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STUDY THE LEVELS OF SERUM GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) AND SOME BIOCHEMICAL PARAMETERS IN LEUKEMIC PATIENTS

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January 2023

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ABSTRACT

STUDY THE LEVELS OF SERUM GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) AND SOME BIOCHEMICAL PARAMETERS IN LEUKEMIC PATIENTS

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Master of Science in Chemistry

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January 2023

In this work, some changes that accompanied the chronic myeloid leukemia disease has been interested. Hematological variations (leukocytes, erythrocytes, and hemoglobin), glucose-6-phosphate dehydrogenase, ferritin, and some elements (iron, sodium, potassium, and zinc) were investigated in the blood and serum of patients. 130 subjects were enrolled in the study, 65 leukemic patients, and 65 healthy controls. The age differences were non-significant between leukemic patients and control group. The count of leukocytes was increased significantly while the count of erythrocytes and hemoglobin were reduced significantly in leukemic patients. The level of serum glucose-6-phosphate dehydrogenase was decreased significantly in leukemic patients. On the other hand, serum ferritin and iron levels were increased significantly and correlated with the count of leukocytes in leukemic patients. The levels of potassium, and zinc were significantly reduced in leukemic patients, while the level of sodium was not changed significantly. In conclusion, chronic myeloid leukemia may associate with anemia, a reduction in the glucose-6-phosphate dehydrogenase, hypozincemia, and hypopotassemia. Glucose-6-phosphate dehydrogenase can be used as fair sensitive biomarker for leukemia prognosis.

2023, 50 pages

Keywords: Glucose-6-phosphate dehydrogenase, Leukemia, Potassium, Zinc, sodium, Iron, Hb, WBC, RBCs

ÖZET

LÖSEMİK HASTALARDA SERUM GLİKOZ-6-FOSFAT DEHİDROJENAZ (G6PD) SEVİYELERİ VE BAZI BİYOKİMYASAL PARAMETRELERİN İNCELENMESİ

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Bu çalışmada kronik miyeloid lösemi hastalığına eşlik eden bazı değişiklikler ele alınmıştır. Hastaların kan ve serumlarında hematolojik varyasyonlar (lökositler, eritrositler ve hemoglobin), glukoz-6-fosfat dehidrogenaz, ferritin ve bazı elementler (demir, sodyum, potasyum ve çinko) araştırıldı. Çalışmaya 130 denek, 65 lösemik hasta ve 65 sağlıklı kontrol alındı. Lösemili hastalar ve kontrol grubu arasında yaş farklılıkları anlamlı değildi. Lösemili hastalarda lökosit sayısı önemli ölçüde artarken eritrosit ve hemoglobin sayısı önemli ölçüde azaldı. Lösemili hastalarda serum glukoz-6-fosfat dehidrojenaz seviyesi önemli ölçüde azaldı. Öte yandan, lösemili hastalarda serum ferritin ve demir seviyeleri önemli ölçüde artmış ve lökosit sayısı ile korele olmuştur. Lösemili hastalarda potasyum ve çinko seviyeleri önemli ölçüde azalırken, sodyum seviyesi önemli ölçüde değişmedi. Sonuç olarak, kronik miyeloid lösemi anemi, glukoz-6-fosfat dehidrogenazda azalma, hipozinsemi ve hipopotasemi ile ilişkili olabilir. Glikoz-6-fosfat dehidrogenaz, lösemi prognozu için oldukça duyarlı bir biyobelirteç olarak kullanılabilir.

2023, 50 sayfa

Anahtar Kelimeler: Glikoz-6-fosfat dehidrogenaz, Lösemi, Potasyum, Çinko, sodyum, Demir, Hb, WBC, RBC'ler

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LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
µL	Microliter
dL	Deciliter
IU	International Unit
kg	Kilogram
L	Litre
m	meter
m^2	Meter square
mg	Milligram
min	Minute
mL	Milliliters
mmol	Milimole
ng	Nanogram
pg	Picogram
rpm	Revolution per minute

LIST OF ABBREVIATIONS

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
BTK	Bruton's tyrosine kinase
CLL	Chronic lymphocytic leukemia
CML	Chronic myelogenous leukemia
CNS	Central nervous system
Fe^{+2}	Iron
G6PD	Glucose-6-phosphate dehydrogenase
GO	Gemtuzumab ozogamicin
GPX	Glutathione peroxidase
GSH	Glutathione monomers
GSSG	Glutathione dimers
HF	Heterozygous females
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplantation
IS	International scale
NADPH	Nicotinamide adenine dinucleotide phosphate
PK	Pyruvate kinase
PPP	Pentose phosphate pathway
RBCs	Red blood cells
ROS	Reactive oxygen species
Ru5P	Ribulose 5-phosphate
SCT	Stem cell transplant
WBCs	White blood cells
Zn	Zinc

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1. INTRODUCTION

Leukemia, a potentially fatal disease that affects millions of people each year, is a cancer of the blood-forming tissues (Sheykhhasan *et al.* 2022). Generalized indications and symptoms of this deadly condition include prolonged fatigue, unexplained weight loss, recurring infections, nosebleeds, enlarged lymph nodes, night sweats, bone pain, and anemia. A general doctor may find it challenging to connect these symptoms to such a fatal illness in clinical practice and recognize an unusual occurrence. Further complicating the disease's early identification is the fact that symptoms do not appear until a specific number of leukemia cells have accumulated in the body (Mittal *et al.* 2022).

WBCs typically increase depending on bodily requirements, however in the case of leukemia, they are produced unnaturally and degenerate. Although they frequently have a dark purple hue that makes them easy to spot, the variety in shape and texture makes examination and subsequent processing quite difficult. Leukocytes are a class of cells that can differ substantially from one another. WBCs are difficult to recognize because of their size and form since they are surrounded by other blood cells including red blood cells and platelets (Sahlol *et al.* 2020).

With 500 million cases globally, glucose-6-phosphate dehydrogenase (G6PD) insufficiency is the most prevalent constitutional erythrocyte enzyme deficiency. The gene that codes for G6PD is mutated, which results in this deficiency. The WHO has discovered more than 200 variations and divided them into five groups based on G6PD activity. The majority of individuals with G6PD deficiency only experience leukemia upon exposure to triggering events, with the exception of class I, which is characterized by a leukemia (Luzzatto. *Et al.* 2020). Measuring the activity of erythrocytic enzymes is the key to diagnosing G6PD deficiency. Patients with leukemia or situations where an enzymatic assay is not appropriate might consider genetic analysis. We are unaware of any cases of acquired G6PD deficiency (Naville *et al.* 2022).

More than 300 distinct enzymes are made of zinc, which is essential for the functioning of cells and the metabolism of proteins, lipids, and carbohydrates. Zinc also plays a crucial role in maintaining the integrity of cell membranes (Jiang *et al.* 2022). Anemia, growth retardation, mental impairment, delayed sexual maturation, anorexia, and decreased immune and visual function can all result from a zinc shortage. Some tumors alter the zinc levels in the blood, and some leukemias may have higher zinc levels (Ghandour *et al.* 2013).

The absorption, storage, and efflux of iron inside cells are changed in leukemia, as is the ferroportin-hepcidin regulatory axis, which is similarly dysregulated. A systematic iron excess in leukemia patients is further exacerbated by repeated red blood cell transfusions given during chemotherapy. Even while iron is necessary for the catalytic generation of ROS necessary to maintain hematological homeostasis, iron buildup and the resulting increased oxidative stress are harmful to normal hematopoiesis (Ghandour *et al.* 2013).

This study's goal is to precisely gauge Glucose-6-phosphate dehydrogenase concentrations (G6PD). It will be explored whether there is any connection between these biochemical testes and leukemia patients.

2. LITERATURE REVIEW

2.1 Leukemia

One of the most prevalent malignant conditions afflicting the global population is leukemia (Bray *et al.* 2018). Leukemia has a global geographic distribution, with more industrialized nations having higher frequency and total mortality. However, the death rate is greater in underdeveloped nations. Leukemia incidence, in instance, has a specific pattern that is well-documented (Bray *et al.* 2018). The age-standardized in the United States in 2018, incidence rates for leukemia in females and males were 6.1 and 4.3 per 100,000, respectively. Similarly, the death rate for males was greater than that for females, coming in at 4.2 per 100,000 people as opposed to 2.8 per 100,000 (Bray *et al.* 2018).

One or more hematopoietic cell-line subgroups may be affected by acute leukemias, which are malignant clonal illnesses of the blood-forming organs. Due to the extensive replacement of the bone marrow by undifferentiated hematopoietic cells, aberrant, and immature, these diseases are characterized by decreased erythrocyte and platelet counts in the peripheral circulation. These diseases can be categorized into lymphoid, myeloid, mixed, and undifferentiated cells. On the other hand, division of differentiated, mature cells in the hematological system characterize a wide variety of disorders known as chronic leukemias.

2.2 Risk Factors of leukemias

2.2.1 Age and race

Age and race have a big impact on how frequently leukemias occur. For instance, 42.8% of leukemia cases in the United Kingdom are among those over 65 (Deschler and Lübbert 2008). For Asian/Pacific Islander groups, the incidence was 7.8 per 100,000, while for American Indian/Alaskan Native people, it was 8.3 per 100,000 (Bispo *et al.*

2020). Similar racial and ethnic trends in death rates per 100,000 individuals were discovered (Bispo *et al.* 2020).

2.2.2 Genetics

Leukemia's genesis is unquestionably heavily influenced by genetics. The relationship between their transition to acute leukemias and genetic variables in normal hemopoiesis, as well as their role in leukemogenesis, is the subject of a wide amount of recent research; nevertheless, it is outside the purview of this broad study to provide a synopsis of this. Identical twins are the ones who exhibit genetic impacts in acute leukemia etiology the greatest. The likelihood of the second identical twin contracting the illness is twice as high as it is for the general population if one twin gets the illness before the age of seven. In time, there is a decreased risk of leukemia. The risk of leukemia in the twin who survives to the age of 15 does not appear to be higher than that of the general population (Aquino 2002).

2.2.3 Environment and occupations

There are many different environmental elements that might contribute to leukemia development. Most of these include a lifetime of exposure to cancer-causing agents, such as radiation, viruses, chemicals (Kleinerman 2006). Leukemia risk has been linked to pastimes, specific exposures, jobs, workplace dangers (Finch 2007). Depending on the sort of industry, there is a documented variance in the incidence of leukemia. It is uncertain and occasionally debatable how particular jobs and the prevalence of acute leukemias are related. Crop production, agricultural and forestry employment, and other occupations have all been linked to an elevated risk for leukemias (Blair *et al.* 2001) construction, animal slaughter and poultry operations, jobs in the oil and gas sectors, and occupations that expose workers to benzene (Blair *et al.* 2001), petrochemicals and oil refining (Wong *et al.* 2010), careers in the electrical utility industry, auto mechanic work, jobs that expose workers to magnetic fields, nuclear power industry jobs that expose workers to ionizing radiation, furniture manufacturing and repair jobs, and nursing and healthcare jobs that expose workers to infectious diseases and viruses.

