



بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**  
**College of Graduated Studies**



**Measurement of some coagulation parameters among  
Pediatric Leukemic Patients Attending Radiation and  
Isotopes Centre of Khartoum**

قياس بعض مكونات تخثر الدم في الأطفال المصابين بسرطان الدم في مركز الخرطوم للعلاج  
بالأشعة والنظائر المشعة

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# الآيه

بسم الله الرحمن الرحيم

قال تعالى:

{ لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاعْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلَانَا فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ } .

صدق الله العظيم

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## **Dedication**

To ...

Those who gave us love and kindness. Those who learned us the meaning of life and humanity and became like the candle burnt it self to light for me.

My parents...

Those who were with me step by step in this research, and lived with me unforgettable moments.

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My sister who always stand with me and be there when I need her, and give me support and encouragement to concise this research.

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I send my deep love and sincere appreciation to my favorite and only brother.... Osman Faisal

I eventually send my deepest thanks to every body helped, supported and encouraged me to complete this research to be delivered as it appearance.

God bless for all.

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## **Abstract**

Among the malignant hematological disorders, the incidence of thrombosis is higher in patients with lymphoma or with acute leukemia. Significant morbidity and high mortality in acute leukemia due to complications of bleeding and infection frequently overshadow thromboembolic events. Disseminated intravascular coagulation is an important cause of bleeding and thrombosis in various benign and malignant diseases.

This is a descriptive prospective analytical case control study, conducted at Radiation and Isotopes Center Khartoum (RICK) during the period between March 2012 to July 2012.

The study aimed to determine prothrombin time, activated partial thromboplastin time, fibrinogen level and platelets count among Sudanese pediatric leukemic patients under 12 years old attending Radiation and Isotopes Center Khartoum (RICK).

Individuals recruited for this study were fifty pediatric leukemic patients (25 acute lymphoblastic leukemia, 15 acute myeloid leukemia, 5 chronic myeloid leukemia and 5 chronic lymphocytic leukemia).

Among the patients, 17 were males and 33 were females, also thirty healthy pediatric individuals were selected as a control group.

80 blood samples were collected from both patients and control used to measure prothrombin time PT, Activated partial prothrombin time APTT, platelet count and fibrinogen level.

The results of this study showed significant prolongation in PT(15 sec), APTT(39.1 sec) and INR(1.1) compared to control group.

Platelet count was significantly decreased compared to control group and the difference was statistically significant in ALL, CLL and AML

patients, but it significant increase compared to control group in CML patients.

Fibrinogen level was normal and there were insignificant difference compared to control. (Reference range 150 - 400 g/dl).

The present results conclude those leukemic patients are at risk of bleeding.

## مستخلص البحث

من بين اضطرابات الدم الخبيثة يعتبر حدوث تجلط الدم أعلى في المرضى الذين يعانون من سرطان الغدد الليمفاوية أو من سرطان الدم الحاد. المراضة الكبيرة وارتفاع معدل الوفيات في سرطان الدم الحاد بسبب مضاعفات النزيف والعدوى وفي كثير من الأحيان تطغي أحداث الانصمام الخثاري. تجلط الدم داخل الأوعية الدموية هو سبب مهم لحدوث النزيف والتجلط في مختلف الأمراض الحميدة والخبيثة.

هذه دراسته وصفية تحليلية أجريت بالمركز القومي للعلاج بالأشعة والطب النووي بالخرطوم في الفترة من مارس الي يوليو 2012 .

هدف هذه الدراسة هو قياس آلية عوامل التخثر الدم عند الأطفال المصابين بسرطان الدم بالمركز القومي للعلاج بالأشعة والطب النووي الخرطوم. وذلك بقياس زمن الثرومبوبلاستين، زمن الثرومبوبلاستين الموضعي النشط، عدد الصفيحات الدموية ومعدل الفيبرينوجين.

الاشخاص المشاركون في هذه الدراسة هم خمسون مريضا دون الثانية عشر سبعة عشر انثي وثلاثة وثلاثون ذكراً خمسة وعشرون مريضا بسرطان الدم الليمفاوي الحاد، خمسة عشر مريضا بسرطان الدم النقوي المزمن، خمسة مرضي بسرطان الدم الليمفاوي المزمن، خمسة مرضي بسرطان الدم النقوي المزمن. وثلاثون طفل من الاصحاء ظاهريا تم اختيارهم كمجموعه ضابطه.

جمعت ثمانون عينة دم من المرضى والاشخاص الاصحاء، لقياس زمن البروثرومبين، زمن الثرومبوبلاستين الموضعي النشط، وعدد الصفيحات الدموية معدل الفيبرينوجين.

اوضحت نتائج هذه الدراسة ان هناك زياده ذات دلالة احصائية في زمن البروثرومبين(15 ثانية)، وزمن الثرومبوبلاستين الموضعي(39.1ثانية) عندي مرضي السرطان مقارنة بالمجموعه الضابطه.

اما عدد الصفائح الدموية كان يوجد به انخفاض ذا دلالة احصائية مقارنة بالمجموعه الضابطه في مرضي سرطان الدم الليمفاوي الحاد(182 ) ، وسرطان الدم الليمفاوي المزمن (116) وسرطان الدم النقوي الحاد(126). كما كان هناك زياده ذات دلالة احصائية عند مرضي سرطان الدم النقوي المزمن (660) مقارنة بالمجموعه الضابطه. وان معدل الفيبرينوجين طبيعيا(215) عند مرضي السرطان مقارنة بالمجموعه الضابطه.

خلصت نتائج هذه الدراسة ان مرضي سرطان الدم معرضين لمخاطر التعرض للنزف.

## Abbreviations

ADP:	Adenosine Di Phosphate
ALL:	Acute lymphoblastic leukemia
AML:	Acute myeloid leukemia
APL:	Acute promyelocytic leukemia
APTT:	Activated partial thrombolastion time
BCR:	Breakpoint cluster region.
CBC:	Complete blood count
CGL:	Chronic granulocytic leukemia
CLL:	Chronic lymphocytic leukemia
CML:	Chronic myeloid leukemia
DIC:	Disseminated intravascular coagulation
FDP:	Fibrin degraded product
FFP:	Fresh frozen plasma
FSP:	Fibrin split product
GP:	Glycoprotein
HMWK:	High molecular weight kininogen
INR:	International normalization ratio
PF3:	Platelet factor 3
PG:	prostaglandins
PIVKA:	Protein independent vitamin k antigen
PT:	Thromplastion time
RICK:	Radiation and isotopes center Khartoum
SPSS:	Statistical package for social science program
TKI:	Tyrosine kinas inhibitors
VWD:	Von will brand disease
VWF:	Von will brand factor.



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# **Chapter One**

Introduction and literature review

# Chapter One

## Introduction and literature review

### 1.1 Introduction

Among the malignant hematological disorders, the incidence of thrombosis is higher in patients with lymphoma or with acute leukemia. Significant morbidity and high mortality in acute leukemia due to complications of bleeding and infection frequently overshadow thromboembolic events. Disseminated intravascular coagulation is an important cause of bleeding and thrombosis in various benign and malignant diseases. (Sarris *et al.*, 1992).

Malignancy is associated with a "hypercoagulable" state and a high risk for thrombohemorrhagic complications. Clinical complications may range from localized thrombosis to bleeding of varying degrees of severity because of DIC. Life-threatening bleeding is frequent in acute leukemias, particularly in acute promyelocytic leukemia (APL). An important pathogenetic role is attributed to the leukemic cell properties interfering with the haemostatic mechanisms. However, chemotherapy and intercurrent infections also contribute to the bleeding risk in the patient with leukemia. (Barbui and Falanga. 2001).

Chronic myeloid leukemia (CML) is infrequent in children. The best-known treatment is stem cell transplant. In a country with limited resources like Sudan, such expensive therapy is not available. Alternative approaches are needed to help these children. The tyrosine kinase inhibitor-imatinib-might be an answer to this problem. Six (19%) patients died with AML or sepsis. Side effects to imatinib were infrequent, observed in 4 out of 29 (13.7%) patients, and mild. One patient only needed dose modification. No resistance was observed during this period.



