemoglobin A2 were significantly higher in steady state than during VOC. (C. E. Omoti, 2005)

The aim of the current study done in sudan by Malik Hassan Ibrahim and his team was to determine the sickle cell trait frequency in Sudanese patient living in Heglig area in Western of Southern Kordofan state from November 2008 to February 2009. An analytical, descriptive and
cross-sectional study conducted for one hundred participants who had confirmed patient diagnosed as (Hb S disease) homozygosis patient. 2-5 mL of venous blood was collected for the measurements of complete blood count, sickling test and Hb electrophoresis. Demographic data and family history were collected in a pre-designed questionnaire with written consent all participants. In this study the frequency of sickle cell trait and sickle cell disease were (52%) and (14%) respectively and 34% were normal. The sickling test showed that 71% of the study population were negative sickling test, the remaining 29% were positive. The total erythrocytes was significantly decreased in sickle cell disease (p< 0.000) compared with normal and sickle cell trait, also the hemoglobin concentration and packed cell volume were significantly lower than that of normal individuals and sickle cell trait patients. The frequencies of sickle cell trait was higher among the participants and patients of sickle disease showed lower values of red blood cells parameters, but higher values of white blood cells and platelets compared to haemoglobin phenotype AA control participants.

The clinical, hematological and biochemical features of 50 Sudanese patients with sickle cell disease (SCD) were determined. 23 patients with complete family data, 21 had sickle cell anemia (homozygous HbSS), 2 had sickle-cell/beta-thalassaemia but none had sickle cell/beta Othalassemia. The remaining 27 patients had HbSS phenotype. 84% of patients were from the Baggara tribe in western Sudan, where HbS is a natural extension of the West African HbS belt. 21 patients were children under 2 years old; 19 were 3-10 years old; and the remaining 10 were over 10 years old. Young patients presented mainly with painful vaso-occlusive crisis, severe anemia, hand and foot syndrome, fever, underweight, malnutrition and various infectious diseases. All patients had mild to moderate cardiac enlargement; 42% had a moderately enlarged spleen but only 10% had an enlarged liver; 20% had infarctive lesions of long bones and another 8% had Salmonella osteomyelitis. Leg ulcers, priapism, enuresis and cholelithiasis were not observed. Patients had a mean hemoglobin concentration of 7.3 g/dl; reticulocyte count of 15.1%; serum bilirubin of 2.1 mg/dl; HbA2 level of 2.8% and HbF of 7%. Thus, the observed pattern of SCD in Sudan is comparable to the severe type described for Africans and not comparable to the benign form found in Shiite Moslem Arabs of Saudi Arabia. 6 adults with mild SCD had HbF levels below 5%. Amelioration of the disease, therefore, does not seem to be related to HbF levels; nor was it possible to relate it to high levels of erythrocyte 2,3-diphosphoglycerate. (Bayoumi, 1988)
2.7 Rationale (Justification):
Sickle cell disease is a major public health disease that has great impact on both individuals and society. Sickle cell disease is also associated with serious morbidity and mortality under unusual circumstances
The aim of the current study was to determine the sickle cell anemia and their hematological parameter in Sudanese patients

2.8 Objectives:
2.8.1 General Objective:
To detect the hematological parameters among Sudanese sickle cell anemia patient with reference to their tribal affiliation in Khartoum teaching hospital

2.8.2 Specific Objectives:
1. To determine complete blood count, sickling test, solubility test and hemoglobin electrophoresis of sickle cell anemia patients in steady state.
2. To identify the patients' distribution on different Sudanese tribes.
3. To correlate between sickle cell anemia and gender.
4. To correlate between sickle cell anemia and age group.
5. To identify the hemoglobin types among gender and age group.
Chapter Three

3. Material and Method

3.1 Study Design:
Descriptive cross-sectional study

3.2 Study Area:
Khartoum Teaching Hospital's in Sudan

3.3 Study Duration:
The study was carried during

3.4 Study Population:
sickle cell anemia patients who were following in the hospitals clinics

3.5 Inclusion Criteria:
sickle cell anemia patients in steady state who were following in the hospitals clinics at the time of the study

3.6 Exclusion Criteria:
newly diagnosed patients with sickle cell anemia, any patient with other sickle cell following in clinics at the time of the study

3.7 Data Collection and Analysis:
Data was collected from hospital records of the laboratory, for patients whose records including their, gender, age, tribe, their complete blood count analysis, sickling test, solubility test, hemoglobin electrophoresis

3.8 Data Analysis:
description and analysis was performed using Statistical Package for Social Sciences version 21 (SPSS).