Hairdressing and hair colouring, painting, laundry work, teaching, those working in the shoe and boot manufacturing business, as well as taxi, bus, truck, and train conductors, are other professions associated with an elevated risk of leukemia. occupations that expose employees to formaldehyde and alkylating chemicals, textile producers and workers, as well as semiconductor workers (Kim *et al.* 2011) are discovered to be at an increased risk for leukemia.

2.2.4 Effects of radiation

Leukemia development has been associated with exposure to ionizing radiation at several points in life, including preconception, in utero, and postnatal exposures. Leukemia incidence and radiation exposure have been found to be related, according to research (Freedman *et al.* 2001). Leukemia cases among survivors who were within 1000 meters of the bombings after Hiroshima and Nagasaki, Japan, were 20 times more common than in the general population.

2.3 Prior Immunosuppressive and Chemotherapy

There is a higher risk of leukemia among those who have used chemotherapy for cancer treatment, whether or not they also underwent radiation. Acute leukemias can also become more likely to develop after using a range of immunosuppressive treatments. Alkylating drugs, platinum derivatives, and topoisomerase II inhibitors are a few chemotherapy medications linked to an increased risk of this illness (Tebbi *et al.* 2007).

2.4 Treatment/Management of Leukemias

Hematologist-oncologists should be consulted to begin treating leukemia patients. Based on the patient's characteristics and subtype of leukemia, treatment differs greatly. Treatment for acute leukemias typically involves hospitalization, necessitating intensive care, regular vitals checks, evaluation for opportunistic infections, and monitoring for electrolyte imbalances. Finding the potential of APL, which has a therapy that is very

different from that of the rest of AML, is the main obstacle at the time of diagnosis of acute the myeloid leukemia.

APL: Patients with APL frequently have bleeding diathesis, high PT and aPTT, and low fibrinogen levels. Myeloid blasts with Auer rods are predominant in the peripheral smear. When APL is suspected, it is crucial to begin therapy with ATRA (all trans retinoic acid) rather than waiting for FISH confirmation. ATRA causes mature granulocytes to develop from stalled promyeloblasts, which can cause differentiation syndrome (Mantha *et al.* 2017). Differentiation syndrome might appear 48 hours after the commencement of ATRA or even three weeks after the start of APL treatment. Delaying the administration of dexamethasone might cause it to mimic sepsis. 10 mg every 12 hours is the standard initial dose until symptoms improve and counts (Montesinos *et al.* 2009). Another notable side effect of ATRA is elevated intracranial pressure, which can cause headaches and substantial abnormalities in vision due to papilledema.

Whether a patient is considered to be at low, moderate, or high risk for developing APL depends on whether their WBC count is below 10,000/mcL or above 10,000/mcL. Platelets above or below 40,000/mcL further distinguish low- or intermediate-risk APL.

- Standard-risk APL: Without the necessity for an allogeneic stem cell transplant, patients react effectively to ATO and ATRA, with fewer problems during induction and recovery (SCT). Patients must be constantly watched for electrolyte abnormalities while receiving ATO, as well as for changes in QTc prolongation on the ECG (Framingham formula).

- High-risk APL: Idarubicin is added to ATRA + ATO in high-risk patients to improve their outcomes. Recent trials have paired ATRA + ATO with the CD33-targeted drug combination (GO) as part of the induction treatment (Abaza *et al.* 2017).

Patients with APL have better overall survival and prognosis without a transplant than those with other forms of AML.

AML: The "7+3" regimen, which consists of a 3-day anthracycline course followed by a 7-day course of continuous cytarabine infusion, is well known as the standard treatment for AML (either daunorubicin or idarubicin). Molecular markers are increasingly being used to risk-stratify patients, which has consequences for prognosis and treatment, thanks to the development of cytogenetics and NGS testing.

ALL: Based on the origin of the lymphoblasts and the presence of more than 20% lymphoblasts in the peripheral smear or BM, ALL is classified as either B or T lymphocyte variations. The most significant molecular marker with therapeutic significance in treating ALL is the presence or absence of the Ph chromosome.

The patient's response to induction treatment and existence or absence of the (MRD) necessitating further BMT and therapies determine the ultimate result.

CML: One of the first malignancies to be completely transformed by targeted treatment and TKIs that target the Ph chromosome was CML. Except in cases of accelerated phase/blast crisis, patients had a considerable response to TKIs, removing the necessity for rapid chemotherapy. A patient's risk can be calculated using a variety of calculators, including EUTOS score, the Sokal score, and EUTOS long-term survival score (ELTS) (Pfirrmann *et al.* 2016). Second-generation TKIs (nilotinib, bosutinib, dasatinib) are used as first-line therapy for patients with high-risk diseases in order to reach the therapeutic milestones more quickly and with deeper responses (Yun *et al.* 2016). Imatinib can be started as the first-line treatment for individuals with low and moderate risk. However, regardless of the TKI generation employed, there is no appreciable difference in the overall survival.

Patients must be kept under observation for resistance mutations, particularly the T315I mutation, which is treatable with the drugs ponatinib, asciminib, and omacetaxine

(Cortes *et al.* 2018). Patients who can undergo SCT may nonetheless acquire resistance to numerous TKIs.

CLL: Compared to the other leukemia subtypes, CLL progresses more slowly, having little to no effect on the patient's longevity. Patients who do not satisfy the requirements for therapy do not benefit from early treatment. Patients who have substantial B symptoms, a quick lymphocyte doubling time, deteriorating cytopenias, growing spleen size causing abdominal discomfort, and these conditions benefit from therapy. Knowing the status of the IGVH mutation, as well as whether or not del17p and TP53 mutations are present, is crucial to the therapy of CLL. It's common to acquire (11:14) to rule out mantle cell lymphoma.

It is possible to try treatment with FCR or BR for individuals with IGVH mutations who have a reasonably favorable prognosis since they can achieve extended disease-free survival for more than 10 years. Targeted treatment with venetoclax or in combination with rituximab or obinutuzumab or (BTK) inhibitors, either as a single drug, is highly beneficial for high-risk patients with the del17p/TP53 mutation (Sharman et al. 2020). BTK inhibitors are more tolerable in older people with concomitant conditions.

Acute aggressive lymphadenopathy in CLL/SLL patients with a latent course is uncommon. To exclude out Richter transition into aggressive the diffuse large B cell lymphoma and, less frequently, T cell lymphomas or Hodgkin lymphoma, they require an immediate biopsy of bone marrow or the lymph node (Howard *et al.* 2016).

2.5 Glucose-6-Phosphate Dehydrogenase (G6PD)

2.5.1 Gene structure of glucose-6-phosphate dehydrogenase

Prokaryotes and mammals both have the X-linked cytosolic enzyme known as the G6PD (EC 1.1.1.49). The gene encoding G6PD is part of a collection of genes that also includes fragile X, congenital dyskeratosis, hemophilia A, and the telomeric region of

the distal arm of the X chromosome, which is widely known as a hot spot (band Xq28) (Allahverdiyev *et al.* 2012).

2.5.2 Epidemiology G6PD deficiency

With about 400 million cases globally, G6PD deficiency has been identified as the most prevalent enzymopathy. G6PD insufficiency manifests more frequently in men than in females and is most prevalent in Africa, Asia, the Mediterranean region, and the Middle East. With a frequency of about 10% in the United States, this deficit mostly affects men with ethnic backgrounds from Africa and the Mediterranean. G6PD deficiency may provide some partial protection against this infection because of an intriguing correlation between its prevalence and the geographic spread of malaria (Nikhoma *et al.* 2009).

2.5.3 Biochemical aspects of the G6PD

The tetramer or dimer forms of the G6PD enzyme are active in (RBCs). A complicated interface of -sheets divides the two subunits of the enzyme into dimer structures, and for structural stability, each subunit binds to a NADP+ molecule. This enzyme, which degrades glucose and promotes the conversion of d-glucono-1,5-lactone-6-phosphate from -d-glucose-6-phosphate, is the rate-limiting enzyme of the pentose phosphate pathway (PPP). It also generates a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) as a byproduct in the oxidative phase (Howes *et al.* 2013). The enzyme 6-phosphogluconate dehydrogenase (6PGD) decarboxylates d-glucono-1,5-lactone-6-phosphate to produce 6-phosphogluconate, which is subsequently hydrolyzed to produce the five-carbon molecule (Ru5P), a precursor to ATP, DNA, and RNA, as well as another NADPH molecule. It is important to highlight that, in contrast to other cell types, RBCs lack mitochondria, making the PPP route the only source of NADPH, which is essential for shielding cells from oxidative damage caused by reactive oxygen species (ROS). NADPH is implicated in at least three antioxidant processes, including glutaredoxin cycles, thioredoxin, the glutathione, due to its crucial role in scavenging cellular ROS. The glutathione reductase enzyme catalyzes the first route, which results

in two reduced the glutathione monomers, the line firstly of defense against ROS, by transferring the electron from NADPH to glutathione dimers (GSSG). Additionally, glutathione peroxidase (GPX) uses GSH as its substrate to remove peroxide from RBCs, whereas NADPH is needed to decrease oxidized GSSG and the sulfhydryl groups of the several essential proteins for the body's defense against oxidative stress. Hemolysis results for RBCs that are unable to recover from this stress (McNicholas *et al.* 2011).

2.5.4 The role of G6PD in development and cell growth

For sustaining growth and development, G6PD is the prototypical housekeeping enzyme. Normal cell proliferation, as well as embryonic and organismal development, are all prevented by reduced G6PD activity or a broken PPP. Tumorigenesis is linked to abnormal activation of the PPP or G6PD (Kathagen-Buhmann *et al.* 2016). In order to meet the biological requirements for nucleic acid synthesis, NADPH generation and fatty acid, rapidly expanding cancer cells have developed a variety of ways to activate G6PD. For instance, G6PD activity is increased when pro-oncogenic pathways like Ras, Src, and PI3K/Akt are activated. By deacetylating G6PD, the SIRT2 gene-encoded NAD-dependent deacetylase Sirtuin 2 enhances NADPH synthesis and leukemia cell proliferation. G6PD is deacetylated and activated as a result of heat shock protein 27's (HSP27; also known as HSPB1) enhancement of the interaction between G6PD and SIRT2. In glioma cells, HSPB1 triggers G6PD via SIRT2 to maintain cellular NADPH and pentose synthesis. IDH2 and G6PD are desuccinylated and deglutarylated by SIRT5, which activates both NADPH-generating enzymes and gives tolerance to oxidative stress (Zhou *et al.* 2016).

