University of Karbala
College of Education for Pure Sciences
Department of Biology

A Physiological and Biochemical Study of Acute Lymphoblastic Leukemia in Iraqi Children before and after Chemotherapy

A Dissertation
Submitted to the Council of the Faculty of Education for Pure Sciences / University of Karbala
In Partial Fulfilment of the Requirements for the Ph.D. Degree In Zoology (Animal Physiology)

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Dedication

In The Name of Allah, Most Gracious and Most Merciful

I Dedicate My Modest Efforts to….

Our Sir … Our Lord ... "God" of Pride, Glory and Mercy … Almighty "Allah"
Master of Prophets and Apostles … The Light of Guidance "Mohammed"... and his Inspired Household and Loyal Companions (peace be upon him and his Household) …

My First Tutor … My Affectionate Corner ... The Virtuous Educator …
… My Beloved and Kind Father …

The Spring of Sympathy and Warmth … Who Surrounded me with Her Luxurious Shadow … Even in Her Absence from Me …
When I missed Her by Surprise …
My Beloved and Kind Mother...May Her Soul Rest in Peace
"God Bless Her Soul and Accommodate Her in a Vast Paradise of a "Sincere Seat"
Dear Brother … Dear Sisters ...For their;
"Affection, Inspiration, Prayers and Support"

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God will reward everyone for the best compensation of the benefactors

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Term	Abbreviation
1,25 Dihydroxy Vitamin D_3	1,25 $DHVD_3$
5-hydroxytryptamine receptor 1	5-HTR1
Absorbance	Abs
Acute Lymphoblastic Leukemia	ALL
Acute Myeloid Leukemia	AML
Albumin	Alb
ammonium ion	NH^{4+}
Analysis of Variance	ANOVA
Antibody	Ab
Antigen	Ag
Atomic Absorption Spectrophotometer	AAS
Blood-Brain Barrier	BBB
Bovine Leukemia Virus	BLV
Bromocresol Green	BCG
Calcium ion	Ca^{2+}
Carbohydrate-Recognition Domains	CRDs
Catalase	CAT
Central Nervous System	CNS
Cerebrospinal Fluid	CSF
Chronic Lymphoblastic Leukemia	CLL
Chronic Myeloid Leukemia	CML
Cluster of Differentiation	CD
Cobalt	Co
Competitive Enzyme Linked Immune Sorbent Assay	Competitive ELISA

Complete Blood Count	CBC
Complete Remission	CR
Concentration	Conc.
Copper	Cu
C-reactive protein	CRP
Cresol Phtalein Complexone	CPC
Deoxyribonucleic acid	DNA
Dilution Factor	DF
Ellman's Reagent (5,5-dithio-bis- [2-nitrobenzoic acid])	DTNB
endothelial NOs	eNOs
Epstein-Barr Virus	EBV
Erythropoietin	EPO
Ferritin	FT
Figure	Fig
French-American-British system	FAB
Galectin-9	Gal-9
Gastrointestinal Tract	GIT
Glutathione Peroxidase	GPx
Glutathione Reductase	GR
Hematocrit	HCT
Hematopoietic Stem Cells	HSCs
Hemoglobin	Hgb
Horseradish Peroxidase	HRP
Human T- Lymphotropic Virus	HTLV

Hydrogen Peroxide	H_2O_2
Identity Document	ID
Immunoglobulins	Igs
inducible NOs	iNOs
Ion Selectivity Electrode	ISE
Iron	Fe
Malondialdehyde	MDA
Manganese	Mn
Mannose associated serine proteases	MASPs
Mannose-Binding Lectin	MBL
Matrix Metalloproteinase	MMP
Mean Corpuscular Hemoglobin	MCH
Mean Corpuscular Hemoglobin Concentration	MCHC
Mean Corpuscular Volume	MCV
Methotrexate	MTX
mitochondrial NOs	mtNOs
Mucin 1	MUC1
Natural Killer	NK
neuronal NOs	nNOs
Nicotinamide adenine dinucleotide phosphate,	NADPH
Nitric Oxide	NO
Nitric Oxide synthases	Nos
Non-Hodgkin's Lymphoma	NHL
Optical Density	OD
Oxidative Stress	OS

oxidized form of Iron	Ferrous (Fe^{2+})
Oxidized Glutathione	GSSG
Parathyroid hormone	PTH
parts per million	ppm
Pediatric Intensive Care Unit	PICU
Peroxiredoxins	Prxs
Platelets	Plts
Poly morphonuclear	PMN
Reactive Oxygen Species	ROS
Reactive Species	RS
Reagent 1	R1
Reagent 2	R2
Red Blood Cells	RBCs
reduced form of Iron	Ferric(Fe^{3+})
Sandwich-Enzyme-Linked Immune Sorbent Assay	Sandwich-ELISA
Serotonin	ST
Serotonin Transporter	SERT
Standard Deviation	SD
Statistical Package of Social Science (SPSS)	SPSS
Superoxide Dismutase	SOD
T-Immunoglobulin and mucin-domain containing protein-3	TIM-3
Total Iron-Binding Capacity	TIBC
Total Protein	TP
Total ReducedGlutathione	T-GSH

