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Evolution the effect of *Nucleophysmin* 1(*NPM*1) Mutations on Liver and Kidneys Functions in Patients with Chronic Myeloid Leukemia

OBJ

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By

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿وَإِذَا مَرِضْتُ فَهُوَ يَشْفِيْنِ﴾

صدق الله العليّ العظيم

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Supervisor Certification

We certify that this thesis entitle **(Evolution the effect of *Nucleophysmin* 1(*NPM*1) Mutations on Liver and Kidneys Functions in Patients with Chronic Myeloid Leukemia)** by (**Ghaniya Haider Obaid**) was prepared under our supervision at the department of Biology, College of Science for Women, University of Babylon as a partial requirement for the degree of Master in Biology.

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Dedication

I dedicate this humble effort...

To those whom God Almighty has associated His worship with being kind to them.

To whom God Almighty has placed their gratitude in the same position as His gratitude

My dear father and mother, may God prolong their lives

To my support and pride... my brother and sister

To the martyrs of the homeland who irrigated the pure land of Iraq with their pure blood.

To all of you... I dedicate this humble effort

Summary

The main objective of this study and the fact that research on leukemia is scarce in terms of molecular and clinical research and its relationship to the course of the disease and its impact on some important vital organs such as the liver and kidney, where it became clear that the behavior of the cancer cell is affected by the changes that occur at the level of DNA. This research sought to examine the process of liver and kidney damage in Iraqi leukemia patients who have a mutation in the *nucleophosmin* protein 1 (*NPM*1) and compare it with patients who do not have this mutation.

The current study dealt with one of the most important malignant blood diseases, chronic myelogenous leukemia. Early diagnosis and monitoring of disease progression may greatly contribute to avoiding disease complications. Chronic myelogenous leukemia is a myeloid neoplasm associated with the BCR-ABL1 fusion gene based on the Philadelphia chromosome.

In Marjan Medical City / Babil Governorate, Baghdad Hospitals Unit (Medicine City) and the Leukemia Center, samples were taken from patients with myeloid leukemia. Samples were collected from 1/September 2022 to 30/September 2023. During this period, (72) samples were collected, but 60 samples were taken from patients and 12 patients were excluded because they were suffering from type 1 diabetes, hepatitis, or kidney failure. Also, any patient within another line of treatment was excluded, including autologous bone marrow transplantation. The study also included 30 samples from a healthy control group.

Blood samples (5 ml) were taken from each person participating in the study (both patients and healthy subjects) by venipuncture, 2 ml were placed in EDTA tubes and 1 ml was used for CBC analysis and 1 ml was placed at -20 °C for later use in the molecular study, while the 3 ml remaining in gel-containing tubes for the purpose of serum separation.

The current study included two main parts: the chemical-physiological functional part and the molecular aspect. In the first part, the levels of serum biomarkers (urea, creatinine, IL-18, and kidney injury molecule-1 (KIM-1) were estimated as markers of kidney function, and the level of GPT, and GOT and vitronectin (VTN) as markers of liver damage function , in addition to studying some important blood parameters (red blood cells, white blood cells, and platelets) in both healthy and sick groups. The relationship among the levels of these biomarkers, with hematological parameters, was also examined.

The results showed through statistical analysis that the disease showed that there were statistically significant differences, and the differences were high between the patients and the control group with regard to (red blood cells, white blood cells, and platelets at the level of $p \leq 0.05$. While chemotherapy leads to a

significant increase $p \leq 0.05$ in the levels of these criteria compared to healthy people. and healthy group with respect to kidney function test (urea, creatinine, and IL18), while there was a significant non-significant difference between patients and healthy group with respect to kim-1 at $p \leq 0.05$.

The second section of the study included the investigation of the presence of the mutation in the nucleofosmin protein. It was found through the genetic study that there are 14 patients in whom this mutation (*NPM*1) is present. As for the healthy group, this mutation does not exist at all. The results indicated that there are statistically significant differences between those Those with a mutation and a sub with respect to white blood cells. Whereas, non-significant differences existed between those with and without the mutation with respect to RBCs and platelets at p-value ≤ 0.05. As for the indicators of hepatic liver function depending on the mutation, it was found that there is a significant difference between those with and without the mutation with respect to (VTN). While there is a non-significant difference $p \geq 0.05$ between those with and without the mutation with respect to AST and ALT at p value ≤ 0.05. As for kidney biomarkers, depending on the mutation, it was found that there is a significant difference between those with and without the mutation with regard to (IL18). While there is a non-significant difference $p \geq 0.05$ between those with and without the mutation with respect to urea, creatinine and KIM-1 with a value of $p \leq 0.05$. It has been concluded that the disease is more common in females than in males for several reasons, including (the use of collagen in cosmetics, hormonal irregularities such as estrogen).

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Abbreviations

ALT	Alanine Transaminase Test
AST	Aspartate transaminase
ASCT	Autologous Stem Cell Transplant
BMI	Body Mass Index
BM	Bone Marrow
BRM	Biological Response Modifiers
BPs	Bis Phosphonates
BRC	Bone Remodeling Compartment
CBC	Complete Blood Count
CT	Computed Tomography
CR	Complete- Response
CDR3	Complementarity-Determining Regions
CTX- Type-1	Terminal Cross-Linking Telopeptide of Type -1- Collagen
Crab	Calcium Renal Anaemia Bone
CNNs	Convolutional Neural Networks
DKKI	Dikkopf-1
EMM	Extramedullary Multiple Myeloma
ECM	Extracellular Matrix
FBLN1	Fibulin-1
FISH	Fluorescence-In-Situ Hybridization
FLCs	Free Light Chain
FDG	Fluor Deoxy Glucose
GEP	Gene Expression Profiling
Hb	Haemoglobin
HDC	High Dose Chemotherapy
HSCT	Hematopoietic-Stem-Cell Transplant

IgG	Immunoglobulin G
IgM	Immunoglobulin M
KIM-1	Kidney Injury Molecule-1
IL	Interleukin
IL-6	Interleukin 6
IMWG	International Myeloma Working Group
LC	Lineal Collider
LDH	Lactate Dehydrogenase
MGUS	Monoclonal
MM	Multiple Myeloma
MBO	Metabolic Bone Disease
MPCs	Malignant Plasma Cells
MRI	Magnetic- Resonance- Imaging
MMP	Matrix Metalloproteinase
MRD	Minimal Residual Disease
NADH	Nicotinamide Adenine Dinucleotide Dehydrogenase
OC	Calcium Renal Anemia Bone
OB	Osteoblast
OS	Overall Survival
PR	Partial- Response
PCL	Plasma Cell Leukemia
PCs	Plasma Cells
PET	Positron Emission Tomography
RANKL	Receptor Activator of NF Kappa B-Ligand
RBC	Red Blood Cell
R-ISS	Revised International Staging
SPEP	Serum Protein Electrophoresis
SMM	Smoldering Multiple Myeloma
VTN	Vitronectin
WBC	White Blood Cell

Chapter One

Introduction

Chapter One

Introduction

1.1 Introduction

Leukemia is a member of a larger class of malignancies known as tumors of the hematopoietic tissues that also affect the bone marrow and blood, Acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML) are the four main kinds of leukemia (Kitchlu *et al.*,2020). Leukemia can entail a significant degree of kidney and liver dysfunction (Pavlovsky and Mela,2018).

The leukemia pathophysiology Because of the Philadelphia chromosome or a reciprocal translocation of chromosomes 9 and 22, hematopoietic cells might develop CML. BCR-ABL1 is the gene product that is produced when the breakpoint cluster region (BCR) gene on chromosome 22 (region q11) and the Abelson (ABL1) proto-oncogene on chromosome 9 (region q34) merge (Pavlovsky and Mela Osorio, 2018). A tyrosine kinase protein, which is mostly produced by this fusion gene, is called p210BCR-ABL1 (Amarante-Mendes *et al*., 2022).

Interleukin-18 (IL-18) In addition to playing a critical part in the immune response against infection and inflammation, The cytokine interleukin-18, sometimes known as IL-18, is responsible for promoting inflammation. Pathogens or inflammatory signals cause a range of cells, including macrophages, dendritic cells, and epithelial cells, to be stimulated to create IL-18. (Yasuda *et al*., 2019).

Kidney injury molecule-1 (KIM-1) is a promising indicator of renal tubular damage (Dumnicka *et al*.,2020). It is a membrane protein that is

split into two parts: the extracellular portion and the cytoplasmic portion. KIM-1 has significant effects on immune system disorders and kidney damage. The amount of KIM-1 in urine can be a good indicator of how much renal tissue damage—possibly caused by leukemia cells infiltration—has occurred (Song *et al*., 2019).

Vitronectin (VTN): In addition to being present in the extracellular matrix of various tissues, A "serum spreading factor" that is also present in blood is known as vitronectin (VTN). The regulation of blood coagulation and fibrinolysis, as well as cell adhesion, migration, and proliferation, are just a few biological processes that VTN is known to have a role in. The compound was also known as "epibolin" and "S protein," and it was shown to be an inhibitor of the complement membrane attack complex. (Shen *et al*., 2020).

The nucleophosmin (NPM1) gene produces a multifunctional protein with significant nucleolar localisation that shuttles between the nucleus and cytoplasm. NPM1 mutations, which function deterministically to produce the aberrant cytoplasmic delocalization of NPM1 mutants, are responsible for around one-third of adult cases of acute myeloid leukemia (AML) (Jishi, 2019)).

1.2 Aim of the Study

In view of the limited studies available on leukemia disease, especially the molecular and clinical studies in Iraq, changes at the DNA level determine how a cancer cell behaves.

This study aimed to provide a practical approach to the study of liver and kidney damage in Iraqi leukemia patients with Nucleophosmin 1 (*NPM1*) mutation_and compare with leukemia patient without (*NPM1*) mutation. This is achieved by measuring the following:

- Determine *Nucleophosmin* 1 (*NPM1*) mutation
- indicators for kidney disease include serum creatinine, urea, IL-18, and kidney injury molecule-1 (KIM -1).
- serum GPT, GOT and vitronectin (VTN) as markers for liver damage.
- Finding the correlation between the studied variables.

Chapter Two

Literature Review

Chapter Two

Literature Review

2-1 Leukemia

Hematological malignancies include leukemia as a diverse group. that develops when blood or bone marrow components become malignant (Chennamadhivuni *et al.*, 2022)

A surplus of abnormal or immature white blood cells are produced by the bone marrow and other blood-forming organs during the development of leukemia, while the production of healthy blood cells is constrained. In other words, they are aberrant white blood cells, also known as blasts or leukemia cells, that have not fully grown (Ahmed *et al.*, 2019). Leukemia cells develop more quickly than normal cells do. They gradually replace the normal WBC and RBC population, and they may disperse to other organs and lymph nodes as well. (Whiteley *et al.*, 2021).

Leukemia and other cancers are similar biologically in that they are clonal. The molecular alterations required for the emergence of a malignant disease are a rare phenomenon when one considers the vast majority of target cells that are vulnerable to this state. To put it another way, a malignant tumor rarely develops as a result of a single genetic alteration. (Wiggins and Stevenson, 2020).

Studies have established that the word "leukemia" is Latin in origin, having its roots in the Greek words "leukos," which means "white," and "haima," which means blood, "white blood," respectively.In Edinburgh, John Hughes Bennett penned "Case of Hypertrophy of the Spleen and Liver in which Death Took Place From Suppuration of the Blood" in 1845. which was the first scientific study to describe leukemia , the earliest

description of chronic myeloid leukemia (CML) was likely provided here (Bennett, 1845).

Regardless of Bennett, Rudolf Virchow published his article "Weisses Blut" in the same year that described a patient with CML (Virchow and Beneke, 1910). In 1872, Ernst Neumann made the earliest scientific discovery that leukemia starts in the bone marrow. In Neumann (1872). Eosinophils, basophils, and neutrophils were recognized as granulocytes by Paul Ehrlich in 1880, and he separated between myeloid (originating from granulocytes) and lymphoid (originating from lymphocytes) categories of leukemia at that time (Ehrlich, 2013). Leukemia can be categorized as acute or chronic, depending on how quickly the leukemic cells multiply.

2-1-1 Leukemia Causes

Scientists do not yet understand the exact causes of leukemia. It seems to develop from a combination of genetic and environmental factors. However, this type of cancer is not hereditary. Different forms of leukemia have unclear underlying causes. Any person can get leukemia and have it spread to a lethal illness stage for a variety of reasons and risk factors. These are the underlying reasons:

Smoking and alcohol use, family history, prior treatment, inherited disorders, ionizing radiation, Occupational exposure to chemicals and the human T-cell leukemia type I virus (HTLV-I) are two risk factors. (Huang *et al*., 2022).

2-1-2 Risk Factors

The following factors may increase the risk of developing leukemia:

• Exposure to high energy radiation from an atomic bomb or a nuclear power plant.

• Exposure to certain chemicals, such as benzene or formaldehyde.

• Blood disorders.

• Exposure to chemotherapy or radiation therapy.

• Genetic disorders such as Down syndrome.

• Some types of viruses can cause tumors, such as the hepatitis B virus and the HIV virus.

• Family history of leukemia, but this is very rare. (Rock *et al*, 2020).

2-1-3 Type of Leukemia

Based on the afflicted white blood cell type (lymphoid vs. myeloid) and the disease's characteristics (acute vs. chronic), leukemia are divided into 4 primary categories: According to a disease's characteristics (Chennamadhivuni *et al.*, 2022), it is categorized as:

· Acute Leukemia

· Chronic Leukemia

- Depending on the damaged WBCs' kinds (Viswanathan *et al*., 2020):

· Lymphocytic Leukemia

· Myelogenous Leukemia

2-1-3-1 Acute Leukemias

Early cells known as "blasts" are the source of acute leukemia. Young cells that divide are called blasts. Normally, bone marrow stem cells (immature cells) are produced by the body and develop into mature blood cells (Blackburn *et al*., 2019).

2-1-3-2 Chronic Leukemia

Leukemia cells originate from mature, aberrant cells in chronic leukemia. For too long, the cells flourish and multiply. Cells develop slowly (Mukkamalla *et al.*, 2022).

2-1-3-3 Acute Lymphocytic Leukemia (ALL)

Occurs as a result of hemopoetic stem cells' aberrant malignant transition into primordial undifferentiated cells with a lengthy lifespan (Whitely *et al*., 2021) Anemia, thrombocytopenia, and granulocytopenia are caused by these lymphoid acute lymphocytic leukemia (ALL) or myeloid acute myelogenous leukemia (AML) cells multiplying and swapping out healthy bone marrow tissue and hematopoietic cells. The liver, spleen, lymph nodes, central nervous system, kidneys, and gonads are just a few of the organs and regions they can infiltrate because they are bloodborne. (Medinger *et al*., 2019).

The malignant transformation and multiplication of lymphoid progenitor cells in the bone marrow, blood, and extramedullary locations is known as acute lymphoblastic leukemia (ALL). Although 80% of ALL cases in children, ALL in adults is a severe disease (Terwilliger *et al*, 2017).

2-1-3-4 Acute Myeloid Leukemia (AML)

Is a diverse collection of hematological malignancies that impact one or more cell lines and are defined by aberrant clonal proliferation of myeloid blast cells in the bone marrow, peripheral blood, and/or other tissues (Bouchacourt *et al*, 2020).

This type of cancer characterized by clonal cells of the myeloid lineage infiltrating the bone marrow, blood, and other organs with little to no ability for differentiation (Kaleka and Schiller, 2022).

2-1-3-5 Acute Myeloid Leukemia (AML) Classification

The two techniques most frequently used to classify AML are (FAB) and (WHO). (AML). The FAB technique was the initial attempt to distinguish between the numerous subtypes of AML. It was created in the 1970s and is based on morphology and cytochemistry. (Saultz and Garzon 2016). The FAB classification divides AML into eight different subtypes. (Table 1).

Table 1: AML Classification According to FAB (Kumar 2011)

FAB subtype	Morphological classification	
AML-M0	Undifferentiated acute myeloblastic leukemia	5
AML-M1	Acute myeloblastic leukemia with minimal maturation	15
AML-M2	Acute myeloblastic leukemia with maturation	25
AML-M3	Acute promyelocytic leukemia	10
AML-M4	Acute myelomonocytic leukemia	20
AML-M4eos	Acute myelomonocytic leukemia with eosinophilia	5
AML-M5	Acute monocytic leukemia	10
AML-M6	Acute erythroid leukemia	5
AML-M7	Acute megakaryoblastic leukemia	5

2-1-3-6 Chronic Lymphocytic Leukemia (CLL)

Small, rounded to cell slightly irregular B cells make up this neoplasm (Mukkamalla *et al.*, 2022).

Chronic lymphocytic leukemia (CLL), a mature B-cell malignancy, is the most prevalent adult leukemia in the West. It is characterized by proliferation and survival signals associated to chronic active B-cell receptor (BCR) signaling2, which Bruton tyrosine kinase (BTK) is crucial for. Understanding the function of BTK in the pathogenesis of the disease led to the development of ibrutinib, a covalent BTK inhibitor that improved progression-free survival (PFS) and overall survival (OS) in CLL patients compared with standard therapy. (Farrukh, *et al.*, 2019).

2-1-3-7 Chronic Myelomonocytic Leukemia (CML)

proliferation of myeloid neoplasms, is brought on by a clonal process involving an early progenitor hematopoietic stem cell. It is also related to the BCR-ABL1 fusion gene, which is based on the Philadelphia (Ph) chromosome. (Thomopoulos *et al.*, 2020).

Massive splenomegaly accompanied with leukocytosis was first reported in two individuals in 1845, and it seemed to be a novel condition that could not be explained by the other splenomegaly-causing conditions that were already well-known in the 1840s, such tuberculosis (Pophali *et al.*, 2018).

2-1-4 Epidemiology

In Sweden, the annual incidence of chronic myelomonocytic leukemia (CML) is 5.7 per 100,000 individuals, or 90 new cases every year. (Foulon, *et al*., 2019). The incidence of CML varies significantly between nations, with China reporting a reported incidence of 0.4 per 100 000 people and the United States reporting an incidence of 1.75 per 100

000 people (Dong *et al*., 2020; Lin *et al.,* 2020). In Sweden, CML accounts for 15% of all adult leukemia cases. The typical age at diagnosis is 60, there are 1.2 times as many men as women, and and CML is somewhat more common in men. Age affects the occurrence, and 30% of adult CML patients were diagnosed when they were under 50 years old (Foulon *et al.*, 2019). CML affects between 0.6 and 1.2 children per million annually, making it even less frequent in children. (Suttorp *et al*., 2021).

2-1-5 Etiology

The cause of CML is largely unknown. High ionizing radiation exposure is the only recognized risk factor (Suttorp *et al*., 2021). According to reports, chemotherapy with DNA-damaging side effects is responsible for 10–20% of all instances of acute myeloid leukemia (AML), Myelodysplastic syndrome (MDS), and occasionally cases of chronic myeloid leukemia (CML). (Fan *et al.*, 2020; Senapati and Sasaki, 2022). However, radiation appears to increase the risk of developing AML, MDS, and sporadically CML (Fan *et al*., 2020; Yang *et al*., 2018). When compared to controls, Musselmann *et al*. found that there is minimal evidence connecting current and former smokers (who smoke less than one pack per day) to the emergence of CML. despite the fact that additional research has not demonstrated a link between smoking and CML (Qin *et al.*, 2022).