CML patients present at an earlier age than in other parts of the world. Imatinib is safe and effective in treating pediatric CML where stem cells transplant is not available. Further cytogenetics is important to monitor response and proper management. (Alkhatib , 2011).

## 1.2 Literature review

### 1.2.1 Hemostasis

Haemostasis is balance process that halts bleeding after blood vessel have traumatized or prevention of blood loss through injuries.

The five major components of normal hemostasis involved are platelet, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels. (Hoffbrand *et al.*, 2006).

Major component involved in maintaining hemostasis are vascular integrity, platelet function, fibrin formation system and lysis “breakdown of fibrin clot” (Hoffbrand *et al.*, 2006).

Haemostasis is the result of several interactive systems designed to prevent or stop bleeding. Integral parts of the active haemostatic process include platelet activity, intrinsic and extrinsic coagulation, release of vasoconstrictor or vasodilatation and systemic removal of clots by fibrinolysis. (Hoffbrand *et al.*, 2006).

Normal hemostasis:

The normal haemostatic response to vascular damage depends on closely linked interaction between blood vessel wall, circulating platelet and blood coagulation factors (Hoffbrand *et al.*, 2006).

An efficient and rapid mechanism for stopping bleeding from sites of vessel injury is clearly essential for survival.

#### 1.2.1.1 Component of normal haemostasis:

The haemostatic mechanism have several important functions like to maintain blood in a fluid state, or to arrest bleeding at the site of injury by formation of haemostatic plug , or to ensure eventual removal of the plug when healing is complete. (Lewis *et al.*, 2006).

#### **1.2.1.1.1 Blood platelet:**

The blood platelet is fragments of cytoplasm of megakaryocyte; hence they are non nucleated and formed in the bone marrow. The platelets are having normal life span ranged between 8 to 14 days depending on physiological factors but also on the method used for its measurement.

Newly released platelet appears to be sequestered in spleen for 24—48 hours before reaching the general circulation. The normal spleen contains around 10—20 % of total number of platelet count. Constant level ranges between 150,000 cell/ micro liter to 400,000 cell/ micro liter in normal subject. Some lower platelet count is seen in the new born, also occur during menstrual cycle. (Lewis *et al.*, 2006).

#### **1.2.1.1.2 Blood vessels function and structure:**

The basic structure of blood vessels can be broken down into three layers: the intima is the inner most layer, its surface cover by single layer of endothelial cells, which rest on basement membrane of sub endothelial micro fibril. The media or middle layer contains circulatory smooth muscle cell and collagenous fibrils. And the adventia or outer layer is composed of endothelial cell vary according to their location in vascular tree. They play role as a barrier to micro molecule and particulate matter circulating in blood stream. (Lewis *et al.*, 2006).

Blood vessel functions:

#### **Vasoconstriction:**

Vasoconstriction is the initial vascular response to injury, even at the capillary level. It is dependent upon local contraction of smooth muscle that has a reflex response to various stimuli. The initial vascular constriction occurs before any platelet adherence at the site of injury. Adherence of endothelial cells to adjacent endothelial cells may be sufficient to cause cessation of blood loss from the intravascular space. Vasoconstriction is subsequently linked to platelet plug and fibrin

formation. Thromboxane A<sub>2</sub> (TXA<sub>2</sub>), which results from the release of arachidonic acid from platelet membranes during aggregation, is a powerful vasoconstrictor. By contrast, prostacyclin, which is also secreted during the platelet release reaction, is a potent vasodilator. Serotonin, 5-hydroxytryptamine (5-HT), released during platelet aggregation, is another vasoconstrictor. Bradykinin and fibrinopeptides in the coagulation schema are also capable of contracting smooth muscle. Some patients with mild bleeding disorders and a prolonged bleeding time have, as their only abnormality, capillary loops that fail to constrict in response to injury. (David Schwartz *et al.*, 2012).

#### **1.2.1.2 Primary hemostasis:**

Platelet has three main functions include in primary hemostasis, they are adhesion, release reaction and aggregation.

##### **1.2.1.2.1 Platelet adhesion:**

Platelet adhere to endothelial cell and this may be a consequence of the high local concentration of PG<sub>12</sub> which bind to specific receptors on the platelet membrane stimulatory adrenal cycle activity and causing rise of cyclic adenosine monophosphate.

Platelets escaping from an injured blood vessel come into contact with each other and adhere to number of endothelial micro fibrils, which bind to larger multimers of VWF and through these, react with membrane GP IIa IIb complex receptor becomes exposed and form secondary binding site with VWF, and lysyl groups on the alpha chains of collagen. (Lewis *et al.*, 2006).

##### **1.2.1.2.2 Platelet Change shape:**

Following adhesion, platelets become spherical and extrude long pseudopodia that help in interaction of platelet with each other.

#### **1.2.1.2.3 Release reaction:**

Platelet release their granules substance “alpha and dense granules” which facilitate platelet adhesion and aggregation (Lewis *et al.*, 2006).

#### **1.2.1.2.4 Aggregation:**

Platelet attach to each other through fibrinogen which acts as bridge between adjacent platelet. Fibrinogen binds to platelet through GP IIb IIIa receptor. Also ADP, collagen thrombin, thromboxane A2 induce Platelet aggregation (Lewis *et al.*, 2006).

#### **1.2.1.2.5 Platelet procoagulant activity:**

After Platelet aggregation and release, the exposed membrane phospholipids PF3 is available for reaction of coagulation protein complex formation. (Lewis *et al.*, 2006).

#### **1.2.1.3 Secondary haemostasis:**

Secondary haemostasis involves a series of blood protein interactions through cascade like process that conclude with the formation of insoluble fibrin clot. This system involved multiple enzymes and several cofactors as well as inhibitors to keep system in balance.

Coagulation factor are produced in liver, except for VWF believed to be produced in the endothelial cells. Usually the factors are in a precursor form, the enzyme or zymogene is converted to an active enzyme or protease. (Betty, 2007).

##### **1.2.1.3.1 The coagulation factors:**

The coagulation factors are classified into groups:

- Thrombin group:

Consist of fibrinogen factor 1 and factor V, VIII, and XIII. They are consumed during the process of coagulation and absent in serum and plasma. There an increased concentration of the factors of this group during inflammatory response and pregnancy. (Lewis *et al.*, 2001).

Vitamin K dependent group:

Include prothrombin factor II and factors VII, IX, and X. vitamin K is necessary for their synthesis, which take place in the liver. Coumarin drugs, which compete with vitamin K, cause functional decrease in these factors by producing abnormal proteins that can not bind to calcium and are unable to function normally(Lewis *et al.*, 2006).

These abnormal proteins called PIVKA “protein induces by vitamin K antagonist”. These factors present in the serum as well as in the plasma.

- The contact group:

Composed of factors XI, XII, prekallikrein and high molecular weight kininogen HMWK. These factors are not consumed during coagulation, they are relatively stable. (Betty, 2007).

- The labile group:

Composed of factor VIII and V. they are rapidly lost from stored blood or heated plasma.

- Fibrinogen factor I:

It is synthesized in the liver, it is made up of three pairs of peptide chains named alpha, beta, and gamma. It converted to fibrin. The normal concentration of fibrinogen is 200 to 400 mg/dl; it has a half life 3 to 4 days (Lewis *et al.*, 2006).

- Prothrombin factor II:

It is synthesized in the liver and almost consumed in coagulation process. It converted to thrombin. It's half life about 60 hours.

- Tissue factor factor III:

It is a lipoprotein found in most of body tissue, with high concentration in the lungs and the brain. It acts as cofactor in activating extrinsic pathway.

- Calcium factor IV:

In the ionized state is necessary for coagulation. It is essential for coagulation, which binds calcium and therefore inhibits coagulation.

