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A Study of Hepcidin Activity in Acute Leukemic Patients

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By
Narjiss Fadhil Obaid Rady
(B.Sc. Biology / Babylon University, 2019)

Supervised by
Assist. Prof. Dr. Walaa Salih Hassen Thaher

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وزارة التعليم العالي والبحث العلمي
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رسالة

مقدمة إلى مجلس كلية العلوم/ جامعة بابل كجزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

{ يَرْفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمْ وَٱلَّذِينَ أُوتُواْ ٱلْعِلْمَ دَرَجَٰتٍ وَٱللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾ }

صدق الله العظيم

سورة المجادلة الآية (١١)

Dedications

I dedicate this work

To the Lord of the worlds, who asks him for help at every moment …

To our Master Muhammad (PBUH), the Seal of the Messengers and Prophets, and to every member of his family…

To my brother, the martyr who is absent from my eyes and present in my heart..

To my mother and father, who worked incredibly hard to get me here...

To my beloved husband, who made an effort to walk alongside me at every turn...

To my brothers and sisters who have worked constantly for my success...

NARJISS

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Summary:

The present study was done in department of biology, faculty of science, university of Babylon. The sample collection and practical work of the study extended through the period November 2021 to May 2022, samples of acute leukemia collected from Merjan Teaching Hospital in Babil ,the Imam Hussein Center for Oncology and Hematology in Karbala and from Baghdad Teaching Hospital in the Medical City. Our study aimed to evaluate the activity of Hepcidin in erythropoiesis in patients with acute leukemia.

The samples are male and female with age ranged 9 month - >41 years old. Which include 101 blood samples ,classified to groups as following: control group 36 samples including 20 males and 16 females and patients group 65 samples including 34 males and 31 females, collected from patients with acute leukemia of both types lymphoid and myeloid (ALL,AML). The samples are divided to three group according to the age, they include first 9 months - 20 age group, second 21 - 40 age group and third > 41 age group .

The present study is divided to three parts: demographic, hematologic and biochemical studies. and the correlation of age and gender with all studied parameters. The results for hematological parameter found that a significant increase ($p \leq 0.05$) in white blood cells between patients with acute leukemia (ALL,AML) compared to the control group.

The results appeared that there is a significant differences ($p \leq 0.05$) in red blood cells between patients with acute lymphoid leukemia and acute myeloid leukemia patients . In addition to a significant decrease between patients with acute myeloid leukemia and the control group. The mean platelet count was significantly increased ($p \leq 0.05$) in patients with acute leukemic group compared to the control group. The results also showed a significant increase in AST and ALT liver in leukemic patients compared with the control group.

The results showed that the statistical analysis of Hepcidin in acute leukemia patients is significantly increased ($p \leq 0.05$) than healthy subjects. In addition, there are a significant increase ($p \leq 0.05$) for Ferroportin, iron and Ferritin concentration in acute leukemic patients compared with the healthy subjects. Also correlation results indicated positive correlation among acute lymphoid leukemic patients (ALL), as a positive relationship is found between Hepcidin and Ferroportin with a significant difference in its value (.646**), as well as there are a direct proportional relation between AST and ALT . While Ferroportin is inversely proportional to AST, Ferritin ,Iron and directly proportional to ALT, with no significant differences. AST is directly proportional to ALT Ferritin and Iron with a significant difference of its value (.554**) with ALT. Relationship between Lymphocytes and PLT it is inversely proportional with a significant difference of its value (-.475**). While relationship between Hemoglobin and Red Blood Cells it is directly proportional with a significant difference of its value (.765**). Among myeloid leukemic patients (AML),there are a negative correlation relationship between White Blood Cells and Ferritin with a significant difference in its value (-.641 *) ,While positive correlation between Lymphocytes and iron with a significant difference its value (.646*) , and there were a directly proportional between RBC and Hb with significant difference its value (.812**)

While Hepcidin is directly proportional to Ferroportin ,AST, ALT ,Iron with significant difference its value (.936**) with Ferroportin and inversely correlation with Ferritin .Ferroportin is directly proportional with AST, ALT ,Iron and inversely with Ferritin . As for Ferritin, it is directly proportional to iron. Whereas for AST is directly proportional to ALT, iron and inversely proportional to Ferritin with significant difference its value (-.641*). The results of our study with respect to gender appear that men are more likely to develop leukemia than females. with

regard to age assessment, the prevalence of acute lymphoblastic leukemia (ALL) was higher in the group of patients aged 9 months to 20 years.

List of Abbreviations

	Name
ALL	Acute Lymphoblastic Leukemia
ALT	Alanine Transaminase Activity
AML	Acute Myeloblastic Leukemia
ANOVA	Analysis of Variance
AST	Aspartate Teansaminase
BCR-ABL1	Fusion Protein
CBF	Core Binding Factor
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myelocytic Leukemia
CSF	Cerebrospinal Fluid
DMT1	Divalent Metal Transporter 1
EBV	Epstein-Barr Virus
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Linked Immune Sorbent Assay
ETV6-RUNX1	Fusion Gene
FAB	French-American-British
Fpn	Ferroportin
FSL1	a synthetic lipoprotein
FTH, FTL	Ferritin Is Composed of Light And Heavy Chains
GOT	Glutamic Oxyaloacetic Transaminase
GPT	Glutamic-Pyruvic Transaminase
HIV	Human Immunodeficiency Virus
HSCs	Haemopoietic Stem Cells

HTLV-1	Human T-Lymphotropic Virus
IFN	Interferon
IL-6	Interleukin 6
IRE	Iron-Responsive Element
JNK	Jun N-Terminal Kinase
LEAP-1	Liver Expressed Antimicrobial Peptides
LPS	Lipopolysaccharides
LYM	Lymphocyte
Pam3CSK4	a synthetic triacylated lipopeptide
PAX5	Paired Box 5 Gene
Plt	Platelet
ROS	Reactive Oxygen Species
S.E	Standard Error
SARS	Severe Acute Respiratory Syndrome
SPSS	Statistical Package for Social Science
TCA	Trichloroacetic Acid
TCF3-PBX1	Fusion Protein
TLR2\6	Toll-like receptors
WHO	World Health Organization

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CHAPTER ONE

Introduction

1-1 Introduction

The term "leukemia" comes from the Greek words "leukos" and "heima," which refer to an excess of white blood cells (WBC) in the body. Leukemia, which was once thought to be a single disease, was first recognized around the fourth century. Leukemia were divided into four subtypes by the end of the nineteenth century: acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia, and chronic lymphocytic leukemia. The prognosis for leukemia is currently known to include a variety of complex and unique hematopoietic tumors. Morphological differences, cytogenetic abnormalities, immune phenotype and clinical features further distinguish each subtype **(** Bryant.,2014).

A malignancy of immature lymphocytes is known as acute lymphocytic leukemia ,also known as acute lymphoid leukemia and acute lymphoblastic leukemia. One class of mature WBC that is a crucial component of the immune system are lymphocytes. An immature WBC called a lymphoblast develops into a lymphocyte, which is present in both the blood and the lymphatic system. The term "leukemic cells" can also refer to abnormal or malignant lymphoblasts. These cancerous cells multiply and divide, displacing healthy cells (Blackburn *et al*.,2019). The most prevalent childhood cancer is acute lymphoblastic leukemia , which continues to be a leading cause of morbidity and mortality in both children and adults despite having a cure rate of over 90% in young patients **(**Iacobucci and Mullighan,2017). Acute lymphoblastic leukemia is a kind of leukemia that affects lymphoid progenitor cells in the bone marrow, blood, and extramedullary areas (ALL). Adults are also at significant health risk, even though children are involved in 80% of ALL cases. According to projections, 1.6 cases of ALL will occur in the US for every 100,000 people. In 2016, there were around 6590 new instances of

ALL recorded, and more than 1400 individuals died from the condition A bimodal distribution of ALL incidence may be seen, with the first peak appearing in babies and the second peak appearing in those over 50 years of age (Howlader *et al* .,2016). While dosage intensification methods have greatly improved children patients' outcomes, the outlook for older patients is still dismal. Despite the high response rate to induction chemotherapy, 30–40% of adult ALL patients will go into long-term remission (Jabbour *et al* .,2015).

Acute myeloid leukemia (AML) is a cancer of the stem cells that give rise to the myeloid lineage of blood cells, which includes platelets, B and T cells, and other white blood cells (Pelcovits and Niroula ,2020). AML is a bone marrow disorder characterized by an overproduction of neoplastic clonal myeloid stem cells as a result of genetic changes in blood cell precursors (Grimwade *et al*.,2016). Furthermore is a kind of malignancy that is distinguished by the infiltration of proliferative, clonal, abnormally differentiated, and occasionally poorly differentiated hematological system cells into the bone marrow, blood, and other organs (Döhner *et al*.,2015). In the United States, the age-adjusted incidence of AML is 4.3 for every 100,000 people each year (US). Incidence rises with aging, with 68 years old in the United States as the median age at diagnosis. Heterogeneity exists in the etiology of AML. Most cases of AML still lack a clear etiology, but in some patients, a history of therapeutic, occupational, or environmental DNA-damaging agents is implicated. Adults with AML have the highest prevalence of acute leukemia and the shortest median survival time (Shallis *et al*.,2019).

The amount of total body iron is determined by the iron-regulatory hormone hepcidin. Hepatocytes secrete hepcidin, which regulates the activity of ferroportin, a cellular iron exporter that transports iron from stores and intestinal iron

absorption to plasma. Hepcidin concentration in plasma is increased by iron loading and inflammation, and it is suppressed by erythropoietic stimulation and during pregnancy. Iron overload in hemochromatosis and anemias with ineffective erythropoiesis are brought on by a hepcidin deficiency. Iron-restrictive anemias, such as anemia of inflammation, are brought on by an excess of hepcidin (Nemeth and Ganz, 2022).

A variety of proteins also control the metabolism of iron. Hepcidin, a 25-amino acid protein released largely by hepatocytes, is the main regulator of iron homeostasis . Hepcidin, the only cellular iron exporter, controls iron absorption and release from tissue stores by targeting ferroportin and inhibits the entrance of iron into the plasma by downregulating ferroportin. Research on the biological processes involved in the release of circulating ferroportin (FNP-1) from the cell membrane as well as its role in the body is ongoing. It is understood that the membrane version of this protein functions as a transporter for the transfer of iron from cells to the circulation, where it is broken down by hepcidin (Coates, 2019; Ginzburg, 2019). A peptide hormone having antibacterial effects is hepcidin. The liver created it in response to the stimulation of inflammation and an excess of iron. In the beginning, hepcidin is discovered in human serum and urine. It is first found in the ultrafiltrates of plasma and it exhibits in vitro bactericidal action under the name liver expressed antimicrobial peptides (LEAP-1) (Ganz ,2013). When iron reserves increase, which is mostly caused by inflammation, hepcidine is produced in a manner that is stimulated; otherwise, anemia, hypoxia, and an increased erythropoiesis urge prevent it from being expressed in that way (Ganz and Nemeth, 2006). In the pathophysiology of many diseases, including anemia in malignancies, damage regulators play a role. Current research on hepcidin expression in acute leukemia is lacking (Ragab *et al.*,2016). Erythropoiesis and

iron metabolism are closely related, and problems in iron metabolism have been connected to a variety of malignancies, such as leukemia (Kanda *et al*.,2008; Wang and Pantopoulos,2011).

1-2 Aims of the study: evaluate the hepcidin activity in patients with acute leukemia of both types lymphoid and myeloid by:

1- Studying epidemiological factors through questionnaire such as family history for cancer,sex,age...etc.
2- Complete blood count examination by using a hematology analyzer.
3- Estimating for hepcidin activity.
4- Measuring the ferroportin concentration
5- Determining of serum ferritin and iron concentration
6- Estimating of (AST) GPT and (ALT) GOT enzyme.

CHAPTER TWO

Literatures review

2- Literatures Review

2-1 Acute Leukemia

Aggressive diseases called acute leukemia are marked by an overgrowth of immature malignant cells in the bone marrow (NØrgaard *et al*.,1996). Rapid onset, rapid progression, and unavoidable death are characteristics of acute leukemias. They are distinguished by a buildup of early hemopoietic cells, or blast cells (Ah-Moye *et al* .,2014). The types of acute leukemia include:

2-1-1 Acute Lymphoblastic Leukemia (ALL)

A feature of the heterogeneous hematologic condition known as acute lymphoblastic lymphoma (ALL) is the proliferation of immature lymphoid cells in the bone marrow, peripheral blood, and other organs (Jabbour et al., 2005). According to a bimodal distribution of ALL, the first peak appears in individuals around the age of 5, and the second peak appears around the age of 50. It is primarily a pediatric leukemia because 20% of adult cases and 80% of child cases involve children. The average patient is 14 years old when they are given their diagnosis, and 60% of patients do so before they are 20 years old, 25% before they are 45 years old, and 11% before they are 65. In the late stage, ALL is relatively uncommon (Paul and Kantarjian ,2016). As with other leukemia, a variety of variables, have been postulated to have a role in the development of ALL. Environmental variables such as parental preconception, in utero and post-natal ionizing radiation exposure have all been studied. Nonionizing radiation, chemicals, diseases, hydrocarbons, and pesticides have also been studied its consequences on the development of ALL in their children have been studied. The significance of genetics in the development of leukemia in general, and acute lymphoblastic leukemia in particular, cannot be overstated. Concordance studies

on identical twins with leukemia highlight the relevance of genetics (Zipf *et al*.,2000; Greaves *et al*.,2003).

Acute lymphoblastic leukemia (ALL) is categorized into FAB L1 (children), L2 (older children and adults), and L3 (those with leukemia related to Burkitt's lymphoma). These categories are categorised based on two factors: (1) the presence of distinct cytologic characteristics, and (2) the degree of heterogeneity among the leukemic cells. Cell size, chromatin, nuclear structure, nucleoli, level of cytoplasmic basophilia, and cytoplasmic vacuolation are among the characteristics taken into account (Bennett *et al*., 1976; Harris *et al* .,1999).

2-1-2 Acute myeloid leukemia (AML)

The malignancy known as acute myeloid leukemia (AML) is characterized by the invasion of proliferative, clonal, incorrectly differentiated, and sometimes poorly differentiated hematological system cells into the bone marrow, blood, and other organs. Despite being incurable 50 years ago, AML is now treatable in 35 to 40% of adult patients who are 60 years of age or younger and in 5 to 15% of those who are older than 60. The prognosis is still grim, with a median longevity of just 5 to 10 months in senior patients who are unable to get vigorous therapy without unpleasant side effects (Döhner *et al*.,2015).

An abnormal proliferation of abnormal myeloid progenitors in the bone marrow and blood, as well as a block in myeloid differentiation-the process that normally results in the production of mature blood cells from haemopoietic stem cells (HSCs)-are characteristics of acute myeloid leukemia (AML), a type of cancer. Although most cases develop spontaneously, some cases are caused by other haematological conditions or as a consequence of genotoxic therapy for other malignancies (Østgård *et al*.,2010). Acute myeloid leukemia (AML) is a

malignancy of the stem cells that make up the myeloid lineage (red blood cells, platelets and lymphocyte other than B and T cells). Similar to other cancers, it is brought on by genetic defects that result in neoplastic changes and clonal growth. Despite still being a rare disease, AML accounts for more than one-third of all leukemia diagnoses and just 1.2% of all new cancer diagnoses in the United States each year (Pelcovits and Niroula ,2020).

AML is a clonal disease characterized by the growth of immature myeloid cells and failure of the bone marrow. Cytogenetics and mutation testing continue to be crucial prognostic tools after induction treatment. AML treatment has remained steady over the last three decades despite significant improvements in the field, such as new drug targets and more biological understanding, with the majority of patients ultimately relapsing and passing away from the condition (Saultz and Garzon , 2016). Children with acute myeloid leukemia have a rare and varied disease in terms of morphology, immunophenotyping, germline and somatic cytogenetic abnormalities, and genetic abnormalities. Results have significantly improved over the last three decades, although survival rates are still about 70% and recurrence rates are at 30% (Quessada *et al* .,2021).