2-1-6 Pathophysiology

Hematopoietic cells develop CML as a result of the Ph chromosome or a reciprocal translocation between chromosomes 9 and 22. The joining of the breakpoint cluster region (BCR) gene on chromosome 22 (region q11) and the Abelson (ABL1) proto-oncogene on chromosome 9 (region q34) results in the gene product known as t(9;22) (q34;q11),(Moretti *et al*.,2023). This fusion gene mostly generates the p210BCR-ABL1 tyrosine

kinase protein. Tyrosine is produced when ATP is phosphorylated by a tyrosine kinase enzyme. Tyrosine kinase activity is constitutively present in CML. which causes the phosphorylation of several substrates in various signaling pathways. This results in increased cell growth, decreased apoptosis, and aberrant cellular adhesion, allowing the adhesion, which permits the malignant clone to grow, inhibit, and take the place of healthy hematopoiesis (Amarante-Mendes *et al.*, 2022). When the BCR-ABL1 gene was transplanted to mice, a condition similar to CML was observed in those animals (Soverini *et al.,* 2018). Although the underlying cause of the creation of the Ph chromosome is not fully understood, it is hypothesized that genomic instability may be a contributing factor (Senapati and Sasaki, 2022). There is a lot of evidence that CML has a chance of turning into AML, ALL, or MDS.. In individuals with ALL and AML, the Ph chromosome is also present to different degrees (2–20%) and is a poor prognostic indicator (Weiss and Sellon, 2022).

2-1-7 Trématent

Leukemia comes in both acute and chronic forms, and each has a wide range of subtypes with varying responses to therapy (Kaleka & Schiller, 2022). As a result, there are generally four main methods used to treat leukemia:

- ❖ Immunotherapy
- ❖ Radiation Therapy Chemotherapy
- ❖ Stem Cell Transplantation
- ❖ Surgery

2-1-7-1 Immunotherapy

Cancer vaccines work by boosting the immune system's reaction to boost the body's defenses against the disease. Our immune system offers a flexible defense against illness brought on by invading pathogens and

aberrant bodily cells. In essence, cancer cells are healthy body cells that have undergone repeated mutations and are no longer functional (Mercadante and Kasi, 2022). Typically, tumor vaccines contain proteins made by or present on cancer cells. The vaccination therapy tries to engage the patient's own defenses in the struggle to eradicate cancer cells by giving them various versions of these proteins and other substances that have an impact on the immune system (Hollingsworth and Jansen, 2019). Many techniques are being tested in clinical trials as part of the emerging discipline of immunotherapy for the treatment and prevention of cancer. Among the class of proteins known as interferons, released by cells with a virus. They assist healthy cells in producing antiviral proteins. While boosting the body's immune response, interferons also aid in reducing leukemia cell proliferation (growth and reproduction) (Jorgovanovic *et al*., 2020)

2-1-7-2 Radiation Therapy Chemotherapy

One of the many methods used to treat cancer is radiation treatment. High-energy waves, like x-rays, are used in radiation treatments to kill cancer cells. To treat or stabilize cancer, radiation can be used alone or in combination with other therapies (such as chemotherapy and surgery) (FitzGerald *et al.,* 2022).

2-1-7-3 Stem Cell Transplantation

Multipotent hematopoietic stem cells are transplanted during hematopoietic stem cell therapy (HSCT), which is often done using bone marrow, peripheral blood, or umbilical cord blood (Khaddour *et al.,* 2022). It is a hematology and oncology procedure that is typically carried out on patients who have certain blood or bone marrow malignancies, like multiple myeloma or leukemia. Prior to the transplant in these situations, the recipient's immune system is typically wiped off using radiation or

chemotherapy. Graft-versus-host disease is a significant HSCT consequence (Zhao *et al.*, 2019).

2-1-7-4 Surgery

surgery to remove an enlarged spleen or to put in a venous access device (a big plastic tube) so that you can take blood samples and administer drugs (Pophali *et al.*, 2018).

2-2 The Effect of Leukemia on Kidney Development

Leukemia can have various different effects on the kidneys, which can lead to kidney damage or dysfunction and have a detrimental effect on a person's chance of survival (Kitchlu *et al.*, 2019). Acute kidney injury (AKI), acute tubular necrosis (ATN), renovascular diseases, extra renal obstruction, glomerulonephritis or glomerular diseases, tumor lysis syndrome (TLS), electrolyte imbalances like hypercalcemia, drug side effects, also known as chemotherapy-associated nephrotoxi, are just a few of the mechanisms through which kidney problems can develop, depending on the type of leukemia present (Kitchlu *et al.*, 2019; Rose *et al.*, 2019; Wanchoo *et al.*, 2018)

A common occurrence in hematologic malignancies is leukemic infiltration by tumor cells, which is seen in 60% to 90% of leukemia patients. The cells may be harmed by this infiltration, which could result in kidney disease. Hematuria, frothy urine, and flank pain are signs of kidney invasion (Sharma *et al*., 2020).

Prerenal acute kidney injury (AKI), which is the most common type of kidney damage seen in leukemia patients, is also cause for serious concern because a decline in kidney function might make it difficult or impossible to administer effective cancer treatments. AKI is frequently accompanied with decreased renal blood flow because of conditions including volume

depletion brought on by inadequate oral intake, diarrhea, anorexia, or early satiety. Leukemia is one of the cancers with the greatest 5-year acute kidney incidence among patients starting systemic cancer therapy, with a percentage of 15.4% (Kitchlu *et al.*, 2019).

Moreover, lysozyme-induced tubular necrosis and tumor lysis syndrome are two common types of kidney injury in people with leukemia, and acute tubular necrosis (ATN), which frequently develops during the course of the disease like sepsis, is another common type (Rose *et al.*, 2019).

Additionally, leukemia frequently exhibits glomerular disease, a collection of disorders that damage the glomeruli and are present in a variety of hematological malignancies. Minimal change disease, which can develop from lysozymuria-induced tubular damage, including glomerulonephritis and thrombotic microangiopathy, and localized segmental glomerular sclerosis, which is more frequently seen in children, are the two main forms of glomerular lesions seen in leukemia. Proteinuria, hematuria, and kidney destruction are all symptoms of glomerular disorders (Wang *et al.*, 2021).

2-3 The Effect of Leukemia on the Development of Liver

The largest and most crucial organ for controlling intermediate metabolism, the liver, plays a crucial part in preserving metabolic balance. One of the detrimental effects of leukemia's manifestation on the liver is the disruption of liver function tests like bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)he effects of Leukemia on Liver development (Islam et al., 2020; Mekonnen and Wondmeneh, 2022).

The liver is the largest and most important parenchymatous organ in the body for the regulation of intermediate metabolism. Due to strict requirements and the need to maintain the metabolic balance, administering chemotherapy presents a challenge. Processes. The majority of medications have a lipophilic nature, which makes the liver easily absorb them. Chemotherapy can cause hepatic steatosis in up to 85% of individuals. The more dangerous condition is steato-hepatitis, especially when there is an increase in serum bilirubin levels (Islam *et al.*, 2020). Hepatotoxicity caused by chemotherapy is mostly mediated by reactive metabolites produced by oxidation processes, immune system damage. liver damage is linked to pediatric acute lymphoblastic leukemia Hypersensitivity, pancreatitis, thrombosis, encephalopathy, and liver failure are just a few of its toxicities (Schmidt *et al.*, 2022).

The liver is the primary location of hematopoiesis throughout the first few weeks of fetal development. The liver still performs this job even as an adult, but the bone marrow eventually replaces it as the fetus matures more (Cenariu *et al.*, 2021). As the liver is a component of the reticuloendothelial system, lympho-proliferative cancers frequently affect it, and hepatic symptoms of hematologic illnesses are therefore not uncommon. In this regard, Murakami and Shimizu studied hepatic manifestations in various hematologic disorders and noted that jaundice and hepatosplenomegaly are frequently seen in hemolytic anemias and may be mistaken for symptoms of primary liver diseases (Alexandra *et al.*, 2022).

While there are a variety of leukemia-related liver consequences, it is important to note how chemotherapy for leukemia affects liver function. Because most of the medications used in chemotherapy are lipophilic and therefore easily absorbed by the liver, their administration in the treatment

of many forms of leukemia can disturb the delicate metabolic balance of the liver. As a result, up to 85% of chemotherapy patients experience liver steatosis; steatohepatitis is a more serious side effect, especially when it's accompanied by a rise in serum bilirubin levels (Islam *et al.,* 2020). Hepatotoxicity is another outcome that has been linked to the creation of reactive metabolites as a result of oxidation processes, immune system damage (Mekonnen and Wondmeneh, 2022).

In addition, chemotherapy used to treat leukemia can result in veno-occlusive disease (VOD), commonly known as hepatic sinusoidal obstruction syndrome (SOS). It happens when the veins in the liver become inflamed, obstructing blood flow. Short-term exposure to 6-TG during leukemia therapy has been linked to SOS, and individuals with low-activity TPMT genotypes are more likely to experience this syndrome's manifestations (Stanulla *et al.,* 2021)

Moreover, it could harm the liver as a result of obstructive, toxic, and infectious factors. Moreover, the invasion of leukemic cells into the liver is one of the primary impacts of leukemia on the liver. Chemotherapy drug-degrading liver enzymes are released in response to the invasion of these cancerous cells into the liver and the resulting tissue damage. This occurrence can further protect leukemia cells from traditional treatments. It should be noted that up to 75% of leukemia patients have leukemia cells infiltrate their liver (Ye *et al.,* 2021). Furthermore, it has been demonstrated that leukemia, which primarily impairs the immune system, is a predictor of the development of acute hepatitis, particularly hepatitis B and C. (Kaya *et al.,* 2018).

2-5 The study's markers for evaluating kidney functions

2-5-1 Interleukin-18 (IL18):

Interleukin-18 (IL-18), an immunostimulatory cytokine with structural similarities to IL-1, is a relatively recent discovery. However, in addition to being expressed by kupffer cells, T cells, B cells, keratinocytes, astrocytes, and osteoblasts, IL-18 is primarily generated by activated macrophages (Kaplanski, 2019). Because of its effects on natural killer (NK) cells, monocytes, dendritic cells, T cells, and B cells, IL-18 can control both innate and adaptive immune responses (Yasuda *et al.,* 2019).

Interleukin-18 (IL18) is marker for kidney damage and Liver. The cytokine IL-18 was initially described as a pro-inflammatory substance that caused interferon gamma and promoted the proliferation of T cells (Hiroka and Nozaki, 2021). Similar to IL-1, IL-18 is a member of the IL-1 superfamily that is generated as a 24-kDa precursor protein (pro-IL-18) that is physiologically inactive and lacks the signal peptide required for secretion. The IL-1-converting enzyme, commonly known as caspase-1, is an intracellular cysteine protease that transforms pro-IL-18 into its mature and active form (Yasuda et al., 2019). Caspase-1 activation is mediated by multiprotein complexes called inflammasomes. The nucleotide-binding oligomerization domain-like receptor (NLR) family member that has been the subject of the most research is the NLR family pyrin domain-containing 3 (NLRP3) inflammasome. Numerous endogenous or exogenous danger signs, such as infections and sterile triggers, can be detected by it. (Talley *et al.*, 2019).

Although other cell types also make IL-18, macrophages are the main source of it. IL-18 activates a variety of cell types and has pleiotropic effects. Type 1 reactions are aided in their beginning by proinflammatory IL-18. It functions in conjunction with interleukin-12 to promote cell-

mediated immunity following infection with lipopolysaccharide (LPS) or other microbial substances. IL-18 and IL-12 stimulate the production of type II interferon (IFN) on CD4, CD8 T cells, and NK cells. The activation of macrophages and other cells requires IFN (Kaplanski, 2018).

Numerous researchers think that cytokines are crucial for both immunoregulation and immunological dysfunction, even if it is unclear how specifically they are involved in the pathophysiology of chronic HCV infection. Cirrhosis and liver disease are linked to interleukin 18 (Flisiak-Jackiewicz *et al.*, 2018). It was found that IL-18 levels were a reliable predictor of the liver inflammation and damage caused by HCV. Due to its increased production, chronic hepatitis becomes more quickly converted to cirrhosis. Because T helper 1 (Th1) cells have been linked to the beginning of hepatitis C virus infection, interleukin 18 (IL-18) may be implicated in the pathogenesis of tissue injury in HCV. (El-Hendawy *et al*., 2018).

Improved liver endothelial cell sensitivity to apoptosis and increased cytokine production by type 1 helper cells (Th1) are two crucial roles of IL-18 in the development of liver cirrhosis. Both ligand-mediated Th1 cells and perforin-dependent hepatic natural killer cells are more lethal when exposed to IL-18 (Vandenhaute *et al*., 2019). Clinical relevance Because it causes IFN-, nitric oxide (NO), and reactive oxygen species (ROS) in phagocytes, IL-18 is crucial for host defense against both intracellular and external pathogens. Additionally, IL-18 directly activates CD8+ T lymphocytes, which are necessary for the removal of viruses. When IL-12 is not present, IL-18 protects against helminth infection by boosting the production of Th2 cytokines and granulocytes. (Yasuda *et al.*, 2019).

It was found that sustaining intestinal homeostasis requires the synthesis of IL-18, which is produced by intestinal epithelial cells (Mahapatro *et al*., 2021). IL-18 helps the intestinal barrier work properly.

When the barrier is breached, microorganisms activate lamina propria macrophages, which subsequently release interleukin-18 for caspase-1 to break down, resulting in inflammation. Additionally, the VCAM-1 (vascular cell adhesion molecule-1) antibody natalizumab blocks macrophages and other myeloid cells from accessing intestine and brain regions in persons with multiple sclerosis and Crohn's disease. (Wadea *et al.*, 2022).

2-5-2 Kidney Injury Molecule-1 (KIM-1)

The cell transmembrane glycoproteins like KIM-1 are made in the kidneys and are undetectable in healthy individuals. By regulating mRNA in the proximal tubule, it was found that renal ischemia or toxic damage raised the amount of KIM-1.

Most research looked at KIM-1's potential as a biomarker for several types of acute kidney injury (AKI), particularly damage brought on by cisplatin. (Seibert *et al.*, 2018). The KIM-1 gene produces a protein that functions as a membrane receptor for the T cell immunoglobulin and mucin domain and the human hepatitis A virus (Song *et al.*, 2019).

In healthy individuals, KIM-1, a marker indicating kidney injury, cannot be expressed discovered. In the event of acute kidney damage, there is a noticeably higher excretion of it in proximal tubule cells in response to acute kidney injury (AKI) (Griffin *et al*., 2019). While NGAL is a marker of both proximal and distal tubule damage in response to different forms of injury (such as ischemia, toxic, and inflammatory), KIM-1 predominantly acts as a marker of proximal tubular injury. Studies on the effectiveness of NGAL and KIM-1 as prognostic markers for the early stages of chronic kidney damage are inconclusive. (Seibert *et al.*, 2018; Song *et al., 2019).* Renal function has decreased as a result of the intensive

multimodal therapy used to treat children solid tumors. Potential indicators for early renal injury include urine kidney injury molecule-1 (KIM-1), urine neutrophil gelatinase-associated lipocalin (NGAL), and urine. (Latoch *et al.,* 2021).

2-5-3 Acute Renal Damage and KIM-1

KIM-1 is a sensitive biomarker for proximal tubular damage that occurs over time (Wajda *et al.*, 2020). When compared to healthy people, patients with acute renal tubular injury had significantly higher levels of KIM-1 expression in their kidneys (Schmidt *et al.*, 2022). The ERK1/2 and STAT3 phosphorylation is thought to be started by acute renal injury, according to the hypothesized mechanism. Following this, nuclear STAT3 binds to the KIM-1 promoter and raises the protein and mRNA levels of KIM-1 (Song *et al.*, 2019). After a kidney transplant, extracellular domain shedding causes significantly higher levels of KIM-1 in the blood and urine, which can be utilized to detect acute renal tubular failure (Shahbaz *et al.*, 2019). Kidney damage and the estimated glomerular filtration rate (eGFR) reduction are both closely related to the KIM-1 gene. (Schulz *et al.*, 2020).

2-6The study's markers for evaluating Liver functions

2-6-1 Vitronectin (VTN):

Other organs such the brain, heart, skeletal muscle, lung, uterus, testis, and thymus have also been found to contain VTN mRNA. Only the male genital tract has been shown to contain VTN mRNA in normal tissues, but tumors are where it is most abundantly expressed, suggesting that it may play a role in cancer. (Mohamed *et al.*, 2022).

Lower plasma VTN levels were found in patients with chronic hepatitis, compensated cirrhosis, and decompensated cirrhosis compared to healthy

individuals. As hepatic dysfunction grew worse in chronic liver diseases, the amount of plasma VTN decreased (Su and Riesbeck, 2018). Vitronectin (VTN) is an essential coagulation regulator (Wang et al., 2020).

The sticky role that vitronectin, a multifunctional glycoprotein, plays in processes like cell proliferation in a variety of human malignancies is well-known (Burgos *et al*., 2019).

Vitronectin (VTN), a multifunctional plasma glycoprotein, is produced by hepatocytes. VTN has been the subject of much research as a cell adhesion molecule. The position of it in the liver's extracellular matrix, however, hasn't received much study. (Burgos-Panadero *et al.*, 2019).

Heart Failure (HF) and end-stage liver disease have previously been connected to vitronectin buildup. (Frangogiannis, 2019; Mohamed *et al.*, 2022). To the results of the present investigation, subtler alterations in vitronectin expression take place prior to the start of fibrosis and hepatic decompensation. A glycoprotein called vitronectin (VTN) is found in plasma and serum at a concentration of 200–300 g/ml and is also found in the extracellular matrix of different organs (Wang *et al.*, 2020). Although other healthy organs and diseased tissues also produce VTN, the liver emerges as the principal source (Biasella *et al.*, 2022; Burgos-Panadero *et al.*, 2019). In developing Drosophila embryos, the adhesion and migration of keratinocytes and neural crest cells, as well as the stimulation of neurite outgrowth and differentiation and myocyte differentiation, are all dependent on the multifunctional protein known as vitronectin (Olson and Nechiporuk, 2018). Vitronectin interacts with a number of coagulation and fibrinolysis-related proteins, such as PAI-1, u-PA, and its receptor (Sillen and Declerck, 2021). The RGD sequence included in vitronectin binds both the platelet receptor IIb3 and the v-integrin family of receptors (v3, v5, v1, and v8) (Huang *et al*., 2019).

The liver has been shown to be the primary source of plasma vitronectin, as evidenced by the fact that people with severe hepatic disease had lower levels of VTN in their blood. (Watson *et al.*, 2022).

2-7 Nucleophosmin-1 (*NPM*1) Mutations

The multifunctional protein known as *nucleophosmin* 1 (*NPM*1, also known as B23) is involved in a variety of cellular processes, such as ribosome maturation, centrosome replication, the maintenance of genomic stability, cell cycle regulation, and apoptosis. *NPM*1 is the most frequently mutated gene in adult acute myeloid leukemia (AML), accounting for around 40% of all cases. The actions of mutant *NPM*1 (*NPM*1mut) during leukemogenesis are unclear. An overview of the structure, physiological roles, pathophysiology of *NPM*1-mutated AML, and potential therapeutic importance of *NPM*1 is given in this article. The action of *NPM*1 as a protein chaperone, which stops leukemia stem cells from developing and regulates non-coding RNAs, has been suggested to have a role in the pathogenesis of AML. A dysfunctional *NPM*1 may, in addition to conventional chemotherapies, have a role in the pathogenesis of AML through its function as a protein chaperone that stops leukemia stem cells from developing and regulates non-coding RNAs. In addition to conventional chemotherapies, *NPM*1 is a promising therapeutic target against AML that requires further study. Examples of *NPM*1-based treatment strategies include exploiting NPM1 as an immune response target, causing nucleolar relocalization of NPM1 mutants, and blocking *NPM*1 oligomerization. (Xue *et al.*, 2022).

Located on chromosome 5q35, *nucleophosmin* 1 (NPM1) encodes *nucleophosmin*, a primarily nucleolar protein that transports cells' nuclei and cytoplasm. At the cellular level, *nucleophosmin* performs a number of crucial functions, including as chaperoning, building proteins in the

ribosome, and inhibiting protein aggregation in the nucleolus. What's more, nucleophosmin is essential for controlling tumor suppressor pathways, including the ARF-p53 pathway (Branford *et al.,* 2018 and Shamanna *et al.,* 2021).