This study discusses the signaling pathways that regulate cancer cell survival in relation to the G6PD state, including p21-activated kinases (PAK), AMP-activated protein kinase (AMPK), Wnt/β-catenin, STAT.

2.5.5 Structure of G6PD protein

A single active site is present in each subunit of the dimer G6PD. The pH-dependent dimer $ tetramer equilibrium is where the active human enzyme may be found. The structural NADP molecule binding site, which is important for human enzyme function alone as well as for long-term G6PD stability under physiological settings, is absent from the bacterial enzyme. Asp421—one of the NADP1 ligands—is in the core of the dimer interface and each NADP1 is completely bonded within one subunit. The alignment revealed two sequence motifs that are entirely conserved: the dinucleotide-binding fingerprint GxxGDLx and the eight-residue peptide RIDHYLGK, which corresponds to the substrate-binding site (residues 38–44). The coenzyme-binding domain, which has the fingerprint GASGDLA and is made up of residues 31 to 200, is the last component (Wang *et al.* 2008).

2.6 Favism

The most frequent clinically important enzymopathy in humans is G6PD Deficiency. This disorder, which affects more than 400 million individuals globally, may lead to favism, drug-induced acute hemolytic anemia, severe chronic nonspherocytic hemolytic anemia, newborn jaundice, and hemolytic anemia linked to viral or microbial infections, among other symptoms. The locations with the greatest rates of G6PD deficiency include mostly those in the Middle East, tropical Africa, Papua New Guinea, tropical and subtropical Asia, and different Mediterranean areas, such as Sardinia island. Due to the afflicted individuals' higher resistance to severe Plasmodium falciparum infection in certain places, G6PD deficiency may represent a selection advantage (Cappellini and Fiorelli 2008).

All favism patients are G6PD deficient, yet many G6PD deficient people can consume fava beans on a daily basis while displaying a wide range of clinical symptoms, often at preclinical and/or undetectable levels. These findings imply that a number of variables, including age, lifestyle, health state, and, lastly, the cumulative intake of fava beans, influence the clinical phenotype. After consuming fresh beans or, less frequently, after

ingesting dry or frozen beans, hemolytic crises can also happen. Even though the whole processes by which these effects are determined have not yet been fully established, it has been hypothesized that the glycosides divicine and isouramil are the parts of the bean that are responsible for the hemolysis. Because of this, hemolysis is caused by a situation where an enzyme deficit is a required but not sufficient component. Determining G6PD Deficiency Enzymatically G6PD deficiency is diagnosed using universal testing, mostly based on the production of NADPH from NADP, in accordance with WHO standards (Rigattieri *et al.* 2008).

1. The following are of semiquantitative assays: (A) the quick, easy, affordable, sensitive fluorescent spot test. Before beginning therapy with antimalarial medications like primaquine, in nations where malaria is widespread and G6PD deficiency is frequent, this test can be used. The huge population screening in tropical places is made possible by a spot test version that uses just the naked eye rather than a UV light; It is possible to do screening tests other than (b) by, for example, directly detecting the G6PD activity in individual RBCs with a _75% accuracy or indirectly determining the NADPH concentration by measuring the decreased methemoglobin levels created after NADPH oxidation. (c) Finally, to identify G6PD-deficient from normal persons, the Heinz body examination and GSH stability test may be used (Tantular and Kawamoto 2003).

2. A quantitative measurement of G6PD activity is necessary for the biochemical conclusive diagnosis. The rate of NADPH production is then spectrophotometrically assessed at wavelength of 340 nm to determine the quantitative measurement of the G6PD activity. The assay mixture must first comprise the substrate (glucose-6-phosphate) and its cofactor NADP. Finally, the G6PD activity is quantified as the ratios of RBCs from G6PD IU and hemoglobin from G6PD IU. When evaluated at 30 8C in normal RBCs, the G6PD activity ranges from 7 to 10 IU/g Hb. When G6PD activity is assessed after or during an incident of acute hemolysis or in the presence of a high blood reticulocyte count, the activity of the reticulocytes is around five times higher than that of old RBCs, which might lead to a false negative result in the diagnostic process. The activity of other enzymes and G6PD, such the Pyruvate Kinase, steadily

declines with RBC aging because protein synthesis does not occur in RBCs, which will result in their selected destruction. People with the G6PD A2 variation may have their activity wrongly classified as normal if the test is performed soon after a posthemolytic episode, for example (Rigattieri *et al.* 2008).

Given that newborns have a young RBC population, further inconsistencies in the evaluation of neonatal screening may arise. Additionally, the X-inactivation phenomena needs to be carefully considered. Males must first be either normal or G6PD-deficient since they are hemizygotes. Females, on the other hand, can either be normal, intermediate (heterozygous) or defective (homozygous). Female heterozygotes (HF) with severely skewed X inactivation exhibit hemizygote to normal levels of activity. The occurrence of genetic mosaics has the most therapeutic impact on women in reality. As a result, heterozygote may exhibit varying degrees of biochemical and clinical manifestations of G6PD deficiency. Quantitative and fluorescence tests should both be within normal ranges during an acute hemolytic event in carrier females since they only monitor the activity of the usual surviving RBC population. If an acute intravascular hemolytic G6PD-dependent anemia is found, all potentially dangerous medicines must be stopped and the test must be repeated 10-15 days later, or later if the patient has had blood transfusions. When available, genetic analysis or family history can help with the diagnosis in certain situations. To remove interferences caused by white blood cell G6PD activity, especially when leukocytosis is present, the G6PD activity assay must be conducted on the deleucocyteized blood (Wang *et al.* 2008).

Although K2-EDTA is the best anticoagulant for G6PD stability and assures adequate blood cell preservation, other anticoagulants such as ACD, sodium citrate, CPD, and lithium heparin may also be utilized. All samples should be shipped in heat-insulated bags and refrigerated transport containers to avoid a drop in G6PD activity. G6PD activity must be measured in the blood collected in tubes containing fluoride oxalate anticoagulants or ammonium oxalate within 12 hours, maintaining the sample at 4 8C until utilized. A full diagnostic assessment of the G6PD insufficiency rate also requires consideration of the following laboratory variables: RBC and reticulocyte counts, indirect and total plasma bilirubin, lactate dehydrogenase levels and plasma iron, ferritin

concentrations and serum aptoglobin, and lastly, urine hemoglobin concentration. In order to rule out the concurrent occurrence of Gilbert Syndrome when indirect bilirubin levels are greater, the UGTA-1 gene promoter research should be taken into consideration. Additionally, (PK) activity assay could offer more direction. Another crucial tool for improved laboratory practice may be the appropriate reference interval selection, which should take into account each population's characteristics such as gender, age, and other factors (Castro *et al.* 2005).

2.7 Other Related Parameters

2.7.1 Sodium

Numerous hematological illnesses, both benign and malignant, can cause hyponatremia (Li *et al.* 2020). Furthermore, there was a strong correlation between hyponatremia and neurologic problems as well as the presence of central nervous system leukemia at diagnosis. Following hematopoietic stem cell transplantation, 40% of the 140 pediatric patients in another single center investigation with 140 patients experienced the hyponatremia (HSCT). According to a large retrospective cohort study of people with certain cancer types, patients with lymphoma had an incidence rate of hypervolemic hyponatremia and euvolemic of 395 per 1000 person-years (Castillo *et al.* 2016). It is noteworthy that a recent experimental investigation showed that oxidative stress, proliferation, and invasion-related molecular pathways are upregulated when extracellular sodium content is low, which may promote carcinogenesis in vitro. Hyponatremia in hematologic patients is crucial to identify, assess, and treat because it is a standalone predictor of poor outcomes in patients with both benign disorders and neoplastic, such as lymphomas, allogeneic hematopoietic stem cell transplants (SCT) (AlloSCT), hemolytic uremic syndrome, sickle cell anemia. Furthermore, signs of hyponatremia including weariness, confusion, or even falls may be mistakenly attributed, in the context of the underlying hematological disease, to other disorders including neutropenic sepsis or (CNS) involvement. In the context of the range of hematological illnesses, this review examines the pathophysiological, clinical, and

clinical features of hyponatremia. Also provided is the appropriate therapy for hyponatremia (Wei *et al.* 2010).

2.7.2 Pottasium

False hyperkalemia manifests as an increased potassium level without the clinical symptoms that are often present in this condition, such as muscular weakness, paralysis, arrhythmia, and irregular ECG (Garwicz and Karlman 2012). Falsely elevated blood potassium levels are known as pseudohyperkalemia, and they frequently develop in hematologic illness patients, particularly in those with severe leukocytosis and thrombocytosis. In these individuals, measuring heparin-anticoagulated plasma prevents this artifact. In reverse pseudohyperkalemia, the serum potassium level isn't actually raised; instead, the plasma potassium level is (Avelar 2014). Heparin in the plasma collecting tube may harm cell membranes when processing samples using delicate leukemic cells. Heparin may alter the permeability of the leukocyte cell membrane, leading to lysis through the activation of phospholipases, albeit the precise process is not entirely known. Potassium may "leak" from intact cells as a result of mechanical stresses, such as the usage of pneumatic transport systems, presumably through mechanosensitive potassium channels (Asirvatham *et al.* 2013). Most cases of reverse pseudohyperkalemia have been observed in people with mantle cell lymphoma and chronic lymphocytic leukemia (Garwicz and Karlman 2012).

2.7.3 Zinc

Zn plays a clear-cut role in the prevention of illness. However, nothing is known about how it contributes to carcinogenesis. A suitable DDR is particularly important in the setting of the hematopoietic system because of the prolonged life span of (HSC) (Ho 2004). The DDR prevents the buildup of DNA damage, which might change the ability of these stem cells to self-renew and their capacity for regeneration, resulting in functional decline and faulty hematopoiesis (Ho 2004). Acute myeloid leukemia (AML) DDR changes have recently been discovered, and they are connected to leukemogenesis (Nilles and Fahrenkrog 2017). Additionally, a noticeable drop in serum Zn levels is a

typical finding in leukemia patients (Zhu *et al.* 2017). The molecular implications of this discovery in leukemogenesis and its connection to DDR in AML cells are unknown despite the fact that it is frequently observed. In this context, our goal was to comprehend how Zn affected both normal and AML lymphocytes' DDR modulation. The effects of Zn and DDR changes in AML should be given greater consideration in order to comprehend the condition and enhance therapy methods.

3. MATERIALS AND METHODS

3.1 Design and Specimens Collection in This Study

The study included 65 cases of leukemia patients and 65 healthy subjects. Cases were collected from different regions of Baghdad Governorate, Iraq. Evidence was gathered from April 12th through August 23rd.

We had two groups in the study:

- Grup A: 65 cases of leukemia patients.
- Grup B: 65 healthy subjects.