Traumatic Lumber Puncture	TLP
Vascular Endothelial Growth Factor	VEGF
Vit D Binding Protein	Vit DBP
Vitamin B_{12}	vit B_{12}
Vitamin D_3	vit D_3
White Blood Cells	WBCs
World Health Organization	WHO
Zinc	Zn

During the extended period (from the beginning of March 2019 until the end of February 2020) in the Center of Oncology of Hematology in the Medical City of Imam Al-Hussain in Holy Karbala. 31 children with newly diagnosis of Acute Lymphoblastic Leukemia (ALL), their ages were ranged from 2-12 years old (18 males and 13 females). ALL patients were follow-up until they received four doses of chemotherapy. The control group included 40 healthy control with age range 2-12 years old (20 males and 20 females).

The present study was designed to evaluate the levels of galectins (Galectin-9), and mannose-binding lectin (MBL), as well as numerous routine hematologic parameters and indices. Additionally, evaluated the levels of the index for oxidative stress which involved Nitric oxide (NO). Besides, the determination of total antioxidant parameters included (T-GSH and GSSG). The data of the present study comprised monitoring the levels of certain hormones; such as Erythropoietin (EPO), Serotonin (ST), and Parathyroid (PTH), as well as Total Protein (TP), Albumin (Alb), and Ferritin (FT); furthermore, vitamins, including vit D_3 and vit B_{12}, in addition; certain trace elements (Zn, Co, Fe, Mn, and Cu), as well as other electrolytes were assessed in the sera of children with ALL (at diagnosis) until patients receiving four doses of chemotherapy and the recorded outcomes of patients were compared to the results of control.

The results of the current study were exhibited a significant increase ($p<0.05$) at the levels of WBCs, Gal-9, MBL, NO, EPO, ST, vit D_3, vit B_{12}, FT, Mn, and Ca^{2+}, while the outcomes showed a statistically significant decrease ($p<0.05$) at the levels of RBCs, Hgb, HCT, Plts, MCHC, T-GSH, GSSG, PTH, TP, Alb, Co, and K^+ in the sera samples of patients with ALL (pre-treatment) when compared to the control group, whereas the levels of MCH, Zn, Fe, Cu, Mg^{2+}, Na^+, and CL^- did not vary significantly, but there was a non-significant increase in MCV level in blood samples of patients. On the other hand, the data have shown decreased levels in each of the following parameters in ALL patients after receiving chemotherapeutic doses: WBCs, Gal-9, MBL, NO, ST, vit D_3, FT, Fe, Cu, Ca^{2+}, Mg^{2+}, Na^+, CL^-, and K^+, however; RBCs showed a fluctuating decrease, while other hematological parameters presented a slight and gradual rise than their levels at diagnosis; such as Hgb, HCT, Plts, MCV, MCH, MCHC, T-GSH, GSSG, TP, Alb, Zn, and Co, while each of EPO, vit B_{12}, and Mn levels remain constantly rising

after chemotherapy, whereas PTH also remains at a low level after treatment, as it was at the time of diagnosis.

The present work showed acceptable statistical correlations at the ($p<0.05$ and $p<0.01$) levels (positive or negative) among the evaluated parameters in the study. A positively significant correlation was observed for Gal-9 and MBL in the sera of ALL patients and control group with the NO, ST, and EPO levels, as well as a high positive correlation, was recorded within antioxidants molecules (T-GSH, GSSG) and trace elements (Zn, Co, Mn, and Cu), moreover; between Zn with Co, Mn, and Cu on the one hand, as well as Co with Mn and Cu on the other side, while the study recorded a negative correlation relationship when comparing Gal-9 and MBL with levels of antioxidants (T-GSH and GSSG), as well as with trace elements (Zn, Co, Mn, and Cu), while a high negatively correlation was noticed between EPO with trace elements (Zn, Co, Mn, and Cu).

