Sudan University of Science and Technology

Collage of Graduate Studies

Assessment of Complete Blood Count of Sickle Cell Anemia Patients Attending Some Hospitals in Khartoum State

قياس تعداد الدم الكامل لمرضى الأنيمياء المنجلية المترددين على بعض مستشفيات ولاية الخرطوم

A Dissertation Submitted in Partial Fulfillment for Requirements of M.Sc.
Degree in Hematology and Immunohematology

Submitted by:

Rana Kamal Yagoob Babiker

B.Sc. Medical Laboratory Science Hematology (Omdurman Islamic University) 2011

Qualifying Year (University of Khartoum) 2012

Supervisor:

Dr. Khalda Mirghani Hamza

2014

الآية

بسم الله الرحمن الرحيم

[إِنَّ اللهَ فَالِقُ الْحَبِّ وَالنَّوَى يُخْرِجُ الْحَيَّ مِنَ الْمَيِّتِ
وَمُخْرِجُ الْمَيِّتِ مِنَ الْحَيِّ ذَلِكُمُ اللهُ فَأَنَّى
تُؤْفَكُونَ]

صدق الله العظيم

سورة الأنعام الأية(95)

Dedication

This dissertation is dedicated to our fathers, who taught us that the best kind of knowledge to have is that which is learned for its own sake.

It is also dedicated to our mothers, who taught us that even the largest task can be accomplished if it is done one step at a time.

Acknowledgement

First grateful thanks to Allah….

Then to my Supervisor...Dr/ Khalda Mirgani Hamza for all the support and tolerance she showed with every one…

All lab staff of National Health Center, hematology department.

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

A lot of thanks to the members of my family and all my friends…

Abstract

This is case control study, conducted in some Khartoum State Hospitals during the period from March 2014 to May 2014. This study aimed to determine the complete blood count and frequency of sickle cell anemia among patients in some Khartoum State Hospitals.

Two hundred (200) blood samples were collected, one hundred fifty for sicklers and fifty for apparently healthy individuals as control. A questionnaire was designed to collect information about the study, 3ml of venous blood was collected in EDTA anticoagulant container and Automated Hematological Analyzer -Sysmex KX 21N- was used to investigate the hematological parameters. Blood sample was washed for preparing it for hemoglobin electrophoresis.

total erythrocyte count, hemoglobin level, packed cell volume and mean cell volume of the sickle cell anemia patients were (7.6± 2.1 g/dl), (2.8± 0.9×10^{12}/l), (23± 6.6 %) and (84±11.6 fl), respectively, was significantly decreased when compared with normal individual.

No significant differences in sickle cell anemia patients in mean cell hemoglobin concentration (32±2.6%), mean cell hemoglobin (28 ±6.2pg) and platelet ($330.5\pm142\times10^{3}/mm^{3}$), ($P<0.47$, $P<0.07$, $P<0.13$) respectively when compared with normal individuals.

The total leucocyte ($15.6\pm10.3\times10^{9}$/l) ($P<0.00$) was significantly elevated in sickle cell anemia when compared with normal individual.

The mean age of the sickle cell anemia patients was (5.2 year). The results showed that percentage of sickle cell trait and sickle cell disease were (25.5%) and (49.5%) respectively.

According to social data inter-marriage played an important role in spreading of sickle cell anemia and sickle cell anemia is more common in Meseria and Bargo tribes.

مستخلص البحث

هذه دراسة مقارنة مجموعة ضابطة أجريت في بعض مستشفيات الخرطوم في الفترة من شهر مارس 2014 إلى مايو 2014. تهدف هذه الدراسة إلى قياس تعداد الدم الكامل ونسبة التردد لمرضى الأنيميا المنجلية في بعض مستشفيات ولاية الخرطوم.

قد تم أخذ 200 عينة من دم من الاشخاص المتطوعين،150 عينة دم من المصابين بالانيميا المنجلية و50 عينة دم من اصحاء ظاهريا. صمم استبيان لجمع المعلومات من المشاركين في الدراسة. أخذت 3 مل من الدم غي حاوية تحتوي على مانع من التجلط وجهاز السيسمكس لقياس تعدد الدم الكامل . بعد ذلك تم غسل العينة لتحضيرها وتحليلها في اختبار خضاب الدم الممرحل كهربائيا.

كان متوسط خضاب الدم ،العدد الكلي لكريات الدم الحمراء ،كريات الدم الحمراء المتراكمة و متوسط حجم الخلية (7.6±2.1g/dl)،(.82±0.9x10^{12}/l)،(23±6.6%) و(84 ±11.6fl) على التوالي لمرضى الانيميا المنجلية زات نقصان بقيمة معنوية مقارنة بمجموعة الاصحاء.

كما لم توجد فروقات ذات قيمة معنوية في متوسط تركيز خضاب الدم للخلية (32± 2.6 g/dl) ومتوسط خضاب الدم للخلايا (28±6.2 pg) والصفائح الدموية (330 ±142×10^3/mm^3) عند مرضى الأنيميا المنجلية مقارنة مع الاصحاء.

ووجدت زيادة ذات قيمة معنوية في مجموع تعداد خلايا الدم البيضاء (l/ 15.6 x10^9±10.3) مقارنة بمجموعة الأصحاء.

متوسط عمر المرضى بالأنيميا المنجلية (5.2) سنة. أظهرت النتائج ان النسبة المنوية لحاملي مرض الأنيميا المنجلية والمصابين بالمرض كانت (25.5 %)(49.5 %) على التوالي.

كما أن نتائج الاستبيان أشارت إلى أن زواج الأقارب يلعب دورا كبيرا في زيادة مرض الأنيميا المنجلية وان الأنيميا المنجلية أكثر انتشارا في قبائل المسيرية والبرقو.

Contents

Topic		Page
الأية		I
Dedication		II
Acknowledgment		III
Abstract		IV
ملخص البحث		V
Table of contents		VI- VIII
List of Tables		IX
List of Figure		X
Abbreviations		XI
Chapter one **Introduction and Literature Review**		
1.1	Introduction	1
1.2	Review of Literature	2
1.2.1	Blood Constituents and Function	2
1.2.1.1	Plasma	2
1.2.1.2	Red Blood Cells	3
1.2.1.3	Hemoglobin	4
1.2.1.4	Hematocrit (HCT, packed cell volume, PCV)	5
1.2.1.5	Red Blood Cells and Indices	5
1.2.1.6	White Blood Cells	6
1.2.1.7	Blood Platelets	6
1.2.2	Blood disorders	7
1.2.2.1	Anemia	7
1.2.2.2	Classification of Anemia	8
1.2.2.3	Sickle Cell anemia	11

1.2.2.3.1	Sickle cell disease (Hb SS)	11
1.2.2.3.2	Sickle cell traits (Hb AS)	12
1.2.2.3.3	Combination of hemoglobin S with other genetic defects of hemoglobin	12
1.2.2.3.4	Molecular study of Sickle cell anemia	13
1.2.2.3.5	Laboratory diagnoses of sickle cell anemia	15
1.2.2.3.6	Red blood cells in sickle cell disease	16
1.2.2.3.7	White blood cells in sickle cell disease	17
1.2.2.3.8	Platelets in sickle cell disease	17
1.2.3 1.2.3.1	Previous Studies Previous studies in Africa	18
1.2.3.2	Previous Studies in Sudan	18
1.3	Rational	20
1.4 1.4.1	Objectives General objectives	21
1.4.2	Specific objectives	21
Chapter Two **Materials and Methods**		
2.1	Study Design	22
2.2	Study Areas and Duration	22
2.3	Study Populations	22
2.5	Inclusion and Exclusion criteria	22
2.5.1	Inclusion criteria	22
2.5.2	Exclusion criteria	23
2.6	Sample Size	23
2.7	Data analysis	23
2.8	Ethical Considerations	23
2.9	Method of Sample Collection	23