The most frequent acquired genetic abnormalities and important prognostic indicators in patients with acute myeloid leukemia (AML) are nucleophosmin-1 (*NPM*1) mutations. The most common acute leukemia in adults is acute myeloid leukemia (AML), and its prevalence increases with age. Immature hematopoietic cells undergo a malignant transformation as a result of a multistep, intricate process that requires the collaboration of various types of genetic defects. The classification of AML subtypes aids in the creation of novel therapy modalities (Blackburn *et al.,* 2019). Nucleophosmin-1 (*NPM*1), which is present in about one-third of newly diagnosed cases in both younger and older adults, is one of the most frequently mutated genes in AML. (Brunetti *et al.*, 2019). Nucleophosmin-1 (*NPM*1) is a nucleus cytoplasm shuttling protein that is universally expressed, highly conserved, and has been demonstrated to contribute to a variety of fundamental cellular functions, including ribosome production, centrosome function regulation, genome stability, and many others. transcriptional control, DNA duplication. Additionally, to taking part in the (ARF-P53) tumor suppressor pathway and reducing protein aggregation in the nucleolus, it also prevents DNA damage. 1 A recent study discovered that the human NPM1 gene, a multifunctional phosphoprotein that is largely found in the granular regions of the nucleolus, is situated on chromosome 5q35 and has 12 exons with sizes ranging from 58 to 358 bp. (Chen, 2020).

Chapter Three

Materials and Methods

Chapter Three

Materials and Methods

3. Materials and Methods

3-1 Chemicals

3-1-1 Chemicals and biological materials used in this study are listed in Table (3-1).

Table (3-1): Chemicals and biological materials and its Origin Company

No	**Substance**	**Company and Origin**
1	100 bp DNA ladder	Intron, USA
2	Agarose	MBI Fermentas
3	DNA primer 100nmol	Microgen, Korea
4	DNA Extraction kit	Favorgen – Korea
5	Ethanol	Biosolv company (USA)
6	Ethidium bromide	Promega, USA
7	AST kit	Sanymed (Italy)
8	ALT kit	Sanymed ((Italy)
9	Urea kit	Linear (a spain)
10	Creatinine	Biolabo, France
11	Human vitronectin (VTN) ELISA Kit	Melsin Medical (Chaina)
12	Human Interleukin 18 (IL-18) ELISA Kit	Melsin Medical (Chaina)
13	Human kidney Injury Molecule (Kim-1) ELISA Kit	Melsin Medical (Chaina)
14	Nuclease free water	Biolabs – EnglandAWQQ2

15	Loading dye (bromophenole blue)	Biolabs – England
16	PCR Master Mix Kit	Macrogen (Korea)
17	Proteinase K	Biolabs – England
18	TBE buffer	Promega, USA

3-1-2 Instruments:

Apparatus and instruments used in this study are listed in Table (2).

Table (3-2): Instruments and its Supplying company

No	**Instruments**	**Supplying company and Origin**
1	Centrifuge;Cooling Centrifuge	Hettich / Germany
2	Bench centrifuge	Hettich / Germany
3	Deep Freeze	GFL / Germany
4	electronic Sensitive Balance	Denevr Instrument/ Germany
5	ELISA reader and washer	Biotek- USA
6	Incubator	Memmert – Germany
7	Vortex	Bioneer/ Korea
8	Water bath	GFL / Germany
9	Thermo Cycler	UK Prime
10	Microwave oven	Gosonic,China
11	Spectrophotometer	Biotech, USA
12	Biometra Tadvanced	Analytik jena, German company
13	Power Pro, Gel electerphoresis systems	Bioneer, Korea
14	Micro plate Reader ELISA	Paramedical,Italian

15	Gel ducumination	Major Science, Taiwan
16	OWL Electrophoresis System	Thermo, USA
17	Nano drop	Thermo Scientific/ UK
18	Vortex	CYAN/ Belgium
19	Micropipettes (different volumes)	Eppendorf / Germany
20	Eppendorf tubes	Sigma (England)
21	Exispin vortex centrifuge	Bioneer/ Korea
22	Refrigerator	Concord/ lebanon
23	Digital camera	Sony (Japan)

3-2 Subjects and Methods

3-2-1 Study Setting and Data Collection Time

This study (patients and control) was conducted during the period from October (2022) to January (2023) and it was performed in marjan teaching hospital (hematology unit) in Babylon governorate and medical city hospital (hematology unit) in Baghdad governorate/ Iraq.

3-2-2 Study subjects (Patients and Healthy)

The study subjects comprised of102 samples were collected from 72 patients, but 60 samples were taken from patients and Other types of leukemia were excluded, The numbers of leukemia patients were male and female (male 27 and female33), age (mean ± SD) = 40.32±18.418 (ranging from12 to 85 years) . these patients were suffered from leukemia and were referred to the Hematology Consultation Clinic for treatment or diagnosis. Those leukemia cases then have been diagnosed by a specialized haematologist. Diagnosis of leukemia patients through Bone marrow

analysis and complete Blood Counts (CBC). The healthy group included 30 individual (15male, 15female) age (mean± S.D) = (28.97±8.463) (range 21 to 54) not suffer from any disease, served as a control group and this groups matched with patient group. All subjects in this study were taken consent before participation in this study.

3-2-3 Exclusion Criteria

The cases of insulin intake (Type 1 Diabetes Mellitus), infection with Hepatitis, renal failure, were ruled out in this study. Also, other line treatment and autologous transplantation bone marrow myeloma were also excluded.

3-3 Data collection

3-3-1 Questionnaire

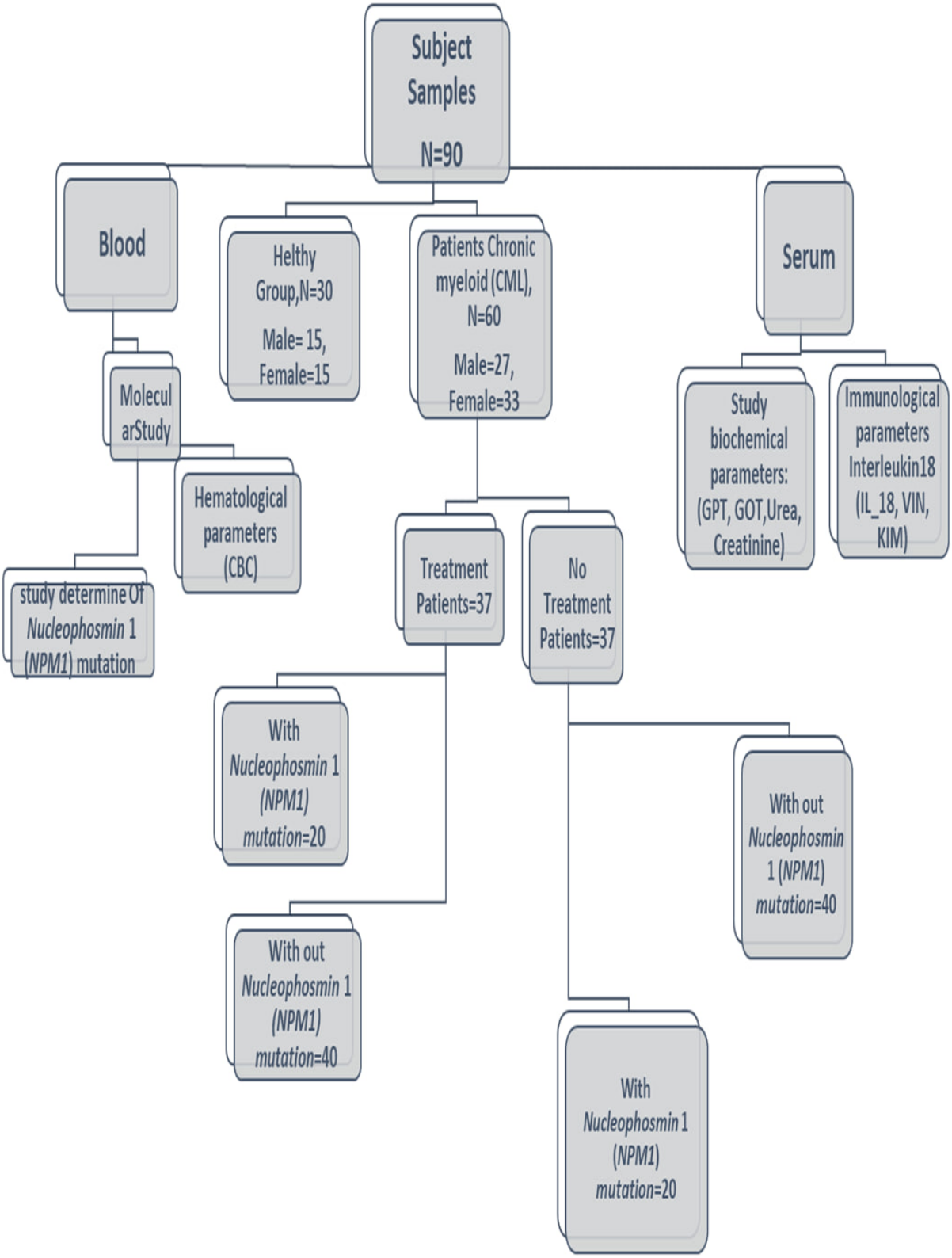
A questionnaire was taken from the patients and case sheets including: name, age, sex, onset of disease, other diseases, and medication.

3-3-2 Collection of blood samples

Venous blood samples were drawn from patients and control subjects by using disposable syringes. 5 ml of blood was obtained from each subject, 2 ml was placed into EDTA tubes and the remaining (3 ml) pushed slowly into disposable gel containing tubes. Blood in the EDTA tubes was used to determine CBC, the remain from blood stored in (-20˚C) in order to be used later in and molecular part (determine *NPM-1* mutation) of the study, while blood in the gel containing tubes was allowed to clot at room temperature for 15 minutes and then centrifuged at 3000 rpm for approximately 10-15. minutes, after that sera was obtained (Barbara and Anna,2015) and stored at -20˚C until used.

3-4 Experimental Design: as in figure (3-1)

Figure (3-1): Experimental design of study



3-5 Determine level of parameters:

3-5-1 Estimation of Hematological Parameters

The hematological parameters which included (RBC cont, WBC count, and platelets count) were estimated by using Analyzer Ruby (Abott Diagnostic Company, USA) in Lab of Hematology of Marjan Hospital in Al–Hilla, Iraq. The principle of this Device, technique for CBC was shown "Appendix A".

3-5-2 Estimation of biochemical parameters

Biochemical parameters (creatinine, urea, aspartate-aminotransferase (AST) (or glutamic-oxaloacetic transaminase/GOT) (AlT) or GPT, for study subjects were conducted by AST and AlT kits from Sanymed (Italy) and urea Kit from Linear (aspain)and creatinine Biolabo, France as flowing:

3-5-2-1 Principle (AST)

Aspartate-aminotransferase (AST) or glutamic-oxaloacetic transaminase/ GOT) catalyzes reaction between alpha-ketoglutarate and L-aspartate giving glutamate and oxaloacetate. In presence of malate dehydrogenase (MDH), oxaloacetate reacts with NADH giving malate and NAD*. The absorbance is proportional to the AST activity of the sample (Reitman and Frankel,1957).

3-5-2-2 Principle (ALT)

In the reaction, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD (Reitman and Frankel,1957).

3-5-3 Preparation of Reagents (AST/ALT)

Reagents are liquid and ready to use. About using as monoreagent ("sample-starter" procedure) add to every 4 ml of R1 reagent, 1 ml of R2

reagent. Keep out the reagents from the refrigerator only for the use and recap them immediately.

Procedure (AST/ALT)

Wavelength	λ:340 nm
Working temperature	37°C
Optical path	"Kinetic" (decreasing)

Reaction

Bring the reagents at 15-25°C before using them.

Monoreagent Procedure "Sample Starter

	Blank	Sample
Working Reagent	1000 µl	1000 µl
Distilled Water	100 µl	--
Sample	--	100 µl

CALCULATION (AST/ALT)

$$ALT, AST\ [U/1] \quad = \quad \Delta E/min\ (3) \quad x \quad 1746$$

3-5-4 Principle/ Creatinine

Colorimetric reaction (Jaffe reaction) of creatinine with alkaline picrate measured kinetically at 490 nm (490-510), without any pre-treatment step. This reaction has been improved (specificity, speed and adaptability) by the development of an initial-rate method. "Appendix E".

Reagents Preparation

Mix 1 volume of R1 and 1 volume of R2

Procedure

Manual method

Let stand reagent and specimens at room temperature.

Working Reagent (R1+R2)	1000 µl
Specimen (Note 3)	100µ l

Mix well. Perform kinetic tests at 37°C (verify constant temperature). After 30 seconds read absorbance A1 and exactly 120 sec after read absorbance A2 at 490 nm (490-510) against distilled water. Test tube by tube with water, calibrator, controls and then assays as specimen

Calculation

Serum or plasma

Result = $\frac{(A.SAMPLE\ 2 - A.SAMPLE\ 1)}{(A.STANDERD\ 2 - A.STANDERD\ 1)}$ * standerd concentration (2 mg/dl)

Urines diluted with 1+19: Multiply the above result by dilution factor 20

GFR (by creatinine clearance determination):) (Fabiny *et al*,1971).

Using 24 h urine and serum creatinine

Corrected Creatinine Clearance (mL/min) = $\frac{UCr\ x\ V\ x\ 1.73}{SCr\ x\ BSA}$

UCr = Urine Creatinine in mg/dL or µmol/L

SCr = Serum Creatinine in mg/dL or µmol/L

V = Urine volume excreted in mL/min (24 h urine volume/1440) BSA = Body Surface Area in m2

OR

Using only serum creatinine (by Cockcroft and Gault formula)

Creatinine Clearance = $\frac{140 - age\ in\ years\ x\ 2.12\ x\ weight\ in\ Kg\ x\ K}{Serum\ Creatinine\ (\mu mol/L)\ x\ BSA\ (m2)}$

K = 1.00 for men or K = 0.85 for women

3.5.5 Principle/ Urea

Urea is hydrolyzed by urease1,2 into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a green cromophore. The intensity of the color formed is proportional to the concentration of urea in the sample. "Appendix E".

Uera + $H_2O \xrightarrow{Urease} 2NH_3 + CO_2$

NH_4^+ + Salycilate + NaCIO $\xrightarrow[OH^-]{NITOPRUSSIDE}$ Indophenol + NaCl

Reagent preparation

Working reagent. Mix 1 volume of **R1** + 24 volumes of **R2**. Stable

for 4 weeks at 2-8^{O}C and for 7 days at 15-25^{o}C.

Procedure

1. Bring reagents and samples to room temperature.

2. Pipette into a cuvette:

TUBES	Blank	Sample	CAL.Standard
Working reagent	1.0 mL	1.0 mL	1.0 mL
Sample	-	10 µL	-
CAL.Standard	-	-	10 µL

3. Mix and incubate for 5 minutes at 37^{o}C or for 10 minutes at room temperature (16-25^{o}C).

4. Pipette:

R3	1.0 mL	1.0 mL	1.0 mL

5. Mix thoroughly and incubate the tubes for 5 minutes at 37^{o}C or for 10 minutes at room temperature (16-25^{o}C).

6. Read the absorbance (A) of the samples and the standard at 600 nm against the reagent blank.

The color is stable for at least 2 hours protected from light.

CALCULATIONS

Serum, plasma

$$\frac{A\ Sample}{A\ Standard}\ X\ C_{Standard} = mg/dL\ urea$$

Samples with concentrations higher than 300 mg/dL (50 mmol/L) should be diluted 1:5 with saline and assayed again. Multiply the results by 5. Urine

Dilute the sample 1:50 with distilled water and multiply the result by 50.

If results are to be expressed as SI units apply: mg/dL x 0.1665 = mmol/L

To convert urea mass units to those of urea nitrogen apply: mg/dL x 0.467 = mg/dL BUN (Friedman and Young,2000).

3.5.6 Human Interleukin 18 (IL-18) ELISA KIT

Quantitative detection of IL-18 in serum was done according to the industrial company Melsin Medical (China), that depended on the technique of the quantitative sandwich enzyme immunoassay. Its procedure is explained in "Appendix B".

Human Vitronectin (VTN) ELISA KIT

Assessment of VTN was done according to the industrial company Melsin Medical (China), that depended on the technique of the quantitative sandwich enzyme immunoassay, a procedure is explained in "Appendix C"

Human Kidney Injury Molecule 1 (Kim-1) PROCEDURE

Human Kim-1 was measured according to the industrial company Melsin Medical (China) , that depended on the technique of the quantitative sandwich enzyme immunoassay, a procedure is explained in "Appendix D"

3.6. Genetic Study (Determine of *NPM-1* mutation)

3.6.1: DNA Extraction

Genomic DNA from white blood cells (WBCs) for both leukemia patients and control groups were extracted by using DNA extraction kit (Favorgen/ Korea) containing the following component (table (3-3) :

Table (3-3): DNA extraction kit component

Item	Quantity
FATG Buffer	30 ml
FABG Buffer	40ml
W1 Buffer	45 ml

*Wash Buffer Concentrated	25 ml
Elution Buffer	30 ml
RBC Lysis Buffer	135 ml
FABG Column	100 pcs
2 ml collection tube	200 pcs

manufacturer protocol (Favorgen / Korea) was followed for extraction the DNA from frozen blood sample and this protocol could be summarized as follows:

1- Two hundred µl of blood was added to a 1.5 ml micro centrifuge tube.
2- Forty µl of Proteinase K (10 mg/ml) was added to the 1.5 ml micro centrifuge tube and mixed briefly. The mixture is incubated at 60ºC for 15 minutes.
3- Two hundred µl of FABG buffer was added to the 1.5 ml micro centrifuge tube and mixed by shaking vigorously.
4- The mixture was incubated in a 70ºC water bath for at 70 Co 15 minutes. During incubation, the tube was inverted every 3 minutes.

5- At this time the required volume of Elution buffer was pre-heated (100 µl/sample) to 60ºC (for DNA Elution).
6- Two hundred µl of absolute ethanol was added to the sample lysate and immediately mixed by shaking vigorously for 10 seconds. If precipitate appeared, it was broken up by pipetting.
7- A FABG Column was placed in a 2 ml collection tube and the entire mixture (including any precipitate) was transferred to the FABG column.
8- The samples were centrifuged at 5000 rpm for 5 minutes and 2 ml collection tube containing the flow-through was discarded and the

FABG column placed in a new 2 ml collection tube.

9- Four hundred µl of W1 buffer was added to the FABG column and centrifuged at 5000 rpm for 30 seconds and the flow-through was discarded and the FABG column was placed back in the 2 ml collection tube.

10- Six hundred µl of wash buffer (add 100 ml / 200ml of ethanol (96-100%) to wash Buffer when first open) was added to the FABG column and centrifuged at 5000 rpm for 30 seconds and the flowthrough was discarded and the FABG column was placed back in the 2 ml collection tube and centrifuged again at 5000 rpm for 3 minutes to dry the column matrix.

11- The dried FABG column was transferred to a clean 1.5 ml micro centrifuge tube.

12- One µl of pre-heated elution buffer was added to the center of the column matrix.

13-Stand for at least 3 minutes to ensure the Elution buffer was absorbed by the matrix.

14-The tubes were centrifuged at 5000 rpm for 30 seconds to elute thepurified DNA DNA solution stored at (-20) C.

3.6.2. DNA Spectrophotometry

The DNA quality and quantity were estimated by Nano- drop , through using scanning ability of Diode array from (200 – 320) nm wave length, the absorption profile was then analyzed to determine the DNA quality and quantity by calculating the 260/230 & 260/280 ratios. If a sample showed 260/230 ratio lesser than 2 or 260/230 ratio lesser than 1.8, it should be Re -extracted.

