بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Evaluation of Some Hematological Parameters in Sickle Cell AnemiaChildren in Omdurman Locality

تقويم بعض مكونات الدم عند الأطفال المصابين بالأنيميا المنجلية في محلية أم درمان

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بِسْمِ اللّهِ الرّحْمَنِ الرّحِيمِ

قال الله تعالى :

اللَّهُ لَا إِلَهَ إِلَّا هُوَ الْحَيُّ الْقَيُّومُ لَا تَأْخُذُهُ سِنَةٌ وَلَا نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ مَن ذَا الَّذِي يَشْفَعُ عِندَهُ إِلَّا بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلَا يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلَّا بِمَا شَاءَ وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالْأَرْضَ وَلَا يَئُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ

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Dedication

- *To …my husband who encourage me.*

- *To… my beloved and blessed parents who did everything for me.*

- *To…my dear (all members of my family who spent more time to encourage and support me.*

- *To …my wonderful supervisor prof: Babiker Ahmed Mohamed who was with me when I need him.*

- *To … my teachers and very special friends and colleagues who were an integral part of my support group.*

- *Special dedications to all sicklier children whom sample are collected.*

For all of you
I dedicate this work
Samah

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First of all thank my God for helping and supporting my research and lighting the way for us.

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Abbreviations

CBC	Complete blood count
EDTA	Ethylene Diamin Tetra Acetic Acid
Epo	Erythropoietin
ICSH	International committee for standardization in Hematology
MCH	Mean corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
nRBC	Nucleated red blood cell
PCV	packed red cell volume
PLT	platelets
RBC	Red blood cell
RNA	Ribonucleic Acid
WBCs	white blood cells

Abstract

This is a case control study, conducted in Omdurman locality during the period from April to May 2015, to evaluate (Hb , RBCs, HCT, MCV, MCH, MCHC, Platelets, WBCs) of 70 Sudanese Sickle cell anemia children as case and 30 as a control group . children □s parents have been informed about the study and agreed forparticipation.

Aquestionnaire was designed to collect demographic data about the study group. Two and half ml venous blood was collected in EDTA anticoagulant container and analyzed in automated hematological analyzer (Sysmex(F-820).) to measure Some hematological parameters, and the results were analyzed using SPSS computer programme.

The results showed that:RBCs (2.6±0.9×1012), Hb(6.9±1.4g/dl), HCT(20.8±4.3%), MCV(79.9±11.8fl), MCH(26.9±4.7pg), MCHC (33.5±1.6%) WBCs (19.2±8.9×109/L), ,platelets count (348±1.57×109/L) in case and RBCs (4.1±0.4×1012), Hb (12.6±0.4g/dl) HCT(36.7±2.3%), MCV (90.6±6.2fl), MCH (28.9±1.9 pg) MCHC (33.6±1.3%) WBCs(5.2±1.3×109/L) platelets (237.3±48.3×109/L) were found in control group. RBCs, Hb, HCT, MCV,and MCH lower in patient than control group (Mean±SD: RBCs(2.6±0.9×1012),Hb(6.9±1.4g/dl),HCT(20.8±4.3%),MCV(79.9±11.) and MCH(26.9±4.7pg) .The difference was statistically significant(p=0.00,0.00,0.00,0.00,0.02), Insignificant in mean of MCHC (P=0.70),Platelets count and WBCs weare increased inpatientsthancontrolgroup(Mean±SD:platelets(237.3±48.3×109/L),WBCs(5.2 ±1.3×109/L) .The difference was statistically significant (P=0.00,0.00).Sever anemia (51.4%) was most frequent compared with moderate (44.3%) and mild anemia (4.3%) in sickle cell anemia children.

There was insignificant in mean of WBCs, RBCs, Hb, HCT,MCV, MCH, MCHC and platelets in patient with crisis (P = 0.36,0.64,0.43,0.43,0.58,0.69,0.77,0.33) when compared to those without crisis in sickle cell anemia children .

RBCslower in female than male (Mean±SD:

RBCs(2.403±0.610×1012)in femle,(2.834±1.025×1012) in male).The

difference was statistically ignificant(p=0.05) but theMCH, MCHClower in male than female(Mean±SD:MCH (26.125±5.459) pg in male,(28.330±2.683)pginfemale)MCHC(33.261±1.781%)in male,(34.100±1.082%) in female).The difference

wasstatisticallysignificant (p=0.05,0.03), Insignificantin means of WBCs, Hb, HCT,MCV and platelets in male(P=0.38,0.49,0.40,0.08) compared with female in sickle cell anemia children. There was insignificant in mean of WBCs, RBCs, Hb, HCT,MCV, MCH, MCHC and plateletin patient with age less than 5years(P=0.11,0.55,0.65,0.67,0.43,0.36,0.36,0.71) compared with patient with age 5-10 years and more than 10 years in sickle cellanemia children**.**

المستخلص

اجريت هذه الدراسة بطريقة الحالات الإفرادية المقترنة بحالات ضابطة في محلية ام درمان في الفترة من ابريل2015 إلي مايو 2015 لقياس بعض مقاييس الدم عند الاطفال المصابين بالانميا المنجليه. تم إختيار 70 من الاطفال المصابين كعينات إختبارية بعد اخذ موافقه والديهم، وتم إختيار 30 طفل من غير المصابين كعينات ضابطة.تم اخذ(2.5ملي لتر)عينة دم وريدية من كل متبرع ووضعت فى إناء بلاستيكى يحتوى على مانع تجلط(EDTA) وتم اختبارها لقياس بعض مقاييس الدم بإستخدام جهاز تحليل الدم الآلي. وتحليل النتائج بنظام الحزم الاحصائية للتحليل الاحصائي.

اظهرت النتائج أن:متوسط عدد كريات الدم الحمراء ($2.6\pm0.9\times10^{12}$/L) ومتوسط الهيموقلوبين.(6.9 ± 1.4g/dl) .ومتوسط حجم كريات الدم الجمراء المضغوطة ($20.8\pm4.3\%$)ومتوسط حجم كرية الدم الحمراء (79.9 ± 11.8fl)ومتوسط الهيموقلوبين في الكرية الحمراء(26.9 ± 4.7pq) ومتوسط تركيز الهيموقلوبين فى الكرية الحمراء ($33.5\pm1.6\%$) ومتوسط عدد كريات الدم البيضاء ($19.2\pm8.9\times10^{9}$/L) ومتوسط عدد الصفائح الدموية ($348\pm1.57\times10^{9}$/L) فى الاطفال المصابين بالآنيمياء المنجلية ومتوسط عدد كريات الدم الحمراء ($4.1\pm0.4\times10^{12}$) ومتوسط الهيموقلوبين (12.6 ± 0.4g/dl)ومتوسط حجم كريات الدم الجمراء المضغوطة ($36.7\pm2.3\%$)ومتوسط حجم كرية الدم الحمراء(90.6 ± 6.2fl) ومتوسط الهيموقلوبين في الكرية الحمراء(28.9 ± 1.9pg)ومتوسط تركيز الهيموقلوبين فى الكرية الحمراء($33.6\pm1.3\%$) ومتوسط عدد كريات الدم لبيضاء ($5.2\pm1.3\times10^{9}$/L)ومتوسط عدد الصفائح الدمويه($237.3\pm48.3\times10^{9}$/L)فى الاطفال الغير المصابين بالآنيمياء المنجلية.

عدد كريات الدم الحمراءو الهيموقلوبين وحجم كريات الدم الحمراء المضغوطةوحجم كرية الدم الحمراءو الهيموقلوبين في الكرية الحمراء اقل في الاطفال المصابين مقارنه بالاطفال الغير مصابين متوسط عدد كرياتالدم الحمراء ($2.6\pm0.9\times10^{12}$/L) ومتوسط الهيموقلوبين .(6.9 ± 1.4g/dl)ومتوسط حجم كريات الدم الحمراء المضغوطة ($20.8\pm4.3\%$)ومتوسط حجم كرية الدم الحمراء (79.9 ± 11.8fl)ومتوسط الهيموقلوبين في الكرية الحمراء(26.9 ± 4.7pq) وهذا الاختلاف ذات دلاله معنويه,($p=0.00,0.00,0.00,0.00,0.02$) ولا يوجد اختلاف في متوسط تركيز الهيموقلوبين فى الكرية الحمراء,($P=0.70$) وان هنالك زياده في عدد كريات

الصفائح المويهو عدد كريات الدم البيضاءفي الاطفال المصابين مقارنه بالاطفال الغير مصابينمتوسط عدد الصفائح الدمويه($237.3\pm48.3\times10^9$/L)ومتوسط عدد كريات الدم البيضاء($5.2\pm1.3\times10^9$/L)وهذا الاختلاف ذات دلاله معنويه(p=,0.00,0.00).

الانميا الحاده (51.4%)الاكثر انتشارا مقارنه بالانميا المتوسطه(44.3%) والانميا البسيطه(4.3%) في الاطفال المصابين.

وليست هنالك دلاله معنويه في متوسط عدد كريات الدم البيضاء ومتوسط عدد كريات الدم الحمراء و ومتوسط الهيموقلوبين و ومتوسط حجم كريات الدم الجمراء المضغوطة ومتوسط حجم كرية الدم الحمراء ومتوسط الهيموقلوبين في الكرية الحمراء ومتوسط عدد الصفائح الدمويه في الاطفال الذين لديهم اعراض واضحه(P=0.36,0.64,0.43,0.43,0.58,0.69,0.77,0.33) مقارنه بالاطفال الذين ليس لديهم اعراض واضحه المصابين بالانميا المنجليه.

هنالك نقص في عدد كريات الدم الحمراء في النساء مقارنه بالرجال متوسط كريات الدم الحمراء في النساء($2.403\pm0.610\times10^{12}$) وفي الرجال ($2.834\pm1.025\times10^{12}$) وهذا الاختلاف ذات دلالة معنوية (p=0.05) ولكن الهيموقلوبين في الكرية الحمراء وتركيز الهيموقلوبين في الكريه الحمراء اقل في الرجال مقارنه في النساء متوسط الهيموقلوبين في الكريه الحمراء في الرجال (26.125±5.459 pg) وفي النساء (28.330±2.683pg) ومتوسط تركيز الهيموقلوبين في الكريه الحمراء في الرجال (33.261±1.781%) وفي النساء (34.100±1.082%), هذا الاختلاف ذات دلاله معنويه(p=0.05,0.03) وليس هنالك دلاله معنويه في متوسط عدد كريات الدم البيضاء و متوسط الهيموقلوبين و ومتوسط حجم كريات الدم الجمراء المضغوطة ومتوسط حجم كرية الدم الحمراء في الرجال (P=0.38,0.49,0.40,0.08) مقارنه بالنساء في الاطفال المصابين بالانميا المنجليه.

وليست هنالك دلاله معنويه في متوسط عدد كريات الدم البيضاء ومتوسط عدد كريات الدم الحمراء و ومتوسط الهيموقلوبين و ومتوسط حجم كريات الدم الجمراء المضغوطة ومتوسط حجم كرية الدم الحمراء ومتوسط الهيموقلوبين في الكرية الحمراء ومتوسط عدد الصفائح الدمويه في الاطفال الذين اعمارهم اقل من 5 سنين(P=0.11,0.55,0.65,0.67,0.43,0.36,0.36,0.71) مقارنه بالاطفال الذين اعمارهم بين 5-10 سنين وما فوق 10 سنين.

Chapter One

Introduction and Literature Review

Chapter one

Introduction and Literature Review

1.1Introduction

.Sickle cell anemia is an inherited, long life disease. People who have the disease are born with it. They inherit two genes for sickle hemoglobin one from each parent. People who inherit a sickle hemoglobin gene from one parent and a normal gene from the other parent have a condition called sickle cell trait. (Quinn *at el*., 2004).

Sickle cell trait is different than sickle cell anemia. People who have sickle cell trait don't have the disease. Like people who have sickle cell anemia, people who have sickle cell trait can pass the sickle hemoglobin gene to their children(Quinn *at el*., 2004).

Sickle cell disease is caused by a genetic abnormality in the gene for hemoglobin, which results in the production of sickle hemoglobin. When oxygen is released from sickle hemoglobin, it sticks together and forms long rods, which damage and change the shape of the red blood cell. The sickle red blood cell causes the symptoms of sickle cell disease (Yawn *at el*., 2014).

Transfusions of red blood cells are given for severe anemia, to prevent strokes, and before surgery. Sometimes an exchange transfusion is performed with a special machine that removes the abnormal sickle red blood cells and replaces them with normal red blood cells (Olujohungbe and Howard , 2008).

1.2 Literature Review

1.2.1Erthropoiesis

The process of erythropoiesis includes all steps of haemopoiesis, starting with the initial specification of haemopoietic stem cells from mesoderm during embryogenesis. This continues with the decisions of these cells to undergo self-renewal or differentiation, through the process of lineage specification and proliferation to form committed erythroid progenitors. Finally, erythroblasts undergo terminal differentiation and post-mitotic maturation as they develop into red blood cells. Many cytokines, growth factors and hormones also influence erythroid proliferation, differentiation and maturation (Hoff brand *et al*., 2005).

Over the past 10 years, key transcription factors controlling the internal programs of erythroid progenitors have been identified and some insights into their roles in lineage specification and erythroid differentiation have been discovered. Understanding the basic biology of erythropoiesis provides a logical basis for the diagnosis and treatment of the inherited and acquired anemia (Hoff brand *et al*., 2005).

1.2.1.1Morphological stages of the red cell

There are six stages of maturation in the red cell series:
pronormoblast, basophilic normoblast, polychromatophilic normoblast, orthochromic normoblast,reticulocyte, and mature red cell. In general, several morphological clues mark the red cell maturation series.
When the red cell is nucleated, the nucleus is “baseball” round. mias that are so frequently encountered in clinical practice. In the bone marrow and in the peripheral smear, each of these clues is helpful in enabling the technologist to stage a particular red cell. Identification of immature red cells should be systematic and based on reliable morphological criteria.

Each red cell maturation stage will be described using size, N:C ratio, nuclear chromatin characteristics, and cytoplasm descriptions. N:C ratio implies the amount of nucleus to the amount of cytoplasm present (Firkin *et al*.,1989).

1.2.1.1.1 Pronormoblast

- Size: 18 to 20 μm, the largest and most immature, the "mother cell" N:C ratio: 4:1 Nuclear chromatin: 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: Dark marine blue definitive areas of clearing immature is the cell. (Firkin *et al*., 1989).

1.2.1.1.2 Basophilic Normoblast

- Size: 16 μm,
- N:C ratio: 4:1,
- Nuclear chromatin: Round nucleus with crystalline chromatin appearance.
- Cytoplasm: Cornflower blue with indistinct areas of clearing (Firkin *et al*.,1989).

1.2.1.1.3 Polychromatophilic Normoblast

- Size: 13 μm,
- N:C ratio: 2:1,
- Nuclear chromatin: Chromatin is condensed, moderately compacted,
- Cytoplasm: A color mixture, blue layered with tinges of orange red. (Firkin *et al*., 1989).

1.2.1.1.4 Orthochromic Normoblast-Nucleated (nRBC)

- Size: 8 μms,
- N:C ratio: 1:1,
- Nuclear chromatin: Dense, velvet-appearing homogenous chromatin,
- Cytoplasm: Increased volume, with orange-red color tinges with slight blue tone (Firkin *et al.*,1989).

1.2.1.1.5 Reticulocyte

- Size: 8 μm,
- Appearance: Remnant of RNA visualized as reticulum, filamentous structure in chains .(Firkin *et al.*, 1989).

1.2.1.1.6 Mature Red Cell

- Size: 6 to 8 μm,
- Appearance: Disk-shaped cell filled with hemoglobin, having an area (Firkin *et al.*, 1989).

1.2.2 Anemia

Anemia is a condition where there is a lower than normal number of red blood cells in the blood, usually measured by a decrease in the amount of hemoglobin. Hemoglobin is the amount of the oxygen carrying part of red blood cells their red color.

The causes of anemia fall into three major path physiological categories:

1. Blood loss

2. Impaired red cell production

3. Accelerated red cell destruction (hemolysis in excess of the ability of the marrow to replace these losses) Anemia may be a sign of an underlying disorder. Delusional anemia with normal or increased total red

cell mass may occur with pregnancy, macroglobulinemia, and splenomegaly.

Some anemia's have more than one pathogenesis mechanism and go through more than one morphological state, such as blood loss anemia. In the case of accelerated red cell destruction, hemolysis in excess of the ability of the marrow to replace these losses occurs (Turgeon, 2010).

The cause varies with the type of anemia, potential causes Include blood loss, poor diet, and many diseases. Mediation reactions, and various problems with the bone morrow where women who have heavy menstrual periods Risk factors include heavy periods, pregnancy, older age and disease that causes anemia (Smith, 2010).

1.2.2.1Clinical signs and symptoms of anemia.

The clinical signs and symptoms of anemia can result from diminished delivery of oxygen to the tissues. Signs and symptoms of anemia are related to the lowered hemoglobin concentration. In addition, clinical signs reflect the rate of reduction of hemoglobin and blood volume. If anemia develops slowly in a patient who is not otherwise severely ill, a hemoglobin concentration of as low as 6 g/dL may develop without producing any discomfort or physical signs if the patient is sedentary.

The usual complaints of an anemic patient are easy fatigability and dyspnea on exertion. Other general manifestations can include vertigo, faintness, headache, and heart palpitations.

The most common physical expressions of anemia are pallor, low blood pressure, a slight fever, and some edemas (Saborio and Scheinman ,1999).

1.2.2.2Classification of anemia

Many different types of anemias exist, with many causes and manifestations.

These classifications group anemia's based on erythrocyte morphology, physiology, or probable etiology.

The method based on red cell morphology, which was originally proposed by Wintrobe, categorizes anemias by the size of the erythrocytes. Anemia also may be classified by red cell morphology as M**acrocytic, normocytic,** or **microcytic.**

The major limitation of such a classification is that it tells nothing about the etiology or reason for the anemia. Several schemes have been proposed to categorize anemias by etiology. None of this classification is entirely satisfactory because within each classification the various Subdivisions are not completely inclusive. However, the basic mechanism or probable mechanism responsible for the anemia(Turgeon, 2010).

Although many classification schemes exist, a classification system that divides the major pathophysiological characteristics into three major categories is easier to understand than other systems. The three major categories in this system are accelerated erythrocyte destruction, blood loss, and impaired RBC production. (Turgeon, 2010).

1.2.2.3 Laboratory findings

Although the red cell indices will indicate the type of anemia, further useful information can be obtained from the initial blood sample.

1.2.2.3.1 Leukocyte and platelet counts

Measurement of these helps to distinguish 'pure' anemia from 'pancytopenia' (a drop in red cells, granulocytes and platelets) which suggests a more general marrow defect (e.g. caused by marrow Hyperplasia or infiltration) or general destruction of cells (e.g. hypersplenism). In anemia's caused by hemolysis or hemorrhage, the neutrophil and platelet count are often raised; in infections and leukemia's

the leucocytes count is also often raised and there may be abnormal leucocytes or neutrophil precursors present (Hoff brand *et al.,* 2006).

1.2.2.3.2 Reticulocyte count

The normal percentage is 0.5-2.5%, and the absolute count 25-125 x $10^9/L$. This should rise in anemia because of erythropoietin increase and be higher the more severe the anemia. This is particularly so when there has been time for elytroid hyperplasia to develop in the marrow as in chronic hemolysis.

After an acute major hemorrhage there is an erythropoietin response in 6 hr, the reticulocyte count rises within 2-3 days, reaches a maximum in 6-10 days and remains raised until the hemoglobin returns to the normal level. If the reticulocyte count is not raised in an anemic patient this suggests impaired marrow function or lack of erythropoietin stimulus (Hoff brand *et al.,* 2006).

1.2.2.3.4 Blood film

It is essential to examine the blood film in all cases of anemia. Abnormal red cell morphology or red cell inclusions may suggest a particular diagnosis. When causes of both microcytosis and macrocytosis are present (e.g. mixed iron and foliate or B12 deficiency) the indices may be normal but the blood film reveals a 'dimorphic' appearance. During the blood film examination the white cell differential count is performed, platelet number and morphology are assessed and the presence or absence of abnormal cells (e.g. normoblasts, granulocyte precursors or blast cells) (Hoff brand *et al.,* 2006).

1.2.2.3.5 Bone marrow examination

This may be performed by aspiration or trephine biopsy .During bone marrow aspiration a needle is inserted into the marrow and a liquid sample of marrow is sucked into a syringe. This is then spread on a slide for microscopy and stained by the usual Roman wsky technique. A great

deal of morphological information can be obtained by examining aspirate slides. The detail of the developing cells can be examined (e.g. normoblastic or megaloblastic), the proportion of the different cell lines assessed (myeloid: erythroid ratio) and the presence of cells foreign to the marrow (e.g. secondary carcinoma) observed (Hoff brand *etal.*,2006).

1.2.3 Sickle cell anemia

Sickle cell disease is a group of hemoglobin disorders in which the sickle globin gene is inherited.

Homozygous sickle cell anemia (Hb SS) is the most common while the doubly heterozygote conditions of Hb SC also cause sickling disease. Hb SS is insoluble and forms crystals when exposed to low oxygen tension.

The red cells sickle and may block different areas of the microcirculation or large vessels cause infarcts of various organs (Hoff brand *et al.,* 2006).

1.2.3.1 Clinical features

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 *(Oz)* to tissues relatively easily 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 adults. Crises may be vase-occlusive, visceral, plastic or hemolytic (Hoff brand *et al.,* 2006).

1.2.3.1.1 Painful vase-occlusive crises

These are the most frequent and are precipitated by such factors as infection, acidosis, dehydration or deoxygenating (e.g. altitude, operations, obstetric violent exercise). Infarcts can occur in a variety of organs including the bones (hips, shoulders are commonly affected), the lungs and the spleen. The most serious vase occlusive crisis is of the brain (a stroke occurs in 7% of all patients) or spinal cord. Delivery, stasis of

the circulation, exposure to cold, this predicts for strokes in children. This can be largely prevented presentation of the disease and may lead to digits of varying lengths `by regular blood transfusions in these cases. The 'hand-foot' syndrome (painful dactylitis caused by infarcts of the small bones) is frequently the first (Hoff brand *et al.,* 2006).

1.2.3.1.2 Visceral sequestration crises

These are caused by sickling within organs and pooling of blood, often with a severe exacerbation of anemia. The acute sickle chest syndrome is a feared complication and the most common cause of death after puberty. It presents with dyspnoea, falling arterial, chest pain and pulmonary infiltrates on chest X-ray. Treatment is with analgesia, oxygen, exchange transfusion and ventilator support if necessary. Hepatic and girdle sequestration crises and splenic sequestration may lead to severe illness requiring exchange transfusions. Splenic sequestration is typically seen in infants and presents with an enlarging spleen, falling hemoglobin and abdominal pain. Treatment is with transfusion and patients must be monitored at regular intervals as progression may be rapid. Attacks tend to be recurrent and splenectomy is often needed (Hoff brand *et al.,* 2006).

1.2.3.1.3 Aplastic crisis

These occur as a result of infection with parvovirus or from folate deficiency and are characterized by a sudden fall in hemoglobin, usually requiring transfusion. They are characterized by a fall in reticulocytes as well as hemoglobin (Hoff brand *et al.,* 2006).

1.2.3.1.4 Hemolytic crises

These are characterized by an increased rate of hemolysis with a fall in hemoglobin but rise in reticulocytes and usually accompany a painful crisis (Hoff brand *et al.,* 2006).

1.2.3.2 Other clinical features

Ulcers of the lower legs are common, as a result of vascular stasis and local ischaemia. The spleen is enlarged in infancy and early childhood but later is often reduced in size as a result of infarcts (autosplenectomy). A proliferative retinopathy and priapism are other clinical complications.

Chronic damage to the liver may occur through micro infarcts. Pigment (bilirubin) gallstones are frequent. The kidneys are vulnerable to infarctions of the medulla with papillary necrosis. Failure to concentrate urine aggravates the tendency to dehydration and crisis, and nocturnal enuresis is common. Osteomyelitis may also occur, usually from Salmonella spp (Hoff brand *et al.*, 2006).

1.2.3.3 Laprotory Findings

1. The hemoglobin is usually 6-9 g/dL-low in comparison to symptoms of anemia.

2. Sickle cells and target cells occur in the blood Howell Jolly bodies may also be present.

3. Screening tests for sickling are make blood is deoxygenated (e.g. with dithionate and *Naz* $HP0_4$)·

4. Hemoglobin electrophoresis in Hb SS is detected (Hoff brand *et al.*, 2006).

1.2.3.4 Sickle cell trait

This is a benign condition with no anemia and normal appearance of red cells on a blood film. Haematuria is the most common symptom and is thought to be caused by minor infarcts of the renal papillae. Hb S varies from 25 to 45% of the total hemoglobin. Care must be taken with anesthesia, pregnancy and at high altitudes given microspherocytes. The spleen is enlarged. The carriers show a few target cells only. (Hoff brand *et al.*, 2006).

1.2.3.5 Combination of hemoglobin S with other genetic defects of hemoglobin

The most common of these are Hb S*I* B-thalassaemia, and sickle *cell/C* disease. In Hb S/B-thalassaemia, the MCV and MCH are lower than in homozygous Hb SS. The clinical picture is of sickle cell anemia; splenomegaly is usual. Patients with Hb SC disease have a particular tendency to thrombosis and pulmonary embolism, especially in pregnancy (Hoff brand *et al.*, 2006).

In general, when compared with Hb SS disease, they have a higher incidence of retinal abnormalities, milder anemia, splenomegaly and generally a longer life expectancy. Diagnosis is made by hemoglobin electrophoresis, particularly with family studies (Hoff brand *et al.*, 2006).

1.2.3.6 Prenatal diagnosis of genetic hemoglobin disorders.

It is important to give genetic colffiselling to couples at risk of having a child with a major hemoglobin defect.

When both partners show an abnormality and there is a risk of a serious defect in the offspring, particularly B-thalassaemia major, it is important to offer antenatal diagnosis. Several techniques are available, the choice depending on the stage of pregnancy and the potential nature of the defect (Hoff brand *et al.*, 2006).

1.2.3.7 DNA diagnosis

The majority of samples are obtained by chorionic villous biopsy although amniotic fluid cells are sometimes used. Techniques to sample maternal blood for fetal cells or fetal DNA-are being developed. The DNA is then analyzed using one of the following methods.

Polymerase chain reaction (PCR) is the most commonly used technique Gap-PCR analysis is useful for detecting gene deletions in a-thalassaemia &B thalassaemia, and Hb Lepore. Small deletions and point mutations are diagnosed by cycle-sequencing of PCR product using fluorescent labels

and analysis of the fragments on a capillary-based automatic sequencer. This approach is useful for rare and unknown mutations, for confirming prenatal diagnosis of B-thalassaemia and sickle cell disorders by other PCR methods, and for the rare non-deletion

a+-thalassaemia mutations that result in severe Hb H hydrops fetalis syndrome.

Pre-implantation genetic diagnosis which avoids the need for pregnancy termination involves performing conventional in *vitro* fertilization, followed by removing one or two cells from the blastomeres on day 3. PCR is used to detect thalassaemia mutations so that lmaffected blastomeres can be selected for implantation. HLA typing can also be used to select an HLA matching blastomere matching a commonly used technique and may be performed by using primer pairs that only amplify previous thalassaemia major child. Ethical considerations are important in deciding to use these applications (Hoff brand *et al.*, 2006).

1.2.4 Complete blood cell count

1.2.4.1 Hemoglobin

Hemoglobin is the life-giving substance of every red cell, the oxygen-carrying component of the red cell.

Each red blood cell is nothing more than a fluid-filled sac, with the fluid being hemoglobin. In 4 months, or 120 days, red cells with normal hemoglobin content submit to the rigors of circulation. Red cells are stretched, twisted, pummeled, and squeezed as they make their way through the circulatory watershed (Firkin *etal* .,1989).

Each major organ in the human body depends on oxygenation for growth and function, and this process is ultimately under the control of hemoglobin. (Firkin *etal* .,1989).

1.2.4.2 Red blood cells

Red blood cells circulate in the blood and carry oxygen throughout the body. They are produced in the bone marrow and then released into the bloodstream as they mature. RBCs have a typical lifespan of about 120 days and are continuously renewed and replaced as they age and degrade or are lost through bleeding. A relatively stable number of RBCs is maintained in the circulation by increasing or decreasing the rate of production by the bone marrow. (Bunn,2000).

1.2.4.3 Hematocrit.

The hematocrit or packed red cell volume (PCV) refers to the proportion of the volume of red cells, relative to the total volume of the blood.

- Normal Range:

  36 to 48% for women

  40 to 55%for men (Turgeon, 2010).

1.2.4.4 Total white blood cell count (WBC)

A WBCs count is a test that measure the number of white blood cell in the body.This test is often included with a complete blood count (CBC) .The blood contains a percentage of each type of white blood cell cell. (Ciesla , 2007)

1.2.4.5 Platelet count

Platelets can be counted in whole blood using the same techniques of electrical detection as is used for counting red cells, normal range in women $280 \pm 130 \times 10^9$/L (Dacie and Lewis , 2011).

1.2.4.6 Red cell indices

1.2.4.6.1. Mean corpuscular volume

The mean volume of red cells (MCV) was formerly determined by dividing the total volume of red cells. (Derived from the packed cell volume, PCV) by the number of red cells in that particular sample of blood. The accuracy of the total volume determination by the manual

haematocrit method provided little difficulty, but manual estimation automated electronic-particle counting devices have revolutionized the estimation of the MCV .

- Normal Range:

  From 80 to 100 FL (Turgeon, 2010).

1.2.4.6.2 Mean corpuscular Hemoglobin

The mean amount of hemoglobin per red cell (MCH) is also rapidly and reliably estimated by automated electronic counting devices by dividing the total amount of hemoglobin by the number of red cells in a sample of blood..

- Normal Range:

  27 to 31pg (Turgeon, 2010).

1.2.4.6.3 Mean corpuscular hemoglobin concentration

· The mean concentration of hemoglobin within red cell (MCHC) reflects an entirely different parameter than the MCH. It is derived by dividing, the concentration .o f hemoglobin in g/ dl by the volume of red cells in ml/dl. Both measurements are readily and reliably obtained by manual subnormal MCHC is usually indicative of an abnormality where interference with the synthesis of hemoglobin is greater than that of other constituents of the red cells, as in thalassaemia or iron deficiency.

- Nomal Range:

  31 to 36%. (Turgeon, 2010).

1.2.5. previous studies:

the result revealed that there were significant decreased in previous study of hematological profile in sickle cell anemic children in Oxfourd,UK,by (Bain2006), the result revealed that there were significant decreased in Hb, PCV, MCH and MCHC compard with non sickler children .but

platelets count and White blood cell count were normal. (Abdelgader *et al*., 2014).

In other previous study of complete blood count in sickle cell anemic children (Platt ,2006), the result revealed that there were significant decreased in Hb, PCV, MCH and MCHC compard with non sicklier children , platelets count and White blood cell count were significant increased . (Abdelgader *et al*., 2014).

In other Previous study of complete hem gram in sickle cell anemic children in United State,(Abbott et al.,2002) , the result revealed that there were significant decreased in Hb, PCV, MCH and MCHC compard with non sicklier children ,White blood cell count and plateles count were increased . (Abdelgader *et al*., 2014).

1.3 Rationale

Sickle cell anemia is widely distributed in Sudan with high rate of morbidity and mortality and associated with chronic crisis and inflammatory state, so routine measurement of hematological parameter of sickle cell patient is important to detect complication earlier, The result of the study could be a base line data about some hematological parameters of Sickle cell anemic children, since few data are available in Sudan.

1.4 Objectives.

1.4.1-General objective

Study of Some Hematological Parameters in children sickle cell anemia patients in Omdurman locality.

1.4.2-Specific objectives

-To measure the change in Hb, RBCs count, PCV in children with sickle cell anemia patient

-To classify patients according to severity of sickle cell anemia.

-To compare result of hematological parameter with crisis.

-To compare result of hematological parameter with gender and age.

Chapter Two

Materials and Methods

Chapter Two

Materials and Methods

2.1. Study design

This study is case control study conducted in the period from April to May 2015 to measure the effect of Some Hematological parameters in sickle cell anemia children.

2.2. Study area

Area of study is Omdurman hospital.

2.3. Study population

Seventy children with sickle cell anemia were enrolled in the study and thirty non sickler as control.

2.4. Inclusion criteria

Children with Sickle cell anemia with different age groups were included.

2.5. Exclusion criteria

Presence of any diagnostic disease, previous blood transfusion (3 months) may affect the parameter under study was excluded.

2.6. Data collection

Data was collected using aquestionnaire which was specifically designed to obtain information about demographic and clinical data.

2.7. Sample collection

Blood collection with care and adequate safety precaution to ensure test result are reliable, contamination of sample avoided.

Sampling: EDTA venous blood was collected . International committee for standardization in hematology (ICSH) recommends the use of (EDTA K3) at concentration of (1.5+-0.20mg/ml) of blood.

2.8. Materials Required

-Syringe and container with (EDTA) anticoagulant

-Alcohol (75%) disinfectant

-Cotton

Equipment: Sysmex(F-820).

2.9. Method of Diagnosis

Automated method by (sysmexF.820) .There are two transducer chambers one used to count WBCs and Hb together and other used to count RBCs and platelet.Apportion of blood separated aspirated whole blood and mixed with diluents in pre-test ratio. A defined amount of this dilution is sent to detection chamber and passed through a small opening known as aperture. There are also electrodes on each side of aperture- and direct current pass through these electrodes. The direct current resistances between the electrodes changes as the blood suspension pass through aperture. This resistance cause an electrical pulse change proportional to the size of blood cell. These electrical data are converted into graphical displays of volume distribution curve, or histogram.

2.10. Ethical consideration

An informal consent from their parents of selected individuals study was taken after being informed with all detailed objective of study.

2.11. Staistical analysis

Data were entered in to computer and analyzed by SPSS using independent T test. The significant P value was set at $P \leq 0.05$.

Chapter Three

Results

Chapter Three

Results

This study was carried out at Omdurman locality during the period from April to May 2015 to measure Some Hematological Parameters of sickle cell anemia children and compared with control.

There was asignificant decreased in mean of RBCs,HCT,MCV, Hb and MCH when compared with control , Insignificant in means of MCHC when compared with control. significant increased in mean of platelets count and WBCs more than control. (tables 3.1).

Sever anemia was most frequent compared with moderate and mild anemia in sickle cell anemic children.(table 3.2).

There was insignificant in mean of WBCs, RBCs, Hb, HCT,MCV, MCH, MCHC and platelets in patient with crisis when compared to those without crisis in sickle cell anemia children.(table 3.3)

There was asignificant increased in mean of RBCs in male compared with female, asignificant decreased in mean of MCH, MCHC in male compared with female, Insignificant in means of WBCs, Hb, HCT,MCV and platelets in male compared with female in sickle cell anemia children. (table 3.4).

There was insignificant in mean of WBCs, RBCs, Hb, HCT,MCV, MCH, MCHC and platelets in patient with age less than 5 compared with patient with age 5-10 years and more than 10 in sickle cell anemia children.(table 3.5).

Table (3.1).Some Hematological parameters compare between Sickle cell anemia children and control

Test	Sample	No	Mean±sd	P value
WBC×10^9/l	Control	30	5.2±1.3	0.00
	patient	70	19.2±8.9	
RBCs ×10^{12}/l	Control	30	4.1±0.4	0.00
	patient	70	2.6±0.9	
Hb g/dl	Control	30	12.6±0.4	0.00
	patient	70	6.9±1.4	
HCT	Control	30	36.7±2.3	0.00
	patient	70	20.8±4.3	
MCV fl	Control	30	90.6±6.2	0.00
	patient	70	79.9±11.8	
MCH pg	Control	30	28.9±1.9	0.02
	patient	70	26.9±4.7	
MCHC %	Control	30	33.6±1.3	0.70
	patient	70	33.5±1.6	
PLT×10^9/l	Control	30	237.3±48.3	0.00
	patient	70	348±157.1	

Table (3-2) frequencies and percentage of patient distribution according to Anemia

Anemia	Gender		
	male	femal	Total
moderate	19 27,1%	12 27,1%	31 44,3%
sever	23 32,9%	13 18,6%	36 51,4%
mild	2 2,9%	1 1,4%	3 4,3%
Total	44 62,9%	26 37,1%	70 100,0%

Table (3-3) Comparison of parameter in patients with crisis

Test	Crisis	No	Mean±sd	P value
WBC×10^9/l	Yes	56	18.792±8.614	0,36
	No	14	21.257±10.240	
RBCs ×10^{12} /l	Yes	56	2.700±.852	0,64
	No	14	2.571±1.156	
Hb g/dl	Yes	56	7.010±1.356	0,43
	No	14	6.678±1.674	
HCT	Yes	56	21.008±4.223	0,43
	No	14	19.985±5.075	
MCV fl	Yes	56	79.519±11.825	0,58
	No	14	81.471±12.176	
MCH pg	Yes	56	26.833±4.767	0,69
	No	14	27.385±4.702	
MCHC %	Yes	56	33.600±1.590	0,77
	No	14	33.464±1.709	
PLT×10^9/l	Yes	56	346.000±143.475	0,33
	No	14	389.714±182.451	

Table (3-4) Comparison of parameter in patients with gender

parameters	gender	N	Mean±sd	P value
WBC×10^9/l	male	44	18.572±8.590	0.38
	female	26	20.492±9.547	
RBCs ×10^{12} /l	male	44	2.834±1.025	0.05
	female	26	2.403± .610	
Hb g/dl	male	44	7.034±1.464	0.49
	female	26	6.792±1.352	
HCT	male	44	21.143±4.371	0.40
	female	26	20.230±4.438	
MCV fl	male	44	78.036±1.709	0.08
	female	26	83.080±7.175	
MCH pg	male	44	26.125±5.459	0.05
	female	26	28.330±2.683	
MCHC %	male	44	33.261±1.781	0.03
	female	26	34.100±1.082	
PLT×10^9/l	male	44	359.840±145.251	0.71
	female	26	346.115±164.444	

Table (3-5) Comparason of parameter with age .

parameters	gender	N	Mean±sd	P value
WBC×10^9 /l	Less than 5 years	53	18.911±8.929	0.11
	5-10 years	14	22.428±8.796	
	More than 10 years	3	11.233±3.162	
	Total	70	19.285±8.938	
RBCs ×10^{12} /l	Less than 5 years	53	2.739±1.005	0.55
	5-10 years	14	2.442± .512	
	More than 10 years	3	2.600± .529	
	Total	70	2.674± .913	
Hb g/dl	Less than 5 years	53	6.969±1.556	0.65
	5-10 years	14	6.721±.892	
	More than 10 years	3	7.533± .602	
	Total	70	6.944±1 .418	
HCT	Less than 5 years	53	20.984±4.850	0.67
	5-10 years	14	19.914±2.424	
	More than 10 years	3	21766± 2 .468	
	Total	70	20.804±4 .386	
MCV fl	Less than 5 years	53	78.894±11.881	0.43
	5-10 years	14	82728±12.470	
	More than 10 years	3	84.700± 5 .678	
	Total	70	79.910±11 .833	

MCH pg	Less than 5 years 5-10 years More than 10 years Total	53 14 3 70	26.513±4.656 28.021±5.184 29.533±2 .977 26944± 4 .725	0.36
MCHC %	Less than 5 years 5-10 years More than 10 years Total	53 14 3 70	33.471±1.648 33.692±1.449 34.800± 1 .311 33.572± 1 .602	0.36
PLT×10^9 /l	Less than 5 years 5-10 years More than 10 years Total	53 14 3 70	348.056±152.986 366.785±157.190 416.666±126.816 354.742±151.626	0.71

Chapter Four

Discussion, Conclusion and recommendation

Chapter Four

Discussion, Conclusion and recommendation

Discussion

The current study aimed to determine Some hematological parameters of the Sickle cell anemia children.

The result showed that there was a significant decreased in mean of RBCs, HCT, PCV, Hb(P=0.00) and MCH (P=0.02) when compared with control. According to genetic changes . (Caboot ,2008).

This finding agrees with(Bain,2006) in Oxfourd,UK, and(Platt OS.preven tion and mangement of strok in sickle cell anemia ,2006)) and agreed with(Abbott et al.,2002) in United State whom reported that decreased in RBCs, HCT, PCV, Hb and MCH in Sickler children.

This study showed that there was an insignificant in MCHC(p=0.70) between sickle cell anemia children and control group.

A significant increased in means of platelets count and significant increased in means of WBCs (P=0.00) more than control according to infection (Stuart and Nagel, 2004).

This finding agrees with (Bain2006) in Oxfourd , UK, and (Platt OS. prevention and mangement of strok in sickle cell anemia, 2006) and agrees with(Abbott et al.,2002) in United State whom reported that increased in platelets and WBCs in Sickler children.

Sever anemia was most frequent compared with morderate and mild anemia in sickle cell anemia children.

There was insignificant in mean of WBCs(P=0.36), RBCs(P=0.64), Hb (P=0.43), HCT(P=0.43),MCV(P=0.58), MCH(P=0.69), MCHC (P=0.77) and platelets(P=0.33) in patient with crisis when compared to those without crisis in sickle cell anemia children.

There was asignificant increased in mean of RBCs(P=0.05) , asignificant decreased in mean of MCH(P=0.05), MCHC(P=0.03) , Insignificantin meansofWBCs(P=0.38),Hb(P=0.49), HCT (P=0.40) , MCV (P =0.08) and platelets (p=0.71) in male compared with female in sickle cell anemia children.

The study also showed insignificant in mean of WBCs(P=0.11), RBCs (P=0.55), Hb (P=0.65), HCT(P=0.67),MCV(P=0.43), MCH(P=0.36), MCHC(P=0.36) and platelets(P=0.71) in patient with age less than 5 compared with patient with age 5-10 years and more than 10 in sickle cell anemia children.

Conclusion:

This study concluded:

1- There is asignificant decreased in mean of RBCs,HCT,PCV, Hb and MCH ,Insignificant in means of MCHC ,Significant increased in mean of platelets count and WBCs when compared with control

2- Sever anemia was most frequent compared with moderate and mild anemia in sickle cell anemia children.

3- There is insignificant in mean of WBCs, RBCs, Hb, HCT,MCV, MCH, MCHC and platelets in patient with crisis when compared to those without crisis in sickle cell anemia children.

4- There is asignificant increased in mean of RBCs , asignificant decreased in mean of MCH, MCHC , Insignificant in means of WBCs, Hb, HCT,MCV and platelets in male compared with female in sickle cell anemia children.

5- There is insignificant in mean of WBCs, RBCs, Hb, HCT,MCV, MCH, MCHC and platelets in patient with age less than 5 compared with patient with age 5-10 years and more than 10 in sickle cell anemia children.

Recommendation

-The sickler children should be regularly visits the doctor to check thier health.

-Ask government to facilitate drugs for sicklier children in a cheap price.

-Also further studies must be done to establish data base for sickler children

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Appendices

Appendices

Appendix (1)

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire to measure Some Hematological Parameters in Sickle cell anemia children in Omdurman locality

NO ( )

Age……

Gender…….

Residence……

Clinical feature……...

Visit to clinic yes ( ) No ( )

Suffer from other disease: yes ( ) No ( )

Previous blood transfusion: yes ( ) When ( ) No ( )

Result: WBC….....RBC…….HGB……...……..

HCT…….....MCV……...MCH……..……

MCHC……..…PLT…….

Appendix (2)

Informed consent

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

ماجستير مختبرات طبية

تخصص علم امراض الدم ومبحث المناعة الدموية

اقرار موافقه بالمشاركه في البحث

الإسم :.....

سوف يتم أخذ عينة من الدم (3 مل) من الوريد بواسطة حقنة طعن وذلك بعد مسح منطقة العينة بواسطة مطهر.

كل الأدوات المستخدمة لأخذ العينة معقمة و متبع فيها وسائل السلامة المعملية

أوافق أنا المذكور اعلاه أخذ عينة لإجراء الدراسة

الإمضاء.....

التاريخ:.....