After obtaining the consent of the patient and through the clinical results conducted by the patient and confirming that he had leukemia, 6 mL of venous blood was withdrawn and divided into two parts, the first 5 mL was placed in gel tubes and the second 1 mL was placed in EDTA tubes.

Gel tubes were isolated and placed in a centrifuge at 4700 speed for 7 minutes. After that, the serum was withdrawn and placed in 4 tubes of 500 microml Ependorf to measure the levels of iron, G6PD, ferritin, zinc, sodium and potassium. The samples were then stored in a refrigerator at -24 °C.

As for the EDTA tubes, the tube was shaken for a minute to measure the levels of WBC, RBC, and Hb.

3.2 Exclusion Criteria in a Group of Healthy Subjects

- Cases suffering from chronic diseases such as high blood pressure, atherosclerosis, or diabetes were excluded from the healthy group.

- ❖ Cases who suffer from obesity or renal failure were excluded from the healthy group.

- ❖ Cases of those who had a course of treatment or used one of the treatments for chronic diseases were excluded.

3.3 Materials

3.3.1 Tools in this study

Table 3.1 details the assortment of research instruments used in this investigation.

Table 3.1 Materials used for this research

NO.	TOOLS	COMPANY	ORIGIN
1	Micropipette (100-1000 µL)	Eppendorf	Germany
2	Fincubator (heating block)	Finecare	Balgium
3	Eppendorf Tube (500 µL)	Eppendorf	Germany
4	Incubator	Human	Germany
5	Micropipette (10-100 µL)	Eppendorf	Germany
6	EDTA tube	Afco	Jordan
7	Gel Tube	Afco	Jordan
8	Timer	Wondfo	China
9	Plain tube	Afco	Jordan
10	Vortex	VELP	Germany
11	Multichannelpipette(50-300 µL)	Eppendorf	Germany
12	Different Glasses	-	China

3.3.2 Instrument used

Table 3.2 details the assortment of research implements that were used.

Table 3.2 List of instrument

NO.	INSTRUMENT	MODEL	COUNTRAY
1	Water bath	Lab Tech	Korea
2	Deep freeze	Hettich	Japan
3	Spincell/Haematology	Spincell 3	Spain
4	Spectrophotometer	PD-303 APEL	Japan
5	Water bath	Memmert	Germany
6	ELISA reader	Human	Germany
7	Spinreact/Chemistry	Automatic biochemistry analyzer SPIN120	Spain
8	Electronic balance	AIK Instruments Ltd	China
9	Refrigerator	Kelon	Korea
10	ELISA washer	Human	Germany
11	Centrifuge	Beckman model TJ-6	Germany

3.3.3 Kits and reagents used

Kits utilized in this investigation are listed in Table 3.3.

Table 3.3 Kits and reagents list for this experiment

No.	Kits and Reagents	Company	REF
1	Human Glucose-6-Phosphate Dehydrogenase ELISA Kit	ELISA	MBS2515491
2	Determination of Zinc	SPINREACT	1001350
3	Determination of Urea	SPINREACT	1001331
4	Sodium Reagent Kit	Bio Research	CE006
5	Determination of Creatinine	SPINREACT	MD1001111
6	Potassium Reagent Kit	Bio Research	CE005
7	Determination of Iron	Linear Chemicals	1135005
8	Determination of Ferritin	Linear Chemicals	3140010
9	Determination of CBC	SPINCELL	-

3.3.4 Human glucose-6-phosphate dehydrogenase ELISA kit

Principle of the Assay: In this ELISA kit, the Sandwich-ELISA Principle is utilized. The micro ELISA plate that comes with the kit has already been coated with an antibody specific to human G6PD. Micro ELISA plates have wells for containing standards, samples, and the appropriate antibody. Each microplate well is subsequently treated with a biotinylated detection antibody specific for human G6PD and an Avidin-Horseradish Peroxidase (HRP) combination. During washing, loose pieces are eliminated. Applying the substrate solution to each well. Only the wells that contain Human G6PD, biotinylated detection antibody, and Avidin-HRP conjugate will show any coloring. When the enzyme-substrate reaction is stopped by adding the stop solution, the hue changes to a bright yellow. Spectrophotometric measurement of the optical density (OD) is performed at a wavelength of 450 nm 2 nm. The OD value is negatively associated with increasing human G6PD levels. The concentration of human G6PD in samples may be calculated by comparing their optical densities to those on the standard curve.

Calculate the average optical density of each standard and sample by subtracting the average of the zero standard's duplicate values from those readings. OD values should be plotted on the y-axis of a piece of log-log graph paper, and standard concentration should be plotted on the x-axis, to create a four-parameter logistic curve. If the samples were diluted, it is necessary to multiply the concentration calculated from the standard curve by the dilution factor. You should retest the sample with the appropriate dilution if the OD exceeds the top limit of the standard curve. To get the real concentration, multiply the calculated concentration by the dilution factor (Figure 3.1).

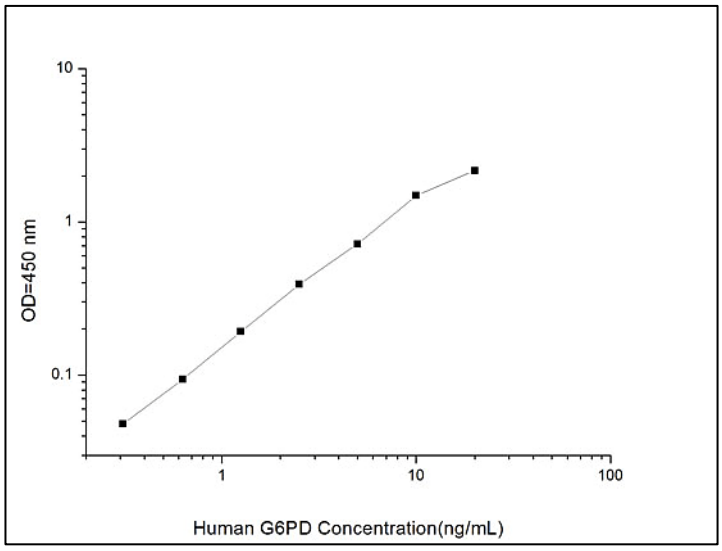

Figure 3.1 Standard curve of G6PD

3.3.5 Determination of zinc

Principle of The Method: Direct colorimetric analysis without sample deproteinization A persistent colored complex is created when zinc combines with the particular complexant 5-Br-PAPS at pH in a buffered medium. The amount of zinc in the sample has a direct relationship with the color intensity.

Clinical Significance: Around the world, nutritional zinc insufficiency in people is rather common. It is characterized by immune system impairment, moderate dermatitis, low appetite, delayed wound healing, incorrect dark adaption, growth retardation in children and adolescents, and hypogonadism in males. Clinical diagnosis should incorporate both clinical and other laboratory evidence; it shouldn't be based just on one test result.

Calculations (Equation 3.1):

$$\frac{(A2\text{-}A1)\text{Sample}\text{-}(A2\text{-}A1)\text{Blank}}{(A2\text{-}A1)\text{Standerd}\text{-}(A2\text{-}A1)\text{Blank}} \text{x200(Standerd conc.)}=\mu\text{g/dL} \quad (3.1)$$

3.3.6 Determination of potassium

Principle of The Method: A colloidal suspension is created when potassium combines with sodium tetraphenol boron in a carefully prepared buffer. The quantity of turbidity created is directly inversely related to the potassium content of the sample.

Clinical Significance: An electrolyte that is essential to cell metabolism is potassium. It facilitates the movement of nutrients into cells and the removal of waste from cells. It aids in the communication of signals between neurons and muscles, which is essential for proper muscular function. Hyperkalemia, or elevated potassium levels, are frequently linked to renal failure, dehydration shock, or adrenal insufficiency. This test analyzes the level of potassium in the blood. Low potassium levels, or hypokalemia, are linked to malnutrition, a negative nitrogen balance, fluid loss from the gastrointestinal tract, and hyperactivity of the adrenal cortex.

Normal Range: Serum/Plasma: 3.5 - 5.5 mEq/L.

Calculations (Equation 3.2):

$$\text{Conc. of Potassium (mEq/L)} \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standerd}} x5(\text{Standerd conc.}) \quad (3.2)$$

3.3.7 Determination of sodium

Clinical Significance: Extracellular fluid's predominant cation is sodium. The regular distribution of water and the osmotic pressure in the different fluid compartments are maintained by it in a crucial way. Foods that contain sodium chloride are the major source of body sodium. Because extracellular bodily fluids make up the majority of the sodium in the body, only around one-third of it is found in the skeleton. Severe polyuria, metabolic acidosis, Addison's disease, diarrhea, and renal tubular disease are just a few disorders that can result in hyponatremia (low blood sodium levels). Hypernatremia (raised blood sodium level) is seen in the following conditions:

hyperadrenalism, severe dehydration, diabetic coma following insulin therapy, and excessive sodium salt use.

Principle: The process is based on the interaction between sodium and a specific chromogen, which results in a chromophore whose absorbance is exactly proportional to the sodium content in the sample.

Normal Range: Serum/Plasma: 135 - 155 mEq/L.

Calculation (Equation3.3):

$$\text{Conc. of Sodium (mEq/L)} \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standerd}} x150(\text{Standerd conc.}) \quad (3.3)$$

3.4 Analyzed Statistically

The experimental data was evaluated statistically using SPSS version 26.0, and a t-test was performed to compare the means of the groups. Excel is used for the shape design process. The Pearson test was used to assess the correlation, while receiver operating characteristics were used to analyze the diagnostic tests' sensitivities (ROC).

4. RESULTS

4.1 Age

The age of the participants (patients and control) was no significant (P>0.05), as shown in Table 4.1 and Figure 4.1, change of age between leukemic patients (54.22±10.53 year) and healthy people (52.25±10.99 year).