Amplification of Sampling

The *NPM1* gene exon 12 fragments were amplified using the polymerase chain reaction (PCR).

Utilizing the forward (NPM11-F) and reverse (NPM12-R) oligonucleotide primers (5'-ACCACATTTCTTTTTTTTTTCCAGGCT-3') of the 5'-CCTGGACAACATTTAT CAAACACGGTA-3' format.

The 1.5µl of primers and 1.5 µl of genomic DNA, amplified In a 50 µl reaction mixture with (47 µl) of Taq DNA polymerase, PCR buffer, dNTPs, gel loading dyes, and new green are all components of the ready-to-use PCR reaction combination known as PCRTM. It also contains a fluorescent dye that is directly detectable on a blue light transilluminator or UV epi-illuminator following DNA electrophoresis.

A negative control was added to every positive reaction, which was evaluated twice.

PCR amplifying was carried out using a PCR Thermal Cycler. The amplification method involved 35 cycles of 1 minute at 94 degrees, 30 seconds at 61 degrees for annealing, 1 minute at 72 degrees for extension, and 7 minutes at 72 degrees for the final extension. Each reaction was verified on a 1.5% agarose gel electrophoresis.

Statistical Analysis

The Statistical Package for Social Sciences- SPSS version 21. Numerical data were tested for normal distribution using the Shapiro– Wilk test. And statistically significant of the data were analyzed by using descriptive analysis to show the mean ± standard deviation of variables . The significance of difference between mean values was estimated by one way ANOVA by used least significant difference –LSD test to significant comparison between means. And use T-test .Significance was assumed for P values ≤ 0.05. Person or Spearman Rho Correlation analysis was used to test the liner relationship between parameters in this study, And the figures

construction by using excel program of Microsoft office 2010. Multivariable logistic regression was used to determine independent indicators of SNPS and Allele By use OR and CI 95% . All P values were less than 0.05 considered statistically significant (Al-Rawi, 2000).

Chapter Four

Results and Discussion

Chapter Four

Results

4.1. The demographical characteristics of study groups

This chapter presents the results of the data analysis systematically in (tables and figures) and these corresponded with the objectives and aims of the current study as follows:

Figure 4.1 Show a statistical study of the types of leukemia in the Iraqi society within the period of sample collection , the results shows that CML had the highest rate, amounting to 82%, while (AML, ALL, CLL) came in very low rates, ranging between 4 , 6, 8 % respectively .

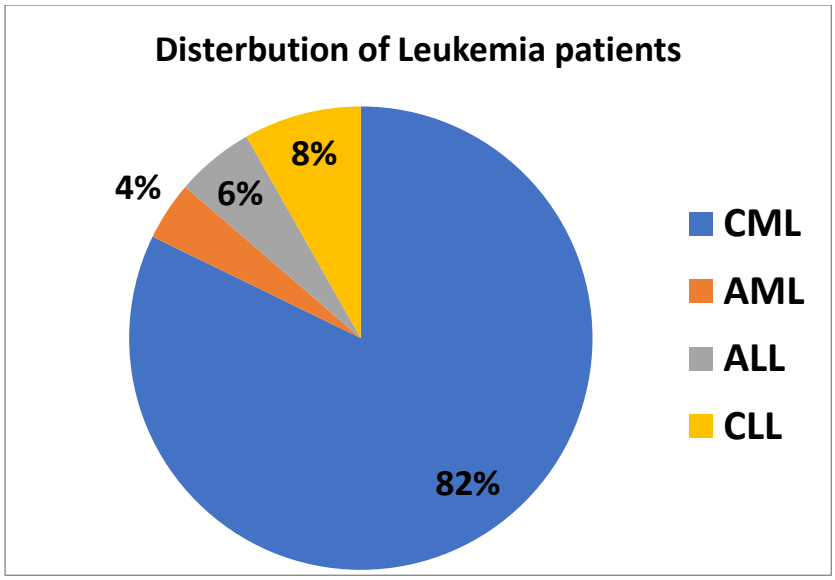


Figure 4.1:Disterbution of Leukemia patients .

The results showed that the majority of leukemia patients were over the age of 25 years , Figure 4.2 .

Regarding the distribution of CML patients according to gender, the results recorded that the majority of CML patients were women, and this was recorded for the first time, as scientific reports indicated that this disease affects males more than female. Figure 4.3.

Body mass index-wise, Fig. 3 showed that the proportion of people who are normal weight (18.5-25) and obese (BMI greater than 30) tended to be lower in the control group when compared with the patients' group (53.3% versus 56.6% and 6.7% versus 10.8 %, respectively). Contrarily, the patients' group (56.6% versus 53.3%, respectively) and the control group (25.1-30) both tended to be overweight, but the differences did not reach the significance level 4.4.

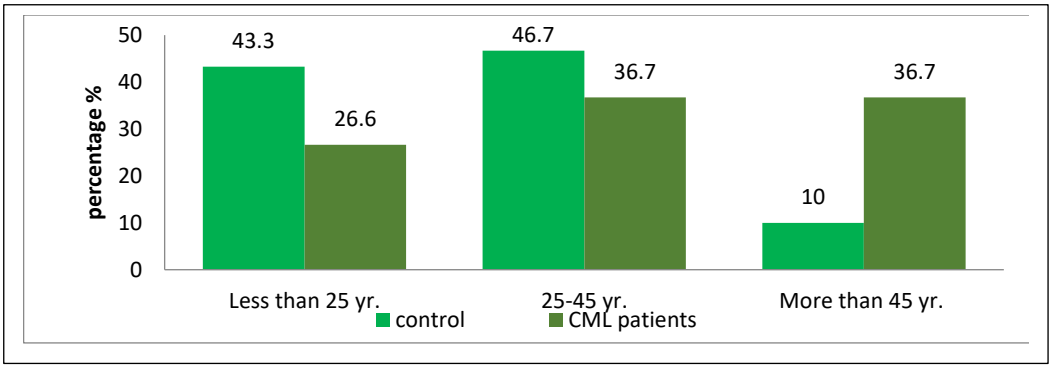


Figure 4.2 : Percentage of CML patient and control (within a study) according to age.

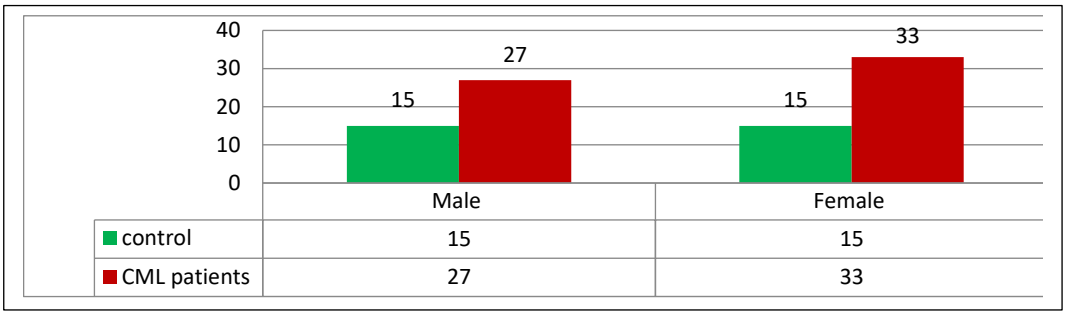


Figure 4.3: Percentage of study CML patient and control according to gender

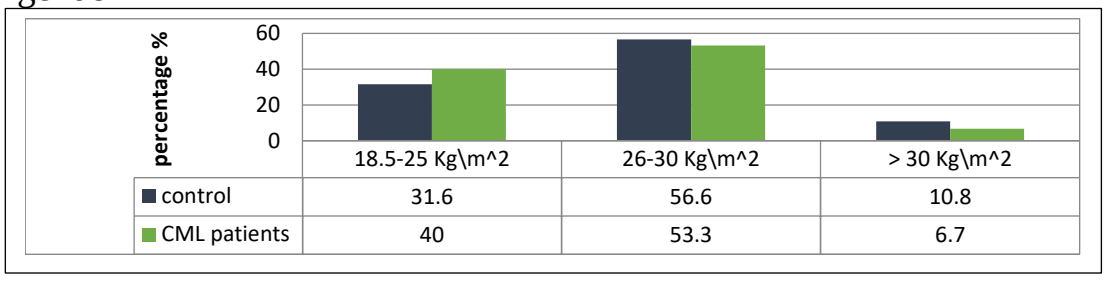


Figure 4.4: Percentage of study CML patient and control according to Body Mass Index (BMI) , 18.5-25 Kg\m^2 (normal weight) , 26-30 Kg\m^2 (overweight) , > 30 Kg\m^2 (obesity) , value represented percentage (%).

As for *Nucleophosmin NPM-1* the mutation, the results of the study recorded the occurrence of the mutation in 20 patients as a percentage of about 23.3%. table (4.1) .Also in current study The number of patients who did not receive chemotherapy was 20, with a percentage of 33.3%, while those who received chemotherapy were 40, with a rate of 66.6%. Table 4.1

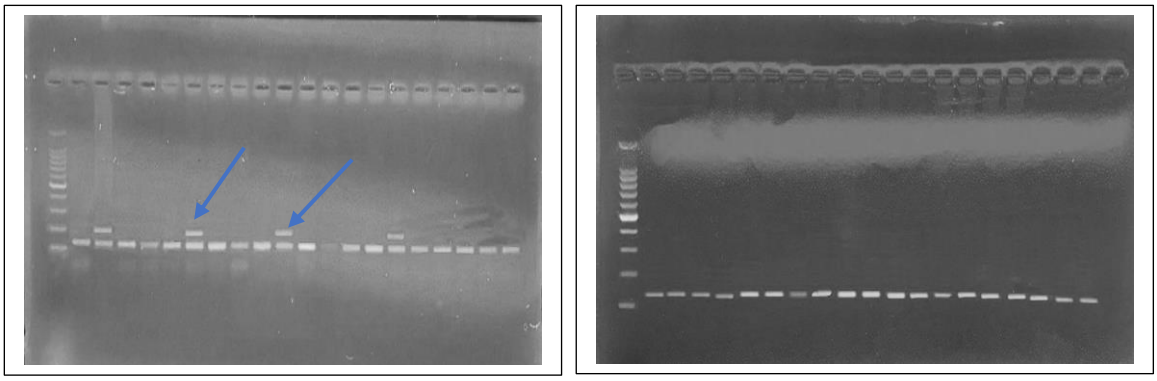


Figure 4.5: PCR product electrophoresis on 2.5% agarose gel for CML patients to identify *NPM-1* mutation. An augmented version of the healthy control product (on the right). Amplified patient products (on the left) display the *NPM-1* band (with an arrow). Wild-type DNA from patients was amplified by Other Lanes (approximately 133 bp). Marker: M.

Table 4.1: demographical characteristics in control and CML patients.

			Groups			
			Patient n=(60)	Control n=(30)	Total	p-value
Age	Less than 25	F	16	13	29	
		%	26.6	43.3	32.2	
	25-45	F	22	11	33	
		%	36.7	36.6	36.6	
	More than 45	F	22	6	28	.004
		%	36.7	20.0	31.1	(sig)
Total		F	60	30	90	
		%	100.0	100.0	100.0	
	Mean ± SD		40.12±7.417	28.97±8.463		
	Min- Max		12-85	12-54		
Gender	Male	F	27	15		
		%	45.0%	50.0%		
	Female	F	33	15		.656
		%	55.0%	50.0%		(N.S)
Total		F	60	30		
		%	100.0%	100.0%		
Mutation	No	F	40	30		
		%	66.6%	100.0%		
	Yes	F	20	0		.004
		%	33.3%	0.0%		(sig)
Total		F	60	30		
		%	100.0%	100.0%		
Treatment	No	F	23	-		
		%	38.3%	-		
	Yes	F	37	30		
		%	61.6%	100%		

P< 0.05 statistically significant

4.2. The Levels of parameters in studied groups (CML patients and control)

4.2.1. Levels of hematological parameters in study groups(CML patients and control):

Table 4.2 show that a highly- significant differences between patients and control group regarding to (RBCs, WBCs, and platelets at p-value ≤ 0.05**.**

Table 4.2: Differences between CML patients and control group regarding to hematological aspects.

Hematological parameters	Groups	N	Mean	± SD	p-value
RBC count (10^6 \mm^3)	Patient	60	2.83	±1.132	0.001* (H.S)
	Control	30	3.90	±.595	
WBC count (10^3 \mm^3)	Patient	60	16.37	±7.006	0.001* (H.S)
	Control	30	5.55	±1.298	
Platelets count (10^3 \mm^3)	Patient	60	225.87	±12.49	0.001* (H.S)
	Control	30	280.8	±9.573	

t – test at P≤ 0.05(statistically significant) , Mean ± S.D.* significant

4.2.2: Levels of liver function biomarkers in study groups:

Table 4.3. show levels of some biomarkers of liver function in CML patients and control groups. The results showed that the GPT level was not significant increase , in contrast to the (GOT, VTN) It show statistical significance. Also These results are shown in Fig 4.6.

Table 4.3: Levels of some liver function biomarkers in CML patients and control groups

Liver function test	Groups	N	Mean	SD	p-value
ALT (GPT) (U\L)	Patient	60	10.419	±2.218	0.132 (N.S)
	Control	30	11.18	±5.09	
AST(GOT) (U\L)	Patient	60	26.49	±7.15	0.001* (H.S)
	Control	30	10.63	±3.22	
VTN (pg\ml)	Patient	60	48.02	±6.66	0.001* (H.S)
	Control	30	31.940	±6.30	

Valuels are means ± SD

4.2.3. Levels of kidney function biomarkers in studied groups(CML patients and control) :

Table 4.4 related to the levels of some biomarkers of kidney function in CML patients and control groups, we note that there is a

statistical significance (urea, creatinine, IL18) while there is insignificant increase (p≥0.05) in Kim-1 marker.

Table 4.4: Levels of some kidney function biomarkers in CML patients and control groups

Kidney function test	Groups	N	Mean	SD	Min	Max	p-value
Urea (mg\dl)	patient	60	13.07	±2.36	3.58	22.28	0.001 (H.S)
	control	30	6.92	±1.35	4.13	8.65	
Creatinine (mg\dl)	patient	60	1.83	±.26	.08	2.08	0.001 (H.S)
	control	30	0.96	±.411	.34	1.98	
IL-18 (pg\ml)	patient	60	37.72	±3.36	7.12	333.39	0.001 (H.S)
	control	30	26.93	±4.12	11.24	41.13	
kim-1(ng\ml)	patient	60	4.88	±.135	.27	7.93	0.135 (N.S)
	control	30	2.76	.184	.43	3.23	

Valuels are means ± SD

4.3. The Levels of parameters in CML patients according to *NPM-1* mutation:

4.3.1. Levels of hematological parameters in CML patients according to *NPM-1* mutation:

In Table (4.5), relied on a comparison of hematological aspects based on the NPM1 mutation in chronic myeloid leukemia patient groups. It became clear from a sample of (60) that there are (40) do not have a mutation in all blood measurmants and (20) had a mutation). Also, the study in WBC gave a statistical significance, and this is clear from the value (Sig. = 0.004 < 0.05). As for platelets, the study did not give any statistical significance, and this is evident from the value of (Sig. = 0.6 > 0.05). That is, it is observed in Table 4.5 demonstrated that the WBCs of those with and without the mutation differed in a substantial way. While there were statistically significant non-significant differences between those with and without the mutation with respect to RBCs and platelets at p-value ≥ 0.05.

Table 4.5: Comparison between hematological parameters depending on *NPM 1*mutation in CML patients' groups.

Variables	Mutation	N	Mean	SD	Min	Max	p-value
RBC count (10^6 \ml)	No	40	2.81	±1.15	1.09	5.63	0.350 (N.S)
	Yes	20	2.89	±1.09	1.28	4.48	
WBC count (10^3 \ml)	No	40	17.35	±7.08	5.50	125.70	0.004* (sig.)
	Yes	20	13.46	±3.30	8.70	19.50	
Platelets count (10^3 \ml)	No	40	207.63	±13.26	212.23	322.02	0.605 (N.S)
	Yes	20	243.78	±17.426	249.00	352.00	

t – test at P≤ 0.05(statistically significant). * significant

4.3.2. Levels some of liver function biomarkers in CML patients according *NPM-1* mutation:

In Table (4.6) below, show the levels of some biomarkers of liver function in chronic myeloid leukemia patients and control groups, and in Table (4.6) study a comparison between a liver function test based on the *NPM-1* mutation in groups of chronic myeloid leukemia patients. The table shows from a sample size of (60) that there are (40) patients that do not have a mutation and (20) cases that have a mutation. For both of the ALT and AST there were no significant differences between the averages, and this is evident through the P. values. As for the VTN level , show statistical significance through the value of (Sig. = 0.007 < 0.05).

Table 4.6: Comparison between liver function test depending on *NPM 1*mutation in CML patients' groups**.**

Liver function Test	Mutation	N	Mean	SD	Min	Max	p-value
ALT (U\L)	No	40	10.26	±7.17	1.16	30.23	0.956 (N.S)
	Yes	20	10.93	±7.61	2.91	30.83	
AST(U\L)	No	40	26.96	±16.16	3.49	76.78	0.308 (N.S)
	Yes	20	24.95	±9.70	3.49	82.02	
VTN(pg\ml)	No	40	49.10	±8.27	12.64	88.17	0.007* (sig.)
	Yes	20	44.48	±6.61	33.60	53.66	

t – test at p≤ 0.05(statistically significant). * significant

4.3.3. Levels some of kidney function biomarkers in CML patients according *NPM-1* mutation:

Table (4.7), showed a comparison of renal function test in CML patients according *NPM 1*mutation. also for both (creatinine and urea) did not give a statistical significance ($p \geq 0.05$). While (IL18 and Kim-1) there is a significant difference between those with and without the mutation with at $p \leq 0.05$.

Table 4.7: Comparison between kidney function test depending on *NPM*1 mutation in CML patients' groups.

Kidney function test	Mutation	N	Mean	SD	Min	Max	p-value
Urea(mg\dl)	No	40	13.51	±4.62	.58	22.28	0.129 (N.S)
	Yes	20	12.62	±3.47	7.64	18.11	
Creatinine (mg\dl)	No	40	1.75	±.26	.08	1.98	0.471 (N.S)
	Yes	20	1.92	±.27	.17	1.68	
IL18(pg\ml)	No	40	38.85	±4.93	7.12	33.39	0.009* (sig.)
	Yes	20	34.03	±4.95	28.39	48.55	
KIM-1 (ng\ml)	No	40	5.95	±1.06	.27	7.93	0.003* (sig)
	Yes	20	3.82	.158	.58	4.11	

t – test at ≤ 0.05(statistically significant). * significant

4.4. Levels of parameters in study groups (CML patients) according age

4.4.1. Levels of hematological parameters in CML patients according to age :

In Table 4.8 , when comparing the hematological aspects of chronic myeloid leukemia patients and control groups according to age, notice that the average cases increase from RBCS test to WBCS test and then to Platelets for all age groups. Also, the different age groups with (RBCS, WBCS, Platelets) did not show any statistical significance, meaning that the relationship is not significant.

That is, to note Table 4.8 show that a non-significant difference among age groups regarding to hematological aspects in patients .

Table 4.8: Comparison hematological aspects in CML patients according to age **.**

Age	**RBC count (10^6 \ml)**	**WBC count (10^3 \ml)**	**Platelets count (10^3 \ml)**
	CML Patients	CML Patients	CML Patients
Less than 25 years N=13	2.88±1.21	12.90±3.09	203.22±13.78
25-45 years N=22	3.13±0.98	17.36±6.1	207.38±14.39
More than 45 years N=22	2.57±0.76	12.40±2.87	248.33±6.08
***p-value**	.209 (N.S)	.484 (N.S)	.413 (N.S)

*significant differences at ($p<0.05$) , ANOVA test ,Mean± SD , N.S (non-significant).