3.9 Ethical Consideration:
Permission will be obtained from the authorities and ethical clearance was taken from the Ethical Committee at the University of and Khartoum Teaching Hospital.

3.10 Sample Size:
There were 55 samples fitting the criteria .The collected data was computerized through Microsoft Excel.
3.11 Sampling Technique:
non randomize technique

3.12 Blood Sample Collection:
A blood sample of 2-5 mL was collected from all participants into EDTA container for the measurements of complete blood count using SysmexKx 21N automated hematological analyzer, sickling test and Hb electrophoresis following laboratory routine procedures as described in Barbara J Bain,(2006);Lewis M,et.al.,(2006). Demographic data and family history were collected in a pre-designed questionnaire.

3.13 Study Measure:
All data were entered into the SPSS database and coded by using anumerical system. The questionnaire contained four categories of race identification for participants to identify tribe and Gender was coded as 1) male and 2) female. Age was determined by students filling in the blank. Ten sickle cell disease questions were listed on the data collection.

3.14 Methods:
1. The complete blood counts (CBC): were analyzed using the automated SysmexKx 21N automated hematological analyzer,. The CBC includes haemoglobin, haematocrit, total white blood cell count and differential, platelet count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and red cell distribution width.

2. Solubility tests for sickle cell

Principle:
Hemoglobin S is less soluble in a reducing agent than other forms of hemoglobin.

Comments:
The solubility test is the most common screening test for sickle cell or presence of HbS. It is based on the relative insolubility of HbS when combined with a reducing agent such as sodium dithionite. When anticoagulated blood is mixed the reducing agent, the red cells will lyse due to the presence of saponin and the hemoglobin in the red cells will be released. If HbS is present, it will form liquid crystals and give a cloudy or turbid appearance to the solution. If HbS is not present, the solution will appear transparent, with rare exceptions (see below). The solubility test cannot be used to differentiate sickle cell disease (homozygous for HbS) from sickle cell trait (heterozygous for HbS).
Hemoglobin electrophoresis is considered the diagnostic procedure and is especially important considering that there are other hemoglobin variants that will also cause a positive solubility test, such as HbC Harlem.

**Sources of error:**
1. A patient with an exceptionally high hematocrit may give a false positive result, while an individual with a very low hemoglobin may give a falsely negative result.
2. Unstable hemoglobins may give a false positive result.
3. False positives can occur with elevated plasma proteins and lipids.

### Electrophoreses

#### 1. PRINCIPLE

Very small samples of hemolysates prepared from whole blood are applied to the Titan III® Cellulose Acetate Plate. The hemoglobins in the sample are separated by electrophoresis using an alkaline buffer (pH 8.2-8.6), and are stained with Ponceau S Stain. The patterns are scanned on a scanning densitometer, and the relative percent of each band determined.

### REAGENTS

#### 1. Supre-Heme® Buffer (Cat. No. 5802)

**Ingredients:** The buffer contains Tris-EDTA and boric acid.

**Warning:** for in-vitro diagnostic use only. Never pipette by mouth. Do not ingest. Ingestion of sufficient quantities of boric acid and EDTA can be toxic.

**Preparation for Use:** Dissolve one package of buffer in 980 mL deionized water. The buffer is ready for use when all material is dissolved and completely mixed.

**Storage and Stability:** The packaged buffer should be stored at 15 to 30°C and is stable until the expiration date indicated on the package and box. The buffer solution is stable two months when stored at 15 to 30°C.

**Signs of Deterioration:** Do not use packaged buffer if the material shows signs of dampness or discoloration. Discard the buffer solution if it shows signs of bacterial contamination.

#### 2. Hemolysate Reagent (Cat. No. 5125)

**Ingredients:** The reagent contains 0.005 M EDTA in deionized water with 0.07% potassium cyanide added as a preservative.