The FT, EPO, Gal-9 showed the highest single sensitivity (100%) among the criteria assessed followed by MBL (77%), while the lowest sensitivity level for patients was 39%, 29%, and 10% for T-GSH, PTH, GSSG, separately in children with ALL compared to the control group.

It was also noted that the variance capacity of assessed standards generally increased when they were linked. The maximum sensitivity rate (100%) was observed when the FT, EPO, and Gal-9 were combined to the PTH, MBL, T-GSH, GSSG, NO, and ST, as well as the sensitivity of diagnostic efficiency of MBL increased to 94% and 90% when evaluating its levels with NO and GSSG respectively, as well as the diagnostic sensitivity for T-GSH was reached (87%) when assessing its levels with MBL and NO, while the lowest allergic rate was 39% and 35% for PTH with GSSG and ST.

Keywords: Acute Lymphoblastic Leukemia (ALL), Galectin-9 (Gal-9), Mannose Binding Lectin (MBL), Hematologic Parameters and Indices, Oxidants and Antioxidants, Vitamins D_3 and B_{12}, Hormones & Proteins, Trace Elements, Chemotherapeutic Doses

Chapter One

Introduction and Literature Review

1.1: Leukemia

1.1.1: Definition and Characterization of Leukemia

The term ‘’leukemia’’ is derived from an ancient Greek word *leukos* (white) and *haima* (blood) that means ‘’white blood’’ therefore it refers to a wide range of malignant disorders in hematopoietic stem cells (HSCs) in the bone marrow due to the accumulation of abnormal white blood cells (WBCs). The duration of this disease varies depending on the type of leukemia, therapeutic methods and patients’ response [Eloranta *et al.,* 2021]. Thus, leukemia is considered one of the most aggressive, dangerous hematologic disorder types of cancers all over the world [Simioni *et al.,* 2019].

The earliest description of leukemia was accurately made by a French physician (Alfred Velpeau) in the year 1827, who was referred to as the primary forms of leukemia cells through analysis and studying the blood of patients after their death [Mehranfar *et al.,* 2017]. In 1845 The British physician (John Bennett) defined leukemia as a blood disorder and it was diagnosed as one of the clinical diseases, while the German physician ‘’Rudolf Virchow’’ in the year 1847 noticed relatively few patients suffering from abnormally high levels of leukocytes and he had named the disease as leukemia, and then classified it into splenic leukemia and lymphatic leukemia according to the location of its appearance in the different organs [Thomas, 2019]. At the end of the 19th century and until the middle of the 20th century, scientific studies on leukemia included a clearer in definition and classification into several different subtypes, besides, the well-defined types of leukemia have been used to develop the type of effective chemotherapy which represents the most accurate knowledge and scientifically advanced in leukemia studies [Thomas, 2019].

The medical discovery of leukemia with an early application is a continuation through observations, descriptions and research that are based on ‘’chronology’’ as a definition of outlines and structures of leukemia [Mehranfar *et al.*,2017; Thomas, 2019].

At the present time, many studies have been carried out in the diagnosis of leukemia and its subtypes with numerous recent techniques have been attributed to facilitating the classification of leukemia, as well as study the cellular pathways within the bone using different methods including; immunohistochemistry, genetic cellular methods, and molecular genetics studies, besides immunological tests to distinguish cells via the specific surface markers [Mehranfar *et al.*, 2017; Al-Mafragy, 2019]. The complete blood count (CBC) is an important test in the primary diagnosis and follow-up patients with leukemia, when this analysis provides a lot of information about the health of the bone marrow by the normal number and presence of cells in peripheral blood [Al-Mafragy, 2019].

1.1.2: Epidemiology of Leukemia

The prevalence of all types of leukemia appears to be various according to age, gender, race and geographical distribution worldwide. It’s globally, the incidence of leukemia would be close by the limited of 10 out of 100,000 people per year, almost half of this percentage is acute leukemia type [Hade *et al.*, 2018]. In particular, leukemia in males is more common than females, when the male: female ratios are 3:2, 2:1, and 1.8:1 in Acute Lymphoblastic Leukemia (ALL), Chronic LymphoblasticLeukemia (CLL), and Chronic Myeloid Leukemia (CML), respectively [Moorman, 2016]. On the other hand, the highest rate of ALL in children could be common between 3-10 years, as well as adults in the age-range between 40 and 60 years are most likely to

develop CML and the incidence rate increases with age progression [Moorman, 2016; Scheurera *et al.*, 2018].