Table 4.9 show that a non-significant differences in patients' group. Regarding to liver function tests (ALT and AST) While there is significant difference (p-value = 0.04< 0.05) among age groups in relation to VTN (as indicator for liver cells) in CML patients.

Table 4.9: Comparison liver function biomarkers in CML patients according to age **.**

Age	**ALT(U\L)**	**AST(U\L)**	**VTN(pg\ml)**
	CML Patients	CML Patients	CML Patients
Less than 25 years N=13	11.43 ± 6.05^a	21.09 ± 10.23^a	42.56 ± 8.31^a
25-45 years N=22	10.84 ± 2.21^a	25.96 ± 5.02^a	56.47 ± 11.09^b
More than 45 years N=22	9.74 ± 1.67^a	28.67 ± 5.87^a	42.84 ± 9.32^a
***p-value**	0.783 (N.S)	.513 (N.S)	0.04 (sig)

*significant differences at (p<0.05) , ANOVA test ,Mean± SD , N.S (non-significant), Different symbols mean significant .

Table 4.10 show that a difference significant at (p≤ 0.05) among age groups in relation to Creatinine and IL-18 in CML patients. While there is a non-significant difference among age groups in relation to urea and, and KIM-1 in patients and control groups.

Table 4.10: Comparison kidney function biomarkers in CML patients according to age **.**

Age	**Urea (mg\dl)**	**Creatinine (mg\dl)**	**IL18 (pg\ml)**	**kim-1 (ng\ml)**
	CML Patients	CML Patients	CML Patients	CML Patients
Less than 25 years N=13	11.44±7.21[a]	1.67±0.31[a]	35.48±7.06[a]	4.81±2.41[a]
25-45 years N=22	13.88±5.76[a]	1.47±0.89[a]	45.47±11.12[b]	4.10±1.09[a]
More than 45 years N=22	13.11±7.09[a]	2.61±0.76[b]	32.08±7.41[a]	4.79±1.09[a]
***p-value**	.949 (N.S)	.04 (Sig.)	.04 (Sig.)	.505 (N.S)

*significant differences at (p<0.05) , ANOVA test ,Mean± SD , N.S (non-significant), Different symbols mean significant .

4.5.Level of study parameters in CML patients with and without *NPM-1*mutation depending on the treatment (responses)

The results also included a study of the parameters included in the current study among CML patients with a *NPM-1*mutation and comparing them with CML patients without the *NPM-1*mutation to find out the effect of the mutation in a neuclophosmin protein on the response to chemotherapy.

4.5.1: Levels of hematological parameters in CML patients in with and without *NPM-1*mutation depending on the treatment(responses):

The results of the statistical analysis showed that the response to chemotherapy was equal in CML patients with or without a *NPM-1*mutation in relation to blood parameters, as the results recorded a decrease in the number of white blood cells in all CML patients after chemotherapy .Table 4.11.

4.11: Levels of hematological parameters in CML patients in with and without *NPM-1*mutation depending on the treatment.(Mean±S.D)

hematological parameters	**CML patients with *NPM-1*mutation N=20**			**CML patients without *NPM-1*mutation** N=40		
	Before N=8	After N=12	p-value	Before N=15	After N=25	p-value
RBC count (10^6\ml)	2.17±0.23	3.62±1.08	0.521	2.49±1.21	3.140.52	0.65
WBC count (10^3\ml)	17.03±5.62	10.34±2.31	0.04*	19.57±6.71	15.13±3.74	0.04*
Platelets count (10^3\ml)	229.67 ±11.76	257.89 ±23.06	0.18	187.42± 18.05	227.84 ±11.29	0.12

t – test at P≤ 0.05(statistically significant). * significant

4.5.2: Levels of liver function biomarkers in CML patients in with and without *NPM-1*mutation depending on the treatment(responses):

The study's findings indicated that, in terms of liver function markers, CML patients with an NPM-1 mutation had significant decreases in their levels of AST and VTN at a p-value of less than 0.05 following chemotherapy, but non-significant decreases in their levels of ALT as compared to before chemotherapy. In contrast, the data showed that CML patients without the NPM-1 mutation had a non-significant decrease in AST and VTN levels and a substantial decrease in ALT levels (p≤0.05) following chemotherapy as compared to before chemotherapy. table 4.12

4.12: Levels of liver biomarkers in CML patients in with and without *NPM-1*mutation depending on the treatment.(Mean±S.D)

Liver function Test	**CML patients with *NPM-1*mutation N=20**			**CML patients without *NPM-1*mutation** N=40		
	Before N=8	After N=12	p-value	Before N=15	After N=25	p-value
ALT (GPT) (U\L)	11.45±5.21	10.48±6.02	0.73	12.96±4.75	7.56±2.07	0.05*
AST(GOT) (U\L)	30.2±7.29	19.7±7.18	0.03*	29.02±9.43	24.90±2.64	0.31
VTN(pg\ml)	50.48±4.61	38.46±9.05	0.02*	51.76±9.71	47.04±11.54	0.17

t – test at p≤ 0.05(statistically significant). * significant

4.5.3: Levels of kidney function biomarkers in CML patients in with and without *NPM-1*mutation depending on the treatment(responses):

The results of the statistical analysis showed that the level of (creatinine ,IL-18 and Kim-1) decreased significantly at P≤ 0.05 in CML patients with a *NPM-1*mutation after chemotherapy, while the level of urea decreased significantly P≥ 0.05. As for CML patients without *NPM-1*mutation, chemotherapy caused a significant decrease P≤ 0.05 in (Kim-1) and a non-significant decrease P≥ 0.05 in (urea ,creatinine and IL-18) level .table 4.13

4.13: Levels of kidney biomarkers in CML patients in with and without *NPM-1*mutation depending on the treatment.(Mean±S.D)

Kidney function test	**CML patients with *NPM-1*mutation N=20**			**CML patients without *NPM-1*mutation** N=40		
	Before N=8	After N=12	p-value	Before N=15	After N=25	p-value
Urea (mg\dl)	14.83±6.07	10.41±2.76	0.27	14.85±9.31	12.19±3.60	0.53
Creatinine (mg\dl)	2.44±1.12	1.40±0.94	0.04*	1.96±0.6	1.54±0.84	0.09
IL-18 (pg\ml)	38.15±8.20	29.91±8.41	0.01*	40.97±7.82	36.73±10.7	0.72
kim-1(ng\ml)	5.93±1.97	1.71±0.29	0.02*	7.16±2.94	4.74±1.43	0.04*

t – test at P≤ 0.05(statistically significant). * significant

4.6. Correlation between study biomarkers in CML patient and control

4.6.1: Correlation between study biomarkers in CML patient:

Table 4.14 shows the correlation between the study indicators in the patient groups, and it shows that there is a weak direct correlation between RBC and (Platelets) by (32.1%), which is a significant correlation under the level of significance of 0.05. It also shows that there is a weak direct correlation between RBC and ALT by (29%), which is significant below the significance level of 0.05. Additionally, it was shown that there was a modest (27%) direct association between ALT and Urea that was statistically significant below the threshold of 0.05. The table also shows that there are very high correlations between IL18 and VTN by 98.4%, and between IL18 and KIM-1 by 97.9%, and there is a correlation between VTN and KIM-1 by 98.6%, and these correlations indicate a significant relationship under the level of significance of 0.01.

Table 4.14: Correlation between study markers in patients groups

		RBC	WBC	Platelets	ALT	AST	Urea	Creatinine	IL18	VTN	KIM-1
RBC	R		.060	.321*	.290*	.011	.040	.042	.149	.125	.148
	Sig		.650	.013	.025	.935	.759	.748	.256	.339	.260
WBC	R			.034	-.200	-.045	-.080	-.059	.031	.055	.024
	Sig			.796	.126	.734	.543	.656	.811	.679	.854
Platelets	R				.072	-.032	.045	-.027	-.063	-.083	-.071
	Sig				.584	.806	.731	.839	.631	.528	.588
ALT (GPT)	R					.052	.270*	-.019	-.047	-.081	-.052
	Sig					.695	.037	.883	.719	.537	.696
AST(GOT)	R						-.061	.014	-.074	-.103	-.106
	Sig						.646	.913	.576	.435	.420
Urea	r							.067	-.075	-.115	-.120
	Sig							.609	.570	.382	.361
Creatinine	r								.003	.025	.045
	Sig								.982	.849	.733
IL18	r									.984**	.979**
	Sig									.000	.000
VTN	r										.986**
	Sig										.000
KIM-1	r										
	Sig										

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table (4.15) shows the linear regression between the variables (RBC, Platelets, ALT). Through the table, the significance of the parameters for (〖β_1 = 0.003〗 _) and (β_2 = 0.042) can be seen through the value of Sig. < 0.05 and significant constant parameter β_0=1.761.

Table 4.15: Liner regression between RBC and (platelets and ALT) in CML patient group.

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
RBC	(Constant)	1.761	.338		5.206	.000
	Platelets	.003	.001	.301	2.496	.015
	ALT	.042	.019	.268	2.221	.030

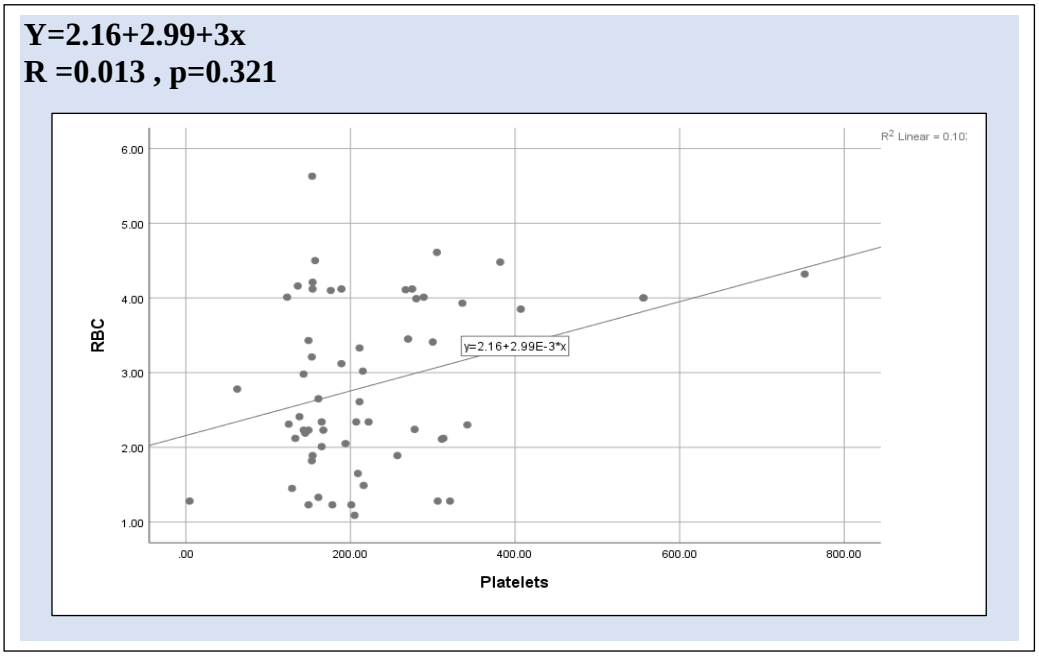


Figure 4.8: correlation between RBCS count and platelets count in CML patients

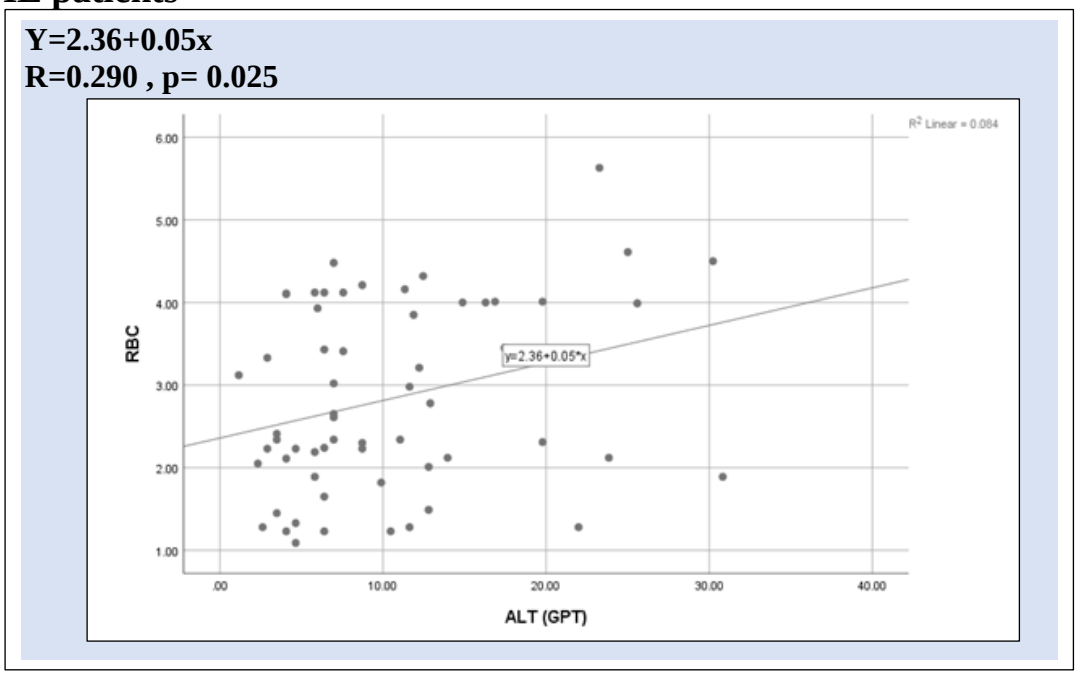


Figure 4.9: correlation between RBC count and ALT(U\L) level in CML patients

Table (4.16) shows the linear regression between the variables (Urea, ALT, GPT). Through the table, the significance of the constant parameter β_0=11.371 can be seen. and the significance of the parameters for (〖β_1=0.164〗 _) through the value of Sig. < 0.05.

Table 4.16: Liner regression between urea and ALT in patient group.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
Urea	(Constant)	11.371	.967		11.761	.000
	ALT (GPT)	.164	.076	.270	2.140	.037

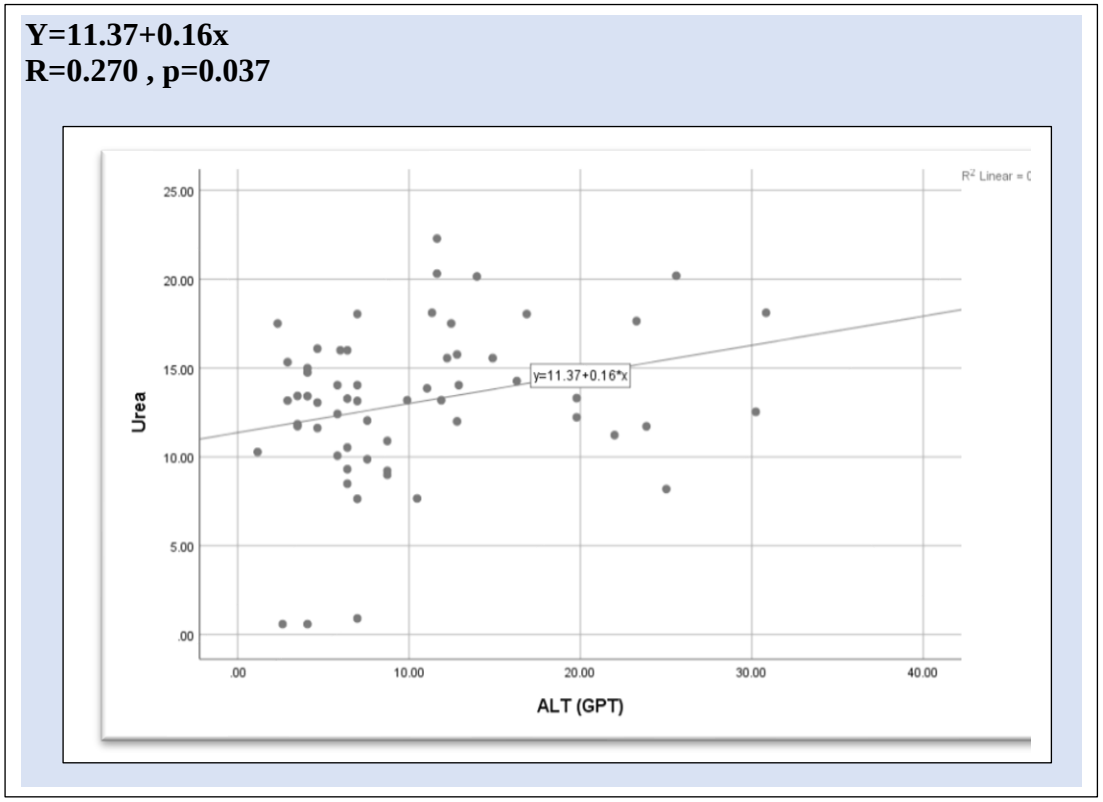


Figure 4.10: correlation between urea (mg\dl) level and ALT(U\L) level in CML patients

Table (4.17) shows the linear regression between the variables (IL18, VTN, KIM-1), and through the table, the significance of the parameters for (〖β_1 = 0.0572〗 _) and (β_2 = 13.055) can be seen

through the value of Sig. < 0.05 and the constant parameter β_0 is not significant.

Table 4.17 show that a significant regression between IL18 and VTN and kim-1 in patient group.

Table 4.17: Liner regression between IL18 with VTN and kim-1 in patient group.

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
IL18	(Constant)	-1.827	1.291		-1.415	.163
	VTN	.572	.115	.678	4.956	.000
	kim-1	13.055	5.750	.310	2.270	.027

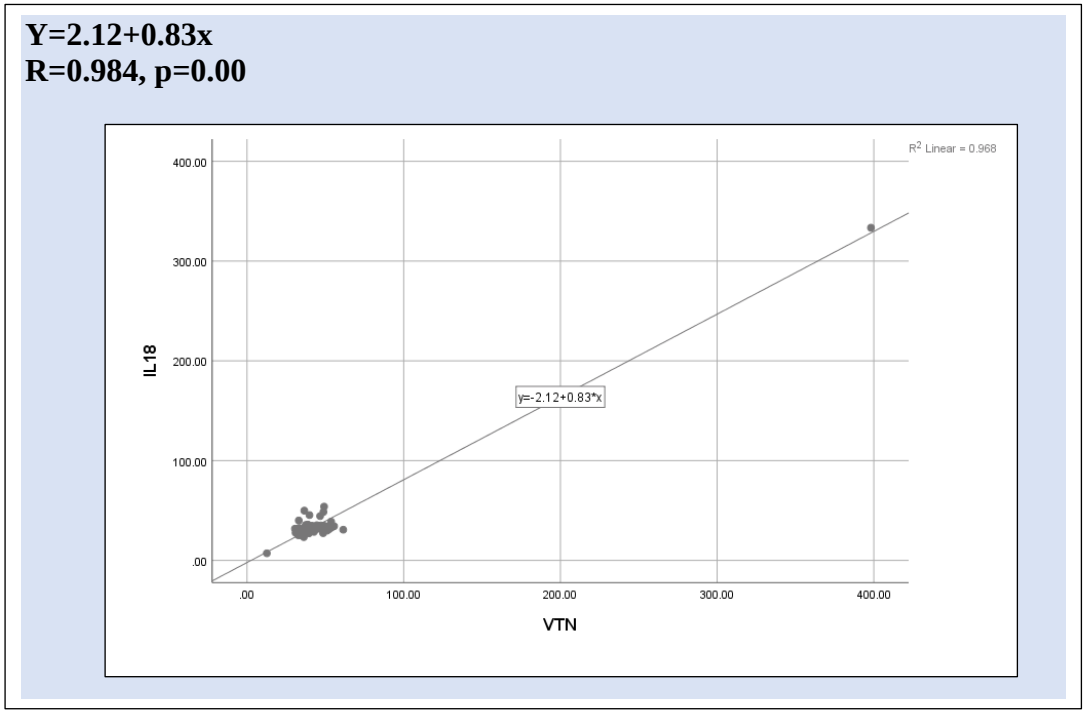


Figure 4.11: correlation between IL-18 (pg\ml) level and VTN (pg\ml) level in CML patients

Table (4.18) explains the linear regression between the two variables (VTN and KIM-1). It shows the significant value of the slope parameter (β_1), and this is evident from the value of Sig. < 0.05). The constant parameter is not significant.