Table 4.1 The age (year) comparison between leukemic and non-leukemic participants

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	52.25	54.22	0.299
SD	10.99	10.53	
SE	1.36	1.31	

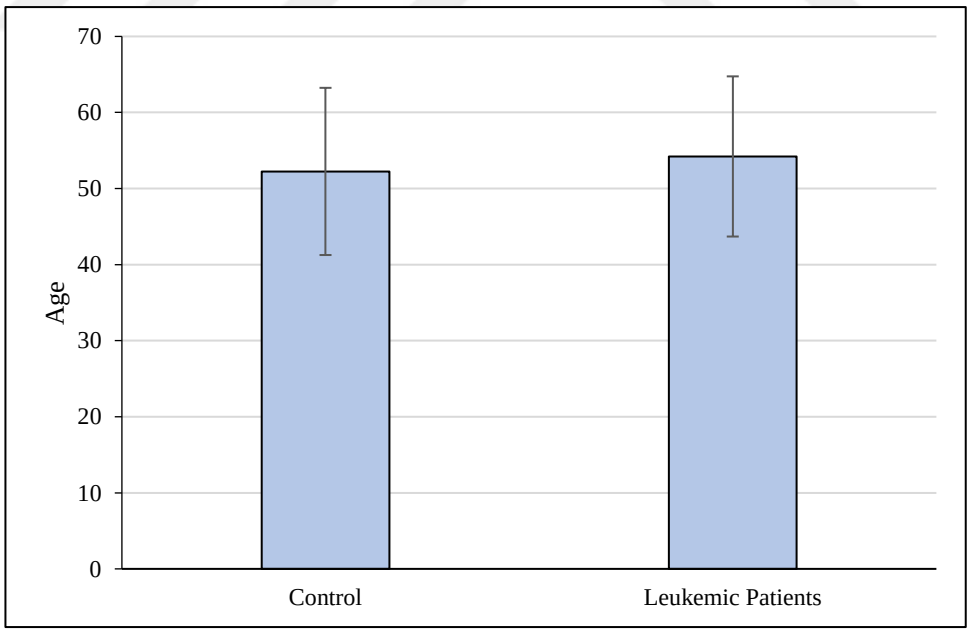

Figure 4.1 Leukemia patients and healthy controls were compared according to age

4.2 Leukocytes

The white blood cells (WBCs) count of the participants is shown in Table 4.2 and Figure 4.2. There was significant (P<0.05) increase in the count of WBCs for leukemic patients (9.26±2.72 $\times 10^3$ cell/µL) compared to healthy people (5.43±0.54 $\times 10^3$ cell/µL).

Table 4.2 The WBC (cell/µL) comparison between leukemic and non-leukemic participants ($\times 10^3$)

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	5.43	9.26	<0.001
SD	0.54	2.72	
SE	0.07	0.34	

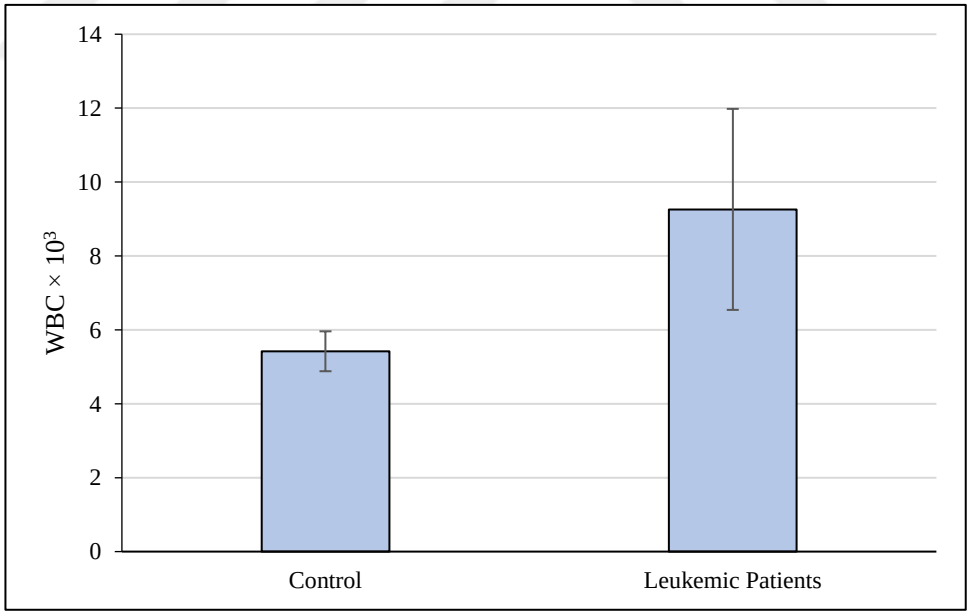

Figure 4.2 The comparison of WBC count between leukemic patients and control

4.3 Erythrocytes

The red blood cells (RBCs) count of the participants was significant (P<0.05), as shown in Table 4.3 and Figure 4.3. There was decrease in the count of RBCs for leukemic patients (4.38±0.56 $\times 10^6$ cell/µL) compared to healthy people (4.62±0.52 $\times 10^6$ cell/µL).

Table 4.3 The RBC (cell/µL) comparison between leukemic and non-leukemic participants ($\times 10^6$)

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	4.62	4.38	0.013
SD	0.52	0.56	
SE	0.06	0.07	

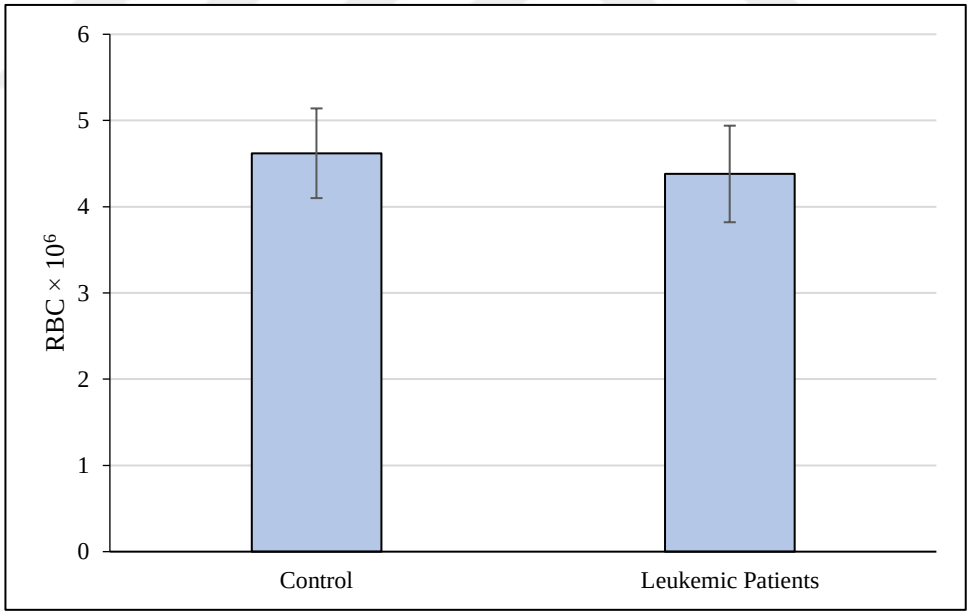

Figure 4.3 The comparison of RBC count between leukemic patients and control

4.4 Hemoglobin

The hemoglobin (Hb) level of the participants was significant (P<0.05) as shown in Table 4.4 and Figure 4.4. There was decrease in the levels of Hb for leukemic patients (12.52±1.76 g/dL) compared to healthy people (13.92±0.93 g/dL).

Table 4.4 The Hb (g/dL) level comparison between leukemic and non-leukemic participants

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	13.92	12.52	<0.001
SD	0.93	1.76	
SE	0.12	0.22	

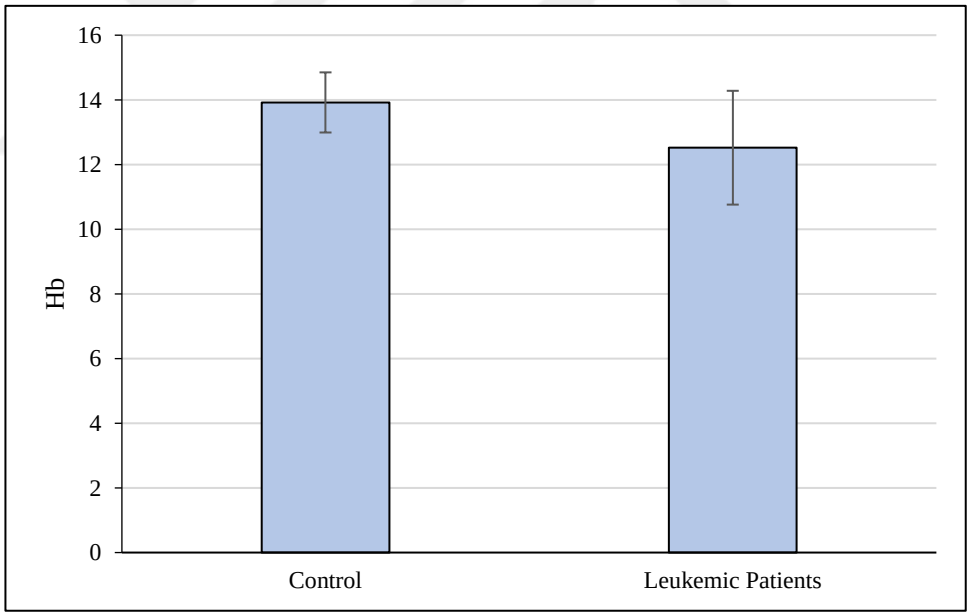

Figure 4.4 The comparison of Hb level between leukemic patients and control

4.5 Glucose-6-Phosphate Dehydrogenase

The glucose-6-phosphate dehydrogenase (G6PD) level of the participants is shown in Table 4.5 and Figure 4.5. Decrease in the level of G6PD for leukemic patients

(1.84±1.39 ng/mL) compared to healthy people (3.43±1.50 ng/mL), there was significant (P<0.05).

Table 4.5 The G6PD (ng/mL) level comparison between leukemic and non-leukemic participants

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	3.43	1.84	<0.001
SD	1.50	1.39	
SE	0.19	0.17	

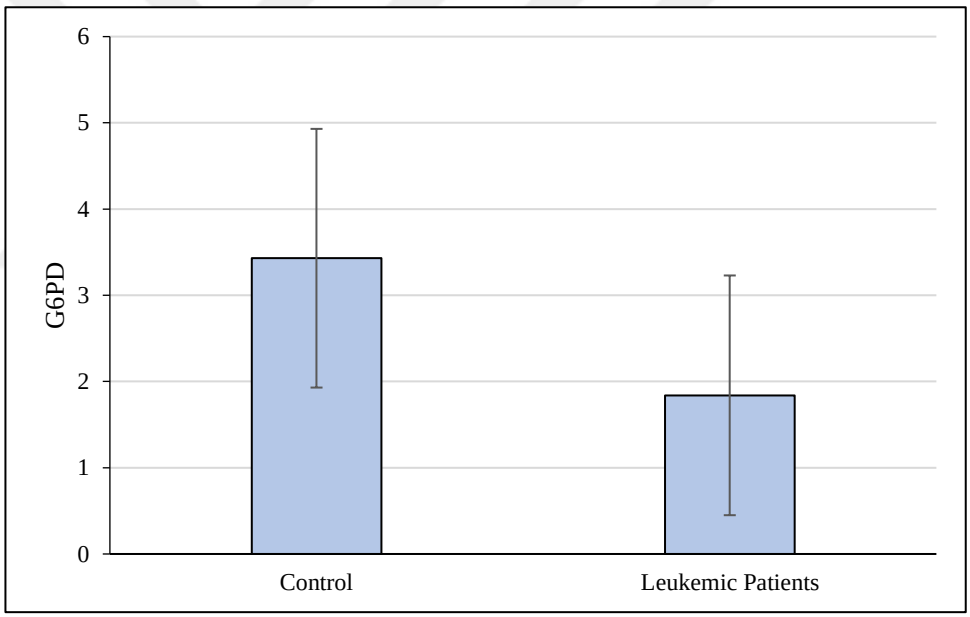

Figure 4.5 The comparison of G6PD level between leukemic patients and control

4.6 Ferritin

The ferritin level of the participants is shown in Table 4.6 and Figure 4.6. Increase in the levels of ferritin for leukemic patients there was significant (P<0.05) (225.23±201.40 ng/mL) compared to healthy people (97.63±64.44 ng/mL).