2-2 Etiology of Acute Leukemia

2-2-1 Age and Race

The incidence of leukemias is significantly influenced by race and age. For instance, in the United Kingdom, those over 65 years account for 42.8% of all leukemia cases (Deschle and Lübbert, 2008). According to a study of the topic conducted in the United States, the age-adjusted incidence of leukemia is generally 15 for every 100,000 in the White population, followed by 11 for every 100,000 in the Black population and 10.6 for every 100,000 in the Hispanic population.

Asian/Pacific Islanders and American Indians/Alaskan Natives both had incidence rates of 7.8 for every 100,000 and 8.3 for every 100,000, respectively. Age-adjusted mortality rates for every 100,000 people were found to follow similar racial and ethnic patterns, with Whites having the highest rate at 7, followed by Blacks at 5.6, Hispanics at 4.8, Asian/Pacific Islanders at 3.8, and Indian/Alaskan Natives at 3.3 (Bispo *et al*.,2020). All age groups are impacted by leukemia, however the disease's spread differs depending on the kind. Some leukemias, like ALL, are more common in the White population than the Black population. According to a 2011 research by the American Association for Cancer Research, among pediatric patients diagnosed in the United States, Hispanic youngsters had one of the lowest survival rates and the greatest prevalence of ALL. According to the findings, the overall mortality risk for ALL is, respectively, 46 and 45 percent higher for Blacks and Hispanics than it is for Whites. In comparison to the White population, these two groups had a 12 and 6 percent greater rate of rise in AML-related deaths (Smith *et al*., 2011).

In individuals who are 15 years old or younger, AML accounts for just 15-20% of instances (Aquino,2002) Up to the age of four years, the incidence rate gradually declines after reaching its peak in the first year of life. Childhood and the early years of adulthood see very little change in the incidence rate (Gurney *et al*.,1995). So, AML is a condition that affects elderly people . 42.8% of leukemia patients in the UK are over 65 years old, according to the distribution of common cases of all leukemia (Forman *et al* .,2003). The median age of those receiving an AML diagnosis is 65 (Sekeres *et al*.,2002). Prior to the age of 40, it is uncommon; however, after that, the prevalence rises steadily with age. AML had an age-adjusted incidence rate of 3.6 for every 100,000 for both sexes in the United States

of America (USA) in 2004, 4.5 for every 100,000 for men, and 3.0 for every 100,000 for women (Ries *et al* .,2004).

2-2-2 Genetic Factors

Genetics is a significant factor in the genesis of leukemia. There is a substantial amount of recent material on the connection between genetic variables in normal hemopoiesis and the development of acute leukemias as well as the processes of leukemogenesis. Leukemia may be inclined to develop in patients with chromosomal abnormalities and impairments of DNA repair. In the absence of extramedullary characteristics, some hereditary mutations may increase the chance of developing leukemia. Leukemia is more common in certain families despite no known genetic abnormalities for the disease (Stieglitz and Loh ,2013 ; Heuser *et al*.,2016).

Leukemic transformational events can include several factors. In the pediatric age range, the ETV6-RUNX1 fusion gene, for instance, is acquired in utero but requires a subsequent somatic mutation to be activated. It is present in around 25% of ALL cases. Genetic changes in B-cell ALL include hyperdiploidy, hypodiploidy, BCR-ABL1, ETV6-RUNX1 or TCF3-PBX1 fusions, PAX5 or ETV6 mutations, MLL rearrangements, or intrachromosomal amplification of chromosome 21 (iAMP21), which is unique to B-cell ALL. These genetic changes are specific to each ALL immunophenotype. T-cell ALL is characterized by changes in LMO2, TAL1, TAL2, TLX1, TLX2, or HOXA (Andersen *et al*.,2001; Pui *et al*., 2019). Important risk factors for AML in childhood include genetic abnormalities and constitutional genetic flaws (Deschler and Lübbert,2008). Acute leukemia is more likely to occur in children with Down syndrome by a factor of 10 to 20 (Bhatia and Neglia, 1995). Klinefelter's syndrome, Li-Fraumeni syndrome

(Pötzsch *et al*.,2002). Fanconi anemia and neurofibromatosis are further hereditary conditions linked to AML. (Aquino, 2002).

2-2-3 Environment and Occupations

Leukemia development has been linked to a wide range of environmental factors. These mostly entail being exposed to cancer-causing substances throughout one's life, such as chemicals, infections, and radiation (Sandler ,1992) Leukemia risk has been linked to certain exposures, jobs, workplace dangers, and pastimes (Wong *et al*., 2010) . It is uncertain and sometimes debatable how particular jobs and the prevalence of acute leukemias are related. Agriculture, forestry labor, and crop production including exposure to pesticides and fertilizers are among the occupations said to be linked to an elevated risk for leukemias (Descatha *et al*.,2005; Terry *et al*.,2005) . Occupations in the oil and gas sector that expose workers to benzene, positions in the electrical utility sector, and jobs that expose workers to magnetic fields (Törnqvist *et al*., 1991). Working in the nuclear power business and being exposed to ionizing radiation, as well as nursing and healthcare jobs that expose one to infectious diseases and viruses (Descatha *et al*., 2005). It has been hypothesized that parental use of alcohol and smoking throughout pregnancy, neonatal development, and childhood may contribute to the onset of leukemia in their kids. According to Pang *et al*., (2003), the risk may be correlated to the intensity, frequency, duration, and scope of the exposure. The risk of ALL and AML is said to be 10 times higher in mothers who use marijuana before, during, or after pregnancy.

2-2-4 Effects of Radiation

Ionizing radiation exposure throughout several stages of life, such as preconception, in utero, and post-natal exposures, has been linked to the development of leukemia, and many cases have been documented. Leukemia incidence and radiation exposure dosage have been shown to be correlated (Ron , 1998). Leukemia was 20 times more common among survivors who were within 1000 meters of the blasts in the aftermath of the bombings of Hiroshima and Nagasaki, Japan, than it was among the general population. It has been investigated how the Chernobyl nuclear power plant catastrophe has affected society (Mahoney *et al*., 2004).

Data on the connection between diagnostic x-ray exposure and a person's chance of developing leukemia are inconclusive. Ionizing radiation exposure is related to AML (Wartenberg *et al*.,2008) .An elevated incidence of AML was seen among those who survived the nuclear explosions in Japan, with a peak around 5-7 years following exposure. Additionally, it has been shown that therapeutic radiation raises the incidence of secondary AML (Kossman and Weiss , 2000).

2-2-5 Infections

Leukemogenesis has been linked to several types of infections, such as bacterial, viral, and fungal ones, both alone and in association with genetic alterations. Acute leukemias in particular and cancer in general have been linked to infections, it has been hypothesized (Tebbi *et al*., 2021). There was been speculation and investigation into the role that various infectious agents, such as the Epstein-Barr virus (EBV), herpesvirus, human immunodeficiency virus (HIV), severe acute respiratory syndrome (SARS) and human T-lymphotropic virus (HTLV-1), among others, may play in the development of leukemia (Belson *et*

al.,2007; Maia and Wunsch, 2013; Tebbi *et al*., 2021). Although a link between the exposure to certain viruses and the development of AML has been suggested, no definitive retrovirus has been shown to be the cause of AML in people. The pathogenesis of AML may thus be influenced by parvovirus B19. However, it has not yet been shown that a simple infection with a virus that is either RNA- or DNA-based alone is the cause of AML (Kerr *et al.,* 2003).

2-3 Regulation of iron metabolism

Iron is an essential nutrient. It is required to maintain normal cell metabolism. Iron is necessary for the iron-containing enzymes involved in ATP synthesis, DNA synthesis, oxygen transport, and many other physiological processes. Iron is able to participate in reactions that produce free radicals because of its capacity to gain and lose electrons (Dixon and Stockwell , 2014). Among these is the Fenton reaction, in which ferrous iron (Fe^{2+}) transfers an electron to hydrogen peroxide to produce a very invasive reactive oxygen species (ROS) known as the hydroxyl radical (Fischbacher *et al*., 2017). Multiple cellular signaling pathways that are essential for cell survival, proliferation, and differentiation are affected by ROS (Lambeth *et al*., 2014). However, abnormal iron buildup and the resultant overproduction of ROS result in oxidative stress, which damages DNA, proteins, lipids, and other macromolecules and may even induce cell death (Dixon and Stockwell, 2014). Studies have linked irregular iron metabolism to a variety of disorders, including cancer, neurological diseases, and atherosclerosis (Vinchi and Platzbecker ,2020). Iron is necessary for the development of cancer and its oxidative effects on oncogenesis (Ward *et al*., 2014). A class of diverse hematopoietic stem cell (HSC) cancers known as leukemia. It is characterized by an abnormal buildup of undifferentiated blasts that may proliferate unchecked in the bone marrow, interfering with the development of

healthy blood cells. Leukemia is one of the most often fatal malignancies, particularly acute leukemia (AL) (Siegel *et al* ., 2019).

2-3-1 Hepcidin

The liver produces hepcidin, a 25-amino-acid (a.a) peptide hormone that controls iron levels. Hepatocytes are the main producers of hepcidin, although it may also play a role in the local autocrine and paracrine regulation of iron fluxes in other tissues and cells, including macrophages, adipocytes, and the brain, due to its low levels of expression in these tissues and cells (Valore and Ganz,2008). It controls the amount of iron that is taken up by absorbing intestinal cells, erythrocyte-recycling macrophages, iron-storing hepatocytes and supplied to blood plasma. Ferroportin, the lone cellular iron exporter that carries iron from all iron-transporting cells to the plasma, is attached to by hepcidin and rendered inactive. Iron storage and plasma iron act as a traditional endocrine feedback mechanism to induce hepcidin production. The fact that inflammation also stimulates hepcidin suggests that it could be involved in innate immunity. The suppression of hepcidin by increased erythropoietic activity results in greater iron absorption and release from storage, balancing the supply of iron with the rise in demand. Hepcidin is suppressed by the hormone erythroferrone, which is produced by erythroblasts driven by erythropoietin (Ganz, 2016).

The accumulation of radiolabeled hepcidin in ferroportin-rich tissues shows that hepcidin is eliminated from those tissues by receptor-mediated endocytosis. (Rivera *et a l.,* 2005). Hepcidin controls ferroportin-mediated cellular iron export to plasma and extracellular fluid. The hepcidin receptor and ferroportin are the only two known cellular iron exporters in vertebrates. The body's professional iron

handlers, such as the duodenal enterocytes that absorb dietary iron, the liver and spleen macrophages that recycle old erythrocytes, the hepatocytes that store iron, and the placental trophoblasts that transfer iron to the fetus during pregnancy, are cells that contain ferroportin. (Donovan *et al*., 2005).

Hepcidin plays a crucial role in iron metabolism because it may regulate the export of iron from cells. The primary iron export protein ferroportin (FPN), which is responsible for hepcidin's activity by breaking down FPN, hepcidin prevents iron export from cells like macrophages and enterocytes. (Ganz and Nemeth, 2012). An 84-amino-acid precursor called prepropeptide is created by the hepcidin gene. A mature peptide of 25 amino acids is produced from the precursor by processing it via two cleavages. (Poli *et al*.,2014) . A FPN extracellular loop important in ferroportin ubiquitination, disulfide bridging, and proteasomal degradation is where hepcidin-25 interacts. FPN1 is downregulated as a consequence of the dissolution of the hepcidin/FPN1 complex, which induces intracellular iron sequestration. (Qiao *et al*., 2012).

2-3-2 Hepcidin Regulation By Erythropoiesis

Because of the high iron requirements for hemoglobin synthesis, erythropoiesis dominates regulation of iron metabolism requiring significant crosstalk. For instance, iron absorption increases, often dramatically, during stress erythropoiesis to accommodate the higher iron demand. Recent data provides mechanistic evidence of an iron restriction response, demonstrating regulation of erythroid precursor proliferation and differentiation during iron restriction (Bullock *et al.*, 2010; Khalil *et al.*, 2018), in addition to an expected decrease in per cell and total hemoglobin synthesis. Conversely, disease states of excess iron are often

associated with expanded RBC size and higher cellular hemoglobin concentrations as a way of sequestering iron into a non-toxic compartment (McLaren *et al*., 2007). Furthermore, diseases in which anemia and excess iron coexist exhibit complicated regulation schema that remain incompletely understood. Such diseases of concurrent iron overload and expanded or ineffective erythropoiesis (e.g., β-thalassemia, myelodysplastic syndromes, and dyserythropoietic anemias) exhibit lower than expected hepcidin expression, despite increased iron stores. Because hemoglobin formation requires a large amount of iron, erythropoiesis predominates in the control of iron metabolism, necessitating substantial crosstalk. For instance, to meet the increased iron need under stress, iron absorption rises, often drastically. Recent research demonstrates the modulation of erythroid precursor proliferation and differentiation under iron limitation, providing mechanistic evidence of a response to iron restriction (Bullock *et al.*, 2010; Khalil *et al.*, 2018), in addition to a predicted decline in total and per-cell hemoglobin production. On the other hand, illness conditions with excess iron tend to sequester iron into non-toxic compartments by enlarging RBC size and increasing cellular hemoglobin concentrations .Additionally, conditions where anemia and too much iron coexist show complex regulatory systems that are yet poorly understood. Even though they have higher iron reserves, illnesses including -thalassemia, myelodysplastic syndromes, and dyserythropoietic anemias that simultaneously have enlarged or inefficient erythropoiesis show lower than predicted hepcidin expression (McLaren *et al*., 2007).

2-4 Hepcidin-Ferroportin Axis Regulates Iron Flows

Iron export via ferroportin into plasma is primarily controlled at the basolateral surface of the duodenal enterocyte to limit iron intake. Divalent metal transporter 1 (DMT1) transports iron into the duodenal enterocyte on the apical membrane, where it is either stored or excreted over the course of a few days. When duodenal enterocytes are shed in the gastrointestinal system, iron that is kept inside the enterocyte but not exported will be lost. Macrophages also express ferroportin. By participating in the erythrophagocytosis of senescent RBCs, macrophages degrade hemoglobin and recycle iron. The two main macrophage populations responsible for steady-state erythrophagocytosis are kupffer cells, which are found in the liver sinusoids, and red pulp macrophages, which are found in the spleen (Willemetz *et al.*,2017).

Iron released from hemoglobin is either exported through ferroportin or secluded inside cytosolic ferritin, much as in duodenal enterocytes. Hepcidin binds to the ferroportin receptor, which causes ferroportin to be endocytosed and degraded, which reduces iron export. Hepcidin controls the acquisition of dietary iron as well as the release of iron from macrophages. In conditions associated with high hepcidin, iron absorption and recycling are decreased, which results in a decrease in circulating serum iron concentration and transferrin saturation as well as an increase in serum ferritin concentration, the latter proportional to the increased intracellular ferritin, which is traditionally driven by increase in hepcidin (Arezes *et al*., 2015).

2-5 Disorders of Hepcidin

2-5-1 Hepcidin Deficiency

Hepcidin deficiency causes an excess of iron and is common in hematological conditions marked by ineffective erythropoiesis, including thalassemias, dyserythropoietic anemias, and myelodysplastic syndromes (Ginzburg and Rivella , 2011; Camaschella and Nai , 2016). Inefficient erythropoiesis is characterized by increased proliferation of bone marrow erythroblasts due to decreased synthesis of mature RBCs. Due to an increase in iron need, hepcidin suppression occurs despite systemic iron surplus. The positive iron signals are clearly outnumbered in this situation by the negative erythropoietic signals. Erythropoietic suppression of hepcidin causes iron overload in non-transfused patients with mild forms of iron loading anemia, and it exacerbates secondary iron overload in transfused patients. In the first case, replenishing hepcidin could prevent iron overload, but in the second, it might boost the effectiveness of iron chelation therapy (Ginzburg and Rivella , 2011).

2-5-2 Hepcidin Excess

Chronic inflammatory conditions brought on by viral or autoimmune illnesses, as well as cancer, often show excessive hepcidin expression (Weiss, 2015; Wang and Babitt , 2016). Hepcidin induction is mainly mediated by IL-6, which results in hypoferremia in tissue macrophages as a result of ferroportin degradation and iron sequestration. Iron metabolism-remodeling pathways that are hepcidin-independent may make this phenotype even worse. In this way, ferroportin expression is decreased by LPS and IFN-, which in turn limits iron efflux from monocytes (Ludwiczek , 2003). Toll-like receptor ligands FSL1 or

PAM3CSK4 (TLR2/6) decrease ferroportin transcription in tissue macrophages, resulting in hypoferemia (Guida *et al*., 2015).