**Warning:** for in-vitro diagnostic use only. Do not pipette by mouth. The reagent contains a small amount of potassium cyanide.
Preparation for Use: The reagent is ready to use as packaged.

Storage and Stability: The reagent should be stored at 15 to 30°C and is stable until the expiration date indicated on the bottle.

Signs of Deterioration: The reagent should be clear and colorless.

3. Ponceau S Stain (Cat. No. 5526)

Ingredients: The reconstituted stain is 0.5% (w/v) Ponceau S in an aqueous solution of 10% (w/v) sulfosalicylic acid.

warning: for in-vitro diagnostic use. do not ingest. harmful if swallowed.

Preparation for Use: Dissolve one vial of stain in 1 L of deionized water.

Storage and Stability: The stain should be stored at 15 to 30°C and is stable until the expiration date indicated on the container. It may be stored in the bottle or in a tightly closed staining dish and may be reused multiple times if properly stored.

Signs of Deterioration: Do not use the stain solution if excessive evaporation occurs, or if large amounts of precipitate appear.

4. Clear Aid (Cat. No. 5005)

Ingredients: The reagent contains polyethylene glycol.

warning: for in-vitro diagnostic use. do not ingest.

Preparation for Use: Clear Aid is used as the clearing solution which is prepared as follows:

30 parts glacial acetic acid
70 parts absolute methanol
4 parts Clear Aid

Storage and Stability: Store the prepared clearing solution at 15 to 30°C in a tightly closed container to prevent evaporation of the methanol. When evaporation occurs, the plates may delaminate. Water contamination from over-use of the clearing solution will cause the plate to be cloudy. The reagent is stable until the expiration date indicated on the bottle.

Signs of Deterioration: Clear Aid should be a clear, colorless liquid, although it may appear cloudy when cold. Do not use the material upon evidence of gross contamination or discoloration. Discard the prepared Clear Aid if plates appear cloudy after the clearing procedure.

5. PermaClear Solution (Cat. No. 4950) - Optional

Ingredients: N-methyl pyrrolidinone and PEG.

warning: for in-vitro diagnostic use - irritant – do not pipette by mouth. vapor harmful. In case of contact, flush affected areas with copious amounts of water. Get immediate attention for eyes.
Preparation for Use: Add 55 mL PermaClear to 45 mL deionized water and mix well.

Storage and Stability: PermaClear should be stored at 15 to 30°C and is stable until the expiration date on the bottle.

Signs of Deterioration: Discard the PermaClear Solution if the plates turn white and do not clear as expected.

6. Titan III-H Plates (Cat. No. 3021, 3022)

Ingredients: Cellulose acetate plates.

warning: for in-vitro diagnostic use.

Preparation for Use: The plates are ready for use as packaged.

Storage and Stability: The plates should be stored at 15 to 30°C and are stable indefinitely.

step by step method

A. Preparation of the Titan® III-H Plate

1. Dissolve one package Supre-Heme® Buffer in 980 mL deionized water.
2. Properly code the required number of Titan® III-H Plates by marking on the glossy hard side with a marker.
3. Soak the required number of plates in Supre-Heme® Buffer for 5 minutes. The plates should be soaked in the bufferizer according to the instructions provided.
   Alternately, the plates may be wetted by slowly and uniformly lowering a rack of plates into the buffer.
   The same soaking buffer may be used for soaking up to 12 plates or for approximately one week if stored tightly closed. If used for a prolonged period, residual solvents from the plates may build up in the buffer and cause poor separation of the proteins or, evaporation may cause greater buffer concentration.

B. Preparation of Zip Zone ® Chamber

1. Pour approximately 100 mL of Supre-Heme® Buffer into each of the outer sections of the Zip Zone® Chamber.
2. Wet two chamber wicks in the buffer and drape one over each support bridge being sure it makes contact with the buffer and that HELENA there are no air bubbles under the wicks.
3. Cover the chamber to prevent buffer evaporation. Discard the buffer and wicks after use.

C. Sample Preparation and Application

1. Prepare a hemolysate of the patient samples as follows:
a) Using whole blood: Add 1 part whole blood to 3 parts Hemolysate Reagent. Mix well and allow to stand 5 minutes.

b) Using packed cells: Mix 1 part packed red blood cells to 6 parts Hemolysate Reagent. Mix well and allow to stand 5 minutes.

