بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Assessment of Serum Electrolytes and Trace Elements in Leukaemia Patients in Sudan.

تقييم الإلكتروليتات والعناصر الندرة ببلازما الدم لمرضى اللوكيميا بالسودان

A Thesis Submitted in the Fulfilment for the Requirements for The Degree of Doctor of Philosophy in Chemistry

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بسم الله الرحمن الرحيم

﴿ وَالْأَرْضَ مَدَدْنَاهَا وَأَلْقَيْنَا فِيهَا رَوَاسِيَ وَأَنْبَتْنَا فِيهَا مِنْ كُلِّ شَيْءٍ مَوْزُونٍ (19) وَجَعَلْنَا لَكُمْ فِيهَا مَعَايِشَ وَمَنْ لَسْتُمْ لَهُ بِرَازِقِينَ (20) وَإِنْ مِنْ شَيْءٍ إِلَّا عِنْدَنَا خَزَائِنُهُ وَمَا نُنَزِّلُهُ إِلَّا بِقَدَرٍ مَعْلُومٍ (21) ﴾ {الْحِجْرِ21- 19}

صدق الله العظيم

DEDICATION

TO MY PARENTS SOUL

FOR MY HUSBAND HAFIZ, SONS, SISTERS AND BROTHER.

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ABSTRACT

The aim of this study is to measure the levels of serum electrolytes, trace elements and total iron binding capacity (TIBC) in Sudanese patients with leukaemia and to investigate the levels of these elements in leukaemia subtypes, in age, sex and related differences. Total of 201 persons were included; 79 (control group) and 122 (leukaemia patients). Serum electrolytes Na^{+},K^{+}, Ca^{2+}, Mg^{2+} and PO_4^{3-} levelsanalysed by spectrophotometer. Trace elements Zn^{2+}, $Cu^{2+,}$ Mn^{2+}, Co^{3+} and TIBC by atomic absorption spectroscopy. Results showed a significant increase in Mg^{2+} level in CML, AML and ALL than control group. Age groups 16-30 and 31-45 having significant interaction with this increased of Mg.Serum Ca^{+} and Zn^{2+} showed lower levels in CLL, CML and ALL. Age groups 31-45 and Over 45 showed significant interaction of the age limits with this decrease in Ca^{2+} concentration. Age groups; less than one year -15 , 31- 45, over 45 years having significant interaction with decreased of Zn. $Cu^{2+,}$ Mn^{2+} and Co^{3+} showed significant decrease in all subtypes of leukaemia. All age groups showed a significant interaction of the age factor on the decreased level of Co^{3+} and three age groups 16-30, 31-45 and Over 45 showed significant interaction of the age limit with this decrease in Cu^{2+} level, while Mn^{2+} level showed no significant difference between age groups. Serum TIBC level was lower in patients with ALL, CML and AML than control. Age groups less than one year -15, 16-30 and 31-45 showed significant interaction with decreased of TIBC, while older patients' ≥46 showed non significant interaction with decreased level of TIBC. Female patients showed significant lower level of Ca^{2+} and higher level of Cu^{2+} compared to male patients. The

difference in levels found between the groups may be associated with factors such as stage of disease, diet and drugs associated with treatment.

المستخلص

الهدف من هذه الدراسة قياس تركيز الإلكتروبتات والعناصر الندرة والقدرة الإجمالية للارتباط بالحديد (TIBC)ببلازما الدم لمرضى سودانيين مصابين بمرض اللوكيميا (مرض إبيضاض الدم)، و قياس مستوى هذه العناصر في الأنواع المختلفة للوكيميا وعلاقتها بالعمر والنوع. شملت الدراسة 201 شخص ؛ المجموعة الضابطة (79 شخص) ، و مرضى اللوكيميا (122 شخص) .تم قياس تركيز كل من : Na^{+},K^{+}, Ca^{2+}, Mg^{2+} and PO_4^{3-} باستخدام طريقة المطياف اللوني. و Zn^{2+}, $Cu^{2+,}$ Mn^{2+}, Co^{3+}, TIBCباستخدام تقنية الامتصاص الذري. أظهرت النتائج زيادة معنوية في تركيز Mg^{2+} للأنواع CML, AML , ALL مقارنة بالمجموعة الضابطة. المجموعات العمرية من عمر (30-16) ومن عمر(45-31) أظهرت تأثرها بالزيادة المعنوية للماغنسيوم. أوضحت الدراسة إنخفاضًامعنويًافي تركيز الكالسيوم والخارصين بالمجموعات CLL, CML, ALL . المجموعات العمرية (اكبر من عمر 45) اظهرت تأثرها بانخفاض Ca^{2+}.وثلاث مجموعات عمرية من (اقل من عمرعام –15) و من عمر (31 -45) ، و(اكبر من عمر 45)،اظهرت تأثرها بانخفاض$^{+}$ Zn^{2}. إنخفض تركيز كل من النحاس ،الكوبالت والمنجنيز ، إنخفاضًا معنويًا في جميع أنواع اللوكيميا الأربعة ، وتأثرت جميع المجموعات العمرية بانخفاض الكوبالت ، وتأثرت ثلاثة مجموعات عمرية بانخفاض النحاس و هي (over 45),(45 – 31), (30 – 16). لم تتأثر المجموعات العمرية بانخفاض المنجنيز.

أوضحت الدراسة إنخفاضًا معنويًا في تركيز TIBC بالمجموعات ALL و CML و AML كما تأثرت كل المجموعات العمرية بتركيز TIBC ، سجلت المجموعات إنخفاضًا معنويًا من عمر (31-45) ، ومن عمر (16-30) و (اقل من عمر عام – 15)،بينما لم تسجل المجموعة (الأكبر عمرا من 46) فرق معنوي في TIBC .

وجد ايضا إنخفاضًا معنوياً في تركيز الكالسيوم بالمرضى الإناث مقارنة بالذكور، وزيادة معنوية في تركيز النحاس ، ايضا بمجموعة الإناث المرضى مقارنة بالذكور.

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CHAPTER ONE

INTRODUCTION

CHAPTER I

INTRODUCTION

1.1 Background:

Cancer is becoming a global health problem and the number of cancer cases in Africa is rising. Being an African country, Sudan has its share of cancer burden (Intisar *et al.,* 2014). Sudan, the largest and most diverse country in Africa, is experiencing a growing cancer problem but it is little known on tumors patterns, cancer epidemiology and ethnic or environmental risk factors (Awadelkarim *et al.,* 2012). There were 322 children with cancer in Sudan from hospital registry for the period 1999 to 2007, 83.26% are diagnosed as leukemic (Abuidris *et al.,* 2008). Cancers form one of the major causes of death in children between the ages of one and 15 years. They differ markedly from adult cancers in their nature, distribution and prognosis. The patterns of childhood cancers in America and Europe are almost the same, with leukaemia and central nervous system tumour accounting for over one-half of the new cases (Mahfouz *et al*.,2006). In study conducted in Gezira state, Central Sudan to determine the patterns of childhood cancers, in the periods from May 1999 - December 2004. The authors found that lymphoma is the most common cancer in children (42.8%) followed by acute lymphoblastic leukaemia (19.8%) and kidney tumour (12.8%) (Mahfouz *et al*.,2006).

Leukaemia is a common type of cancer after lung, breast and prostate, accounting for about 30% of all cancers. IT is estimated that 47, 150 patients (26,830 men and 20,320 women) will be diagnosed with leukaemia in 2012. These patients frequently exhibit acid base and electrolyte disturbances that complicate their stay in hospital (Farah *et al.,*

2012). Leukaemia is considered as the most common type of cancer in children. Its annual incidence has increased over the last decades. Today, due to research and new treatments, nine out of ten children will survive. (Whitehead *et al.*, 2016). Also is the most common cancers in adults (Atieh *et al*.,2012). Leukaemia can arise in lymphoid cells or myeloid cells, Leukaemia that affects lymphoid cells is called lymphocytic leukaemia. Leukaemia that affects myeloid cells is called myeloid leukaemia or myelogenous leukaemia. The types of leukaemia are grouped by how quickly the disease develops and gets worse. Leukaemia is either chronic (gets worse slowly) or acute (gets worse quickly). There are four common types of leukaemia: Chronic Myelogenous Leukaemia (CML), Acute Myelogenous leukaemia (AML), Chronic Lymphocytic Leukaemia (CLL) and Acute Lymphocytic Leukaemia (ALL) (Catarine *et al.*, 2009).

Patients with cancer frequently exhibit acid-base and electrolyte disturbances that complicate their management and prolong their hospitalization. (Miltiadous *et al.*,2008). The most common electrolyte abnormality in clinical practice is Hyponatremia, yet little is known about its frequency in patients with cancer or its impact on their clinical outcomes (Doshi ,2012). Electrolyte and acid-base disturbances can cause kidney disease, it occur frequently and often with cancer are associated with an ominous prognosis, and acute kidney injury. Tumorlysis syndrome is a potentially life-threatening condition that frequently occurs in patients with a high tumour burden and high cellular turnover after cytotoxic therapy (including steroids in steroid-sensitive hematologic malignancies). Electrolyte and acid-base disturbances are the consequence of neoplastic spread, anticancer treatment, or, more rarely, paraneoplastic phenomena of all types of tumours (Lameire *et al.*, 2010).

1.2 Hematopoietic system:

Blood is not immediately thought of as an organ, but it is one of the largest in the body. The volume of blood in adults is approximately 4.5–5 litres and marrow cells form about 40% of this volume. Haemopoiesis is the name given to the production of marrow cells and this is further subdivided into erythropoiesis (production of red blood cells), leucopoiesis (production of white blood cells) and thrombopoiesis (production of platelets). (Marvelle and Tracey ,2012).

This system is uniquely susceptible to toxic insults, and ranks alongside the liver and kidney as one of the most important target organs of toxicity. Circulating blood cells perform many vital functions, and even mild injury can impair tissue oxygenation, immune function, or haemostatic function. The rapid rate of proliferation required to support systemic demands for blood cells also makes hematopoietic tissue particularly sensitive to toxic injury by, for example, radiation, or cytoreductive or antimitotic agents. Even in health, the on-going senescence of mature blood cells requires that new cells be generated at a very rapid rate: ~1.5×10^6 cells per second in humans. Haematopoiesis is a highly regulated, complex, and dynamic process that is susceptible to disruption by xenobiotic. (Lila *et al.*, 2013).

1.3 Classification of Leukaemia:

Leukaemia can be either acute or chronic. There are several different types, classed according to the way the cancer develops and the kind of white blood cells they affect. Acute leukaemia progresses quickly, whereas in chronic leukaemia, the symptoms take longer to develop and progress much slower (Fatemeh *et al.*, 2015). They are further divided

into lymphoid, myeloid and biphenotypic leukaemias, the latter showing both lymphoid and myeloid differentiation. The four main types of leukaemia are: Acute lymphoblastic leukaemia (ALL), Acute myeloid leukaemia (AML), Chronic myeloid leukaemia (CML),Chronic lymphocytic leukaemia (CLL)((Barbara, 2003).

The risk of lymphoid leukaemia significantly increased for working in chemical laboratories, while the risk of myeloid leukaemia increased for working in the shoe or other leather goods industry. Exposure to benzene seemed to be associated with both acute and chronic myeloid leukaemia (Saberi *et al.*, 2013).

1.3.1 Acute leukaemias:

The acute leukaemia are the result of accumulation of early myeloid or lymphoid precursors in the bone marrow, blood and other tissues, and are through to arise by somatic mutation(s) of a single cell within a minor population of stem or early progenitor cells in the bone marrow or thymus (Pettit, 2001). Also defined as a malignant disorder of white blood cells caused by a Failure of normal differentiation of haemopioietic stem cells and progenitors into mature cells (Alea *et al.*, 2013). Acute leukaemia may arise de novo or be the terminal event in number of pre-existing blood disorders for instance polycythaemia , chronic myeloid leukaemia or one of the myelodysplastic syndromes at presentation at least 30% and usually more than 80% of marrow cells are blasts. The disease are divided into two main subgroups a cute myeloid leukaemia (AML) and acute lymphoblastic leukaemia a(ALL) (Pettit, 2001). Most cases of acute leukaemia (AL) can be classified as acute myeloid leukaemia (AML) or acute lymphoid leukemia (ALL) using the French-American-British (FAB) and World Health Organization (WHO) classification (

Alea *et al.,* 2013). In study conducted by Tahir *et al.,* (2015) to study the relation between trace elements and leukaemia; they found a significant association between trace elements (Fe, Zn, Cu and Ni) and acute leukemia. Also, the imbalance of serum trace elements contributes to pro and anti-oxidative stress via regulation of ROS and SOD in acute leukemia compared to the normal healthy subjects.

1.3.1.1 Acute Myeloid Leukaemia:

Acute myeloid leukaemia (AML) is a heterogeneous group of neoplastic disorders characterized by the proliferation and accumulation of immature myeloid cells in the bone marrow and blood (Miller and Pilichowska, 2014). Another definition for (AML) is a disorder of hematopoietic malignancy due to the accumulation of the blast and uncontrolled proliferation factors which really affects differentiation (Mahmood *et.al* ,2015). The incidence of AML increases steadily with age . More than 50% of patients with a diagnosis of AML are older than 60 years and the median age at the onset is over 64 years (Aurelio *et. al.*,2001). AML is infrequent, yet highly malignant neoplasms responsible for a large number of cancer-related deaths. The incidence has been near stable over the last years. It continuously shows 2 peaks in occurrence in early childhood and later adulthood. With an incidence of 3.7 per 100,000 persons and an age-dependent mortality of 2.7 to nearly 18 per 100,000 persons, there is a rising awareness in the Western world of AML's special attributes resulting from an ever-aging population (Deschler and Lübbert, 2006).

AML in the setting of community hospitals is one of the most challenging diseases to manage, involving prolonged hospitalizations, high readmission rates and severe infectious complications (Ali *et al.*,2014).

1.3.1.2 Acute lymphoid leukaemia:

Acute lymphoid leukaemia (ALL) is the most common cancer diagnosed in children (Mahmood *et.al,* 2015). The precise pathogenetic events leading to development of acute lymphoid leukaemia are unknown. Only a few cases (<5%) are associated with inherited, predisposing genetic syndromes, such as Down's syndrome, Bloom's syndrome, ataxia-telangiectasia, and Nijmegen breakage syndrome, or with ionizing radiation or exposure to specific chemotherapeutic drugs. Although accumulating published work on high birth weight as a risk factor for childhood acute lymphoblastic leukaemia is becoming increasingly convincing, there exists an extensive list of conflicting or isolated reports of factors purported to confer an increased risk for this disease, including parental occupation, maternal reproductive history, parental tobacco or alcohol use, maternal diet, prenatal vitamin use, exposure to pesticides or solvent, and exposure to the highest levels (>0.3 or 0.4 μT) of residential, power-frequency magnetic fields (Paui *et al.,* 2008).

ALL is an incurable disease due to the resistance toward treatment. There are many factors which are involved to cause resistance from chemotherapy which include oxidative stress due to the generation of reactive oxygen species (ROS) and presence of hypodiploid cells. Most common antioxidants include vitamins A, C, and E, glutathione (GSH), and the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx). Chemotherapy resistance is a basic hurdle in the treatment of ALL patients (Miguel *et al.*, 2012).