2-6 Ferroportin

The only known iron exporter in vertebrate cells is ferroportin (Fpn), which controls cytosolic iron levels and exports iron to plasma to preserve iron homeostasis. Iron and other transition metals may influence ferroportin1 expression (FPN1). Fpn posttranslationally may be changed by internalization and degradation by hepcidin (Troadec *et al.*,2010). For iron to enter plasma, ferroportin (Fpn), the only known mammalian iron exporter is necessary (Wessling-Resnick,2006; Harmse, 2017). The cells that express Fpn at the greatest quantities include syncytial trophoblasts, hepatocytes, and duodenal enterocytes. However, a variety of distinct cell types may create Fpn in response to heme (Delaby *et al.*, 2008; Marro *et al.*,2010) or iron (Delaby *et al.*,2005; Aydemir *et al.*,2009; Yang *et al.*,2002) . Iron may influence transcriptional and translational mechanisms that control the expression of Fpn. Iron-responsive element (IRE) on the ferroportin1 (FPN1) gene, which codes for Fpn, enables iron regulatory proteins to control the translation of this gene. The increased expression of Fpn by iron is thought to be a reaction to cellular iron load since more cytosolic iron results in improved iron export. The only known iron exporter in mammals is ferroportin (FPN), which controls both iron absorption from meals and iron outflow from macrophages and other cells. But rather of working alone, it relies on a sophisticated system with a number of partners (Yang *et al.*,2020).

Ferroportin, the only known mechanism for the export of intracellular non-heme associated iron, is stabilized by the hormone hepcidin (Pinnix *et al.*,2010).

All physiologically relevant iron-exporting organs, such as the placenta, macrophages, hepatocytes, and the duodenum of the intestine, contain ferroportin. Fpn is the receptor for hepcidin, a polypeptide hormone generated by the liver in response to iron accumulation and inflammation (Ganz and Nemeth,2006).

Fpn is internally absorbed and destroyed when hepcidin binds to it (Nemeth *et al*.,2004). The interaction of Fpn and hepcidin may explain the majority of inherited iron overload diseases; inadequate hepcidin synthesis in response to body iron burden will result in continuous iron export. Hereditary hemochromatosis type IV, commonly known as Fpn sickness, is a genetic kind of iron overload disease that is not brought on by a deficiency in hepcidin production. The Fpn gene is mutated, which results in the disease (Montosi *et al*.,2001). Mutant Fpn either does not reach the cell surface or is not responsive to hepcidin on the cell surface, according to studies in culture cells (De Domenico *et al.*,2005; Schimanski *et al.*,2005; Drakesmith *et al.*,2005; Yang *et al.*,2002). Contrary to the other iron overload illnesses, which are inherited through recessive mechanisms, this sickness is dominant (Montosi *et al*.,2001). Fpn, a transmembrane protein present in many tissues, exports iron (Donovan *et al.*,2000; McKie *et al.*,2000; Abboud and Haile,2000) . Fpn is the receptor for hepcidin, a hormone that the liver produces in response to iron and inflammation. Hepcidin binds to Fpn and induces its internalization and degradation in lysosomes, which decreases the outflow of iron (Nemeth *et al.*,2004). The absence of iron export activity is shown by either reticuloendothelial iron surplus or a relative iron shortfall in the plasma of patients with ferroportin disease (Pietrangelo,2004). or elevated transferrin saturation and excess iron in parenchymal cells (Sham *et al.*,2005).

According to in vitro research, some Fpn mutations made in cultured cells are resistant to hepcidin and have increased iron export activity rather than

decreased iron export activity (Drakesmith *et al*.,2005; Schimanski *et al*.,2005; De Domenico *et al*.,2005;Liu *et al*.,2005). The hormone's receptor is called Fpn. In response to iron and swelling, the liver makes hepcidin. The body releases less iron when hepcidin binds to Fpn, internalizing it, and breaking it down in lysosomes (Nemeth *et al*.,2004).

2-7 The Relationship Between Hepcidin and Ferroportin

Experimental and clinical data support the notion that iron is a major metal that plays a substantial role in cancer biology. Thus, substances like as hepcidin and ferroportin (Fpn) regulate and maintain iron metabolism. In macrophages and hepatic hepatocytes, respectively, hepcidin, a peptide hormone that encourages the breakdown of the only iron exporter, Fpn, in the target cells, limits recycled and stored iron fluxes to the blood stream (Shibabaw *et al*.,2020). FPN controls Fe availability at the cellular level as well. Higher FPN expression at the plasmalemma is associated with lower intracellular Fe levels (Knutson *et al*.,2005). The oligopeptide hepcidin performs two functions in mammals by deactivating ferroportin, which regulates iron export from absorptive enterocytes, hepatocytes, and macrophages into the circulation, hepcidin maintains iron homeostasis. Additionally, hepcidin is a naturally occurring antimicrobial agent that is triggered by pathogen invasion and kills bacteria by lowering iron levels in plasma and extracellular fluids. The sole known cellular iron exporter, ferroportin, is bound and destroyed by hepcidin, which prevents cellular iron outflow. Hepcidin production is decreased by anemia and hypoxia, and is homeostatically boosted by iron loading. Hepcidin levels increase during infections and inflammation, causing a decline in serum iron levels and aiding in the emergence of inflammation-related anemia (Nemeth and Ganz , 2006).

2-8 Alanine Amino Transaminase (ALT) and Aspartate Amino Transaminase (AST) liver enzymes

The liver is the primary iron storage organ. Furthermore, it is the main source of transferrin and ferritin (Soliman *et al* ., 2014). The liver is the body's largest and most important parenchymatous organ in the body for regulating intermediate metabolism. Aminotransferase levels, a sensitive indicator of liver cell damage, contribute in the diagnosis of hepatocellular illnesses such as hepatitis. Two amino transferases, alanine transaminase (ALT) and aspartate transaminase (AST), are frequently found in blood at low quantities, generally less than 30 to 40 U per liter (U/L). Oxaloacetate is produced in the liver by the transfer of amino groups, which is mediated by ALT. It is abundant-about 3000 times more so than in the serum-in the cytosol of the hepatocyte. Hepatocellular injury and its faulty catabolism lead to the release of ALT into the blood (Fey, 2007).

Functions of these enzymes as a biochemical catalyst, converting the amino acids aspartate and alanine into the compounds oxaloacetate and pyruvate, respectively (Lin *et al*., 2010). Chemotherapy administration is challenging because to the stringent regulations and requirement to preserve the equilibrium of the metabolic processes. The primary mechanisms causing chemotherapy-related liver damage are reactive compounds created by oxidation processes (Rasool *et al* ., 2015).

The liver is one of the main suppliers of these enzymes, which are extensively disseminated throughout the body's tissues and found in varied levels in many of them (Goorden *et al*,. 2013). ALT is a better predictor of hepatic damage than AST, which is found in similar amounts in the liver, heart, skeletal muscle, erythrocytes, and brain tissue (Hyder *et al.*, 2013). Both enzymes are

released into the blood at increasingly higher amounts when the liver cell membrane is ruptured. It is believed that liver transaminases are the best method for detecting hepatocellular necrosis (hepatitis). Compared to AST, ALT is a more specific sign of liver injury since it is only found in the cytosol of hepatocytes and is mostly found in liver tissue (Pratt *et al*.,2000).

Over the course of their treatment, the majority of acute lymphoid leukemia patients usually encounter aberrant liver biochemistry, which typically denotes hepatic damage caused by therapeutic drug interactions, infections, veno-occlusive disease, or ischemia. Elevated aminotransferases are a common sign of acute leukemia patients with (AML/ALL). This is most likely brought on by leukemic infiltrates, which induce liver damage (Segal, 2010).

CHAPTER THREE

Materials and Methods

3- Materials and Methods:

3-1 Materials

3-1-1 Equipment and Kits

The table (3.1) shown list of the kits are used in the present study

Table (3.1): Kits used in the present study

No.	Kits	The Company (Origin)
1	Hepciden- Kit	BT-LAB/China
2	Ferritin- Kit	Mindray /China
3	Ferroportin- Kit	BT-LAB/China
4	ALT- Kit	Mindray /China
5	AST- Kit	Mindray China
6	Iron- Kit	Cobas Germany

3-1-2 The equipment and tool

Table (3.2) shown list of the equipment and tool commonly used the present study

Table (3.2): The equipment and tool

No.	Devices and Tools	Company	Origin
1	EDTA tube 5ml	Gongdong Medical	China
2	Gle tube	Gongdong Medical	China
3	Micro pipette	Slamid	United States
4	tips	Jippo	Japan
5	Centrifuge	Hettich	Germany
4	Microcentrifuge Tubes 2ml	Luckmedical	China

5	Hitachi cup	Luckmedical	China
6	Sterile Syringes 5ml	Easymed	Germany
7	Refrigeratore	Arcelik	Turkey
8	Water Bath	Memmert	Germany
9	Roller Mixer	Medispec	India
10	Gloves	Kleenhand	Malaysia
11	Mindray BS-240	Mindray	China
12	Mindray CL-900i	Mindray	China
13	STEL3	LINEAR	Spain

3-2 Subjects:

3-2-1 Study Design

The study included 101 (65 patient ,36 control) individuals . Hepciden and ferroportin are calculated by ELISA in both patient and control groups . Hematological investigation including (Hb , Platelet , WBC, RBC, LYM). Chemical examinations Ferritin, Iron ,AST and ALT were calculated and compared between these groups . As in the following figure.

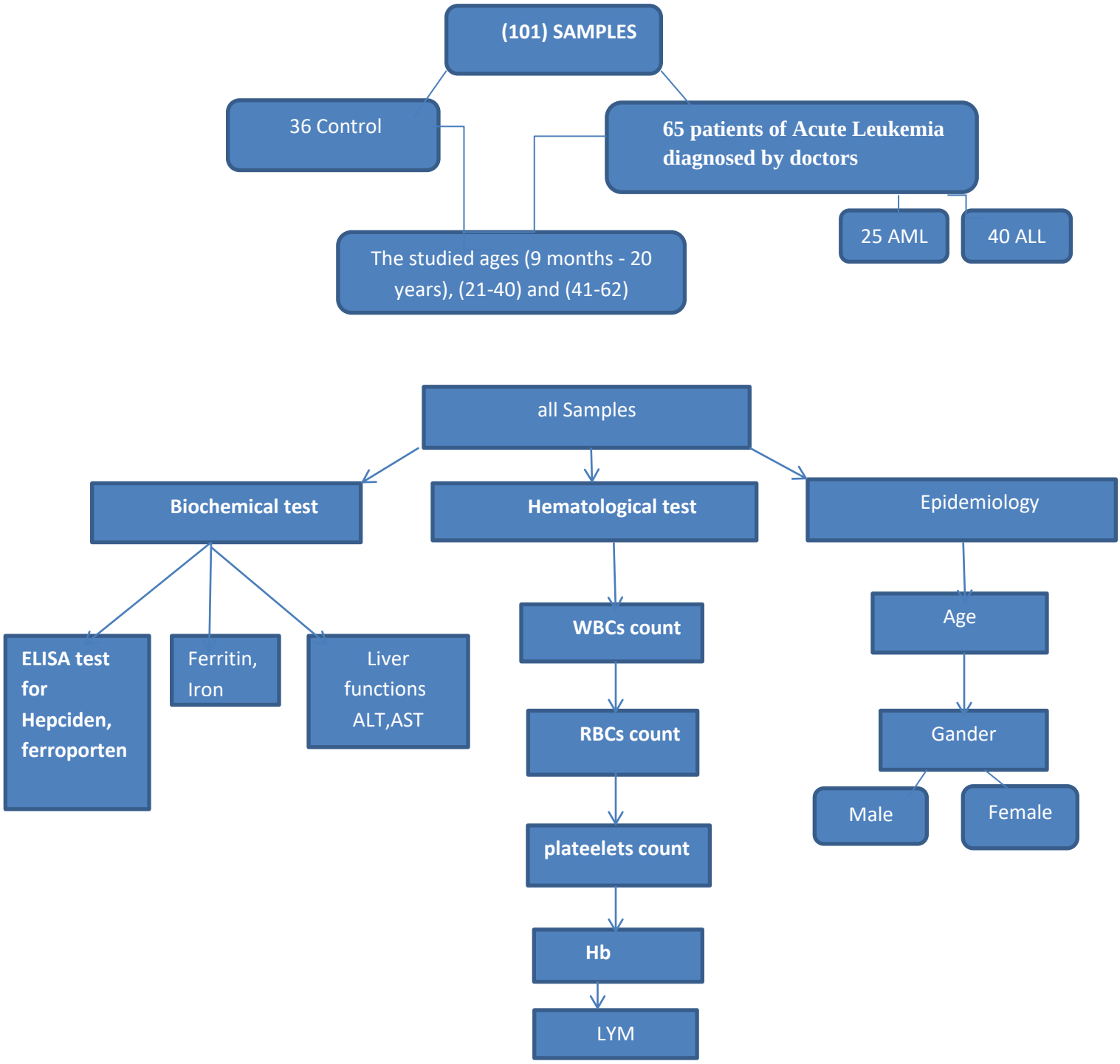

Figure (3-1): Experimental Design

3-2-2 The study is conducted on the following main groups:

3-2-2-1 Patient group

Sixty-Five samples of leukemia patients were collected , including 40 from patients with lymphoid leukemia and 25 from patients with myeloid leukemia. at the Imam Hussein Center for Oncology and Hematology (Imam Al Hussein Hospital) in Karbala and Teaching Hospital in the Medical City of Baghdad. Samples were collected from November 2021 to May 2022. The patients were divided into three groups according to age 9mounth-20; 21-40; >41 years.

3-2-2-2 Control group

The control group include 36 individuals that involved children and adults who appeared to be in good health and did not complain of any blood problem. These samples were collected if the subjects did not receive any medication and did not have any chronic or acute blood diseases.

3-3 Collection of blood

Blood samples were collected from patients by drawing 5 ml of venous blood in the arm using a compressor around the arm, and withdrawing it using a sterile syringe after sterilizing the place of withdrawal with alcohol using a cotton. They were divided into two groups: the first blood was placed in a 3 ml gel tube, then after coagulation for (15-20) minutes to obtain serum at room temperature, then centrifuged at 3000 rpm for ten minutes, and second section was placed 2 ml of blood in an EDTA tube. The blood is gently mixed and then used for blood tests.

3-4 Survey Form

The questionnaire form (Fig. 3-2) was designed, which included information about the residential address, the variation in the distribution of patients between villages and cities, the patient's profession, the educational level of the family, as well as the economic and social status. The form was filled out directly while asking questions to the patients and their families**.**

Questionnaire Form		
Age:	Educational level:	
Sex:	social situation:	
Home address:	City center:	Villages/ countryside:
Using Drug:	disease history:	
Patients' family history		
Father and Mother:	Brothers and sisters:	
Uncles and aunts:	Relatives:	

Figure (3-2) Questionnaire Form

3-5 Methods

3-5-1 Complete blood count (CBC)

A complete Blood Count Test or CBC examination is performed in the laboratory of Imam Al-Hussein Hospital, Oncology Department, in Karbala were collected from November 2021 to May 2022, and a blood sample is taken in an EDTA tube to avoid clotting for CBC examination. A cylindrical mixer mixes the blood well before placing it in an automated analyzer that counts the amount and different types of cells in the blood. STEL3 LINEAR scanned the result before it was printed and/or transferred to a computer where white blood cells, lymphocytes, red blood cells, hemoglobin and platelets are measured. According to the working principle of the device mentioned in the kit , and according to the method of work described in the appendices section.

3-5-2 Biochemical Tests

3-5-2-1 Liver Function Tests Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

Liver function tests (ALT, AST) are carried out in the laboratory of Imam Al-Hussein Hospital, Department of Oncology, in Karbala, Using a device (Mindray BS-240) where the gel tube is placed after centrifugation for 10 minutes in the appropriate place in the device and then the result is read and printed by the computer according to the way the device works as shown in the method of Kit. and according to the method of work described in the appendices section .