However; in Iraq, the incidence rates of cancer have been risen dramatically, and in particular, leukemia was in the seventh-ranked in 1993, while it represents the second-ranked among other ten common cancers in 2017 [Ministry of health, Iraqi, 2017]. Besides the ALL has been characterized as the first-ranked out of the cancers types which common in children [Ministry of health, Iraqi, 2008].

1.1.3: Etiology and Risk Factors of Leukemia

The initiating mechanism of leukemia is completely unclear yet, but it usually results from the destruction of the mechanism that controls the process of normal cell division and prevents the process of differentiation, as well as, its' not succumbing to the mechanism of apoptosis [Chen *et al.*, 2015]. However, leukemia is similar to other types of cancers in the terms of modifications that happen in the cell, which frequently starts with genetic mutations in the DNA within the cell or more of HSCs, therefore; the growth and division of cells become uncontrolled and unorganized, at this time, the mutations change permanently the common behaviour of the cell, thus it is often the occurrence of genetic mutations in DNA of one or more hematopoietic cells, which causes a disturbance of development and cellular differentiation at this stage [Fieg, 2016].

HSC is produced as a particular type of blood cells may become a functional disorder as the uncontrollable proliferation of cells due to increasing the development of more abnormal blood cells which are made in the bone marrow, then these cells move to the bloodstream, and begin to spread or invade to the various tissues and organs such as lymph nodes, liver, spleen, brain and even skin.

At the same time, the production of functionally effective normal blood cells in bone marrow would be decreased, causing unbearable complications for the patients' health such as anemia, severe bleeding and infections, as well as splenomegaly and hepatomegaly that often lead to death [Chen *et al.*, 2015; Fieg, 2016].

Overall, numerous risk factors could lead to leukemia, for instance; environmental factors, radiation, atomic explosions, exposure to irritants, carcinogenic chemicals, pesticides, smoking and alcohol intake [Pereira *et al.*, 2017; Hade, 2018]. In addition, viral infections are associated with the incidence of leukemia such as; Epstein-Bar, Bovine, Feline, and Human T-Lymphotropic Virus (HTLV) [Prado *et al.*, 2018]. Moreover, some genetic disorders have a main role in the occurrence of leukemia for instance; neurofibromatosis, Shwachman syndrome, Down's syndrome, and Bloom syndrome [Iacobucci and Mullighan, 2017].

Additionally, the use of chemotherapy particularly compounds called alkalinizing agents that may enhance the appearance of new types of leukemia which has been associated with increased rates of leukemia, especially leukemia subtypes of ALL [Quah, 2017].

1.1.4: Classification of Leukemia

The classification of leukemia is depending upon the French-American-British (FAB) system which is based on several criteria as the following [Hoffbrand and Moss, 2015]:

The first classification depends on the type of tissue from which leukemic cells are derived, as this category includes the following:

Myeloid Leukemia is the type that created from the myeloid stem cell in the bone marrow.

Lymphoid Leukemia is the leukemia cells are made from the lymphoid stem cell in the bone marrow.

The second classification is depending on the clinical path of the disease and the stage of maturation of the leukemic cells, this class is involved:

Acute Leukemia has influence on immature leukocytes on both of myeloid and lymphoid stem cells and generally this disease developed suddenly as a result of short-course of exposure with may last for a short period of time, therefore. It considers the most dangerous disease for the patients and the chance of survival is low.

Chronic Leukemia, unlike to the acute leukemia, chronic leukemia affects mature leukocytes and develops slowly and gradually for a long duration that may reach the period of 5-10 years. The patient can live for several years after appearance of symptoms, hence it is less serious than the first type and may worsen the patient's condition over a long period of time.

Overall, there are four basic types of leukemia, included the following:

Chronic Myeloid Leukemia (CML): This is the type of myeloproliferative disturbances growth of myeloid progenitor cells in bone marrow and associated with an integrated gene called **BCR-ABLI** which is based on Philadelphia chromosome. Therefore; it is considered as a clonal malignant disorder. CML affects both sexes and represents a ratio of 15-20% in adults with the highest age of 40-60 years. However, it rarely occurs in children and newborns [Hochhaus *et al.*, 2017; Jenna *et al.*, 2017].

Chronic Lymphocytic Leukemia (CLL): is a type of lymphoproliferative disorders growth of lymphoid progenitor cells in the bone marrow. CLL is characterized by accumulation of abnormal mature B-lymphocytes in the bone marrow, blood, lymph nodes, and spleen due to the incidence of a defect in the apoptosis process of cells, thus these cells become inactive and lose

their normal functions, and then causes to enlargement of the lymph nodes ‘’lymphadenopathy’’, splenomegaly, hepatomegaly, as well as anemia [Hoffbrand and Moss, 2015; Jenna *et al.*, 2017]. Besides, most patients usually do not complain of any specific symptoms due to the disease, but they are diagnosed by accident when they have done a routine blood test [Baliakas *et al.*, 2019].