Table 4.18 show that a significant regression between VTN and kim-1 in patient group.

Table 4.18: Liner regression between VTN and kim-1 in patient group.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
VTN	(Constant)	2.454	1.434		1.711	.092
	kim-1	49.162	1.094	.986	44.958	.000

Table 4.27: Liner regression between VTN and kim-1 in patient group.

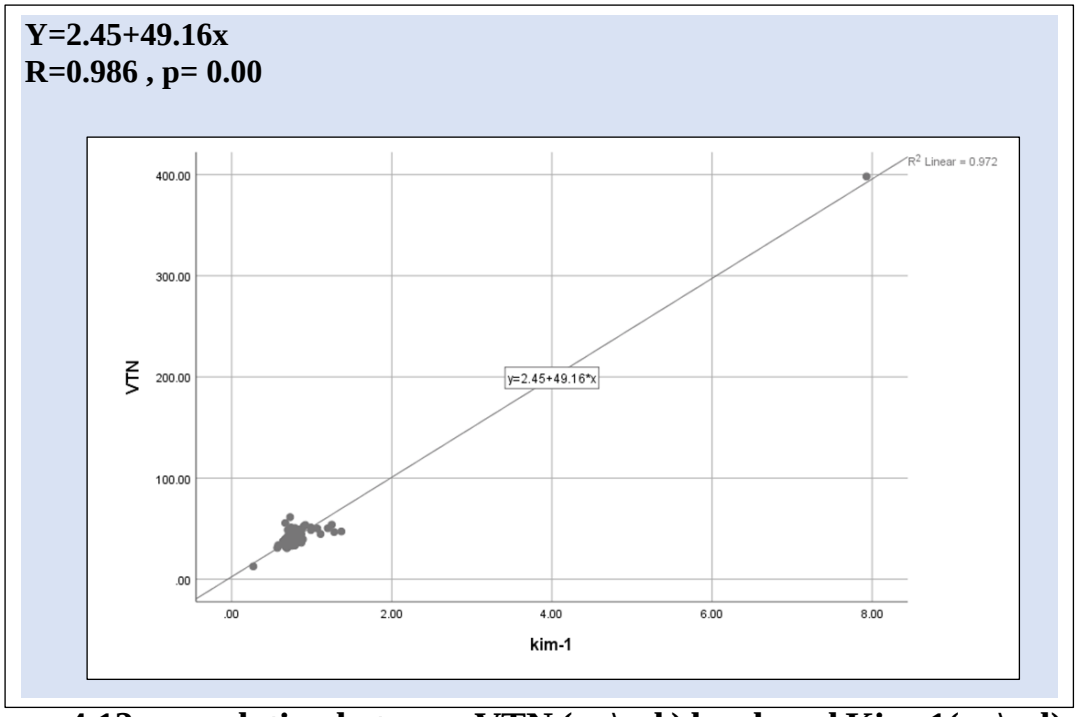


Figure 4.12: correlation between VTN (pg\ml) level and Kim-1(ng\ml) level in CML patients

4.6.2. Correlation between study biomarkers in healthy control:

Table (4.15) demonstrates the relationship between the study results for the patient groups, and it demonstrates that there is an average inverse relationship between WBC and ALT of (52.5%), which is a significant relationship under the level of significance of 0.01. It also shows that there is a weak direct correlation between IL18 and KIM-1 by (44.6%), which is significant below the level of significance of 0.05. Additionally, it was discovered that there was an average direct correlation of (66.2%) between VTN and KIM-1, which is statistically significant below the significance level of 0.01. The rest of the variables did not show a statistically significant association.

Table 4.19: Correlation between study markers in control groups

		RBC	WBC	Platelets	ALT	AST	urea	creatinine	IL18	VTN	kim-1
RBC	r		.158	-.163	-.158	.140	-.011	.102	.065	.234	.010
	Sig		.405	.390	.403	.461	.952	.594	.735	.213	.957
WBC	r			-.128	-.525**	.255	.146	-.058	.093	.047	.103
	Sig			.501	.003	.174	.441	.762	.625	.804	.588
Platelets	r				.129	.220	-.073	-.325	.183	.175	.121
	Sig				.498	.243	.701	.080	.333	.354	.525
ALT (GPT)	r					-.280	-.143	.175	.126	-.035	-.167
	Sig					.134	.452	.356	.506	.856	.377
AST (GOT)	r						.100	-.068	-.130	-.206	-.230
	Sig						.600	.722	.492	.274	.221
Urea	r							.122	.098	.042	-.085
	Sig							.522	.608	.826	.653
Creatinine	r								.109	-.200	-.186
	Sig								.567	.289	.324
IL18	r									.303	.446*
	Sig									.104	.014
VTN	r										.662**
	Sig										.000
kim-1	r										
	Sig										

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

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

Table (4.19) explains the linear regression between the variables (ALT, WBC). Through the table, the significant parameters of (〖β_0 = 22.638〗 _) and (β_1 = -2.062) can be seen through the value of Sig. < 0.05.

Table 4.20: Liner regression between ALT and WBC in control group.

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
ALT	(Constant)	22.638	3.597		6.293	.000
	WBC	-2.062	.631	-.525	-3.268	.003

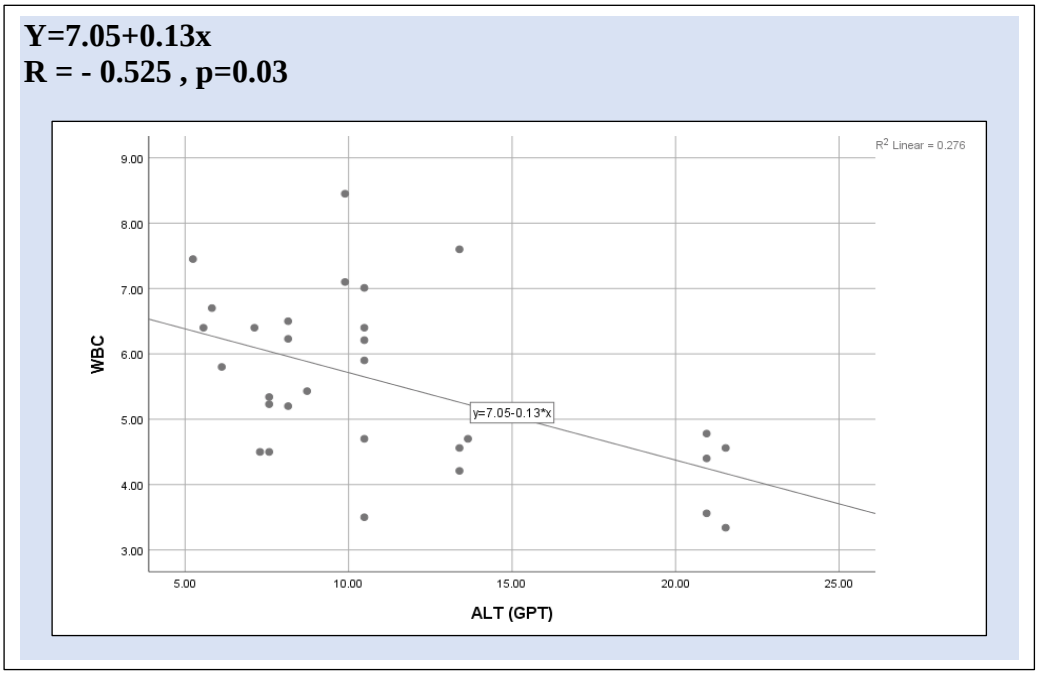


Figure 4.13: correlation between WBC count and ALT(U\L) level in control health .

Table (4.20) shows the linear regression between the variables (Kim-1, IL18, VTN). Through the table, it is clear that the parameter is significant (〖β_2 = 0.017〗 _) and the non-significance of the rest of the parameters, and it is evident through the value of Sig.

Table 4.20 show that a significant regression between kim-1 and VTN and a non-significant regression with IL18 in control group.

Table 4.21: Liner regression between kim-1 with IL18 and VTN in control group.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
Kim-1	(Constant)	-.099	.184		-.539	.595
	IL18	.012	.006	.270	1.900	.068
	VTN	.017	.004	.580	4.076	.000

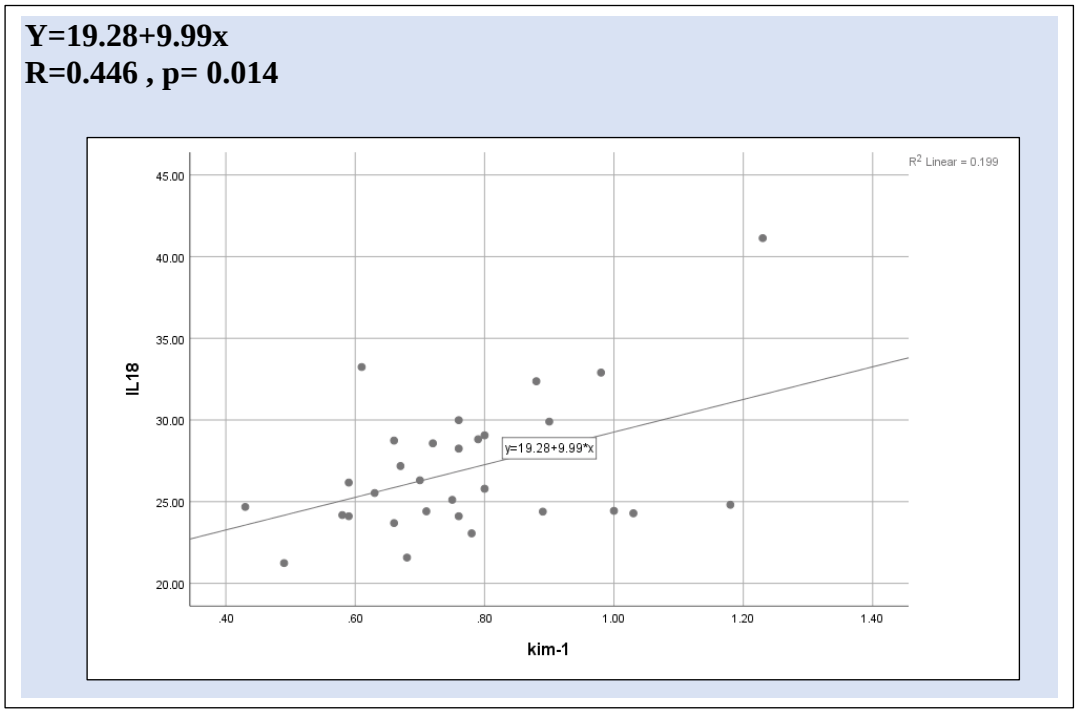


Figure 4.14: correlation between IL-18 (pg\ml) level and Kim-1(ng\ml) level in healthy control.

Y=14.58+22.64x
R=0.662 , p= 0.00

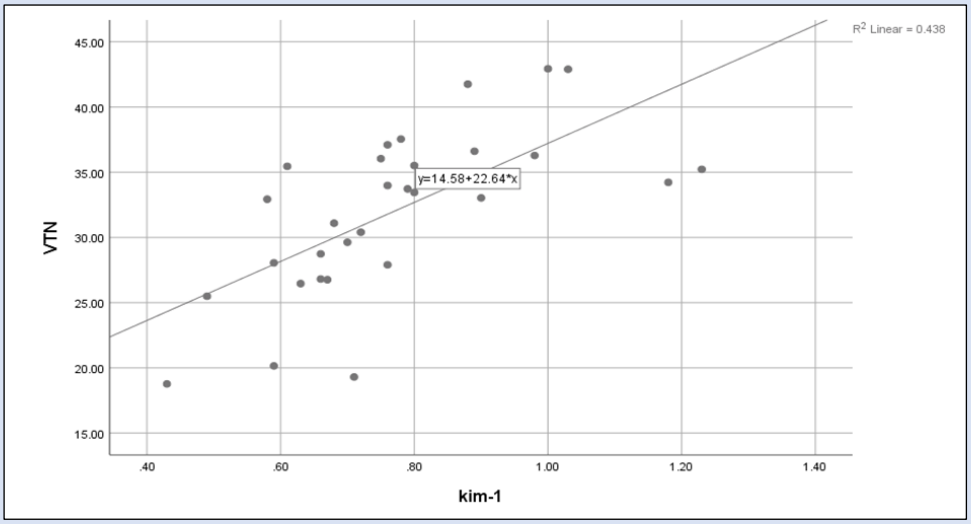


Figure 4.15: correlation between VTN (pg\ml) level and Kim-1(ng\ml) level in healthy control.

Chapter Five

Discussion

Chapter Five

Discussion

5.1.The general characters of study groups

5.1.1. Distribution of Leukemia patients

The leukemia patients were distributed in Figure (4.1) to the four types of leukemia, and it was found that the percentage of the AML type was (4%), while the ALL type was (6%), and for the CLL type it was (8%) and for the CML type it was (82%) It was found through our study that CML is the most common type compared to the other three types. In another study , it was found another region of Iraq, Karbala Governorate, where chronic lymphocytic leukemia (CLL) was the most prevalent type at 41%, followed by chronic myeloid leukemia (CML) at 24.1%, and chronic myeloid leukemia (CML) at 24.1%. (AML) 19.2%, chronic lymphocytic leukemia (CLL) 15.7% (13) and Sulaymaniyah governorate. It was the most common type of leukemia with 44% of all cases, chronic myeloid leukemia was the second type with 20% of cases, followed by chronic lymphocytic leukemia (CLL) with 18% and Acute myelogenous leukemia (AML) with 17% (Mjali *et al*., 2019) , Although chronic myeloid leukemia (CML) is a rare condition with an estimated frequency of 1-2 occurrences per 100,000 people. Around 15% of newly diagnosed adult leukemia cases are related to people's presence (Alves *et al*,2021) .

5.1.2. Age

The current study showed an increase in leukemia cases, as more than 72 samples were collected in about three months from the city of Baghdad and Babylon only, and this is logical because of the many wars and crises that occur in Iraq. During the past 31 years (1980-2010), Iraq witnessed three wars, (the Iran-Iraq War, 1980-1988), (the Gulf War, 1990-1991), and (the Iraq War, 2003), in addition to economic sanctions (1990-

2003), these types of wars and crises have a negative impact on people's health. (AL-Hashimi and Wang ,2013). Hematologic and general cancer incidence has been shown to rise with age (Jurczyszyn *et al*., 2018). Leukemia can emerge at any age, whether it has an acute or chronic origin (Juliusson *et al*., 2020). Leukemia can manifest at any age, from infants to the elderly, however the diverse kinds have varying age distributions. In the current study, The highest incidence of the disease was over the age of 25 years Table 4.1 and figure 4.2.

This is comparable to what has been reported elsewhere regarding the leukemia hypothesis with age, which states that older individuals may develop leukemia more frequently than younger individuals due to advancing age, as many environmental exposures to carcinogens, irradiation, and malignant mutations due to clonal expansion occur more frequently (Konieczny *et al*., 2018; Cagnetta *et al*., 2018). In contrast to Noone *et al*., 2017, 67% of diagnoses were given to patients above the age of 65. The incidence rate is 26.4 per 100,000 for those aged 65 and older and 35.8 per 100,000 for people aged 85 and older, given that aging is typically associated with lower overall survival, it may be possible to anticipate a decline in treatment tolerance that leads to treatment stopping, which lowers the cumulative dose.

5.1.3. Gender

Multiple diseases have seen an increase in the realization of gender-specific disparities in presentation and outcome of signs and symptoms (Migliore *et al*., 2021). Incidence and clinical outcomes of a number of cancer forms vary by gender; gender may have an impact on the genesis and natural history of various cancers. According to Miranda-Filho *et al*. (2018), nations with high and very high Human Development Index ratings have a greater percentage of adult males with chronic myeloid leukemia. The effects of gender on chronic myeloid leukemia (CML) have not been

thoroughly researched. According to the study's findings, men made up (45%) and women made up (55%), as indicated in table 4.1 figure 4.3 . (Ahmad *et al*.,2019) discovered that men were 64.5% more likely to get leukemia than women were, and they stated that this was because men were exposed to more carcinogens at work and in the environment. While Miranda-Filho *et al*. (2018) noted that males have higher incidence rates of chronic lymphocytic leukemia were low throughout Asia, especially in Japan. Iraqi population in other region of Iraq , Karbala Province also reported a dissimilar finding that accounted 58.2% for males and 41.8% for females between November 2011 to May 2018 (Mjali *et al* .,2019).

5.1.4. Body mass index (BMI):

Additionally, obesity has been linked to a higher risk of leukemia-related mortality. More than 900,000 US people participated in a sizable prospective cohort study (Tentolouris *et al*., 2023). A statistically significant 70% greater risk of leukemia death was found in men with a BMI of 35 kg/m2 or above; no such association was found in women (Dong *et al*., 2023). According to findings from a different cohort study with 35,420 US people, there is a positive dosage response connection between BMI and leukemia mortality in both men and women (Berger, 2018). (Rivera-Izquierdo *et al*, 2021). reported that on a continuous scale, a 5 kg/m2 increment in BMI was associated with a statistically significant 13% increased risk of leukemia. In current study three groups of BMIs showed in table 4.1 and figure 4.4 , Normal weight was (31.6%), Overweight was (56.6%), Obesity (10.8%). However, BMI is an important tool for describing the nutritional status of leukemia sufferers before chemotherapy.

5.1.5. Mutation (*NPM*1)

In the current study, 33.3% of the case sample has the mutation (*NPM1*), which is statistically significant (P value > 0.05), while 66.6% of the case sample does not. According to Kunchala *et al*. (2018), *NPM1* mutations are present in about 35% of all AML patients. According to another study, while *NPM*1 mutations are much less common in children, occurring in only 8–10% of all AML cases and in roughly 25% of those with an NK, and are the most common genetic abnormality in adult acute myeloid leukaemia (AML), accounting for about 35% of all cases and up to 60% of patients with normal karyotype (NK) AML (Rau *et al*., 2009). As per Yusoff *et al*., (2019), He found them in between 24 and 45% of all patients. Aberrant NPM1 mutations at exon 12 were the most common genetic variants in AML. After observing that the NPM1 gene is mutated in more than 50% of cases of normal karyotype-AML (Belfield *et al*., 1971), Gallagher concluded that it had the greatest incidence of any mutation in AML. According to Mencia-Trinchant *et al*. (2017), half of their CML patients have normal cytogenetics while 40% to 50% of them have *NPM1* mutations. According to Othman *et al*. (2021), the *NPM1*-A mutation was discovered in 24.8% of AML patients.The majority of intracellular *NPM*1 is oligomeric and interacts with other proteins, such as tumor suppressor proteins (Chen *et al*., 2020). In addition, NPM1 is a versatile phosphoprotein that participates in a variety of processes including chromatin remodeling, mRNA processing, ribosome biogenesis, and embryogenesis (Gupta *et al*., 2020). NPM1 mutations are strongly associated with newly identified de novo acute myeloid leukemia (AML) cases in human hematologic malignancies, which make up about one-third of all AML patients and have unique genetic, pathologic, immunophenotypic, and clinical characteristics (Reichard *et al*., 2022). Notably, the majority of patients have mutant *NPM*1, which is a strong

indicator of their AML status (Ivey *et al*., 2016). Even many years after the initial diagnosis, NPM1 mutations can still be found in AML upon relapse (Vosberg *et al*., 2019). In the most recent revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia, NPM1-mutated AML has been identified as a separate molecular leukemia entity with different biological and clinical characteristics (Hasserjian, 2021). However, the biological diagnosis, prognostic classification, and monitoring of minimal residual disease (MRD) in hematologic malignancies are all significantly impacted by *NPM*1 abnormalities. A reasonable foundation for the development of molecularly targeted treatments for leukemia and lymphoma has been provided by the identification of NPM1 gene mutations (Bailey *et al*., 2019; Cocciardi *et al*., 2019).