3-5-2-2 Ferritin Test

A ferritin test is carried out in the laboratory of Al-Husseini Hospital, Department of Oncology, in Karbala. Using a device (Mindray CL-900i) where the blood serum is placed in a Hitachi cup and then it is placed in its appropriate place in the device and the result is read and printed by the computer according to the working principle of the device mentioned in the kit and according to the method of work described in the appendices section.

3-5-2-3 Iron Test

The iron examination test was carried out in the laboratory of Imam Hussein Hospital, Department of Oncology in Karbala, using the (Cobas) device, where the gel tube is placed after centrifugation for 10 minutes in the appropriate place in the device, and then the result is read and printed by the computer according to the method of the device as well. Described in the Kit method. And according to the method of work described in the appendices section.

3-5-2-4 Hepciden and Ferroportin Test by ELISA

Hepcidin and ferroportin assay is performed using a sandwich type ELISA technique according to the working principle described in Kits and according to the method of work described in the appendices section.

3-6 Statistical Analysis

The data are statistically analyzed using SPSS version 23.0, a statistical package for the social sciences. When comparing acute leukemia patient groups with the control group at a significant level ($p \leq 0.05$), one-way ANOVA is used to display the mean and standard error (S.E.), and the correlation coefficient is used to display linear relationships using a linear regression test for each relationship (Hoshmand , 2018).

CHAPTER FOUR

Results and Discussion

4 Results and Discussion

4-1 Sociodemographic Characteristics:

Table (4-1) showed the age and gender distribution of the patient groups and the type of disease in addition to the control group included in this study. Sixty-five 65 samples of acute leukemia patients and thirty-six 36 samples of control group. The patients group subdivided according to gender 34 males and 31 females. While control group were matched by 20 males and 16 females. Then classified to three groups according to age .There were 34 patients of the age group 9 months-20 corresponding to 15 of the control group and 23 patients of the age group 21-40 corresponding to 11of the control group , and 8 patients of the age group >41corresponding to 10 of the control group. As for the type of disease 40 patients were diagnosed with acute lymphoid leukemia and 25 with acute myeloid leukemia.

The results of our study with respect to gender appear that men are more likely to develop leukemia than females. This is consistent with Dorak and Karpuzoglu,(2012) study that confirmed this, which can be related to physiological and genetic factors differences between gender. With regard to age assessment, the prevalence of acute lymphoid leukemia (ALL) was higher in the group of patients aged 9 months to 20 years, Among children, acute lymphoblastic leukemia is a frequent form of cancer. Children are affected at a rate of 2-4 per 100 kids under the age of 15. The majority of malignant tumors in children are this one than in the other age groups (Pui *et al.*, 2004; Krull *et al*., 2019). Acute lymphoid leukemia (ALL), continues to be the leading cause of cancer-related mortality in children and teenagers because since ALL relapse is frequently unsolvable (Inaba *et al* ., 2013). While AML is more common in the elderly, these results are agreed with

the results of the Webster and Pratz , (2018) study that showed that acute myeloid leukemia (AML) is more common in the old age group.

Table (4-1) sociodemographic characteristic for studied groups.

Group Variables	Control	Patient	p≤0.05
	No. (%)		
	36	65	
Gender			
Male	20	34	**0.754**
Female	16	31	
Age (years)			
9 months-20	15	34	**0.023***
21-40	11	23	
≥41	10	8	
Type of disease			
ALL		40	**0.043***
AML		25	

(ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia

4-2: Hematological Parameters Concentrations among ALL, AML and control groups:

The results in table (4-2) showed that a significant increase (p≤0.05) in white blood cells in patients with acute leukemia (ALL,AML) compared to the control group respectively. The results also showed no significant differences in the average white blood cells between lymphoid and myeloid leukemia patients. The results appeared that there was a significant increase (p≤0.05) in lymphocytes in the patients with acute leukemia (ALL,AML) compared to the control group respectively. While there are no significant differences in lymphocytes between

acute lymphoid and myeloid leukemia patients. The results appeared that there was a significant differences ($p \leq 0.05$) in red blood cells between patients with acute lymphoid leukemia and acute myeloid leukemia patients respectively, in addition to a significant decrease between patients with acute myeloid leukemia and the control group respectively, while there is no significant difference between patients with acute lymphoid leukemia and the control group as shown in the table below .The current study appeared a significant decrease ($p \leq 0.05$) in Hb among patients with acute myeloid leukemia respectively compared to the control group. While the results revealed no significance differences in the mean hemoglobin concentration among patients (AML , ALL). While there is a significant difference between lymphoid leukemic patients and the control group. The results of appeared for the platelet counts in patients groups when compared with the control group. The mean platelet count was significantly increased ($p \leq 0.05$) in patients with acute lymphoid leukemia and acute myeloid leukemia respectively when compared to the control group. there was no Statistically significant differences can be observed in the mean platelet count between patients with acute lymphoid leukemia and acute myeloid leukemia.

Leukocytes are one of the signs of inflammation, thus an increase in them may be a reaction to the inflammation that preceded a leukemia (Hutter, 2010). This finding gives support to the hypothesis that stem cell DNA abnormalities produce a large number of abnormal leucocytes known as blasts that circulate and begin to compete with normal, healthy blood cells, limiting their ability to perform their functions (Hutter, 2010 ; Zainulabdeen, 2014). The results in table (4-2) showed an increase in leukocytes, which is similar with the findings of Xu *et al*., (2009), who suggested that chemotherapy may be responsible for the rise in total leukocytes.

platelet count interact closely with tumor cells and play a much broader role in tissue regeneration and repair. On the one hand, tumor cells cause platelet aggregation (TCIPA), which is known to be the starting point for cancer-related thrombosis. The release of a variety of growth factors, such as angiogenic and mitogenic proteins, by platelets recruited to the tumor microenvironment, on the other hand, interacts directly with tumor cells to promote their proliferation. They have been demonstrated in the context of cancer to support a number of stages of tumor growth, including local growth, migration into and out of the bloodstream, and metastasis establishment. Platelets are crucial for immune system evasion during several of those steps (Goubran *et al.*,2014). It has become clear that platelets also serve as immune cells in addition to their well-known and significant roles in hemostasis and wound healing (Maouia *et al.*,2020). In numerous distinct tumor entities, high platelet counts were found to be a risk factor for unfavorable outcomes (Lu *et al.*,2020). According to Alberts *et al.,* (2008) the blast cells that quickly multiply begin in the bone marrow and force out the healthy cells. The most often afflicted organs are lymph nodes, the spleen, the liver, and the skin. After a period, they spill over into the blood stream and may be discovered in key organs of the body. sometimes the kidney, brain, or other nervous system components.

Table (4-2) Comparison of Some Hematological Parameters among ALL, AML and Control Groups.

Groups Parameters	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control
	Mean±S.E		
WBC(10^9/L)	8.03±0.7 b	9.10±1.7 b	6.06±0.3 a
Lym (%)	43.79±3.1 b	45.74±4.3 b	35.50±1.9 a
RBC (10^{12}/L)	4.08±0.9 b	3.52±0.2 a	4.33±0.1 b
Hb (g/dL)	10.67±0.3ab	9.81±0.4 a	11.83±0.2 b
Plt (10^9/L)	261.78±19.0b	265.14±14.2b	220.08±16.2 a

*Similar letters is not significant difference at (P≤0.05) level. Different letters is significant difference at (P≤0.05) level. (ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia.

4-3: Hematological Parameters among ALL, AML and control groups according to gender.

Table (4-3) show the relationship of some hematological parameters with gender. the results of showed there were WBC for males, there is no significant difference between patients with acute leukemia compared to the control, but for females, there is a significant increase in patients with acute myeloid leukemia compared to patients with lymphoid leukemia and the control. With regard to the lymphocytes of males there was a significant increase in patients with acute myeloid leukemia compared to the control while females, there were no significant differences between acute leukemia patients and the control. There is a significant increase in red blood cells for males in patients with lymphoid leukemia compared to patients with acute myeloid leukemia and the control. As for females, there is a significant decrease between the patients and the control. In regarding hemoglobin (Hb), it was found in males that there is a slight significant difference between acute leukemic patients. But when comparing the control group with respect to the

patients, there is a significant difference in the patients with acute myeloid leukemia. Platelets for males and females, there is no significant difference in acute leukemia patients compared to the control. so our results proved that gender had no effect of some hematological studied parameters.

Michallet *et al*., (2013) appeared in his study the decrease in erythrocytes may be attributed to their lysis when it be higher than its production, the lysis may be occurred due to free radicals which interact with all cell components such as the membrane lipids that is oxidized by a process called lipid peroxidation to give malondialdehyde. This process cause damage in the cell membrane structure and thus leads to tearing membrane and cells death (Kwon *et al.,* 2009; Cabanillas *et al.,* 2012). Erythrocytes are considered the ideal cell to determine the imbalance between oxidation and antioxidants because break it continuously by free radicals that considered toxins which stimulates the bone marrow to produce leukocytes but they are abnormal as a result the mutation in DNA of hematopoietic stem cells, this explains the increase in the leucocytes count and decrease in normal erythrocytes number. Men have somewhat greater hemoglobin levels than women, which is assumed to be related to androgens' stimulating influence on bone marrow (Dasgupta and Wahed , 2021). A drop in hemoglobin (Hb) concentration is connected with anemia, which is defined by a decrease in erythrocyte count and hence a decrease in hemoglobin concentration in the blood. This results are in agreement with findings by (Mehde *et al*., 2014).

Hemoglobin levels in male and female children are similar, and they grow at the same pace throughout adolescence. In adolescence, gender disparities in hemoglobin concentration arise. In girls, the hemoglobin level hits a plateau throughout early adolescence, but in males, the hemoglobin level rises throughout puberty to higher levels characteristic of adult men. The difference is due to the

stimulating impact of testosterone secretion. Indeed, androgenic steroids are used to treat bone marrow failure in some cases (Garvin,2010).

Table (4-3) Comparison between Male and Female in Some Blood Parameters among ALL, AML Patients and Control Groups.

Groups(A) / Parameters	Gender(B)	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control	$LSD_{(0.05)}$
		Mean±S.E			
WBC(10^9/L)	**Male**	7.01±0.2	5.30±0.3	5.76±0.2	**for (A)=1.426 for(B)=1.064 for(A*B)=2.167**
	Female	4.71±0.1	12.08±0.4	6.43±0.2	
Lymo (%)	**Male**	39.80±1.2	46.15±1.8	35.64±2.4	**for (A)=4.571 for(B)=3.221 for(A*B)=6.136**
	Female	44.49±1.4	38.27±2.1	37.58±1.6	
RBC (10^{12}/L)	**Male**	4.33±0.2	3.46±0.1	4.29±0.1	**for (A)=0.311 for(B)=0.142 for(A*B)=0.515**
	Female	3.73±0.1	3.57±0.3	4.37±0.1	
HGB (g/dL)	**Male**	11.15±0.6	9.52±0.2	11.90±0.2	**for (A)=0.971 for(B)=0.516 for(A*B)=1.210**
	Female	10.01±0.7	10.03±0.5	11.75±0.1	
PLT (10^9/L)	**Male**	207.13±3.4	241.38±1.6	194.40±2.1	**for (A)=20.555 for(B)=17.691 for(A*B)=38.468**
	Female	288.65±2.9	230.24±1.2	252.19±2.4	

(ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia.

4-4: Hematological Parameters among ALL, AML Patients and control groups according to age groups.

Table (4-4) demonstrated the relationship between age and some hematological parameters in patients with acute lymphoid and myeloid leukemia and the control group. The results showed that with regard to white blood cells, there was no significant difference between patients with acute leukemia and controls for the age group 9 months -20 and for the age group21-40. where the results were shown there was no significant difference for the two age groups 9 months-20 years and 21-40 of acute leukemic group for white blood cells in comparison to control group. As for the age group >41, there is a significant increase in patients with myeloid leukemia compared to patients with acute lymphoid leukemia and control group.

The results for lymphocytes, showed the age group from 9 months to 20 years had no significant difference between leukemic patients, but there is a significant difference between acute myeloid leukemic group and control. Age group 21-40 there is a significant increase in patients with acute lymphoid leukemia compared to patients with acute myeloid leukemia and the control group. As for the age group >40, there is a significant increase in patients with acute myeloid leukemia compared to patients with acute lymphoid leukemia and the control group. The results for the red blood cells count were shown a non-significant difference for all studied age groups .

As for hemoglobin, The results appeared that there is a significant difference between patients with acute leukemia and control groups ,for the age group from 9 months - 20 years and from >41. As for the age of 21-40, there is a significant increase in acute lymphoid leukemia patients compared to the acute myeloid leukemia patients and the control. The results involved for platelets for the age

group 9 months - 20 years there is a significant increase between acute lymphoid leukemic patients , but there were no significant difference between patients with lymphoid leukemia and control, while there is a significant difference between patients with acute myeloid leukemia and control groups. There is a significant difference in the age group 21-40 between acute myeloid leukemia patients compared to acute lymphoid leukemia patients and controls. As for the age group from >41, there is a significant increase in patients with acute myeloid and lymphoid leukemia compared with the control group.

Elevated levels of lymphocytes are associated with the mutations in lymphoid progenitor cell that alter regulation of cellular proliferation, differentiation and apoptosis, which rapid accumulate in a lymph nodes causing lymphadenopathy, as well as, can be found in the peripheral blood. The results of this study are in agreement with results by Huang *et al*. (2015) and Wang *et al*. (2011). When compared to the lymphocytes in patients with myeloid leukemia .In lymphoid leukemia, the cancerous change takes place in a type of marrow cell that normally goes on to form lymphocytes which are infection fighting immune system cells (Ritu *et al*., 2013). Previous studies conducted on patients with acute lymphocytic leukemia indicated that the increase in the number of white blood cells is a bad fate for patients (Digel *et al*.,1998). The increase of blast cells in the bone marrow may be the cause of the decline in platelet count. As a result, bone marrow cannot produce the essential elements of blood (Trafalis *et al*.,2008). The findings of Schmied *et al*. (2021) are in agreement with the our results.

As a result of disorders, particularly those linked to the process of platelet synthesis, the number of platelets decreases. When this happens, little red spots may develop on the skin or mucous membranes. These petechiae spots may put patients at risk for lung or brain bleeding, which can be dangerous or deadly.

Minor bleeding, such nosebleeds and blood in the urine, typically precedes major hemorrhage (Trafalis *et al*.,2008 ; Schmied, *et al.* 2021). The decrease in platelets number occurs as a result of injury by diseases especially the diseases associated with the process of its formation when there is decrease in the platelets count, minute red dots may appear in the skin or mucous membranes. These dots, called petechiae which are may predispose patients to bleed in the brain or lung which can be serious or fatal. Such bleeding is usually preceded by minor bleeding such as nosebleeds and blood in the urine (Ghoshal and Bhattacharyya , 2014; Wachowicz *et al*., 2016). Most patients with lymphoid leukemia have pallor as a result of low hemoglobin levels caused by a low number of red blood cells caused by a low number of the erythroblastic cell line caused by the accumulation and proliferation of abnormal bone marrow cells at the expense of the other lines, resulting in bone marrow failure (Hoffbrand, 2005). Anemia may arise due to a shortage of key materials required by the bone marrow to generate normal blood cells, or it can be caused by a problem in the bone marrow itself, such as an inability to produce normal erythrocytes or a failure of the bone marrow to produce a sufficient number of erythrocytes (Veena *et al*., 2012).

Singh and Hertello, (2005) and Mehde and Yousif, (2014) reported reduced Hb concentration increases the requirement for oxygen in individuals with leukemia, causing the heart to pump blood more forcefully and at a faster pace to compensate for the lost oxygen, resulting in left ventricular hypertrophy, dilatation, and heart failure.

Table (4-4) Comparison between the Three Age Groups in Some Blood Parameters among ALL, AML Patients and Control Groups.