➢**Acute Myeloid Leukemia (AML):** This type of leukemia is the most mutual in adults and has the risk of increasing with the age of individuals, it is regarded as the greater number of myeloblasts in the bone and blood stream than before, these cells lose their ability to differentiate and develop as normal blood cells, as well as to acquire the characteristic of self-regeneration. Thus these cells could not undergo to apoptosis process, and then their numbers would be increased at the expense of other blood cells types. Besides, there are some medical symptoms in patients with AML; such as failure of bone marrow functions and infiltration of organs [Estey, 2018].

➢**Acute Lymphoblastic Leukemia (ALL):** it is one of the greatest common hematological cancers, especially during the stage of childhood by which mainly affects children with a high percentage (80-85%), principally those under 13years, the agewhich is the most commonly affected group of children, while the incidence of ALL in adults which it does not exceed (20-25%) of patients [Terwilliger and Abdul- Hay, 2017]. ALL is characterized by a steadily increase in the number of immature lymphocytes well-known as lymphoblasts in the bone marrow, therefore; the lymphoblasts replace the normal components of blood in the bone marrow and with a noticeable reduction in the production of normal blood cells, as well as a decreasing number of platelets, besides; the existence of anemia in variable forms. The lymphoblasts circulate around the body and begin to proliferate in many other organs mainly in the spleen, liver and lymph nodes [Pandey *et al.*, 2017].

1.1.5: Hypotheses of Acute Lymphoblast Leukemia

Several hypotheses have been put forward to explain the cause of ALL, the most important of which are:

Leo Kinlen (Integrating of Population) Hypothesis: it was developed in 1988, is indicated that one of the most common causes of ALL due to exposure pathogenic infection factors after mixing the community as a result of their transmission from one region to another. Thus the children exposed to carriers of disease, but this hypothesis was achieved very little by international recognition because it did not identify any specific virus or cause of infection among the population [Bartenhang *et al.,* 2017].

The Birth Infection Hypothesis by Smith: it is indicated that one of the reasons for the increased incidence of ALL in children may be due to their exposure to infection during the intrauterine life due to the transmission of infection. According to ‘’Smith’s hypothesis’’, the pathogen works directly in particular on B-lymphocytes through unspecified mechanisms, and with the start of the process of cellular transformation, then it could stimulate with directly affect the ‘’oncogenes’’ either in the embryonic stage within the uterus or after birth [Mejía-Aranguré, 2016]. In addition, the risk of incidence of ALL in children is increased if their mothers are infected, particularly by ‘’Epstein-Barr virus’’ prenatal [Hussein and Alkhayat, 2021]. On the other hand, many studies have noticed that when assessed the maternal exposure to infection during the gestation period which observed that the increased children’s risks of leukemia [Bartenhang *et al.,* 2017; Arellano-Galindo *et al.,* 2017; Hussein and Alkhayat, 2021].

The Delayed Infection Hypothesis by Graves: this hypothesis indicated that ALL requires at least two genetic mutations to onset in children and the existence of genetic disorders which could be indirectly caused by the infection, that is possibly stimulated the achievement of further genetic

disturbances in children with ALL [Quesada *et al.*, 2021]. Graves's hypothesis also showed that the exposure of the common infectious agents during the early stages of children's life is an important factor for the healthy development of a child's immune system, in which maybe as a result of abnormal immune responses due to delayed infections in corresponding with the development of lymphocytes in the bone marrow. it could be caused a potential postpartum genetic disorders or as known ''oncogenic hits''. Consequently, Graves's viewpoint shows that the infection factors have an important role in the emergence of leukemia in children, and that's by studying the ages of infected children with delayed infections as compared to their peak period of leukemia, which could be associated with abnormal responses leading to undeveloped in immune system in children [Hade, 2018; Quesada *et al.*, 2021].

1.1.6: Characteristics of Acute Lymphocytic Leukemia Types

According to the FAB system, ALL is classified into three different subtypes which are mainly based on the set of characteristics observed in lymphoblasts within the bone marrow under the microscope and how these blasts are stained by cytochemical stains [Daniel *et al.*, 2017]. **Table 1-1** shows some essential cellular features to differentiate the different subtypes of ALL.