5.2: The Levels of parameters in study groups (CML patients and control)

5.2.1. Levels of Hematological Parameters

A complete blood count (CBC) is a blood test used to assess general health and identify a number of illnesses, such as leukemia, anemia, and infection. The results of a CBC test reveal the types and quantities of blood cells such platelets, white blood cells, and red blood cells. When compared to a normal, healthy blood cell count, this blood cell count indicates the presence of problems. Your blood's various components and characteristics are measured by a complete blood count test, including:Red blood cells, which carry oxygen, Infection-fighting white blood cells, Red blood cells contain three components: hemoglobin, a protein that carries oxygen, hemocrit, which measures the ratio of hemoglobin to plasma, the liquid component of the blood, and platelets, which aid in blood clotting. A complete blood count that reveals abnormal increases or declines in cell

counts may point to an underlying medical problem that necessitates additional testing.

The rapid generation of aberrant white blood cells is what leads to leukemia, a type of cancer that affects the blood and bone marrow. The production of red blood cells and platelets by the bone marrow is hampered by these aberrant white blood cells, which are unable to fight infection (Heidari and Gobato [a], 2019). Whole blood was subjected to a complete blood count (CBC) within 24 hours following sample collection. At a p-value of 0.05, Table 4.2 reveals extremely significant differences in RBCs, WBCs, and platelets between the patient and control groups.

Heidari and Gobato [b], (2019) explained the decline in RBC and platelet counts by stating that leukemia caused a rapid production of abnormal white blood cells, and that an abundance of abnormal white blood cells makes it difficult for cancers of the bone marrow to produce red blood cells and platelets. (Rosmarin, 2019). Also keep in mind that leukemia and lymphoma are two malignancies that might drop your platelet count. In the bone marrow, which produces platelets, the aberrant cells in these malignancies can displace healthy cells.

Patients without symptoms may be diagnosed with chronic leukemias, which are characterized by an increase in more mature WBCs (e.g., more circulating mature lymphocytes in chronic lymphocytic leukemia or more mature myeloid cells in chronic myeloid leukemia) (Ware, 2020).

5.2.2: Levels of Biochemical Parameters (Markers of liver function)

Table 4.3 show that a highly- significant differences between patients and control group regarding to (AST and VTN). While there are a non-significant significant difference between patients and control group regarding to ALT at p-value ≤ 0.05.

a. aspartate-aminotransferase (AST) (or glutamic-oxaloacetic transaminase/GOT) (ALT) or GPT Level :

This study found that the majority of liver enzyme biochemical tests (AST, ALT) were significantly higher in the CML. Our results are in line with liver enzyme levels that were discovered to be markedly higher in leukemia patients (Burhan [a]*et al*., 2016). AlMashhadani *et al*, (2011) and Burhan *et al*, (2016) conducted a second study that found that the ALT and AST levels in the serum of leukemia patients had significantly increased. The hepatic infiltration of these enzymes in people with leukemia is one of the main reasons for the level increase. A blood test would be required to detect the blood AST level due to the recognized defect in the cytoplasmic and mitochondrial membranes (Burhan [b] *et al*., 2016).

Leukemia and its subsequent liver damage usually result in elevated levels of the ALT and AST enzymes (Segal *et al*., 2010). A rise in the number of leukemic cells could lead to an increase in the transaminase enzyme concentration as a side effect of chemotherapy (Burhan *et al*., 2016). The reason leukemia patients had high levels of the AST and ALT enzymes was explained by Gao [a] *et al*. (2002), who highlighted that chemotherapy has a deleterious impact on liver function. These results are in line with other studies on serum AST activity in patients with ALL, AML, and lymphoma (Gao [b] *et al*., 2020; Scolyer *et al*., 2020). According to Abdalla *et al*. (2018), patients with acute lymphocytic

leukemia (ALL) had significantly higher levels of AST and ALT when compared to healthy individuals. Male patients with ALL also had significantly higher levels of AST and ALT than female patients with ALL. There was also a positive correlation between the level of AST and age in (ALL) patients, but there was no correlation between the level of AST and age in controls.

The known flaw in the cytoplasmic and mitochondrial membranes would prompt a blood test to determine the blood AST level. (Burhan [a],2016). Elevated levels of ALT and AST enzymes are frequently found in the beginning of liver damage is caused by leukemia and that in turn (Segal *et a*,2010). Because of the side effects of chemotherapy, a rise in leukemic cell count could result in an increase in transaminase enzyme concentration (Burhan [b],2016). Gao (20), who noted that chemotherapy has a negative impact on liver function, explained why leukemia patients have high levels of the AST and ALT enzymes. These findings are consistent with prior research on the serum AST activity in ALL, AML, and lymphoma patients (Gao [a],2020)

This conclusion was consistent with that of a prior study by Al-Hammami (2015), which found that patients with ALL had higher AST and ALT due to the infiltration of leukemic cells. It also concurred with Segal *et al*. (2010), who claimed that increased transaminases are typical at the time of the initial presentation of ALL and are most likely caused by hepatic damage brought on by leukemic infiltrates.

According to this study, the majority of biochemical tests for liver enzymes (AST, ALT) were considerably higher in the CML before chemotherapy also, a significant increase after chemotherapy when compared with the time of diagnosis (day 1) of the disease . Our findings are consistent with liver enzymes were found to have considerably elevated levels in leukemia patients (Burhan, 2016) . Another study carried out by

(Jhalla, and Alamin , 2017 ; Pandey *et al* .,2018) revealed a considerable rise in both ALT and AST levels in the serum of leukemia patients. One of the primary causes of the level increase is the hepatic infiltration among those with leukemia of these enzymes.

The known flaw in the cytoplasmic and mitochondrial membranes would prompt a blood test to determine the blood AST level (Burhan [c], 2016). Elevated levels of ALT and AST enzymes are frequently found in the beginning of liver damage is caused by leukemia and that in turn Because of the side effects of chemotherapy, a rise in leukemic cell count could result in an increase in transaminase enzyme concentration , Gao (2002) who noted that chemotherapy has a negative impact on liver function, explained why leukemia patients have high levels of the AST and ALT enzymes. These findings are consistent with prior research on the serum AST activity in ALL, AML, and lymphoma patients (Hijiya and Van Der Sluis ,2016).

b. Vitronectin (VTN):

Vascular walls, tumor cells, and various extracellular matrix sites are all discovered to be connected with vitronectin, an abundant sticky glycoprotein in blood plasma, which is especially present during tissue remodeling, injury/repair, or disease situations (Gupta *et al*., 2023). According to Preissner (1991), VTN is largely produced in the liver and released into the blood on its high level of gene expression, especially in hepatocytes (Keasey *et al*., 2022). Additionally, the results of the current study record a high significant increase in level VTN in CML patients compared into healthy control. This explains why leukemia patients' liver cells are damaged. also, the results of current study record high significant increase in level VTN in CML patients compared into healthy control , According to Preissner (Preissner,1999) VTN is largely produced in the liver and released into the blood on its high level of gene expression,

especially in hepatocytes(Keasey [a] *et al* .,2022) . This explains the damage to liver cells in leukemia patients .On other hand the results record high significant increase in level VTN in CML patients compared into healthy control , and after receive chemotherapy . According to Preissner (1991) VTN is largely produced in the liver and released into the blood on its high level of gene expression, especially in hepatocytes(Keasey[b] *et al*.,2022) . This explains the damage to liver cells in leukemia patients.

5.2.3: Kidney function markers

Acute kidney injury (AKI) is a syndrome characterized by the rapid loss of kidney function. Recently acute kidney injury was described and classified by KDIGO (Kidney Disease: Improving Global Outcomes) guideline (Lameire[a] *et al*.,2021). Acute kidney injury is a common complication and causes to increase in mortality rate significantly in hospitalized and critically ill patients (Jia *et al*,2022). It was reported that AKI may be responsible for 2 million deaths per year in the United States, and furthermore, 50% of critically ill patients in intensive care may develop AKI (Farrar[a],2018).

Drug Imatinib and nilotinib were observed to decrease urea levels while increasing serum creatinine levels in the diseased group as compared to healthy participants, according to Arsalan et al.'s findings from 2022. When compared to healthy participants, the sick group's serum creatinine level was higher according to the results of the renal profile (serum urea and serum creatinine) (Arsalan [a]*et al*., 2022). According to other studies (Schrezenmeier [a] *et al*., 2017), an increase in IL-18 urine concentrations happens quite quickly in response to renal tubular damage. After a 50% decrease in glomerular filtration rate, there is a significant increase in serum creatinine as well (Leem *et al*., 2017). In contrast to prior studies, it was discovered that there was a considerable rise in urine KIM-1 as soon as 6 hours after ICU admission, and it persisted at this level for 48 hours.

Furthermore, a higher level of KIM-1 was found in the patients who passed away (Tu *et al*., 2014).

Rapid loss of kidney function is a phenomenon known as acute kidney injury (AKI). By KDIGO (Kidney Disease: Improving Global Outcomes) guidelines, acute kidney damage was recently characterized and categorized (Lameire [b]*et al*., 2021). Acute renal impairment is a prevalent complication that significantly increases mortality rates in hospitalized and critically ill patients (Jia *et al*., 2020). AKI may be responsible for 2 million fatalities yearly in the United States, and it occurs in 50% of critically sick patients getting intensive care, according to reports (Farrar[b], 2018).

(Abdalla *et a*l.,2018), revealed that as compared to healthy people, patients with acute lymphocytic leukemia (ALL) had significantly higher levels of urea and creatinine. However, there was no association between urea and creatinine levels and age. This conclusion was consistent with that of a prior study by Al- Hammami (2015), which found that patients with ALL had higher urea and creatinine levels as a result of the infiltration of leukemic cells.

a. Indicators of renal status (urea level):

It has long been believed that urea, a sign of uraemic retention in chronic kidney disease (CKD) and of adequate intradialyte solute clearance, is physiologically inactive. Nevertheless, a number of recent experimental findings imply that urea is hazardous at levels typical of CKD. First off, according to at least five studies, urea causes molecular alterations in the body that affect insulin resistance, the formation of free radicals, apoptosis, and the integrity of the intestinal barrier. Second, urea is the source of the production of cyanate, ammonia, and carbamylated chemicals, all of which have been associated with alterations in biological processes. Post-translational protein modifications linked to atherogenesis

and other functional alterations have been attributed, in particular, to carbamylation. These carbamylated substances were linked to cardiovascular and general morbidity and mortality in observational clinical trials (Vanholder *et al.*, 2018).

The biological significance of urea in uraemic syndrome and chronic kidney disease (CKD) is still up for dispute. For a long time, the conventional view held that urea, despite being used globally to calculate Kt/Vurea as a measure of the sufficiency of dialysis, is a molecule with low toxicity that is comparatively inert. This theory was supported by a number of acute animal trials, the most from the 19th century, which showed that urea had no detectable harmful effects (Lau *et al.*, 2017).

When compared to healthy participants, the sick group's serum creatinine level was higher according to the results of the renal profile (serum urea and serum creatinine) (Arsalan[b] *et al*., 2022). After a 50% decrease in glomerular filtration rate, there is a significant increase in serum creatinine as well (Leem *et al*., 2017).

The quantity of urea in the serum affects the most frequently determined clinical indices for predicting renal function. BUN levels are seen to be higher in conjunction with renal disease or failure. Blood urea nitrogen (BUN) levels are reduced under conditions such fluid overload, trauma, and starvation (Cheng *et al*., 2020).

According to recent findings (Table 3), there is no statistically significant association between BMI and kidney function tests in the patient and control groups. This criticism of (Gerchman *et al*, 2009). who claimed that there was a positive correlation between BMI and creatinine clearance (P 0.001). In their investigation of the relationship between BMI and creatinine and urea values, Abeadalla *et al*. (2018) found that while there was no discernible relationship between BMI and urea, there was a definite relationship between BMI and creatinine levels. Urea and age do not

correlate significantly, which is inconsistent with Rajdev *et al*. (2023). Urea is shown to be extremely significant in all age group examined (P 0.001). When compared to healthy people, Abdalla *et al*. (2018) discovered that patients with acute lymphocytic leukemia (ALL) had significantly higher levels of AST, ALT, urea, and creatinine.

b. Renal Status indicators (Creatinine level):

A commonly used indicator of renal function is creatinine. When serum creatinine exceeds the upper limit of the normal range, the diagnosis of renal failure is frequently suspected. Leukemia is observed to have higher values (Hantoosh *et al*., 2019).

Acute kidney injury (AKI) has been mostly diagnosed in the last 50 years based on serum creatinine levels. Serum creatinine levels may cause a delay in the therapy of AKI and result in negative results. It is well known that an increase in serum creatinine level occurs after a considerable decline in glomerular filtration rate (GFR).

When specific conditions are present, such as rhabdomyolysis or exposure to drugs like cephalosporins and sulfa, an increase in serum creatinine level can overstate changes in GFR. Other diseases, such as cirrhosis, hyperbilirubinemia, fluid overload, older patients, and those with an abrupt loss of muscle mass, might cause blood creatinine levels to underestimate changes in GFR. Therefore, the specificity, sensitivity, and timeliness of serum creatinine level are limited. Oliguria, a less severe form of renal dysfunction, may appear long before the level of serum creatinine rises (Alge *et al*., 2015; Schrezenmeier[b] *et al*., 2017).

Age and creatinine in the current study had a substantial correlation in the control group but a non-significant correlation in the sick group.

Patients using imatinib for chronic myeloid leukemia have reported a rise in creatinine, however the underlying cause is yet unknown. Imatinib entirely reduces the amount of creatinine that is secreted from the tubules,

according to Vidal *et al*.'s (2016) findings. Independent of any glomerular dysfunction, this inhibition raises serum creatinine and is completely reversible upon stopping imatinib.

c. Interleukin 18 (IL-18) Level:

Inflammatory events cause the pro-inflammatory cytokine IL-18 to become more active. It is a modulator of hypoxic-induced tissue injury (Kumar, 2020).

According to Rizvi *et al*. (2017), urine IL-18 is an early, quick, and affordable marker that enables the diagnosis of early kidney injury brought on by ischemia or nephrotoxins. As evidenced by (Bi *et al*., 2019), patients with greater serum IL-18 levels tended to have lower BMI. Serum IL-18's potential as an AKI biomarker is, however, not well understood. Although serum levels of IL-18 were much greater in AKI patients than in controls, this difference was barely statistically significant (Zdziechowska *et al*., 2020).

According to Saadi *et al*.'s (2021) findings, IL-6 and IL-18 expression levels were assessed in AML patients based on how well they responded to treatment based on CR. According to the findings, IL-6 and IL-18 gene expression levels were higher in AML patients who did not react to therapy than in individuals who did.

The relevance of IL-18, IMT-1, and beta2-microglobulin in the diagnosis of chronic renal disease in cancer patients following the completion of therapy was investigated by Zubowska *et al*. in 2013. The author came to the conclusion that early indicators of persistent damage to the proximal tubules in children after chemotherapy include beta2-microglobulin and, in particular, IL-18. According to other studies (Schrezenmeier[d] *et al*., 2017), an increase in IL-18 urine concentrations happens quite quickly in response to renal tubular damage.

Using an ELISA method, Khakroo Abkenar *et al*., (2019) were able to detect the presence of IL-18 in the plasma of patients who had different forms of leukemia. IL-18 plasma levels in leukemia patient groups with ALL or CML were considerably higher than those in the group of healthy volunteers. Although there was no statistically significant difference between the groups with CLL or AML, comparatively higher levels of IL-18 were seen in a few of the individuals in these groups. The IL-18 levels in the normal control group were extremely low, but the ELISA measured values that were unquestionably detectable.

d. Kidney Injury Molecule 1 (Kim-1) level:

Kidney injury molecule-1 (KIM-1) is a type I membrane protein with extracellular and cytoplasmic domains that is only very weakly produced in healthy kidneys. After kidney damage, the extracellular part can split and quickly enter tubule lumens, where it can then be seen in the urine. It has been established that renal tissue damage is connected with the urine KIM-1 level, which is closely related to the tissue KIM-1 level. KIM-1 has been shown to be an early indicator of acute kidney injury and may potentially play a role in the prognosis of long-term renal outcomes. The connections between KIM-1 and kidney injury, particularly in chronic kidney disease, are summarized in this review (Yin *et al*., 2016). In recent years, novel biomarkers, like kidney injury molecule-1 (KIM-1), a type 1 trans-membrane glycoprotein, have been employed to identify acute kidney injury at an early stage. KIM-1, a marker for kidney damage, cannot be found in healthy people. Acute kidney injury, on the other hand, causes a marked rise in its excretion in proximal tubule cells in response to AKI (Griffin et al., 2019; Li et al., 2020).

In contrast to prior studies, it was discovered that there was a considerable rise in urine KIM-1 as soon as 6 hours after ICU admission, and it persisted at this level for 48 hours. Furthermore, a higher level of

KIM-1 was found in the patients who passed away (Tu et al., 2014). Similar to prior studies, it was found that there were no appreciable variations in KIM-1 excretion between the obese, overweight, and control groups (Gul et al., 2020). KIM-1 and IL-18, according to Connolly et al. (2018), were not effective early predictors of acute kidney injury. According to Latoch et al. (2020), urine KIM-1 levels were shown to be considerably higher in ALL survivors compared to healthy controls using commercial Immunoenzymatic ELISA kits. Elevated NGAL and KIM-1 excretion in the urine was seen in adults and adolescents receiving treatment for various malignancies either immediately after the infusion of cisplatin or ifosfamide or several days later. The severity of the tubular injury was correlated with a rise in their concentrations (Westhoff *et al*., 2017; Maeda *خ*., 2017; George *et al*., 2018).

Male, obese, and hypertensive patients in a research on children with hyperuricemia showed greater urine excretion of NGAL and KIM-1. According to the scientists, this was most likely caused by endothelial dysfunction, local kidney inflammation, or a deleterious impact of hyperuricemia on renal tubules (Tomczak *et al*., 2013).

5.3. Correlation Results Study

From the results of the correlation shown in Tables (4.14-4.15), we found positive and significant correlation between the numbers of RBC and the numbers of platelets ,these results are consistent with (Chaitra *et al*., 2023) those that found a highly significant positive correlation in anemia patients , Munker *et al* (2007) suggest that both thrombopoietin and erythropoietin belonging to the same hematopoietic growth factor subfamily, are majorly produced in the kidney and act similarly by activating the JAK/STAT pathway and Ras signal transduction on their respective precursors. GATA-1, a transcription factor is expressed in primitive and definite erythroid and megakaryocytic cells and expression

of both lineages are dependent on the presence of an intact 40 41 GATA site. Thus a large body of data supports the concept that megakaryocytic and erythrocytic cell lineages share a common progenitor**.**

The correlation results also showed a significant positive correlation between the number of red blood cells and the level of ALT , in a previous study, it was found that there is a relationship between liver function enzymes and the number of red blood cells(Ohshima and Toyama ,1987). The results of the statistical analysis also showed that there is a positive significant correlation between ALT level and the level of urea and these results are consistent with the results of a previous study (Lin *et al*., 2022) that included more than four thousand patients with cirrhosis of the liver. There is a relationship between the level of urea and the severity of the disease. The results of the current study recorded a positive significant correlation between il18 with kim -1 and VTN, Both IL-18 and KIM-1 are specific to the proximal tubule and have been implicated in ischemia-reperfusion injury to the kidney (Bonventre and Yang ,2010). The results of the current study recorded a positive significant correlation between IL-18 with kim-1 and VTN, which indicates damage to liver and kidney tissue in leukemia patients.