Table 4.6 The ferritin (ng/mL) level comparison between leukemic and non-leukemic participants

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	97.63	225.23	
SD	64.44	201.40	<0.001
SE	7.99	24.98	

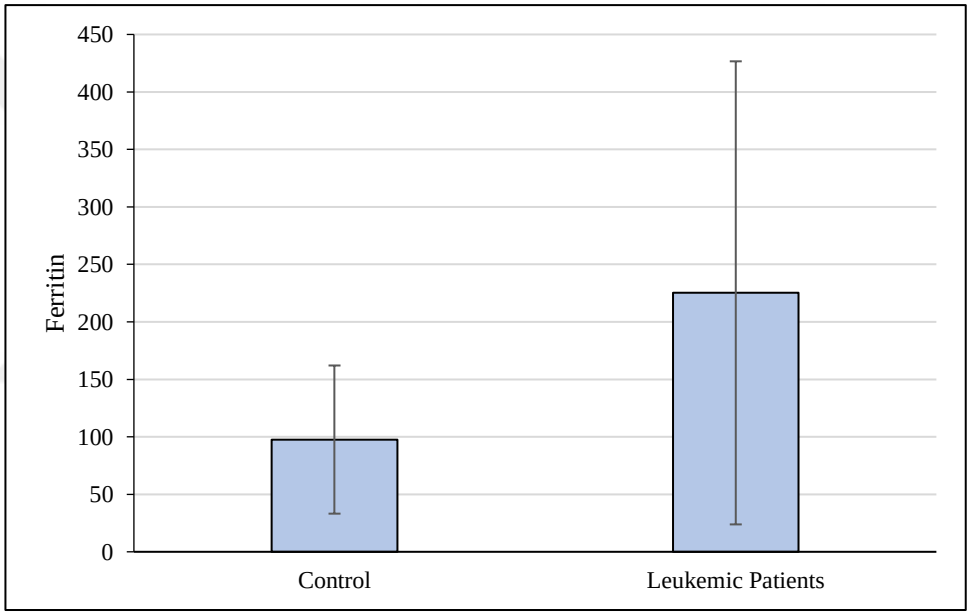

Figure 4.6 The comparison of ferritin level between leukemic patients and control

4.7 Iron

The iron level of the participants is shown in (Table 4.7) (Figure 4.7). Increase in the level of iron for leukemic patients (116.04±22.78 µg/dL) compared to healthy people (82.44±12.74 µg/dL), there was significant (P<0.05).

Table 4.7 The iron (µg/dL) level comparison between leukemic and non-leukemic participants

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	82.44	116.04	
SD	12.74	22.78	<0.001
SE	1.58	2.83	

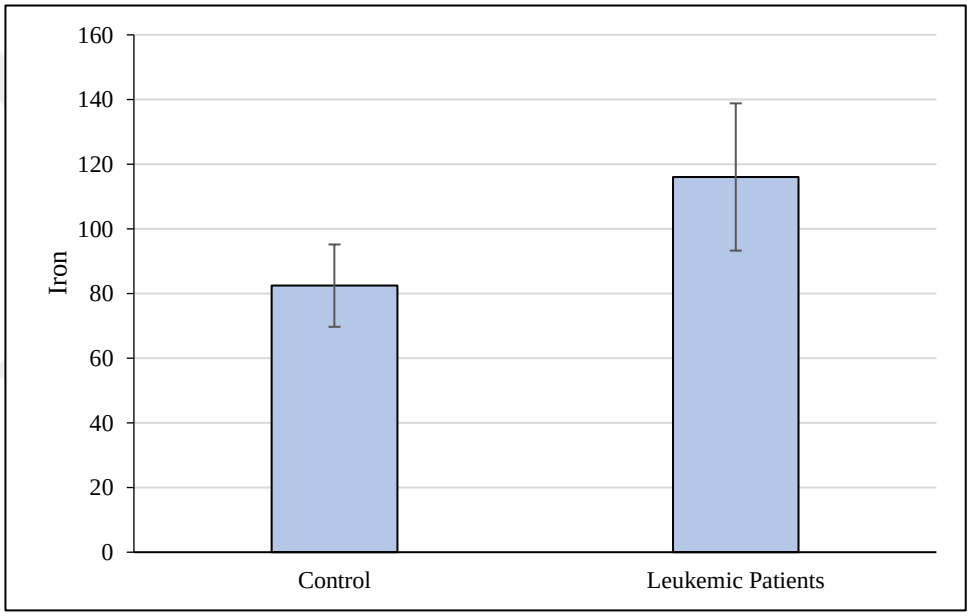

Figure 4.7 The comparison of iron level between leukemic patients and control

4.8 Sodium

The sodium level of the participants is shown in (Table 4.8) (Figure 4.8). Change in the levels of iron between leukemic patients (140.22±4.06 mEq/L) and healthy people (139.48±2.88 mEq/L), there was no significant (P<0.05).

Table 4.8 The sodium (mEq/L) level comparison between leukemic and non-leukemic participants

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	139.48	140.22	
SD	2.88	4.06	0.238
SE	0.36	0.50	

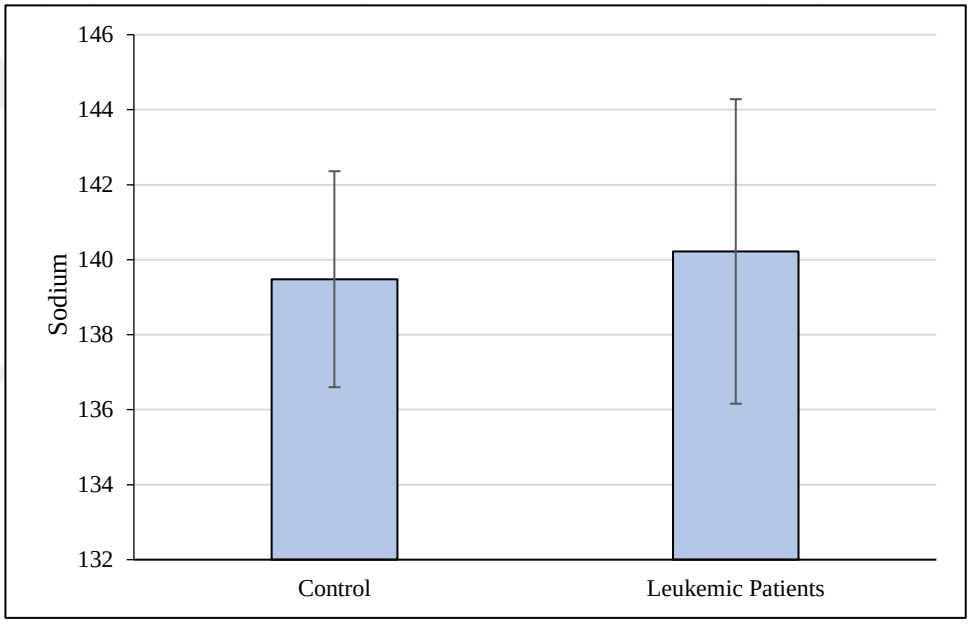

Figure 4.8 The comparison of sodium level between leukemic patients and control

4.9 Potassium

The potassium level of the participants is shown in (Table 4.9) (Figure 4.9). Decrease in the potassium levels for leukemic patients (3.92±0.68 mEq/L) compared to healthy people (4.39±0.48 mEq/L), there was significant (P<0.05).

Table 4.9 The potassium (mEq/L) level comparison between leukemic and non-leukemic participants

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	4.39	3.92	
SD	0.48	0.68	<0.001
SE	0.06	0.08	

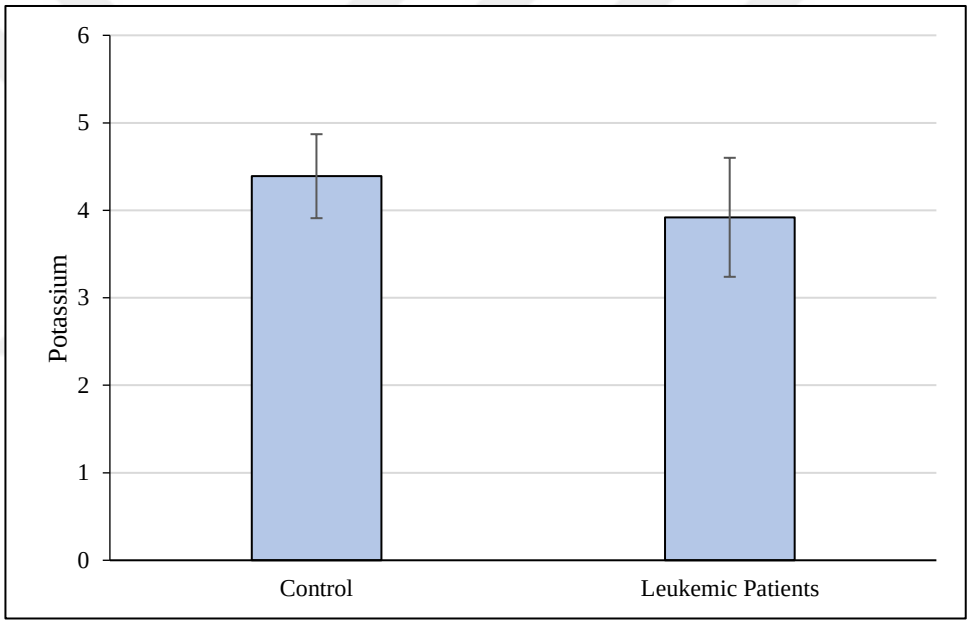

Figure 4.9 The comparison of potassium level between leukemic patients and control

4.10 Zinc

As shown in (Table 4.10) (Figure 4.10), the zinc level of the participants. There was significant (P<0.05), decrease in the levels of zinc for leukemic patients (77.49±10.86 µg/dL) compared to healthy people (98.31±14.22 µg/dL).