Groups Parameters	Age (years)	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control
		Mean±S.E		
WBC(10^9/L)	**9 months-20**	6.12±0.6	5.23±0.1	7.31±0.9
	21-40	6.29±0.1	8.20±0.2	5.80±0.1
	>41	5.67±0.5	14.92±0.4	4.91±0.2
$LSD_{(0.05)}$			**2.575**	
Lym (%)	**9 months-20**	44.23±1.2	51.26±1.3	39.05±1.2
	21-40	40.33±1.7	38.86±0.9	30.87±2.1
	>41	34.70±2.4	38.45±1.1	36.20±2.4
$LSD_{(0.05)}$			**8.565**	
RBC (10^{12}/L)	**9 months-20**	4.03±0.1	3.26±0.1	4.43±0.9
	21-40	4.38±0.2	3.86±0.4	4.21±0.1
	>41	4.11±0.2	3.04±0.7	4.27±0.2
$LSD_{(0.05)}$			**N.S**	
HB (g/dL)	**9 months-20**	10.52±0.5	9.41±2.2	11.90±0.3
	21-40	12.77±0.3	10.25±0.6	11.87±0.3
	>41	10.42±0.1	9.25±0.4	11.79±0.4
$LSD_{(0.05)}$			**1.033**	
PLT (10^9/L)	**9 months-20**	249.00±5.6	116.32±3.3	277.47±7.1
	21-40	114.00±7.1	272.22±4.7	165.17±6.4
	>41	261.89±4.3	273.63±5.1	184.67±5.9
$LSD_{(0.05)}$			**34.534**	

(ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia

4-5: Hepcidin, Ferroportin, Ferritin and Iron concentration among acute leukemic patients

The results of our study appeared an increase in the concentration of Hepcidin in the serum of leukemia patients in general. The results showed an increase ($p<0.05$) in Hepcidin in the serum of patients with lymphoid leukemia when compared to myeloid leukemia and the control group respectively. The results also showed that there was a significant increase ($p<0.05$) in the concentration of Hepcidin among patients with lymphoid and myeloid leukemia. According to table (4-5) below. The results of the current study showed an increase ($p<0.05$) in the concentration of ferroportin in the serum of leukemia patients. The results showed an increase ($p<0.05$) in ferroportin in the serum of patients with lymphoid leukemia compared with myeloid leukemia and the control group respectively.

The results of our study appeared that there is a significant increase ($p\leq0.05$) in the concentration of ferritin in the blood for patients. The results showed that acute myeloid leukemic patients had a high ferritin concentration when compared to lymphoid leukemia patients and the control group. The results also showed that there was an increase in ferritin concentration among patients with myeloid and lymphoid leukemia. The current study showed an increase in iron concentration in the serum of patients when compared to the control group and involved that there is a noticeable increase in iron in the serum of leukemic patients when compared to the control respectively. There is also a clear significant difference between lymphoid and myeloid leukemic patients. According to table (4-5) below.

4-6: Hepcidin, Ferroportin, Ferritin and Iron concentration in leukemic patients groups (AML, ALL) and control group according to gender

In the table (4-6) showed Hepcidin, Ferroportin, Ferritin and Iron concentration in leukemic patients groups (AML, ALL) and control group according to gender. The results for males were that there was a significant increase in the Hepcidin concentration among the lymphoid and myeloid patients in male. There was also a significant increase between the patients and the control. In the case of females, there was also a significant difference between the leukemic patients and between the patients and the control. According to table (4-6), females with acute myeloid leukemic have higher significant differences in Hepcidin concentrations than males.

As for the relationship of ferroportin with gender, there is a significant increase among patients with lymphoblastic and myeloid leukemia, and there is also a significant increase among patients groups and control for males and females. The table showed that the concentration of ferroportin in females has higher significant differences than in males among patients with acute lymphoid leukemia. For the relationship of ferritin with gender, there is a significant increase between lymphoid and myeloid leukemia patients, and there is a significant increase between patients and control for males. As for females, there is no significant difference between patients with leukemia, but there is a significant difference between leukemic patients and control. The table involve the concentration of ferritin in males has a higher statistically significant difference than in females between patients with acute myeloid leukemia. The table also appears the relationship of iron to gender, and there was a significant increase among patients with lymphoblastic and myeloid leukemia, and there was a significant increase between patients and control for males and females. According

to the table below the iron concentration in males has a statistically significant difference than in females between patients with acute lymphoid leukemia.

4-7: Hepcidin, Ferroportin, Ferritin and Iron concentration in acute leukemic patients groups (AML, ALL) and control group according to age groups

Tables (4–7) displayed the relationship between the Hepcidin parameter and age in leukemic patient groups and the control group. where the results showed for the age groups from 9 months-20 years was a significant increase in leukemia patients compared to the control group, the group from 21-40 there was a significant increase among myeloid leukemia patients compared to acute lymphoid leukemia and the control group, and the age group from >41years that there was a high significant difference between cancer patients and between patients and control.

The relationship of the ferroportin parameter with age in the groups of leukemia patients and the control group, where the results showed for the age groups from 9 months - 20 years, there was a significant increase in leukemia patients compared to the control group, while the group from 21-40, there was a significant increase among myeloid leukemia patients compared to acute lymphoid leukemia and the control group, but there was no significant difference between acute lymphoid leukemia patients and control. As for the age group of >41years, there is a high significant difference between cancer patients and between patients and control. As for the relationship of Ferritin parameter with age in leukemia patient groups and the control group, where the results showed for the age groups from 9 months-20 years, the group from 21-40 and the age group from >41years

that there was a high significant difference between cancer patients and between patients and control.

As for the relationship of iron parameter with age in the group of leukemia patients and the control group, where the results for the age groups from 9 months to 20 years showed a high significant difference between leukemia patients and between patients and control, However the group from 21-40 significant increase among patients with acute myeloid leukemia compared to patients with lymphoid leukemia and the control group. The age group is >41 years, significant increase in patients with acute lymphoid leukemia compared to the control group.

Our results showed high concentration for Hepcidin hormone in acute leukemic patients (AML,ALL) $p<0.05$ in contrast with control group which consistant with (Hamad *et al.*,2019). Hepcidin and serum ferritin typically react with inflammation and changes in iron stores in a similar manner (Kemna *et al.*,2005) . It is known that Hepcidin binds to ferroportin, causing external iron linked to transferrin or serum ferritin to decrease and intracellular iron to be retained in macrophages (Muckenthaler *et al.*, 2008). It thus brings the intriguing issue of whether higher Hepcidin concentrations in the presence of transfusional iron loading may prevent excessive parenchymal iron overload and consequent organ damage. Iron issues are often brought on by variations in Hepcidin concentrations, as opposed to ferritin, a marker of iron storage. The dysregulation of Hepcidin-ferroportin axis showed high Hepcidin and uncontrolled ferroportin (FPN) hyperactivity at childhood ALL which may point to the impaired down-regulation of FPN by Hepcidin (Hsi-Che *et al.*, 2020). Nemours studies that discovered significantly higher concentration of Hepcidin in patients compared to the healthy group provided an explanation for the same outcomes, reporting that the increase in Hepcidin concentration may protect the body from excessive iron

parenchymal and organ damage in the presence of iron loading (Liu, *et al* ., 2016; Vinchi *et al* .,2020; Ali *et al.,* 2022).

All living things need iron for their essential metabolic processes. A multitude of comorbidities with poorer prognoses and more expensive treatment choices are linked to iron imbalance, which still affects over 25% of the global population. This is despite several public health programs and in-depth research over the years. Hepcidin is largely responsible for controlling the homeostasis of systemic iron. By binding to ferroportin, the only known iron exporter, the highly conserved Hepcidin lowers plasma iron concentrations and induces iron retention in macrophages and enterocytes (Nemeth *et al*., 2004).

Iron and erythropoietic activity govern the homeostatic concentration of Hepcidin. Hepcidin synthesis is increased by iron overload and inflammation, but Hepcidin expression is decreased by anemia and hypoxia.(Chauhan *et al* .,2014). Other investigations demonstrated that chronic disorders like leukemia, which can lead to iron malfunction with hypoferremia and anemia, greatly elevated serum Hepcidin concentration during inflammation and infection (Langer and Ginzburg, 2017);(Sonkar *et al*.,2018).

The current study showed an increase in iron in the blood serum of patients, and these results were similar to the studies he conducted (Wande *et al.*,2020). Iron is an essential element for biological processes. Iron homeostasis is regulated through several mechanisms, from absorption by enterocytes to recycling by macrophages and storage in hepatocytes. Iron has dual properties, which may facilitate tumor growth or cell death (Coates,2019). Cancer cells exhibit an increased dependence on iron compared with normal cells. Macrophages potentially deliver iron to cancer cells, resulting in tumor promotion. Mitochondria

utilize cellular iron to synthesize cofactors, including heme and iron sulfur clusters. However, highly increased iron concentrations result in cell death through membrane lipid peroxidation, termed ferroptosis (Olcay *et al.*, 2017). Therefore, the risk of an imbalance in the entire iron homeostasis should be taken into account. The toxicity of iron results from the Fe^{2+} forms of iron, which are highly reactive and cause rapid oxidant damage of proteins and DNA, permanently changing the structure of proteins and genetic material (Koskenkorva-Frank *et al*., 2013).

The intensity of chemotherapy results in an increase toxic forms of iron, occurring simultaneously with increased serum iron, ferritin, Hepcidin, and ferroportin concentrations (Coates, 2014). Elevated cellular iron concentration are a result of increased iron absorption and reduced iron efflux in leukemia cells. Leukemia patients also have an elevated systemic iron pool, which is made worse by repeated red blood cell transfusions. Numerous experimental and epidemiological investigations have shown a connection between iron metabolism dysregulation and the development and progression of leukemia (Kennedy *et al.*, 2014; Benadiba *et al*.,2017; Hagag *et al* .,2018). Due to their high need for iron to support their fast multiplication, leukemia cells are far more vulnerable to iron deprivation than normal cells (Callens *et al* .,2010).

The significance of iron in the development and spread of cancer has been apparent in recent years (Shen *et al*., 2011;Torti and Torti.,2013). In particular, iron chelating drugs constitute a viable anticancer therapy since tumor cells use more iron than healthy cells do (Forciniti *et al*.,2020). In both hematological and solid malignancies, iron chelators have been shown by several authors to decrease proliferation and trigger apoptosis (Calabrese *et al.*,2020; Amano *et al.*,2020).

Iron intake, storage, and export indicators frequently coexist with iron excess. TFR1 expression, also known as CD71, is often higher in leukemic cells than in normal counterparts. The amount of CD71 expression in AML, whether it is minimally differentiated or not, may be directly connected with the degree of differentiation (Liu *et al*., 2014). In this study, serum ferritin was increased in patients when compared with the control group. These results are similar with those reported (Genena *et al*.,2015; Hamodat *et al* .,2020). A significant difference in ferritin concentration between healers and non-healers reflecting the concentration of serum acute phase ferritin, which is typically linked to iron storage, can aid in disease stage prediction (Luznik *et al.*,2008; Armand *et al*.,2007;Hamad *et al.,* 2019). The increase in ferritin concentrations for all patients is caused by broken cancer cells releasing ferritin into the bloodstream. (Kemna *et al*., 2005)**.**

Serum ferritin, the iron storage analogue, is commonly elevated in leukemic patients and is detrimental to overall and relapse-free survival in chemotherapy-treated patients (Baker *et al* .,2014; Tachibana *et al* .,2018; Bertoli *et al*.,2019; Ihlow *et al*.,2019) as well as in recipients of allogeneic stem cell transplants (Artz *et al.,* 2016). Ferritin is made up of 24 polypeptide heavy chain (FTH) and light chain subunits (FTL). FTH appears to be an NF-B downstream effector that, in an inflammatory setting, inhibits Jun N-terminal kinase (JNK) to decrease TNF-driven apoptosis (Kamata *et al* .,2005) NF-B and pro-oxidant pathways are included in a gene expression profile linked to FTH overexpression in AML patients, which causes resistance to chemotherapy (Bertoli *et al*.,2019). AML frequently exhibits dysregulation of the ferroportin-Hepcidin axis, which results in decreased iron outflow. Notably, core binding factor (CBF) AML subgroups are

regularly reported to have low ferroportin expression, which appears to be associated with better outcomes and increased chemotherapeutic sensitivity .

Table (4-5) Hepcidin, Ferroportin, Ferritin and Iron concentration acute in leukemic patients groups (Acute Myeloid Leukemic Patients, Acute lymphoid Leukemic Patients) and control group.

Groups Parameters	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control
	Mean±S.E		
Hepcidin	745.24±9.8 c	634.80±2.6 b	261.79±21.3 a
Ferroportin	11.13±1.8 c	7.95±0.6 b	5.58±0.4 a
Ferritin (ng/ml)	712.43±8.8 b	833.10±3.0 c	48.86±2.2 a
Iron (mg/dL)	117.25±2.8 c	107.67±1.8 b	91.50±6.9 a

*Similar letters is not significant difference at (P≤ 0.05) level. Different letters is significant difference at(P≤ 0.05) level. (ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia

Table (4-6) Hepcidin, Ferroportin, Ferritin and Iron concentration in leukemic patients groups (AML, ALL) and control group according to gender.

Groups Parameters	Gender	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control	$LSD_{(0.05)}$
		Mean±S.E			
Hepcidin	**Male**	664.48±12.5	271.29±6.3	251.68±3.9	**for (A)=6.771 for(B)=4.238 for(A*B)=9.206**
	Female	849.76±9.7	816.56±5.8	276.40±2.5	
Ferropoten	**Male**	8.04±0.6	5.04±0.4	4.75±0.6	**for (A)=1.206 for(B)=0.971 for(A*B)=2.832**
	Female	15.12±0.3	9.40±0.3	6.78±0.7	
Ferrintin (ng/ml)	**Male**	822.90±7.4	1500.00±11.2	54.79±5.6	**for (A)=74.544 for(B)=50.233 for(A*B)=206.519**
	Female	569.47±8.2	499.64±4.6	42.89±7.2	
Iron (mg/dL)	**Male**	135.00±7.4	107.67±8.1	99.20±1.6	**for (A)=16.114 for(B)=11.525 for(A*B)=24.706**
	Female	64.00±9.2	110.23±6.4	83.80±2.7	

(ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia

Table (4-7) Hepcidin, Ferroportin, Ferritin and Iron concentration in acute leukemic patients groups (AML, ALL) and control group according to age groups.

Groups / Parameters	Age (years)	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control
		Mean±S.E		
Hepcidin	**9 months-20**	913.26±3.3	331.29±3.4	245.38±2.6
	21-40	213.32±2.9	448.15±4.1	225.22±3.7
	≥41	536.92±5.2	1066.55±3.7	301.65±6.1
$LSD_{(0.05)}$			**59.973**	
Ferroportin	**9 months-20**	13.22±0.9	5.95±0.6	2.93±0.3
	21-40	4.93±1.1	6.23±0.2	4.96±0.1
	≥41	9.05±0.7	11.53±1.7	6.73±0.02
$LSD_{(0.05)}$			**1.261**	
Ferritin (ng/ml)	**9 months-20**	655.27±25.6	166.23±12.3	50.00±15.6
	21-40	639.99±15.1	1008.47±9.7	47.22±11.2
	≥41	767.17±14.0	903.47±10.7	49.37±10.2
$LSD_{(0.05)}$			**66.74**	
Iron (mg/dL)	**9 months-20**	139.00±6.4	109.50±8.2	97.50±5.2
	21-40	89.50±5.8	104.00±7.7	82.50±1.3
	≥41	114.00±11.6	99.14±6.9	79.11±2.6
$LSD_{(0.05)}$			**28.175**	

(ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia

4-8: liver function tests Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activity in acute leukemic patients groups (ALL,AML) and control group:

The results of our study in table (4-8) showed Aspartate Teansaminase (AST) activity in all patient groups compared to the control group. The results showed an increase in (AST) in the blood among acute leukemia patients compared to the control group respectively. As for the levels of Alanine Transaminase (ALT) in the blood serum, the results showed a significant increase. in the serum (ALT) level of acute leukemia patients in comparison with the control group respectively. As illustrated in the table below.

4-9: Aspartate Transaminase and Alanine Transaminase activity in leukemic patients groups (ALL,AML) and control group according to gender:

Table (4-9) shows the relationship between gender and Aspartate Transaminase (AST) activity between acute leukemia patients and the control group. The results showed that there is no significant difference for (AST) activity between males and females among leukemia patients, but there is a significant increase between acute leukemia patients and the control group. Table (4-9) also shows the comparison between gender and Alanine Transaminase (ALT) activity between leukemia patients and control group. The results showed that there is no significant difference for (ALT) activity between males and females among acute leukemia patients, but there is a significant increase between acute leukemia patients and the control group. According to the table below.