1.3.2 Chronic Leukaemia:

1.3.2.1 Chronic Lymphoid leukaemia:

Chronic lymphocytic leukaemia is primarily a disease of older adults, with more than 90% of the cases occurring in persons older than 50 years; however, CLL has also been described in young adults. CLL is found in twice as many males as females and is slightly more common in whites than in blacks. Unlike the signs and symptoms of acute leukaemia, those of CLL develop gradually, and the onset of the disease is difficult to pinpoint. In fact, it is not unusual for the disease to be accidentally discovered because of an elevated lymphocyte count during the course of a routine visit to a physician (Kedar and Carlos, 2007).

Chronic lymphocytic leukaemia is the commonest form of leukaemia in Europe and North America, and mainly, though not exclusively, affects older individuals. It has a very variable course, with survival ranging from months to decades. Major progress has been made in identification of molecular and cellular markers that could predict disease progression in patients with chronic lymphocytic leukaemia. Available treatments generally induce remission, although nearly all patients relapse, and chronic lymphocytic leukaemia remains an incurable disease (Dighiero,2008).

Chronic Lymphoid leukaemia is characterized by the clonal expansion of lymphocytes that accumulate in the peripheral blood , lymph nodes, bone marrow, liver and spleen. Although significant advances have been made in the treatment of CLL in the last decade, it remain incurable (John and John , 2013).

1.3.2.2 Chronic Myeloid Leukaemia:

In contrast to the acute monocytic leukaemia, chronic monocytic leukaemia is seldom seen (Ryder,1966)**.** The incidence of chronic myeloid leukaemia (CML) ranges between 10 and 15 cases/106/year (age adjusted) without any major geographic or ethnic differences. The median age at diagnosis ranges between 60 and 65 years in Europe, but is considerably lower in countries where the population is younger. The prevalence of CML is steadily rising due to the very substantial prolongation of survival that has been achieved with targeted therapy (Baccarani *et al.*,2012).Chronic myeloid leukemia (CML) is characterized by the BCR-ABL fusion gene originating in a hematopoietic stem cell. The BCR-ABL oncoprotein ($p210^{BCR-ABL}$) encoded by this gene displays constitutively elevated tyrosine kinase (TK) activity that drives the pathogenesis of the disease by perturbing multiple signaling pathways (Min *et al.*,2013). The incidence of chronic myeloid leukemia (CML) increases with age, but it is unclear how the characteristics of the disease vary with age. In children, where CML is very rare, it presents with more aggressive features, including huge splenomegaly, higher cell count and higher blast cell percentage (Seki *et al.*, 2013). In absence of treatment of CML, the outcome is fatal and the course varies from few months to many years. Also with very effective treatments, as tyrosine kinase inhibitors (TKIs), about 20% of patients are still at risk of dying of leukemia . The prognosis of CML is still based on three scoring systems including clinical and hematologic variables, such as spleen size, platelet count, and the percentage of blast cells, eosinophil and basophils in the peripheral blood. The Sokal and EURO scoring systems, based on the overall survival (OS) of CML patients treated with conventional chemotherapy and with recombinant interferon-α (rIFN-α), respectively,

also include the age: some patients are classified in the high Sokal and EURO risk category mainly because of old age, and some patients are not classified in the high Sokal and EURO risk category mainly because of young age (Castagnetti *et al.*, 2105).

1.4 Aetiology of Leukaemia:

A variety of factors probably are etiologically involved in leukaemia, not only in different cases but also within individual cases. Certain chemicals may be important etiologic factors in a few cases. Many different studies have established that ionizing radiation in large doses may be leukemogenic. Whether small doses of irradiation are dangerous has not been demonstrated (Shafer,1966).Various chromosomal abnormalities, both in structure and in number, have been found in acute leukaemia. The changes have been unique in each case, and it has not been possible to correlate the changes with any clinical picture. In individual cases, however, the abnormalities have been consistent irrespective of the stage of the disease or the response to therapy (Shafer ,1966).

Investigations on association between environmental hazards and the development of various types of leukaemia are reviewed. There are many risk factors like; exposure to ionizing radiation, strong magnetic field and occupational handling of benzene (Brandt, 1985).

No consistent evidence is available to link chronic lymphocytic leukaemia with environmental exposure to either radiation or chemicals, except in the case of agricultural workers and herbicides. On Jan 23, 2003, the US National Academy of Sciences' Institute of Medicine published a report concluding that there is “sufficient evidence of an association between exposure to Agent Orange, a herbicide used in Viet Nam, and the development of chronic lymphocytic leukaemia”. Although

ionizing radiation has traditionally been absolved from causing chronic lymphocytic leukaemia, recent studies have suggested that this may be unwarranted (Dighiero,2008).

On association between maternal coffee consumption and childhood leukemia risk and provide indications for a similar role of maternal cola intake. In contrast, an inverse association with tea was found, implying that other micronutrients contained in this beverage could potentially counterbalance the deleterious effects of caffeine. Further research should focus on the intake of specific micronutrients, different types of coffee and tea, specific immunophenotypes of the disease, and the modifying effect of genetic polymorphisms (Thomas *et al.*, 2015).

1.5 The Importance of the Mineral Elements:

Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Although they yield no energy, they have important roles to play in many activities in the body . Every form of living matter requires these inorganic elements or minerals for their normal life processes (Soetan *et al.*, 2010).

Minerals may be broadly classified as macro (major) or micro (trace) elements. The macro-minerals include Ca, K, Mg, Na, Cl and S, while the micro-elements include Fe, Cu, Zn Cr, Fe, Co, Cu, Zn, Mo, Mn, F, and I. They occur in physiological concentrations, play key roles in living processes, and either an excess or deficit can disturb biochemical functions in both humans and animals. The trace elements mentioned above are widely found in nature, particularly in various mineral deposits, plants, and soils, meaning that they are available to be taken up by plants and animals that serve as food sources for humans. Each of these mineral

nutrients has their own role for a proper body function and health status (Amente, 2016). Deficiencies of these minerals are often present in hospitalized patients. Deficiencies occur due to inadequate or inappropriate administration, increased or altered requirements, and increased losses, affecting various biochemical processes and resulting in organ dysfunction, poor wound healing, and altered immune status with deleterious squealae (Sriram and Lonchyna, 2009).

1.6 Cancer and Electrolytes:

Patients with malignancies commonly experience abnormalities in serum electrolytes, including hyponatraemia, hypokalaemia, hyperkalaemia, hypophosphatemia, and hypocalcaemia. In many cases, the causes of these electrolyte disturbances are due to common aetiologies not unique to the underlying cancer. However, at other times, these electrolyte disorders signal the presence of paraneoplastic processes and portend a poor prognosis. Furthermore, the development of these electrolyte abnormalities may be associated with symptoms that can negatively affect quality of life and may prevent certain chemotherapeutic regimens. Thus, prompt recognition of these disorders and corrective therapy is critical in the care of the patient with cancer (Mitchell and Alan, 2014).
Electrolyte disturbances in leukaemia can be the result of the disease process or drug therapy. One group of electrolyte abnormalities is related to the stage of the leukemic process. Included in this group are newly diagnosed patients who may show elevated serum potassium, phosphorus, and magnesium, a result of their release from malignant cells after cytotoxic therapy or their accumulation due to urate nephropathy. Patients in remission usually have normal serum electrolyte concentrations, but acute leukaemia patients during relapse may have hypokalaemia,

hypophosphatemia, and hypomagnesaemia . Nonspecific factors related to the disease process which may aggravate the electrolyte imbalance include gastrointestinal loss through nausea, vomiting, and malnutrition (O'Regan *et al.,* 1977). Cancer patients developing a variety of fluid and electrolyte disturbances caused by the disease process or by complications from therapy. An understanding of the pathophysiology of these potential abnormalities allows the clinician to manage patients expectantly and to avoid severe metabolic disarray by correcting imbalances promptly (Kapoor and Chan, 2001). The mechanisms of acid-base and electrolyte disturbances encountered for these abnormalities are multifactorial in origin. Both the underlying disease and the therapeutic interventions can contribute to the development of these disturbances. An understanding of the mechanisms involved in their pathogenesis is of paramount importance for their prevention and treatment in cancer patients (Miltiadous *et al.*,2008).

Recognition and appropriate management of fluid and electrolyte disorders in critical patients is extremely important. In many cases, these secondary problems are more complicated and more serious than the initiating disease process. Proper fluid therapy and treatment of electrolyte abnormalities make a major difference in the survival rate of critically ill animals (Garvey,1989).

A prospective study was carried out to evaluate the pattern of mineral changes in children treated for acute lymphoblastic leukaemia (ALL) before and after induction chemotherapy. Serum calcium, phosphate, alkaline phosphatase, albumin and creatinine and urinary creatinine and calcium were estimated in cases and control. The mean calcium level at presentation and post induction was 9.50±1.48 mg/dl and 9.08±1.30

mg/dl. Serum phosphate was higher in preinduction than post induction value and it was statistically significant. Serum alkaline phosphatase was higher in the post induction period. It was not statistically significant. No statistically significant difference was found in pre and post induction urinary calcium and creatinine (Jamal *et al.,* 2011).

Hyponatremia in patients with cancer is associated with longer hospital stay and higher mortality . Serum sodium levels categorized as eunatremia (serum sodium, 135-147 mEq/L) and mild (134-130 mEq/L), moderate (129-120 mEq/L), and severe (<120 mEq/L) hyponatremia. . Whether long-term correction of hyponatremia would improve these outcomes remains to be determined (Doshi ,2012). Others abnormalities in serum electrolytes, including hypokalaemia, hyperkalaemia, hypophosphatemia, and hypocalcaemia. In many cases, the causes of these electrolyte disturbances are due to common aetiologies not unique to the underlying cancer. However, at other times, these electrolyte disorders signal the presence of paraneoplastic processes and portend a poor prognosis. Furthermore, the development of these electrolyte abnormalities may be associated with symptoms that can negatively affect quality of life and may prevent certain chemotherapeutic regimens. Thus, prompt recognition of these disorders and corrective therapy is critical in the care of the patient with cancer (Rosner and Dalkin, 2014). Hypokalaemia, hyperkalaemia, hyponatremia, hypernatremia, hypocalcaemia, and hypercalcaemia are commonly seen in emergency medicine. Severe abnormalities in any of these electrolytes can cause potentially life-threatening consequences to the patient. It is essential that the clinician understand and correct (if possible) the underlying cause of each disorder and recognize the importance of the rates of correction, especially with serum sodium disorders (Schaer , 2008).

1.7 Electrolytes:

Minerals are inorganic and needed for the wide range of function.

A nutritional element essential for the successful growth and reproduction of an organism is called essential elements. The major essential elements for the life are carbon , hydrogen, oxygen, nitrogen, sulfur, phosphorus, potassium, sodium, magnesium, calcium and chlorine. In addition ,certain elements, the trace elements are essential in trace amounts (a few parts per millions) of these all organism require manganese, iron, cobalt, copper and zinc. Some also require combination of molybdenum , vanadium, chromium and other heavy metals as well as boron, silicon, fluorine and iodine (Taylor,1994).

Trace elements have some fundamental roles within human body like protection against cellular oxidative stress and synthesis and structural stabilization of proteins and nucleic acids .According to the etiology of neoplastic disease and alteration in antioxidants levels, many researches over recent years have studied if trace elements have any modifying effect in clinical outcome (Atieh *et al*.,2012).

Most children with ALL have alterations in bone metabolism and bone mass when first examined. These data suggest defective mineralization as the mechanism for decreased bone mass and implicate the leukemic process as causative (Halton *et al*., 1995).

The measurement of levels of minerals and electrolytes is of considerable interest in the management of cancer (Brodzki *et al*., 2004). Another study measured the levels of Iron (Fe) and copper (Cu) and electrolytes such as sodium (Na) and potassium (K) levels in patients diagnosed with primary brain tumour , it there was significant decrease in serum iron and sodium, serum copper and potassium were increased. Measurement of serum level of sodium, potassium, iron and copper level significance in

the assessment of primary brain tumour (Amareshwara, 2011). Reduction in bone mineral content (BMC), hypomagnesemic and hypermagnesuric are noticed in study which undertaken the effect of chemotherapy in bone mass and biochemical mineral status in children receiving therapy (Halton *et al.*, 1996).

1.7.1 Sodium:

A major extracellular electrolyte is sodium. The normal sodium level in human serum is 135–145 mmol/L. Potassium is the major intracellular electrolyte . The normal potassium level in serum is usually considered to be 3.5–5.1 mmol/L. The balance between intracellular and extracellular electrolytes is maintained by a sodium–potassium ATPase pump present in cell membranes.Four major electrolytes of the human body :sodium, potassium, chloride, and bicarbonate play important roles in human physiology, including: Maintaining water homeostasis of the body, maintaining proper pH of the body 7.35 to 7.45 ,maintaining optimal function of the heart, participating in various physiological reactions and Co-factors for some enzymes (Amitava and Amer, 2014).

The concentration of serum sodium, the primary extracellular cation, is tightly regulated, and a level lower than the lower limit of the laboratory range, usually 135 mEq/L, constitutes hyponatremia. Often, the presence of hyponatremia indicates excess of total body water relative to sodium. The converse is also true in that water depletion leads to hypernatremia The pituitary-secreted antidiuretic hormone (ADH) (vasopressin) plays a key role in maintaining water balance primarily by regulating water excretion through the kidneys. When water content is low, the concentration of serum sodium increases vis-à-vis serum osmolality

triggering the secretion of ADH. ADH in turn binds to the V2-receptors causing water reabsorption from the tubular lumen (Ala *et al.*,2104).

Hyponatremia is defined as a serum sodium concentration less than 136 mEq/L (<136 mmol/L). Hyponatremia may occur in the presence of low, normal, or high total body sodium. Therefore, natremic status cannot be assessed without regard to the fluid and water status of the patient. The most common causes of hyponatremia can be broken down into two types: sodium depletion in excess of total body water loss (e.g., severe dehydration with true depletion of total body sodium) and dilutional hyponatremia i.e., water intake greater than water output (Whelton ,2015).

1.7.2 Potassium:

The human body contains about 3500 mmol of potassium only about 2% of this is in the extracellular fluid and the rest is intracellular. This 98 % is held in cells by a set of complex mechanisms and is pumped in and out of them by Na/ K-ATPase pump . Potassium has many biological function . It is cofactor for many enzymes and it is required for insulin secretion, creatine phosphorylation , carbohydrates metabolism and proteins synthesis. The ratio of intracellular to extracellular potassium is the major determinant of muscular and neuronal excitability and if this balance is disturbed , various pathological states can develop (Ringer and Yvette, 2007).

Disturbance in serum potassium is common in cancer patients through a variety of mechanisms. One of the most common clinical settings is tumour lysis syndrome (TLS), which is caused by the disintegration of malignant cells, usually following the initiation of chemotherapy or rapidly growing tumour, especially hematologic malignancies such as

acute leukaemia with high white cell counts at presentation or diffuse Burkitt-type non-Hodgkin lymphoma (Kevin, and Scott 2013).