Table 4.10 The zinc (µg/dL) level comparison between leukemic and non-leukemic participants

	CONTROL	LEUKEMIC PATIENTS	P-VALUE
Mean	98.31	77.49	
SD	14.22	10.86	<0.001
SE	1.76	1.35	

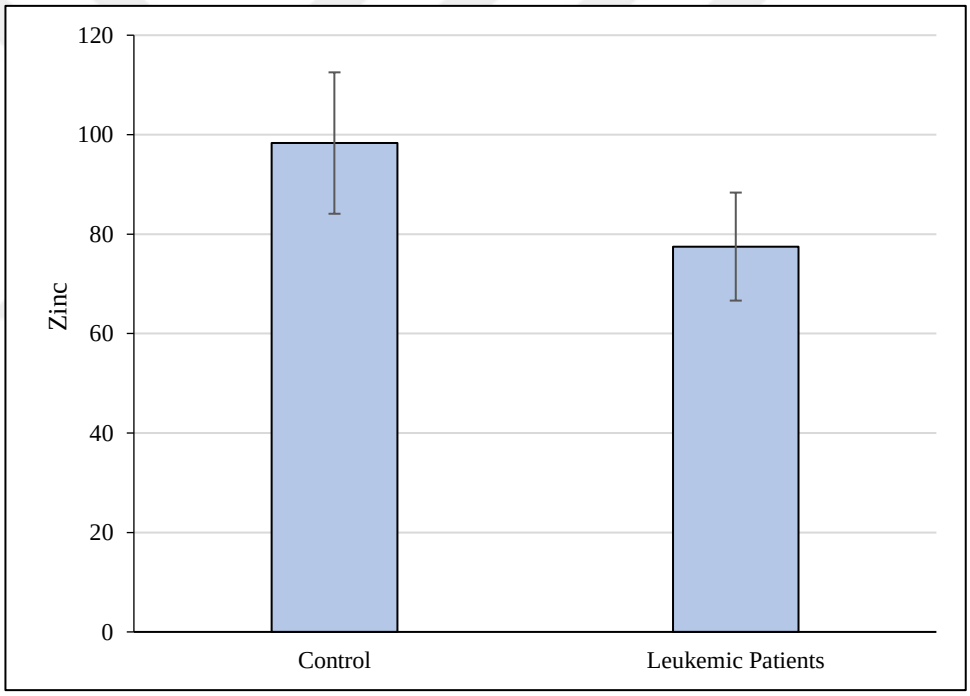

Figure 4.10 The comparison of zinc level between leukemic patients and control

4.11 Correlation

The effect of each variable on the other variables was investigated for G6PD, ferritin and zinc in leukemic patients as shown in Table 4.11.

Table 4.11 Correlation results of G6PD, ferritin and zinc in leukemic patients

VARIABLE	G6PD		FERRITIN		ZINC	
	r	P	r	P	r	P
Ferritin	-0.081	0.523	-	-	-0.056	0.660
Zinc	-0.106	0.400	-0.056	0.660	-	-
Age	0.309*	0.012	-0.007	0.958	-0.116	0.356
WBC	-0.080	0.528	0.503*	0.0001	0.070	0.579
RBC	-0.120	0.342	0.180	0.152	-0.148	0.238
Hb	-0.005	0.971	0.024	0.852	-0.009	0.946
Iron	0.064	0.613	0.407*	0.001	-0.249*	0.045
Sodium	0.072	0.567	0.095	0.452	-0.103	0.416
Potassium	0.123	0.327	-0.058	0.645	-0.204	0.102

Four connections were observed among the study main variables in leukemic patients, a correlation between age and G6PD (Figure 4.11), a correlation between ferritin and WBC (Figure 4.12), and iron (Figure 4.13), and a correlation between zinc and iron (Figure 4.14).

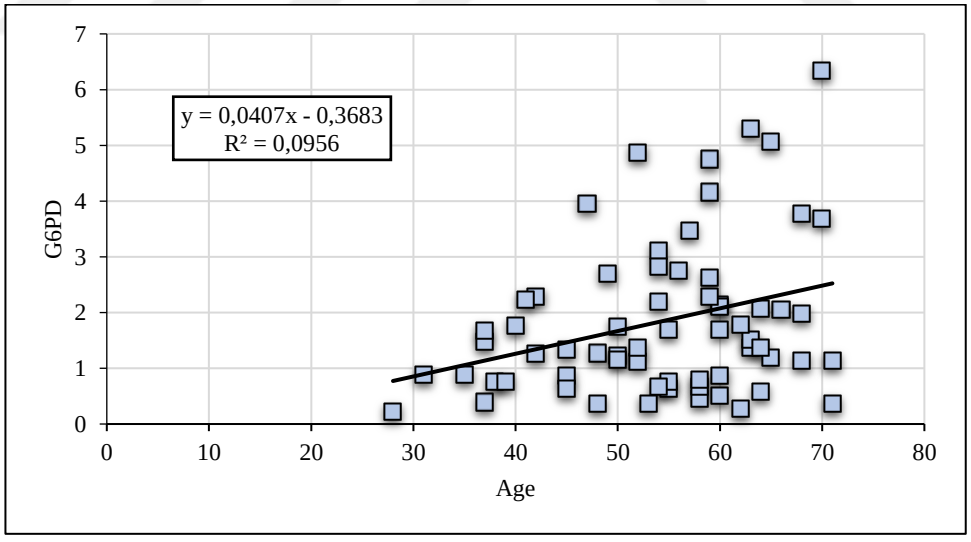

Figure 4.11 Age and G6PD correlation in leukemic patients

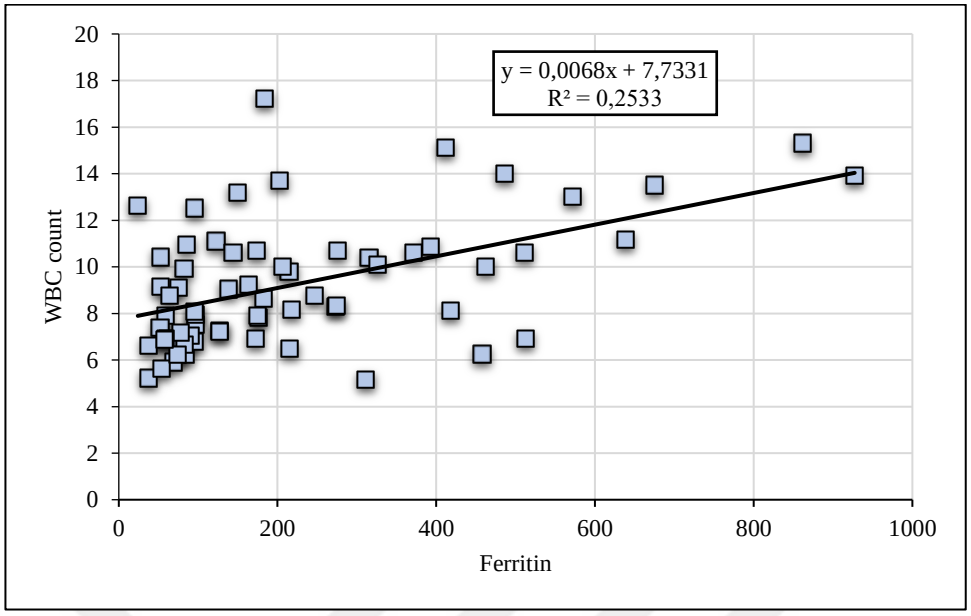

Figure 4.12 Ferritin and WBC count correlation in leukemic patients

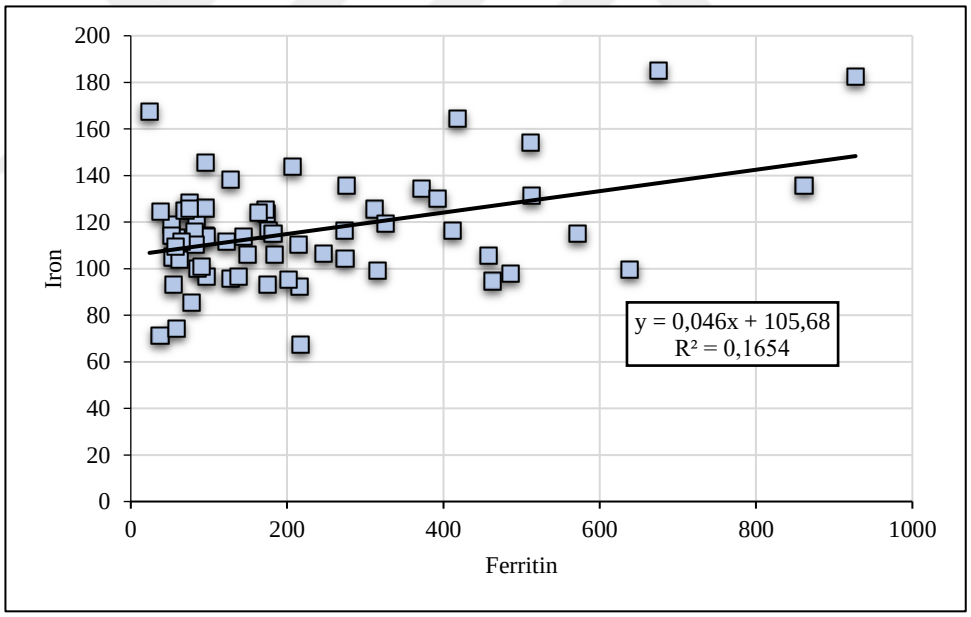

Figure 4.13 Ferritin and iron correlation in leukemic patients

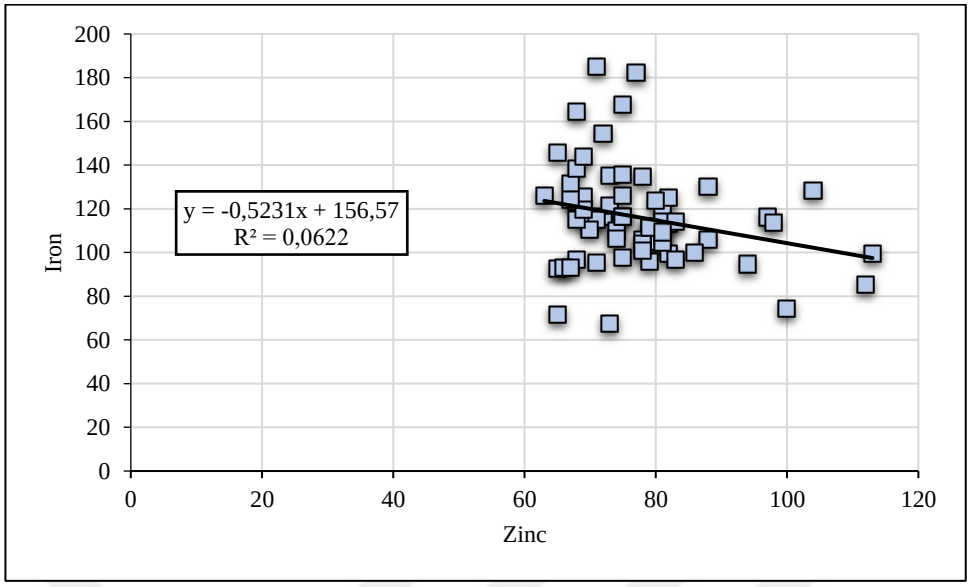

Figure 4.14 Zinc and iron correlation in leukemic patients

4.12 Receiver Operating Characteristics

Glucose-6-phosphate dehydrogenase, ferritin, and zinc were tested for the possibility of using them in the prognosis of leukemia (Table 4.12).