- Proaccelarin factor V:

It is synthesized in the liver also released from platelets. Is a cofactor in converting prothrombin to thrombin. Has life span about 12 to 14 hours.

- Proconvertin factor VII:

It is synthesizing in the liver and requires vit K for its production. Life span about 4 to 5 hours.

- Antihemophilic factor C or factor VIII:

It is single chain glycoprotein, circulate in the blood bound to vWF, it is a cofactor to enhance activation of factor X by IXa.

- Christmas factor factor IX:

It is synthesized in liver, required vit K for production. It is decrease in plasma of patients with Christmas disease. Has half life 23 hours.

- Stuart factor (factor IX):

It synthesized in the liver, it is necessary for converting prothrombin to thrombin. Half life 40 hours.

Plasma thromboplastin antecedent (factor XI):

Is a beta globulin, synthesized in the liver, it activated by thrombin and factor XII. It is stable at room temperature. Half life about 45 hours.

- Hageman factor:

Is single chain polypeptide, synthesized in the liver, involved in activation of XI and in converting of plasminogen to plasmin.

- Fibrin stabilizing factor (factor XIII):

It is synthesized in the liver and also present in platelets. Converts fibrin polymer to stable in soluble fibrin.

- Prekallikrein Fletcher:

Is a single chain gamma globulin, produced in the liver.

High molecular weight kininogen HMWK:

Is a single chain glycoprotein, has a half life 5 to 6 days. It is produced in the liver but vit k independent. (Edward *et al.*, 1995).

- Von willebrand factor VWF:

It is protein synthesized by endothelial cells and megakaryocyte. It acts as a carrier protein for factor VIII and other function as bridges between platelet and sub endothelial components. (Edward *et al.*, 1995).

#### **1.2.1.3.2 Coagulation cascade:**

The classic coagulation cascade was introduced by Biggs and Macfarlane in the early part of 1964. The clotting factors circulate in the blood stream in the form of inactive precursors. According to the coagulation mechanism is composed of extrinsic pathway. (Hoffbrand *et al.*, 2002)

- Intrinsic coagulation pathway:

Initiation of intrinsic pathway occurs when factor XII, HMWK and prekallikrin are exposed to negatively charged surface. This is termed as the contact phase; occur as result of interaction with phospholipids of circulated lipoprotein.

The assemblage of contact phase component results in conversion of prekallikrin to kallikrein which in turn to activate factor XII to XIIa.

Factor XIIa can then hydrolyzed more prekallikrin to kallikrein. Factor XIIa also activates factor XI to factor XIa and lead to release of bradykinin.

In the presence of calcium, factor XIa activate factor IX to IXa. Factor IX lipoenzyme contain vitamin K dependent carboxyglutamate residues.

Active factor IXa with factor VIII in the presence of calcium and phospholipids cleaves factor X to Xa.

The activation of factor VIII occurs in the presence of minute of quantities of thrombin. As the concentration of thrombin increased, FVIII is ultimately cleaved by thrombin and in activated



- Extrinsic pathway:

The extrinsic pathway is initiated at the site of injury in response to release of tissue factor. Tissue factor is a cofactor in the factor VIIa catalysis activation of factor X.

Factor VIIa contains serine protease, cleaves factor X to factor Xa. The activation of F VII occurs through the action of thrombin or factor Xa. (Hoffbrand *et al.*, 2002)

- Common pathway:

The common pathway consists of four different steps:

- Conversion of prothrombin to thrombin by active factor X
- Cleavage of fibrinogen to fibrin by thrombin.
- Polymerization of fibrin.
- Stabilization of fibrin polymer by factor XIII. (Ernst *et al.*, 2001)

- Fibrinolysis:

-Fibrinolysis is the enzymatic breakdown of fibrin. It is a normal function of blood. Fibrinolysis produces by plasmin enzyme, which derived from stable plasma precursor, plasminogen. In addition to fibrin it can digest certain clotting factors, including fibrinogen, factor V and factor VIII.

-Plasmin is serine protease hydrolyze susceptible arginine and lysine bonds in many proteins, include fibrinogen, fibrin, factors V and VIII.

-When fibrin is digested by plasmin, a series of fragments known as fibrin degradation product FDP or fibrin split product FSP are produced. (Hoffbrand *et al.*, 2006).

#### **1.2.1.4 Regulation of hemostasis:**

Regulation of normal hemostasis and blood flow involve complex interaction between plasma proteins and blood cells, including platelets, leukocyte, and endothelial lining of blood vessels. (Walker, *et al.* 1992).

Protein C is a plasma, vitamin K-dependent zymogen of a serine protease that can inhibit blood coagulation. Protein C is regulated by a series of

reactions known as the protein C pathway. The importance of this pathway is seen in the occurrence of thrombosis in individuals with deficiencies in elements of the pathway like protein C and protein S. Work on several steps in this pathway has revealed that mechanisms involved in activation of protein C and the expression of its anticoagulant activity have features that allow for the expression of the anticoagulant activity away from sites in which procoagulant reactions occur, but not systemically. Thrombin, the principal procoagulant enzyme at the site of an injury, is converted to an anticoagulant enzyme at distant sites through its interaction with the endothelial cell protein thrombomodulin. ( Walker and Fay., 1992).

The fibrinolysis activator like tissue plasminogen activator TPA and urokinase enzymes. Also the fibrinolysis inhibitor like plasminogen activator inhibitor PAI and alpha 2 antiplasmin inhibitor. These inhibitors block adsorption of plasminogen to fibrin, there by reducing the amount of plasminogen available. (Oscar and Chalies., 1991).

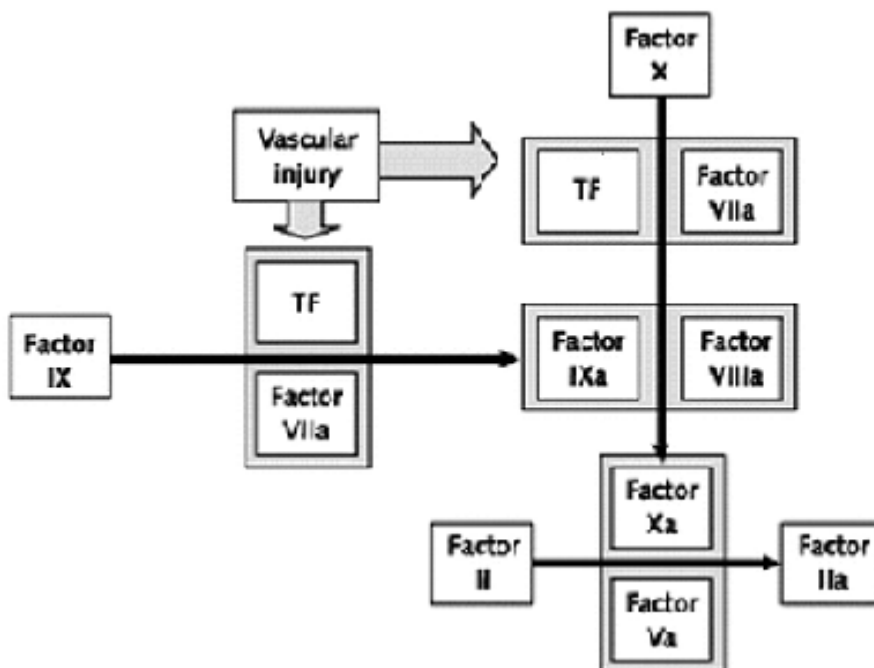


Figure 1: coagulation cascade. (Mark *et al.*, 2003)

## **1.2.2 Leukemia**

Leukemia is disease in which abnormal proliferation of hemopoietic cells cause progressively increasing infiltration of bone marrow, although in certain forms the lymphatic tissues are partially affected.

The process of differentiation in leukemic cell is often abnormal, and these commonly result in immature morphological appearance. Leukemic cell are usually present in the peripheral blood, where the count range from very low to very high values. These cells very commonly have myeloid or lymphocytic characteristics, but occasionally cells with feature of erythroid precursors or megakaryocytic part of the disease process.