Hypokalemia is the most pronounced electrolyte abnormality in patients with Acute Leukaemia . Its frequency ranges from 43% to 64% in this population . Although frequently noted, hypokalemia remains a poorly illuminated metabolic disturbance, primarily described in patients with acute monocytic and acute myelomonocytic leukemia . leukemic patients had a significantly lower potassium concentration per kilogram of body weight, per kilogram of lean body mass and total body water when compared with healthy volunteers. This was evident during disease relapse as well as during remission. patients with acute leukemia excrete large quantities of lysozyme in the urine, thus suggesting a possible causal relationship between lysozymuria and potassium depletion in leukemic patients (Filippatos *et al.,* 2005).

1.7.3 Magnesium:

Magnesium is the fourth most abundant cation in the body. Intracellular magnesium, like phosphate, is necessary for a wide range of cellular functions. It is an essential cofactor in enzymatic reactions, including most of the same glycolytic, kinase, and phosphatase pathways that also involve phosphate. (Shlomo *et al.*,2011). Magnesium is the wonder element that is a cofactor for many metabolic enzymatic reactions in the body, either as an integral part of a metalloenzyme or as an activator. It has essential roles in the regulation of cell growth, division and differentiation . This second major intracellular cation (after potassium) is also effective on several steps in immune reactions (Merza *et al.,* 2009).

Magnesium plays a pivotal role in muscle contraction, protein, fat , and carbohydrate metabolism, methyl group transfer, oxidative

phosphorylation, functional properties and stabilization of membranes, cell division, and immune responses. Magnesium regulates ribosomal RNA and DNA structure, thereby affecting cell growth and membrane structure (Thomas , 1997).

Derangement in magnesium metabolism or body content is rare in the general patient population but fairly common in patients with cancer, often related to renal toxicity of drugs used for chemotherapy, hematopoietic stem cell transplantation (HSCT) or both. The average daily magnesium intake is 360 mg (15 mmol). Forty to fifty percent of dietary magnesium is reabsorbed in the gastrointestinal tract mediated by a channel encoded by the TRPM6 gene. Total body magnesium in the body is 25 g with 60- 65% in the bone and the rest in the intracellular muscle and soft tissue. One percent of total body magnesium is present in the extracellular fluid compartment. Serum magnesium in a healthy individual is closely maintained at 1.50- 2.30 meq/L (0.7 to 1.0 mmol/L). Only 20% is protein bound, therefore variations in serum albumin have less of an effect (Ala *et al.*,2104).

1.7.4 Calcium:

Calcium plays an important role in a great variety of biological compounds, processes, and systems, occurring in mammals, plants, and a number of living species in the oceans. It regulates many key biochemical and physiological processes, such as enzymic reactions, muscle action, blood coagulation, and secretion. Its compounds are central to conferring rigidity and to maintaining membranes. In vertebrates, the majority of the calcium is in the skeleton. Adult humans have 1 to 1.5 kg in their skeleton and only a few grams elsewhere in the body. However, the relatively

small amounts in cells and body fluids are of enormous importance in a multitude of ways (John and Emma, 2009).

Calcium (Ca^{2+}) functions as a critical intracellular second messenger that regulates numerous cellular functions, including processes as diverse as hormonal secretion, muscle contraction, neuronal excitability, glycogen metabolism, and cell division . Many of these functions are the result of the interaction of $Ca^{2+}$with its intracellular binding proteins, e.g., calmodulin, and the consequent activation of enzymes and other intracellular effectors systems . The free cytosolic calcium concentration (Ca_i^{2+}) in cells under resting conditions is on the order of 100 nM. The level of (Ca_i^{2+}) as controlled by diverse channels, pumps, and other transport systems that regulate the movements of Ca^{2+} into and out of the cytosol and between various intracellular compartments (Edward, 2001). Calcium intake, unless extremely low or extremely high, does not influence the levels of total serum calcium in blood. Serum calcium levels influence vital physiologic processes such as heart rate and nerve conduction and therefore are under tight physiologic control. The skeleton is the reservoir for calcium in blood. When levels of ionized calcium in serum drop below their set point, the calcium-sensing receptor on the parathyroid glands signals parathyroid cells to manufacture and release parathyroid hormone (PTH) into the circulation. PTH acts to conserve calcium by driving the conversion of 25-hydroxyvitamin D (25-OHD) to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) in the kidney; reducing calcium excretion in the urine, and by liberating calcium from the skeleton into the circulation. The resulting increase in ionized calcium in blood restores calcium balance and inhibits further release of PTH (Mridul and Gary, 2013). Hypercalcemia is a frequent and well recognized complication of many malignancies, but only a few cases have been reported in association with leukemia (Georgew, 1966).

1.7.5 Phosphorus:

Phosphate in the mammalian body is present predominantly (90%) as hydroxyapatite [$Ca_{l0}(PO_4)_6(OH)_2$] in the mineralized matrix of bone, with most of the remaining 10% occurring intracellularly in soft tissues. Phosphate is the major intracellular anion existing in organic (e.g., phospholipids, nucleic acids, phosphoproteins, ATP) or inorganic forms and plays an integral role in many metabolic processes (e.g., energy metabolism, delivery of O_2 to tissues, muscle contraction, and skeletal integrity) (Thomas, 1997).

The deficient levels of serum calcium, phosphorus and magnesium might contribute to the development of cancer (Abdelgawad *et al.,* 2015). Recent studies in both rodents and humans suggest that elevated serum phosphorus, in the context of normal renal function, potentiates, or exacerbates pathologies associates with cardiovascular disease, bone metabolism, and cancer (Lin *et al.*, 2015).

High serum concentration of phosphate can cause adverse renal effects, cardiovascular effects including vascular or volvuli calcification, and stimulate bone reabsorption. In addition, to it is affects organs in developing mice, and is related to tumorigenesis . Maintenance of normal Pi concentration (2.7-4.5 mg/dL) are important for health and prevention of diseases caused by inadequate Pi intake (Hong *et al.*, 2015).

Acute severe hypophosphatemia can be life threatening and is associated with mortality and impaired cardiac and respiratory function. Several conditions including decreased absorption or increased urinary phosphate excretion, shifts from the extracellular to intracellular compartments, and phosphate consumption by rapidly proliferating cells are known to induce

moderate to severe acute hypophosphatemia. Although hypophosphatemia and/or phosphate depletion in patients with acute or chronic myeloid leukaemia have been reported in the literature, hypophosphatemia due to acute lymphoblastic leukaemia (ALL) is very rare. The hypophosphatemia may induced by a shift of phosphorus into leukemic cells that rapidly replicated in the tissues and excessive cellular phosphate consumption by rapidly proliferating cells. Serum phosphate levels should always be monitored, especially in suspected life-threatening manifestation in relapsed ALL (Yasemin *et al.,* 2014).

1.8 Trace elements:

A useful definition describes the trace elements as those that constitute less than 0.01% of the dry weight of the body. Elements may be essential or non-essential but all can be toxic at certain levels of exposure (Taylor,1994). Iron, fluorine, and zinc, the most abundant trace elements, comprise 0.006%, 0.0037%, and 0.0033% of the body, respectively. By comparison, the least abundant bulk elements, magnesium and silicon, make up 0.027% and 0.026%, respectively, of the body. There are two general classes of abnormalities associated with trace elements: specific deficiency from dietary inadequacies, imbalances, or secondary to other diseases-and accumulation of innately toxic trace elements from the environment, which can either displace essential elements from their metabolically active sites and cause conditioned deficiency, or act directly as cellular toxins. Both kinds of abnormalities can be diagnosed by analyses of trace elements in plasma or serum, red blood cells, and urine. Furthermore, secondary changes occur as a result of systemic disease; they are not understood (Henry *et al.*,1971).

Trace elements at optimum levels are required for numerous metabolic and physiological processes in the human body. Zinc (Zn), Copper (Cu) and Manganese (Mn) are important cofactors for several enzymes that play a role in maintaining DNA integrity. In addition, they are involved in membrane transport, nerve conduction and muscle contraction and also in the function of sub-cellular systems such as mitochondria. Cu, Zn, Mn and selenium also act as antioxidants. Therefore, imbalances in the optimum levels of these trace elements may adversely affect biological processes and are associated with many fatal diseases, such as cancer. (Cengiz *et al.*, 2011).

1.8.1 Zinc:

Zinc is known as an essential micronutrient for human health because of its structural and biochemical functions, influencing growth and affecting multiple aspects of the immune system. Zinc has been extensively studied in neoplastic processes but its role in children with leukaemia still remains to be elucidated in several aspects (Consolo *et al.*, 2013).

Zinc is an essential component of more than 300 metalloenzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. At the cellular level, the function of zinc can be categorised into Catalytic, Structural and Regulatory. Various enzymes depend on zinc for their ability to catalyse vital chemical reactions within body. Zinc dependent enzymes can be found in all known classes of enzymes. Zinc plays an important role in the structure of proteins and cell membrane. The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their functions. Zinc

finger proteins have been found to regulate gene expression by acting as transcription factors. Zinc also plays a role in cell signalling and has been found to influence hormone release and transmission of nerves impulse (Bimola *et al.,* 2014).

Zinc is an essential element whose significance to health is increasingly appreciated and whose deficiency may play an important role in the appearance of diseases. zinc deficiency can be induced by continued use of low mineral purified foods (minerals are lost during purification), foods containing additives with chelating activity. Gastrointestinal surgery, Crohn's disease, ulcerative colitis, short bowel syndrome, and other digestive diseases can all decrease zinc absorption and increase zinc loss from the body (Jayant *et al.,* 2013) .

1.8.2 Copper:

Copper is an essential nutritional element. It is required for normal cell metabolism and is present in all human cells and tissues . The highest concentrations of copper are found in the liver, brain and hair (Gordon, *et al.,* 1997) . It's also consider as an essential trace element that participates in metabolic pathways involving cellular respiration, peptide biogenesis, connective tissue biosynthesis and antioxidant defence. It is an acute phase reactant increasing in response to infection, injury and in chronic inflammatory conditions. Copper exists in the human body in both oxidation states, oxidized Cu (II) and reduced Cu (I)((Georgia *et al.*,2012). It serves as a co-factor of the antioxidant enzyme, superoxide dismutase which detoxifies the toxic superoxide radical. It is proven that measuring serum Cu level could be useful in assessing disease activity and response to treatment in some kinds of lymphoma and acute leukaemia (Atieh *et al*., 2012).

Copper is an essential trace element in mammalian nutrition as a component of metalloenzymes in which it acts as an electron donor or acceptor. Conversely, exposure to high levels of copper can result in a number of adverse health effects. Acute copper toxicity is generally associated with accidental ingestion; however, some members of the population may be more susceptible to the adverse effects of high copper intake due to genetic predisposition or disease . Copper status has also been associated indirectly with a number of neurological disorders, including Alzheimer's disease and prion diseases. Depending on the source of the biological material, copper content ranges from parts per billion (ppb) to parts per million (ppm). Copper-deficient diet produce insufficient red blood cells (Bonnie *et al*., 2007).

1.8.3 Manganese:

Manganese (Mn) is necessary for optimal biological function that is required as a cofactor for many enzymes (Nancy and Michael, 2016).

Manganese is an essential trace element. It is a component of various metalloproteins and a normal constituent of almost all tissues. Manganese is a cofactor essential for the activity of a variety of enzymes, e.g. pyruvate carboxylase, arginase, phosphatase, superoxide dismutase, glutamine synthetase and manganese-dependent ATPase (Parmalee and Michael , 2016).

Manganese-assisted enzymatic antioxidant Mn-superoxide dismutase (Mn SOD) is an endogenous antioxidant enzyme it is function neutralize free radicals and prevent cellular damage by catalysis the dismutation of superoxide radicals, producing hydrogen peroxide and oxygen (Afridi *et al*., 2011). Reactive oxygen species (ROS) have been shown to be involved in tumor promotion, while antioxidant defences may have an

anticarcinogenic action. Evidence suggests that superoxide or hydrogen peroxide can influence the growth, as well as, animal cells death. Studies demonstrated that relatively low levels of oxidative stress promote cellular proliferation rather than causing degeneration or death. The increase in free radical generation in leukemic patients and the decrease in antioxidant defences are indicative of oxidative stress involvement in the pathogenesis of human leukaemia (Ana Bela *et al.*, 2012) .

1.8.4 Cobalt:

Cobalt is an essential element, as it is a co-factor of vitamin B12 (hydroxycobalamin), and its metabolism is the same as for vitamin B12, it is widely distributed throughout the environment (Nicole *et al.*, 2015). Cobalt is essential to mammals in the form of cobalamin (vitamin B_{12}) In humans, dietary intake of cobalt varies between 5 and 50 μg/day and most of it is inorganic with vitamin B_{12} representing only a small fraction. Cobalt does not accumulate in the organism and is rapidly excreted, mainly in urine (Dominique, 2015). In addition to its role in vitamin B12, cobalt is also a cofactor of enzymes involved in DNA biosynthesis and amino acid metabolism, also plays a role in methylating choline and thamine. The latter is required for the synthesis of DNA, which regulates cell division and growth. Co is readily absorbed into the bloodstream and excreted primarily in the urine. Co deficiency manifestation in humans; toxicity disease or symptoms include goitre, hypothyroidism and heart failure (Soetan, 2010). There was an increase of many symptoms and problems related to B12 deficiency, particularly pernicious anaemia, and nerve damage. with hip prosthesis, there was a significant increase in the incidence of lymphatic and hematopoietic malignancies, and significant deficits of breast and colorectal cancer. (Lingamaneni *et al.*, 2015).

1.9 Total Iron-Binding Capacity (TIBC) :

Iron has a pivotal role in homeostasis due to its participation in virtually all of the body's oxidation reduction processes (Juan, 2011). Also consider as a cofactor of a number of cellular enzymes it maintain their functions. Excess iron has been suggested as a risk factor in cancer development mostly due to increased production of reactive oxygen species (ROS) (Mi-Kyung and Yun-Jung,2014). Due to its ability to accept and donate electrons, while conversion amid ferric (Fe^{3+}) and ferrous (Fe^{2+}) oxidation states, iron is a critical component of sensor, transporter and storing molecules and enzymes involved in energy production and intermediate metabolism. Iron is also vital for the cell division process once the enzyme responsible for the synthesis of deoxyribonucleotides, ribonucleotide reductase (RR), is iron dependent (Oriana *et al.,* 2014).

Iron is transported to body tissues by a protein, transferrin, in plasma. This protein has two high-affinity binding sites for iron. Total iron-binding capacity (TIBC) indicates the maximum amount of iron needed to saturate plasma or serum transferrin (TRF).Virtually all plasma iron (P1) normally is bound to transferrin, and measurement of P1 is assumed to reflect the amount of transferrin iron. The expression "transferrin saturation," expressed as percent [(P1/TIBC) x 100], indicates the availability of iron to tissues. As transferrin saturation increases, there is an increase in the amount of diferric transferrin , which has a greater capacity to deliver iron than does monoferric transferrin (Huebers *et al.*, 1987). Measurements of TIBC, serum iron, and the percentage of iron saturation of transferrin are useful for the clinical diagnosis of iron-deficiency anemia and chronic inflammatory disorders and as screening tests for other clinical conditions . TIBC is routinely determined by

saturation of transferrin with an excess predetermined amount of iron, removal of the unbound iron, and measurement of the iron that is dissociated from transferrin (Yamanishi *et al.*,2003).