Table 4.12 Correlation results of G6PD, ferritin and zinc in leukemic patients

VARIABLE	G6PD	FERRITIN	ZINC
AUC	0.798	0.723	0.876
SE	0.039	0.044	0.033
P-value	<0.001	<0.001	<0.001

The results have indicated fair sensitivity for G6PD (Figure 4.15), fair sensitivity for ferritin (Figure 4.16), and good sensitivity for zinc (Figure 4.16) in the prognosis of leukemia disease. Together, these tests can provide efficient prognosis for leukemia.

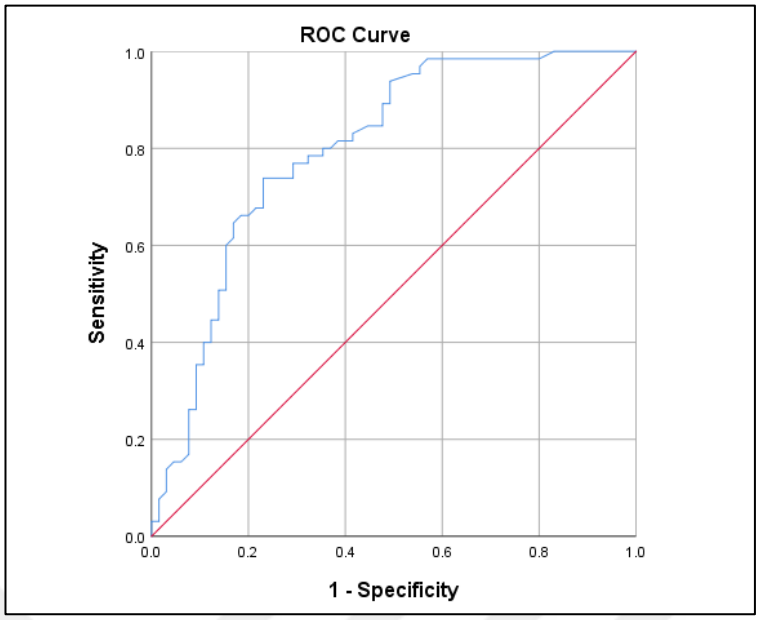

Figure 4.15 ROC of G6PD for leukemia prognosis

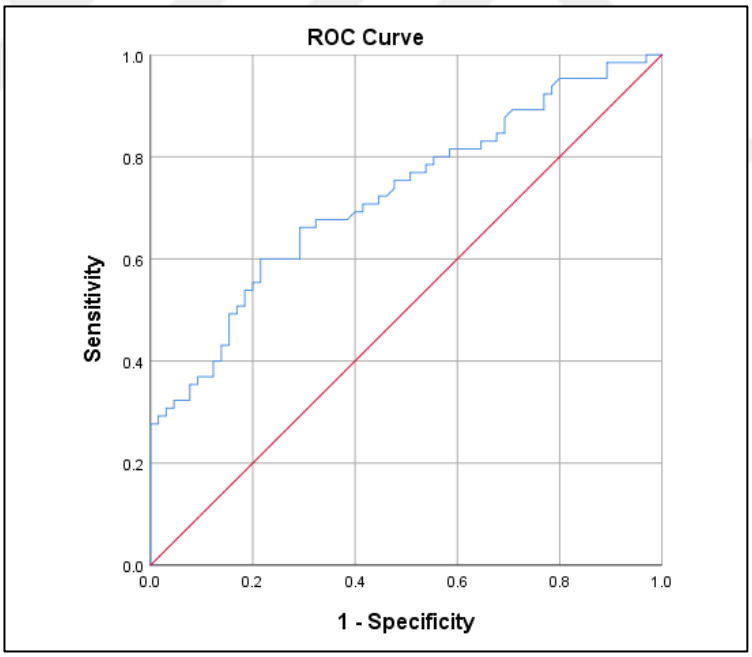

Figure 4.16 ROC of ferritin for leukemia prognosis

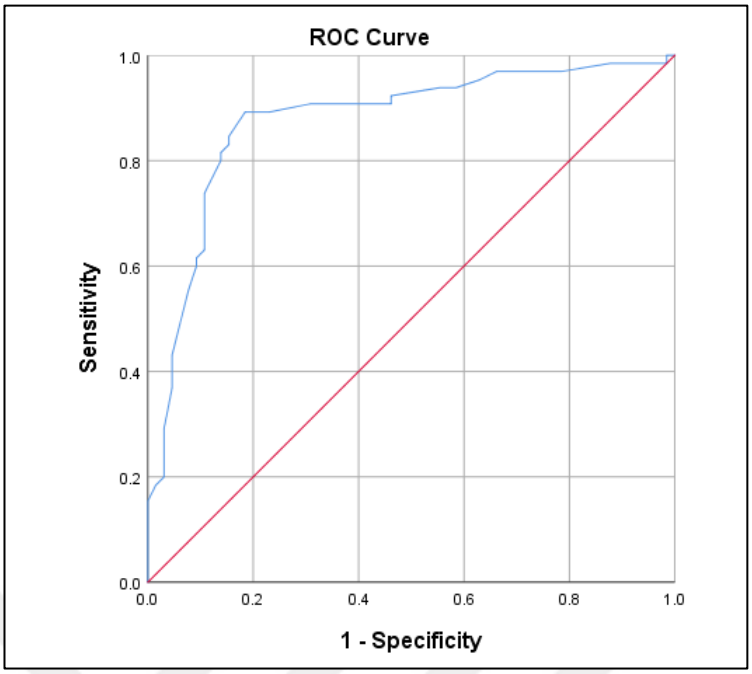

Figure 4.17 ROC of zinc for leukemia prognosis

5. DISCUSSION

In this study chronic myeloid leukemia disease was designed to be experimented. Hematological variations (leukocytes, erythrocytes, and hemoglobin), glucose-6-phosphate dehydrogenase, ferritin, and some elements (iron, sodium, potassium, and zinc) were investigated in the blood and serum of patients. The leukemic patients and the healthy control were selected in matched age category to avoid the effect of aging on the variables of the study.

The count of WBC was increased significantly in the blood of leukemic patients compared to healthy control, while the count of RBC and the level of Hb were reduced significantly. Essentially, leukemia is defined as a state of increased unmatured WBCs production or a production of abnormal WBCs. Therefore, the count of WBC is a nascency test for leukemia prognosis (Quintás-Cardama and Cortes, 2006). Khazaal *et al.* have reported increased leukocytes counts with decreased erythrocyte count and Hb level in patients with chronic myeloid leukemia disease (Khazaal *et al.* 2019). Majumder *et al.* have reported significant alterations occur in the morphology of RBC in patients with leukemia. These morphological changes would result in unmature degradation of RBCs which results in the development of anemia (Majumder *et al.* 2006).

The level of serum glucose-6-phosphate dehydrogenase was decreased significantly in leukemic patients compared to healthy control. The authors have found that almost third of patients with acute myeloid leukemia disease were exhibited G6PD deficiency. Since this enzyme involved in the manufacturing of NADPH that participate in the oxidative burst of forging microbodies, the deficiency of this enzyme would result in the increase of infectious catch of the patients (Sanna *et al.* 2017). In a recent review article, it has been documented that deficiency in G6PD arises from mutations in the genes coding for G6PD, these mutations can evolved easily in leukemia disease (Naville *et al.* 2022). On the contrary, Devi *et al.* have reported significant increase of G6PD activity in patients with chronic myeloid leukemia compared to control, but the activity of G6PD was

decreased in acute non-lymphocytic leukemia patients (Devi *et al.* 1997). Our results disagreed with Devi *et al.* results.

The levels of ferritin and iron were increased significantly in leukemic patients compared to healthy control. Tachibana *et al.* have reported that increased ferritin in leukemic patients can be used to determine the inflammatory condition of the patients that associates with the malignancy (Tachibana *et al.* 2018). Saurabh *et al.* have reported significant increase of ferritin level in patients with chronic myeloid leukemia compared to people without leukemia (Saurabh *et al.* 2017), which agreed with our results. Wang *et al.* have indicated significant alterations in the iron metabolism during leukemia disease (Wang *et al.* 2019). Weber *et al.* have reported that red blood cell transfusions frequently lead to systemic iron overload in leukemic patients (Weber *et al.* 2021).

Potassium and zinc levels were decreased significantly in the serum of leukemic patients, while sodium level was at normal range. Salman *et al.* have reported significant decrease of both sodium and potassium levels in the serum of leukemic patients (Salman *et al.* 2013). Their results are in agreement with ours regarding potassium only. The authors have attributed this reduction of potassium to renal-related consequences in which the clearing of potassium through urination is increased in leukemic patients (Salman *et al.* 2013). In the study of Valadbeigi *et al.* the authors have shown significant decrease in the level of zinc in leukemic patients (Valadbeigi *et al.* 2019). Also, Ahmadi-Faghih *et al.* have shown significant reduction in the level of serum zinc in leukemic patients compared to people without leukemia. They have linked this reduction to the antioxidant system, since the trace elements are essential in the oxidoreductase enzymes that generate or detoxify free radicals (zinc is necessary for superoxide dismutase which detoxify superoxide radical) (Ahmadi-Faghih *et al.* 2013).

6. CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

1. Patients with leukemia have an abnormally high white blood cell (WBC) count, low red blood cell (RBC) count, and low hemoglobin (Hb) level.

2. Leukemic patients have shown significant increase in ferritin and iron levels.

3. Hematological tests and iron status tests revealed an increase in the inflammatory status with probable anemia associated with chronic myeloid leukemia.

4. The level of G6PD was decreased significantly in leukemic patients compared to control.

5. Potassium level in the serum of leukemic patients was reduced significantly compared to control.

6. Zinc level in the serum of leukemic patients was reduced significantly compared to control, which probably indicate a reduction in the antioxidant system.

7. Ferritin was associated with WBC count and iron in leukemic patients.

8. Ferritin and G6PD can be used as fair sensitive biomarkers for leukemia prognosis, while zinc can be used as good biomarker.

6.2 Recommendations

1. Check up the iron status in leukemic patients is very important to avoid the anemia complications.

2. Using G6PD as a prognostic biomarker for chronic leukemia disease.

3. Studying the effect of G6PD in bone and breast malignancy and find out the role of this enzyme in the progression of cancer.

4. Studying the effect of zinc supplementation on leukemic patients' signs and symptoms.

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