The rate of progression varies considerably in different types of leukemia. But death is usual outcome in untreated disease as a result of compromised production of mature blood cells.

Leukemia account for about four percent of all deaths from malignant disease, although the proportion is greater in childhood.

(Frank *et al.*, 1991).

### **1.2.2.1 Aetiology of leukemia:**

Leukemia is generally considered to be neoplastic disorder originating in hemopoietic cell which has undergone an intrinsic change, causing it to escape from the normal restraints impose on proliferative activity. Many contributory factors have been incriminated in the development of neoplastic change and include inherited predisposition effect of viruses and effect of radiation and chemicals. (Tefferi and Louis, 2006).

#### **1.2.2.1.1 Chromosome abnormalities:**

Chromosome abnormalities are very common in leukemia and occur in all major categories of the acute and chronic form of the disorder.

Usually the abnormality is maintained in a constant manner in a particular case, in keeping with defect that is replicate with high degree of

fidelity in the clone of cells arising from the cell in which malignant behavior first developed. (Hoffbrand *et al.*, 2002)

Sometimes additional changes occur at the first time of characteristics of the leukemia alter the abnormality of the chromosomes are directly involved in abnormal cellular behaviors.

Not all cases of leukemia have chromosome abnormalities detectable at the microscopic level with currently available techniques.

A number of correlations exist between laboratory feature or clinical behavior of leukemic process and specific chromosome abnormalities, such as the translocation between the long arm of chromosome 15 and 17 in acute leukemia. (Frank *et al.*, 1991).

#### **1.2.2.1.2 Radiation:**

There are evidence that ionizing radiation especially x-radiation, the incidence of leukemia increase with the cumulative dose received.

An increase in the incidence of leukemia was first noted in irradiated survivors of the atom bomb last three years of event.

The mechanism linking irradiation with development of leukemia up to many years later is un clear, but it is perhaps relevant that irradiation produces chromosome abnormalities. (Frank *et al.*, 1991)

#### **1.2.2.1.3 Chemicals:**

Leukemia is more common in people who have been exposed for substantial periods to benzene vapor, and the risk caused leukemia developoement has been suggested to increase with the extent of cumulative exposure.

(Frank *et al.*, 1991).

#### **1.2.2.1.4 Family history of leukemia:**

It's rare for more than one person in a family to have leukemia. When it does happen, it's most likely to involve chronic lymphocytic leukemia. However, only a few people with chronic lymphocytic leukemia have a

father, mother, brother, sister, or child who also has the disease. Having one or more risk factors does not mean that a person will get leukemia. Most people who have risk factors never develop the disease. (Frank *et al.*, 1991).

#### **1.2.2.1.5 Viruses:**

Some leukemogenic retroviruses do not contain oncogenes, retroviruses HTLV-1 affect T cells and these cells evolved into T lymphocyte leukemia. (Frank *et al.*, 1991).

#### **1.2.2.1.6 Genetic factors:**

These are inherited abnormalities of nuclear DNA associated with increase incidence of leukemia. Inherited disease like Fanconi anemia, Bloom syndrome and ataxia, are associated with increase incidence of leukemia.

#### **1.2.2.2 Classification of leukemia:**

According to type of stem cell leukemia is classified into myeloid and lymphoid. And according to clinical course it is classified to chronic and acute. French American British FAB classified leukemia into acute myeloid leukemia and acute lymphoid leukemia. (Hoffbrand *et al.*, 2002)

##### **1.2.2.2.1 Acute myeloid leukemia (AML):**

Acute myeloid leukemia (AML) is one of the most common types of leukemia among adults. This type of cancer is rare under age 40. It generally occurs around age 60. AML is more common in men than women.

Persons with this type of cancer have abnormal cells inside their bone marrow. The cells grow very fast, and replace healthy blood cells. The bone marrow, which helps the body fight infections, eventually stops working correctly. Persons with AML become more prone to infections and have an increased risk for bleeding as the numbers of healthy blood cells decrease. (Hoffbrand *et al.*, 2002)

The following are risk factors for AML:

A weakened immune system (immunosuppression) due to an organ transplant

Blood disorders, including:

-Polycythemia Vera

-Essential thrombocythemia

-Myelodysplasia (refractory anemia)

-Exposure to radiation and chemicals

**Symptoms:**

Bleeding from the nose, bleeding gums, bruising, bone pain or tenderness, fatigue, fever, heavy menstrual periods, Pallor, Shortness of breath (gets worse with exercise), Skin rash or lesion, swollen gums (rare) and Weight loss.

• **Types of AML:**

**-M0:**

Un differentiated leukemia.

**-M1:**

Is myeloblastic leukemia without maturation. Myeloblast with non granular cytoplasm or rare azurophilic granules.

**-M2:**

Myeloblastic leukemia with maturation. Myeloblast with promyelocytes and more mature myeloid cells. Myeloperoxidase positive.

**-M3:**

Hyper promyelocytes leukemia. Promyelocyte with heavy cytoplasmic granulation and renifore nuclei, multiple Auer rods in parallel bundles.

**-M4:**

Myeloplasyic leukemia, immature and mature cells of both myeloid and monocytic series.

**-M5:**

Monocytic leukemia classified into:

**M5a:** poorly differentiated type, monoblast with non granular cytoplasm or rare azurophilic granules

**M5b:** Differentiated type, monoblast promonocyte and monocyte.

**-M6:**

Erythroblastic leukemia, contain erythroblast more 50% of marrow nucleated cells.

**-M7:**

Megakaryoblastic leukemia (Tefferi and Louis, 2006).

**-Treatment:**

Treatment involves using medicines to kill the cancer cells. This is called chemotherapy. But chemotherapy kills normal cells, too. This may cause side effects such as excessive bleeding and an increased risk for infection. Your doctor may want to keep you away from other people to prevent infection.

Other treatments for AML may include Antibiotics to treat infection, Bone marrow transplant or stem cell transplant after radiation and chemotherapy, Red blood cell transfusions to fight anemia, Transfusions of platelets to control bleeding, most types of AML are treated the same way (Hoffbrand *et al.*, 2002).

**1.2.2.2.2 Acute lymphoid leukemia (ALL):**

Acute lymphocytic leukemia (ALL) is a fast-growing cancer of a type of white blood cells called lymphocytes. These cells are found in the bone marrow and other parts of the body. Acute lymphocytic leukemia (ALL) occurs when the the body produces a large number of immature white blood cells, called lymphocytes. The cancer cells quickly grow and replace normal cells in the bone marrow. Bone marrow is the soft tissue in the center of bones that helps form blood cells. ALL prevents healthy

blood cells from being made. Life-threatening symptoms can occur. This type of leukemia usually affects children ages 3 – 7 years. It is the most common childhood acute leukemia. However, the cancer may also occur in adults. Most of the time, there is no obvious cause. However, the following may play a role in the development of leukemia in general:

- Certain chromosome problems
- Exposure to radiation, including x-rays before birth
- Past treatment with chemotherapy drugs
- Receiving a bone marrow transplant
- Toxins such as benzene
- The following increase your risk for ALL:
  - Down syndrome or other genetic disorders
  - A brother or sister with leukemia (Tefferi and Louis., 2006).

- **Types of ALL:**

- L1:** Small uniform lymphoblast, with scanty cytoplasm and moderately basophilic regular round nucleus with in visible nucleoli.

- L2:** Lymphoblast varying in size, nucleus membrane irregular and nucleoli one or more.

- L3:** Lymphoblast large uniform. Regular cell membrane with clear vacuoles, nucleoli one or more.

Blood tests may include:

Complete blood count (CBC), including white blood cell (WBC) count

Platelet count and Bone marrow aspiration and biopsy.