Table 1-1: Specific Cellular Characteristics of ALL Subtypes [Hade, 2018]

	L1	L2	L3
Cell Size	Usually small	Various and large in mass	Usually large
Nuclear Chromatin	Homogenous and possibly dense in some cells	Varied	Homogenous, finely and stippled
Shape of The Nucleus	Regular	Irregular and twisted or folded	Regular, oval or round
Nucleoli	Invisible or too small and usually indistinct	Usually visible and large in mass	Clear and regularly prominent
The Amount of Cytoplasmic	Scanty in amount	Heterogeneous	Available in a moderate quantity
Presence of Cytoplasmic Vacuoles	Diverse in number	Various in number	Noticeable and obvious

In children, the first subclass of ALL (L1) is represented around 70% of cases, while the second (L2) and the third (L3) categories represent 25% and 5% of children cases suffered ALL [Itzykson *et al.*, 2018].

However; in the recent time, the World Health Organization (WHO), 2016 has proposed a new system to classify ALL which involves some groups to divide ALL into several sets:

(1)T-cell of ALL (Premature T-cell precursor lymphoblastic leukemia)

(2)B-cell of ALL with specific genetic factors abnormalities

(3)B-cell of ALL without specific genetic factors abnormalities

(4) Mixed heredity acute leukemia, which is also known as either mixed phenotype acute leukemia or acute undifferentiated leukemia, which it constitutes a very low percentage of around 2-5% of acute leukemia [Wohlfahrt *et al.*, 2015].

1.1.7: Symptoms of Acute Lymphocytic Leukemia

General symptoms of ALL are included: frequent infections, fever, easy bruising, bleeding that is hard to stop, flat, dark-red skin spots ‘’petechiae’’

due to haemorrhage under the skin, pain in the bones or joints, lumps in the neck, underarm, stomach or groin, pain or fullness below the ribs, weakness, fatigue, paleness, loss of appetite, and shortness of breath [Kaplan, 2019].

1.1.8: Diagnosis of Acute Lymphocytic Leukemia

The Immunophenotyping technique using a cluster of differentiation(CD) and flow cytometry play a fundamental role in the diagnosis of the three sub-types of ALL, also in the patient's prognosis [Narang *et al.,* 2016].

1.1.9: Treatment of Acute Lymphocytic Leukemia

According to the importance and specificity of this type of cancer, the treatment process is more complicated and rather than different from another form of leukemia routine treatment (its varies in manner) depending upon the therapeutic regimen, as well as it differs from one patient to another [Sas *et al.,* 2019]. The programme of therapy is determined by some overlapping aspects of cancer cells such as; their behaviour, density, degree of widespread, along with the patient's age, health condition, and physical structure of the patient [Plummer *et al.,* 2016]. Hence, the cases of leukemia could be characterized according to the severity of disease into ''Standard Risk'' category (this group responds to treatment and achieves relatively high recovery rates) and ''High-risk'' category, as well as ''Very High-risk'' category (which are required a complex and intensive based therapeutic regimen) [Amarullah *et al.,* 2018]. Therefore, the early treatment is a very essential step in dealing with this disease, and one of the main targets of treatment is the blockade of leukemic cells are known as ''blasts'', then it's important to be in a stable stage that identified by the ''Remission Induction Phase'', and finally eliminating the blasts [Plummer *et al.,* 2016; Amarullah

et al., 2018]. Consequently; the treatment protocols are used with leukemia which varied and could comprise the following:

- **Chemotherapy:** is a number of different medication doses, is given according to the specific strategy that depends mainly on the general patient's health and the severity of the disease [Yoong and Poon, 2018]. The strategy of chemotherapy would go through several steps, like the following:

(1) Remission-Induction: This stage is organised to destroy leukemic cells into bone marrow and given the patient particular drugs with strong effects for a long period which could be specified for 4-5 weeks of treatment [Khan *et al.,* 2017]. The remission period also represents the stage of diagnosis and initial chemotherapy treatment of the central nervous system (CNS) of leukemia patients, which is very important to organise it in the long term consequence in order to protect CNS from spread leukemic cells, in which prepares as Traumatic Lumbar Puncture (TLP) through intrathecal treatment and it often consists of a triple therapy of hydrocortisone methotrexate (MTX) and cytarabine [Abdelmabood *et al.,* 2020].

