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Measurement of CBC and some Haemostatic Parameter among Patients with Sickle Cell Anemia in Khartoum State.
قياس التعداد الكامل الدم وبعض عوامل تخثر الدم لدى مرضى الأنيميا المنجلية بولاية الخرطوم

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قال تعالى:

{ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ }

صدق الله العظيم
سورة البقرة
الآية 32

Dedication

To the candle which burns to light my life

… my mother .

To the one who I 'm alive for making his dreams become true

… my father

To my second half …. my husband.

To my kids…… source of happiness.

To those who have made it possible …. my teachers.

To those who encouraged me .. my sister, my brothers

and

To all suckle cell children.

Acknowledgement

First thanks to my God

It gives me great pleasure to conduct this study, and I would like to thanks everyone who has made this possible.

It is most appropriate that I begin by expressing my undying gratitude to my supervisor Dr. Selma E. Abdalla, for her invaluable guidance with her super talent, professional expertise and immense patience; showing great care and attention to details and without her guidance this study would have been impossible.

My thanks extend to laboratory staff at Laboratory Administration who performed investigations and measurements.

My thanks also go to everyone, who helped me in this work to see the light.

ABSTRACT

The study (case control) aimed to measurement the mean levels of platelet count, Prothombin time (PT), activated Thromboplastin time (APTT) and fibrinogen in patients with homozygous sickle cell disease (HbSS), sickle cell trait (HbAS) and normal controls (HbAA) . The study period from the May to Aug 2015, at Khartoum State in the Sickle Cell Clinic of Gaffer Ibn Auf Children Specialized Hospital, Teaching Hospital and Mohamed Elamin Paediatric Hospital. The study population comprises two groups of children in different age-groups and gender. The first group of 50 patients included the children who are known to have sickle cell anemia (HbSS). The second group of 50 patients included normal-health (HbAA) siblings of those patients which as saved as a control group. The data was collected using laboratory investigations to obtain coagulation tests. Also data collected using a questionnaire.

The Hb concentration (7.334 g/dl), PCV (19.84 %) and RBCs(2.2494 x 10^6 /µl) values in anaemic patients were decrease significantly (P ≤ 0.000) compared to control values. The platelet (504.1c/cm) and fibrinogen (5.3112 g/l) concentration were increase significantly (P ≤ 0.000) compared to control values. While no significant changes in MCV, MCHC, MCH, PT and aPTT parameters among patients with sickle cell diseases.

المستخلص

هذه دراسة حالة تهدف إلى قياس مستويات متوسط مؤشرات الصفائح الدموية، مدة التخنثر ، الوقت النشط لتجلط الدم والفيبرينوجين في المرضى الذين يعانون متماثل مرض فقر الدم المنجلي بالمقارنة مع الأصحاء من أقاربائهم . فترة الدراسة من مايو إلى أغسطس 2015، في ولاية الخرطوم في عيادة الدم المنجلي بمستشفى جعفر بن عوف للأطفال التخصص ي، المستشفى التعليمي ومستشفى محمد الأمين للأطفال. ويتكون مجتمع الدراسة من مجموعتين من الأطفال في مختلف الفئات العمرية، كلا الجنسين. وضمت المجموعة الأولى 50 مريضا من الأطفال المصابون بفقر الدم المنجلي. ضمت المجموعة الثانية 50 من الأطفال الأصحاء (مجموعة مقارنة). تم جمع البيانات باستخدام الفحوص ات المختبرية للحصول على اختبارات التخثر ، أيضا جمعت البيانات بواسطة استبيان.

أظهرت هذه الدراسة، إن متوسط خضاب الدم وحجم خلايا الدم المضغوطه وعدد كريات الدم الحمراء في مرضى فقر الدم انخفضت انخفاضا ذات دلاله معنويه مقارنة بمجموعة المقارنة من الأصحاء. وكان تركيز الصفائح الدموية والفيبرينوجين ارتفعت مقارنة بمجموعة المقارنة من الأصحاء . في حين لا توجد تغييرات في معايير متوسط حجم الخلية، متوسط حجم الخضاب فى الخليه الوحده ، متوسط تركيز خضاب الدم في الخلايا، وقت التخثر، الوقت النشط لتجلط الدم بين الأطفال المصابون بفقر الدم المنجلي.

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LIST OF ABBREVIATIONS

APTT	Activation Partial Thromboplastin time
CBC	Complete Blod Count
GRAN	Granulocyte
Hb	Haemoglobin
HbA$_1$	Haemoglobin A$_1$
HbA$_2$	Haemoglobin A$_2$
HbF	Haemoglobin F
HbS	Haemoglobin S
HbC	Haemoglobin C
LYM	Lymphocytes
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
PCV	Packed Cell Volume
Plts	Platelets
PT	Prothrombin time
RBCs	Red Blood Cells
SCD	Sickle-Cell Disease
SPs	Sample Persons
WBC	White blood cell count

CHAPTER ONE
1. INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION:

Khartoum town lies along the left bank of the Blue Nile, and forms a huge triangle. Its vertex at the confluence of then two Niles, the White Nile on its west side and the Blue Nile on its east and the base bordering Gezira State some 30 K. Southward. It is situated on latitude 15 36 N, and longitude 31 32 E, and it is 1352 ft. above sea level. Its population has grown to over 5 million people. Khartoum, together with the two cities, Omdurman and Khartoum North (Bahri), these cities jointly called the tri-capital, constitute the National Capital of the republic of Sudan (Federal Ministry of Health, 2013).

Sickle cell disease is the major haemoglobinopathy seen in Khartoum, the capital of Sudan. This may be attributed to the migration of tribes from western Sudan as a result of drought and desertification in the 1970s and 1980s, and the conflicts in Darfur in 2005. The rate is highest in Western Sudanese ethnic groups particularly in Messeryia tribes in Darfur and Kordofan regions (Federal Ministry of Health, 2013). Among 632 patients attending various clinics at the Khartoum Teaching Hospital, there were 5.1% with Hb AS and 0.8% with Hb SS (Elderdery et al., 2011). In the Blue Nile area, where groups of indigenous population live, the prevalence ranges from 0-5% in addition to a rate of 16% among some immigrant tribes from western Sudan and West Africa in the area (Elderdery et al., 2011; Federal Ministry of Health, 2013). The SCA presentation is usually severe and accompanied with major complications, and could be fatal in early childhood (El-Hazmi, et al., 2011; Majdi and Sabahelzain ,2014).

1.2. Literature Review:

1.2.1. Normal blood constituents:

1.2.1.1. Red cell count (erythrocytes):

Cells in tissues need a steady supply of oxygen to work well. Normally, hemoglobin in red blood cells takes up oxygen in the lungs and carries it to all the tissues of the body (Boudreaux, 2016). Normal range in children 3.8 – 4.8 cell/cumm.

Before the advent of reliable, automated electronic counting devices in the routine diagnostic laboratory, the red cell count were estimated visually in a haemocytometer on diluted samples of blood. The number of red cells that it is feasible to count under diagnostic laboratory conditions by 'the latter method is insufficient to yield a highly reproducible value. For this reason, the red cell count was formerly rarely employed in clinical practice as an index of the adequacy, or otherwise, of red cells in the blood. Vastly greater numbers of red cells can be counted in a brief interval by currently available electronic devices, and the error in the red cell count is consequently reduced to an order comparable to, or even less than, that of the haemoglobin level (Frank, et al., 1996).

1.2.1.2. Blood haemoglobin:

Haemoglobin is a conjugated protein of molecular weight 64000, consisting of two pairs of polypeptide chains to each of which a haem is attached. Human haemoglobin exists in a number of types, which differ slightly in the structure of their globin moiety. However, the haem is identical in all types (Hoffbrand, et al., 2001).

1.2.1.2.1. Haemoglobin types:

Haemoglobin A (Hb-A) comprises about 97% of the haemoglobin of adult red cells. It consists of two alpha (α) and two beta (β) chains with the structural formula $\alpha_2\,\beta_2$. The α chain contains 141 amino acids, and the β

chain, 146 (Hoffbrand, et al 2001). Small amounts of Hb-A are detected in the fetus as early as the eighth week of life. During the first few months of post-natal life, Hb-A almost completely replaces Hb-F, and the adult pattern is fully established by six months (Hoffbrand, et al 2001).

Haemoglobin A-i (Hb-A;) is the minor haemoglobin in the adult red cell. It has the structural formula of $\alpha_2 \delta_2$, the delta (δ) chain containing 146 amino acids (Hoffbrand, et al 2001). The α chain is identical to that of Hb-A. Hb-A_2 is present in very small amounts at birth and reaches the adult level of 1.5-3.2 per cent during the first year of life. Elevation of Hb-A_2 is a feature of some types of thalassaemia and occasionally occurs in megaloblastic anaemia and unstable haemoglobin disease. Hb-A_2 may be reduced in iron deficiency (Hoffbrand, et al., 2001).

Haemoglobin F (Hb-F) is the major respiratory pigment from early intra-uterine life up to term. It has the structural formula $\alpha_2 \gamma_2$ each gamma (γ) chain consisting of 146 amino acids. At term Hb-F accounts for 70-90 percent of the total haemoglobin. It then falls rapidly to 25 percent at I month, and 5 per cent at 6 months. The adult level of about one per cent is not reached in some, children until puberty (Hoffbrand, et al 2001). Hb-F is elevated in some haemoglobinopathies and thalassaemia syndromes. It may be elevated in occasional cases of congenital and acquired aplastic anaemia, megaloblastic anemia, paroxysmal nocturnal haemoglobinuria sideroblastic anemia, and in some forms of leukaemia. It is also occasionally raised in early pregnancy (Hoffbrand, et al 2001). The acid elution test indicates that Hb-F is unevenly distributed in the red cells in these conditions (Hoffbrand, et al 2001). Hb-F is measured by the alkali denaturation technique. Weatherall et al (1974) provide a more comprehensive list of hereditary and acquired conditions associated with raised Hb-F.

Hb-Gower 1 and Hb-Gower 2 are confined to the embryonic stage of development. They contains epsilon (ε) and zeta (ζ) chains, Hb-Gower 1 being $\zeta_2\varepsilon_2$ and Hb-Cower 2, $\alpha_2\varepsilon_2$. Hb-Portland is found in trace amounts throughout intra-uterine life and in neonates. It has the structural formula $\zeta_2\gamma_2$. Hb Bart's is also found in small amounts in cord blood if sensitive techniques are used. Both Hb-Portland and Hb-Bart's are increased in the cord blood of neonates with α-thalassaemia (Hoffbrand, et al 2001).

1.2.1.2.2. Hemoglobin electrophoresis:

Although the sickle and haemoglobin solubilitytests detect the presence of Hb-S, haemoglobin electrophoresis is mandatory for precise diagnosis of the sickle haemoglobinopathies. Hb-5 may be demonstrated by electrophoresis on cellulose acetate at pH 8.6 in a position between Hb-A and Hb-A$_2$. Although nearly 50 haemoglobin variants have a similar mobility to Hb-S, only practical problem of any frequency caused by identical morbidities is the differentiation of homozygous sickle-cell disease from sickle-cell Hb-D disease. Red cells from patients with both conditions sickle, and as Hb-S and Hb-D migrate in the same position on cellulose acetate, the two diseases appear to be identical. The problem may resolved by electrophoresis in agar gel at pH 6.0 on this medium, Hb-S and Hb-D separate widely. Thus, a two-band pattern on agar gel would confirm sickle-cell Hb-D disease, and a one-band patteri homozygous sickle-cell disease. The difficulty of differentiating the electrophoretic pattern of some cases of sickle-cell ft thalassaemia from homozygous sickle-cell disease (Papana, 1998).

Normal results:

Normal reference values can vary by laboratory, but are generally within the following ranges:

Adults:

- HbA$_1$:   95-98%.

- Hb A$_2$: 2-3%.

- Hb F: 0.8-2.0%.

- Hb S: 0%.

- Hb C: 0%.

Child (Hb F).

- 6 months: 8%

- Greater than 6 months: 1-2%

- Newborn (Hb F): 50-80%

Abnormal results:

Abnormal reference values can vary by laboratory, but when they appear within these ranges, results are usually associated with the conditions that follow in parentheses (Papana, 1998).

Hb A$_2$:

- 4-5.8% (-thalassemia minor).

- Under 2% (Hb H disease).

Hb F:

- 2 - 5% (-thalassemia minor).

- 10-90% (-thalassemia major).

- 5–35% (heterozygous hereditary persistence fetal haemoglobin, or HPFH).

- 100% (homozygous HPFH).

- 15% (homozygous HBS).

Homozygous HbS:

- 70 - 98% (Sickle cell disease).

Homozygous HbC:

- 90-98% (Hb C disease).

1.2.1.2.3. Abnormal haemoglobin and the haemoglobinopathies:

Each arises from a mutation affecting the gene directing the structure of the particular pair of polypeptide chains, and they are classified as α^-, β^-, γ^- or δ^- chain variants depending on the chains involved. The mutant gene is situated at the same chromosomal locus as (i.e. is an allele of) the normal gene controlling production of the corresponding normal chain. When the possession of a haemoglobin variant gives rise to a dearly defined disease state, the affected person is said to have a haemoglobinopathy (Frank, et al., 1996). It is important to appreciate, however, that the great majority of abnormal haemoglobins confer no harmful effect, and the individual remains asymptomatic and unaware of the abnormality within the red cell (Frank, et al., 1996).

1.2.1.4. Haematocrit:

The haematocrit or packed red cell volume (PCV) refers to the -proportion of the volume of red cells relative to the total volume of the blood. High-speed centrifugation in the microhaematocrit procedure used to sediment- the red cells yields highly reproducible results. The values do not correspond strictly to those obtained by electronic automated devices which derives a result from a formula involves multiplying the red cell count by the mean red cell volume. The microhaematocrit procedure is of value in providing a reliable and simple means for rapid determination by the clinician of the red cell content of the blood. Normal range in children 31% - 41% (Frank, et al., 1996) .

1.2.1.5. Red cells indices:

1.2.1.5.1. 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 of the red cell count was laborious and unreliable, so that determination of MCV, like the red cell count, was formerly rarely performed in the routine diagnostic laboratory Normal range in children 75-87fl (Frank, et al., 1996).

Automated electronic-particle counting devices have revolutionized the estimation of the MCV (Frank, et al., 1996). Most devices measure the electrical impedance caused by each red cell as it passes through the counting mechanism, and the extent of the impedance provides an accurate indication of the volume of each cell. Such machines not only indicate the profile of the distribution of the volume of red cells, but also provide a highly reproducible value for the MCV. The MCV derived by this mean therefore, provides a reliable index of the average size of red cells, which is a guide of considerable importance to the nature of the disorder underlying an abnormality in the haemoglobin level. A subnormal MCV is indicative of microcytosis, and an elevated MCV indicative of macrocytosis (Frank, et al., 1996).

1.2.1.5.2. Mean corpuscular haemoglobin:

The mean amount of haemoglobin per red cell (MCH) is also rapidly and reliably estimated by automated electronic counting devices by dividing the total amount of haemoglobin by the number of red cells in a sample of blood. A subnormal MCH occurs in microcytosis, but is even lower when microcytosis occurs in conjunction with subnormal concentrations of

haemoglobin in the red cell, as in thalsssaemia minor or iron deficiency (Frank, et al., 1996). Normal range in children 32 -37pg.

1.2.1.5.3. Corpuscular haemoglobin concentration:

The mean concentration of haemoglobin within the red cell (MCHC) reflects an entirely different 'parameter than the MCH. It is derived by dividing the concentration of haemoglobin in g/dl by the volume of red cells in ml/dl. Both measurements are readily and reliably obtained by manual methods, and the result is expressed in gram haemogobin/dl packed red cells (Frank, et al., 1996). Normal range in children 24-32%.

A subnormal MCHC is usually indicative of an abnormality where interference with the synthesis of haemoglobin is greater than that of other constituents of the red cells, as in thalassaemia or iron deficiency. Elevated values reflect dehydration of the erythrocyte and one of the relatively few important clinical causes of this phenomenon is spherocytosis. Values obtained by automated electronic counting devices are indirectly determined, and not only fail to correspond strictly to those obtained when the microhaematocrit method is employed to estimate, the PCV, but also fail to display a shift to the same extent in abnormal states as values determined by the traditional method (Frank, et al., 1996).

1.2.1.6. Reticulocyte count:

Reticulocyte are immature red blood cells, the normal count is 0.5-2.5%, and the absolute 25-125 $\times$ 10^9/L. This should rise in anaemia because of erythropietin increase and be higher the more severe the anaemia. This is particularly so when there has been time for erythroid hyperplasia to develop in the marrow as in chronic haemolysis. After an acute major haemorrhage, there is an erythropoietin response in 6 hours, the reticulocyte count rises within 2-3 days, reaches a maximum in 6-10 days and remains raised until the haemoglobin returns to the normal level. If the

reticulocyte count is not raised in an anaemic patient this suggests impaired marrow function or lack of erythropoetin stimulus (Hoffbrand, 2001). But Slavov et al., (2011) reported that during SCD, reticulocyte count dropped dramatically causing reticulocytopenia and rapid turnover of red cells leading to the drop in haemoglobin. This crisis takes 4 days to one week to disappears.

1.2.1.7. Blood film:

It is essential to examine the blood film in all cases of anaemia. 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 folate or B_{12} deficiency, the indices may be normal but the blood film reveals a 'dimorphic' appearance (a dual population of large, well-haemoglobinized cells and small, hypochromic cells). 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, is noted (Hoffbrand, 2001).

1.2.1.8. Platelet (Plts):

Platelets, also called thrombocytes (thromb- + -cyte, "blood clot cell"), are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clotting blood vessel injuries (Furie, 2008). Platelets have no cell nucleus: they are fragments of cytoplasm that are derived from the megakaryocytes (Yip J , 2005) of the bone marrow, and then enter the circulation. These unactivated platelets are biconvex discoid (lens-shaped) structures (Yip J, 2005) 2–3 μm in greatest diameter.[5] Platelets are found only in mammals, whereas in other animals (e.g. birds, amphibians) thrombocytes circulate as intact mononuclear cells (Machlus, 2014).

1.2.2. Haemostasis:

Coagulation (also known as clotting) is the process blood changes from a liquid to a gel, forming a clot. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves activation, adhesion, and aggregation of platelets along with deposition and maturation offibrin. Disorders of coagulation are disease states which can result in bleeding (hemorrhage or bruising) or obstructive clotting (thrombosis) (David et al., 2009).

Coagulation is highly conserved throughout biology; in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component (Alan, 2006). The system in humans has been the most extensively researched and is the best understood (Schmaier, et al., 2011).

Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium lining the vessel. Exposure of blood to the space under the endothelium initiates two processes: changes in platelets, and the exposure of subendothilial tissue factor to plasma Factor VII, which ultimately leads to fibrin formation. Platelets immediately form a plug at the siteof injury; thisiscalled *primaryhemostasis. Secondary hemostasis* occurs simultan-eously: Additional coagulation factors or clotting factors beyond Factor VII (listed below) respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Furie, 2005).

The coagulation cascade of secondary hemostasis has two initial pathways which lead to fibrin formation. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor *pathway* (also known as the extrinsic pathway) which both lead to the same fundamental reactions that produce fibrin. It was previously thought that the two pathways of coagulation cascade were of equal importance, but it is now known that the primary pathway for the initiation of blood coagulation

is the *tissue factor* (extrinsic) pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase *a* appended to indicate an active form (Pallister et al., 2010).

The coagulation factors are generally serine proteases (enzymes), which act by cleaving downstream proteins. There are some exceptions. For example, FVIII and FV are glycoproteins, and Factor XIII is a transglutaminase (Pallister et al., 2010). The coagulation factors circulate as inactive zymogens. The coagulation cascade is therefore classically divided into three pathways. The *tissue factor* and *contact activation* pathways both activate the "final common pathway" of factor X, thrombin and fibrin (Hoffbrand, 2002).

1.2.2.1. Tissue factor pathway (extrinsic):

The main role of the tissue factor pathway is to generate a "thrombin burst", a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released very rapidly. FVIIa circulates in a higher amount than any other activated coagulation factor. The process includes the following steps (Pallister et al., 2010):

- Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).
- TF-FVIIa activates FIX and FX.
- FVII is itself activated by thrombin, FXIa, FXII and FXa.

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- The activation of FX (to form FXa) by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).

- FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.

- Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which forms a complex with FIX), and activates and releases FVIII from being bound to vWF.

- FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues.

1.2.2.2. Contact activation pathway (intrinsic):

The contact activation pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that patients with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder. Instead, contact activation system seems to be more involved in inflammation (Pallister et al., 2010) and innate immunity. Despite this, interference with the pathway may confer protection against thrombosis without a significant bleeding risk (Long, et al., 2015).

1.2.2.3. Final common pathway:

The division of coagulation in two pathways is mainly artificial, it originates from laboratory tests in which clotting times were measured after the clotting was initiated by glass (intrinsic pathway) or by thromboplastin (a mix of tissue factor and phospholipids). In fact thrombin is present from

the very beginning, already when platelets are making the plug. *Thrombin* has a large array of functions, not only the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it is the most important platelet activator and on top of that it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers (Pallister et al., 2010).

Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down-regulated by the anticoagulant pathways (Pallister et al., 2010).

1.2.3. Prothrombin time (PT) and activated partial thromboplastin time (APTT):

APTT is a test that characterizes blood coagulation. A part from detecting abnormalities in blood clotting (Kort et al., 200). It is also used to monitor the treatment effects with heparin. A major anticoagulant APTT is a performance indicator of the efficacy of both the "intrinsic" (now referred to as the contact activation pathway) and the common coagulation pathways. It is used in conjunction with the prothrombin time (PT) which measure the extrinsic pathway (Langdell., 1953).

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are used to test for the same functions; however, in APTT, an activator is added that speeds up the clotting time and results in a narrower reference range. The APTT is considered a more sensitive version of the PTT and is used to monitor the patient's response to heparin therapy. The reference range of the APTT and PT are 30-40 and 11-16 seconds respectively.

Critical values that should prompt a clinical alert are as follows: APTT: More than 70 seconds (signifies spontaneous bleeding). PTT: More than 100 seconds (signifies spontaneous bleeding) (Fischbach, 2009; Pagana, 2010).

Aprolonged APTT result may indicate the following (Fischbach, 2009; Pagana, 2010): Congenital deficiencies of intrinsic system clotting factors such as factors VIII, IX, XI, and XII, including hemophilia A (Christmas disease) and hemophilia B (two inherited bleeding disorders resulting from a deficiency in factors VIII and IX, respectively). Congenital deficiency of Fitzgerald factor (prekallikrein). Von Willebrand disease, which is the most common inherited bleeding disorder, affecting platelet function owing to decreased von Willebrand factor activity. Hypofibrinogenemia. Liver cirrhosis (the liver makes most of the clotting factors, including those that are vitamin K-dependent ones); diseases of the liver may result in an inadequate quantity of clotting factors, prolonging the APTT. Vitamin K deficiency: The synthesis of some clotting factors requires vitamin K, so vitamin K deficiency results in an inadequate quantity of intrinsic system and common pathways clotting factors, as a result the APTT is prolonged. Disseminated intravascular coagulation (DIC): The clotting factors involved in the intrinsic pathway are consumed, prolonging the APTT. Heparin therapy, which inhibits the intrinsic pathway at several points (eg, prothrombin II), prolonging the APTT (Yie, et al., 2015). Coumarin therapy, which inhibits the function of factors I, IX and X,prolonging the APTT. Nonspecific inhibitors, such as lupus anticoagulant and anticardiolipin antibodies, which bind to phospholipids on the surface of platelets. Specific circulating anticoagulants, inhibitor antibodies that specifically target certain coagulation factor, such as in individuals with hemophilia after many plasma transfusions, systemic lupus erythematosus,

rheumatoid arthritis, tuberculosis, and chronic glomerulonephritis. A shortened APTT result may indicate the following Early stages of DIC: circulating procoagulants exist in the early stages of DIC, shortening the APTT. Extensive cancer (e.g., ovarian cancer, pancreatic cancer, colon cancer). Immediately after acute hemorrhage. An acute-phase response leading to high factor VIII levels. (Fischbach, 2009; Pagana, 2010).

Many drugs can change the results of the activated partial thromboplastin time (APTT), including nonprescription drugs (Pagana, 2010). No specific preparation is required. However, since lipemia may interfere with photoelectric measurements of clot formation, specimens should not be obtained after a meal (Estridge, 2000).

If the patient is receiving heparin by intermittent injection, the sample should be drawn 30-60 minutes before the next dose, while, if the patient is receiving a continuous heparin infusion, the sample can be drawn at any time (Pagana, 2010).

Factors that interfere with the APTT test included the following (Fischbach, 2009; Pagana, 2010): Drugs that may prolong the test values, including antihistamines, ascorbic acid, chlorpromazine, heparin, and salicylates. Incorrect blood-to-citrate ratio. Hematocrit that is highly increased or decreased. Blood samples drawn from heparin lock or a heparinized catheter.

1.2.4. Related tests:

Related tests include the following (Pagana, 2010; Daniels, 2009).

- Prothrombin time (PT).
- Coagulation factor concentration.
- Fibrinogen testing.
- Thrombin time testing.
- Platelet counts.

1.2.5. Fibrinogen:

Fibrinogen is a soluble protein in the plasma that is broken down to fibrin by the enzyme thrombin to form clots.

The reference range for the different fibrinogen tests are as follows: Fibrinogen antigen: 149-353 mg/dL. Fibrinogen: 150-400 mg/dL. Fibrinogen antigen/functional ratio: 0.59-1.23.

Normal fibrinogen activity results usually reflect normal blood-clotting ability.

Decreased fibrinogen levels (< 100 mg/dL) are associated with the afibrinogenemia: Chronic, hypofibrinogenemia: Chronic, end-stage liver disease: Chronic, severe malnutrition: Chronic, disseminated intravascular coagulation (DIC): Acute abnormal fibrinolysis: Acute and large-volume blood transfusions: Acute (Pagana, 2010).

Fibrinogen is an acute-phase reactant, meaning that elevated fibrinogen levels can be seen the following conditions (Fischbach, 2009; Pagana, 2010): Inflammation, tissue damage/trauma, infection, cancer, acute coronary syndrome, strokes and inflammatory conditions.

1.2.6. Definition of sickle cell anaemia:

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 (Deshmukh, 2006). Sickle cell anaemia (HbSS) results from homozygosity 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 (Ingram, 1958; Inwald 2000, Buseri 2007).

Sickle-cell anaemia is a form of sickle-cell disease (SCD) in which there is homozygosity for the mutation that causes HbS (Buseri 2007). It is also

referred to as "HbSS", "SS disease", "haemoglobin S and Butany " or permutations of those names. About 300,00 children are born with form of sickle-cell disease every year mostly in Sub-Saharan Africa and India (GBD, 2013). In heterozygous people, that is, those who have only one sickle gene and one normal adult haemoglobin gene, the condition is referred to as "HbAS" or "sickle cell trait (Buseri 2007).

The author added that both normal red blood cells and sickle-shaped cells are present in human blood (Buseri 2007).

Sickle cell disease (SCD) is a potentially devastating condition that is caused by an autosomal recessive inherited hemoglobinopathy which results in the vaso-occlusive phenomena and hemolysis.

1.2.6.1. Sickle-cell disease and other haemoglobin disorders:

Cells in tissues need a steady supply of oxygen to work well. Normally, hemoglobin in red blood cells takes up oxygen in the lungs and carries it to all the tissues of the body. Red blood cells that contain normal hemoglobin are disc shaped. This shape allows the cells to be flexible so that they can move through large and small blood vessels to deliver oxygen (Boudreaux, 2016**).**

Approximately 5% of the world's population carries trait genes for haemoglobin disorders, mainly, sickle-cell disease and thalassaemia. Haemoglobin disorders are genetic blood diseases due to inheritance of mutant haemoglobin genes from both, generally healthy, parents. Over 300 000 babies with severe haemoglobin disorders are born each year. The health burden of haemoglobin disorders can be effectively reduced through management and prevention programmes (Kauf, 2009). The total healthcare cost associated with sickle cell disease estimated $2 billion annually Medicaid patients total and health care costs: $1,400 per month

($1,700*). Annual costs in children ages 0-9: $10,700 (12,900*), at ages 30-39: $34,300 (41,000*) (Kauf, 2009).

Mean medical expenditures (2005) for children (<20 years) $11,000 for those enrolled in Medicaid ($13,300*). $14,800 for those with private insurance ($17,700*) in 2010 dollars (Amendah, 2010).

Sickle-cell anaemia (SCA) or sickle cell disease (SCD), is a hereditary blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape (Wellems, 2009). Sickling decreases the cells' flexibility and results in a risk of various complications and the sickling occurs because of a mutation in the haemoglobin gene (Wellems, 2009). The author added that individuals with one copy of the defunct gene display both normal and abnormal haemoglobin. Specifically, humans with one of the two alleles of sickle-cell disease show less severe symptoms when infected with malaria.

SCD is characterized by two cardinal pathological manifestations – chronic hemolytic anaemia and vaso-occlusive crisis (Amin 2004; and Raffini, 2006). Though patients with SCD are characterized by a hypercoagulable state, nearly every component of hemostasis, including platelet function and the procoagulant, anticoagulant and fibrinolytic systems, is altered in this disease (Yilmaz, 2008).

Many studies have observed platelet activation and aggregation markers in homozygous SCD, but there is paucity in literature regarding the importance of simple parameters like mean platelet volume (MPV) and platelet distribution width (PDW) in this disease (Westerman, 2002). Patients with homozygous SCD are in a hypercoagulable state, it is still debatable whether further increment occurs in this state during crisis. Also it remains to be elucidated whether this hypercoagulable state is involved in pathogenesis of crisis or is merely a secondary effect following crisis

(Yilmaz, 2008). This issue is further complicated by certain reports suggesting the presence of a hypercoagulable state in steady state sicklers and any evidence of hypercoagulability may be an early indication for an onset of crisis (Amin 2004; Westerman 2002).

In the current study we sought to estimate mean levels of various haemostatic parameters like platelet indices, mean prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen levels in children with sickle cell cell anaemia. These parameters were compared with those in normal HbAA controls.

1.2.6.2. Etiology and epidemiology:

Sickle cell disease (SCD) is a potentially devastating condition that is caused by an autosomal recessive inherited hemoglobinopathy, which results in the hallmark clinical sequelae of vasoocclusive phenomena and hemolysis. The genetic abnormality is due to a substitution of the amino acid valine for glutamic acid at the sixth position on the beta globin chain and was first described over one hundred years ago (AAP, 2002).

Hemoglobin S (HbS), the hemoglobin that is produced as a result of this defect, is a hemoglobin tetramer (alpha2/beta S2) that is poorly soluble and polymerizes when deoxygenated (Darbari, et al., 2006).

SCD results from any combination of the sickle cell gene with any other abnormal β-globin gene and there are many types of SCD. The most common types include sickle cell anemia (Hb SS), the sickle beta-thalassemias (Hb Sβ0 and Hb Sβ+), hemoglobin SC disease (Hb SC) and sickle cell disease with hereditary persistence of fetal hemoglobin (S/HPFH) (Reiter, et al., 2002). HbSS is the most common form of sickle cell disease. Patients with Hb SS and Hb Sβ0, in general, have the most severe forms of SCD including lower hemoglobin levels and more frequent vasoocclusive and hemolytic complications (Reiter, et al., 2003). Sickle-C

(Hb SC) disease is the second most common form of SCD. Patients with this type of SCD generally have a more benign clinical course than do patients with Hb SS or sickle β0-thalassemia (Reiter, et al., 2002; Reiter, et al., 2003).

Overall, the incidence of sickle cell disease exceeds that of most other serious genetic disorders, including cystic fibrosis and hemophilia (Rahimy, 2004; Quinn, 2004). It is seen worldwide but occurs most frequently in Africans and less commonly in those of Mediterranean, Latino, East Indian, and Arab descent. It is estimated that 16% of the population in Africa has a sickle hemoglobinopathy which is the highest proportion worldwide. The Americas and the East Mediterranean region represent the next highest proportion of sickle cell hemoglobinopathy as delineated by the WHO (Manci, et al., 2003).

The highest frequency of sickle cell diseases was found in tropical region, where malaria is common, carry a signal SC allele converse selective advantage (Wdle, 2009).

European country sickle cell disease over taken more familiar genetic condition such as hemophilia and cystic fibrosis (Roben and deMontale, 2007). In 20013 it resulted in 176,000 deaths due to SCD comparing to 113,000 deaths in 1990 (GBD, 2013). Prevalence of sickle cell haemoglobin in Wardha district, India was reported to be 2.9% (Deshmukh, 2006).

With regard to children with SCD, in the developed world, the mortality rate is estimated to be as low as 0.5-1.0 per 100,000 children. This is in contrast to higher rates in developing countries such as the Republic of Benin which recently reported a mortality rate of 15.5 per 1,000 children or 1,550 per 100,000 children (Vichinsky, et al ., 2003). The most common

causes of death in childhood from sickle cell disease are infection, acute chest syndrome and stroke (Strouse et al., 2007; Field et al. 2010).

1.2.6.3. Pathophysiology:

There is a large amount of heterogeneity in the expression of sickle cell disease which is not fully explained by the single mutation or different variants of hemoglobin S. This variability is manifest by a wide spectrum in both frequency and intensity of painful vaso-occlusive crises as well as highly variable degrees of organ dysfunction (Reiter, et al., 2002; Reiter, et al., 2003). The pathophysiologic processes that lead to sickle cell disease related complications result from a combination of hemolysis and vaso-occlusion. Hemolysis occurs as a result of repeated episodes of hemoglobin polymerization/depolymerization as sickle red blood cells pick up and release oxygen in the circulation (Reiter, et al., 2002). Red blood cell membranes become abnormal from this process and red blood cells have a shortened lifespan. Hemolysis can occur both chronically and during acute painful vaso-occlusive crises and also results in the release of substantial quantities of free hemoglobin into the vasculature (Reiter, et al., 2003). This resultant free ferrous hemoglobin likely consumes significant quantities of nitric oxide (NO) which in turn, leads to abnormal regulation in vascular homeostasis.

1.2.6.4. Common morbid complications:

1.2.6.4.1. Vaso-occlusion:

Vaso-occlusive painful events are the most common morbidity seen in patients (both children and adults) with sickle cell disease. Vaso-occlusion is caused by sickled red cell that obstructed capillaries and restrict blood flow to an organ resulting in pain, ischemia and necrosis and organ damage (Olujohungbe and Bumett, 2013).

Previous studies in 209 patients who were over 20 years of age when they died, indicator that 22% of deaths occurred during a pain episode. Acute chest episodes were temporally related to hospitalization for pain in 77% of patients who had them, and individuals older than 20 years of age with a higher rate of painful episodes had an increased risk of premature death when compared to those with a lower rate of pain (Dampier, et al 2002; Smith, et al., 2008).

1.2.6.4.2. Bacteremia/Sepsis:

In patients suffering from SCA, the spleen is usually affected because of its narrow vessels and function in clearing defective red blood cells (Wong, et al., 1992). Children with sickle cell disease are at increased risk for bacteremia that can result in sepsis and death; due in large part to functional of spleen that develops over time in these children. In developed countries and recently in Africa, the most common organisms involved include *Streptococcus pneumoniae*, *Salmonella* species, and *Haemophilus influenza* (Gladwin et al., 2004; Williams, et al., 2009).

1.2.6.4.3. Acute Chest Syndrome:

The specific definition of what constitutes acute chest syndrome (ACS) varies but usually refers to a new pulmonary infiltrate accompanied by fever and/or symptoms or signs of respiratory disease in a patient with (SCD) (Strouse, et al., 2007). It is a relatively common cause of frequent hospitalizations and death and a common indication for transfusion and treatment with hydroxyurea (Scothorn, 2002). Several studies suggest that the case fatality rate is lower in children (1.1–1.5%) than adults (4.3–9%), but ACS accounts for a significant proportion of mortality in both groups. Over half of the patients who developed ACS were hospitalized for another reason prior to developing ACS, usually a vaso-occlusive painful crisis (Brandow, et al., 2009). It accounted for about 25% of death in patient

with SCD, majority of cases present with vaso- occlusive crisis, and then they developed to ACS (Paul, et al., 2011). Brandow, et al., (2009) reported that infection, fat emboli, and pulmonary infarction are all commonly associated with the development of ACS but many episodes of ACS develop without an obvious cause.

1.2.6.4.4. Pulmonary hypertension:

Gladwin, (2004) and Aliyu, (2008) noted that pulmonary hypertension in adults with sickle cell disease is 25-32% in both the United States and Africa.

1.2.6.4.5. Central nervous system disease:

Central nervous system disease is common in sickle cell disease and usually manifests as stroke and/or vasculopathy in those with the disease. Overt stroke occurs in up to 10% of children with the disease and usually involves large cerebral vessels that affect large regions of the brain (Price, 2002; Pegelow, 2002). The author added that without treatment, there is a high risk of recurrence. With transfusion therapy, this risk remains substantial at 22%. Silent stroke, defined as an infarct on imaging studies with a normal neurological examination, occurs in at least 22% of those with sickle cell disease.

1.2.6.4.6. Renal effects:

Microalbuminuria and albuminuria are common in the more severe genotypes of sickle cell disease and can occur in up to 80% of patients resulting in a glomerulopathy (McKie, 2007; Alvarez, et al 2008). Approximately 15% of patients will advance to end stage renal disease by their third decade of life. About 25% of patients with hemoglobin SS disease have renal insufficiency defined as a reduced creatinine clearance of < 90 ml/min (Guasch, 2006).

1.2.6.4.7. Avascular necrosis:

Avascular necrosis is one of the few complications that is more common with Hb SC than Hb SS and its prevalence has been reported to be as high as 41% of adults with sickle cell disease. With the advent of newer imaging such as magnetic resonance imaging, however, true prevalence remains unknown (Pegelow, 2002). Vascular necrosis of the hip and other major joints may occur as a result of ischemia (Marti, et al., 2004).

- **Stroke:** A stroke can occur if sickle cells block blood flow to an area of your brain. Signs of stroke include seizures, weakness or numbness of your arms and legs, sudden speech difficulties, and loss of consciousness. If your baby or child has any of these signs and symptoms, seek medical treatment immediately. A stroke can be fatal.

- **Organ damage:** Sickle cells can block blood flow through blood vessels, immediately depriving an organ of blood and oxygen. In sickle cell anemia, blood is also chronically low on oxygen. Chronic deprivation of oxygen-rich blood can damage nerves and organs in your body, including your kidneys, liver and spleen. Organ damage can be fatal.

- **Blindness:** Tiny blood vessels that supply your eyes can get blocked by sickle cells. Over time, this can damage the portion of the eye that processes visual images (retina) and lead to blindness.

- **Skin ulcers:** Sickle cell anemia can cause open sores, called ulcers, on your legs.

- **Gallstones:** The breakdown of red blood cells produces a substance called bilirubin. A high level of bilirubin in your body can lead to gallstones.

- **Priapism:** Men with sickle cell anemia may experience painful, long-lasting erections, a condition called priapism. As occurs in other parts of

the body, sickle cells can block the blood vessels in the penis. This can damage the penis and eventually lead to impotence.

1.2.6.5. Sickle-cell crisis:

The terms "sickle-cell crisis" or "sickling crisis" may be used to describe several independent acute conditions occurring in patients with SCD. SCD results in anemia and crises that could be of many types including the vaso-occlusive crisis, aplastic crisis, sequestration crisis, haemolytic crisis, and others. Most episodes of sickle-cell crises last between five and seven days.[14] "Although infection, dehydration, and acidosis (all of which favor sickling) can act as triggers, in most instances, no predisposing cause is identified" (Kumar, et al., 2009).

1.2.6.5.1. Vaso-occlusive crisis:

The vaso-occlusive crisis is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ resulting in ischaemia, pain, necrosis, and often organ damage. The frequency, severity, and duration of these crises vary considerably. Painful crises are treated with hydration, analgesics, and blood transfusion; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients manage on NSAIDs (such as diclofenac or naproxen). For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia devices are commonly used in this setting. Vaso-occlusive crisis involving organs such as the penis (Olujohungbe, et al., 2013), or lungs are considered an emergency and treated with red-blood cell transfusions. Incentive spirometry, a technique to encourage deep breathing to minimise the development of atelectasis, is recommended (Glassberg, 2011).

1.2.6.5.2. Splenic sequestration crisis:

Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected (Green, 2012). It is usually infracted before the end of childhood in individuals suffering from sickle-cell anemia. This spleen damage increases the risk of infection from encapsulated organisms;[19][20] preventive antibiotics and vaccinations are recommended for those lacking proper spleen function.

Splenic sequestration crises are acute, painful enlargements of the spleen, caused by intrasplenic trapping of red cells and resulting in a precipitous fall in hemoglobin levels with the potential for hypovolemic shock. Sequestration crises are considered an emergency. If not treated, patients may die within 1–2 hours due to circulatory failure. Management is supportive, sometimes with blood transfusion. These crises are transient; they continue for 3–4 hours and may last for one day (Khatib, et al., 2009).

1.2.6.5.3. Acute chest syndrome:

Acute chest syndrome (ACS) is defined by at least two of the following signs or symptoms: chest pain, fever, pulmonary infiltrate or focal abnormality, respiratory symptoms, or hypoxemia (Glassberg, 2011). It is the second-most common complication and it accounts for about 25% of deaths in patients with SCD, majority of cases present with vaso-occlusive crises then they develop ACS. Nevertheless, about 80% of patients have vaso-occlusive crises during ACS (Mekontso, et al., 2008; Paul, et al., 2011).

1.2.6.5.4. Aplastic crisis:

Aplastic crises are acute worsening of the patient's baseline anaemia, producing pale appearance, fast heart rate, and fatigue. This crisis is normally triggered by parvovirus B19, which directly affects production of

red blood cells by invading the red cell precursors and multiplying in and destroying them (Kumar, et al., 2009).

Parvovirus infection almost completely prevents red blood cell production for two to three days. In normal individuals, this is of little consequence, but the shortened red cell life of SCD patients results in an abrupt, life-threatening situation. Reticulocyte counts drop dramatically during the disease (causing reticulocytopenia), and the rapid turnover of red cells leads to the drop in haemoglobin. This crisis takes 4 days to one week to disappear. Most patients can be managed supportively; some need blood transfusion (Slavov, et al., 2011).

1.2.6.5.5. Haemolytic crisis:

Haemolytic crises are acute accelerated drops in haemoglobin level. The red blood cells break down at a faster rate. This is particularly common in patients with coexistent G6PD deficiency (Balgir, 2012). Management is supportive, sometimes with blood transfusions (Glassberg, 2011).

1.2.7. Previous studies:

A Study on the Changes in the First-line Coagulation Screening Tests in Sudanese Sickle Cell Disease Patients Submitted by Eiman et al., (2011).

Forty patients with SCD of both sexes with ages ranged between 9 months and 18 years, admitted to Um Durman Children Emergency Hospital from January to March 2011, were included in this cross-sectional study. These were either during crisis state or in the steady state. Eleven healthy controls matched with ages and genders of patients were used for comparison. Blood samples from both groups were collected and investigated for CBC, PT, APTT, TcT, and Fbg concentrations.The study revealed that, in comparison with control means, the mean of Plts count was elevated (P=0.003), TcT and APTT were significantly low (P=0.00) (P=0.013) respectively, while there were no significant changes in PT clotting times

(P=0.641) and fibrinogen concentrations (P=0.212). These results were the same in patients during crisis and those in steady state. The study concluded that these is hypercoagulable state in SCD patients indicated by shortened TcT and APTT clotting times, these findings were not associated with severity of the anaemia (Eiman et al., 2011).

Another study on haemostatic alterations in patients of sickle cell trait and homozygous sickle cell disease: A hospital based case control study conducted by Tatkare, et al., in India, aim was to determine the mean levels of platelet indices, Prothombin time (PT), activated Thromboplastin time (APTT) and fibrinogen in patients with homozygous sickle cell disease (HbSS), sickle cell trait (HbAS) and normal controls (HbAA) and their role as prognostic markers. The study included 321 cases of sickle cell haemoglobinopathies (118 HbSS and 203 HbAS) and 321 normal controls. Platelet indices were determined by automated cell counter. PT, APTT and fibrinogen levels were estimated by using commercial agents and BK coagulometer. The results showed mean fibrinogen levels were 275.56, 357.37 and 522.24 mg/dl respectively in HbAA controls, HbAS and HbSS patients. The fibrinogen levels in HbSS patients were found to be raised even more in those in crisis. Mean platelet volume (MPV), Platelet distribution width (PDW) and PT and APTT values were also significantly prolonged in these patients. In conclusions: Since, fibrinogen levels showed a higher increase in crisis, its estimation can be used as a parameter to monitor progression of sickle cell crisis. We obtained high MPV and PDW in HbSS patients as compared to controls; larger platelets are more thrombogenic, we propose a hypothesis that larger platelets in HbSS patients may predispose them to vaso-occlusive crisis (Tatkare, et al., 2014).

1.3. RATIONALE:

Strong relationship between (SCA) and the coagulation system (SCA) are one of the high risk factors for bleeding and venous thromboembolism. In Sudan there is a paucity of data regarding the disorders of coagulation system in children suffering from (SCA). So this study was undertaken to assess the coagulation system state during (SCA). Nearly every component of hemostasis is altered in patients with sickle cell disease (SCD). Though these patients are known to be in hypercoagulable state, increased risk of peri-operative bleeding complications has also been observed in these patients.

1.4. OBJECTIVES:

1.4.1 General objective:

Measurement of CBC and some Haemostatic Parameter (PTAPTT& Fibrinogen) among Patients with Sickle Cell Anemia in Khartoum State.

1.4.2. Specific objective:

1- To measure Hb, PCV, RBCS and PLT in control group and patients.
2- To measure PT, APTT and Fibrinogen in control group and patients.
3- To compare between patients according to age gender.
4- To compare between HbSS and HbAS groups.

CHAPTER TWO
2. MATERIALS AND METHODS

**

2.1. Study design:

This is case & control study was conducted through the period from the of May to of Aug 2015.The study was carried out at Khartoum State in the Sickle Cell Clinic of Gaffer Ibn Auf Children Specialized Hospital, Teaching Hospital and Mohamed Elamin Paediatric Hospital.

2.2. Study population:

The study population comprises two groups of children in different age-groups, both sexes. was included of 35 patients included the children who are known to have sickle cell anemia (HbSS) &15of patients included the children who are known to have sickle cell triat (HbAS) & 50 of healthy children (HbAA) siblings as control group. The diagnosis of both groups was confirmed by hemoglobin electrophoresis.

2.2.1.. Inclusion criteria:

Children already confirmed SCA based on hemoglobin electrophoresis.

1.2.2. Exclusion criteria:

Patients underwent exchange transfusion in the last 3 months.

Patients who parents/caregiver refused to participate in the study.

2.3. Data collection tools:

Laboratory investigations were performed to obtain CBC and coagulation tests. Data was collected using questionnaire included age and sex.

2.4. Samples collection:

Five ml of venous blood samples was collected. 2.5 ml was added slowly to 0.25 ml of 0.38% trisodium citrate for coagulation tests. The rest (2.5 ml) was added to EDTA tube for CBC.

2.5. Methodology:

Complete blood count (Hb, PCV, RBC, MCV, MCH, MCHC, TWBC and PLT) was done using SWELAB. The coagulation test (PT –PTT and fibrinogen) was performed using STAGO.

2.5.1. Principle of Swelab:

A sample volume of a whole blood specimen is aspirated into the analyzer where a portion of it is automatically diluted with Diaton-SWE Diluent. A portion of this first dilution is further diluted with Diaton-SWE Diluent. This second dilution of the sample is then introduced into impedance particle analyzer where the red blood cell counts (RBC) and the thrombocyte count (PLT) is measured. To the remainder of the first dilution a lysing reagent (Dialyse-SWE) is added for the measurement of hemoglobin (HGB), white blood cell count (WBC), lymphocytes count (LYM), mid cell (MID), granulocyte count (GRAN). Consult your specific instrument Operators Manual for additional information with respect to procedures and principles for whole blood hematological analysis (Beckman, 1992).

Clinical Relevance and Summary of Test Principle Perform a complete blood count (CBC) in duplicate on all Sample Persons (SPs) age 1 and older. Perform the CBC on the Beckman Coulter MAXM. Run a CBC on home exam SP's EDTA blood tubes after returning to the MEC (Beckman, 1992).

2.5.2. Procedure:

The Beckman Coulter method of sizing and counting particles uses measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte. A suspension of blood cells passes through a small orifice simultaneously with an electric current.

A smallopening (aperture) between electrodes is the sensing zone through which suspended particles pass. In the sensing zone, each particle displaces its volume of electrolyte. Beckman Coulter measures the displaced volume as a voltage pulse, the height of each pulse being proportional to the volume of the particle.

The quantity of suspension drawn through the aperture is for an exact reproducible volume. Beckman Coulter counts and sizes individual particles at a rate of several thousand per second. This method is independent of particle shape, color, and density.

The MAXM is a quantitative, automated, differential cell counter for in vitro diagnostic use.

The methods used to derive CBC parameters are based on the Beckman Coulter method of counting and sizing, in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scattergram plots the cells based upon the measurements of these three parameters (Beckman, 1992).

2.5.2.1. Prothrombien time (PT):

2.5.2.1.1 Principle of Stago:

The principle of the test consists of the use of calcium thromboplastin to measure the clotting time of the patient's plasma and to compare it with that of a normal standard (for example: EtaIoquick or Unicalibrator). The test measures, as a whole, the activity of extrinsic coagulation factors: factor II (prothrombin), factor V (proaccelerin), factor VII (proconvertin), and factor X (Stuart factor) (Hirsh, et al., 2001).

2.5.2.1.2Procedure:

Changing Sampling Modes

1. The Compact Stago can operate in two different patient loading modes, auto mode or manual mode. To change loading modes, select **Loading** from the main menu.

2. Select **Samples**. The mode will be displayed near the top of the screen. If you wish to change modes press ESC for options. Choose the desired mode.

Loading Patient Samples in Manual Mode:

1. Choose **Loading** from the main menu.

2. Select Samples (the sample drawer will open)

3. Ensure that the sample mode is set to manual.

4. Scan or type the accession and press enter on the keyboard.

5. Load the tube into the sample drawer.

6. Select tests from the test menu.

7. Press **F10** to save.

8. Continue loading samples until all samples have been loaded, pressing **F10** after each sample.

9. After loading the last sample, press **ESC**. The drawer will close and processing will begin.

Loading Patient Samples in Auto Mode:

1. Choose **Loading** from the main menu.

2. Select **Samples** (the sample drawer will open).

3. Ensure that the sample mode is set to auto.

4. Scan or type the accession and press enter on the keyboard.

5. Load the tube into the sample drawer.

6. After loading the last sample, press **ESC**. The drawer will close and processing will begin.

Standardized:

Microsample pour-offs:

• Select **F8** to indicate volume once drawer has opened. Be sure to turn off "micro volume" before running normal volume samples again.

Instrument Processing:

• As soon as the sample drawer closes, the "**Test Status**" screen will appear. If there are insufficient reagents to run the test, the "Blocked Sample Pipetting" will appear in red with the amount of deficiency. This deficiency will **BLOCK** the sample pipettor. When this occurs, add the necessary reagents and run QC.

• Dilutions are automatically prepared by the analyzer, according to the established parameters that are entered in each **TEST SETUP** at time of installation. If the patients' results fall outside the assay range, the analyzer will automatically retest that sample at an appropriate dilution, if the test has been previously defined for this response.

Results:

• All patient results display on the **TEST PANEL** screen of analyzer's monitor.

• Results will automatically transmit to the LIS.

• Results may automatically print or be batch printed at the end of each day.

• For results in question that need operator intervention, cursor to the identification number in the "Test Panel" screen; <enter>. This will display the **File Processing** screen. Follow the options in the left hand corner of the screen, (i.e. re-run test).

2.5.2.2. Activate partial thromboplastin time (APTT):

 2.5.2.2.1 Principle:

The APTI involves the recalcification of plasma in the presence of a standardized amount of platelet substitute and a specific activator. This

procedure minimizes test variables by standardizing the contact activation and optimizes the concentration of platelet-like phospholipids.

The APTT explores the intrinsic coagulation pathway (factors XII, XI, IX, VIII, X, V, II and I) except the platelets. The test is not sensitive to quantitative or qualitative platelet abnormalities, deficiencies in factors VII and XIII.

2.5.2.3. Fibrinogen:

Principle:

In the presence of an excess of thrombin, the clotting time of diluted plasma has a direct bearing on the level of plasma fibrinogen.

2.6. Questionnaire data:

Questionnaire design was used to perform CBC and coagulation test (Appendix).

2.7. Ethical approval:

- Ethical clearance from the ethical committee of the Sudan University of Science and Technology, College of Graduate Studies.
- Consent was taken from parents /care givers of all children participated in the study.
- Permission was obtained from Administration of the study area to conduit the study.

2.8. Data analysis:

The data was extracted from the questionnaires and the lab reports into a major spread sheet and then fed on the statistical software SPSS version 11.5. Descriptive statistics and probability testing was done by using independent T-test. The results obtained were presented in tables and figures. Level of significance was set at <0.05. The mean was calculated $\pm$SD.

CHAPTER THREE
3. RESULTS

3.1. Demographic Date:

This study aimed to evaluate the haemostatic parameters among patients with homozygous sickle cell disease (HbSS), sickle cell trait (HbAS) and normal controls (HbAA) and their role as prognostic markers. A total of 100 samples were investigated by performed haeoglobin electrophoresis test. Figures 1, 2 and 3 show the distribution of children with sickle cell disease according to gender, age and type of SCD respectively.

3.2. Laboratary Date:

The mean values of CBC **(Table 3.1)** shows the haemoglobin concentration value in SCD patients was 7.334 g/dl compared to 12.262 g/dl in control group. The data indicated significant (P $\geq$ 0.000) lower value in cas compared with normal group.

The PCV value in case patients was 19.84% compared to 39.98% in normal group (control). The data indicated significant (P $\leq$ 0.000) lower value in anaemic compared with normal group.

The RBcs value in anaemic patients was 2.2494 $\times$ 10^6/µL compared to 4.2198$\times$ 10^6/µL in normal group (control). Case patients had significantly (P $\leq$ 0.000) lower values compared with normal group.

The Plts value in anaemic patients was 504.1 c/cm compared to 295.44 c/cm in normal group (control). The platelet was significantly (P $\leq$ 0.000) higher value in anaemic compared with normal patients.
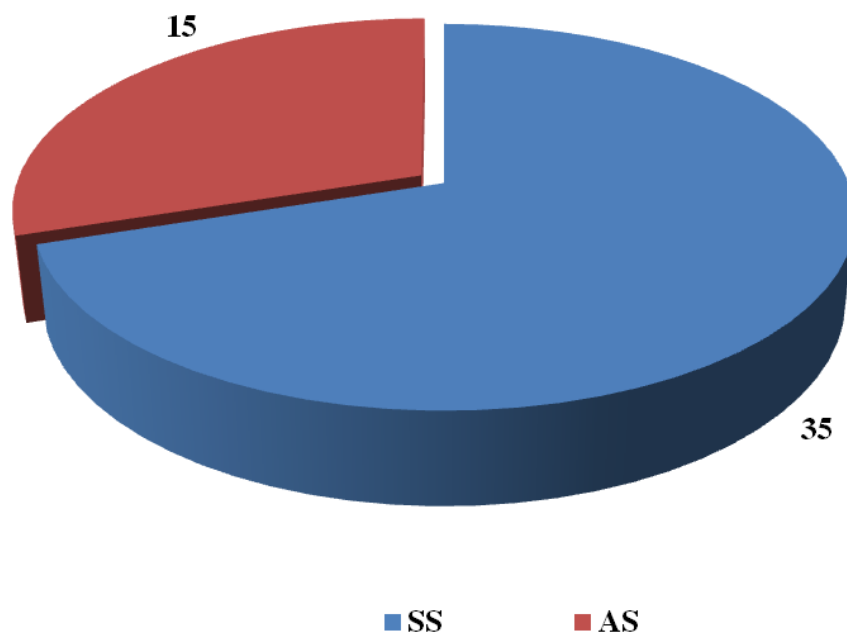
also shows there was slight difference in MCH, MCV and MCHC between anaemic and normal group **Table(3.1)**.
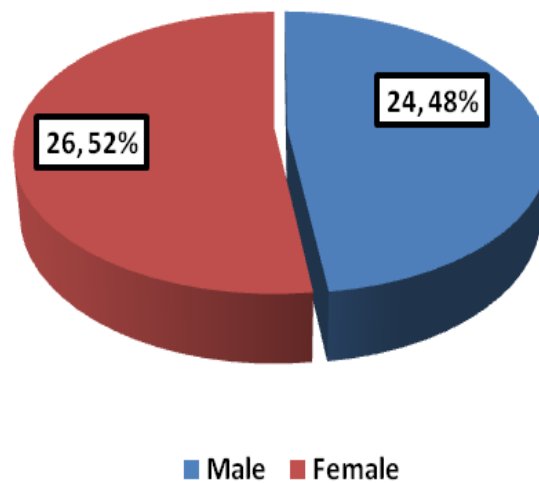
Figure (3-1): Distribution of patients with SCD according to gender

(n = 50)
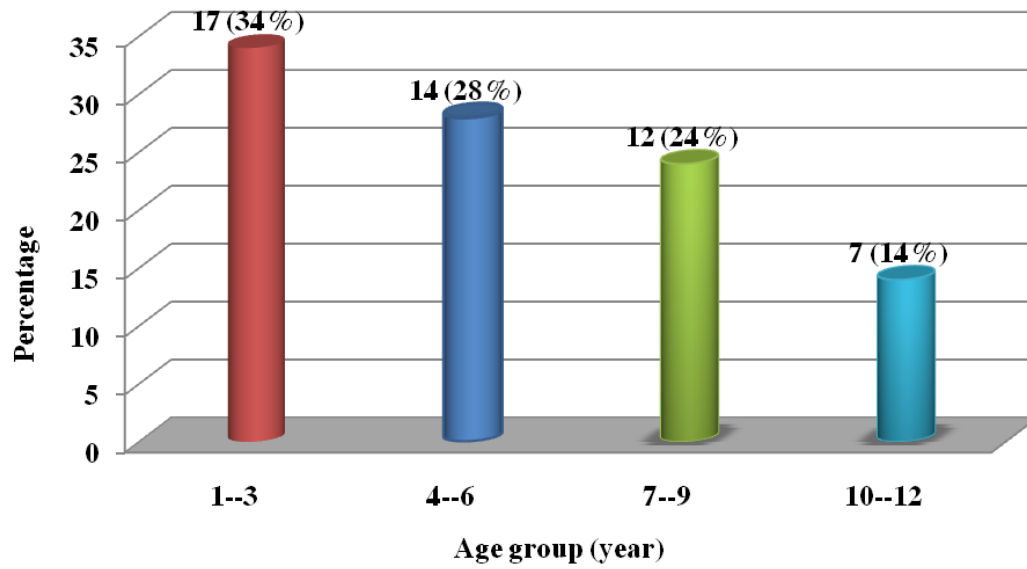
Figure (3-2): Distribution of patients with SCD according to age group

(n = 50)

Figure (3-3): Distribution of patients with SCD & sickle cell trait with study population (n = 50)

Table 3-1: Mean of CBC in case and control in the study

(n = 100)

	Sample	N	Mean	P. value
HB **g/dl**	Control	50	12.26 ± .76	
	Anaemic	50	7.33 ± 1.58	0.000
RBC **c/cm**	Control	50	4.21 ± .339	
	Anaemic	50	2.24 ± .553	0.000
PCV**%**	Control	50	39.98 ± 3.479	
	Anaemic	50	19.84 ± 3.026	0.000
MCV fl	Control	50	87.88 ± 8.484	
	Anaemic	50	88.62 ± 4.861	0.594
MCHpg	Control	50	29.74 ± 2.456	0.317
	Anaemic	50	29.28 ± 2.100	
MCHC**%**	Control	50	33.32 ± 1.731	
	Anaemic	50	33.12 ± 1.637	0.554
Plts **c/cm**	Control	50	295.44 ± 67.093	
	Anaemic	50	504.10 ± 186.776	0.000

Table (3.2) shows the fibrinogen values in normal and anaemic patients, the fibrinogen value in anaemic patients was 5.3112 g/L compared to 2.2140 g/l in control group. The data indicated significantly ($P \leq 0.000$) higher value in anaemic compared with normal patients.

The result of **PT** and APTT values indicated that there were no significant differences related to SCD ($P = 0.472$; $P = 0.739$) respectively.

Table 3-2: Mean of coagulation profile of case and control group

(n = 100)

	Sample	N	Mean	P. value
PT sec	Control	50	13.32 ± 1.708	
	Anaemic	50	13.08 ± 1.614	0.472
APTT sec	Control	50	37.62 ± 3.917	
	Anaemic	50	37.36 ± 3.864	0.739
Fibro g/l	Control	50	2.2140 ± .59932	
	Anaemic	50	5.3112 ± 2.23105	0.000

CHAPTER FOUR

4. DISCUSSION-CONCLUSION& RECOMMENDATIONS

4.1. DISCUSSION:

Sickle cell anaemia results in a myriad of metabolic nutritional, haematological and clinical effect. The study evaluated the effect of sickle cell anaemia on blood constituents, estimating mean levels of various haemostatic parameters like platelet indices, mean prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen levels in children with sickle cell cell anaemia. These parameters were compared with those in normal HbAA controls.

In this study the most common types of SCD was HbSS &HbAS of the study population. This result agrees with previous studies in which reported that the most common types include sickle cell anemia (Hb SS), the sickle beta-thalassemias (Hb Sβ0 and Hb Sβ+), hemoglobin SC disease (Hb SC) and sickle cell disease with hereditary persistence of fetal hemoglobin (S/HPFH) (Reiter, et al., 2003). HbSS is the most common form of sickle cell disease. Patients with Hb SS and Hb Sβ0, in general, have the most severe forms of SCD including lower hemoglobin levels and more frequent vasoocclusive and hemolytic complications (Reiter, et al., 2002).

The present there was significant (P ≤ 0.000) decrease in Hb, PCV, RBC in patients with sickle cell anaemia compared with control group. This may be associated with increase haemolysis rate which associated with SCD. Haemolysis is resulting from the damaged red cell may be occurred in intravenously or extravenously (Tesr, et al., 1991).

Sherwood, et al (1987) reported that there is also a blunted response to erythropoietin secretion in SCD. Morris, et al (1991) suggested that the drop in erythrocyte parameters in anaemic patients may be related to right

shift haemoglobin dissociation curve. The mean Hb concentration and PCV obtained in this study were similar to values obtained in previous studies by Iwalokun, et al (2011) and Ahmed et al., (2010).

The present results indicated that platelet and fibrinogen had higher value in patients with sickle cell anaemia compared with normal (P ≤ 0.000) control group. The results indicated that platelet count increased in anaemic patients. Evaluated platelet could be associated with spleenic sequestration. The increase of platelet activation may associate with high haemolytic rate in anaemic patients (Serry et al., 2001).

Celik et al., (2015) reported higher platelet count and mean platelet volume which increase the incidence of blood viscosity and crises in children with sickle cell anaemia. Ibanga (2006) reported significant activation of platelet insickled patients suggesting the contribution of platelet in the vaso-occlusive phenomena was found in the sickle cell anaemia. The present results are in general agreement with studies (Amin et al., 2004; Chinawa et al., 2013) which reported increase platelet in sickle cell anaemia patients. In the current study, an increase level in fibrinogen level was observed It possible that the hyper-fibrinogenaemia observed in anaemic patients was partly responsible for the increase of blood viscosity. A good correlation between plasma and serum viscosity and fibrinogen concentration has been reported by Lane and Anson (1994). The increase in fibrinogen level in SCD patients may be an added factor in severity of vascular occlusion during SCD crisis (Famod and Reid, 1987).

Buseri et al (2007) noted that the estimation of plasma fibrinogen level in anaemic patients might be a useful indicator of hyper-coagulability, while early diagnostic may help to prevent vaso-occlusive crisis in the sicklers.

In this study, PT and APT was normal. These results were compared with previous obtained in a study conducted in Nigeria in which reported that

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prothrombin time (PT) was normal, Activated partial thromboplastin time (APTT) was increase (Chinawa, eta l., 2013).

The data indict slightly significantly change in MCV, MCHC, MCH, PT, APTT among patients with sickle cell diseases.

PT, APTT among patients with sickle cell diseases slightly significant when compared with other studies, this may be due to small sample size, severity of the disease.

Raffini et al (2006) and Buseri et al (2007) noted that there was elevated PT or both PT and aPT in SCD patients in studies. There are certain possible explanations for the significantly increase PT and APTT values observed in their studies the first explanation could be haepatocytic dysfunction frequently observed in SCD. Decreased synthesis of clotting factors has also been reported in SCD. The third possible explanation for the abnormal PT and APTT values found in their studies could be an associated vitamin K deficiency due to decreased dietary intake in sicklers. (Reiter, et al., 2002)

The above results indicated that the possibility of identify and diagnosis the disease through the laboratory investigation and importance of continuous investigation and avoidance of consanguinity marriage.

4.2. CONCLUSION:

- The study revealed that the Hb, PCV, RBC had lower value in patients with sickle cell anaemia compared with normal.

- There was statistically significant (P ≤ 0.000) association with sickle cell diseases. Plts and fibrinogen had higher value in patients with sickle cell anaemia compared with normal; there was statistically significant (P ≤ 0.000).

- There were no significant differences among patients with sickle cell diseases in MCV, MCH, MCHC, PT and APTT.

4.3. RECOMMENDATIONS:

- Improving the provision of health care for affected individuals by providing laboratory diagnostic parameters.

- Early diagnosis and treatment to decrease thrombin generation or platelet activation may be beneficial of sickle cell disease and prevention of complications that characterize the disorder.

- Fibrinogen measurement during different phases of crisis with measures of fibrinolysis as a protective mechanism from increase fibrinogen production.

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Appendix (1)

Questionnaire

**Measurement of CBC and some Haemostatic Parameter among Patients with
Sickle Cell Anemia in Khartoum State.**
قياس تعدد حلايا الدم وبعض عوامل تخثر الدم لدى مرضى الأنيميا المنجلية بولاية الخرطوم

Date: /   / 2015

Sample No.:

 Age: ……… yrs

Gender…………   Male……….. Female……..

Duration of disease ……… yrs

Last transfusion ………………………………………

Family history ………………………………………

……………………...............................

Complications ………………………………………

Medical test ……………………………………

Appendix (2)

Swelab

Appendix (3)

Stago