Transfusions with packed erythrocytes is a common practice in paediatric patients with acute lymphoblastic leukaemia (ALL) who are on chemotherapy. Since there is no physiological excretion mechanism for iron, the iron related to erythrocyte transfusions accumulates and may contribute to late cardiac, hepatic and endocrine complications in these patients (Unal *et al.*,2014). Although repeated transfusions are needed during the treatment of most cancers, paediatric patients are not routinely screened for Iron overload IO. The evaluation of IO can be achieved by various approaches, the most appropriate being still a matter of active research. Percutaneous liver biopsy remains the gold standard to diagnose liver iron overload (LIO). Serum ferritin (SF) is useful as non-invasive-screening parameter as it is easily measured and showed a positive correlation with transfusion burden, morbidity, and mortality (Maëlle *et al.*, 2013). Severe liver iron overload as well as moderate myocardial iron overload can be found one year after cancer treatment and that this overload persists overtime. The patients with higher ITR and those who have received more than a liter of blood red cells per square meter, regardless of their diagnosis or ITR, are at risk of iron overload and should be screened carefully (De Ville *et al.*,2013).

Estimation of iron level in children with malignant disorders at the end of remission induction therapy showed anemia in most of children , so the observation suggests that introduce therapeutic iron supplements to children on therapy for malignant disorders and various hematological and body iron status parameters should be assessed on a case-by-case basis (Bhakhri *et al.*, 2012).

1.10 Objectives:

1.10.1 General Objective:

This study to evaluate the serum level of electrolytes (Na ,K, Mg, Ca and PO_4^{3-}) , trace elements (Zn, Cu, Mn, and Co) and total iron binding capacity (TIBC) in serum of leukemic patients.

1.10.2 Specific Objectives:

1- To measure the levels of electrolytes ,trace elements and Total iron binding capacity in leukemic patients compared to control participants .
2- To study the effect of subtypes of leukaemia in the level of electrolytes, trace elements and total iron binding capacity.
3- Determining age- and sex-related changes in serum electrolytes, trace elements and TIBC in leukemia patients.

CHAPTER TWO

MATERIAL & METHODS

CHAPTER II

MATERIALS AND METHODS

2.1 Study Design:

This was a descriptive laboratory – based study. Conducted to evaluate serum electrolytes, trace elements and total iron binding capacity of leukemic patients ;during the period June 2012 to August 2013.

2.2 Study Subjects:

The study group consisted of 122 who diagnosed as leukemic patients (78 males and 44 females). The diagnosis was made by means of cytochemical stains and bone marrow smears. Age ranged from less than one year to over 45 years. The patients were recruited from Radiation and Isotope Centre in Khartoum State, Sudan (KRIC). All the patients enrolled in the study were receiving treatment before the analysis was made. Among these patients, 70 of them had acute lymphoid leukaemia (ALL), 19 patients had acute myeloid leukaemia (AML), 27 patients had chronic myeloid leukaemia (CML), and six patients had chronic lymphoid leukaemia (CLL).

The control group consisted of 79 healthy subjects (55 males and 24 females) were randomly selected as control from those who attended blood bank as donors in the Al – Shurta hospital in Khartoum or attended to follow up after recovering from disease. They are healthy subjects and don't represent any particular clinical group and without any previous medication with mineral supplement or recent blood transfusion. The study subjects were briefed about the purpose of the study, and written

consent was taken from each of them. Ethical approval was obtained from the ethical and research committee of Ministry of Health in Sudan.

2.3 Sampling (Blood Collection):

Venous blood sample (5 ml) was collected from the Leukaemia patients and healthy group. Samples with signs of haemolysis were discarded. Blood samples were allowed to clot and centrifuged for 15 minutes at 3000 rpm to extract the serum. The serum was transferred to 5 ml polystyrene tube, and stored at -20 ºC (without thawing) until analysis.

Calculated sample size is 162 samples, was calculated according to the following equation :

$$Ss = \frac{Z^2 (p) (1- p)}{C^2}$$

Z = (1.96 for 95% confidence level)

P = estimated prevalence (percentage)

C = confidence interval

P = 0.086 , C = 0.05

2.4 Biochemical Analysis :

A structural questionnaire with students consent was designed to collect demographical and clinical data (Appendix 1).

2.4.1 Ion Selective electrode Measurements:

2.4.1.1 Sodium and Potassium Measurements:

Electrolyte; sodium and potassium were measured by potentiometric method; ion selective electrode (ISE) direct method (EasyLyte analyzer, Medica Corporation, USA). ISE is completely automated microprocessor

controlled electrolyte systems that use current ISE technology to make electrolyte measurements. The EasyLyte product line measures various combinations of sodium, potassium, chloride, lithium, calcium and pH in whole blood serum plasma or urine.

2.4.2 Spectrophotometer Measurements:

Magnesium, calcium and phosphorus levels were measured by spectrophotometer instrument by using kits from Biosystems chemical company.

2.4.2.1 Determination of Magnesium:

Principle:

Magnesium in the sample reacts with xylidyl blue in alkaline medium forming a colored complex that can be measured by spectrophotometry. (Barbour and Davisdon , 1988) and (Chromýa et al., 1973).

2.4.2.2 Determination of Calcium:

Principle:

Calcium in the sample reacts with arsenazo III forming a coloured complex that can be measured by spectrophotometry (Michaylova, 1971) and (Burtis *et al.*,2005).

2.4.2.3 Determination of Phosphorus:

Principle:

Inorganic phosphorus in the sample reacts with molybdate in acid medium forming a phosphomolybdate complex that can be measured by spectrophotometry (Gamst and Try ,1980) and (Muñoz *et al.*, 1983).

2.4.3 Atomic Absorption spectroscopy Measurements:

Serum concentrations of Cu, Zn, Co, Mn, and Total Iron Binding Capacity in both patients and controls were determined by using atomic absorption spectrophotometer (Buck Scientific 210 VGP. USA ,2005).

2.4.3.1 Determinations of copper and zinc in blood serum:

Sample preparation:

For the determination of serum copper, the samples were diluted with an equal volume of deionized water. For the determination of serum zinc the samples were diluted 1:5 with deionized water.

Standard preparation:

Standards for copper (1000 mg/L) are prepared by dissolving 1.000 g of copper metal in a minimum volume of (1+1) HNO_3 and then diluted to 1 litter with 1% (v/v) HNO_3. Standards for zinc (500 mg/L) were prepared by dissolving 0.500 g of zinc metal in a minimum volume of (1+1) HC1 and then diluted to 1 litter with 1% (v/v) HCl.

Procedure:

After samples being diluted with deionized water, the analysis was performed against standards prepared in glycerol to approximate the viscosity characteristics of the diluted samples. The wavelengths for copper and zinc are 324.8 and 213.9 , respectively (Butrimovitz and Purdy , 1977).

2.4.3.1 Determination of Cobalt and Manganese in blood serum:

Sample preparation:

For the determination of serum manganese and cobalt the samples were diluted 1 : 9 with HNO_3 .

Standard preparation:

Standards for cobalt (1000 mg/L) were prepared by dissolving 1.000 g of cobalt metal in a minimum volume of (1+1) HCl and then diluted to 1 litter with 1% (v/v) HCl. Standards for manganese (1000 mg/L) were prepared by dissolving 0.500 g of manganese metal in a minimum volume of 1+1) HNO_3 and then diluted to 1 litter with 1% (v/v) HCl.

Procedure:

The analysis was performed after samples were diluted with Nitric acid against standards prepared. The wavelengths for cobalt and manganese are 240.7 nm and 279.5 nm, respectively.

2.4.3.3 Determinations of total iron binding capacity in blood serum:

Sample preparation:

For the determination of total iron binding capacity (TIBC), 0.2 ml of the serum samples was diluted with an equal volume of ferric chloride solution (containing 5mg/L Fe) , were added then mixed and left to stand for 5 minutes. Then added 200 mg of magnesium carbonate , mixed for 4 times during a 30 minute period and centrifuge of the supernatant was removed and 0.2 ml were transferred to another clean polyethylene tube, added 2.0 ml of a 20% (w/v) trichloro- acetic acid (TCA) solution were added , then mixed and heated at 90°C for 15 minutes. Cooled and centrifuged.

Procedure:

To determine the TIBC, samples were saturated with a ferric chloride solution (containing 5mg/L Fe) which causes all protein not bound with iron to become iron – bound. The excess iron was removed with magnesium carbonate and the sample was centrifuged. The resulting the

supernatant was treated with 20% (w/v) trichloroacetic acid (TCA) solution which precipitated the plasma protein and removed approximately 95% of any hemoglobin present, then heated at 90°C for 15 minutes. Cooled and centrifuged. Reading by AAS at wave length 248.3 nm (Fernandez and Kahn,1971) and (Olson and Hamlin, 1969).

2.5 Statistical analysis:

The Statistical Package of Social Science (SPSS) will be used for statistical analysis. The means and medians of the variables were compared by Student's t test program depending on the sample distribution. Also used Once an Analysis of Variance (ANOVA) Use of multiple comparison analysis tests.

CHAPTER THREE

RESULTS & DISCUSSION

CHAPTER III

RESULTS and DISCUSSION

Statistical data for leukaemia patients during the period 2000 to 2012, from radiation and isotopes center in Khartoum- Sudan, represented in tables (3. 1), (3. 2), (3.3) and (3. 4).

Tables (3. 5), (3. 6) and (3.7) show a summary of results on the serum samples of leukemia patients as divided into different subtypes; ALL, AML, CML and CLL and control subjects analyzed in this work. Tables (3.8), (3.9) and (3.10) show the results of serum concentration of leukemia patients and controls as classified into four groups according to their age. Leukemia and control subjects were divided into two groups according to their gender (Males and Females) results for these groups are depicted in tables (3.11), (3.12) and (3.13).

Figures (3.1) show the percentage of males and females in leukaemia patients in the study sample, figure (3.2) show the percentage according to the age groups and different types of leukaemia male and female. The incidence of leukaemia patients according to place of birth showed in figure (3.5). The concentration of serum electrolytes, trace elements and total iron binding capacity (TIBC) in different types of leukaemia patients and control presented in figures (3.6), (3.7) and (3.8). The concentration of TIBC in different types of leukaemia patients and in males and female show in figure (3.9) and (3.10).

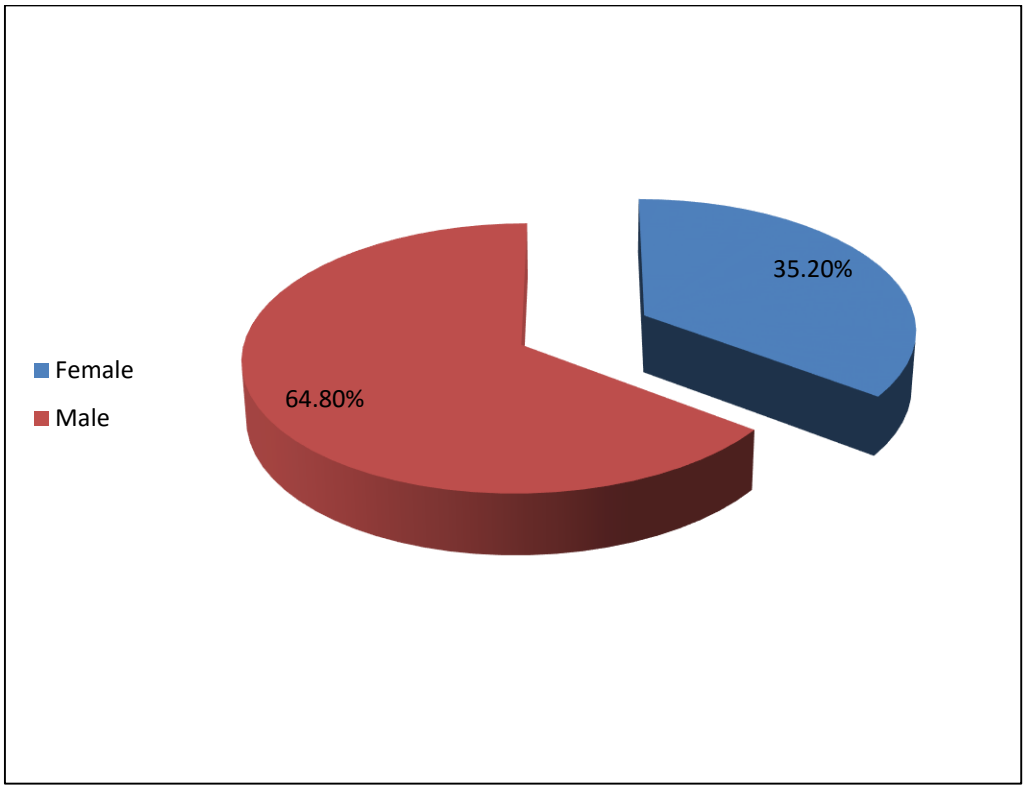


Figure (3.1): The Percentage of Males and Females in Leukemic patients.