(2) Consolidation Phase: is also termed intensification period with aggressive consolidation that is characterized by receiving a high dose of chemotherapy to protect the patient's CNS and avoid later relapse, it may be provided by intensive intrathecal treatment as one or two cycles for five days over a period of schedule management by specialists in which arrange for 14-28 weeks depending upon the risk degree of patients' groups [Plummer *et al.,* 2016; Bhatia *et al.,* 2015]. Therefore, the patients take delivery of successive consolidation and intensification therapy with the intensity and length duration of the chemotherapy regimen in which be determined by the patient's risks group [Robert *et al.,* 2018].

(3)Maintenance Phase: is the last period of chemotherapy, is focused on preventing cancer cells growth over again in which the treatment is less than

previous stages, but it lasts a longer period, that could be extended for 2-3 years or more. It seems too long with essentials to receive daily therapeutic drugs, while the diversity of medications in which given to the patients with the possibility of an interruptions period of treatment could be the main reasons for the increased risk of relapse in leukemia patients [Bhatia *et al.*, 2015].

Many patients could particularly take place a failure during remission-induction and otherwise, they may have undergone a bone marrow transplantation, thus they do not continue the next stages of therapy, however; the maintenance phase is considered as an essential stage in the treatment of ALL [Bhatia *et al.*, 2015; Hallek, 2019]. After the patients delivered several regular chemotherapeutic drugs, it's a possible response to treatment and arrived to be cured. In particular, if they haven't any hard complications, in addition, when the number of lymphoblasts should be less than 5% in the bone marrow and never to be found the blasts in peripheral blood [Hoffbrand and Moss, 2016; Hallek, 2019].

- **Radiotherapy:** is used in the cases of benign and malignant tumours, as it works very effectively in termination tumour cells during the proliferation stage by means of X-ray and/or Gamma-ray, these rays work very effectively to destroy tumour cells by affecting the damage of DNA, and then modify the gene expression [Hallek, 2019]. However; one of the most important problems with radiotherapy is the possibility of its damage to the normal tissue cells, and causing a new type of cancer as a result of DNA mutation lesion of healthy cells, then it has a negative impact to inhibit normal bone marrow functions in which principally has permanent damage [McReynolds and Savage, 2017].

- **Targeted Therapy:** this type of treatment relies on a type of drug that attacks certain types of cancer cells without damaging healthy cells and it has less risk of side effects [Pierro *et al.*, 2017].
- **Interferon Therapy:** it reduces or prevents the growth and spreading of leukemia cells. It has severe side properties and acts obviously in a comparable approach of substances in the immune system which is produced naturally [Mo *et al.*, 2018].
- **Stem Cells Transplantation:** The process of stem cells transplantation is involved in cancerous cells destroyed that exist in bone marrow either by chemotherapy, radiation therapy, or both. Then, they infuse new and healthy hematopoietic stem cells from donors into the bone marrow of patients to create healthy and noncancerous blood cells [Dessie *et al.*, 2020].

1.1.10: Prognosis of Acute Lymphocytic Leukemia

A number of patients may be able to be survived within 5-15 years after the completion of the regular chemotherapeutic regimen cycles. although there are many variations between patients, especially in relation to the patient's response to treatment. There are several factors that determined whether the therapy is either good or poor [Hoffbrand and Moss, 2015; Lee and Cho, 2017]. **Table 1-2** shows the main factors that are determined as prognosis factors of patients with ALL.

Table 1-2: The Prognostic Factors in ALL Patients [Hade, 2018]

Good Prognosis	Poor Prognosis
Children age in between 2-10 years old	Adults age more than 50 years old
ALL blasts from type L_1	ALL blasts from type L_2
More effective in white races than in black	More effective in black races than in white
Hemoglobin value > 10g/dL	Hemoglobin value< 7g/dL
The number of WBCs <$10x10^9$/L	The number of WBCs >$50x10^9$/L
Blast cells are not spread to the CNS	Blast cells are spread to the CNS
Patients have Complete Remission (CR) period within 4-5 weeks of initial therapy	Patients do not have CR period within 4-5 weeks of initial therapy

The prognosis of children with ALL under the age of 13 years old is a usually good prognosis, where there is a decline in symptoms of disease, especially after being treated with a combination of both radiotherapy and chemotherapy besides in some particular cases the patients may require to bone marrow transplantation to achieve almost completely recovery [Hade, 2018]. However; other patients may be in very serious and incurable conditions because they are severely susceptible to infections due to either acute deficiency in leukocytes and/or as a result of uncontrolled bleeding caused by thrombocytopenia [Hade, 2018; Tebbi, 2021].