NOTE: If removal of denatured hemoglobins from the sample is deemed necessary, see the

Alternate Sample

Preparation Procedure.

1. Place 5 μL of the patient hemolysates or 5 μL of the Hemo Controls into the wells of the Sample Well Plates using the Microdispenser. Do not prepare a hemolysate of the Hemo Controls.

2. To prevent evaporation, cover the Sample Well Plate with a glass slide, if the samples are not used within 2 minutes.

3. Prime the applicator by depressing the tips into the sample wells 3 or 4 times. Apply this loading to a piece of blotter paper. Priming the applicator makes the second loading much more uniform. Do not load applicator again at this point, but proceed quickly to thenext step. Remove the wetted Titan® III Plate from the buffer with the fingertips and blot once firmly between two blotters. Place the plate in the aligning base, cellulose acetate side up, aligning the top edge of the plate with the black scribe line marked “CATHODE APPLICATION”. The identification mark should be aligned with sample No. 1. Before placing the plate in the aligning base, place a drop of water or buffer on the center of the aligning base. This prevents the plate from shifting during the sample application.

4. Apply the sample to the plate by depressing the applicator tips into the sample well 3 or 4 times and promptly transferring the applicator to the aligning base. Press the button down and hold it 5 seconds

Alternate Sample Preparation Procedure:

If removal of denatured hemoglobins from the sample is deemed necessary, perform the following steps:

a. Centrifuge the blood sample at 3500 RPM for 5 minutes.

b. Remove the plasma from the sample and wash the red blood cells in 0.85% saline (v/v) three times. After each wash, centrifuge the cells for 10 minutes at 3500 RPM.
c. Add 1 volume deionized water and 1/4 volume toluene (or carbon tetrachloride) to the washed red cells. Vortex at high speed for one minute. Centrifuge the samples at 3500 RPM for 10 minutes.

d. If toluene is used, the top layer in the tube will contain cell stroma and should be removed with a capillary pipette before proceeding to the next step. The clear middle layer contains the desired sample. If carbon tetrachloride is used, all red cell waste material will be contained in the bottom of the tube after centrifugation.

e. Filter the clear red solution through two layers of Whatman #1 filter paper.

**Electrophoresis of Sample Plate**

1. Quickly place the plate in the electrophoresis chamber, cellulose acetate side down, such that the sample end is toward the cathodic(-) side of the chamber. Place a weight (glass slide, etc.) on the plate to insure contact with the wicks.

2. Place the cover on the chamber, and electrophorese the plate for 25 minutes at 350 volts.

**E. Staining the Hemoglobin Bands:**

1. Remove the plates from the electrophoresis chamber and stain in Ponceau S for 5 minutes.

2. Destain in 3 successive washes of 5% acetic acid. Allow the plates to stay in each wash 2 minutes or until the background is white.

3. The plates may be dried and stored for a permanent record at this point. If a transparent background is desired for densitometry, proceed to the next step.

**If using Clear Aid Solution:**

1. Dehydrate, by washing the plate twice in absolute methanol, for two minutes each wash. Allow the plate to drain for 5-10 seconds before placing in the next solution.

2. Place the plate into the Clear Aid solution for 5-10 minutes.

3. Drain off excess solution. Then place the plate, acetate side up, onto a blotter, and into an I.O.D., Micro-Hood, or other drying oven at 50-60°C for 15 minutes or until dry.

**If using PermaClear Solution:**

1. Place the plate(s) into the diluted PermaClear clearing solution for 2 minutes.

2. Drain off excess solution by holding plate(s) vertically for 1 minute. Then place the plate, acetate side up, onto a blotter, and into an I.O.D., or other drying oven at 50-60°C for 15 minutes or until dry.
F. Evaluation of the Hemoglobin Bands

1. Qualitative evaluation: The hemoglobin plates may be inspected visually for the presence of abnormal hemoglobin bands. The Helena Hemo Controls provide a marker for band identification.

2. Quantitative evaluation: Determine the relative percent of each hemoglobin band by scanning the cleared and dried plates in the densitometer using a 525 nm filter.