4-10: Aspartate Transaminase and Alanine Transaminase activity in acute leukemic patients groups (ALL,AML) and control group according to age groups

Table (4-10) showed that a significant increase of AST in acute leukemia patients compared to control groups for the age group 9 months - 20 years and 21-40 years. As for the age group >41year there was a significant increase among patients with lymphoid leukemia compared to patients with myeloid leukemia and the control group. The result revealed a significant increase of ALT in leukemia patients compared to control groups among the age group 9 month- 20 year ,21- 40 year and >41year .While there was insignificant difference (≥0,05) between ALL,AML patients. As shown in the table below.

The results supported (Segal *et al.*,2010) findings that leukemia patients had greater levels of glutamic oxyaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT). Hepatic infiltration may be brought on by increased GOT activity. Infiltrative illness, which is brought on by a problem with the mitochondrial and cytoplasmic membranes, is associated with an increase in Glutamic Oxaloacetic Transaminase (Craig *et al*.,2002). GOT and GPT enzyme levels rose when leukemia first appeared, which led to liver damage from the leukemic stream. Increasing leukemic cell levels are associated by an increase in transaminase enzyme concentration because chemotherapy is dangerous. Additionally, a rise in the quantity of leukemic cells causes a rise in the level of transaminase enzymes (CESUR *et al.*, 2004).

A researcher (Bhojwani, 2019) discovered that there was no primary patient or pathologic feature (age, gender, or genetic cytogenetic subtype) as a significant predictor of response in a study of pediatric patients with acute lymphoblastic

leukemia. AST is found in a variety of tissues in humans, including the heart, liver, kidneys, and skeletal muscles. Thus, any damage to these tissues causes an increase in the concentration of the AST enzyme, or the ALT enzyme, which is found in many tissues but is more abundant in the liver. As a result, any damage to the liver causes an increase in the concentration of ALT, making it a special enzyme for detecting liver disease (Bishop, 2010). It is worth noting that ALT elevations are frequently higher than AST elevations and tend to last longer due to the longer half-life of ALT in serum (16 h-24 h) (Mashhadani *et al*., 2011). Increased levels of ALT and AST in leukemia patients are due to liver injury (Carvalhana *et al*.,2016). In addition, a higher number of leukemic cells leads to an increased concentration of transaminase enzymes (Verma *et al* .,2014).

Table (4-8) Comparison of Some liver function tests (AST,ALT) activity in acute leukemic patients groups (ALL,AML) and control group.

Groups Parameters	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control
	Mean±S.E		
AST (U/L)	56.34±2.6 b	53.42±0.4 b	23.40±1.7 a
ALT (U/L)	45.05±1.8 b	43.22±2.1b	28.39±1.1 a

*Similar letters is not significant difference at P≤ 0.05 level. Different letters is significant difference at P≤ 0.05 level, (ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia, (AST) Aspartate Transaminase (ALT) Alanine Transaminase

Table (4-9) Comparison between Male and Female in Some liver function tests (AST,ALT) among ALL, AML Patients and Control Groups.

Groups(A) Parameters	Gender(B)	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control	$LSD_{(0.05)}$
		Mean±S.E			
AST (U/L)	**Male**	54.36±6.2	57.54±3.2	24.97±2.2	**for (A)=6.187 for(B)=4.228 for(A*B)=9.206**
	Female	58.32±4.7	49.30±2.9	21.81±1.6	
ALT (U/L)	**Male**	47.04±6.8	44.28±6.9	26.40±2.9	**for (A)=3.105 for(B)=2.614 for(A*B)=5.024**
	Female	43.06±5.0	42.15±3.2	30.35±3.1	

(ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia, (AST) Aspartate Transaminase (ALT) Alanine Transaminase

Table (4-10): Comparison between the Three Age Groups in Some liver function tests (AST,ALT) among ALL, AML Patients and Control Groups.

Groups Parameters	Age (years)	Acute Lymphoid Leukemia	Acute Myeloid Leukemia	Control
		Mean±S.E		
AST (U/L)	**9 months-20**	56.36±5.5	52.83±5.2	25.35±3.1
	21-40	53.24±2.3	57.81±4.4	21.63±1.2
	≥41	59.41±7.1	49.61±2.2	23.39±0.7
	$LSD_{(0.05)}$	**6.292**		
ALT (U/L)	**9 months-20**	46.04±2.2	40.82±6.4	30.45±2.5
	21-40	47.03±1.9	46.66±2.9	26.91±1.6
	≥41	42.07±3.3	42.19±3.4	27.82±2.3
	$LSD_{(0.05)}$	**7.846**		

(ALL) Acute Lymphoid Leukemia ,(AML) Acute Myeloid Leukemia, (AST) Aspartate Transaminase (ALT) Alanine Transaminase

4-11 Correlation coefficient of all studied parameters Among acute lymphoid

Our results shown in table (4-11) showed the correlation factors between the variables for patients with acute lymphoblastic leukemia (ALL). Where we found that there is no significant difference between white blood cells (WBC) and the parameters mentioned in the table, and white blood cells are directly proportional to LYM, RBC, Hepicidin, Ferroportin and iron . While inversely proportional to each of Hb ,PLT, AST ALT and Ferritin. With regard to lymphocytes, it is inversely proportional to platelets, with a significant difference of its value (-.475**). It is also inversely proportional to RBC, AST ,ALT ,ferritin and directly to Hb, Hepcidin ,Ferroportin and Iron.

In red blood cells, it is directly proportional to hemoglobin with a significant difference in its value (.765**) as well as inversely proportional to PLT,Hepicidin, AST , iron and inversely with Ferroportin , ALT, Ferritin. While Hemoglobin was directly proportional to PLT, AST and Iron with no significant difference. While inversely compatible with Hepicidin ,Ferroportin ,ALT and Ferritin. Platelets are directly proportional to AST, ALT ,ferritin and inversely proportional to Hepicidin, Ferroportin and iron, with no significant differences.

Hepcidin is directly proportional to Ferroportin with a significant difference in its value (.646**) as well as directly proportional to AST ALT and inversely proportional to Ferritin and iron with no significant differences. While Ferroportin is inversely proportional to AST, ferritin ,Iron and directly to ALT, with no significant differences.

AST is directly proportional to ALT, ferritin and Iron with a significant difference of its value (.554**) with ALT. While ALT is directly proportional to ferritin and

inversely proportional to iron, and there are no significant differences. Ferritin is inversely proportional to iron with no significant difference.

In this study, serum Hepcidin was considerably greater in ALL patients at diagnosis compared to its level after remission, and it was significantly higher in both conditions than the control values. This is in agreement with what was reported by (Ragab *et al*.,2016). Serum Hepcidin exhibited a negative correlation with Hb level in this group as well. which is consistent with (Ragab *et al*.,2016).

indicating that anemia is Hepcidin regulator. Iron load, inflammatory stimuli such as interleukin 6, and unknown erythropoietic signals all influence Hepcidin production. All of these things can occur in acute lymphoid leukemia (Pigeon *et al*.,2001);(Nemeth *et al* .,2003);(Kanda *et al*.,2008). Erythropoiesis and Iron metabolism are intimately related. (Nemeth,2008). Iron metabolism imbalance has been linked to numerous malignancies, including leukemia (Yang, 2015). A trustworthy indicator of bodily iron reserves is considered to be serum ferritin (Piperno, 1998).

Acute leukemia patients at various stages are at risk for iron overload because of frequent blood transfusions (Porter ,2001). In this study, all patient groups had significantly greater blood ferritin and Hepcidin levels than controls. The inflammatory condition may be primarily responsible for the increased ferritin before therapy (Cook *et al*.,2003).

Increased iron overload may have contributed to this increase. The concurrent rise in serum ferritin and Hepcidin in the study populations confirms the significance of increasing iron load in promoting Hepcidin expression. Hepcidin and serum ferritin often react to inflammation and alterations in iron storage similarly, which is shown in the high correlation between the two markers (Kemna *et al*.,2005); (Ganz *et al*.,2008). There is not a lot of information on how

platelets impact blood cancers. This may be due to cancer cells, both myeloid and lymphoid, rarely collecting platelets, and in some cases inhibiting platelet aggregation (Pulte *et al*.,2007). Platelets have been demonstrated to protect adenomas from chemotherapy-induced apoptosis (Radziwon-Balicka *et al*.,2012) . In the pediatric age range, lymphocyte counts following chemotherapy may also be predictive of survival (Lomas *et al*.,2003).

Table (4-11): Correlation Coefficient of All Studied Parameters Among Acute Lymphoid Leukemia ALL

		LYM	RBC	Hb	PLT	Hepicidin	Ferroportin	AST	ALT	Ferritin	Iron
WBC	**R**	.258	.300	-.072	-.066	.047	.020	-.132	-.124	-.193	.047
	Sig.	.112	.063	.662	.691	.775	.905	.423	.450	.239	.774
LYM	**R**	1	-.029	.078	-.475**	.184	.289	-.043	-.190	-.033	.040
	Sig.		.861	.637	.002	.262	.075	.796	.247	.842	.809
RBC	**R**		1	.765**	.219	.111	-.013	.161	-.130	-.113	.116
	Sig.			.000	.180	.500	.936	.328	.430	.492	.481
Hb	**R**			1	.064	-.094	-.096	.274	-.046	-.023	.128
	Sig.				.700	.568	.561	.092	.781	.890	.437
PLT	**R**				1	-.078	-.039	.100	.016	.033	-.121
	Sig.					.635	.812	.543	.924	.840	.462
Hepicidin	**R**					1	.646**	.038	.096	-.061	-.048
	Sig.						.000	.820	.560	.712	.772
Ferroportin	**R**						1	-.049	.027	-.020	-.108
	Sig.							.767	.868	.902	.512
AST	**R**							1	.554**	.135	.206
	Sig.								.000	.411	.207
ALT	**R**								1	.050	-.019-
	Sig.									.763	.910
Ferritin	**R**									1	-.304
	Sig.										.060

**. Correlation is significant at the 0.01 level (2-tailed).

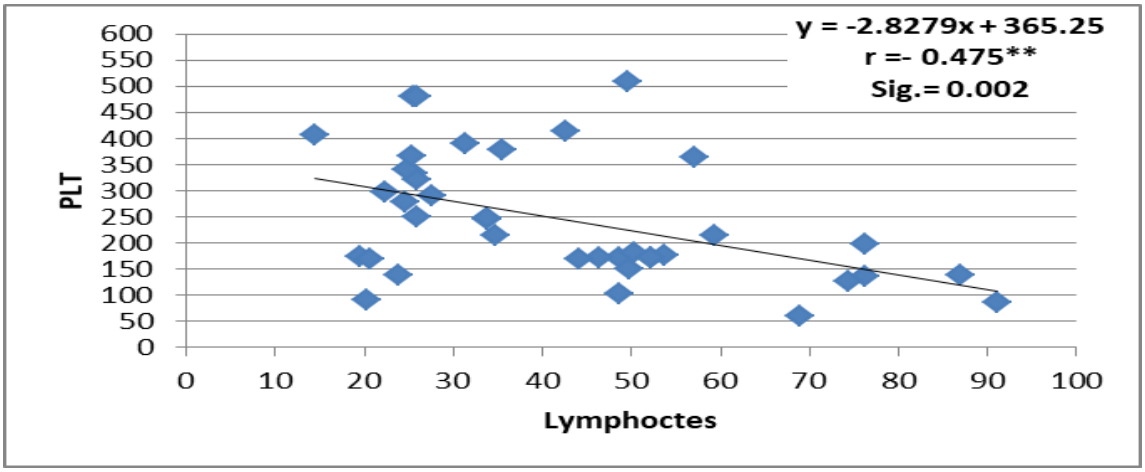

Figure (4-1): Relationship between Lymphocytes and PLT among Acute Lymphoid Leukemia.

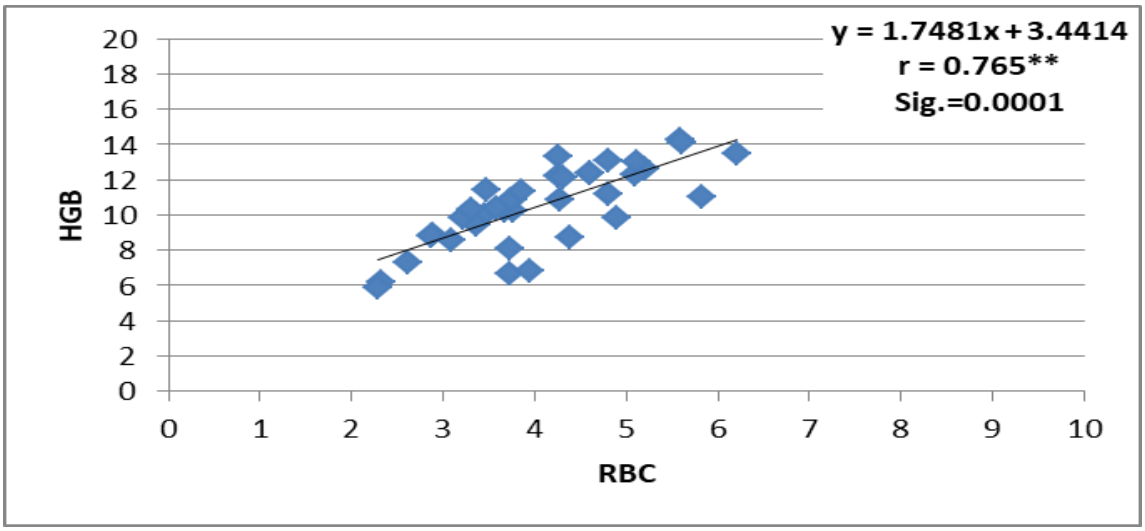

Figure (4-2): Relationship between Hemoglobin and Red Blood Cells among Acute Lymphoid Leukemia.

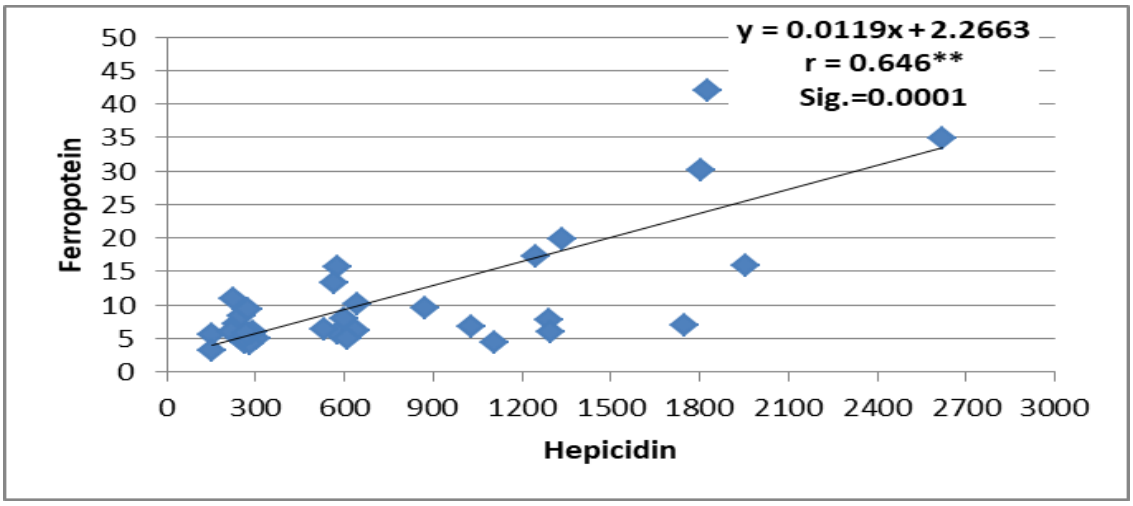

Figure (4-3): Relationship between Hepicidin and ferropotin among Acute Lymphoid Leukemia.