**Bone marrow aspiration and biopsy:**

Lumbar puncture (spinal tap) to check for leukemia cells in the spinal fluid

Tests are also done to look for chromosome changes in the cells of some leukemia. Leukemia's with certain types of chromosome changes have a poor outlook, while those with other types of genes can have a very good



outlook. Having certain chromosome change may determine what kind of treatment you receive. (Tefferi and Louis., 2006).

#### **1.2.2.2.3 Chronic myeloid leukemia (CML):**

Chronic myelogenous (or myeloid) leukemia (CML), also known as chronic granulocytic leukemia (CGL), is a cancer of the white blood cells. It is a form of leukemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which proliferation of mature granulocytes (neutrophils, eosinophils, and basophils) and their precursors is the main finding. It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome. CML is now largely treated with tyrosine kinase inhibitors (TKIs), such as imatinib, dasatinib, or nilotinib, which have led to dramatically improved survival rates since their introduction in the last decade. (Tefferi and Louis., 2006).

##### **1.2.2.2.3.1 Signs and symptoms of CML:**

Patients are often asymptomatic at diagnosis, presenting incidentally with an elevated white blood cell count on a routine laboratory test. In this setting, CML must be distinguished from a leukemoid reaction, which can have a similar appearance on a blood smear. Symptoms of CML may include: enlarged spleen causing pain on the left side, malaise, joint and/or hip pain, low-grade fever, increased susceptibility to infections, anemia, and thrombocytopenia with easy bruising (although an increased platelet count may also occur in CML). (Hoffbrand *et al.*, 2002)

##### **1.2.2.2.3.2 Diagnosis of CML:**

CML is often suspected on the basis on the complete blood count, which shows increased granulocytes of all types, typically including mature myeloid cells. Basophils and eosinophils are almost universally

increased; this feature may help differentiate CML from a leukemoid reaction. A bone marrow biopsy is often performed as part of the evaluation for CML, and CML is diagnosed by detecting the Philadelphia chromosome. This characteristic chromosomal abnormality can be detected by routine cytogenetics, by fluorescent in situ hybridization, or by PCR for the bcr-abl fusion gene. (Hoffbrand *et al.*, 2002)

Controversy exists over so-called Ph-negative CML, or cases of suspected CML in which the Philadelphia chromosome cannot be detected. Many such patients in fact have complex chromosomal abnormalities that mask the (9;22) translocation, or have evidence of the translocation by FISH or RT-PCR in spite of normal routine karyotyping. The small subset of patients without detectable molecular evidence of bcr-abl fusion may be better classified as having an undifferentiated myelodysplastic/myeloproliferative disorder, as their clinical course tends to be different from patients with CML (Tefferi and Louis., 2006).

#### **1.2.2.2.3.3 Pathophysiology of CML:**

CML was the first malignancy to be linked to a clear genetic abnormality, the chromosomal translocation known as the Philadelphia chromosome. This chromosomal abnormality is so named because it was first discovered and described in 1960 by two scientists from Philadelphia, Pennsylvania.

In this translocation, parts of two chromosomes (the 9th and 22nd by conventional karyotypic numbering) switch places. As a result, part of the BCR ("breakpoint cluster region") gene from chromosome 22 is fused with the ABL gene on chromosome 9. The action of the BCR-ABL protein is the pathophysiologic cause of chronic myelogenous leukemia (Hoffbrand *et al.*, 2002).

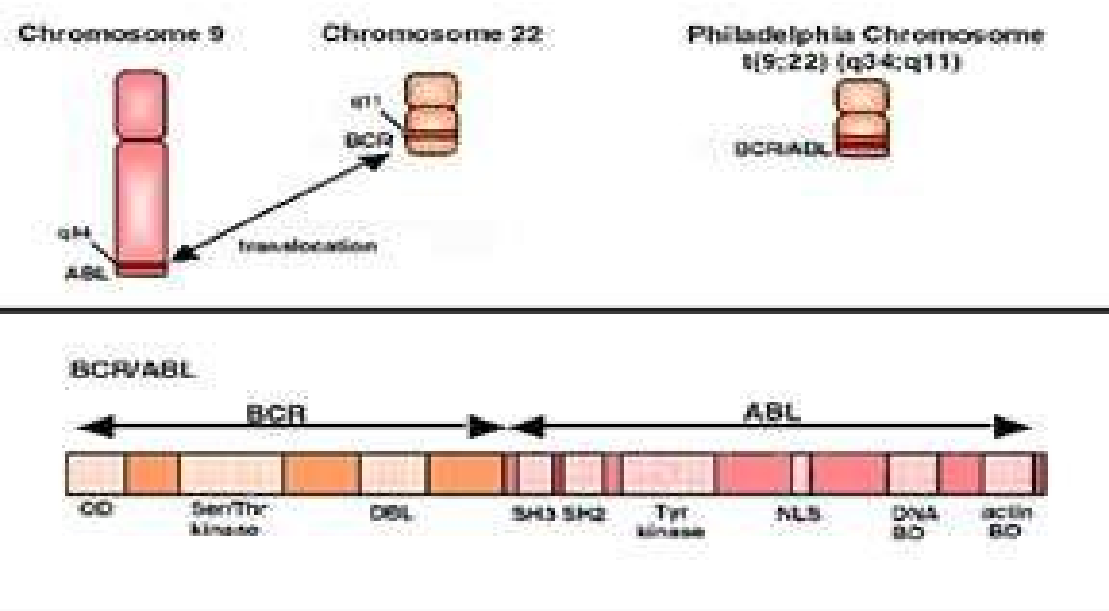


Figure 2 : Philadelphia chromosome (Hoffbrand *et al.*, 2002)

#### 1.2.2.2.3.4 Classification of CML:

CML is often divided into three phases based on clinical characteristics and laboratory findings. In the absence of intervention, CML typically begins in the chronic phase, and over the course of several years progresses to an accelerated phase and ultimately to a blast crisis. Blast crisis is the terminal phase of CML and clinically behaves like an acute leukemia. Drug treatment will usually stop this progression if started early. One of the drivers of the progression from chronic phase through acceleration and blast crisis is the acquisition of new chromosomal abnormalities (in addition to the Philadelphia chromosome). Some patients may already be in the accelerated phase or blast crisis by the time they are diagnosed (Hoffbrand *et al.*, 2002).

- **Chronic phase:**

Approximately 85% of patients with CML are in the chronic phase at the time of diagnosis. During this phase, patients are usually asymptomatic or have only mild symptoms of fatigue, left side pain, joint and/or hip pain, or abdominal fullness. The duration of chronic phase is variable and

depends on how early the disease was diagnosed as well as the therapies used. In the absence of treatment, the disease progresses to an accelerated phase (Frank *et al.*, 1991).

- **Accelerated phase:**

Criteria for diagnosing transition into the accelerated phase are somewhat variable; the most widely used criteria are those put forward by investigators Sokal *et al.* and the World Health Organization. The WHO criteria are perhaps most widely used, and define the accelerated phase by any of the following:

10–19% myeloblasts in the blood or bone marrow

>20% basophils in the blood or bone marrow

Platelet count <100,000, unrelated to therapy

Platelet count >1,000,000, unresponsive to therapy

Cytogenetic evolution with new abnormalities in addition to the Philadelphia chromosome

Increasing splenomegaly or white blood cell count, unresponsive to therapy

The patient is considered to be in the accelerated phase if any of the above is present. The accelerated phase is significant because it signals that the disease is progressing and transformation to blast crisis is imminent. Drug treatment often becomes less effective in the advanced stages.

- **Blast crisis:**

Blast crisis is the final phase in the evolution of CML, and behaves like an acute leukemia, with rapid progression and short survival.

Blast crisis is diagnosed if any of the following are present in a patient with CML:

>20% myeloblasts or lymphoblasts in the blood or bone marrow large clusters of blasts in the bone marrow on biopsy (Hoffbrand *et al.*, 2002)

#### **1.2.2.2.4 Chronic lymphocytic leukemia (CLL):**

Chronic lymphocytic leukemia (CLL) causes a slow increase in white blood cells called B lymphocytes, or B cells. Cancer cells spread through the blood and bone marrow, and can also affect the lymph nodes or other organs such as the liver and spleen. CLL eventually causes the bone marrow to fail. (Kantarjian *et al.*, 2002).