Chapter Six

Conclusions and Recommendations

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6.1 Conclusions

●Leukemia is a heterogeneous malignant disease that affects all age groups, with different clinical symptoms.

● According to the study's findings, CML, followed by CLL, ALL, and AML, had the highest prevalence of leukemia in Iraqi community

●During the study period, the incidence of leukemia was higher in women than in men

●Symptoms of the disease are different from one patient to another for unknown reasons.

● A complete blood count is helpful in the diagnosis of acute leukemia, as are peripheral exams and measurements of bone marrow flow.

● The nucleofosmin protein was mutated in about 33.3% of leukemia patients, according to the study's findings.

6.2 Recommendations

- Investigation of other biomarkers for predication diagnosis of chronic leukemia , and monitoring of progress of disease .
- monitoring the progression of the disease in various age groups by continually testing the liver and kidneys, as the existence of any disruption in these parameters is indicative of harm to these organs.
- A study micro RNA hat has a role as effective biomarkers in stratifying and thus responding to treatment without resorting to surgical interventions.
- Studying ideal biomarkers that have a specific expression in the disease helps in early diagnosis and monitoring the course of the disease.
- it is necessary to conduct an examination of liver indicators before and after giving chemotherapy and periodically to know the effect of the treatment that may cause liver toxicity, which may end the patient's life.

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Appendices

Appendices

Appendix (A)

Hematological Assessments

The counting of the cellular blood components, the Analyzer Ruby, uses the impedance technique only. A cyanide free spectrophotometry method was used to measure hemoglobin by formation of oxyhemoglobin at 555 nm. Packed cell volume platelet count (PLT). For platelet counting a floating threshold was used, whereas for RBC and WBC counts the thresholds are predefined.

Results are provided within 1 minute on the display, printed out on the printer and stored in the resident memory or on a USB key. Results were presented with flags; optionally reference ranges can be reported.

the instrument uses three reagents: a diluent, a lysis reagent and a cleaning solution

Procedure

1. 10 µl of the EDTA blood sample was placed in the aspirator on the instrument.
2. The start key on the instrument was pressed and the blood sample was aspirated.
3. Results were provided within 1 minute on the LCD display, printed out on the printer and stored in the resident memory.

Appendix (B)

Human Interleukin 18 (IL-18) Level:

Assay Principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process to assay Interleukin 18 (IL-18)

in **Human serum, blood plasma, urine, and other biological fluids.**

Add standard, test sample and HRP-labeled Interleukin 18 (IL-18) antibodies to wells which are Pre-coated with Interleukin 18 (IL-18) antibody. After incubation and washing to remove the uncombined enzyme, add Chromogen Solution A and B. The color of the liquid will change into blue. At the effect of acid, the color finally becomes yellow. The color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Interleukin 18 (IL-18) in

the samples is then determined by comparing the O.D. of the samples to the standard curve.

ASSAY PROCEDURE

1. Prepare all r e a g e n t s before starting assay procedure. It is recommended that all Standards and Samples be

added in duplicate to the Microelisa Stripplate.

2. Add standard: Set Standard wells, testing sample wells. Add standards 50µl to standard wells.

3. Add Sample: Add testing sample **10µl. Then** add sample diluent **40µl** to testing sample well; Blank well doesn't add

anything. **(The sample is diluted 5-fold in this step.)**

4. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.

5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with

Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is

essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting.

Invert the plate and blot it against clean paper towels.

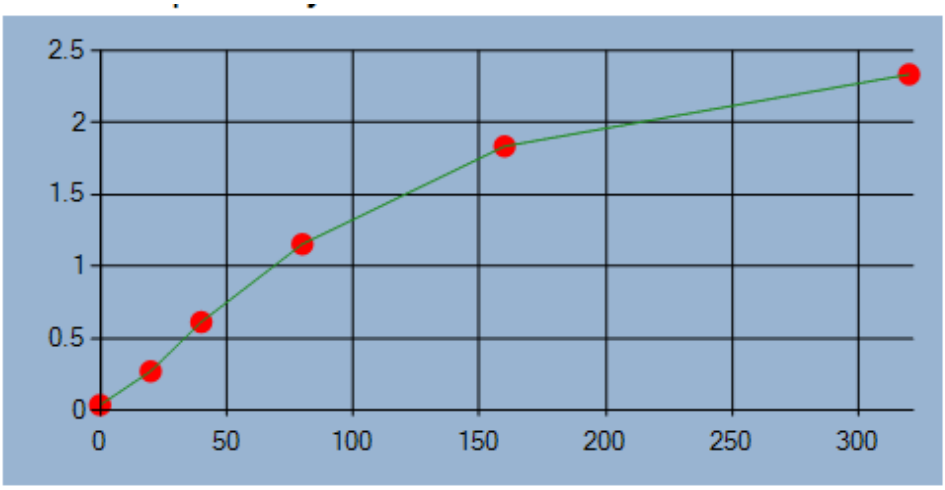
6. Add chromogen solution A 50µl and chromogen solution B 50µl to each well. Gently mix and incubate for 15 minutes

at 37°C. **Protect from light.**

7. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

Standard curve of IL18



Appendix C

Human Vitronectin (VTN) Level:

Assay Principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process to assay Vitronectin (VTN) in

Human serum, blood plasma, urine, and other biological fluids.

Add standard, test sample and HRP-labeled Vitronectin (VTN) antibodies to wells which are Pre-coated with Vitronectin (VTN) antibody. After incubation and washing to remove the uncombined enzyme, add Chromogen Solution A and B. The color of the liquid will change into blue. At the effect of acid, the color finally becomes yellow. The color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Vitronectin (VTN) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

ASSAY PROCEDURE

1. Prepare all r e a g e n t s before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.

2. Add standard: Set Standard wells, testing sample wells. Add standards 50µl to standard wells.

3. Add Sample: Add testing sample 10µl .Then add sample diluent 40µl to testing sample well; Blank well doesn't add anything... **(The sample is diluted 5-fold in this step.)**

4. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.

5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with

Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is

essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting.

Invert the plate and blot it against clean paper towels.

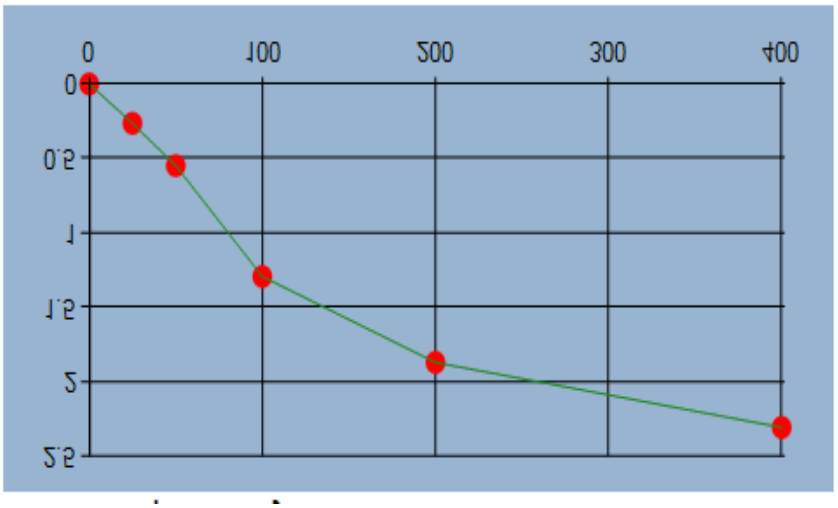
6. Add chromogen solution A 50µl and chromogen solution B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**

7. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells

is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

standard curve of VTN



Appendix (D)

Human Kidney Injury Molecule 1 (Kim-1) level:

Assay Principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process to assay Kidney Injury
Molecule 1 (Kim-1) in **Human serum, blood plasma, saliva,urine, and other biological fluids.**

Add standard, test sample and HRP-labeled Kidney Injury Molecule 1 (Kim-1) antibodies to wells which are Pre-coated with Kidney Injury Molecule 1 (Kim-1) antibody. After incubation and washing to remove the uncombined enzyme, add Chromogen Solution A and B. The color of the liquid will change into blue. At the effect of acid, the color finally becomes yellow. The color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Kidney Injury Molecule 1 (Kim-1) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

ASSAY PROCEDURE

1. Prepare all r e a g e n t s before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.

2. Add standard: Set Standard wells, testing sample wells. Add standards 50µl to standard wells.

3. Add Sample: Add testing sample **10µl** .Then add sample diluent **40µl** to testing sample well; Blank well doesn't add
anything. **(The sample is diluted 5-fold in this step.)**

4. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.

5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with

Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is

essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting.

Invert the plate and blot it against clean paper towels.

6. Add chromogen solution A 50µl and chromogen solution B 50µl to each well. Gently mix and incubate for 15 minutes

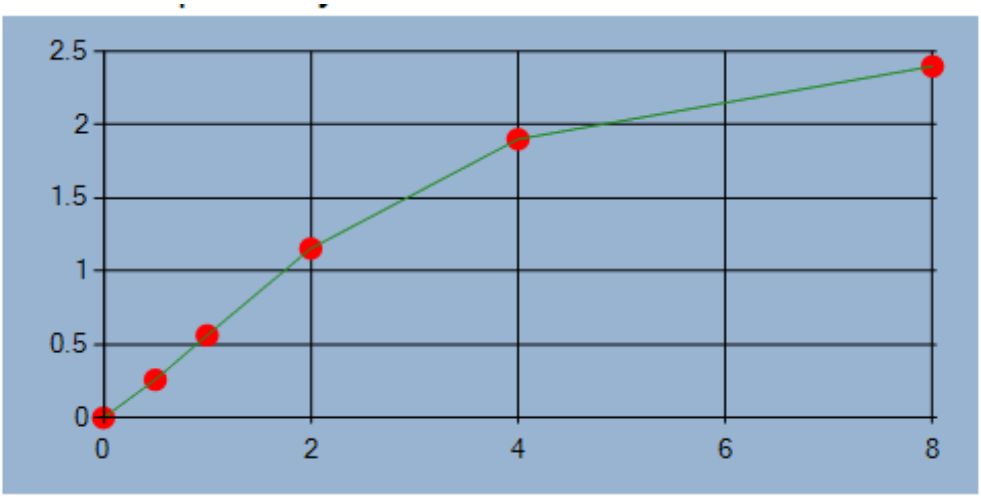
at 37°C. **Protect from light.**

7. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells

is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

standard curve of (KIM-1)



Appendix (E)

●Biochemical Assessments

Urea Level:

Detection principle

In strong acidic and heating condition, urea can react with diacetyl to form red diazine compound. The depth of color is proportional to the content of urea. Because the instability of the diacetyl, the diacetyl oxime usually reacts with the strong acid firstly in the reaction system to generate diacetyl, then react with urea to generate the red diazine compound. The reaction equation is as follows:

2 NH3 + CO2 ↔ NH4 + + NH2-COO - ↔ NH2-CO-NH2 (urea) + H2O**.**

Procedure

1. It is recommended to take 2~3 samples which expected large difference to do

pre-experiment before formal experiment.

2. Bring all the reagents to room temperature before experiment.

3. Centrifuge the serum (plasma) at 12000 rpm for 5 min, take the supernatant for detection.

4. open the water bath in advance and set the temperature to 100℃.

5. Blank tube: add 0.02 mL of double-distilled water into a 10 mL glass tube.

Standard tube: add 0.02 mL of 10 mmol/L urea standard into a 10 mL glass tube.

Sample tube: add 0.02 mL of Sample into a 10 mL glass tube.

6. Add 1 mL of Reagent 1 and 1 mL of Reagent 2 working solution into each tube.

Tight the tubes with preservative film and mix fully with vortex mixer. Incubate the tubes in boiling water for 15 min. Cool the tubes with running water**.**

7. Set to zero with double-distilled water and measure the OD value of each tube with 1cm cuvette at 520 nm**.**

●Creatinine (Cr) Level:

Principle:

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated wit Cr. During the reaction, Cr in the sample or standard competes with a fixed amount of Cr on the solid phase supporter for sites on the Biotinylated Detection Ab specific to Cr. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Cr in the samples is then determined by comparing the OD of the samples to the standard curve.

Assay procedure:

1- Add the Standard working solution to the first two columns: Each concentration
of the solution is added in duplicate, to one well each, side by side (50 uL for each well).

2- Add the samples to the other wells (50 uL for each well). Immediately add 50μL
of Biotinylated Detection Ab working solution to each well. Cover the plate with the sealer provided in the kit.

3- Incubate for 45 min at 37℃. Note: solutions should be added to the bottom of
the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

4- Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper.
5- Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.

الخلاصة

الهدف الأساسي من هذه الدراسة ولكون الأبحاث شحيحة حول اللوكيميا فيما يخص الأبحاث الجزيئية والسريرية وعلاقتها بمسار المرض وتأثيره في بعض اعضاء الحيوية المهمة كالكبد والكلية حيث بات واضحا ان سلوك الخلية السرطانية يتأثر بالتغيرات التي تحدث على مستوى الحمض النووي. سعى هذا البحث لتفحص عملية تلف الكبد والكلى لدى مرضى سرطان الدم العراقيين الذين لديهم طفرة في بروتين نوكليوفوسمين 1 (NPM1) ومقارنتها مع المرضى اللذين ليس لديهم هذه الطفرة.

تناولت الدراسة الحالية أحد أهم أمراض الدم الخبيثة، ابيضاض الدم النقوي المزمن. قد يساهم التشخيص المبكر ومراقبة تطور المرض بشكل كبير في تجنب مضاعفات المرض. ابيضاض الدم النقوي المزمن هو ورم نخاعي مرتبط بجين الاندماج BCR-ABL1 المستند إلى كروموسوم فيلادلفيا.

في مدينة المرجان الطبية / محافظة بابل ووحدة مستشفيات بغداد (مدينة الطب) ومركز سرطان الدم تم أخذ العينات من مرضى سرطان الدم النخاعي، جمعت العينات للفترة من شهر تشرين الاول 2022 وحتى شهر كانون الثاني 2023. خلال هذه الفترة جمعت (72) عينة، ولكن تم أخذ 60 عينة من المرضى واستبعاد 12مريض لأنهم كانوا يعانون من داء السكري من النوع الأول او التهاب الكبد او الفشل الكلوي ايضا تم استبعاد اي مريض ضمن خط آخر للعلاج منها زراعة النخاع العظمي ذاتيًا وشملت الدراسة ايضا 30 عينة من مجموعة المراقبة السليمة

اخذت عينات دم (5 مل) من كل شخص مشارك في الدراسة (مرضى واصحاء) عن طريق ثقب الوريد، ووضع 2 مل في أنابيب EDTA واستخدام 1 مل لتحليل CBC و 1 مل وضعت في -20 م° لاستخدامها لاحقا في الدراسة الجزئية، بينما يتم وضع 3 مل المتبقية في انابيب حاوي على هلام لغرض فصل المصل .

تضمنت الدراسة الحالية قسمين رئيسيين: الجزء الوظيفي الفسيولوجي الكيميائي و الجانب الجزيئي، في القسم الاول تم تقدير مستويات المؤشرات الحيوية في المصل (واليوريا والكرياتينين وIL-18 وجزيء إصابة الكلى -1 (KIM -1) كعلامات حيوية لوظيفة الكلى، ومستوى GPT و GOT و vitronectin (VTN) كعلامات حيوية لوظيفة الكبد بالإضافة الى دراسة بعض معايير الدم المهمة تعداد كل من (كريات الدم الحمراء ،خلايا الدم البيضاء ،والصفيحات الدموية) في كل من مجموعات المرضى والأصحاء. كما تم فحص العلاقة بين مستويات هذه المؤشرات الحيوية، مع المعلمات الدموية.

أظهرت النتائج من خلال التحليل الإحصائي أن المرض أظهر أن هناك فروق ذات دلالة إحصائية وكانت الفروق عالية بين المرضى والمجموعة الضابطة فيما يتعلق بـ (كرات الدم الحمراء، كرات الدم البيضاء، والصفائح الدموية عند مستوى $p \leq 0.05$. بينما يؤدي العلاج الكيميائي إلى زيادة معنوية $p \leq 0.05$ في مستويات هذه المعايير مقارنة بالأشخاص الاصحاء تشير النتائج ان GPT لم يكن ذات دلالة إحصائية أي إنها غير معنوية، على عكس (GOT,VTN) اذ أبدت دلالة إحصائية. اما بالنسبة للمؤشرات الحيوية الخاصة بالكلى فقد تبين وجود فروق ذات دلالة إحصائية بين المرضى ومجموعة الاصحاء فيما يتعلق باختبار وظائف الكلى (اليوريا، والكرياتينين، و IL18). بينما يوجد فرق كبير غير معنوي بين المرضى ومجموعة الاصحاء فيما يتعلق بـ kim-1 عند مستوى $p \leq 0.05$.

القسم الثاني من الدراسة تضمن التحري عن وجود الطفرة في بروتين النيوكلوفوسمين فقد تبين من خلال الدراسة الوراثية ان هناك 14 مريض متواجدة فيهم هذه الطفرة (*NPM*1) اما بالنسبة للمجوعة الاصحاء فلا وجود لهذه الطفرة نهائيا، اشارت النتائج الى ان وجود فروق ذات دلالة إحصائية بين أولئك الذين يعانون من طفرة ودون فيما يتعلق بـ خلايا الدم البيضاء. بينما توجد فروق غير ذات دلالة إحصائية بين أولئك الذين يعانون من طفرة ودون فيما يتعلق كرات الدم الحمراء والصفائح الدموية عند قيمة $p \leq 0.05$. اما بالنسبة لمؤشرات وظيفة الكبد الكبد بالاعتماد على الطفرة فقد تبين أن هناك فرقا كبيرا بين أولئك الذين يعانون من طفرة ودون فيما يتعلق (VTN). بينما هناك فرق غير معنوي $p \geq 0.05$ بين أولئك الذين لديهم طفرة وبدون فيما يتعلق بـ AST و ALT عند قيمة $p \leq 0.05$. وبالنسبة لعلامات الكلى الحيوية بالاعتماد على الطفرة فقد تبين أن هناك فرقا كبيرا بين أولئك الذين لديهم طفرة ودون فيما يتعلق (IL18). بينما هناك فرق كبير غير معنوي $p \geq 0.05$ بين أولئك الذين لديهم طفرة وبدون فيما يتعلق باليوريا والكرياتينين و KIM-1 بقيمة $p \leq 0.05$. وتم استنتاج ان المرض يكون بالإناث اكثر من الذكور للأسباب أهمها استخدام مواد التجميل مثل استخدام الكولاجين وكذلك تغير الهرمونات مثل هرمون الاستروجين.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل – كلية العلوم للبنات

قسم علوم الحياة

دراسة تأثير طفرة النوكليوفوسمين *1 (NPM1)* على وظائف الكبد والكليتين لدى المرضى المصابين بسرطان الدم النخاعي المزمن

رسالة

مقدمة الى مجلس كلية العلوم للبنات، جامعة بابل، وهي جزء من متطلبات نيل

درجة الماجستير في العلوم / علوم الحياة

من قبل الطالبة

غنية حيدر عبيد

(بكالوريوس علوم، علوم الحياة، جامعة بابل ،2019)

بإشراف

أ.م. د. شيماء عبيد عبد الله

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