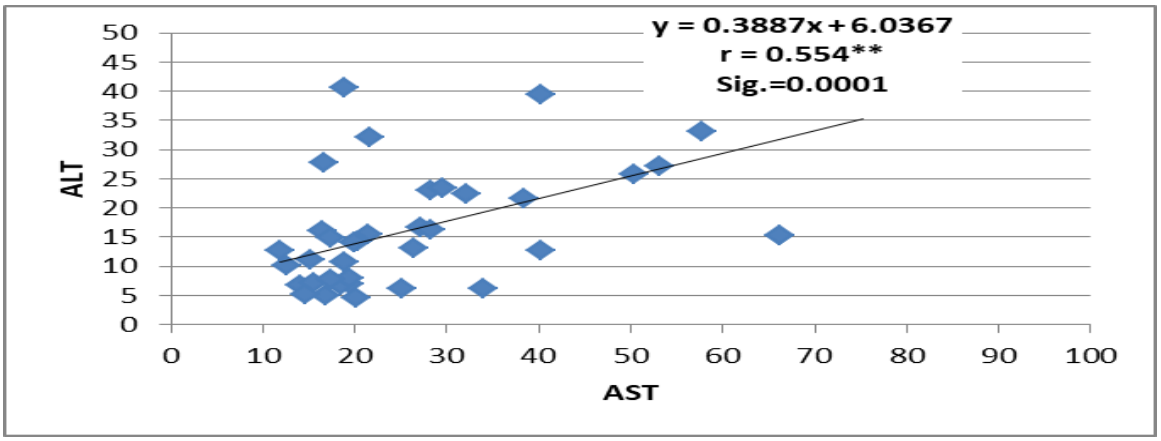

Figure (4-4): Relationship between AST and ALT among Acute Lymphoid Leukemia.

4-12 Correlation coefficient of all studied parameters Among myeloid leukemia (AML)

Our results are shown in the table (4-12). White blood cells were positively associated with RBC, Hb, PLT, Hepicidin and AST, and inversely proportional with LYM, Ferroportin, AST and iron and there is no significant difference. But the correlation between white blood cells and ferritin was inverse, with a significant difference in its value (-0.641 *).

While lymphocytes are inversely proportional to RBC, PLT, Hepicidin, AST and ALT with no significant difference. While directly proportional to each of Hb, Ferroportin ferritin, and there was no significant difference, but there was a significant significant difference with iron, its value (0.646 *).

Red blood cells are directly proportional to LYM, Hb ,PLT, Hepicidin ,AST ,ALT while inversely proportional to ferroportin, ferritin and iron and there is no significant difference between them, we showed through the results a significant difference hemoglobin value (0.812**).

Hemoglobin is directly proportional to LYM, RBC, PLT, Hepicidin, Ferroportin , Ferritin, Iron and inversely proportional to AST and ALT liver tests, and there is no significant difference. Platelets are directly related to Hepicidin, and AST, and inversely to iron, ALT, ferritin, iron, and there is no significant difference between them.

Hepicidin is directly proportional to Ferroportin ,AST, ALT ,Iron and inversely with ferritin. a significant difference in value (0.936**) with Ferroportin. Ferroportin has been directly associated with AST, ALT ,Iron and inversely with ferritin and there is no significant difference for you. As for ferritin, it is directly proportional to iron and there is no significant difference.

Liver function tests AST are directly proportional to ALT, iron and inversely proportional to ferritin with a significant difference in value is (-0.641 *) . while ALT is inversely proportional with ferritin. Ferritin is directly proportional to iron, with no significant differences.

These current findings agreed with prior study findings by (Yokus *et al*., 2021). they found considerably greater Hepcidin concentrations in comparison to healthy controls ,because of the increased Hepcidin synthesis in AML patients. In AML patients the levels of Hepcidin in the blood increase significantly in inflammation and infection in leukemia, can produce iron malfunction and blood insufficiency as a result of chronic disorders (Langer and Ginzburg, 2017) . The iron exporter ferroportin is bound by Hepcidin, which causes internalization and destruction of the protein. Hepcidin expression in acute myeloid leukemia (AML) patients undergoing HCT may be influenced by both genetic and medical variables (Eisfeld *et al*.,2011).

The majority of research into platelet-leukemia interactions has been done in the setting of myeloid leukemia, while a few studies have looked at platelet function in lymphoblastic leukemias (Pulte *et al*.,2007); (Jaime-Pérez *et al*.,2004). Platelets produced from individuals with acute myeloid leukemia (AML) exhibit decreased platelet response to physiological agonists (Pogliani *et al*.,1989), may have platelet storage pool deficit (Gerrard *et al*.,1992), and are frequently associated with illness and treatment-induced thrombocytopenia (Woodcock *et al*.,1984); (Foss *et al* .,2002). According to Cinar *et al*. (2015) and Lamarre *et al*. (2013), the increase and decrease in the number of erythrocytes is changing due to some diseases that affect humans and some physiological changes in the body that lead to a reduction in the number such as anemia, bleeding or an increase in the number such as Polycythemia, Hypoxia, and increased destruction by free radicals.

According to Jemal *et al*. (2009), thrombocytopenia is a major risk factor for bleeding (hemorrhage) and is defined by an unusually low platelet count. Bleeding is a typical adverse effect of several drugs or chemotherapy treatments. Because of this, leukocytes serve a crucial role in defending the body against infection by viruses, bacteria, fungi, and parasites via the phagocytosis process, and are therefore the mobile units of the body's defensive system (Bain, 2017). LYM Because it is created in the bone marrow and thymus, as well as the majority of lymphocytes in the peripheral circulation, many scientists believe that its creation is caused by an antigenic challenge, such as viruses or foreign proteins (Al-Ouqaili *et al*., 2018).

According to Veena *et al*. (2012) anemia is defined by a reduction in both the quantity of red blood cells (RBC) and the concentration of hemoglobin in the blood. Anemia is a symptom of leukemia, a typical indication of bone marrow diseases. WBC count in patients was substantially greater than in healthy controls. According to Wang *et al*. (2011) this conclusion might be explained by the fact that in leukemia there is an increase in clonal proliferation of leukemia cells, which can occur at any stage of maturation in bone marrow as lymphoid, myeloid, or pluripotent. Acute leukemia (AML/ALL) patients frequently have increased aminotransferases. This is probably caused by leukemic infiltrates, which cause liver damage (Segal *et al*., 2010).

ALT is released into the serum as a result of hepatocellular damage and its defective catabolism. Following hepatocellular damage, AST typically rises as well and initially reaches a greater level than ALT. Then, because to its longer plasma half-life, ALT will surpass AST within 24 to 48 hours if the damage is still there. Hepatocellular necrosis is thought to be most easily detected by liver transaminases (hepatitis). These enzymes contribute to gluconeogenesis by giving the TCA cycle

the amino acids it needs. Since ALT is mostly detected in liver tissue and is confined to hepatocytes' cytosol, it is a more accurate diagnostic of liver damage than AST (Pratt and Kaplan , 2000). Rarely is liver involvement associated with acute myeloid leukemia (AML) documented(Mathews *et al*.,2008).

Table (4-12): Correlation Coefficient of All Studied Parameters Among Acute Myeloid Leukemia AML

		LYM	RBC	Hb	PLT	Hepicidin	Ferroportin	AST	ALT	Ferritin	Iron
WBC	**R**	-.162	.555	.257	.300	.068	-.142	.321	-.093	-.641*	-.415
	Sig.	.634	.076	.446	.370	.843	.677	.336	.785	.034	.204
LYM	**R**	1	-.070	.099	-.344	-.044	.048	-.097	-.164	.304	.646*
	Sig.		.839	.771	.300	.899	.889	.777	.631	.363	.032
RBC	**R**		1	.812**	.006	.028	-.124	.315	.143	-.487	-.126
	Sig.			.002	.987	.935	.716	.346	.674	.128	.712
Hb	**R**			1	.238	.095	.082	.349	-.052	-.300	.243
	Sig.				.481	.780	.812	.293	.879	.370	.472
PLT	**R**				1	.041	-.035	.003	-.108	-.214	-.007
	Sig.					.904	.920	.992	.753	.527	.984
Hepicidin	**R**					1	.936**	.152	.281	-.600	.145
	Sig.						.000	.655	.402	.051	.671
Ferroportin	**R**						1	.101	.078	-.353	.241
	Sig.							.768	.819	.288	.476
AST	**R**							1	.312	-.641*	.228
	Sig.								.350	.034	.500
ALT	**R**								1	-.481	.294
	Sig.									.134	.381
Ferritin	**R**									1	.095
	Sig.										.781

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

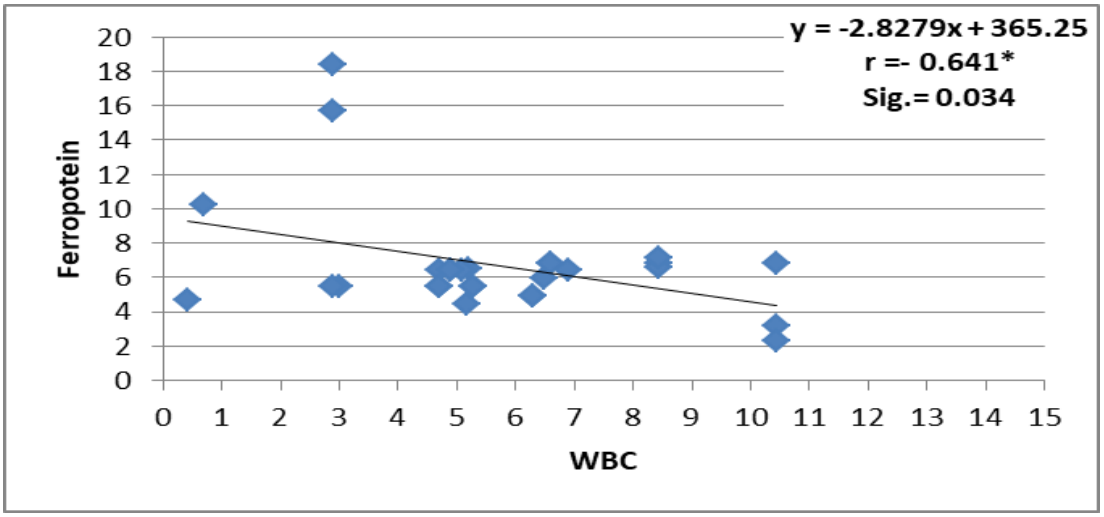

Figure (4-5): Relationship between WBC and ferropotin among myeloid leukemia **.**

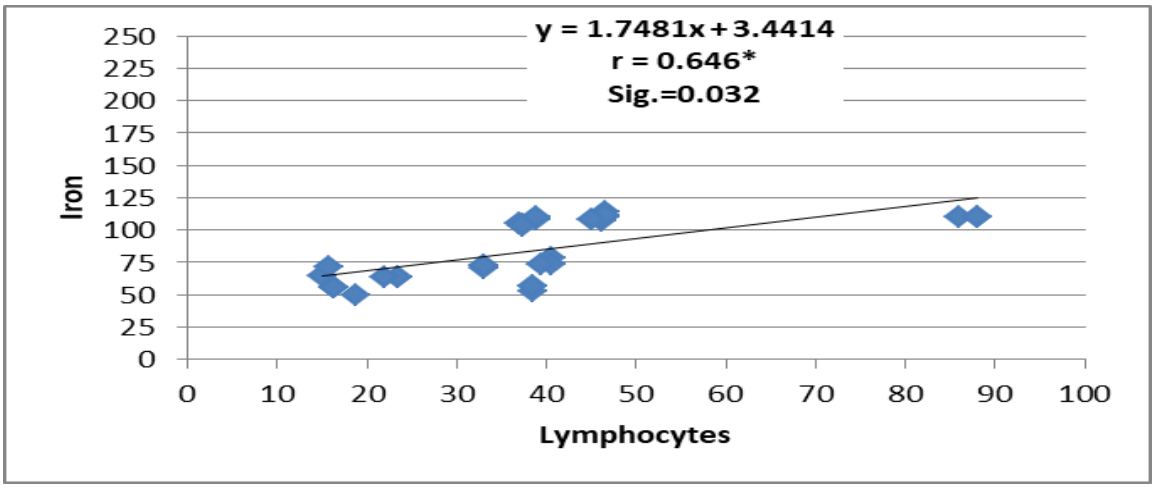

Figure (4-6): Relationship between Lymphocytes and Iron among Acute myeloid leukemia .

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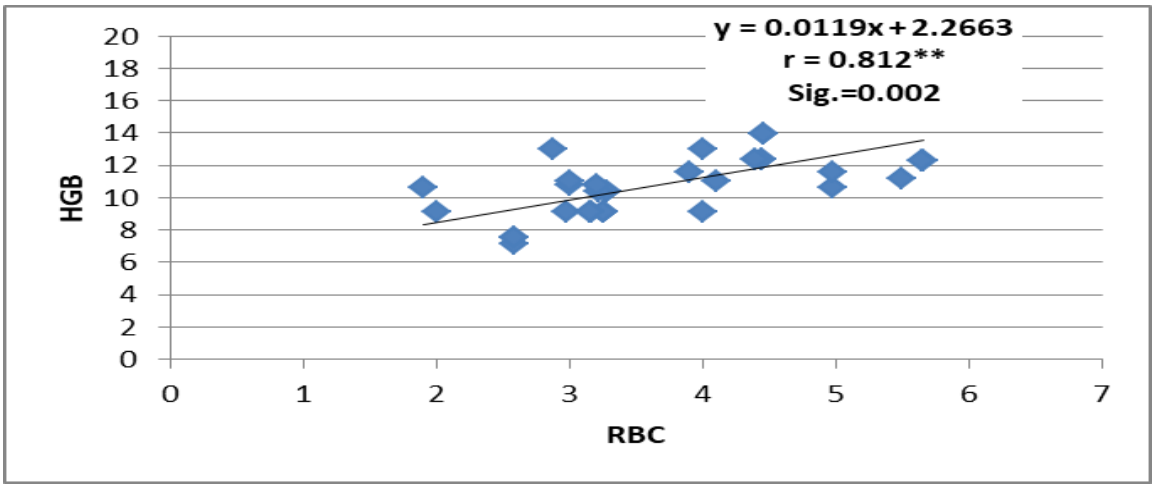

Figure (4-7): Relationship between RBC and HGB among Acute Myeloid Leukemia.

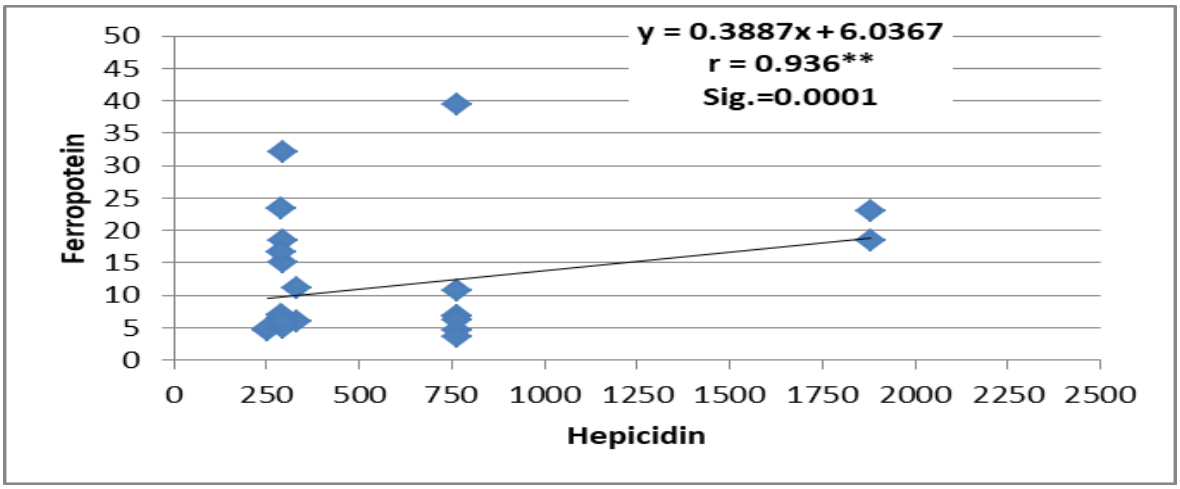

Figure (4-8): Relationship between Hepicidin and Ferropotin among Acute Myeloid Leukemia.