#### **Symptoms:**

Symptoms usually develop slowly over time. Many cases of CLL are detected by blood tests done in people for other reasons or who do not have any symptoms.

#### **Symptoms that can occur include:**

Abnormal bruising (occurs late in the disease), Enlarged lymph nodes, liver, or spleen, Excessive sweating, night sweats, Fatigue, Fever, Infections that keep coming back (recur), Loss of appetite or becoming full too quickly (early satiety). (Kantarjian *et al.*, 2002)

#### **1.2.2.3 Disseminated Intravascular Coagulation and Acute Lymphoblastic Leukemia:**

(DIC) is an important cause of bleeding and thrombosis in various Benign and malignant diseases. DIC was reported in 30% of all patients with an acute leukemia the contribution of DIC to the early fatalities of acute promyelocytic leukemia (led to the introduction of prophylactic heparin to induction regimens with subsequent decrease in early deaths and increase in complete remission rates<sup>4</sup>.’ Others have achieved similar results with intensive use of fresh frozen plasma (FFP) and platelets, but without using heparin (Sarris *et al.*, 1992).

DIC is very common during remission induction of adult ALL and is often associated with significant morbidity and mortality.

The bleeding diathesis in patients with acute promyelocytic leukemia (APL) is generally attributed to disseminated intravascular coagulation

(DIC), initiated by the release of procoagulant activity from leukemic cells. Primary fibrinogenolysis, mediated by the release of leukocyte proteases, may also contribute to this disorder (Sarris *et al.*, 1992).

This DIC syndrome was probably limited, since no prothrombin time decrease, no significant factor V consumption, and normal levels of coagulation inhibitors (antithrombin III and protein C). Evidence of degraded forms of von Willebrand factor in the plasma suggested an extended proteolytic activity.

The present findings provide new arguments for the association of DIC and proteolysis syndromes in APL-associated coagulation disorders. Further prospective studies are needed in order to confirm the persistence of thrombin activation in course of ATRA therapy.(Sarris *et al.*, 1992)

### **1.3 Rationale:**

Leukemic patients suffer from many abnormalities that may affect their haemostatic status and may result into bleeding and thrombosis, which both can affect their survival. So the leukemic patients need to regularly checkup for their haemostatic status.this study was carried out to measure PT, APTT, INR, Platelet count and fibrinogen level in leukemic pateints to help in early detection of any risk that may result in more complications which may lead to death (Hau *et al.*, 2007).

## **1.4 Objectives:-**

### **1.4.1 General objective:**

To measure some haemostatic parameters of pediatric leukemic patients attending Radiation and Isotopes Centre of Khartoum.

### **1.4.2 Specific objectives:**

1. To measure Prothrombin time PT, activated partial thromboplastin time APTT, platelet count and fibrinogen level of Sudanese pediatric leukemic patients compared with control group.
2. To compare the PT, APTT, INR, fibrinogen level and platelet count between different types of leukemia.
3. To compare haemostatic parameters of patients according to age and gender.



# **Chapter Two**

## Materials and methods

## **2. Materials and methods**

### **2.1 Study design:**

This was a descriptive prospective and analytical case control study conducted over a period of time between March 2012 to July 2012. The study was conducted at Radiation and Isotopes centre of Khartoum (RICK).

### **2.2 Study population:**

Samples were selected from 50 leukemic patients and 30 apparently healthy individuals as control with matched age.

#### **2.2.1 Inclusion criteria:**

All patients who confirmed after diagnosis as leukemic, both sexes were included with age less than 12 years old.

#### **2.2.2 Exclusion criteria:**

Patient with age more than 13 years' old or non leukemic patients.

### **2.3 Sample size:**

Samples were selected by simple random sampling method “probability sampling. Total number of samples 50 leukemic and 30 healthy.

### **2.4 Data collection:**

A specifically designed questionnaire was used as a tool of data collection in this study.

### **2.5 Data analysis:**

All data include the questionnaire was coded and listed in master sheet and then computerized (SPSS- statistical package for social science program) was used for data entry and analysis.

### **2.6 Data presentation:**

The data was presented in forms of tables and figures.

## **2.7 Procedure of sample collection:**

### **2.7.1 Sampling:**

5 ml of blood was taken from each patient.

2.5 ml was collected in trisodium citrate container to obtain plasma for PT, APTT, and fibrinogen level determination.

Other 2.5 ml was collected in EDTA container for platelet count.

## **2.8 Laboratory procedure:**

Reagents:

Reagent for platelet count:-

1% Ammonium oxalate solution for measure platelets count

Reagent for Prothrombin Time (PT) Activated Partial Thromboplastin Time (APTT) and fibrinogen level:-

- platelet poor plasma from patient and control, obtained by centrifugation of citrated blood on 3000 rpm for 15 minutes.
- Commercially prepared thromboplastin for PT.
- Kaolin cephalin reagent for APTT.
- Calcium chloride (CaCl<sub>2</sub>) for activation of APTT.

## **2.9 Methods:**

Collection of blood sample:

1. 5 ml blood was drawn from vein using disposable plastic syringe from antecubital vein or from the back of the hand.
2. After cleaning of the skin with 70 % alcohol, the needle was inserted into the vein and blood was slowly with drawn until the sufficient amount of blood was collected (Dacie and Lewis 2006).
3. Then the needle was removed and the site of puncture was covered with adhesive bandage to stop bleeding.
4. The blood was slowly poured into container, EDTA for platelet count

and trisodium citrate for PT, APTT & fibrinogen. (Dacie and Lewis., 2006).

### **2.9.1 Platelet count:**

- 1) 2 ml of 1% Ammonium oxalate solution was placed in glass test tube.
- 2) 0.02 ml of EDTA anticoagulant blood was placed in the same glass test tube.
- 3) Mix well, and left for 3-5 minutes.
- 4) During this time, hemocytometer was prepared.
- 5) The mixture was placed in the hemocytometer chamber
- 6) Waited for 2-3 minutes for platelets cell to be settled, was counted under the microscope by using lens X40. Counted for corner and middle squared and was calculated the number and get the results by this formula:

$$\text{Platelets count} \times 10^9 / \text{L} = \frac{\text{No of the cell counted} \times \text{DF}}{\text{Depth} \times \text{Area of count}}$$

Normal value:

The normal range for platelet falls between 150,000 – 450,000 cell/cumm.

### **2.9.2 Semi automated Coagulometer:**

Coagulometer is an apparatus in the form of a graduated tube, used to determine the rapidity of coagulation of any given specimen of blood, or the coagulating used to measure the ability of the blood to coagulate (and the time taken to do so).

#### **2.9.2.1 Principle:**

The coagulometer clot has an optical measurement, which detect a student variation in optical density when clot is formed. The chronometer

and stirring system are activated by a sudden change of optical density. This permits the initiation of time measurement when the sample is added to the reagents and stop measurement time at moment that the clot is formed. The continues mixing guarantee perfect homogenization which make the measurement possible of low concentration of fibrinogen by grouping the fibrinogen filaments in the centre of optical pass. The system has programmable security time during which variation in optical density, when the reagent and plasma are still in homogenization phase, cannot activate the detection cell.

### **2.9.2.3 Prothrombin Time (PT):**

principle:

The test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract and indicate overall efficiency of the extrinsic clotting system.

Although originally thought to measure prothrombin, the test not known to depend also on reactions with factor V, VII, X and on fibrinogen concentration in the plasma.

Procedure:

- 1- The coagulometer was opened and adjusted to PT location.
2. The reagent and samples were placed in water bath adjusted at 37 c for 3 to 5 minutes which is the part of coagulometer.
3. Astierr was placed in specific cuvettes
4. 0.2 ml of calcified liquiplastin was added to cuvette
5. The cuvette was placed in reader cavity.
6. 0.1 ml of plasma (PPP) was added to cuvette. When the stirrer stopped the result was clearly shown on the screen of coagulometer, the PT was

measured.

Normal value: Normal values for PT are between 11-16 seconds.

#### **2.9.4 Activated Partial Thromboplastin Time APTT:**

Principle:

The test measure the clotting time of plasma after activation of contact factor without addition of tissue thromboplastin, and indicate efficiency of intrinsic and common pathway.

Procedure:

- 1- The coagulometer was opened and adjusted to APPT location.
2. The reagent and samples were placed in water bath adjusted at 37 c for 3 to 5 minutes which is the part of coagulometer.
3. Stirror was placed in specific cuvettes
4. 0.1 ml of plasma (PPP) was incubated with 0.1ml of liquid for 3 minutes at 37C.
5. 0.1 ml of 0.025 mol Cacl<sub>2</sub> was added.

When the stirror stopped the result was clearly shown on the screen of coagulometer, the was APTT measured per second.

#### **2.9.5 Fibrinogen level:**

Dilution of plasma by using buffer (thrombin buffer) then measure the concentration of fibrinogen was measured using semi automated coagulometer.

Normal value:

1.5 - 4.5 g/l

### **2.10 Ethical Consideration:**

All information, specimens and results obtained from patients were considered as highly security data. The participants were provided with information about the study and any risk that may arrive especially when collection technique was applied.

# **Chapter Three**

## Results



## Results

PT of patients with all different types of leukemia insignificantly increased compared to control. In patients with AML PT is  $15.7 \pm 2$  seconds compared to  $13 \pm 1$  seconds of control group.

APTT of patients with different types of leukemia insignificantly increased compared to control. In patients with AML APTT is  $41.6 \pm 4.2$  seconds compared to  $32.8 \pm 2$  seconds of control.

INR of patients with all different types of leukemia insignificantly increased compared to control. In patients with AML INR is  $1.2 \pm 0.1$  compared to  $0.9 \pm 0.1$  of control.

Significant increase in platelet of patients with CML  $660 \pm 121$  cells/cumm compared to  $250 \pm 100$  cells/cumm of control.

Significant decrease in platelet of patients with CLL  $116 \pm 11$  cells/cumm compared to  $250 \pm 60$  cells/cumm of control.

With insignificant decrease in fibrinogen level of leukemic patient compared to control group. In patients with AML  $308 \pm 42$  g/dl compared to  $336 \pm 50$  g/dl of control.

There were insignificant difference in both male and female patients in PT, APTT, INR, Platelet count, Fibrinogen level ( $P$  value=0.12) ( $P$  value=0.06) ( $P$  value=0.11) ( $P$  value=0.23) ( $P$  value=0.81), respectively.

**Table 3.1:****Distribution of patients according to type of leukemia, age and Gender**

Variations		Types of leukemia			
		AML	ALL	CML	CLL
Age	4 - 6	60%	60%	80%	80%
	7 - 12	40%	40%	20%	20%
Gender	Male	24%	47%	40%	40%
	Female	76%	53%	60%	60%

**Table 3.2:**  
**Prothrombin Time, Activated Partial Thromoplastin Time, International Normalization Ratio, Platelets count and Fibrinogen level in different patients and control**

Types of leukemia	PT/ Sec  Mean±SD	APTT/ Sec  Mean±SD	INR  Mean±SD	Platelet count/ cell/cumm Mean±SD	Fibrinogen level/ g/dl  Mean±SD	No
ALL	15±2.6	37.1±6.6	1.0±0.2	182.0±41.0	150.0±20.0	25
AML	17.7±2.0	41.6±4.2	1.2±0.1	126.0±32.0	308.0±42.0	15
CLL	15.1±1.5	41.0±3.4	1.1±0.1	660.0±121.0	212.0±11.0	5
CML	14.2±1.09	36.6±3.9	1.1±0.1	116.0±11.0	188.0±20.0	5
Control	13.0±1.0	32.8±2.0	0.9±0.1	250.0±60	336.0±50.0	30
<i>P.value</i>	0.55	0.06	0.49	0.00	0.45	--

**Table 3.3:**  
**Prothrombin Time, Activated Partial Thromoplastin Time,**  
**International Normalization Ratio, Platelets count and Fibrinogen**  
**level in study group according to Gender.**

Parameter	Gender		<i>P.value</i>
	Male Mean±SD	Female Mean±SD	
PT/sec	15.0±2.2	16.0±1.9	0.12
APTT/sec	37.6±5.7	40.6±3.9	0.06
INR	1.1±0.1	1.1±0.1	0.11
Platelet count/ Cell/cumm	178±13.0	228±21.0	0.23
Fibrinogen level/ g/dl	232±31.0	253±31.0	0.81
Total	33	17	-

# **Chapter Four**

Discussion, conclusion and  
recommendation

## **Chapter Four**

### **Discussion, conclusion and recommendation**

#### **4.1 Discussion**

Among the malignant hematological disorders, the incidence of thrombosis is higher in patients with lymphoma or with acute leukemia. Significant morbidity and high mortality in acute leukemia due to complications of bleeding and infection frequently overshadow thromboembolic events. Disseminated intravascular coagulation is an important cause of bleeding and thrombosis in various benign and malignant diseases.

This was a case control study conducted in Radiation and Isotope Centre Khartoum (RICK) during the period March 2012 to July 2012.

The study populations included 25 patients with ALL, 15 patients with AML, 5 patients with CML and 5 patients with CLL.

PT, APTT and INR were significantly increased in leukemic patients compared to control group. And insignificant difference in platelet and fibrinogen level compared to control group.

AML patients showed significant increase in PT(15.7 sec), APTT(41.6 sec), INR(1.2) and fibrinogen level compare to other types of leukemia.

CML patients showed increase in platelet count but CLL patients showed decrease in platelet count compared to other types of leukemia.

There were insignificant different in PT, INR, APTT, Platelet count, Fibrinogen level compared to different age group and sex of patients.

Similar study conducted by Hung Chang, and co workers in Chang Gung University, different results was obtained. Show that there were High WBC counts, prolonged PT, and APTT. But Fibrinogen levels, platelet counts, were not significantly are associated with clinical bleeding in our

series. Patients with APL are susceptible to DIC and subsequent bleeding events. Prompt ATRA administration is crucial in preventing hemorrhagic events (Chang *et al.*, 2012).

The prolongation of PT, APTT and INR compared to control group results from number of causes, the first cause is leukemic infiltration which leads to decrease of hepatic function and interfere with the synthesis of vitamin K dependent factors. The second cause was the increase consumption of coagulation factors result from DIC that occur in AML (M3) due to excessive fibrinolysis and thrombocytopenia. (Sarris *et al.*, 1992).

Much express of TF and procoagulant present in the outer membrane when apoptosis occur in the inner membrane is exteriorized and the tissue factor initiates coagulation cascade.

The platelet count was significant decrease in ALL, AML and CLL, but there was significant increase in CML patients compared to control group.

Reduction of platelet count in ALL, AML and CLL result from enlargement of spleen (splenomegally) which leads to trapping of platelet in spleen resulting in reduction in number of platelet. Also failure of bone marrow to produce platelets due to infiltration or replacement of normal bone marrow cells with leukemic cells which resulted in reduction of megakaryocytes. (Sarris *et al.*, 1992). Chemotherapy treatment may affect rapidly dividing cells of body besides affecting of cancer cells and make damage of bone marrow and failed to produce blood cells (Sarris *et al.*, 1992).

## 4.2 Conclusion

This study concluded:

- 1- Prothrombin time PT and Activated partial thromboplastin time APTT was significantly increased in ALL, AML, CLL and CML patients compared to control group.
- 2- AML patients showed significant increase in PT, APTT, INR and fibrinogen level compare to other types of leukemia.
- 3- CML patients showed significant increase in platelet count but CLL patients showed decrease in platelet count compared to other types of leukemia.
- 4- Fibrinogen level was statistically insignificant in ALL, AML, CLL and CML patients.
- 5- Platelets counts were decreased significantly in ALL, AML and CLL, but in CML patients there were increased significantly in platelet count.
- 6- There were insignificant differences in both male and female patients in PT, APTT, INR, Platelet count, Fibrinogen level.
- 7- Due to this abnormal haemostatic status, leukemic patients are at risk of bleeding.



### **4.3 Recommendations**

This recommendation included:

- 1- Regular check of coagulation parameters of leukemic patients is recommended to minimize risk of bleeding and thrombosis.
- 2- Health centers should be established in different states for early treatment of patients.
- 3- Health education should be implemented to increase the awareness about the importance of blood donation to leukemic patients.

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# **Appendices**

## Appendix I

بسم الله الرحمن الرحيم

Sudan University of science and technology

College of Post Graduated Studies

A questionnaire about some coagulation parameter among leukemic  
children patients

Date: .....

Number: .....

Name: .....

Age: .....

Sex: .....

Inhabitation: .....

Type of leukemia: .....

Date of disease: .....

Type of treatment: .....

Family history: .....

Test results:

PT: .....sec

INR: .....

APTT: .....sec

Fibrinogen level: .....g/l

Platelet count: .....cell/cumm



## Appendix II

بسم الله الرحمن الرحيم  
جامعة السودان للعلوم والتكنولوجيا  
كلية الدراسات العليا-برنامج الماجستير-مختبرات طبيه  
تخصص عام أمراض الدم ومبحث المناعة الطبية

### براءة أخلاقية

الإسم:.....

سوف يتم أخذ عينة من الدم (2.5 مل) من الوريد بواسطة حقنة طعن وذلك بعد مسح مكان أخذ العينة بواسطة المطهر. كل الأدوات المستخدمه لأخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية.

و أنا أقر بأن هذه العينات سوف يتم تحليلها فقط لغرض البحث.  
أوافق أنا المذكور أعلاه بأخذ عينة لإجراء الدراسة.

الإسم:.....

الإمضاء:.....

## Appendix III

### Requirements:

1. EDTA containers.
2. Trisodium citrate container.
3. Cotton, Alcohol 70%.
4. Syringes, Tourniquet.
5. Working Rack.
6. stierr, disposables cuvettes.
7. Epindorf tube.
8. Automated pipette, tips.
9. Centrifuge, waterpath.
10. Haemocytometer.
11. Cover glass and glass tube.

## Master sheet

No	Type of leukemia	Sex	Age	PT/ sec	INR	APTT/ sec	Platelet count/ cell/cumm	Fibri
1	ALL	Male	10	13.0	0.9	31.6	140	133
2	ALL	Female	9	12.9	0.9	29.0	220	180
3	ALL	Male	7	15.0	1.1	43.0	180	137
4	ALL	Male	11	12.2	0.8	36.6	220	130
5	ALL	Male	9	13.1	0.9	30.5	172	181
6	ALL	Female	8	13.0	0.9	36.6	185	142
7	ALL	Female	5	16.1	1.1	31.6	140	168
8	ALL	Male	9	16.6	1.2	36.6	193	159
9	ALL	Male	8	14.0	0.9	30.3	188	150
10	ALL	Female	11	13.9	0.9	42.6	186	159
11	ALL	Male	6	15.0	1.1	29.8	140	133
12	ALL	Female	6	15.9	1.1	43.0	185	155
13	ALL	Female	7	14.8	1.1	40.0	174	162
14	ALL	Male	5	13.0	0.9	31.6	196	133
15	ALL	Male	5	17.0	1.2	40.0	220	180
16	ALL	Male	9	16.6	1.2	30.5	181	137
17	ALL	Male	4	15.3	1.1	43.0	179	130
18	ALL	Male	7	12.9	0.9	42.1	183	171
19	ALL	Female	12	17.1	1.2	29.8	140	142
20	ALL	Male	9	14.7	1.0	42.6	182	156
21	ALL	Female	8	15.5	1.1	31.6	180	159
22	ALL	Male	11	15.2	1.1	29.0	190	133
23	ALL	Female	12	16.0	1.2	42.1	220	165
24	ALL	Male	12	14.0	0.9	43.0	185	142
25	ALL	Female	8	14.1	1.0	30.3	140	174
26	AML	Male	4	13.7	0.9	43.0	126	308
27	AML	Female	10	15.7	1.1	36.6	140	340
28	AML	Male	10	13.7	0.9	30.5	133	290
29	AML	Male	11	17.1	1.2	36.6	122	298
30	AML	Male	5	15.7	1.1	31.6	120	333
31	AML	Male	4	14.2	1.1	36.6	128	288
32	AML	Male	10	13.7	0.9	30.3	127	279
33	AML	Female	4	14.0	1.0	42.6	121	321
34	AML	Female	4	13.7	0.9	29.8	135	298
35	AML	Male	11	13.5	0.9	43.0	133	333

36	AML	Female	5	15.7	1.1	40.0	140	288
37	AML	Female	4	13.9	0.9	31.6	122	287
38	AML	Male	10	13.7	0.9	40.0	144	320
39	AML	Male	11	17.0	1.2	30.5	133	307
40	AML	Male	8	17.1	1.2	43.0	145	310
41	CML	Male	8	15.1	1.2	42.1	660	212
42	CML	Male	5	15.5	1.1	31.6	555	211
43	CML	Female	7	14.9	1.1	40.0	720	233
44	CML	Male	6	16.0	1.1	30.5	615	205
45	CML	Male	4	14.2	1.0	43.0	679	212
46	CLL	Male	11	14.2	1.0	42.1	116	188
47	CLL	Male	5	15.0	1.1	36.6	119	202
48	CLL	Male	10	13.4	0.8	36.4	113	166
49	CLL	Female	12	14.0	1.0	34.8	120	186
50	CLL	Male	7	14.4	1.0	38.8	110	190
51	Healthy	Female	9	13.0	0.9	31.6	240	308
52	Healthy	Female	10	12.9	0.9	29.0	220	340
53	Healthy	Female	9	12.0	0.8	43.0	180	290
54	Healthy	Male	11	12.2	0.8	36.6	220	298
55	Healthy	Male	10	13.1	0.9	30.5	172	333
56	Healthy	Female	8	13.0	0.9	36.6	185	288
57	Healthy	Female	9	14.1	1.0	31.6	140	279
58	Healthy	Female	9	13.6	0.9	36.6	193	321
59	Healthy	Male	11	14.0	1.0	30.3	188	298
60	Healthy	Male	10	12.9	0.9	35.6	186	333
61	Healthy	Male	8	12.0	0.9	29.8	260	288
62	Healthy	Female	7	12.9	0.9	28.0	185	287
63	Healthy	Female	10	13.8	0.9	30.0	174	320
64	Healthy	Female	8	11.0	0.7	31.6	196	307
65	Healthy	Male	8	12.1	0.8	30.0	220	310
66	Healthy	Female	10	11.6	0.8	30.5	181	186
67	Healthy	Male	12	13.3	0.9	28.9	179	190
68	Healthy	Male	11	12.9	0.9	34.0	183	308
69	Healthy	Male	8	13.1	0.9	29.8	255	340
70	Healthy	Female	9	14.7	1.1	32.6	182	290
71	Healthy	Male	11	15.5	1.1	31.6	180	298
72	Healthy	Male	10	15.2	1.1	29.0	190	333
73	Healthy	Male	8	16.0	1.2	32.1	266	288
74	Healthy	Male	12	14.0	1.0	33.0	185	279
75	Healthy	Male	8	14.1	1.0	30.3	300	321
76	Healthy	Male	11	13.7	0.9	31.6	320	321
77	Healthy	Male	9	12.7	0.9	29.0	333	298

78	Healthy	Male	8	12.7	0.9	30.0	400	333
79	Healthy	Male	11	13.1	0.9	29.6	240	288
80	Healthy	Male	9	13.0	0.9	30.5	256	321