Stability of End Product: The dried plates are stable for an indefinite period of time, and may be stored in Titan Plastic Envelopes.

Calibration: A calibration curve is not necessary because relative concentration of the bands is the only parameter determined.

Quality Control: Four controls for hemoglobin electrophoresis are available from Helena Laboratories: AA2 Hemo Control (Cat. No. 5328), ASA2 Hemo Control (Cat. No. 5329), AFSA2 Hemo Control (Cat. No. 5330), and AFSC Hemo Control (Cat. No. 5331). The controls should be used as markers for the identification of the hemoglobin bands, and they may be quantitated for verification of the accuracy of the procedure. Refer to the package insert provided with the controls for assay values and migration patterns. Use at least one of these controls on each plate run.

RESULTS

Figures 1 illustrates how the combination of cellulose acetate and citrate agar electrophoresis can be used in tandem for the identification of hemoglobins. Figure 2 lists the relative mobilities of various hemoglobin mutants on cellulose acetate and citrate agar plates.

Calculation of Unknown: The Helena EDC, CliniScan® 3 and other Helena densitometers with computer accessories will automatically print the relative percent of the bands.

LIMITATIONS

Some abnormal hemoglobins have similar electrophoretic mobilities and must be differentiated by other methodologies.

Further testing required:

1. Citrate agar electrophoresis may be a necessary follow-up test for confirmation of abnormal hemoglobins detected on cellulose acetate.
2. Isoelectric focusing, high performance liquid chromatography, globin chain analysis (both acid and alkaline) and structural studies may be necessary in order to positively identify some of the more rare hemoglobins.

3. Anion exchange column chromatography is the most accurate method for quantitating HbA2. Helena Laboratories’ Sickle-ThalQuikColumn® Method (Cat. No. 5334) for quantitation of HbA2 in the presence of HbS, or the Beta-Thal HbA2 Quik Column® Procedure (Cat. No. 5341) are recommended. HbA2 quantitation is one of the most important diagnostic tests in the diagnosis of β-thalassemia trait.
CHAPTER FOUR

4. Result

4.1. Distribution of hemoglobin type among case and control

A fifty five patient distributed into case 50 and 5 control, out of 50 case the Hb SS was found (40) 80% and (10) 20% for HbAS while in control group there is no Hb SS case but HbAS (5) 100% among case group. Table (1)

Table 4.1 Distribution of hemoglobin type among case and control

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of Hb</th>
<th>Total</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hb SS</td>
<td>Hb AS</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>Number</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>Control</td>
<td>Number</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Hb SA= hemoglobin AS, Hb SS = hemoglobin SS == Significant of (P.value less than 0.005)

4.2. Correlation between sickle cell anemia and gender:

A total of (22) 44% males and (28) 56% females completed the sickle cell disease included in this study while the control (2) 40% male (3) 60% female. Both genders had a general understanding of sickle cell disease from a medical standpoint. However, results showed that (21) 95.5% of males had Hb SS and (1) 4.5% of them their haemoglobin HbAS. Females had also low number for Hb SS (19) 67.9% compared HbAS (9) 32.1%. All the control group give positive result for HbAS male (2) 40% and female (3) 60% and all of them negative for Hb SS (P.value 0.015). see table (2)
Table 4.2 Correlation between gender and type of hemoglobin:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Type of Hb</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb SS</td>
<td>Hb AS</td>
<td>Hb SS</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>21</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>95.5%</td>
<td>4.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>19</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>67.9%</td>
<td>32.1%</td>
<td>0%</td>
</tr>
</tbody>
</table>

P.value = 0.015

Significant of (P.value less than 0.005)

4.3. Correlation between type of hemoglobin and age group

A fifteen participant divided into age interval group, the group which showed high result for Hb SS was (1-10) years (26) 100.0% AS while the other group result as follow: (11-20) year (14) 93.3% for Hb SS and (1) 6.7% for Hb AS the three other group (21-30)(31-40)(41-50) give negative result for Hb SS while their result to Hb SA was (6) 100%, (2) 100% and 100% respectively.
Table 4.3: Correlation between type of hemoglobin and age group:

<table>
<thead>
<tr>
<th>Age</th>
<th>Type of Hb</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb SS</td>
<td>HbAS</td>
<td>Hb SS</td>
</tr>
<tr>
<td>1-10</td>
<td>Count</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>11-20</td>
<td>Count</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>93.3%</td>
<td>6.7%</td>
</tr>
<tr>
<td>21-30</td>
<td>Count</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>31-40</td>
<td>Count</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>41-50</td>
<td>Count</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

(P.value = 0.00)

4.4. Measurement of the blood parameter participant

Complete blood count was done to the case and control. The result was calculated to detect the mean, standard deviation, and p.value. The significant (p.value) was found in red blood indices Hb(p.value; 0.00), PCV(p.value; 0.00), RBCs(p.value; 0.00), reticulocitye(p.value; 0.00), and ESR(p.value; 0.02). While the white blood indices is not affected TWBC mean 7.720 /cumm (p.value; 0.1) also the platelet is not significant (233150/ l) (p.value; 0.5).
Table 4.5: Measurement of the blood parameter participant:

<table>
<thead>
<tr>
<th>Test</th>
<th>type of Hb</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hb SS</td>
<td>Mean</td>
<td>Std.</td>
<td>Deviation</td>
<td>HbAS</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.4</td>
<td>1.8</td>
<td></td>
<td>10.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Hb</td>
<td></td>
<td></td>
<td>18</td>
<td>6</td>
<td></td>
<td>37</td>
<td>4</td>
</tr>
<tr>
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<td>5980</td>
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<td>279999</td>
<td></td>
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<td>3.3040</td>
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<tr>
<td>MCV</td>
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<td>6</td>
<td></td>
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</tr>
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<tr>
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<td></td>
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<tr>
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<td></td>
<td></td>
<td>39</td>
<td>8</td>
<td></td>
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<td></td>
<td>2</td>
<td>2</td>
<td></td>
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<td>2</td>
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Hb= Hemoglobin , PCV =pack cell volume ,TWBCs= total white blood cells ,RBCs= red blood cells ESR erythrocyte sedimentation rate, MCV mean cell volume , MCH=mean cell hemoglobin , MCHC=mean cell hemoglobin concentration , N= neutrophil ,L= lymphocyte ,M= monocyte ,E= eosinophil ,B= basophil significant of (P.value less than 0.005)

4.5.Distribution of sickle anemia among tribe:
The record information which collected to conduct the study include tribe ,after analysis of date the tribe that had large frequency number of participant tribes were Mesriria(19) 38.0% followed by Hawsa (7) 14%, Barno(5) 10% and Silahab(5) 10% . three of the tribe had same
frequency number of case was Ben.hasain, Mena and Zanda(2)4% for each one. The frequency found in Mahas, Noba, Rizagat and Tama which is(1) 2% for all of them. Table (4)

Table 4.6: Distribution of sickle anemia among tribe:

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Frequency</th>
<th>Percent</th>
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<tr>
<td>Barno</td>
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</tr>
<tr>
<td>Falata</td>
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<td>6.0</td>
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<tr>
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<tr>
<td>Mena</td>
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<td>4.0</td>
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<tr>
<td>Mesriria</td>
<td>19</td>
<td>38.0</td>
</tr>
<tr>
<td>Noba</td>
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<tr>
<td>Rizagat</td>
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<tr>
<td>Silahab</td>
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<tr>
<td>Taisha</td>
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<tr>
<td>Tama</td>
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<td>Zanda</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td><strong>100.0</strong></td>
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CHAPTER FIVE

5. Discussion

5.1. Discussion:

There is so much disparity in the haematological status of sickle cell anaemic patients and haemoglobin AA genotyped persons. The increase (P<0.1) in WBC, Neutrophil, and Lymphocyte and significant decrease (P<0.05) in the mean values of PCV but no significant change (P>0.1) in the mean values of Monocyte when compared between sickle cell anaemic patients and Hb AA subjects. The WBC is close to the reports of Ahmed et al (2006) which was 12.3 *10^9/L, Akinbami et al (2012) which was 10.27 *10^9/L and 11.6*10^9/L by Rao et al (2012). This increase could because of some oxidative stress. The significant decrease in PCV could be as a result of its effect on the bone marrow and on the kidney because of its multi systemic nature. The PCV was lower than what was reported by Ahmed et al(2006) which was 24%, Akinbami et al (2012) which was 24.44%. This could be as a result of changes in diet and management of the patients. Patients with sickle cell disease (SCD) generally have a background rate of red cell sickling, which drastically shortens the life span of red cells leading to a chronic haemolytic anaemia and jaundice even in steady state (Kaul et al, 1996).