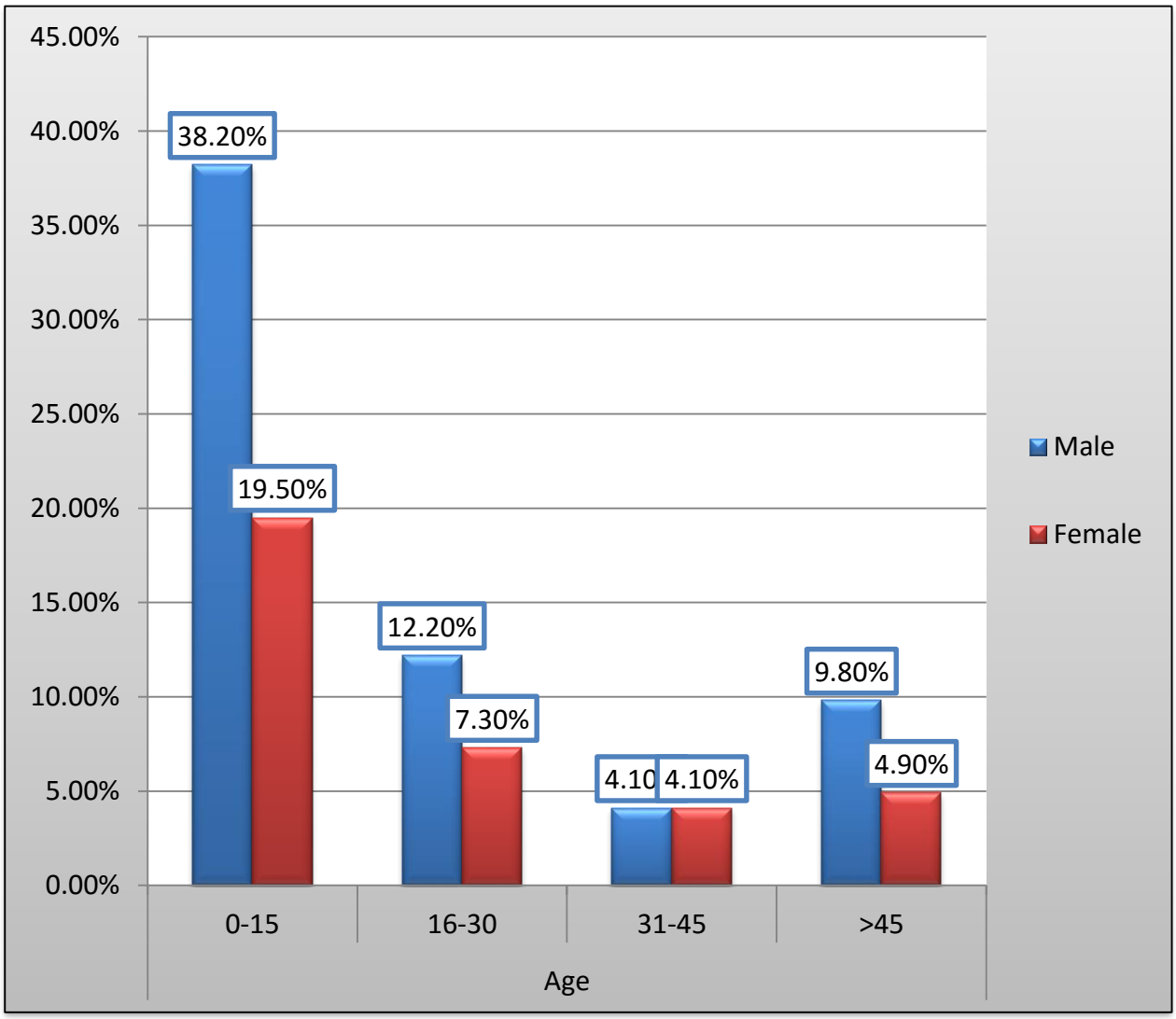


Fig (3.2): The percentage of Males and Females leukaemia patients according to Age groups.

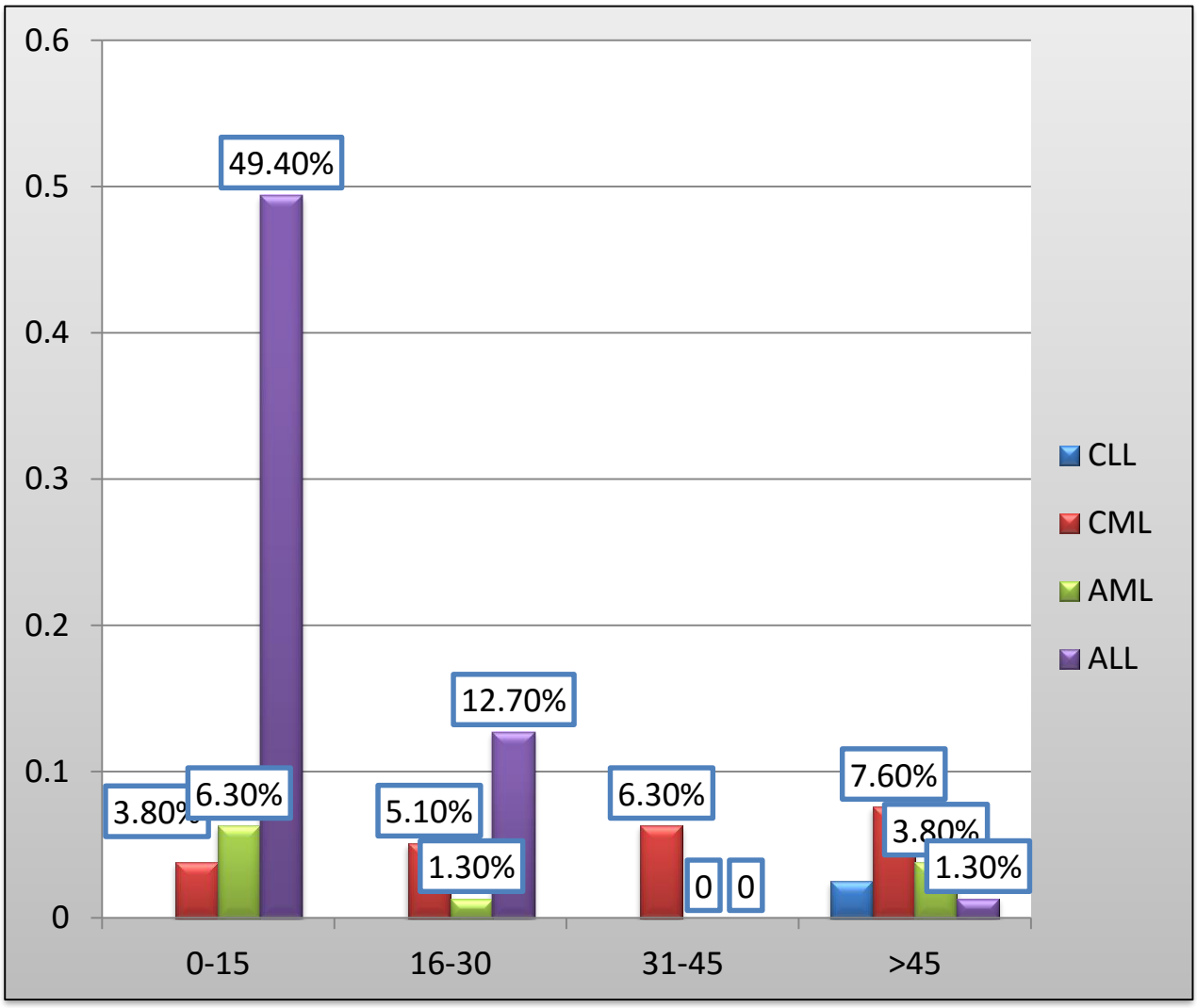


Figure (3.3): The Percentage of Male leukaemia Patients Classified According to the Age Groups and Different Types of leukaemia

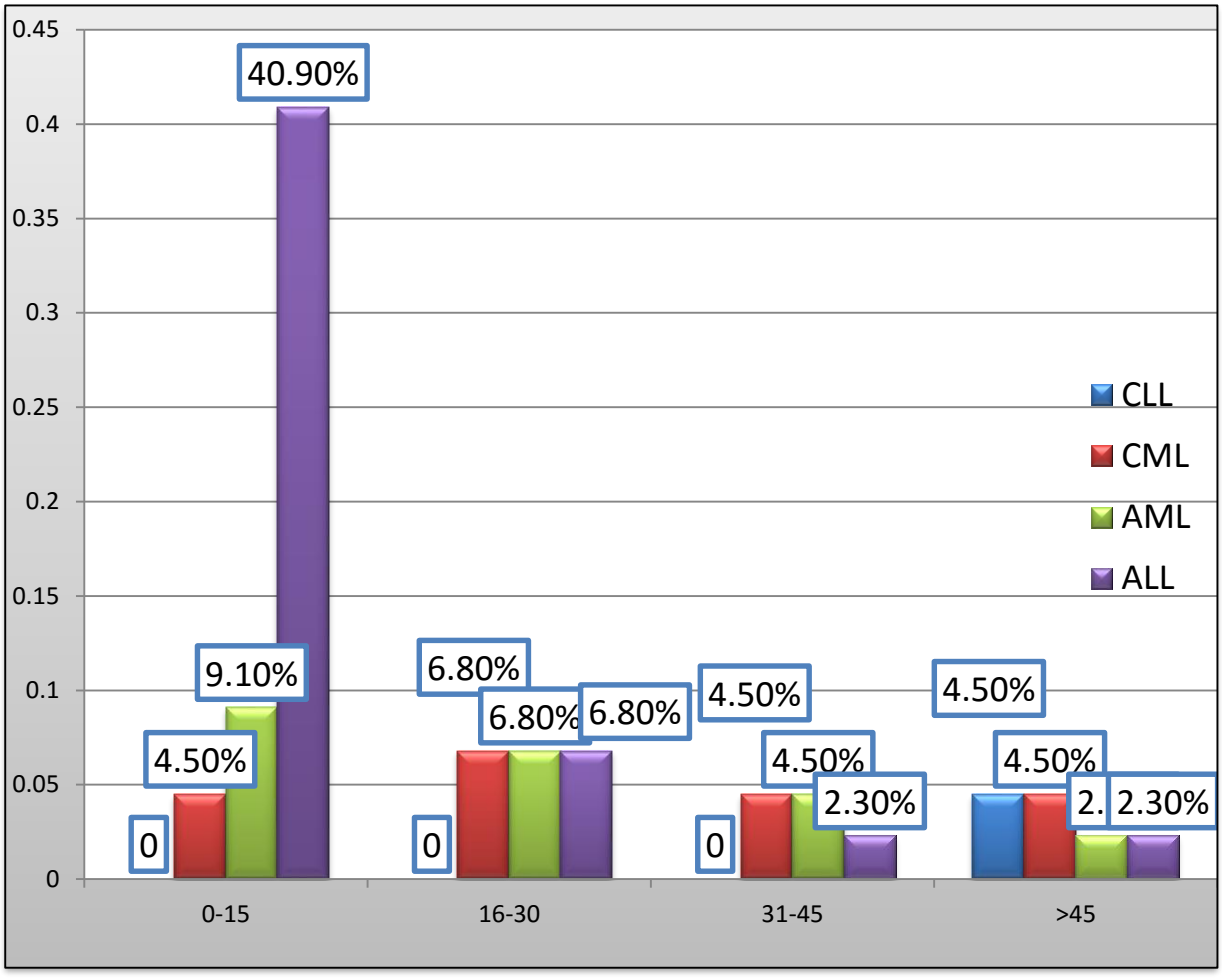


Figure (3.4): The Percentage of Female leukaemia Patients Classified According to the Age Groups and Different Types of leukaemia.

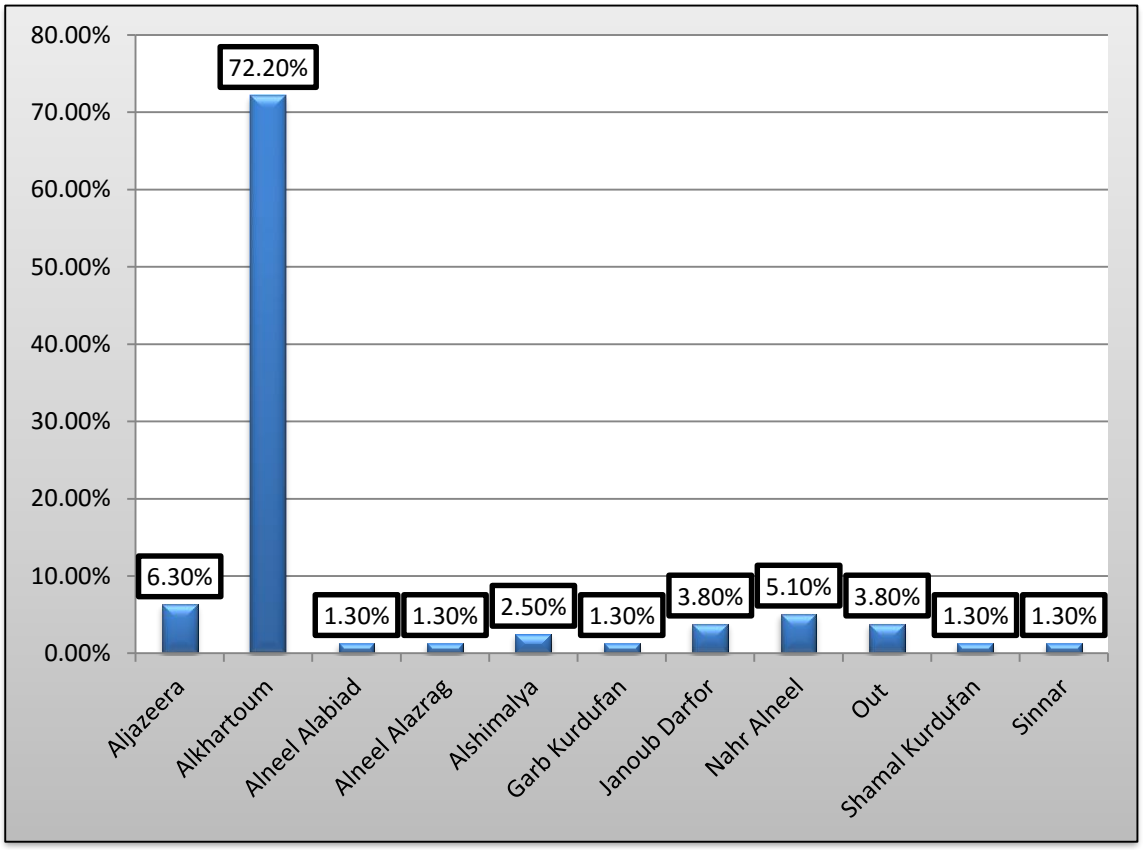


Figure (3.5): Incidences of the leukaemia patients according to the Place of birth

3.1: Incidence of leukaemia patients during (2000 – 2012)*

Table (3.1): The incidence of Acute lymphocytic leukaemia (ALL) patients during the period (2000 – 2012)*

Sex		1- 10	11-20	21-30	31-40	41-50	51-60	61 - 70	over 71	Total
Male	2000	16	18	3	2	1	0	2	0	42
	2001	14	18	6	2	3	3	2	1	49
	2002	20	15	6	5	1	0	1	2	50
	2003	23	13	3	4	2	3	5	1	54
	2004	26	12	10	1	3	3	1	0	56
	2005	21	11	2	2	0	2	0	0	38
	2006	20	19	4	4	1	2	2	0	52
	2007	21	21	9	3	3	2	2	1	62
	2008	11	7	4	1	1	3	0	0	27
	2009	28	16	4	0	4	5	2	5	64
	2010	34	18	6	0	1	3	4	1	67
	2011	32	17	6	3	2	1	4	3	68
	2012	33	16	6	6	4	1	0	1	67
Total		299	201	69	33	26	28	25	15	696
Female	2000	7	3	1	2	1	1	0	0	15
	2001	9	5	6	1	3	0	0	3	27
	2002	9	9	3	1	3	1	0	0	26
	2003	13	4	1	2	1	1	1	0	23
	2004	8	4	1	0	0	0	0	0	13
	2005	10	9	8	1	1	1	1	1	32
	2006	13	8	1	1	5	3	0	2	33
	2007	15	12	3	1	2	0	1	0	34
	2008	10	3	1	0	2	2	0	0	18
	2009	22	8	5	1	3	2	2	2	45
	2010	15	5	1	8	2	3	1	0	35
	2011	26	10	2	4	3	0	3	0	48
	2012	17	6	1	3	2	2	2	4	37
Total		174	86	34	25	28	16	11	12	386

*From radiation and isotopes center in Khartoum - Sudan

Table (3.2): The incidence of Acute myeloid leukaemia (AML) patients during the period (2000 – 2012)*

Sex		1- 10	11-20	21-30	31-40	41-50	51-60	61 - 70	over 71	Total
Male	2000	3	3	2	4	2	1	1	0	16
	2001	6	4	6	2	2	2	4	2	28
	2002	2	7	4	2	4	7	3	0	29
	2003	8	12	6	10	4	3	4	3	50
	2004	5	11	3	3	2	4	1	4	33
	2005	4	9	7	3	1	2	1	0	27
	2006	14	5	6	9	3	2	3	2	44
	2007	4	4	11	4	8	2	2	3	38
	2008	8	8	7	6	10	2	2	2	45
	2009	15	8	4	4	7	12	9	4	63
	2010	11	7	9	14	6	8	5	9	69
	2011	11	12	7	7	6	5	10	4	62
	2012	18	9	12	8	7	10	10	7	81
Total		109	99	84	76	62	60	55	40	585
Female	2000	1	2	3	4	1	1	0	0	12
	2001	1	3	2	2	3	3	3	1	18
	2002	2	4	4	5	2	2	2	1	22
	2003	2	4	1	4	3	1	1	1	17
	2004	4	4	0	3	1	3	1	2	18
	2005	3	6	3	1	0	0	2	0	15
	2006	6	9	2	5	4	5	1	0	32
	2007	9	12	8	3	4	1	2	2	41
	2008	5	4	6	9	5	1	4	2	36
	2009	9	6	11	8	6	6	3	1	50
	2010	8	9	5	12	9	7	2	0	52
	2011	8	11	8	5	7	7	3	2	51
	2012	13	13	8	6	8	11	5	3	67
Total		71	87	61	67	53	48	29	15	431

Table (3.3): The incidence of chronic lymphoid leukaemia CLL patients during the period (2000 – 2012)*

Sex		1- 10	11-20	21-30	31-40	41-50	51-60	61 - 70	over 71	Total
Male	2000	0	0	0	2	1	7	5	0	15
	2001	0	0	0	1	7	10	12	5	35
	2002	1	1	2	3	9	13	17	1	47
	2003	0	0	0	0	3	9	15	6	33
	2004	0	0	0	3	7	8	14	7	39
	2005	1	0	1	4	5	14	24	6	55
	2006	0	0	2	0	7	20	25	9	63
	2007	2	0	1	33	14	22	10	0	82
	2008	4	11	2	5	14	25	22	0	83
	2009	3	6	2	4	10	9	17	12	63
	2010	1	0	2	6	17	30	27	19	102
	2011	0	1	2	5	15	27	29	26	105
	2012	0	0	0	4	10	23	20	18	75
Total		12	19	14	70	119	217	237	109	797
Female	2000	0	0	0	2	1	7	5	0	15
	2001	0	0	0	7	1	7	2	1	18
	2002	0	1	0	3	7	6	5	0	22
	2003	0	0	0	0	6	6	2	1	15
	2004	1	0	0	2	8	9	9	7	36
	2005	0	0	0	2	9	8	4	7	30
	2006	0	0	2	7	9	9	9	3	39
	2007	0	0	0	2	1	5	14	5	27
	2008	1	7	3	4	7	5	15	10	52
	2009	2	0	1	3	7	12	8	2	35
	2010	0	0	1	7	12	19	11	8	58
	2011	0	0	0	3	12	10	12	16	53
	2012	0	0	1	2	8	16	14	7	48
Total		4	8	8	44	88	119	110	67	448

Table (3.4): The incidence of chronic myeloid leukaemia (CML) patients during the period (2000 -2012)*

Sex		1- 10	11-20	21-30	31-40	41-50	51-60	61 - 70	over 71	Total
Male	2000	0	7	7	7	8	3	3	2	37
	2001	1	2	3	6	3	5	8	2	30
	2002	0	1	8	10	12	9	6	1	47
	2003	0	7	8	10	7	9	9	2	52
	2004	5	1	9	13	14	4	5	3	54
	2005	4	6	17	17	14	12	7	1	78
	2006	1	3	14	23	16	8	4	3	72
	2007	1	4	10	2	13	7	7	2	46
	2008	0	12	20	26	15	15	15	9	112
	2009	9	2	13	22	13	13	7	5	84
	2010	4	5	18	28	21	13	11	4	104
	2011	4	7	13	17	34	16	8	3	102
	2012	1	7	19	16	19	16	20	10	108
Total		30	64	159	197	189	130	110	47	926
Female	2000	0	4	9	7	12	5	4	1	42
	2001	1	2	6	6	7	6	4	2	34
	2002	0	2	9	9	10	7	10	0	47
	2003	1	4	10	6	18	10	6	1	56
	2004	0	3	9	17	17	13	13	9	81
	2005	2	5	10	10	11	9	3	1	51
	2006	0	5	11	17	12	4	5	0	54
	2007	1	4	10	2	13	7	7	2	46
	2008	3	5	11	18	13	12	6	5	73
	2009	9	3	11	14	15	10	5	3	70
	2010	1	4	20	16	16	15	13	4	89
	2011	1	8	15	30	25	9	11	5	104
	2012	2	6	17	27	22	16	12	8	110
Total		21	55	148	179	191	123	99	41	857

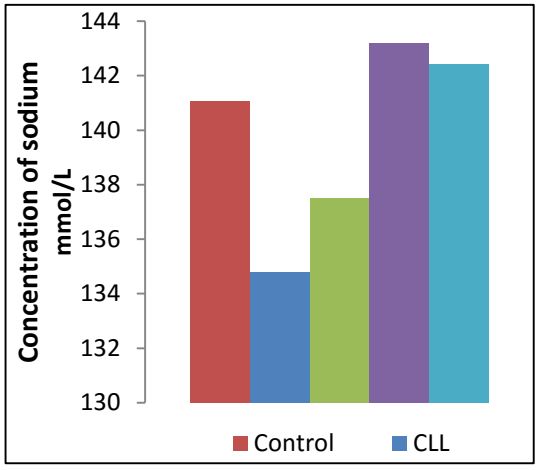
3. 2: Concentration of serum electrolytes in leukaemia patients

Table (3.5): The concentration of serum electrolytes (mg/dl) in different subtypes of leukaemia patients

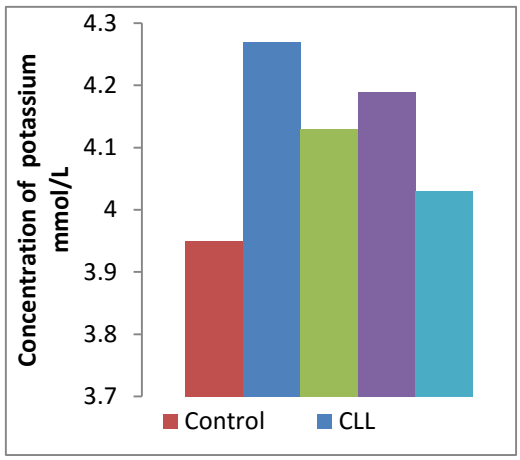
Parameter	Control	CLL	CML	AML	ALL
Na(mmol /L)	141.07 $^{a\,b}$ ±0.57	134.78 ^{a} ±0.66	137.49 $^{a\,b}$ ±0.86	143.18 ^{b} ±2.22	142.41 $^{a\,b}$ ±1.94
K(mmol /L)	3.95^{a} ±0.04	4.27 ^{a} ±0.85	4.13 ^{a} ± 0.13	4.19 ^{a} ±0.17	4.03 ^{a} ±0.09
Ca (mg/dl)	9.62 ^{a} ±0.14	8.82 ^{b} ±0.70	8.40 ^{c} ±0.13	9.48 $^{a\,b}$ ±0.25	9.03 ^{b} ±0.19
Mg (mg/l)	2.15 ^{a} ±0.03	1.83 ^{a} ±0.14	2.73 ^{b} ±0.10	2.52 ^{b} ±0.10	2.48 ^{b} ±0.08
P (mg/dl)	3.74 ^{a} ±0.10	3.89 ^{a} ±1.01	4.14 ^{a} ±0.16	3.82 ^{a} ±0.23	4.03 ^{a} ±0.15

Means with the same letter are not significantly different from each other (p<0.05).

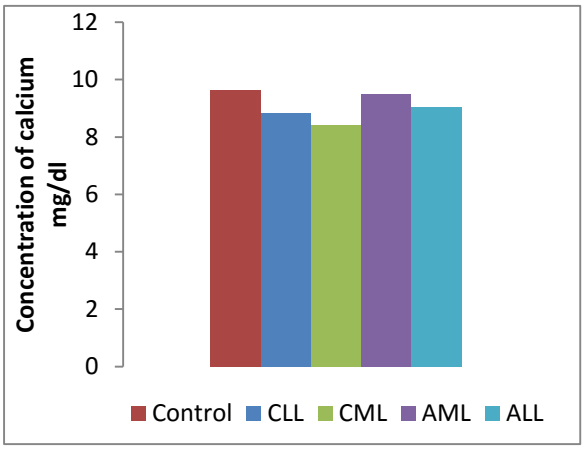
The data presented as the Mean ± SE



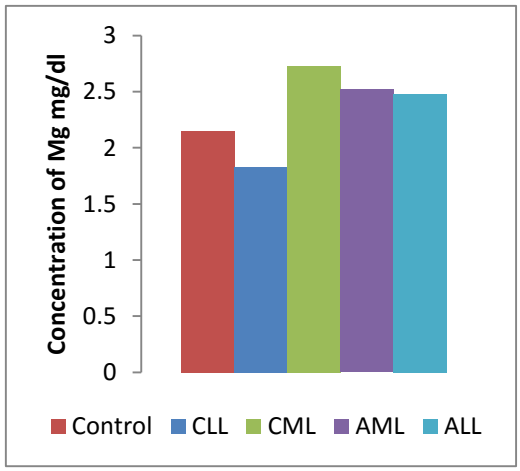
a) Concentration of Na in subtypes of leukaemia



b) Concentration of K in subtypes of leukaemia



c) Concentration of Ca in subtypes of leukaemia



d) Concentration of Mg in subtypes of leukaemia

e) Concentration of phosphorous in subtypes of leukaemia

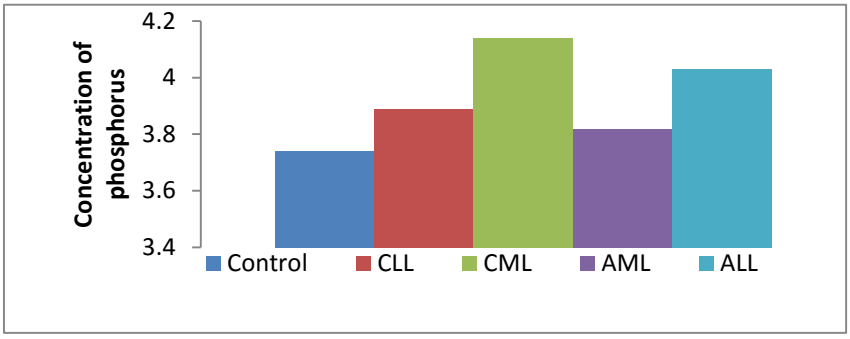


Figure (3.6): The concentration of serum electrolytes in different subtypes of leukaemia patients

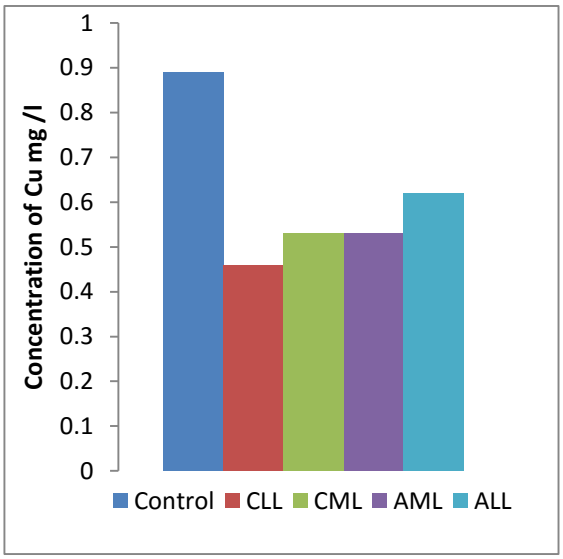
3.3: Concentrations of serum trace elements in leukaemia patients and control

Table (3.6): The concentrations of serum trace elements (mg/ l) in different types of leukaemia patients and control

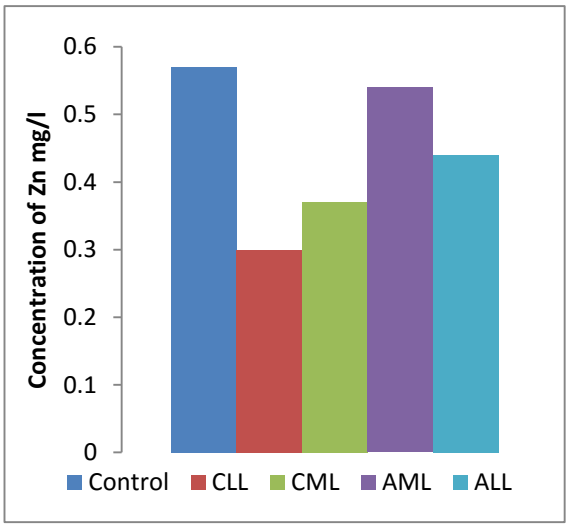
Parameter	Control	CLL	CML	AML	ALL
Cu (mg/ l)	0.89 [a] ±0.02	0.46 [b] ±0.14	0.53 [b] ±0.06	0.53 [b] ±0.08	0.62 [b] ±0.04
Zn (mg/ l)	0.57 [a] ±0.02	0.30 [c b] ±0.00	0.37 [c b] ±0.03	0.54 [a b] ±0.14	0.44 [b] ±.03
Mn (mg/ l)	0.34 [a] ±0.03	0.22 [b] ±0.02	0.21 [b] ±0.03	0.20 [b] ±0.03	0.26 [b] ±0.02
Co (mg/ l)	0.46 [a] ±0.02	0.14 [b] ±0.05	0.12 [b] ±0.02	0.22 [b] ±0.04	0.15 [b] ±0.02

Means with the same letter are not significantly different from each other ($p<0.05$).

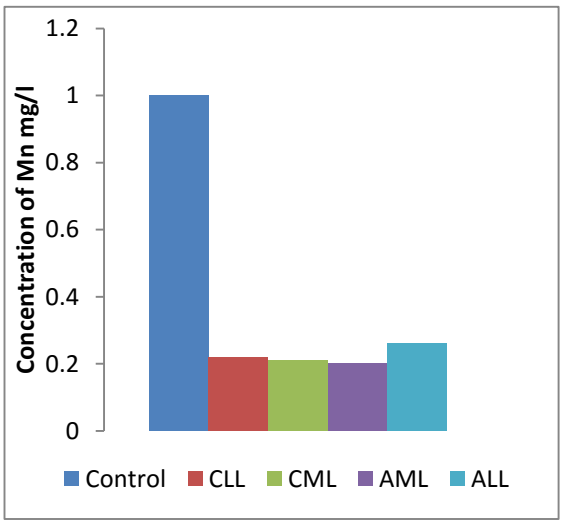
The data presented as the Mean ± SE.



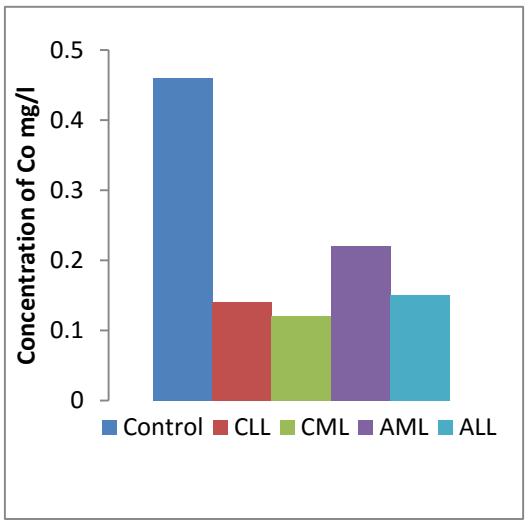
a) Concentration of Cu in subtypes of leukaemia



b) Concentration of Zn in subtypes of leukaemia



c) Concentration of Mn in subtypes of leukaemia



d) Concentration of Co in subtypes of leukaemia

Figure (3.7): The concentrations of serum trace elements (mg/l) in different types of leukaemia patients and control.

Table (3.7): The concentration (mg/ l) of serum Total Iron Binding Capacity (TIBC) in different types of leukaemia patients and control

Parameter	Control	CLL	CML	AML	ALL
TIBC(mg/ l)	2.02 [a] ±0.07	2.25 [a b] ±0.39	1.17 [b] ±0.10	1.66 [b] ±0.09	1.64 [b c] ±0.06

Means with the same letter are not significantly different from each other ($p<0.05$).

The data presented as the Mean ± SE.

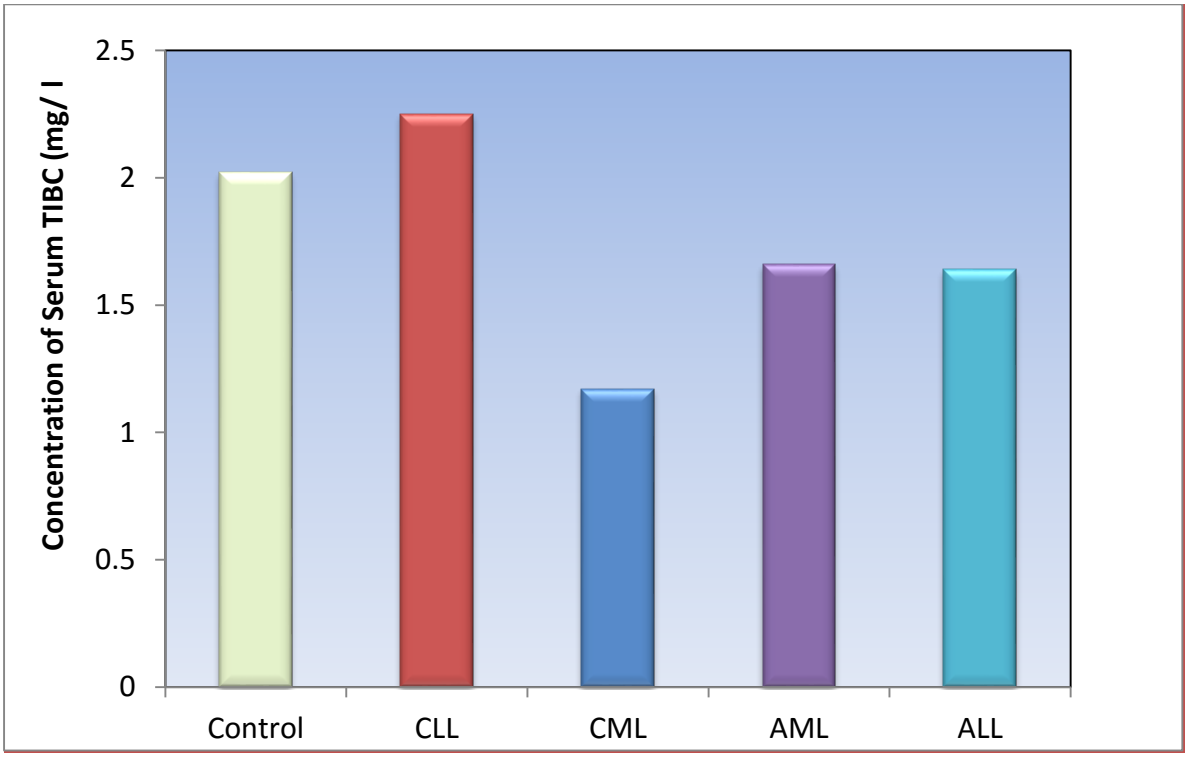


Figure (3.8): The concentration (mg/ l) of serum Total Iron Binding Capacity (TIBC) in different types of leukaemia patients and control.

Table (3.8): The Concentration of serum electrolytes (mg/dl) in Patients and control Classified According to the age.

AGE	parameters / Group	Na	K	Ca	Mg	P
0-15	Case	142.67 [a] ±1.98	4.10 [a] ±0.09	9.08 [a] ±0.19	2.49 [a] ±0.07	3.97 [a] ±0.15
	Control	140.94 [a] ±1.16	4.10 [a] ±0.10	9.23 [a] ±0.17	2.00 [a] ±0.05	4.05 [a] ±0.38
16-30	Case	138.81 [a] ±1.78	3.86 [a] ±0.11	8.92 [a] ±0.25	2.49 [b] ±0.15	4.19 [a] ±0.18
	Control	141.60 [a] ±0.87	3.86 ±0.04	9.57 [a] ±0.33	2.09 ±0.05	3.65 [a] ±0.16
31-45	Case	141.25 [a] ±3.70	4.19 [a] ±0.32	8.87 [b] ±0.53	2.64 [b] ±0.26	3.58 [a] ±0.28
	Control	141.55 [a] ±1.30	4.00 [a] ±0.07	9.73 [a] ±0.14	2.23 [a] ±0.07	3.70 [a] ±0.21
>45	Case	138.73 [a] ±1.40	4.27 [a] ±0.22	8.54 [b] ±0.18	2.59 [a] ±0.14	4.12 [a] ±0.29
	Control	139.29 a ±1.10	3.95 [a] ±0.12	9.78 [a] ±0.25	2.26 [a] ±0.07	3.75 [a] ±0.24

Means with the same letter are not significantly different from each other ($p<0.05$).

The data presented as the Mean ± SE.

Table (3.9): The Concentration of Trace Elements (mg/ l) in Control and Patients Classified According to the age .

AGE	parameters / Group	Cu	Zn	Mn	Co
0-15	Case	0.62 [a] ±0.04	0.42 [b] ±0.02	0.24 [a] ±0.02	0.12 [b] ±0.01
	Control	0.75 [a] ±0.04	0.58 [a] ±0.04	0.19 [a] ±0.03	0.41 [a] ±0.07
16-30	Case	0.52 [b] ±0.07	0.54 [a] ±0.12	0.25 [a] ±0.05	0.18 [b] ±0.05
	Control	0.86 [a] ±0.04	0.57 [a] ±0.03	0.41[a] ±0.06	0.44 [a] ±0.03
31-45	Case	0.44 [b] ±0.09	0.35 [b] ±0.06	0.16 [a] ±0.02	0.19 [b] ±0.05
	Control	0.90 [a] ±0.05	0.59 [a] ±0.04	0.35 [a] ±0.06	0.54 [a] ±0.04
>45	Case	0.51 [b] ±0.06	0.39 [b] ±0.05	0.22 [a] ±0.04	0.19 [b] ±0.03
	Control	1.04 [a] ±0.03	0.54 [a] ±0.03	0.25 [a] ±0.04	0.37 [a] ±0.05

Means with the same letter are not significantly different from each other ($p<0.05$).

The data presented as the Mean ± SE.

Table (3.10): The Concentration(mg/ l) of Total Iron Binding Capacity (TIBC) in Control and Patients Classified According to the age .

parameters / Group	0 -15		16 - 30		31-45		>45	
	Case	Control	Case	Control	Case	Control	Case	Control
TIBC	1.59 [b] ±0.06	2.22 [a] ±0.07	1.73 [b] ±0.12	2.23 [a] ±0.08	1.69 [b] ±0.08	2.07 [a] ±0.13	1.94 [b] ±0.13	1.41 [a] ±0.22

Means with the same letter are not significantly different from each other ($p<0.05$).

The data presented as the Mean ± SE.

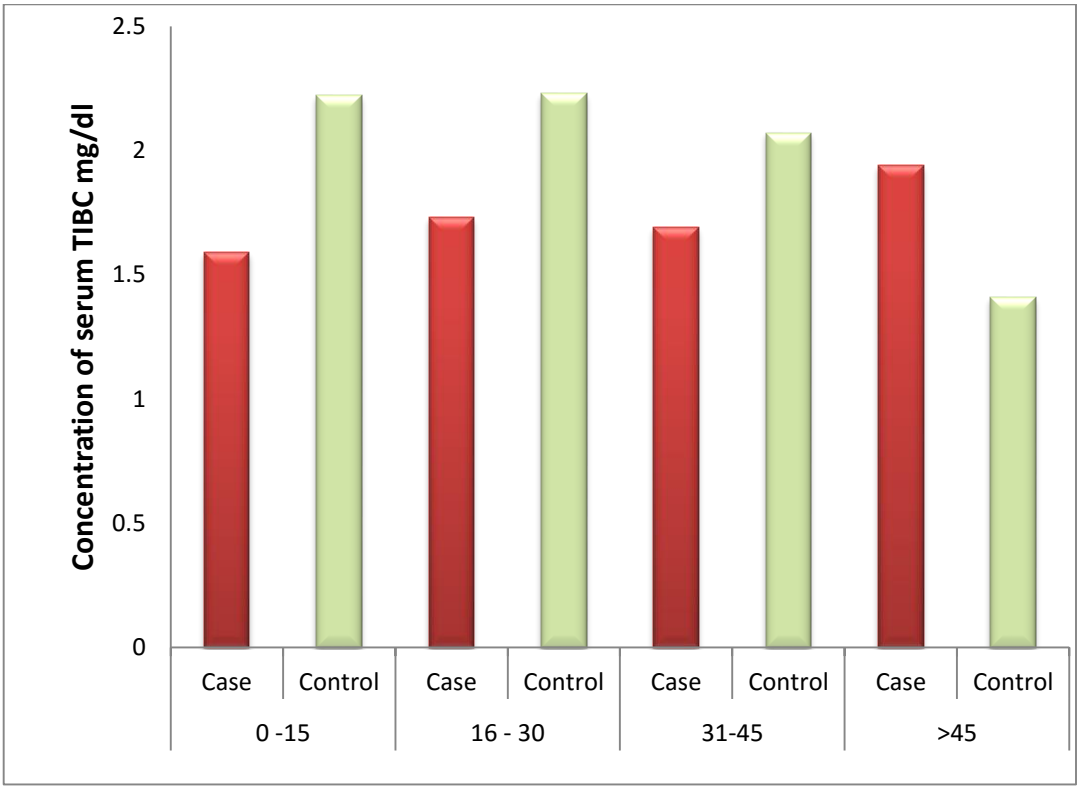


Figure (3.9): The Concentration (mg/ l) of Total Iron Binding Capacity (TIBC) in Control and Patients Classified According to the age.

Table (3.11): The concentration of serum electrolytes (mg/dl) in Males and Females Control and Patients.

Groups		Elements: Na	K	Ca	Mg	P
Male	Case	140.63[a] ±1.17	4.05 [a] ±0.08	9.12 [a] ±0.15	2.38 [a] ±0.07	3.97 [a] ±0.12
	control	140.20 [a] ±0.62	3.91 [a] ±0.04	9.58 [a] ±0.24	2.15 [b] ±0.04	3.76 [a] ±0.14
Female	Case	142.15 [a] ±0.2.89	4.13 [a] ±0.14	8.65 [b] ±0.22	2.75 [c] ±0.08	4.09 [a] ±0.19
	Control	142.27 [a] ±1.05	4.00 [a] ±0.06	9.65 [a c] ±0.13	2.14 [b d] ±0.04	3.69 [a] ±0.16

Means with the same letter are not significantly different from each other ($p<0.05$).

The data presented as the Mean ± SE.

Table (3.12): The concentration of serum trace elements (mg/ l) in Males and Females Control and Patients.

Groups		Elements: Cu	Zn	Mn	Co
Male	Case	0.53 [b] ±0.03	0.43 [b] ±0.04	0.24 [b] ±0.02	0.15 [c] ±0.02
	Control	0.90 [a] ±0.04	0.59 [a] ±0.02	0.37 [a] ±0.04	0.48 [a] ±0.03
Female	Case	0.66 [c] ±0.06	0.42 [b] ±0.02	0.22 [b] ±0.03	0.13 [c] ±0.02
	Control	0.87 [a d] ±0.03	0.54 [a b] ±0.02	0.28 [a b] ±0.04	0.41 [a b] ±0.03

Means with the same letter are not significantly different from each other ($p<0.05$).

The data presented as the Mean ± SE.

Table (3.13): The concentration of serum Total Iron Binding Capacity (mg/ l) in Males and Females Control and Patients.

Group / Parameter	Male		Female	
	case	control	case	control
TIBC	1.74 [c] ±0.06	2.00 [a] ±0.10	1.58 [b] ±0.06	2.04 [a] ±0.12

Means with the same letter are not significantly different from each other (p<0.05).

The data presented as the Mean ± SE.

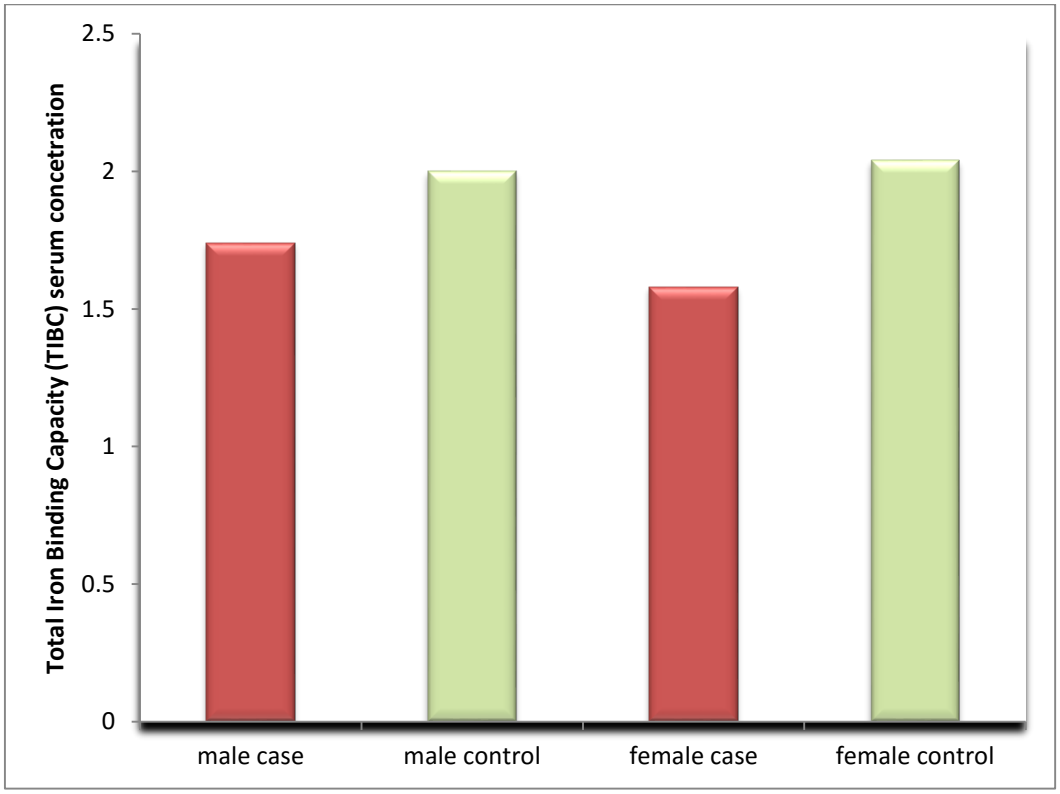


Figure (3.10): The concentration of Total Iron Binding Capacity (TIBC) (mg/ l) in Males and Females Patients and Control

3.4 Discussion

There are relatively few reports on the concentration of electrolytes and trace elements in the serum of patients with leukaemia. It is very difficult to compare the exact values for each element in one subtype with others, as the available literature shows the concentration in one or two types of leukaemia, no study content four types. In addition, no study compared the levels of these elements according to their age or sex. However, a comparison was done between the results obtained in this study and the relative literature data.

Patients investigated were 122 (64.80 %) males and (35.20%) females (figure 1). The frequency of males more than females in all groups of age, except in 31- 45 years group has the same percentage of male and female. Acute lymphoid leukaemia (ALL) has high incidence in children group from less than one year to 15 years; (49.40 %) and (40.90%) for male and female groups respectively (figure 3 and figure 4).

Khartoum state showed high percentage of incidence (72.20%) of leukaemia patients compared with other states according to their place of birth in Sudan (figure 5).

Statistical data from radiation and isotopes center in Khartoum showed that the males predominated in all types of leukaemia. There was 5126 cases during 12 years. Acute lymphoblastic leukaemia 1082 cases (64.33 % male and 35.67 % female), 70 % of cases under 20 years. This agree with Whitehead *et al.*, 2016 who reported that: Leukaemia is considered as the most common type of cancer in children, its annual incidence has increased over the last decades. Stacy and Patrick, 2015 also reported acute lymphoid leukaemia (ALL) is the most common cancer diagnosed in children. The records showed there was 1016 cases of acute myeloid leukaemia during the twelve years (57.58 % male and 42.42 % female), 57 % of cases under 40 years. This agree with study done by A Pinto

et.al. (2001) who reported that the incidence of AML increases steadily with age more than 50% of patients with a diagnosis of AML are older than 60 years and the median age at the onset is over 64 years. The records showed there was 1245 cases of chronic lymphocytic leukaemia ,Of those, 64.02 %cases were in men and 35.98 % were in women. Most of patients 70% were over 50 years. This in agreement with study conducted by Kedar and Carlos (2007), chronic lymphocytic leukaemia is primarily a disease of older adults, with more than 90% of the cases occurring in persons older than 50 years. Another study by Dighiero (2008) conducted that; the

chronic lymphocytic leukaemia is rare in people younger than 50 years, but after this age a fairly rapid rise in incidence takes place. The median age for diagnosis of the disease is 70 years for men and 74 years for women.

1783 cases of chronic myeloid leukemia in the records data (51.93 % male and 48.07 % female) in contrast to the data collected by Baccarani *et al.*,2012 who found decreased in cases of CML and reported that the prevalence of CML is steadily rising due to the very substantial prolongation of survival that has been achieved with targeted therapy.

According to the present study the results showed that serum magnesium level in leukemia patients was significantly higher in CML, AML and ALL ($P > 0.01$) when compared to the control. No significant difference in Mg serum level between control and CLL patients was observed. The concentration of Mg was significantly lower in CLL ($p<0.05$) compared with the CML, AML and ALL patients. In leukemic patients, with ages ranging between 16- 30 and 31- 45 years serum magnesium was significantly higher in patient groups than the control ($p < 0.01$) and ($p < 0.01$) respectively . While other groups less than one year – 15 and Over 45 years was not showed significant difference between patients and

controls .When compared serum magnesium, according to the sex the male and female cases group showed significantly higher levels than the male and female control ($p < 0.01$) and ($p < 0.05$) respectively . No significant difference in concentration level of Mg between male control and female control. This findings agrees with the Study conducted by Merza *et al*. (2009) who observed increased in serum Mg in some leukaemia patients while other patients recorded normal and lower levels. Another study conducted by Alea *et al.* 2013 found increase in serum Mg in ALL patients . Although the increased level of serum magnesium was statistically significant in this study, but it was not considered as case of hypermagnesmia (more than 3.5 mg/dl). Also O'Regan *et al*. (1977) reported that serum magnesium was elevated as a result of its release by malignant cells after cytotoxic therapy or its accumulation due to urate nephropathy.

Serum level of calcium was significantly lower with CML, CLL and ALL than in control ($p < 0.001$, $p < 0.05$ and $p < 0.05$) respectively . The concentration of serum Ca was significantly lower in cases than control in 31- 45 and Over 45 years group ($p < 0.05$) and ($p < 0.001$) respectively. No significant difference in concentrations level of calcium between cases and control in less than one year – 15 and 16- 30 years. The determination of the level of calcium showed statistically significant decrease in female cases compared with other groups ; female control , male control and male cases ($p < 0.01$), ($p < 0.01$) and ($p < 0.05$) respectively. No significant difference between male cases, male control and female control. The concentration of sodium was significantly lower in CML than the ALL patients ($p < 0.05$). No differences in serum sodium and potassium levels were found in all types of leukaemia patients and controls the same result was obtained when measured sodium and potassium levels in four age groups and gender groups. In the

present study serum calcium recorded lower level in leukaemia subtypes, in cases older than 31 years and in female subjects. This result was in agreement with that achieved by Randy and Ursula (2014) who reported : lower level of calcium (hypocalcemia) in hematologic malignancy and might results from various factors, including hypoalbumine-mia, malabsorption, malnutrition, vitamin D deficiency. Also this finding was similar to that obtained by Liamis *et al.* (2016) who reported hypocalcaemia in patients with malignancies associated with the using of anticancer drugs, which is beneficial for patients with malignancies but is frequently associated with the occurrence of electrolyte disorders such as hyperphosphatemia and hypocalcaemia.

All types of leukaemia patients in this study were having normal sodium and potassium level. The same result was obtained when measured the sodium and potassium levels in four age groups and sex groups, this results disagree with Filippatos *et al.* (2005) and Milionis *et al.*, (1999) finding , which showed a low serum level of sodium (hyponatremia) and potassium (hypokalemia). One study by O'Regan *et al.* (1977) reported that patients in remission usually have normal serum electrolyte concentrations.

In agreement with the observations of other studies conducted by Reisi *et al.* (2015) and Salman *et al.* (2013) , serum phosphate levels in the current study showed non significant difference when comparing different subtypes of leukemia patients, age and sex groups to control groups. Similar finding reported by Berman *et al*. (2006), normal phosphate levels in patient with Chronic myeloid leukemia.

With regard to the nutritional role of copper and zinc and their important roles in metabolism regulation and their direct relation with cancers any significant changes in the level of these elements could be harmful to the body (Sanaat *et al.*, 2011).

In current study we observed that the significant decrease in serum zinc concentration in the leukemic group; CLL, CML and ALL ($p < 0.05$) compared with control. The results also showed significantly higher concentration level of Zn in AML group than CML group ($p < 0.05$). Zinc concentration in less than one year – 15, 31- 45 and Over 45 years groups showed significantly lower level than control ($p < 0.05$), *(* $p < 0.05$*)*, ($p < 0.01$) and ($p < 0.05$) respectively. The concentration of zinc was significantly lower in male cases and female cases compared with male control ($p < 0.01$) and ($p < 0.01$) respectively. Whereas no significant difference was found in the female case compared with male case and female control. In this study observed also, no significant difference in concentration level of Zn in male control and female control. Leukaemia patients in this study were having decreased levels of serum zinc concentration in different subtypes, three age groups and sex groups.This in an agreement with Atieh *et al.* (2012) and Ursula *et al*. (2006) who reported that the general trend in malignant diseases towards slight decrease in zinc concentrations, implying that zinc deficiency is associated with the aetiology of cancer. Zuo *et al*. (2006) also reported decreasing of serum zinc in all groups of leukaemia patients and this has been related to general nutrition.

In this study observed decreased in copper concentration ($p < 0.01$) in all types of leukaemia; CLL, CML, AML and ALL compared with control. Serum Cu was significantly lower ($p < 0.001$) in groups of leukemic patient their age group was 16- 30 , 31- 45 and Over 45 years. While Leukemic children aged less than one year to 15 years not showed significant difference in the level of cupper compared with control . Copper serum level was significantly lower in the male cases ($p < 0.001$) and female cases ($p < 0.01$) than the male and female control respectively. Female cases also showed lower level than male control (*p*

< 0.001). Cu level was significantly higher in female case than male case ($p < 0.05$). No significant difference in concentrations level of copper in male control and female control. The variation of serum copper in patients with leukaemia has been described before. However, the changes in serum copper concentration found in some leukemic patients (Zuo *et al.,* 2006) . In the current study copper concentration was decreased in all subtypes of leukaemia , in age groups older than 16 years and in sex groups.This findings of Cu levels are in agreement with study conducted by (Rafallah, 2015). Some investigators combined between copper deficiency and haematological and neurological abnormalities. They consider copper deficiency as an established cause of haematological abnormalities but is frequently misdiagnosed (Thorvardur *et al.,* 2008).

Concentration of manganese in the serum of patients in all groups ; CML, CLL, AML and ALL studied is significantly lower ($p < 0.05$) compared with control. No significant difference in concentration level of manganese in all age groups; less than one year to 15 years , 16- 30 , 31-45 and Over 45 years. The concentration of manganese in serum was significantly lower in male case and female case than in male control ($p < 0.05$) and ($p < 0.01$) respectively . Whereas no difference was found in female case compared with male case and female control. No significant difference in concentrations level of Mn in male control and female control. Manganese (Mn) is necessary for optimal biological function that is required as a cofactor for many enzymes (Parmalee and Michael, 2016). In the current study, serum manganese showed significantly lower concentration . This finding was agree with a study done by Cengiz *et al.* (2011) who reported : significant decrease in the serum concentration of Mn in leukaemia patients. Ana Bela *et al.* (2012) showed that when Mn level was decreased the concentration of an antioxidant enzymes also

decreased and low levels of oxidative stress so increasing free radicals generation, stimulate cellular proliferation and involved in the pathogenesis of human leukaemia.

Significantly lower concentration of cobalt in all groups ; CML, CLL , AML and ALL ($p < 0.05$). Cobalt recorded significantly lower ($p < 0.001$) level in all groups of age; less than one year to 15 years , 16- 30 , 31- 45 and Over 45 years. The concentration of cobalt in serum was significantly lower in male case and female case than in male control ($p < 0.001$). The results also showed lower significant difference in male case and female case than female control ($p < 0.001$). Whereas no difference was found in male case compared with female case. No significant difference in concentrations level of Co in male control and female control. In this study there was low cobalt level in serum of different subtypes of leukemic patients, age and sex groups. This disagree with Cengiz *et al.* (2011) who reported no significant difference in serum level of cobalt between leukaemia patients and control. While Sheppard *et al*. (2007) suggested that in study conducted in Nevada they a temporal correspondence between the onset of excessive childhood leukaemia and elevated levels of tungsten and cobalt. Lingamaneni *et al*. (2015) said that the deficiency of cobalt leads to decreased availability of B12, and developed many symptoms and problems attributed to B12 deficiency, particularly pernicious anemia, nerve damage and a significant increase in the incidence of lymphatic and hematopoietic malignancies.

In the present study we found serum TIBC level was lower in patients with ALL ($p < 0.001$) , CML and AML ($p < 0.05$) than control. While no differences in serum TIBC level was noticed between those of CLL and controls. CLL patients had higher concentration of TIBC in serum

than ALL group ($p < 0.05$). In ages groups ; less than one year -15 , 16-30 and 31- 45 showed significant lower level of TIBC in cases than control ($p < 0.001$), ($p < 0.001$) and ($p < 0.01$) respectively. While odder patients ≥46 revealed higher concentration ($p < 0.05$) than healthy subjects. Serum TIBC level was lower in male case than male and female control ($p < 0.05$). Female cases had significantly ($p < 0.01$) lower level of TIBC than female control. No significant difference in concentrations level of TIBC in male case and female case. Also no differences in serum TIBC levels were found between male control and female control. Determining the total iron binding capacity of serum (a measure of transferrin concentration) provided a diagnostic test for both iron deficiency and iron overload (Worwood, 1997). Total iron-binding capacity (TIBC) concentration in this study recorded variation in its level. Three subtypes of leukaemia (ALL,AML and CML) showed lower levels, while no difference in CLL group. Ages groups younger than 45 years also showed lower levels , while odder patients ≥46 revealed higher concentration than healthy subjects. Male and female cases reported lower level of TIBC. These finding agreed with the studies done by Karp and Merz (1986) and Halonen *et al.*,(2003) who reported that patients with leukaemia have lower concentration of total iron binding capacity or increased transferrin saturation values and iron overload. Osma *et al.* (1980) reported that patients with leukaemia have high concentration of serum iron , which may be at least partially attributed to a decrease in iron consumption caused by diminished bone marrow cell erythropoietic activity. Unal *et al*.(2014) conducted that; transfusion of iron to pediatric patients with ALL lead to iron overload and increased cardiac iron loading.

CONCLUSION

The present study concluded that:

- All leukaemia patients – in Sudan - included in this study recorded significant decrease in serum levels of copper, manganese and cobalt.
- Most of leukaemia patients showed higher magnesium levels and lower concentration of zinc and calcium.
- Measuring of total iron binding capacity in patients , revealed that most leukaemia patients have iron overload.
- Female patients showed decreasing in concentration of calcium and increasing in concentration of copper compared to male patients.
- Acute lymphoid leukaemia (ALL) is most abundant leukaemia in children over the world, and in Sudan also.
- Children group; less than 15 years showed normal levels of most elements such as Mg, Ca, Cu and Mn.
- Leukemic patients showed normal concentrations of Na, K and PO_4^{3-}.

RECOMINDATION

- In this study, determination of serum electrolytes and trace elements in leukemic patients indicates an abnormal level of electrolyte and trace elements.
- This study emphasizes the need for routine measurement of serum electrolytes , trace elements and observed the overload of iron during all phases of the leukemic process.
- Further studies are needed to illustrate the role of these elements in developing the leukaemia.

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