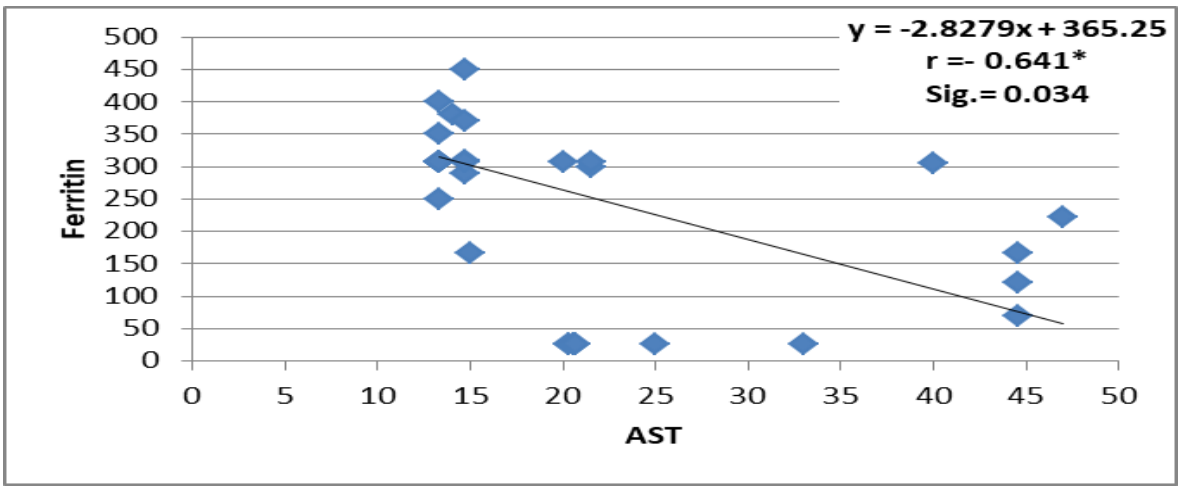

Figure (4-9): Relationship between AST and ferritin among Acute Myeloid Leukemia.

CHAPTER FIVE
Conclusions
and
Recommendations

Conclusions :

1. According to our study, acute lymphocytic leukemia is more common in children than acute myeloid leukemia, which is more common in older people.
2. The individuals with acute leukemia appeared to have an increased concentrations of the hormone hepcidin thus making it a risk factor for leukemia.

3. The findings revealed that patients had higher concentrations of iron, ferritin, and ferroportin than the control group, which may be considered a risk factors for acute leukemia.

Recommendations:

1. Molecular study for hepcidin hormone and ferroportin.
2. Research further biochemical aspects of iron metabolism.
3. Conducting comprehensive studies on one type of acute leukemia.
4. Before administering chemotherapy, iron measurement is crucial.

CHAPTER SIX

Appendices

7- Appendices

7-1 Methods

7-1-2 Complete blood count (CBC)

1- Lyser

Principle

Lyser is used on the STEL 3 Auto hematological analyzer for count, distinguishing, measuring hemoglobin. RBC are destroyed by the Lyser and hemoglobin is released throughout the counting process. Hemoglobin interacts with Lyse, the reaction produces a specific color, allowing the concentration of the solution to be detected using the colorimetric technique. WBCs discharge cytoplasm and shrink at the same time, the size of solid substance, such as the nucleus and granules, left in various types of white blood cells varies. An auto hematology analyzer can classify WBCs into 3 groups based on their electrical nonconductivity such as lymphocyte, intermediate cells, and neutrophil, By detecting change in electrical resistance through the counting hole as they pass through suspended particles in the electrolyte solution.

Reagents

Quaternary ammonium salt salt<0.15%, NaCl<0.15%, Stabilizer<0.12%

2- Diluent

Principle

Diluyent is a kind of balanced electrolyte solution with electrical conductivity which is used in the process of counting blood cells. The STEL 3 auto Hematology analyzer performs a complete blood count based on the electrical

failure of the blood cells and is done by detecting changes in electrical resistance as suspended particles in the electrolyte solution pass through counting hole.

Reagent

NaCl <0.6% , Stabilizer <0.1% and buffer solution

3- Cleaner

Principle

The STEL 3.Cleaner is used in the STEL 3 automatic blood analyzer to clean and wash the tubing system, then eliminates other blood residues and particles to ensure an accurate blood cell count.

Reagent

Buffer solution<0.3%, Protease<0.2%

7-1-3 Biochemical Tests

7-1-3-1 Liver Function Tests Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

- **The Principle of Alanine Aminotransferase (ALT)**

Method

UV-assay according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) without pyridoxal phosphate activation.

Reaction Principle

L.Alanine + α.Oxoglutarate $\xrightleftharpoons{ALT}$ pyruvate + L. glutamate

Pyruvate + NADH + H^+ $\xrightleftharpoons{LDH}$ L. lactate + NAD^+ + H_2O

Reagents

Reagents	**Concentration**	**Concentration**
R1	TRIS buffer L. Alanine LDH NADH	150 mmol/L 750 mmol/L ≥1200 U\L 0.4 mmol/L
R2	α.Oxoglutarate NADH	**90** mmol/L 0.9 mmol/L

Assay Procedure

	Blank	Sample
Reagent 1 Dist Water Sample	1000 µL 100 µL _	1000 µL _ 100 µL
Mix, incubate for 5 min ,then add:		
R2	250 µL	250 µL
Mix thoroughly, read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.		
ΔA/min [ΔA/min sample]-[ΔA/min blank]		

Calculation

The analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process. Conversion factor of traditional units (U/L) into S1-units (μ kat/L):

1 U/L = 16.67×10^{-3} μkat /L, 1 μkat /L = 60 U/L

- **The Principle of Aspartate Aminotransferase (AST)**

Method

UV-assay according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) without pyridoxal phosphate activation.

Reaction Principle

L. Aspartate+ α.Oxoglutarate $\xrightleftharpoons{AST}$ Oxaloacetate+ L.glutamate

Oxaloacetate +NADH+H^+ $\xrightleftharpoons{MDH}$ L. malate +NAD^+ +$\mathbf{H_2O}$

Reagents

Reagents	Content	Concentration
R1	TRIS buffer	100 mmol/L
	L. Aspartate	300 mmol/L
	LDH	≥ 900 U/L
	MDH	≥ 600 U/L
	NADH	0.4 mmol/L

R2	NADH α-oxoglutarate	0.9 mmol/L 60 mmol/L

Assay Procedure

	Blank	Sample
Reagent 1 Dist Water Sample	1000 µL 100 µL _	1000 µL _ 100 µL
Mix, incubate for 5 min ,then add:		
R2	250 Ml	250 µL
Mix thoroughly, read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.		
ΔA/min [ΔA/min sample]-[ΔA/min blank]		

Calculation

The analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process. Conversion factor of traditional units (U/L) into S1-units (µkat/L):

1 U/L = 16.67×10^{-3} µkat /L, 1 µkat /L = 60 U/L

7-1-3-2 Iron Test

Test principle

Colorimetric assay.

Transferrin-Fe-complex $\xrightarrow{pH < 2.0}$ apotransferrin + Fe^{3+}

Fe^{3+} $\xrightarrow{ascorbate}$ Fe^{2+}

Fe^{2+} + FerroZine $\longrightarrow$ colored complex

Under acidic conditions, iron is liberated from transferrin. Lipemic samples

are clarified by the detergent. Ascorbate reduces the released Fe3+ ions to

Fe2+ ions which then react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically.

Reagents - working solutions

R1 Citric acid: 200 mmol/L; thiourea: 115 mmol/L; detergent

R2 Sodium ascorbate: 150 mmol/L; FerroZine: 6 mmol/L;

preservative

R1 is in position A and **R2** is in position B.

7-1-3-3 Ferritin Test

The Principle

Assay Principle The CL - series FERR assay is a two – site sandwich are assay to determine the level of FERR . In the first step , sample , paramagnetic microparticle coated with monoclonal anti - FERR antibody (mouse) and monoclonal anti - FERR antibody (mouse) -alkaline phosphatase conjugate added into a reaction vessel . After incubation , FERR present in the sample binds to both anti - FERR antibody coated microparticle and anti - FERR antibody alkaline phosphatase - labeled conjugate to form a sandwich complex . Microparticle is magnetically captured while other unbound substances are removed by washing . In the second step , the substrate solution is added to the reaction vessel . It is catalyzed by anti- FERR antibody (mouse) -alkaline phosphatase conjugate in the immunocomplex retained on the microparticle . The resulting chemiluminescent reaction is measured as relative light units (RLUS by a photomultiplier built into the system . The amount of FERR present in the sample is proportional to the relative light units (RLUS) generated during the reaction . The FERR concentration can be determined via a calibration curve .

Reagent Components:

Ra	Paramagnetic microparticles coated with monoclonal anti - Ferritin antibody (mouse in TRIS buffer with preservatives
Rb	Monoclonal anti - Ferritin antibody (mouse) -alkaline phosphatase conjugate in TRIS buffer with preservatives
Rc	TRIS buffer with preservatives

7-1-3-4 Hepciden and Ferroportin Test by ELISA

- **Assay Procedure of Hepciden**

1- Prepare all reagents, standard solutions, and samples according to the instructions. Before using any reagents, bring them to room temperature. The test is carried out at room temperature.

2- Count the number of strips needed for the test. To use, place the strips in the frames. Unused strips should be kept at 2-8°C.

3- Fill standard well with 50ul standard. Because the standard solution contains biotinylated antibody, do not add antibody to the standard well.

4- Add 40ul sample to sample wells, followed by 10ul Rat HEPC antibody, and finally 50ul streptavidin-HRP to sample and standard wells (Not blank control well). Combine thoroughly. Apply a sealant to the plate. Incubate at 37°C for 60 minutes.

5- Remove the sealant and wash the plate with wash buffer 5 times. For each wash, soak wells in 300ul wash buffer for 30 seconds to 1 minute. Aspirate or decant each well and wash 5 times with wash buffer for automatic washing. Using paper towels or other absorbent material, blot the plate..

6- Pour 50ul of substrate solution A into each well, followed by 50ul of substrate solution B. Incubate the plate in the dark for 10 minutes at 37°C with a fresh sealer.

7- Add 50ul Stop Solution to each well; the blue hue will soon turn to yellow.

8- Within 10 minutes of applying the stop solution, determine the optical density (OD value) of each well using a microplate reader set to 450 nm.

- **Assay Procedure of Ferroportin**

This procedure is provided for reference only. The product manual may differ slightly. The product should be used as stated on the product manual included and delivered together with the product.

1- Determining the standard, testing samples and checking control wells.
2- Divide 100 ul of diluted standard solution into standard wells.
3- Aliquot 100 ul of the standard diluted buffer into the control well (well zero).
4- Transfer 100 ul of the diluted samples into the sample wells. At 37 °C, incubate for 90 minutes.
5- Detection Reagent A, 100 ul, should be aliquoted into each well. At 37 °C, incubate for one hour.
6- Wash three times.

7- Divide 100 ul of Detection Reagent B evenly across the wells. At 37 °C, incubate for 30 minutes.

8- Wash five times.

9- Divide 90 µl of TMB in half. Add substance to every well. At 37 °C, incubate for 10–20 minutes.

10- Aliquot 50 µl of the Stop Solution.

11- Take a 450 nm reading of the OD.

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الخلاصة :

اجريت الدراسة الحالية في مختبرات قسم علوم الحياة كلية العلوم، جامعة بابل. تم جمع العينات واكمل الجزء العملي لهذه الدراسة خلال المدة الممتدة بين تشرين الثاني 2021 الى شهر ايار 2022. العينات التي تم جمعها كانت من مرضى ابيضاض الدم الحاد بنوعيه اللمفي والنقوي (ALL,AML) من مستشفى مرجان التعليمي في بابل ومستشفى الحسين التعليمي مركز الاورام/كربلاء ومستشفى بغداد التعليمي في مدينة الطب في بغداد. جاءت هذه الدراسة لتقييم فعالية هرمون الهبسيدين (له دور رئيسي في تنظيم تركيز الحديد) في عملية تكوين الدم لدى مرضى ابيضاض الدم الحاد.

تم جمع العينات من الذكور والاناث ضمن اعداد عمرية ممتدة من 9 اشهر الى 41< سنة. وتضمنت 101 عينة دم صنفت الى مجاميع على النحو الاتي: مجموعة السيطرة تشمل 36 عينة (20 عينة ذكور و16 عينه اناث) .اما مجموعة المرضى بابيضاض الدم الحاد بنوعيه اللمفي والنقوي كانت 65 عينة (34 ذكور و31 اناث). ثم صنفت العينات الى ثلاث مجاميع حسب العمر المجموعة الاولى 9 اشهر–20 سنة والمجموعة الثانية 21–40 سنة اما المجموعة الثالثة 41< سنة. قسمت الدراسة الحالية الى ثلاث محاور المحور الاول الديموغرافي والثاني قياس معايير الدم والثالث الدراسة البايوكيميائية ثم التحري عن العلاقة فيما بين جميع المعايير المدروسة.

اظهرت نتائج حساب معايير الدم زيادة معنوية في خلايا الدم البيض و عدد صفائح الدم لدى مرضى ابيضاض الدم الحاد بنوعيه مقارنة مع السيطرة ماعدا عدد كريات الدم الحمر اذ اظهرت انخفاضا معنويا لدى مرضى ابيضاض الدم الحاد مقارنة مع السيطرة. وبينت نتائج الدراسة البايوكيميائية وجود زيادة معنوية ($p\leq 0.05$) في تركيز انزيمات الكبد لدى مرضى ابيضاض الدم الحاد مقارنة مع مجموعة السيطرة. أظهرت نتائج هرمون الهيبسيدين في مرضى ابيضاض الدم الحاد ازديادا معنويا ($p\leq 0.05$) مقارنة بالأشخاص الأصحاء. فضلا عن وجود زيادة معنوية ($p\leq 0.05$) لتركيز الفيروبورتين والحديد والفيريتين لدى مرضى اللوكيميا الحادة مقارنة بالأشخاص الأصحاء. كما أشارت نتائج الارتباط إلى وجود ارتباط ذو علاقة طردية بين مرضى ابيضاض الدم اللمفي الحاد (ALL) بين هيبسيدين والفيروبورتين مع وجود فرق معنوي

قيمته (0.646**) ، بالإضافة إلى وجود علاقة طردية بين ناقلة أمين الأسبارتات و ناقلة أمين الانين. بينما يتناسب الفيروبورتين بعلاقة عكسَية مع ناقل امين الاسبارتات و الفيريتين و الحديد ويتناسب طرديًا مع ناقل امين الالانين ، مع عدم وجود فروق ذات دلالة إحصائية. يتناسب ناقل امين الاسبارتات طرديًا مع الفيريتين و الحديد و ناقل امين الالانين مع وجود فرق معنوي قيمته (0.554 **) مع ناقل امين الانين. اما العلاقة بين الخلايا الليمفاوية و الصفائح الدموية علاقة عكسية مع اختلاف كبير في قيمتها المعنوية (-475. **). في حين أن العلاقة بين الهيموغلوبين وخلايا الدم الحمراء علاقة طردية مع وجود فرق معنوي قيمته (.765 **). اما فيما يخص مرضى ابيضاض الدم النقوي الحاد (AML) ، اوضحت النتائج وجود علاقة ارتباط عكسية بين خلايا الدم البيضاء والفيريتين مع اختلاف معنوي في قيمتها (-0.641 *) ، بينما وجدت علاقة ارتباط طردي بين الخلايا الليمفاوية والحديد مع اختلاف قيمته (0.646*) ، وكان هناك ارتباط طردي بين كريات الدم الحمراء و الهيموغلوبين مع وجود فرق معنوي قيمته (0.812 **) . في حين يتناسب الهبيسيدين طرديًا مع الفيروبورتين و ناقل امين الاسبارتات و ناقل امين الانين والحديد مع اختلاف معنوي كبير قيمته (0.936 **) مع الفيروبورتين. وارتباط عكسي مع الفيريتين. يتناسب الفيروبورتين بعلاقة طردية مع ناقل امين الاسبارتات و ناقل امين الالانين والحديد, وعكسًا مع الفيريتين .اما الفيريتين ، فهو يتناسب بعلاقة طردية مع الحديد. في حين أن ناقل امين الاسبارتات يتناسب طرديًا مع ناقل امين الانين والحديد ويتناسب عكسًا مع الفيريتين مع اختلاف معنوي كبير قيمته (-0.641 *). نستنتج من هذه الدراسة ان هنالك زياده في نسبة المرض لدى الذكور مقارنة مع الاناث كما اشارت الدراسة الى انه خطر المرض يزداد لدى الفئات العمرية الفتية لاسيما المجموعة الاولى (9اشهر-20سنه).