2.9.1	Requirement	23
2.9.2	procedure of sample collection	24
2.10	Principle of Sysmex KX-21 Hematology Analyzer	24
2.10.1	WBCs, RBCs, Platelet Counting and hemoglobin measurement	25
2.11	Hemoglobin electrophoresis	26
2.11.1	Principle	26
2.11.2	Equipments	26
2.11.3	Reagents	26
2.11.4	Procedure	27
Chapter Three Results		
	Result	28
Chapter Four Discussion, Conclusion and Recommendation		
4.1	Discussion	38
4.2	Conclusion	40
4.3	Recommendation	41
References		
	References	42
Appendixes		
	Appendixes	45

List of Tables

Table	Subject	Page
3.1	Distribution of study group according to age.	30
3.2	Distribution of study group according to type of hemoglobin.	31
3.3	Distribution of sickle cell anemia according to gender in study groups.	32
3.4	Distribution of sickle cell anemia according to consanguineous marriage in study groups.	33
3.5	Distribution of sickle cell anemia according to age in study groups.	34
3.6	Hematological parameter of sickle cell anemia compare with normal control.	35
3.7	Hematological parameter of sickle cell anemia (Hb SS and HbAS).	36
3.8	Distruibtion of sickle cell anemia patients according to tribes	37

List of figure

Figure	Subject	Page
1.1	Classification of sickle cell anemia	9
1.2	Sickle-cell disease is inherited in the autosomal recessive pattern.	15
1.3	Hemoglobin electrophoresis patterns	16

Abbreviations

CBC: Complete Blood Count

CO_2: Carbon Dioxide

EDTA: Ethylene Diamine Tetra Acetic Acid

Hb: Hemoglobin

HCT: Hematocrit

Hb S: Sickled Hemoglobin

Hb AS: Sickle Cell Trait

LCD: Liquid Crystal Displayer

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin concentration

MCV: Mean Corpuscular Volume

O_2: Oxygen

PCV: Packed Cell Volume

Plts: Platelets

RBCs: Red Blood Cells

SCD: Sickle Cell Disease

SCA: Sickle Cell Anemia

SPSS: Statistical Package for Social Sciences

SNP: Single Nucleotide Polymorphism

WBCs: White Blood Cells

Chapter One

Introduction and Literature Review

Chapter One

Introduction and Literature Review

1.1 Introduction

Sickle cell anemia is group of hematological disorders in which the β-globin abnormality is caused by substitution of valine for glutamic acid at position 6 in the β-globin. This hemoglobin becomes polymerized and poorly soluble when the oxygen tension is lowered and red cells that contain this hemoglobin become distorted and rigid (Marshall, 2005).

This condition is characterized by fever, chest pain, difficulty breathing, painful crisis, hemolytic crisis, aplastic crises and sequestration crisis (Henry, 2001).

Sickle cell anemia is widely spread in West Africa. Sickle cell anemia is known to be prevalent in Sudan and it has been suggested to be more common in population from Kordofan and Darfur (West Sudan), consanguineous marriage is more common throughout the Sudan and is a significant cause of the spread of the sickle cell anemia (Mustafa *et al*, 2013)

The aim of this study is to measure some hematological parameters and frequency of patients with sickle cell anemia attended some Khartoum State Hospitals, to detect hematological abnormalities associated with sickle cell anemia and compares it with normal individual.

1.2. Literature Review

1.2.1 Blood Constituents and Functions

Blood is a fundamental component of human life. Within the adult body. Approximately 4 to 5 liters of blood circulates continuously through an intricate network of vessels, driven by the powerful contractions of the beating heart. As blood moves away from the lungs and heart, passing through large arteries and winding into increasingly narrower and more complex networks of small vessels, it comes into contact with the individual cell of tissues. At this level, its primary functions is to feed these cells, delivery to them a multitude of nutrients, including oxygen – the most basic element necessary for human survival. In exchange for these beneficial nutrients blood picks up and carries away cellular waste, such as carbon dioxide, that will ultimately be removed from the body as blood travels back to the lungs (Rogers,2011).

In addition, blood plays a vital role in immune system and in maintaining a relatively constant body temperature. Blood is a highly specialized tissue composed of more than 4,000 different kinds of components. Four of the most important components are red cells, white cells, platelets, and plasma (Fox, 2006)

1.2.1.1 Plasma

Plasma consists of organic and inorganic substances dissolved in water. Plasma proteins constitute most of the plasma solutes, by weight, there are three types: albumin, globin and fibrinogen. Cells normally do not take up plasma proteins of, cells use plasma amino acids, not plasma proteins, to make their own proteins. Thus, plasma proteins must be viewed differently formed most the other organic constituents of plasma, use as the medium for transport to and from cells. In contrast most plasma

proteins perform their functions in the plasma itself or in the interstitial fluid (Widmaier *et al* 2006).

1.2.1.2 Red Blood Cells (Erythrocyte)

Erythrocytes have the shape of biconcave disk thicker at the edge than in the middle like doughnut with a center depression on each side instead of a hole. This shape and their small size (7µm in diameter) are important to the erythrocyte, so that oxygen and carbon dioxide can diffuse rapidly to and from the interior of the cell. Erythrocyte plasma membrane contains specific polysaccharides and proteins that differ from person to person and these confer upon the blood it's so called blood type or blood group.

The site of erythrocyte production is the soft interior of bones called bone marrow, specifically the red bone marrow. With differentiation, the erythrocyte precursors produce hemoglobin, but then they ultimately lose their nuclei and organelles- their machinery for protein synthesis. The production of erythrocytes requires the usual nutrient need to synthesize any cell, amino acid, lipid and carbohydrate. In addition both iron and certain growth factors including the vitamins, folic acid and vitamin B12, are essential (Widmaier *et al*,2006).

Erythrocytes are produced by process known as erythropoiesis which is stimulated by the hormone erythropoietin which is produced by the kidney in response to tissue hypoxia. (Baker and Silverton's, 1998)

Erythrocytes lack nuclei and organelles, they can neither reproduce themselves nor maintain their normal structure. The average life span of an erythrocyte is approximately 120 days, which means that almost 1 percent of the body‹s erythrocytes is destroyed and must be replaced every day. This amount to 250 billion cell per day. Erythrocyte destruction normally occurs in the spleen and the liver.

The major function of the erythrocyte is to carry oxygen taken in by the lungs and carbon dioxide produced by the cells. Erythrocyte contain large amount of hemoglobin with which oxygen and, to a lesser extent, carbon dioxide reversibly combined. Oxygen binds to iron atoms (fe^{++}) in the hemoglobin molecules. (Widmaier *et al*, 2006)

The normal range of RBCs:

Male $5.0\pm 0.5x10^{12}/l$

Female $4.3\pm 0.5x10^{12}/l$

Children $4.6\pm0.5x10^{12}/l$ (Dacie and Lewis, 2006)

The normal range in sudan:

Male: $4.2\text{-}5x10^{3}/\mu l$

Female: $4.6\text{-}5.6x10^{3}/\mu l$ (Elbashir and Osman).

1.2.1.3 Hemoglobin

Hemoglobin (Hb), which is contained in red blood cells, the main function of red cells is to carry O_2 to the tissues and to return carbon dioxide (CO_2) from the tissues to the lungs. In order to achieve this gaseous exchange they contain the specialized protein hemoglobin. Each red cell contains approximately 640 million hemoglobin molecules. Each molecule of normal adult hemoglobin (HbA), (the dominant hemoglobin in blood after the age of 3-6 months) consists of four polypeptide chains, $\alpha_2\beta_2$each with its own hem group. Normal adult blood also contains small quantities of two other hemoglobins: Hb F and Hb A2. These also contain αchains, but with contain γ and δchains, respectively, instead of β. The major switch from fetal to adult hemoglobin occurs 3-6 months after birth.

Haem synthesis occurs largely in the mitochondria by a series of biochemical reactions commencing with the condensation of glycine and succinyl coenzyme A under the action of the key rate limiting enzyme

δ-aminolaevulinic acid (ALA) synthase. Pyridoxal phosphate (vitamin B_6) is a coenzyme for this reaction which is stimulated by erythropoietin. Ultimately, protoporphyrin combines with iron in the ferrous (Fe^{2+}) state to form hem, each molecule of which combines with a globin chain made on the polyribosomes. A tetramer of four globin chains each with its own hem group in a 'pocket' is then formed to make up a hemoglobin molecule. (Hoffbrand, 2001)

Normal range of hemoglobin:

Men 15± 2.0 g/dl

Women 13.5± 1.5 g/dl

Children 14 ± 2.5 g/dl (Dacie and Lewis, 2006).

1.2.1.4 Hematocrit (HCT, packed cell volume, PCV)

Hematocrit is the proportion of blood volume that is occupied by red blood cells. Hematocrit measurement is considered an integral part of complete blood count results. Most of the modern automated analyzer has the facility to measure hematocrit. Both elevated and depressed value of hematocrit is suggestive of some malfunctioning on the body. (Singh, 2010).

Reference value of HCT in men (45±5%) and female (41±5%). (Dacie and Lewis, 2006).

1.2.1.5 Red Blood Cells and Indices

From the estimated content, HCT and red blood cell count, it is possible to drive other values, which indicate the red cell volume, hemoglobin content and concentration in the red cell. These values are commonly referred to as red blood cell indices.

The Red blood indices are:

- Mean corpuscular volume (MCV) = (PCV÷ RBCs) x 10

  The volume or size of the average RBC

- Mean corpuscular hemoglobin (MCH) = (Hb ÷ RBCs) x10

  The weight of hemoglobin in the average RBC
- Mean corpuscular hemoglobin concentration (MCHC) = (Hb ÷ PCV) x100

  The hemoglobin concentration or color of the average RBC (Pal and Pal, 2005).

  The normal range of MCV (92±9 fl), MCH (29.5±2.5 pg) and MCHC (33±1.5 g/dl). (Dacie and Lewis, 2006)

1.2.1.6 White Blood Cells

The primary function in the body is to defense the tissue against infection and substance foreign to the body. A normal adult count is about 4000-11000x10^{12}/l WBCs of blood. White blood cells of various types develop by a complex process in the body's red bone marrow. All white blood cells enter the circulation by diapedesis, and some complete their maturation elsewhere.

White blood cells live for several hours or several months, depending on their type and many white blood cells leave the circulation by diapedesis to mingle among the tissue cells.

There are two major groups of white blood cells, the granulocytes and agranulocyes. Granulocytes have granules in their cytoplasm and include neutrophils, eosinophils and basophils. Agranulocyte have no granules in their cytoplasm and include monocytes and the lymphocytes which are T-lymphocytes and B-lymphocytes. (Aclamo and Krumhard, 2004)

Normal range in Sudan:

Male: 4.0-6.2x10^3/µl

Female: 4.2-6.4x10^3/µl (Elbashir and Osman).

1.2.1.7 Blood Platelets

Blood platelet are the smallest cells of blood average about (2-4 µm), although much numerous 150-400x10^9/l than the white blood cells, they occupy a much smaller fraction of the volume of the blood because of their relatively minute size.

They lack nucleus, but they have a more complex metabolism and internal structure. Platelets adhere to each other but not to red cells and white cells. Tiny granules within platelets contain substance important for the clot performing activity of platelet. The function of the platelets is related to hemostasis, the prevention and control of bleeding. (Rogers, 2011).

Normal range in Sudan:

Female: 205.4-350.2x10^3/µl

Male:179.0-324x10^3/µl (Elbashir and Osman).

1.2.2 Blood Disorders

Blood disorders affect one or more parts of the blood and prevent the blood from doing its function. Types of blood disorders include:

- Platelet disorders, excessive clotting, and bleeding problems, which affect how the blood clots
- Anemia, which happens when blood does not carry enough oxygen to the rest of the body
- Cancers of the blood cells, such as leukemia and myeloma
- Eosinophilic disorders, which are problems with one type of white blood cell (www.nlm.nih.gov).

1.2.21 Anemia

This is defined as a reduction of the hemoglobin concentration in the blood. Although normal values can vary between laboratories, typical values would be less than 13.5 g/dL in adult males and less than11.5 g/dL in adult females. From the age of 2 years to puberty, less than 11.0 g/dL indicates anemia. As newborn infants have a high hemoglobin level, 14.0 g/dL which is taken as the lower limit at birth. Reduction of hemoglobin is usually accompanied by a fall in red cell count and packed cell volume (PCV) but this maybe normal in some patients with subnormal hmoglobin levels(and therefore by definition anemic).Mild anemia often causes no symptoms. More severe anemia can cause fatigue, pale skin, and shortness of breath with exertion (Hoffbrand, 2001).

1.2.2.2 Classification of Anemia

1.2.2.2.1 Etiological classification

Impaired RBCS production

Excessive destruction

Blood loss (Martin*et al*, 1998)

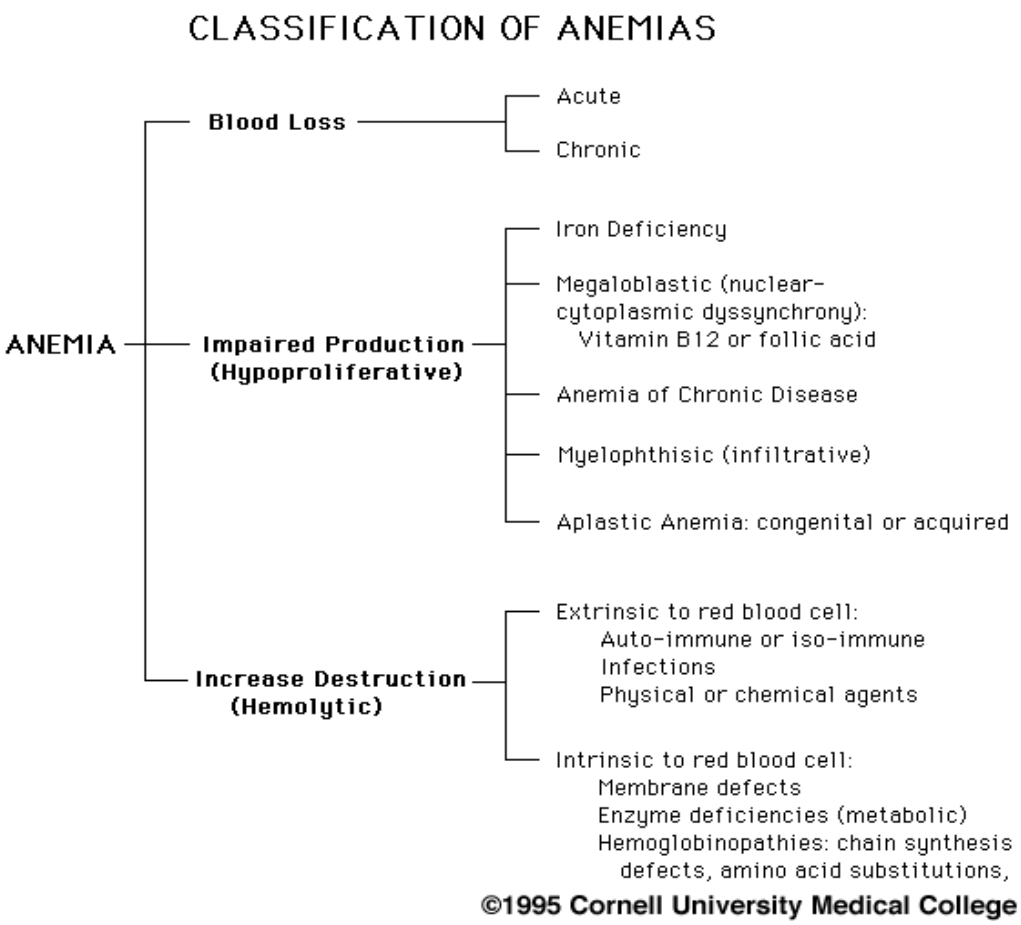

Figure (1.1): Classification of anemia. (http://www.oocities.org/zaferyedi/hematopathology.html)

1.2.2.2.1 Morphological classification

Macrocytic anemia

Macrocytic anemia can be further divided into "megaloblastic anemia" or "non megaloblastic macrocytic anemia". The cause of megaloblastic anemia is primarily a failure of DNA synthesis with preserved RNA synthesis, which results in restricted cell division of the progenitor cells. The megaloblastic anemias often present with neutrophil hypersegmentation (six to 10 lobes). The non megaloblastic macrocytic anemias have different etiologies (i.e. unimpaired DNA globin synthesis,) which occur, for example, in alcoholism (Martin *et al*, 1998).

Microcytic hypochromic

Microcytic anemia is primarily a result of hemoglobin synthesis failure or insufficiency, which could be caused by several etiologies:

- Heme synthesis defect
    - Iron deficiency anemia (microcytosis is not always present)
    - Anemia of chronic disease (more commonly presenting as normocytic anemia)
- Globin synthesis defect
    - Alpha-, and beta-thalassemia
    - HbE syndrome
    - HbC syndrome
    - Various other unstable hemoglobin diseases
- Sideroblastic defect
    - Hereditary sideroblastic anemia
    - Acquired sideroblastic anemia, including lead toxicity (Martin *el al*. 1998).

Normocytic Normochromic Anemia

Normocytic anemia occurs when the overall hemoglobin levels are decreased, but the red blood cell size (mean corpuscular volume) remains normal. Causes include:

- Acute blood loss
- Anemia of chronic disease
- Aplastic anemia (bone marrow failure)
- Hemolytic anemia (Martin *et al*, 1998)

1.2.2.3 Sickle Cell Anemia

The term “sickle cell disease” refers to a collection of autosomal recessive genetic disorders characterized by the Hb S variant of the β-globin gene.

Individuals who are affected with sickle cell anemia have two copies of this variant (Hb SS), and the primary hemoglobin present in their red blood cells is sickle hemoglobin. Individuals affected with other types of sickle cell disease are compound heterozygotes. They possess one copy of the Hb S variant and one copy of another β-globin gene variant, such as Hb C or Hb β-thalassemia. These individuals produce a mixture of variant hemoglobin’s. Carrier individuals have one copy of the sickle variant and one copy of the normal β-globin gene (Hb AS), producing a mixture of sickle hemoglobin and normal hemoglobin. The carrier state for sickle cell disease is often referred to as “sickle cell trait. The distribution of sickle cell anemia provides evidence for origin of the mutation in several locations within Africa (the Senegal, Benin and Bantu haplotypes) and Asia (the Arab–Indian haplotype) (Hoffbrand, 2005). An Hb S gene mutation has provided resistance, but not immunity, from the malaria parasite *plasmodium falciparum*, which is transmitted by mosquitoes. When a red cell containing p.falciprum undergoes the sickling process, the parasite dies.(Martin*et al*, 1998).

1.2.2.3.1 Sickle Cell Disease (Hb SS)

Sickle cell disease is a worldwide disorder that occurs when the sickle (S) gene is inherited from both parents (the homozygous state).When oxygenated, Hb S fully soluble. Sickling occur when oxygen decrease at the tissue level. When oxygen released from the Hb molecule, a comformational change occurs, which results in polymerization of Hb molecule and lead to formation of crystals, which lead to become rigid.

Sickle cell impeded blood flow to tissue and organs, resulting in tissue death, organ infraction and pain (Martin*et al*, 1998).

Clinical features are of a severe hemolytic anemia punctuated by crises. The symptoms of anemia are often mild in relation to the severity of the anemia because Hb S gives up oxygen (O_2) to tissues relatively compared with Hb A. The clinical expression of Hb SS is very variable, Some patients having an almost normal life, free of crises but others develop severe crises even as infants and may die in early childhood or as young adults. Crises may be vaso-occlusive, visceral, aplastic or hemolytic. (Hoffbrand, 2001)

1.2.2.3.2 Sickle cell traits (Hb AS)

Sickle cell trait is the heterozygous (AS), because Hb A is present in higher percentages than Hb S, Hb compensates for HbS, and these patients usually have no symptoms. The disorder may go undetected, but patients may experience the pain-ful crises described for HbSS if they encounter situations that cause extreme tissue hypoxia, such as those caused by severe respiratory infection or exposure to extreme cold.(Martin*et al*, 1998)

This is a benign condition with no anemia and normal appearance of red cells on a blood film. Hb S varies from 25 to 45% of the total hemoglobin. Care must be taken with anesthesia, pregnancy and at high altitudes. (Hoffbrand, 2001).

1.2.2.3.3 Combination of hemoglobinS with other genetic defects of hemoglobin

The most common of these are HbS/β-thalassaemia, and sickle cell /C disease. In HbS/β-thalassaemia, the MCV and MCH are lower than in homozygous HbSS. The clinical picture is of sickle cell anemia; splenomegaly is usual. Patients with HbSC disease have a particular

tendency to thrombosis and pulmonary embolism, especially in pregnancy. In general, when compared with HbSS disease, they have a higher incidence of retinal abnormalities, mild eranemia, splenomegaly and generally a longer life expectancy. Diagnosis is made by hemoglobin electrophoresis,

Particularly with family studies (Hoffbrand,2001)

1.2.2.3.4 Molecular Detection of Sickle Cell Anemia:

Sickle-cell gene mutation probably arises spontaneously in different geographic areas. In people heterozygous for HbS (carriers of sickling haemoglobin), the polymerisation problems are minor, because the normal allele is able to produce over 50% of the haemoglobin. In people homozygous for HbS, the presence of long-chain polymers of HbS distort the shape of the red blood cell from a smooth doughnut-like shape to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries. Carriers have symptoms only if they are deprived of oxygen (for example, while climbing a mountain) or while severely dehydrated. The sickle-cell disease occurs when the seventh amino acid (if the initial methionine is counted), glutamic acid, is replaced by valine to change its structure and function.

The gene defect is a known mutation of a single nucleotide (single-nucleotide polymorphism - SNP) (A to T) of the β-globin gene, which results in glutamic acid being substituted by valine at position 6. Haemoglobin S with this mutation is referred to as HbS, as opposed to the normal adult HbA. The genetic disorder is due to the mutation of a single nucleotide, from a GAG to GTG codon mutation, becoming a GUG codon by transcription. This is normally a benign mutation, causing no apparent effects on the secondary, tertiary, or quaternary structure of hemoglobin in conditions of normal oxygen concentration. Under

conditions of low oxygen concentration, is the polymerization of the HbS itself. The deoxy form of hemoglobin exposes a hydrophobic patch on the protein between the E and F helices. The hydrophobic residues of the valine at position 6 of the beta chain in hemoglobin are able to associate with the hydrophobic patch, causing hemoglobin S molecules to aggregate and form fibrous precipitates.

The allele responsible for sickle-cell anemia is autosomal recessive and can be found on the short arm of chromosome 11. A person that receives the defective gene from both father and mother develops the disease; a person who receives one defective and one healthy allele remains healthy, but can pass on the disease and is known as a carrier. If two parents who are carriers have a child, there is one in four chance of their child developing the disease and one in two chance of their child's being just a carrier. Since the gene is incompletely recessive, carriers can produce a few sickled red blood cells, not enough to cause symptoms, but enough to give resistance to malaria. Because of this, heterozygotes have a higher fitness than either of the homozygotes. This is known as heterozygote advantage (Hoffbrsnd, 2005).

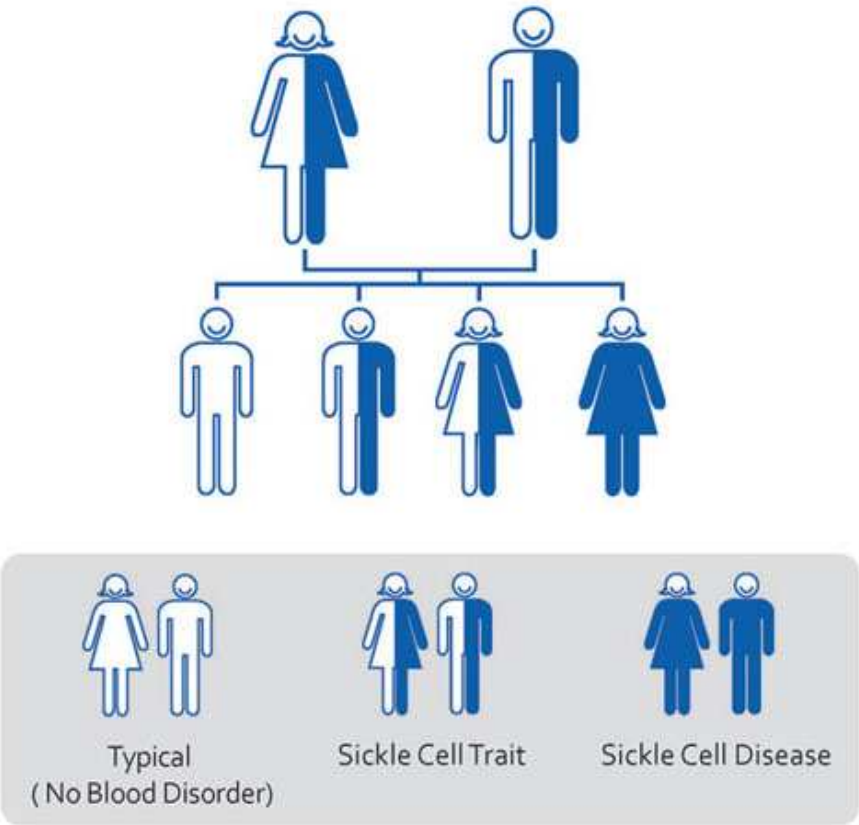

Figure (1-2): Sickle-cell disease is inherited in the autosomal recessive pattern.(http://www.cdc.gov/ncbddd/sicklecell/traits.html)

1.2.2.3.5 Laboratory diagnoses of sickle cell anemia

The hemoglobinis usually 6-9 g/dL-low in comparison to symptoms of anemia, the peripheral blood picture depend upon the type of sickle cell syndrome. In HbSS disease, the red cells are normocytic and normochromic, with polychromasia, many sickle cell and fewer target cells. The average reticulocyte count is 10% (4–20%), and normoblasts may be observed. Red cells are microcytic in the presence of coexisting ß-thalassaemia or iron deficiency. Screening tests for sickling are positive when the blood is deoxygenated (e.g. with dithionate and Na2HP04) · Hemoglobin electrophoresis in HbSS, no Hb A is detected. The amount of Hb F is variable and is usually 5-15%, larger amount are normally

associated with a milder disorder. Prenatal diagnosis is available through direct detection of the GAG→GTG mutation responsible for SCD in the fetal cells (Hoffbrand, 2001)

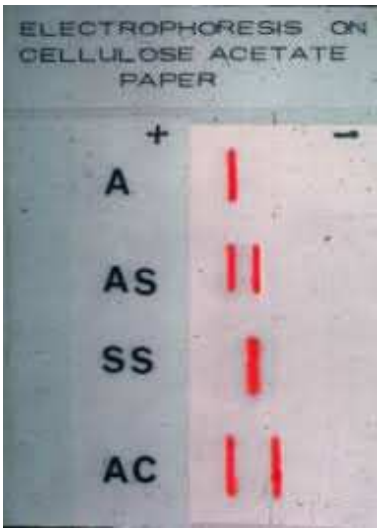

Figure (1-3): Hemglobin eletrophersis patterns.
(http://www1.imperial.ac.uk/departmentofmedicine/divisions/experimentalmedicine/haematology/morphology/bain/images/slide18/)

1.2.2.3.6 Red blood cells in sickle cell disease

Quantitative and qualitative changes in red blood cells have been reported. Hemolysis consequent to the damaged red cell membrane could be intravascular or extravascular. The former results from the lysis of complement-sensitive red cells and hemoglobin lost during sickling-induced membrane damaged. The latter, occurs by phagocytosis of red cells that have undergone sickling and physical entrapment of rheologically compromised red cells. Increased susceptibility to mechanically induced cell fragmentation has been documented in-vitro and in sickle cell patients undergoing vigorous exercise. Degree of hemolysis is inversely related to hemoglobin concentration and packed cell volume in sickle cell anemia patient. Numerous factors affect hemolysis in sickle cell anemia, percentage of irreversible sickle cell is of

greatest significance. The degree of hemoglobin polymer formation, calculated from the mean corpuscular hemoglobin concentration and the relative proportion of hemoglobin fractions also correlates closely with the severity of hemolysis **(**Akinbami*et al*,2012)

1.2.2.3.7 White blood cells in sickle cell disease

Although sickle cell disease is primarily a disease of the red blood cell, leucocytes, because of their sizes obstruct blood vessels more effectively than red blood cells when attached to the endothelium. The red blood cells measures 7.2 μm, while small lymphocytes measures 10 μm, neutrophils 10–14 μm , large lymphocytes 12–16 μm, monocytes 14-20 μm. Bacterial infection associated with leucocytosis is a known predisposing factor to sickle cell disease crises. A high absolute neutrophil count showed statistically significant relationship with clinical severity of sickle cell anemia. Many complications of sickle cell disease are associated with leucocytosis. It is a risk factor for early sickle cell disease –related death. It is implicated in clinically overt stroke. **(**Akinbami *et al*, 2012)

1.2.2.3.8 Platelets in sickle cell disease

Unlike the red and white blood cells, the clinical effects of platelets on sickle cell disease are not well established. However, an association between stroke in sickle cell disease and platelet count > 450,000/μl has been reported. Qualitatively, poor platelet aggregation responses to epinephrine and ADP were also reported in sickle cell disease.**(**Akinbami *et al*, 2012)

1.2.3 Previous Studies

1.2.3.1 Previous studies in Africa

- Okwi*et al*, (2007) established the prevalence of the SCT in Uganda. The established prevalence of the SCT (As) in Eastern Uganda was 17.5% compared to 13.4% and 3% in Bundibugyo and Mbarara/Ntungamo respectively. 1.7% of the children in Eastern Uganda tested positive for hemoglobin SS relative to 3% in Bundibugyo, giving gene frequencies of 0.105 and 0.097 for the recessive gene espectively.
- Taiwo et al (2011) determine The frequency of sickle cell genotype (HbSS) among the Yorubas living in Lagos, Nigeria, who found that normal and sickle cell hemoglobin genotypes were detected in subjects within the age group 1–50 years such that 366 (73.1%) had HbAA genotype, 123(24.5%) had HbAS, while 12 (2.4%) had HbSS.More than half (58.3%) of the subjects with the HbSS were in the 11–30 years age group.

1.2.3.2 Previous Studies in Sudan

- Osman and Alfadni. (2010) estimated the prevalence of sickle cell anemia in (Algadaref State). The study revealed that the majority of sickle cell anemia cases were found among the Masaleet tribe. 20 samples were (HbSS), 55samples were (HbAS) and 25 samples were (Hb AA).
- Munsoor and Alabid.(2011) estimated the frequency of sickle cell trait (HbAS) among patients suffering from sickle cell disease (SCD) who referred to Elobied Hospital. One hundred persons of seventeen different tribes were included (48% males and 52% females) with an age range between 4 to 70 years. The results of hemoglobin electrophoresis showed that, 54% of target samples

were heterozygous carrier (HbAS) while 42% were normal (HbAA) and 4% were diagnosed as sickle cell disease (HbSS). The highest distribution of sickle cell trait was among Bederia tribe 9 (23.1%) followed by Fulani and Selehab 6 (15.4% for each tribe).

- Mustafa *et al*. (2013) determine the sickle cell trait frequency in Sudanese patient living in Heglig area in Western of Southern Kordofan state. In their study the frequency of sickle cell trait and sickle cell disease (52%) and (14%) respectively and 34% were normal. The total erythrocytes, hemoglobin concentration and packed cell volume was significantly decreased in sickle cell disease ($p < 0.000$) compared with normal and sickle cell trait and the higher values of white blood cells and platelets compared to hemoglobin phenotype AA control participants.

1.3 Rational

Sickle cell anemia causes sever complications and it may lead to death particularly during early childhood through painful crisis, hand foot syndrome, stroke, fatigue, and shortness of breath. In Sudan there are many previous studies about the sickle cell anemia done before, for example in Heglig Area -Mustafa *et al*- reported that the frequency of sickle cell trait (52%) and sickle cell disease and (14%) , Osman and Alfadni –Algadaref State- founded that 20% of samples are sickle cell disease and 55% of samples are sickle cell trait and in Elobied Hospitals –Monsoor and Alabid- reported that 54% of target samples are heterozygous carrier and 4% are sickle cell disease. Furthermore the spread of the sickle cell anemia is increased in Sudanese. So that was needed for further study to investigate the disease and to evaluate of complete blood count of disease

1.4 Objectives

1.4.1General objectives:

Assessment of complete blood count of Sudanese patient with sickle cell anemia attended some Khartoum State Hospitals.

1.4.2 Specific objectives:

1. To measure of complete blood count (Hb, RBCs, WBCs, PCV, MCV, MCH, MCHC and Plts) for sickle cell anemia patients and compare with normal individual as control
2. To determine frequency of sickle cell anemia in patients attended some Khartoum State Hospitals
3. To study distribution of sickle cell anemia patients according to age, sex contagious marriage and tribes.

Chapter Two

Material and Method

Chapter Two

Material and Method

2.1. Study Design

This is case control study.

2.2. Study Areas and Duration

This study was conducted in Khartoum State in different laboratory centres (Khartoum Teaching Hospital), (Biochemistry Department, Faculty of Medicine, University of Khartoum), (Haematology Department, National Health Laboratory), (Omdurman Teaching Hospital),(Alboulk Hospital) and (Jafer Ibn Ouf Hospital) during period from March 2014 to May 2014.

2.3. Study Populations

The study populations were patient diagnosed with sickle cell disease based on their hemoglobin electrophoresis on cellulose acetate paper at alkaline pH or sickling test, who attended one of the laboratory centres either for their regular chick up or for diagnosis and evaluation.

Some of control group was selected from relatives. Others were choosing from different hospitals after the apparently health or not suffering from disease that may affect the parameters under study.

2.4. Inclusion and Exclusion criteria

2.4.1 Inclusion criteria

All patients with SCD homozygous and heterozygous from different tribes, with age ranged between 5 months and 17 year. Both sexes were included.

2.4.2. Exclusion criteria

Sickle cell anemia patients treated with blood transfusion at least one month. Older patients with sickle cell anemia (more than 17 years).

2.5. Sample Size

Samples were selected by simple random sampling method. The sample size was two hundred, one hundred fifty patients with sickle cell anemia and fifty controls.

2.6. Data analysis

The collected data was analyzed to obtain the mean, standard division, frequency and excreted P. value of the sampling using statistical package for social science (SPSS) computer programmed version 16.

2.7. Ethical Considerations

It was considered that all information that obtained from patients was kept highly confidential data and specimens. The participatients were provided with information about the study and any risk that may arise especially when the collection technique was applied. Because some populations of the study were children, their parents were asked for consent, and blood sample was collected after the consent of patients or patient's parent.

2.8. Methods of Sample Collection

2.8.1. Requirement

- Ethylene-diamine- tetra-acetic acid EDTA
- cotton
- 70% Alcohol
- Disposable syringes
- Tournique.

- **2.8.2 Procedure of sample collection**

1. Patients were either sat or lid down on an examination table.
2. The arm was positioned on the armrest so that the vein identified become under some tension and its mobility was reduced.
3. The skin was cleaned with 70% ethanol and allowed to dry.
4. Personal details were checked up on the forms and on blood vials.
5. Tourniquet was applied to the arm, tight sufficiently to distend the vein, but not rightly to cause discomfort.
6. 3 ml of blood samples were taken from the superficial vein of the fore arm.
7. Blood was collected in K2EDTA, blood sample was analysed by sysmex. (Dacie and Lewis, 2006).

2.9. Principle of Sysmex KX-21 Hematology Analyzer

The KX-21 performs speedy and accurate analysis of 18 parameters in blood and detects the abnormal samples. To assure easy sorting of abnormal samples in the laboratory, the instrument displays abnormal analysis data with abnormal marks attached on the LCD screen. Thus displayed analysis data allows detecting those samples which are outside the tolerance and need further analysis and reconsideration.

The KX-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method (Sysmex American Inc., 2003).

Reagents and materials

Provide by sysmex manufacture and contain:

1. stromatolyzer
2. Cell pack
3. Detergent
4. Cell cleaner

2.9.1. WBCs, RBCs, Platelet Counting and hemoglobin measurement

This instrument performs blood cell count by DC detection method (Sysmex American Inc, 2003).

DC Detection Method

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both sides of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size are detected as electrical pulses.

Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data (Sysmex American Inc, 2003).

Non-Cyanide Hemoglobin Analysis Method

To analyze hemoglobin by automated methods, the Cyanmethemoglobin method or Oxyhemoglobin method have so far been the main stream (Sysmex American Inc, 2003).

2.10 Hemoglobin electrophoresis

2.10.1 Principle

At alkaline PH, hemoglobin is a negatively charged protein and when subjected to electrophoresis will migrate toward the anode +ve structural variants that have a change in the charge on the surface of the molecule at alkaline PH will be separated from HbA. Hemoglobins variant that have an amino acid substitution that internally sited may not effect on overall charge and it will not separate by electrophoresis (Henry, 2001).

2.10.2 Equipments

- Horizontal electrophoresis tank.
- Power supply capable of delivering 350 V.
- Cellulose acetate membrane paper.
- Applicator.
- Stain jar.

2.10.3 Reagents

- Hemolysing reagent
- Electrophoresis buffer-TEB PH 8.5
- protein stain
- Destaining solution
- Cleaning solution

2.10.4 Procedure

- Lysate was prepared (lyse 1 volume of washed packed cells in 4 volume of lysing reagent consist of 3.8g EDTA +0.7g of potassium cyanide to 1 liter of water).
- With the power supply disconnected, the component was filled of the electrophoresis tank were filled with TEB buffer. Sock and position the wicks.
- In a separate dish the cellulose acetate membrane was socked in TEB buffer for at least 5 minute membrane was immersed slowly, to saturation of the membrane.
- Small volume (10µ) of each diluted sample was placed in to a sample well.
- The applicator was dipped in to sample well.
- The samples were applied to the cellulose acetate approximately 3 cm from one end of the membrane. Allow the applicator tips to remain in contact with the membrane for 3 second.
- Place the membrane upside down cross the bridge of the tank so the cellulose acetate surfaces in contact with the buffer, with the line of application at the cathode end.
- The power supply was applied and run at 250-350 V for 20minute or until a visible separation is obtain.
- The power supply was disconnected, the membrane was removed and stained in ponceau‹s for 3-5 minute.
- The membrane was dehydrated in absolute methanol for 3-2 min. and immersed in a cleaning solution for 6-8 min.
- The membrane was dried at 65c for 4-6 min and Labeled and store in a protective envelope (Hunstman *et al*, 2004).

Chapter Three

Results

Chapter Three

Results

The results of the study showed that the haematological parameter of patients with sickle cell anemia and normal individual as control.

According to the age, the sickle cell anemia patients divided into three group (table 3-1), the most distribution of age group ranged between 5 months and 5 years, while the least distribution of age group ranged between 11 and 17 years,

As showed in table (3-2), the highly distribution of study groups in sickle cell disease (49.5%), followed by sickle cell trait (25.5%) and (25%) as normal control.

In table (3-3) ,the distribution of male is more compared to female in study groups, in HbSS the distribution of female is increased when compared to female, while the distribution of male decreased when compared with female in HbAS.

As showed in table (3-4), 76% of sickle cell anemia patients are relatives. 53.3% of them have HbSS and 22.7% have HbAS.

Meseria tribes constitute 22%, 15.3% of them with Hb SS and 6.7% with Hb AS. Bargo is the next tribes which constitute 8% Hb SS and 6.7% Hb AS, table (3-5).

In this study showed that RBCS of sickle cell anemia ($2.8x10^{12}$ /l) SD (±0.9) decreased significantly P. value 0.00, compared to normal control (4.2) SD (±0.5)in table (3-7).

The means of haemoglobin of sickle cell anemia (7.6 g/dl) SD (± 2.1) decreased significantly (P.value 0.00), when compared to the control groups (12.9g/dl) SD (±1.5),(table 3-7).

PCV (23%) SD (±6.6) and MCV (84fl) SD (±11.6) of sickle cell anemia is significantly decreased compared to normal control (P.value 0.00), (table 3-7).

As showed in table (3-7), no significant difference in MCH (28pg) SD (±6.2), MCHC (32g/dl) SD (±2.6) and plts ($330.5x10^3/mm^3$) SD (±142) when compared with normal control (P.value 0.07, 0.46 , 0.13) respectively, while showed significantly increased of WBCs of sickle cell anemia ($15.6x10^9/l$) SD (±10.3) when compared with normal control.

In table (3-8) showed that significantly decreased of RBCs, PCV and Hb of sickle cell disease compared to sickle cell trait, while WBCs showed significantly increased in sickle cell disease compared to sickle cell trait. NO significant difference in MCV, MCH, MCHC and plts of sickle cell disease when compared with sickle cell trait, table (3-8).

Table (3-1): Distribution of study groups according to age.

Age	Patients		Control	
	Number	Percent%	Number	Percent%
5month-5year	75	50%	23	46%
6-10 year	56	37.4%	20	40%
11-17year	19	12.6%	7	14%
Total	150	100%	50	100%

Table (3-2): Distribution of study group according to type of hemoglobin.

	Frequency	Percent
HbSS	99	49.5%
HbAS	51	25.5%
HbAA	50	25.0%
Total	200	100.0%

Table (3-3): Distribtion of sickle cell anemia according to gender in study groups.

Gender	HbSS		HbAS		Total	
	Number	Percent	Number	Precent	Number	Percent
Female	45	30%	26	17.3%	71	47.3%
Male	54	36%	25	16.7%	79	52.7%
Total	99	66%	51	34%	150	100%

Table (3-4): Distribution of sickle cell anemia according to consanguineous marriage in study groups.

Type of consanguinity	Hb variant				Total	
	HbSS		HbAS			
	Number	percent	Number	percent	Number	percent
Yes	80	53%	34	22.7%	114	76%
No	19	12.7%	17	11.3%	36	24%
Total	99	66%	51	34%	150	100%

Table (3-5): Distribution of sickle cell anemia according to age in study groups.

Age(year)	Type of hemoglobin				Total	
	HbSS		HbAS			
	Number	percent	Number	Percent	Number	percent
5months-5 year	42	28.0%	33	22.0%	75	50.0%
6-10 year	44	29.3%	12	8.0%	56	37.3%
11-17 year	13	8.7%	6	4.0%	19	12.7%
Total	99	66.0%	51	34.0%	150	100%

Table (3-6): Distribution of sickle cell anemia according to tribes in study groups.

Tribes	Type of hemoglobin				Total	
	HbSS		HbAS			
	Number	percent	Number	Percent	Number	percent
Meseria	23	15.35	10	6.7%	33	22.0%
Bargo	12	8.0%	10	6.7%	22	14.7%
Hosa	10	6.7%	6	4.0%	16	10.7%
Falata	7	4.7%	7	4.7%	14	9.3%
Zagawa	6	4.0%	4	2.7%	10	6.7%
Barno	4	2.7%	2	1.3%	6	4.0%
Selihab	2	1.3%	4	2.7%	6	4.0%
Jawama	4	2.7%	1	0.7%	5	3.3%
Salamat	4	2.7%	0	0.0%	4	2.7%
Others	27	18.0%	7	4.7%	34	22.7%
Total	99	66.0%	51	34.0%	150	100%

Table (3-7): Hematological parameters of sickle cell anemia compare with normal control.

Variable	Sickle cell anemia Patients	Normal control	p. value
	Mean ± SD	Mean ± SD	
Hb g/dl	7.6 ±2.1	12.9 ±1.5	0.00
WBCs×10^9cell/L	15.6 ±10.3	8.2 ±2.8	0.00
PCV%	23 ±6.6	38 ±4.5	0.00
RBCs×10^{12}/L	2.8 ±0.9	4.2 ±0.5	0.00
MCV FL	84 ±11.6	91 ±4.6	0.00
MCHPg	28 ±6.2	30 ±1.5	0.07
MCHC g/dl	32 ±2.6	32 ±1.3	0.47
Plts ×10^3/mm^3	330.5 ±142	290.5 ±76.5	0.13

Table (3- 8): Hematological parameter of sickle cell anemia (Hb SS and HbAS).

Variable	Sickle cell anemia Patient		p. value
	HbAS	HbSS	
	Mean± SD	Mean± SD	
Hb g/dl	9.2 ±1.9	6.8 ±1.7	0.00
WBCs×10^9cell/L	13.2 ±6.6	16.9 ±1.7	0.00
RBCs×10^{12}/L	3.4 ±1.0	2.5 ±0.6	0.00
PCV%	27 ±6.3	21 ±5.9	0.00
MCV FL	82 ±13.9	85 ±10.1	0.15
MCHpg	30 ±9.0	28 ±4.0	0.20
MCHC g/dl	32 ±2.0	32 ±2.8	0.80
Plts ×10^3/mm^3	341.7 ±145.8	308.7 ± 134.6	0.16

Chapter Four

Discussion, Conclusion and Recommendation

Chapter Four

Discussion, Conclusion and Recommendation

4.1 Discussion

This study was carried out to determine complete blood count (CBC) and Hemoglobin electrophoresis of patients with sickle cell anemia in Khartoum state during period from March 2014 to May 2014.Age of study population ranged between 5 months and 17 years. Both sexes were included in the study, female included 47.3% and male 52.7%.

Two hundreds (200) individuals from different tribes were investigated for complete blood count and hemoglobin electrophoresis.

Highest frequency and percent of study groups with age ranged between 5months and 5 years (50%), which disagreed with results obtained by Ibrahim *et al* who found the highest age between 6 and 16 years.

The results of this study showed that frequency of sickle cell trait (25.5%) is lower than frequency of sickle cell disease(49.5%) compared with other studies in Nigeria by Taiwo *et al*(2011) and Sudan, (Mansoor and Alabid(2011), Osman and Alfadni(2010), Mustafa *et al*,2013) who reported that highest percent among sick cell traits were 24.5%,54%,55%,52% respectively.

The ratio of male to female in patients with sickle cell anemia was 1:1,

Which agreed with study in Sudan (Mustafa *et al, 2013*) who found the ratio of male to female is 1:1.

Sickle cell anemia detected in 28 tribes but high percent in Meseria (22%) and Bargo trib (14.7%), compared with Osman and Alfadni which reported the majority of sickle cell anemia in masaleet tribe.

Highly percent (76%) of parent of patients are married from relatives, 53.3% of them have HbSS and 22.7% have HbAS.

The total erythrocyte was significantly decreased in sickle cell anemia (P value< 0.00), compared with normal control. Hemoglobin concentration and packed cell volume were significantly lower than normal individual (P<0.00).

The total erythrocyte count, Hb concentration and packet cell volume were significantly decrease in sickle cell disease when compared with sickle cell trait (P<0.00).These results agreed with Mustafa *et al*(2013) who observed that anemia significantly is more often in patients with sickle cell disease compared with sickle cell trait (P<0.00).

The low values of Hb, PCV and RBCs count in sickle cell disease have been associated with short survival rate of erythrocyte and acute splenic sequestration and hemolytic crisis (Martin *el al*,1998).

No significant difference between sickle cell anemia patients and normal individuals in mean cell hemoglobin, mean cell hemoglobin concentration and platelets but observe the significant difference in mean cell volume in sickle cell anemia compare with normal individuals.

No significant difference in MCV, MCH. MCHC in sickle cell disease compared with sickle cell trait, The result agreed with Mustafa *et al* (2013), who found no significant difference of MCV, MCH, MCHC in sickle cell disease when compared with sickle cell trait and normal individuals.

The total leucocyte count was significantly increased in sickle cell disease when compared with normal individual and sickle cell trait (p<0.00). Similar results were obtained by Mustafa *et al* (2013), who reported that, the total leucocyte count was significantly elevated in sickle cell disease when compared with normal individuals and sickle cell trait.

4.2 Conclusion

1. Highest frequency of study groups was observed in patients with age ranged between 5 months and 5 years.
1. Hb AS constitutes 25.5% of study groups, while HbSS constitute 49.5%.
2. Erythrocyte count, hemoglobin concentration packed cell volume and Mean cell volume were decreased in sickle cell anemia patients compared with normal individuals.
3. MCHC, MCHC and Plts in sickle cell anemia patients were insignificantly different compared with normal individuals.
4. Total leucocyte count was increased in sickle cell anemia patients compared with normal individuals.
5. Highest percent of sickle cell anemia were marriage from relatives (76%).
6. Highest percent of sickle cell anemia was showed in Meseria tribe followed by Bargo tribe.

4.3 Recommendation

1. Newborn screening programs should be established by Sudan Federal Ministry of Health in areas with high incidence of SCA.
2. A network health care center program should be designed to include sickle cell center and pediatric intensive care.
3. Parents should be aware about their children health status, and should be learned about the problem and how they can help their children whenever possible.
4. More advanced researches (including molecular technique) should be conducted to evaluate the incidence and prevalence of sickle cell anemia in different regions of Sudan.

References:

Alcamo, I. Edward and Barbara, K. (2004), *Anatomy and Physiology the Easy Way*. 2nd Edition. New York. Barron's Educational series.

Akinbami, A. Dosunmu, A. Adediran, A. Oshinaike, O. Adebola, P. and Arogundade, O. (2011). Haematological Value in Homozygous Sickle Cell Disease in Steady State and Haemoglobin Phenotype AA Control, in Lagos, Nigeria. *BMC Research Notes*, Volume 5. 264-268

Alfadni, M and Osman, N. (2010) The Prevalence of SCA in Northen Areas of Algadaref State, Sudan. *Sudanese Journal of Public Health*, Volume 5. 322-325

Baker, F.J and Silverton's, R.E. (1998) *Introduction to Medical Laboratory Technique*. 7th Edition. UK. Professional Publishing Ltd.

Dacie, JV. and Lewis, SM. (2006) *Practical Hematology*. 10th Edition. Philadelphia. Churchilliving Stone Edinburgh.

Elbashir. Osman, M.M. (2011) Theses. Normal Reference Values of Blood Cells Count In Sudan Khartoum North Area. Sudan University of Science and Technology, Collage of Medical Laboratory Science.

Fox Sturat Ira. (2006) *Human Physiology*. 9th Edition. Boston. Published by McGraw-Hill companies.

Henry. John, B. (2011). Clinical Diagnosis and Management by Laboratory Methods. Philodelpha. W.B Saunders.

Hoffbrand A.V. Moss, P.A.H and Pettit, J.E. (2006) *Essential Hematology*. 4th Edition, Victoria. Black well Science LTD.

Hoffbrand, A.V. Lewis, D, and Edward, G.D.T. (2001) *Post-Graduate Hematology*. 4th Edition. New York. Oxford University.

Hunstman, R.G. Baecaly, G.P.E. Canning, D.M. (2004) A rapid Whole Blood Sickling Test to Differentiate the SCT from SCA. *Journal of Clinical Pathology*. Volume 23. 781-783.

Mashall A. Lichtman, Ernest Bentler, Thomas J. Kipp, UriSeligsohn, Kenneth Ky, J osef . T. Prchal. (2005) *WilliumHematology*.7th Edition. United State of America, McGrow-Hill Professional.

Martin, S. Steininger, L and Koepke. *(*1998) *Clinical Hematology*. 2nd Edition. Philadelphia. Lppincott-Raven.

Munsoor, M and Alabid, A (2011) Sickle Cell Trait Among Relatives of Sickle Cell Patients in Western Sudan. *Candian Journal of Medicine.* Volume 2. 451-454.

Mustafa, M. Eltayeb, E. Elmisbah, T. Babiker, H. Osman, N and Nasir, O. (2011) Frequency of the Sickle Cell Disease and Sickle Cell Trait in Heglig Area, Sudan. *International Journal of Public Health and Epidemiology*. Volume 3. 278-282.

Okwi, A. Byarugaba, W. Ndugw, C. Parkes, A. Ocaido, T andTumwine, T. (2010) An Update on The Prevalence of Sickle Cell Trait in Eastern and Western Uganda. *BMC Blood Disorders*. Volume 10. 392-402

Pal G, and Pal Pravatienti. (2005) *Text Book of Practical Physiology*. 2ndEdition. Delhi. Orient Longman Private Limitted.

Rogers, K. (2011) *Blood Ohysiology and Circulation*. 1st Edition. New York. Beitannica Eductional Publishing.

Singh Mandeep. (2010) *Introduction in biomedical instrumentations*. Eastern Economy Edition. New York. OHI Leading Private Limited.

Sysmex American Inc. (2003) Sysmex K X 21 N, Document number 10.

Taiwo, I.V. Oloyede, O.A. and Dosumu, A.O. (2011) Frequency of sickle cell genotype among the Yorubas in Lagos: implications for the level of awareness and genetic counseling for sickle cell disease in Nigeria. *Journal* of C*ommunity Genetics*.Volume 2. 13-18.

Widmair, E. Raff, H. and Srang, K. (2006) *Human Physiology, The Mechanism of Body*. 10th Edition. Boston. Published by McGraHill Companies.

www.nlm.nih.gov

http://www.oocities.org/zaferyedi/hematopathology.html, Weill Medical Collage of Cornell University Web Site.

http://www.cdc.gov/ncbddd/sicklecell/traits.html, Centre of Disease Control and Prevention Web Site.

http://www1.imperial.ac.uk/departmentofmedicine/divisions/experimentalmedicine/haematology/morphology/bain/images/slide18/, Imperial Collage London Web Site.

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا –برنامج الماجستير – مختبرات طبية

تخصص علم امراض الدم ومبحث المناعة الدموية

براءة اخلاقية

الإسم:.....

سوف يتم اخذ عينة من الدم (3 مل) من الوريد بواسطة حقن طعن وذلك بعد مسح منطقة العينة بواسطة المطهر. كل الادوات المستخدمة لأخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية.

وانا اقر بأن العينات سوف يتم تحليلها فقط لطلب البحث

أوافق انا المذكور اعلاه اخذ عينة لإجراء الدراسة

الإمضاء.....

التاريخ.....

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Questionnaire in Measurement of some hematological parameter and frequency of sickle cell anemia in Khartoum State

Questionnaire

Date: —/—/2014 NO( )

Age:
……
……..

Tribe:……
……..

Gender:……
……..

History of blood transfusion:……
……..

Relationship between parent:……
……..

Diagnosis:

CBC:

HB:……
……

PCV:……
……..

RBCS COUNT:……
……..

MCV:
…….
……..
MCH:……
…….

MCHC:
…….
…….
WBCS COUNT:
…….
……..

Hemoglobin electrophoresis:

1.SS ( ) 2. AS ( ) 3. AA ( )