This study was conducted to investigate the sickle cell disease and sickle cell trait among Meseria tribe in Heglig area in Southern West of Kordofan. One hundred individuals of Meseria were investigated for, sickling test, full blood count and haemoglobin electrophoresis this study done by Malik Hassan et al, 2013 the result finding was agree with our study.

The mean age of the sickle cell disease patients was significantly lower than that of normal individuals (haemoglobin AA) and sickle cell trait patients (p <0.008), there was suggestion of a direct relationship between the young age and the sickle cell disease. The total erythrocytes, haemoglobin concentration and packed cell volume were significantly decreased in sickle cell disease (p < 0.000) when compared with normal and sickle cell trait, which indicated to anaemia in sickle cell disease patients, this results agreed with previous study which reported that anaemia is significantly more often in patients with sickle cell disease compared with sickle cell trait at all ages 3 months and older, Losek JD, et al., (1992).

There were no significantly differences in sickle cell trait and normal individuals in mean cell haemoglobin concentration, mean cell volume and mean cell haemoglobin. This indicated no morphological differences between sickle cell trait (AS) and normal (AA) individuals.
Similar results was obtained by John et al 2003, who reported that, blood pictures in sickle cell trait group and normal individuals were normocytic normochromic and microcytic hypochromic. The total leukocytes count was significantly elevated in sickle cell disease when compared with normal individuals also total and segmented leukocyte numbers were greatly increased during vasoocclusive crisis and ininfection, but only with bacterial infection was there a consistent increase in bands or non-segmented leukocytes (mean, 4,580/μl). On the basis of these data we believe that total and differential leukocyte counts are of value for identifying those children with potentially serious bacterial infections, Buchanan GR & Glader BE, (1978).

Despite a clear role for leukocytes in modulating the pathophysiology of sickle cell disease (SCD), the mechanism by which leukocyte numbers are increased in this disorder remains unclear. Hypothesizing that the chronic inflammatory state, elicited by adhesive interactions involving various cell types, might underlie leukocytosis, Conran N, et al., (2007). The platelet count was significantly increased in sickle cell disease patients suggesting the contribution of platelets in the vasoocclusive phenomena found in sickle cell anaemia, Ibanga IA, (2006). Sickle cell diseases is a genetic abnormality involving the haemoglobin, although, it is primarily a red cell disorders, the white blood cells and platelets are also affected by the mutation, Akinsegun A., et al., (2012). The characteristics of Hb SS disease observed in this study did not differ significantly from those of other studies in Nigeria, Nduka, N, et al., (1993), frequencies lower than that were reported in Bahrain, Sultanate of Oman and Turkey in which SCT frequencies were 7%. 10% and 0.5% respectively, Buhazza MA, et al., (1985); Al Arrayed SS, et al., (1995).

The distribution of the S gene among various ethnic and linguistic groups in the Sudan studied, Mohammed AO A, (2006), they found that, the majority of patients (93.7%) belonged to families of single ethnic descent, indicating the high degree of within-group marriages and thus the higher risk of augmenting the gene.
CHAPTER SIX
6. Conclusion and Recommendations

6.1. Conclusion:

- Meseria tribe was the most tribe contain large prevalent number of SCA.
- Sicklers diagnosed by hemoglobin electrophoresis also tested positive for solubility and sickling test was also positive.
- Mean Hb concentration was 5.4 g/dl with high readings for MCV, MCH, MCHC.
- Hemoglobin parameters are helpful in following up patients in steady state of sickle cell anemia.

6.2. Recommendations:

1. Hemoglobin parameters must included in the follow up of patients in steady state should be studied for vaso-occlusive crisis as well in Sudan.
2. Pre married testing for Genetic sickles to avoid SCA.
3. PCR must be included as diagnostic tool.
4. Education the population about the hazard of relative married.
5. Many research must carried to find solution for SCA treatment.
Reference: