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The impact of hematological parameters, Reticulocyte Parameters and Blood Cell Morphology on Severity of disease Among Sudanese children with Sickle Cell Disease 2018

تاثير مؤشرات الدم العام، مؤشرات الخلايا الشبكيه وشكل خلايا الدم على حدة المرض عند الاطفال السودانين المصابين بمرض الخلايا المنجليه ٢٠١٨

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الآيـــــة

قَالَ تَعَالَىٰ: ﴿ ۞ وَلَقَدْ كَرَّمْنَا بَنِيٓ ءَادَمَ وَحَمَلْنَٰهُمْ فِي ٱلْبَرِّ وَٱلْبَحْرِ وَرَزَقْنَٰهُم مِّنَ ٱلطَّيِّبَٰتِ وَفَضَّلْنَٰهُمْ عَلَىٰ كَثِيرٍ مِّمَّنْ خَلَقْنَا تَفْضِيلًا ﴿٧٠﴾ ﴾ الإسراء: ٧٠.

صدق الله العظيم

Dedication

Every challenging work needs self efforts as well as guidance of elders especially those who were very close to our hearts…

Our humble effort we dedicate to our sweet and loving fathers and mothers, whose affection, love, encouragement and prays of day and night make us able to get such success and honor…

A long with all hard working and respected teachers …

Acknowledgement

First of all, we are grateful to the Almighty ALLAH for giving me strength and ability to complete this study, I would like to express my sincere gratitude to my supervisor **Dr. Munsoor Mohammed Munsoor**, for continuous support, motivation, enthusiasm and immense knowledge, his guidance helped me in all the time of research and writing of this thesis , beside my supervisor, I would like to thank my family, colleagues and friends for their support, help and encouragement, I place on record, my sincere thanks to all doctors and teachers of the faculty of Medical Laboratory Sciences ,Sudan university for science and technology, for learning, helping and supporting along all my higher education journey ,special and grateful thanks to Hematology and Immunohematology Department , finally I would like to thank the patients for their cooperation.

Abstract

This study is analytical Cross-sectional descriptive study conducted to estimate the impact of hematological parameters , reticulocyte Parameters and blood cell morphology on disease severity among hundred sickle cell Sudanese Children , in age group from (1-6) years in Khartoum state, at Jaafer Ibn Aouf Pediatric Hospital from January to November 2018 , a designed questionnaire was used for data collection accompanied with written informed consent for all participant , (2.5 ml) EDTA venous blood was collected under standard phlebotomy procedure, the complete blood count (CBC) was measured by using automated hematological analyzer (Sysmex xp-300) , reticulocyte parameters were calculated and blood films were done for each sample, sample analysis was done in Turkish medical diagnostic center and then data analyzed using statistical package of social science program version 19 , the results showed that the red blood cells count was (2.5 ± 0.5) $\times 10^{12}$ /l, hemoglobin (7.4 ± 1.2) g/dl, hematocrit (21.9± 3.9)%, red cell distribution width (59.9± 15.2) fl , mean cell volume (88.9 ± 11.9) fl , mean cell hemoglobin (30.1 ± 4.1) Pg, mean cell hemoglobin concentration (34.0 ± 2.4)g/dl, white blood cells count (15.3 ±6.6)$\times 10^{9}$ /L, Neutrophils (47.7± 11.6)%, Lymphocytes (42.0 ± 11.3) % , MID% (10.3±2.4) , platelets count (387.0±162.7) $\times 10^{9}$/L, retics (5.4±3.1) %, corrected reticulocyte count (2.5± 1.4) % and reticulocyte production index (0.62±0.39) , peripheral blood picture show variety of cells with diagnostic value, hematological parameters showed significant correlation with anemia severity include red blood cells count (p.value =.000) , hemoglobin concentration (p.value=0.000) , hematocrit (p.value=0.000) , red cell distribution width (p.value=0.000) and white blood cells count (p.value=0.003), while other hematological parameters including mean cell volume , mean cell hemoglobin , mean cell hemoglobin concentration and platelets count showed insignificant correlation with anemia severity (p.value

> 0.05) , retics count show significant correlation with anemia severity (p.value=0.005) , while corrected retics count and reticulocyte production index showed insignificant correlation with anemia severity (p.value > 0.05).

مستخلص البحث

هذه دراسه وصفية مقطعية اجريت لتقييم تاثير مؤشرات الدم العام ، مؤشرات الخلايا الشبكية و شكل خلايا الدم على حدة المرض في مائة من الاطفال السودانيين المصابين بمرض الأنيميا المنجلية والذين تتراوح أعمارهم ما بين عمر السنه و الستة سنوات بمستشفى جعفر بن عوف للأطفال من يناير الى نوفمبر(2018)، تم جمع العينات عن طريق استبيان مرفق باقرار موافقه لجميع المرضى ، حيث تم سحب 2.5 مل من الدم في انبوبة حمض الاسيتيك الايثيلي الديامينى المانعة للتجلط تحت الظروف القياسية لسحب العينات ، ثم تم قياس الدم العام باستخدام جهاز محلل الدم سيسميكس كس-٣٠٠، وتم تعداد الخلايا الشبكية وعمل مسحة الدم لجميع العينات بالمركز التركي الطبي التشخيصي وتم تحليل البيانات باستخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية الاصدار١٩، أظهرت النتائج أن تعداد كريات الدم الحمراء كانت (2.5±0.5) خلية/لتر، خضاب الدم (7.4 ±1.2) جم/دل ، الحجم المضغوط لخلايا الدم الحمراء (21.9- ±3.9)% ، مدى انتشار خلايا الدم الحمراء (59.9±15.2) فيمتولتر ، متوسط حجم كريات الدم الحمراء (88.9±11.9) فيمتولتر ' متوسط الخطاب في الخلية الواحدة (30.1±4.1) بيكو جم ' متوسط الخضاب في لتر من الدم (34±2.4)% ، كريات الدم البيضاء (15.3±6.6) خلية/ميكرولتر ، المتعادلات (47.7±11.6)% ، الليمفاويات (42.0±11.3)% ، اجمالي الخلطيات (10.3±2.4)% ، الصفائح الدموية (387±162.7) خلية/ميكرولتر ، الخلايا الشبكية (5.4±3.1)% ، التعداد المعادل للخلايا الشبكية (2.5±1.4)% ومعامل انتاج الخلايا الشبكية (0.62±0.39) ، كما أظهرت صورة الدم الطرفية أن هنالك تباين في الخلايا ذات القيمة التشخيصية ، أظهرت بعض عناصر تعداد الدم العام أن هنالك ارتباط معتبر بحدة المرض وشملت هذه العناصر كريات الدم الحمراء (القيمة الاحتمالية =0.000) ، خضاب الدم (القيمة الاحتمالية =0.000) ، الحجم المضغوط لكريات الدم الحمراء (القيمة الاحتمالية =0.000) ، مدى انتشار خلايا الدم الحمراء (القيمة الاحتمالية =0.000) وتعداد كريات الدم البيضاء(القيمة الاحتمالية =0.003) ،بينما اظهرت بقيه عناصر تعداد الدم العام التي شملت متوسط حجم كريات الدم الحمراء ، متوسط الخطاب في الخلية الواحدة ، متوسط الخضاب في لتر من الدم ، المتعادلات ، الليمفاويات ، اجمالي الخلطيات و الصفائح الدموية عدم ارتباط بحدة المرض (القيمة الاحتمالية >0.05) ، اظهر تعداد الخلايا الشبكية ارتباط معتبر بحدة المرض (القيمة الاحتمالية =0.005) ، بينما اظهر كل من التعداد المعادل للخلايا الشبكية ومعامل انتاج الخلايا الشبكية عدم ارتباط بحدة المرض (القيمة الاحتمالية >0.05) .

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List of Abbreviations

ACS	Acute Chest Syndrome
ALDH	Aldehyde Dehydrogenase
BFU-E	Burst Forming Unit –Erythroid
CFU-E	Colony Forming Unit – Erythroid
CLPs	Common Lymphoid Progenitors
CMPs	Common Myeloid Progenitors
CRC	Corrected reticulocyte count
CVS	Chronic Villus Sampling
CBC	Complete Blood Count
DC	Direct Current
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetic acid
EPO	Erythropoietin
GAG	Guanine Adenine Guanine
GM-CSF	Granulocyte-Monocyte –Colony Stimulating Factor
GTG	Guanine Thytothine Guanine
Hb	Hemoglobin
HCT	Hematocrit

HSCs	Hematopoietic Stem Cells
IV	Intravenous
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MDR	Multidrug Resistant proteins
PBP	Peripheral Blood Picture
PCV	Package Cell Volume
PLTs	Platelets
PND	Prenatal Diagnosis
RBCs	Red Blood Cells
RDW	Red Cell Distribution Width
RE	Reticuloendothelial
RNA	Ribonucleic Acid
RPI	Reticulocyte Production Index
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SD	Standard Deviation
SLS	Sodium Lauryl Sulfate
SPSS	Statistical Package for the Social

	Sciences
Thy-1	Thymocyte Differentiation Antigen -1
VPC	Vaso-Occlucive Pain Crises
WBCs	White Blood Cells

Chapter One

Introduction and Literature Review

Chapter one

Introduction and Literature Review

1.1 Introduction:

The main function of red cells is to carry oxygen (O2) to the tissues and return the carbon dioxide (CO2) from the tissues to the lungs, in order to achieve this gaseous exchange they contain the specialized protein Hb/, normal adult blood contains three types of hemoglobins, the major component is hemoglobin A with the molecular structure (α2 β2) representing (96-98 %) of total Hb, fetal Hb F (α 2 γ2) representing (0.5 – 0.8%) and hemoglobin A2 (α 2 δ2) forming about (1.5 –3.2%), the major switch from fetal to adult hemoglobin occurs (3-6) months after birth, each red cell contains approximately (640) million hemoglobin molecules (Hoff brand *et al.,* 2006).

Sickle cell disease (SCD) is a common term for a group of haemoglobinopathies characterized by sickle cell anaemia, sickle beta thalassemia syndromes and other haemoglobinopathies in which (HbS) is in association with abnormal haemoglobin, sickle cell anaemia (HbSS) results from homoz-ygosity for (A – T) substitution at codon 6 of β globin gene (GAG-GTG) leading to a glutamic acid to valine (Glu-Val) substitution in the β globin chain of human adult haemoglobin (Tatkare *et al.,* 2014)

Patients with sickle cell anemia are usually diagnosed through neonatal screening programs or between (6 months - 2 years of age) , prior to this time, red cells are protected from sickling with high levels of hemoglobin F, because the switch from the production of hemoglobin F to hemoglobin A occurs between 3 and 6 months of age (Betty, 2007).

Most patients with sickle cell anemia have a chronic hemolytic process, characterized by a hypercellular bone marrow, red cells that live only (10-20) days, a marked reticulocytosis (8% to 12%), and increased bilirubin, the anemia is usually compensated with hematocrits in the range of(20% to 25%), and Patients do well even with these low numbers, complications occur in the form of aplastic anemia or splenic sequestration crisis and acute aplastic anemia may develop as a result of infection, usually parvovirus, when the already over worked bone marrow simply fails to produce cells, the hematocrit may fall by (10% to 15%) per day(10), transfusion is essential because there is no backup

therapy for bone marrow aplasia and death may occur without transfusion intervention (Betty, 2007).

Sickle cell disease is primarily a red cell disorder but significant changes are observed in other hematological parameters such as white cells and platelet which play important roles in pathophysiology of the disease and in prediction of outcome (Iheanacho, 2015).

The SCD patients experience alternating periods of apparent good health (steady state) and acute exacerbation of symptoms (crisis) as well as development of chronic complications, the hematological parameters in these periods vary but at the same time provide evidence-based management information for diagnosis, treatment, monitoring and prognostication, although the hematological parameters of SCD patients vary significantly from those of normal HbA individuals, these patients are able to adapt to their steady state hematological values and remain apparently healthy, the importance of some of the steady state hematological values such as hemoglobin (HB) concentration, white blood cell (WBC) and platelet (PLT) counts in prediction of clinical severity as well as management of SCD has been documented, lower steady state HB is associated with higher risk of stroke, whereas higher values are reported to have higher rates of severe pain, furthermore, red cell transfusions beyond the steady state HB may increase blood viscosity with attendant consequences such as worsening of vaso-occlusion and osteonecrosis (Iheanacho, 2015).

High WBC count (above 11 x 109 /L) is associated with SCD complications including cerebrovascular accidents, some researchers have also shown that lowering the WBC count through the use of drugs such as hydroxyurea improves the clinical outcome of these patients ,other parameters for assessment include the red cell indices such as MCV, MCH and mean cell hemoglobin concentration (MCHC) which are useful in detecting co-existing causes of anemia in the patient, over the years several modalities of treatment of SCD such as blood transfusion, haematincs and hydroxyurea have been used and these modify the hematological parameters of these patients, knowledge of the steady state hematological values in these patients becomes an important asset for the managing physician (Iheanacho,2015).

In addition to variation in hematological parameters, PBP of SCA patients is also vary, PBP of such patients permits interpretation of diagnostically

significant RBCS findings, these include assessment of RBCs shape, size, color ,inclusions and arrangement ,abnormalities of RBCs shape and other RBCs features can provide key information in establishing a differential diagnosis (Ford,2013).

Literature review

1.2 Literature review:

1.2.1. Blood composition:

Blood constitutes (6 - 8) percent of total body weight, it consists of a fluid portion called plasma, and solid portion that includes red blood cells (erythrocytes), white blood cells (leukocytes) and platelets (thrombocytes), plasma makes up to (45-60) percent of blood volume and composed of water (90 %), amino acids, proteins, carbohydrates, lipids, vitamins, hormones ,electrolytes and cellular wastes (Bonita, 2003).

1.2.1.2 Blood function:

The principal function of blood are the transport of oxygen, nutrients and hormones to all tissues and removal of metabolic wastes to the organs of excretion, additional functions of blood include regulation of the temperature by transfer of heat to the skin , regulation of the PH of body fluids through the buffer systems and facilitation of excretion of acids and bases, and defense against infection by transportation of antibodies and other substances as needed (Bonita, 2003).

1.2.2 Hematopoiesis:

1.2.2.1 Definition of hematopoiesis:

Hematopoiesis is the process of making blood cells, the term comes from the Greek haima (mean blood) and poiein (to make), for the average adult, the bone marrow produces (5×10^{11}) cells per day, production of blood cells is highly regulated and balanced (William, 2002).

1.2.2.2 Site of hemopoiesis:

In the first few week of gestation the yolk sac is the main site of hemopoiesis, and by the age of 6 weeks until (6 – 7) months of fetal life the liver and spleen are the major organs and continue to produce blood cells until about 2 weeks after birth, by the age of (6 – 7) months and throughout the life the bone marrow is the main site of hemopoiesis, however liver and spleen can resume their hemopoietic function if needed (Hoffbrand *et al*., 2006).

1.2.2.3 Hematopoietic stem cells (HSC):

Are cells that contain the potentiality in self renew and differentiation into specialized blood cells that function in some biological activities: control homeostasis balance, immune function, and response to microorganisms and inflammation, most HSCs are in quiescent state within the niches that maintain HSC pool and will respond to the signals after the balance of blood cells or HSC pool is disturbed from either intrinsic or extrinsic stimuli (Kamonnaree and Wilairat, 2012).

Phenotypically, murine HSCs are small cells with minimal cytoplasm, and they express high levels of the multi drug resistant (MDR) proteins and high levels ofaldehyde dehydrogenase (ALDH),these cells tend not express surface markers seen on mature HSCs but can express low levels of the thymocyte differentiation antigen (thy-1), HSCs generate the multiple hematopoietic lineages through a successive series of intermediate progenitors, these include common lymphoid progenitors (CLPs), which can generate only B. lymphocyte, T. lymphocyte and natural killer cells, and common myeloid progenitors (CMPs), which can generate only red cells, platelets, granulocytes, and monocytes, downstream of the CLPs and CMPs are more mature progenitors that are further restricted in the number and type of lineages that they can generate ultimately, terminally differentiated cells are produced that cannot divide and undergo apoptosis after a period of time ranging from hours (for neutrophils) to decades (for some lymphocytes) (Clayton, 2003) .

1.2.2.4 Erythropoiesis:

Erythropoiesis is a continuous and dynamic process by which erythrocytes are generated from multipotent hematopoietic stem cells, erythropoiesis is mainly divided into two stages, early erythroid progenitor proliferation and terminal erythroid differentiation (Jieying *et al.*, 2015).

HSCs proliferate and differentiate into the earliest erythroid progenitors: burst-forming-unit erythroid (BFU-E) cells, and then, colony-forming-unit erythroid (CFU-E) cells, subsequently, terminal erythroid differentiation starts with pro erythroblasts, which undergo three mitoses to produce basophilic, polychromatic, and orthochromatic erythroblasts, eventually, orthochromatic erythroblasts expel their nucleus and become reticulocytes, which subsequently become mature erythrocytes, noticeable changes in cellular composition and structure occur during terminal erythroid differentiation, including the filling of

the cells with abundant hemoglobin and the clearance of all intracellular organelles from the cells, such as mitochondria and ribosomes (Jieying *et al*., 2015).

1.2.2.5 Erythropoietin:

Erythropoietin (EPO) consists of (165) amino acids and has a molecular weight of (35,000 D) , the hematopoietic growth factor acts synergistically with other growth factors to cause maturation and proliferation from the stage of burst-forming unit erythroid (BFU-E) and CFU-E (colony-forming unit erythroid) to the normoblast stage of erythroid cell development, thus, EPO acts primarily on apoptosis to decrease the rate of cell death in erythroid progenitor cells in the bone marrow, during the fetal and neonatal period of life, EPO is primarily produced by the liver, after birth EPO production is shifted to the peritubular interstitial cells of the renal cortex, the liver keeps its capability to produce EPO also in the adult, but its contribution is not more than 10 % (Wick *et al*., 2011).

1.2.2.6 Maturation stages of the red cells:

There are six stages of maturation in the red cell series: pronormoblast, basophilic normoblast polychromatophilic normoblast, orthochromatophilic normoblast, reticulocyte, and mature red cell (Betty, 2007).

1.2.2.6.1 Pronormoblast:

The size of the (18 to 20 μm), it consider to be the largest and most immature cell called the mother cell with nuclear cytoplasmic ratio (4:1), has round nucleus With a densely packed chromatin, evenly distributed, fine texture with deep violet Color, nucleoli may be present but are hard to visualize, cytoplasm is dark marine Blue definitive areas of clearing (Betty, 2007).

1.2.2.6.2 Basophilic Normoblast:

The cell size (16 μm) with nuclear cytoplasmic ratio(4:1) , has round nucleus with crystalline chromatin appearance, parachromatin under layer may be visible, red-purple color to chromatin, the cytoplasm cornflower blue with indistinct areas of clearing (Betty, 2007).

1.2.2.6.3 Polychromatophilic Normoblast:

The cell size is (13 μm), with nuclear cytoplasmic ratio (2:1) , nucleus has condensed chromatin, a color mixture cytoplasm of blue layered with tinges of

orange red, (the dawn of hemoglobinization) as hemoglobin begins to be synthesized (Betty, 2007).

1.2.2.6.4 Orthochromic Normoblast:

Cell size is (8 μm) with nuclear cytoplasmic ratio (1:1) , nucleus has dense, velvet appearing homogenous chromatin, and the cytoplasm is large, with orange-red color tinges with slight blue tone (Betty, 2007).

1.2.2.6.5 Reticulocyte:

Cell size is(8 μm) appear as RNA remnant visualized as reticulum, filamentous structure in chains or as a single dotted structure in new methylene blue, when stained with Wrights stain appear as blue red cells known as polychromatophilic macrocytes (Betty, 2007).

1.2.2.6.6 Mature red blood cells:

Red blood cells (also referred to as erythrocytes) are the most common blood cells that deliver oxygen to body tissues via a cardiovascular system, they take up oxygen in the alveoli and exchange it for carbon dioxide and the exchange of gases occurs by simple diffusion (Omar, 2013).

The cell size is (6 to 8 μm), disk-shaped cell filled with hemoglobin, having an area of central pallor of 1 to 3 μm (Betty, 2007).

1.2.2.7 RBC′s Membrane:

The RBC′s membrane comprises of a bipolar lipid layer that anchors integral membrane proteins, surface antigens, and a membrane skeleton (Ankyrin, protein 4.1, spectrin and actin),this membrane helps to hold the shape of the red cell thereby preventing it from being deformed, these proteins contain many sulphydryl (-SH) groups which are necessary for the structure of the cell, a defect on the membrane proteins leads to abnormalities of the shape of the red cell (e.g. elliptocytosis, hereditary spherocytosis and sickle cell anemia)(Mehta and Hoffbrand, 2014).

1.2.2.8 Hemoglobin:

Hemoglobin is made up of(2α and 2γ) polypeptide chains (HbF), whereas in adults it predominantly consists of(2α- and 2β) chains (HbA) with a small portion of (2α and 2δ) chains (HbA2), each of these chains carries a heme as prosthetic group, which in turn is capable of binding an oxygen molecule, the total molecular weight of this tetramer is about(64,500 D), the formation of the normal quaternary structure is dependent on the regular synthesis not only of the peptide chains but also of the heme constituent, and in particular on the adequate adhesion of the heme and protein components by means of iron which also guarantees oxygen binding (Wick *et al*., 2011).

1.2.2.9 Breakdown of the Hemoglobin:

Red cells destruction usually occurs after a mean lifespan of (120 days) when the cells are removed extravascularly by the macrophages of the reticuloendothelial (RE) system, the breakdown of the haem from red cells liberates iron for recirculation via plasma transferring to marrow erythroblasts, the protoporphyrin is then broken down to bile pigments and excreted by the liver, the globin chains are broken down to amino acids which are reutilized for general protein synthesis in the body, haptoglobins are proteins present in normal plasma capable of binding hemoglobin, the hemoglobin-haptoglobin complex is removed from plasma by the RE system, intravascular haemolysis plays little or no part in normal red cell destruction (Hoff brand *et al*, 2006).

1.2.2.10 Hemoglobin abnormalities (Haemoglobinopathies):

The genetic abnormalities of the Hb molecule are mainly divided into two categories: the structural abnormalities and abnormalities due to the reduced synthesis of normal (α- or β) chains of the globin molecule, sickle cell anemia are the prototype of a structural abnormality, whereas in the thalassemias, a reduced synthesis of the globin chains is encountered, other structural abnormalities of the Hb molecule cause HbC, HbE disease, and other disorders, each hemoglo- binopathy occurs in homozygous and heterozygous forms, clinical manifestations are usually only present in the homozygous state, heterozygotes are referred to as having a trait of the hemoglobinopathy (Reinhold *et al*, 2007).

1.2.2.11 Sickle cell anemia:

Sickle cell disease (SCD) is a group of blood disorders typically inherited from a person's parents, the most common type is known as sickle cell anemia (SCA), it results in an abnormality in the oxygen carrying protein hemoglobin (hemoglobin S) found in red blood cells, this leads to a rigid, sickle like shape under certain circumstances, problems in sickle cell disease typically begin around 5 to 6 months of age, a number of health problems may develop, such as attacks of pain ("sickle cell crisis"), anemia, swelling in the hands and feet, bacterial infections, and stroke, Long term pain may develop as people get older, the average life expectancy in the developed world is 40 to 60 years, sickle cell disease occurs when a person inherits two abnormal copies of the hemoglobin gene, one from each parent, this gene occurs in chromosome 11, several subtypes exist, depending on the exact mutation in each hemoglobin

gene, an attack can be set off by temperature changes, stress, dehydration, and high altitude, a person with a single abnormal copy does not usually have symptoms and is said to have sickle cell trait, such people are also referred to as carriers(Vanaja ,2018).

1.2.2.12.1 History and distribution of sickle cell disease worldwide:

Anemia is a medical condition in which the red blood cell count (RBC) or Hb is below the reference range, the sickle cell disease is observed in certain areas of the

Sudan, ranging from(0.8%)in central Sudan to(30.4%) in western Sudan, the Messeria tribe as a branch of Baggara groups in kordofan and Darfur was reported to be has the highest rate of sickle cell disease (Omer *et al* ,2017).

Sickle cell anemia (SCA) was discovered by Herrick in(1910), he described the clinical and hematological manifestations of SCA, globally, this disease affects millions of people, particularly those come from sub-Saharan Africa, Spanish speaking regions (South America, Cuba, Central America), Saudi Arabia, India, and Mediterranean countries such as Turkey, Greece, and Italy, its highest frequency was reported in tropical regions, and the Middle East, in the Unites States, it affects around (72,000) people, most of them come from Africa, the disease occurs in about(1 in every 500) African, American births and(1 in every 1000 to 1400)Hispanic American births, about(2 million)Americans, or(1 in 12) African Americans, carry the sickle cell trait, migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades and in some European countries sickle cell disease has now overtaken more familiar genetic conditions such as hemophilia and cystic fibrosis (Omer *et al* ,2017).

Some studies found that sickle cell disease affects(about 90 x10^7)Americans, the disease was also reported in one out of each five hundreds African, American births and one out of each(36 x 103) Hispanic American births, most infants with SCD, born in the United States of America and some other developed countries are now identified by routine neonatal screening, Forty four states along with the district of Columbia, Puerto Rico and the Virgin Islands currently provide universal neonatal screening for SCD(Omer *et al* ,2017).

1.2.2.12.2 SCA epidemiology:

The highest frequency of Sickle Cell Disease is found in tropical regions, particularly Sub Saharan Africa, India and the Middle-East, migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades, the prevalence of Sickle cell anemia is highly common in the tribal belt of central and southern India, the public health implications of sickle cell anemia are significant leading to poor quality of life, lower life expectancy and higher rates of infant mortality, sickle cell disease (SCD) with an estimated (5,200) live births each year is a major public health problem in India, although SCD has been described in India in numerous ethnic groups, it is most prevalent, prevalence of Sickle Cell gene is(5 to 34 %) in scheduled tribes, who have a high prevalence of socioeconomic disadvantage and are frequently medically underserved, India has also a very huge populations of tribal community about(18crore)and expected to have(1.80 crore)sickle cell trait and (14 lakhs) of sickle cell disease, these show the big burden on the public health of India (Vanaja ,2018).

1.2.2.12.3 Genetics of SCA:

The sickle mutation substitutes thymine for adenine in the sixth codon of the ß gene (GAG, GTG), thereby encoding valine instead of glutamine in the sixth position of the (ß-chain), this ostensibly minor change in structure is responsible for profound changes in molecular stability and solubility, the tendency of deoxygenated Hb S to undergo polymerization underlies the innumerable expressions of the sickling syndromes (John, 2003).

1.2.2.12.4 Inheritance Patterns:

Sickle cell anemia are inherited from parent the same way as blood type, hair color and texture, eye color and other physical traits, the type of hemoglobin a person makes in the red blood cells depend upon what hemoglobin genes are inherited from his parents, if one parent has sickle cell anemia(SS) and the other has sickle cell trait (AS), there is a(50%) chance of a child's having sickle cell disease (SS) and a (50%) chance of a child's having sickle cell trait (AS),when both parents have sickle cell trait (AS), they have a(25%)chance of a child's having sickle cell disease (SS)(Obeagu *etal*,2015).

1.2.2.12.5 Pathophysiology of Sickle cell anemia:

The abnormality in (Hb S)is substitution of valine for glutamic acid at the sixth amino acid position (6Glu→Val), deoxygenated hemoglobin (S) tends to polymerize into long rigid structures, which distort the cell into the characteristic sickle shape anything that causes deoxygenation of hemoglobin predisposes to sickling, including hypoxia, acidosis, and increased temperature, the polymerization of hemoglobin (S) is reversible, and cells that have sickled may return to normal shape with reoxygenation, however, the repeated cycles of sickling and unsickling damage the cell, and, eventually, the erythrocytes becomes irreversibly sickled, the rigid elongated sickle cells obstruct small blood vessels, resulting in tissue infarction, sickled erythrocytes are also "sticky "and adhere to endothelial cells, predisposing to thrombosis, common sites of infarction include the spleen, bone and bone marrow, the medulla of the kidney, mesenteric vessels, and pulmonary vessels (William ,2002).

1.2.2.12.6 Clinical manifestations of SCA:

The clinical course of SCA is typically characterized by variable period of steady state that is periodically punctuated by vaso-occlusive crisis, sickle cell anemia runs a variable clinical course ranging from mild disease diagnosed accidentally to severe crippling disease, patients can be in relative good health termed "steady state" which may be periodically punctuated by acute exacerbations called "crises" which could have sudden onset and eventual fatal outcomes, crisis is the hallmark of sickle cell anemia, although red cell sickling is more prominent during crisis, continuous sickling occurs at lower rate in steady state, the crises are traditionally classified as vaso-occlusive, aplastic, sequestration and hyperhaemolytic crises, vaso-occlusive crisis (VOC) is preceded by a prodromal experience and if uncomplicated is self-limiting, aplastic or hypoplastic, sequestration and hyperhaemolytic crises are natively referred to as acute anemic crises, since they worsen the clinical state(Usman *etal*,2017).

Sickle cell crises: the term "sickle cell crises" is used to describe several independent acute conditions occurring in patients with sickle cell disease, sickle cell disease results in anemia and crises that could be of many types including the vaso-occlusive crises, aplastic crises, sequestration crises, hyper hemolytic crises and others, most episodes of sickle cell crises last between five and seven days and patients have to be hospitalized (Kaur *et al*, 2013).

1.2.2.12.7 Diagnosis of Sickle Cell anemia:

Newborns with sickle cell disease benefit from early detection through early institution of penicillin prophylaxis to prevent pneumococcal sepsis, the prenatal diagnosis (PND) for the disease has opened a window of opportunity for expectant couples to have information about the hemoglobin (Hb) genotype of their unborn child, this gives them the option of termination of the pregnancy in case of positive result and to prepare them psychologically, financially and medically for the arrival of the new child when abortion is not an option, prenatal diagnosis is usually carried out using either chorionic villus sampling (CVS) or amniocentesis and the samples taken have DNA analysis done on them, both procedures are invasive with CVS being done between the (10th and 12^{th}) week of pregnancy while amniocentesis is usually carried out later (between the 14th and 20th week), sickle cell disease can be diagnosed in newborns, as well as older persons, by hemoglobin electrophoresis, isoelectric focusing, high-performance liquid chromatography or DNA analysis, in general, these tests have comparable accuracy, the testing method should be selected on the basis of local availability and cost (Kaur *et al*, 2013).

1.2.2.12.8 Treatment of sickle cell anemia:

Folic acid and penicillin, children having SCD should be kept under close observation of the paediatrician and to be managed by a hematologist to keep them healthy, these patients will take a (1 mg) dose of folic acid daily and up to five years of age penicillin daily because of poor immune system, they are more prone to early childhood illnesses, painful (vaso-occlusive) crises: most people with sickle cell disease get extremely painful episodes called vaso-occlusive crises, the rate, severity, and duration of these crises vary tremendously, these situation are managed by antibiotics, blood transfusion (normal or exchange transfusion), oxygen, pain control with paracetamol-codeine, ibuprofen or morphine analogues, hydroxyurea, was shown to decrease the number and severity of attacks ,bone marrow transplants have proven to be effective in children, at present it is only available curative therapy for sickle cell anemia, while the survival rate from this procedure is roughly (91%)and the cure rate is(82%), blood transfusion is widely used in the treatment of sickle cell anemia, it is estimated that (50%) of all patients receive a red cell transfusion at some point in their lives and that (5%) receive chronic transfusions (Kaur *et al*,2013).

1.3 Previous studies:

Sickle cell disease is the most common genetic disease in the world, (100 to 150)

million people are healthy transmitters and (300,000 to 400,000) children per year

worldwide are born with sickle cell disease (Asmaa *et al*, 2017).

In India study of hematological profile of children affected by sickle cell anemia had been done, their results show low MCV & MCH and normal MCHC values (Vanaja *et al*, 2018).

Hematological and clinical profile in Nigerian sickle cell disease patients had performed the results showed there were no significant differences in the means of PCV, WBC, platelet count and corrected reticulocyte count in the two populations of patients (John *et al*, 2014).

Erythrocyte indices in Pre-school Nigerian children with sickle cell anemia in steady state were estimated, the results show lower mean haemoglobin concentration and packed cell volume, the mean corpuscular volume (MCV) was higher in SS subjects than AA controls (Samuel *et al*, 2015).

Clinical and hematological profile of sickle cell disorder patients in a tertiary care hospital of Central India were studied, the outcome of the study was, mean Hb% value was nearby to other study, values of HCT, total RBC count, MCH and MCHC were found to be low in present study which is comparable to other studies, mean RDW value was found to be high (Vidhyanand *et al*, 2017).

Clinical and laboratory profile of patients with sickle cell anemia in Brazil were evaluated, the results were as follow, increases in hemoglobin and hematocrit values were statistically significant (Phelipe *et al*, 2017*)*.

An observational study of children with sickle cell disease in Kenya had been performed, the results showed that the mean haemoglobin concentration in children with SCD in our study showed higher values (Manish *et al*, 2009).

Hematological profile of sickle cell disease form south India were estimated the results show that, total hemoglobin (Hb) is low in SCD patients, total red cell count ,mean cell hemoglobin(MCH)and mean cell hemoglobin concentration (MCHC) and MCV are low (Sanjeev *et al*, 2012).

Disease severity scores and haemogram parameters in Nigerian sickle cell disease patients were estimated, the results show the following, PCV, Hb, WBC and MCHC significantly correlated with disease severity (Emmanuel *et al*, 2015). Hematological profile of adult sickle cell disease patients in North Maharashtra, India had been measured, the results were as follow, lower levels of haemoglobin, RBC count and PCV in the male and the female patients of sickle cell disease, the mean MCV was high in both the sexes of SCD patients, whereas MCHC values were low (Jadhav *et al*, 2016).

The above studies were international ones, in Sudan many studies had been performed also include the following:

Hematological profile among Sudanese patients with sickle cell anemia had been evaluated, the results show, total hemoglobin (Hb) is low in SCD patient, and total red cell count, mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) are low in our study, mean cell volume (MCV) is low (Mohammed, 2015).

Evaluation of clinical severity of sickle cell anemia in Sudanese patients had been performed, the results show, total white blood cell count, absolute neutrophil count, and hemoglobin (Hb) concentration revealed statistical significant difference among the severity score groups(P values = 0.0001, 0.003 and 0.006 respectively) (Alabid *et al*, 2016).

Hematological parameters in Sudanese children with sickle cell disease had been measured, the results show, the Hb, RBCs and PCV results were generally low in the patients, together with the observable high RDW, TWBCs was observed and there was an observable decrease in MCV and MCH values (Mohammed, 2014).

1.4 Rationale:

Sickle cell disease continues to be a global health problem that presents major challenges to our health care systems, the reviewed SCD literature (international and national ones in Sudan) expresses a dire need for more public education and awareness on SCD, It is essential to know which determinants are associated with severe disease, so that prognostic models can be constructed and appropriate management given, these prognostic models may help to identify patients who are at increased risk for a severe disease course , as SCD affect patient's hematological profile, the measurement of hematological parameters for such patients consider as prognostic marker and will reflect the severity of disease, also SCD characterize by increased rate of hemolysis, so the patient bone marrow try to compensate the loss of RBCs, during this compensation immature RBCs (reticulocyte, nucleated RBCs) appear in PBP, so the calculation of these cells will reflect the activity of bone marrow and also give an indication of anemia severity.

1.5 Objectives:

1.5.1General Objective:

To evaluate the impact of hematological parameters, reticulocyte parameters and blood cell morphology on severity of disease among Sudanese children with sickle cell disease 2018.

1.5.2Specific objectives:

_To estimate CBC parameters (HB, PCV, RBCs, RBCs indices, WBCs, WBCs 5 parts differential and PLTs) and correlate them with severity of anemia.

_To evaluate PBP of each subject, observing morphological abnormalities of cells, cells of diagnostic value, number of these cells and correlate them with anemia severity.

_To calculate reticulocytes and getting out RPI using patient PCV in order to reflect the activity of bone marrow.

_To compare the results obtained with similar measures in other studies finding out the similarities and differences between these measures and the possible causes for differences.

_To compare severity of disease.

Chapter tow

Materials and Methods

Chapter tow

Materials and Methods

2. Materials and Methods:

2.1 Study design:

This study was cross-sectional descriptive hospital based study design.

2.2 Study area and duration:

The study was performed in Khartoum state, at Jaafer Ibn Aouf Pediatric Hospital in a period from January 2018 to April 2019.

2.3 Study population:

100 SCA patients were included.

2.4 Inclusion criteria:

Sudanese patients of both sexes known to have sickle cell disease were included.

2.5 Exclusion criteria:

Non Sudanese sickle cell patients, sickle cells traits, children's having chronic infective diseases, children's having history of blood transfusion ,subjects with massive edema or ketosis and those already on dialysis were excluded.

2.6 Study Variables:

CBC parameters including: hemoglobin, red cell count, red cell indices, red cell distribution width, white blood cell count, differential count and platelet count in addition to reticulocyte profile and peripheral blood picture for each sample.

2.7 Data Collection:

Data was collected using designed questionnaire to obtain information about demographic and clinical data that helped in either including or excluding certain subject, questionnaires were filled by asking the patients about personal informations, medical information was collected from medical file with the help of treating doctors.

2.8 Sample collection:

Tow point five ml of venous blood was collected from individual under study and dispensed in EDTA container for CBC, PBP and retics profile (Dacie and Lewis, 2011).

2.9 Principle of operation:

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has minute hole called the aperture, on both sides of aperture, there are the electrodes between which flows direct current, blood cells suspended in the sample pass through the aperture, causing DC resistance to change between the electrodes. As DC resistance change the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses and a histogram of blood cell sizes is plotted by determining the pulses sizes, also, analyzing a histogram makes it possible to obtain various analysis data. (Sysmex Corporation 2012-2014).

2.10 WBCs principle:

RBCs are lysed with stomatolyser-4DL (dying solution) , at the same time , the reagent act on the WBCs membrane to allow dye passage ,which added to enter the WBCs at the damaged portion of the membrane and stain DNA and RNA, following this reaction the sample is analyzed by flow cytometry using a semiconductor laser to detect forward and side scattered light information (four parameter count lymphocytes, monocytes, eosinophil and granulocytes), and by using the acid hemolytic reagent (stomatolyser-FB) this reagent selectively suppresses the degranulation of basophil resulting in separation from the other WBCs which analyzed by flow cytometry using semiconductor laser to give WBCs/ basophil scattered (Hiroyuki Inoue, 1999).

2.11Hemoglobin principle:

HGB is measured by SLS (Sodium Lauryl Sulfate) hemoglobin detection method (Hiroyuki Inoue, 1999).

The detailed action of SLS is still unknown , but presumably SLS convert Hb into methemoglobin in the order of oxyhemoglobin , hemchrome and methemoglobin , and its oxidative activity .therefore, unlike other, this method

does not need oxidative reagent and does not generate toxic waste such as KCN and NaN3 (Oshiro *et al*., 1982).

2.12 Procedure of complete blood count:

Fully automated multichannel instruments require only that an appropriate blood sample is presented to the instrument and usually measure from (8–20)components for the basic CBC and blood cell differential, impedance counting systems depends on the fact that red cells are poor conductors of electricity, whereas certain diluents are good conductors (Dacie and Lewis, 2011).

2.13 Quality control:

The reliability of this instrument and reagents is monitored by quality control, by use of control blood or control materials the stability of the measured value is monitored over a certain period of time and a quality control should be performed before analyzing sample, after replacement of the reagent, after maintenance, if there is any doubt about the accuracy of the analysis value, as required by regulations (Sysmex Corporation 2012 - 2014).

The control materials are of three types eightcheck-3WP-N (Normal), EIGHTCHECK-3WP-L (Low level) and eightcheck -3WP-H (High level) (sysmex corporation 2012-2014).

This instrument has following 2 quality control methods; choose the control method in accordance to your laboratory internal regulations, this methods are X control which use control blood (EIGHTCHECK-3WP) to monitor an instrument performance over time, and Levey -Jennings (L-J) control , which use the data from a single analysis of control blood as quality control data (sysmex corporation 2012-2014).

Quality control process flow selects quality control method, set control blood information, perform quality control analysis, check and record quality control results (quality control chart screen) (Sysmex Corporation 2012-2014).

2.14 Procedure of reticulocyte count:

Reticulocytes are young red cells, newly released from the bone marrow, that still contain ribosomal RNA. On exposure of unfixed cells to certain dyes, such as brilliant cresyl blue or new methylene blue, the ribosomes are precipitated

and stained by the dye, to appear as a reticular network, as the cells are still living when exposed to the dye, this is referred to as supravital staining, with new methylene blue, red cells stain a pale greenish-blue while the reticulum stains bluish-purple, reticulocytes are usually counted as a percentage of red blood cells, the use of an eyepiece containing Miller ocular micrometer disc facilitates counting, reticulocytes are counted in the large squares and the total red cells in the small squares, which are one-ninth of the size of the large cells, reticulocyte counts have traditionally been ex-pressed as a percentage, if an RBC is available an absolute reticulocyte count, which gives a more accurate impression of bone marrow output, can be calculated, as an alternative, a result that is more meaningful than a percentage can be produced by correcting for the degree of anemia as follows:

Reticulocyte index = reticulocyte percentage ×observed PCV / normal PCV, the reticulocyte production index is calculated by dividing the reticulocyte index by the average maturation time of a reticulocyte in the peripheral blood at any degree of anemia (Barbara ,2006).

2.15 Ethical considerations:

The study was revised and ethically approved by the ethical committee of the faculty of medical laboratory sciences, university of Sudan for science and technology, samples were taken with substitute informed consent from patients or their relatives, all data and results was used for research objectives only.

2.16 Data analysis:

The data was analyzed using Statistical Package for the Social Sciences (SPSS) software program (version 19) using measurements of central tendency, measurements of dispersion and statistical significant testing.

Chapter three

Results

Chapter three

Results

3. Results:

One hundred patients comprising (54) males and (46) females were recruited, the mean age (± SD) of the patients was (3.27±1.15) years, (rang: 1-6 years). Anemia was categorized according to HB level, as mild when the HB was (9.1-11.5) constitute (20%) of total cases, moderate when HB was (6.5-9.00) constitute (70%) of total cases and severe when the HB was (4.2-6.4) constitute (10%) of total cases.

There was significant correlation between sex and anemia severity (P= .000), anemia is more sever in females (17%) than males (3%), on other hand there was also significant correlation between age and anemia (P= .000), the age range from (1-3 years) within group 1(most sever group).

There was no significant correlation between sex and HB concentration (P= .163).

3.1 Mean standard deviation and range of red blood cells, RBCS indices, hemoglobin and hematocrit within all cases:

Parameter	Mean ± 2Standard deviation	Range
RBCs (cell/L)	(2.5 ± 0.5) $\times 10^{12}$	(1.46 –4.38) $\times 10^{12}$
HGB (g/dl)	(7.4 ± 1.2)	(4.2– 11.5)
HCT (%)	(21.9± 3.9)	(13.8– 36.9)
MCV (fl)	(88.9 ± 11.9)	(58–129)
MCH (Pg)	(30.1 ± 4.1)	(20.5–43.2)
MCHC (g/dl)	(34.0 ± 2.4)	(30 – 40.4)
RDW (fl)	(59.9± 15.2)	(31.9 –97.9)

3.2 Mean standard deviation and range of red blood cells, RBCS indices, hemoglobin and hematocrit within mild anemia group:

Parameter	Mean ± 2Standard deviation	Range
RBCs (cell/L)	(3.29±0.59) $\times 10^{12}$	(2.48–4.38) $\times 10^{12}$
HGB (g/dl)	(10.12±0.77)	(9.1– 11.5)
HCT (%)	(29.69±3.31)	(26.4– 36.9)
MCV (fl)	(91.7±11.27)	(74.5–111.2)
MCH (Pg)	(31.2±3.98)	(25.8–37)
MCHC (g/dl)	(34.15±1.66)	(31.1 – 36.1)
RDW (fl)	(46.45±10.6)	(35.5 –70.3)

3.3 Mean standard deviation and range of red blood cells, RBCS indices, hemoglobin and hematocrit within moderate anemia group:

Parameter	Mean ± 2Standard deviation	Range
RBCs (cell/L)	(2.55±0.44) $\times 10^{12}$	(1.48–4.08) $\times 10^{12}$
HGB (g/dl)	(7.54±0.62)	(6.6– 9)
HCT (%)	(22.24±2.19)	(17.6– 28.4)
MCV (fl)	(88.3±13.02)	(58.2–129)
MCH (Pg)	(29.8±4.41)	(20.5–43.2)
MCHC (g/dl)	(34.0±2.44)	(30.3 – 40.4)
RDW (fl)	(58.90±12.9)	(31.9 –87.2)

3.4 Mean standard deviation and range of red blood cells, RBCS indices, hemoglobin and hematocrit within severe anemia group:

Parameter	Mean ± 2Standard deviation	Range
RBCs (cell/L)	(1.94±0.24) $\times 10^{12}$	(1.46–2.40) $\times 10^{12}$
HGB (g/dl)	(5.85±0.68)	(4.2– 6.5)
HCT (%)	(17.22±1.84)	(13.8– 19.7)
MCV (fl)	(89.4±8.17)	(74.7–101.7)
MCH (Pg)	(30.3±2.96)	(22.2–33.8)
MCHC (g/dl)	(34.11±2.69)	(30.0 – 39.2)
RDW (fl)	(70.23±18.3)	(35.5 –97.9)

3.5 Mean standard deviation and range of white blood cells and differential counts within all cases:

Parameter	Mean ± 2Standard deviation	Range
WBCs (cell/L)	(15.3 ±6.6) $\times 10^{9}$	(4.1– 41.8) $\times 10^{9}$
Neutrophils (%)	(47.7± 11.6)	(23.6 –77.5)
Lymphocytes (%)	(42.0 ± 11.3)	(10.1 –69.9)
MID (%)	(10.3±2.4)	(5.7 –17.2)

3.6 Mean standard deviation and range of white blood cells and differential counts within mild anemia group:

Parameter	Mean ± 2Standard deviation	Range
WBCs (cell/L)	(11.8 ±5.8) $\times 10^{9}$	(4.1– 22.3) $\times 10^{9}$
Neutrophils (%)	(55.5± 16.2)	(28.0 –77.0)

Lymphocytes (%)	(34.6 ± 15.5)	(10.1 –59.2)
MID (%)	(9.8±2.3)	(7.0 –14.2)

3.7 Mean standard deviation and range of white blood cells and differential counts within moderate anemia group:

Parameter	Mean ± 2Standard deviation	Range
WBCs (cell/L)	(14.6 ±5.6) $\times 10^9$	(6.2– 41.8) $\times 10^9$
Neutrophils (%)	(47.1± 10.1)	(24.0 –71.0)
Lymphocytes (%)	(42.3 ± 9.8)	(22.9 –68.0)
MID (%)	(10.4±2.6)	(5.9 –17.2)

3.8 Mean standard deviation and range of white blood cells and differential counts severe anemia group:

Parameter	Mean ± 2Standard deviation	Range
WBCs (cell/L)	(19.5 ±8.7) $\times 10^9$	(9.7– 41.7) $\times 10^9$
Neutrophils (%)	(45.5± 13.1)	(23.6–69.0)
Lymphocytes (%)	(44.7 ± 13.2)	(22.4 –69.0)
MID (%)	(9.9±2.1)	(5.7 –12.4)

3.9 Mean standard deviation and range of platelets within all cases:

Parameter	Mean ± 2Standard deviation	Range
PLT (cell/L)	(387.0±162.7) $\times 10^9$	(64–1060) $\times 10^9$

3.10 Mean standard deviation and range of platelets within mild anemia group:

Parameter	Mean ± 2Standard deviation	Range
PLT (cell/L)	(337.7±135.6) $\times 10^9$	(159–617) $\times 10^9$

3.11 Mean standard deviation and range of platelets within moderate anemia group:

Parameter	Mean ± 2Standard deviation	Range
PLT (cell/L)	(400.3±164.0) $\times 10^9$	(130–1060) $\times 10^9$

3.12 Mean standard deviation and range of platelets within severe anemia group:

Parameter	Mean ± 2Standard deviation	Range
PLT (cell/L)	(365.1±170.9) $\times 10^9$	(64–709) $\times 10^9$

3.13 Mean standard deviation and range of reticulocytes, CRC and RPI within all cases:

Parameter	Mean ± 2Standard deviation	Range
Retics (%)	(5.4±3.1)	(0.7–15)
CRC (%)	(2.5± 1.4)	(0.3– 6.2)
RPI	(0.62±0.39)	(0.0–2.3)

3.14 Mean standard deviation and range of reticulocytes, CRC and RPI within mild anemia group:

Parameter	Mean ± 2Standard deviation	Range
Retics (%)	(2.44±1.62)	(0.7–6)
CRC (%)	(1.59± 1.2)	(0.4– 4.5)
RPI	(0.62±0.61)	(0.1–2.3)

3.15 Mean standard deviation and range of reticulocytes, CRC and RPI within moderate anemia group:

Parameter	Mean ± 2Standard deviation	Range
Retics (%)	(5.7±2.8)	(1–12)
CRC (%)	(2.8± 1.4)	(0.4– 6.3)

RPI	(0.70±0.36)	(0.1–1.6)

3.16 Mean standard deviation and range of reticulocytes, CRC and RPI within severe anemia group:

Parameter	Mean ± 2Standard deviation	Range
Retics (%)	(5.95±4.1)	(1–15)
CRC (%)	(2.3± 1.4)	(0.3– 6.2)
RPI	(0.36±0.29)	(0.0–1.0)

3.17 Complete blood count parameters associated with disease severity:

3.17.1 Hemoglobin concentration:

HB level show significant correlation with anemia severity (P= .000), mean HB within group 1(5.8 ±0.68), while it's more than the previous mean within group 2 (7.5±0.62) and group 3(10.1±0.77).

3.17.2 WBCs count:

WBCs count show significant correlation with anemia severity (P= .003), i.e.: as the anemia become severe the WBCs count elevated (positive correlation), mean WBCs count within group 1 (19.5±8.69), while the mean is less within group 2 (14.6±5.63) and group 3 (11.8±5.82).

3.17.3 RDW %:

RDW show significant correlation with anemia severity (P= .000), which reflect that the elevation of RDW indicate that the anemia become more sever, mean RDW within group 1 (70.2±18.3), while the mean is less within group 2 (58.9±12.9) and group 3 (46.4±10.6).

3.17.4 RBCs count:

RBCs count show significant correlation with anemia severity (P= .000), which mean that when the anemia become more sever the RBCs count decreased, mean of RBCs count within group 1 (1.94±0.24),while its higher within group 2 (2.55±0.44) and group 3 (3.29±0.59).

3.17.5 Hematocrit:

HCT % reflect significant value of correlation with anemia severity (p= .000), indicating that the HCT% decreased as the anemia become more sever, mean HCT within group 1 (17.2±1.84), while its higher within group 2 (22.2±2.19) and group 3 (29.6±3.31).

3.17.6 Other CBC parameters:

MCV, MCH, MCHC, PLTs count, neutrophils, lymphocytes and MID, all of them show insignificant correlation with anemia severity (p value > 0.05) in all of these variables.

3.18 Retics profile indication for anemia severity and bone marrow activity:

3.18.1 Retics count: increased within group 1 (5.9±4.09), while it's less in group 2(5.7±02.77) and group 3(2.4±1.62), i.e.: retics count elevated as anemia become more sever (p=0.005).

3.18.2 CRC and RPI: show significant correlation with anemia (p=0.031) for CRC and (p=0.003) for RPI, both of them show higher elevation in the moderate form of anemia (2.8±1.36) for CRC and (0.7±0.36) for RPI and they are less within other groups.

3.19 Bone marrow activity:

RPI reflect the activity of bone marrow, the mean RPI within group 1(0.36±0.29), group 2(0.70 ±0.36) and group 3(0.62±0.61), all of them are less than 2, indicating ineffective erythropoiesis, i.e.: the bone marrow is failed to handle the anemia in all cases.

3.20 Findings on Peripheral Blood Picture:

3.20.1 RBCs:

sickle cells were observed in 100% of cases, in addition to other cells with varying type, shape and size were also observed including: target cells ,fragmented RBCs , polychromatic cells and nucleated RBCs(the presence, distribution and number of these cells is vary among the hundred blood films.

(65%) were normocytic normochromic, (21%) were microcytic hypochromic and (14%) of cells were macrocytic normochromic.

3.20.2 WBCs:

Normal in maturation with hypersegmented neutrophils noted in some cases.

3.20.3 PLTs:

Normal in maturation and morphology, some giant platelets were noted.

Chapter Four

Discussion, Conclusion and Recommendations

Chapter Four

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4.1 Discussion:

This study was conducted in Sudanese patients with sickle cell anemia in age between (1 - 6) years for determination of complete blood count parameters including (RBCs count, HGB, HCT, RBCs indices, RDW, WBCs counts, differential WBCs, PLTs counts, reticulocyte profile with 2SD interval, and PBP in order to evaluate the impact of hematological parameters, reticulocyte parameters and blood cell morphology on severity of disease.

Generally this study revealed that RBCs parameters show the follow: RBCs count was (2.5 ± 0.5) cell /L, Hb level was(7.4 ± 1.2) g/dl, HCT level was (21.9± 3.9) %, MCV(88.9 ± 11.9) fl, MCH(30.1 ± 4.1) Pg, MCHC(34.0 ± 2.4) g/dl, RDW (59.9± 15.2) fl, while the results of WBCs and its differential were as follow , total WBCs count (15.3 ±6.6) $\times 10^9$ cell/L, neutrophils (47.7± 11.6) %, lymphocytes (42.0 ± 11.3) %, MID(10.3±2.4)%, platelets count (387.0±162.7) $\times 10^9$ cell/L, retics count (5.4±3.1)%, CRC (2.5± 1.4)% and RPI(0.62±0.39).

This study revealed that some hematological parameters showed significant correlation with anemia severity including: HB% (P= 0.000), WBCs count (P=0.003), RDW (P=0.000), RBCs count (P=0.000), HCT (P=0.000) in addition to retics count (within severe anemia group (P=0.005) and CRC and RPI showed significant correlation with anemia (p=0.031) for CRC and (p=0.003) for RPI.

In comparison with other studies in the field that had been previously mentioned in literature review I found that some hematological parameters had significant P. value comparing to my such measures p.value including:

HB %(P=0.001), HCT (P=0.001) and WBCs count (P=0.001) (Manish *etal*, 2009). HB %(P=0.001), PCV (P=0.001) and WBCs count (0.001) (Emmanuel *etal*, 2015). WBCs count (p=0.0001) and HB %(P=0.003) (Tyseer *etal*, 2016).

HB %(P=0.001) and HCT (P=0.005) (Phelipe *etal*, 2017*)*.

PCV (P=0.000) and HB %(P=0.001) (Samuel *etal*, 2015).

Some parameters in the above mentioned studies showed insignificant correlation with anemia severity including:

RBCs count (P=0.82) in Emmanuel study, RBCs (P=0.593) count and HCT (P=0.581) in Tyseer study and WBCs count (P=0.121) in Phelipe study.

On the other hand some hematological parameters in the previously mentioned literature showed insignificant correlation with anemia severity comparing to the same measures in my study including:

HB %(P=0.55), HCT (P=0.63), RBCs count (P=0.09) and WBCs count (p=0.14) (Sanjeev *etal*, 2012).

PCV (P=0.91), CRC (P=0.20) and WBCs count (P=0.39) (John *etal*, 2014).

HB %(P=0.56), RBCs count (P=0.08) and PCV (P=0.72) (Mohammed, 2015).

4.2 Conclusion:

- RBCs count, HB% level, HCT, RDW and WBCs count showed significant correlation with anemia severity (P.value was significant in all of them), while the rest CBC parameters showed insignificant correlation with anemia severity.
- In comparison with other studies in the field comparing the parameters that had correlation with anemia severity some studies parameters showed significant correlation with anemia severity as in my study and others showed insignificant correlation.
- Retics count, CRC and RPI showed different correlation with anemia severity within the three different anemia groups.
- Bone marrow failed to compensate anemia in all cases (ineffective erythropoiesis).
- In PBP sickle cells were observed in all patient's blood films, in some cases the anemia was microcytic hypochromic , the majority showed normocytic normochromic anemia , while it was macrocytic in some cases.

4.3 Recommendations:

1-Increase the sample size.

2-Performing of HB electrophoresis because the inheritance pattern will affect the anemia severity.

3-Appropriate and adequate medical supplies must be given in order to limit the complication of anemia (loss of iron and folate) and to assess the bone marrow to deal with anemia.

4-Educational programs about SCA may also be need as well as specialized centers for sickle cell disease survey, diagnosis and continues medical care, such centers may determine easily the affected newborn children.

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5. References

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5.2 Appendices:

5.2.1 Questionnaire:

بسم الله الرحمن الرحيم

Sudan University of science and technology

College of Graduate studies

science Diagnostic Value of Complete Blood Count, Blood Cell Morphology and Reticulocyte Count in Determination of disease severity in Individuals with Sickle Cell Disease 2018

تاثير مؤشرات الدم العام، مؤشرات الخلايا الشبكيه وشكل خلايا الدم على حدة المرض عند الاطفال السودانين المصابين بمرض الخلايا المنجليه ٢٠١٨

_ Date: / /2016

_ Name / Sample number: ……

_ Age: …….. Years

_ Residence: ……

_ Telephone number: …….

_ Nutritional status: good { } poor { }

_ Presence of other diseases:

-Malaria: Yes { } No { } - chronic disease(s): Yes { } No { } -Inflammation: Yes { } No { } -others: { }

_ Family history of disease(s): Yes { } No { }

_ History of blood loss: Yes { } No { }

_ Supplementation of treatment: Yes { } (which type?)……No { }

_ History of blood transfusion: Yes { } Donor { } Recepient { } No { }

_Number of crisis: { }

5.2.2 Informed consent:

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

قسم علم الدم والمناعة الدموية

اقرار موافقه

رقم الهاتف:..... اقر انا:.....(من ينوب عن المشترك)

بأنني اطلعت على بيان مفصل عن البحث من الجهة القائمة عليه والذي يهدف لتقييم الدم العام، تعداد الخلايا الشبكيه وشكل خلايا الدم وتاثيرها على حدة المرض في المرضى السودانين المصابين بمرض الخلايا المنجليه وأنه تم اطلاعي على أنه سيتم اخذ 2.5 مل من الوريد بواسطه حقنة طعن وذلك بعد مسح منطقة الطعن بواسطة مطهر وأن كل الأدوات المستخدمة لاخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية واوافق بكامل اختياري علي المساهمة في هذه الدراسة .