1.2: Lectins

1.2.1: Definition of Lectins

Lectins (from Latin, *Legere*, to select or choose) simply are abundant proteins or glycoproteins that are probably present in all eukaryotic cells and many other bacterial species, as well as in some viruses [Mishraa *et al.*, 2019; Chettri *et al.*, 2021]. They are capable to bind mono- and oligosaccharides with high affinity and usually agglutinate cells or precipitating polysaccharides and glycoconjugates, specifically and reversibly [Chettri *et al.*, 2021].

Broadly, lectins constitute a superfamily of ubiquitously distributed proteins, which are described and characterized in a steadily increasing number of publications [Manikandan *et al.*, 2020].

1.2.2: Classification and Structure of Lectins

The classification of lectins is wide-ranging due to the presence of a great number of diverse lectins. Lectins may be broadly divided into four categories by using different approaches [Coulibaly and Youan, 2017]. Lectins are categorized into 13 types (families) according to their specificity

of sugar, included: Calnexin, F-type, F-box, Selectin, L-type, M-type, P-type, R-type, Ficolins, Galectins and C-type [Mishraa *et al.*, 2019; Li *et al.*, 2019a].

In general, these families are divided into those which act as intracellular and extracellular. The intracellular lectins are localised in luminal compartments of the secretory pathway, while the extracellular lectins are either secreted into the extracellular matrix, body fluids or located in the plasma membrane, as they are mediated by a range of functions [Kumar *et al.*, 2012]. **Figure 1-1** has been exposed to some types of lectins.

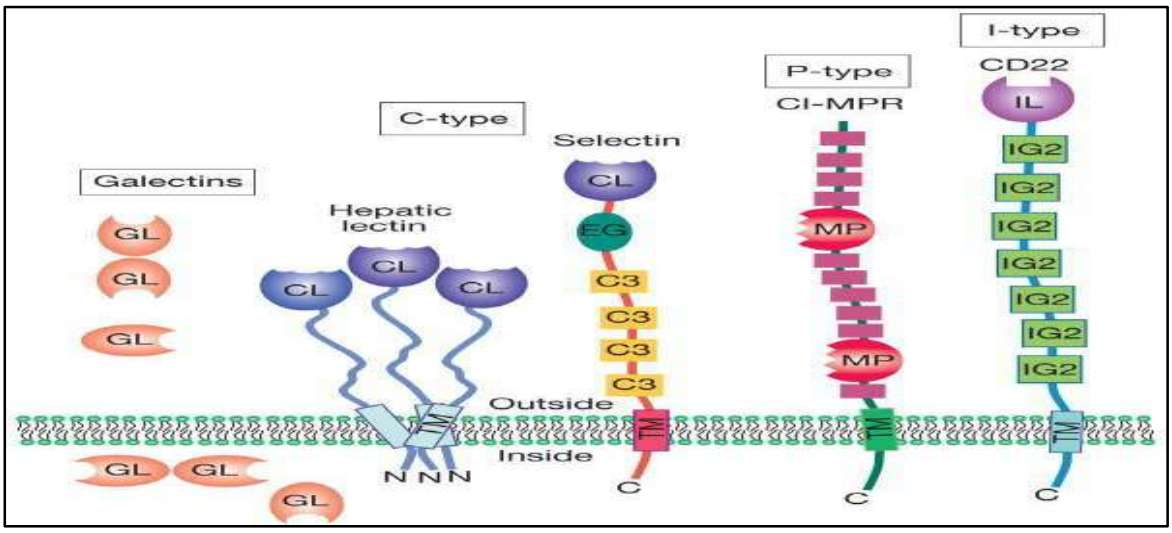


Figure 1-1: Examples of Main Types of Lectins [Kumar *et al.*, 2012]

Lectins consist of two main groups based on their metal dependence: The calcium-dependent (C-type) and the calcium-independent (S-type lectins). C-type family includes both soluble and insoluble or integral membrane proteins, by which are regarded by Ca^{2+} requirement for their activities, and a sequence of patterns with highly conserved amino acids that contain about 15% of their carbohydrate-recognition domains (CRDs) [Majbel, 2020]. S-type lectins are sulfhydryls-dependent, or beta-galactoside binding proteins which are soluble proteins in vertebrates, as these lectins

require for their binding activity to ligands (mostly ß-galactosyl residues) [Cassandra *et al.,* 2017].

1.2.3: Galectins

Galectins are soluble glycoproteins, bind specifically to β-galactosides. galectins have broadly disseminated in nature by their binding to a variety of glycoproteins, by which galectins have central stimuli for this type of communication in the cells, and it's probably to play a crucial role in the involvement of growth and progression of cancer [Hisrich *et al.,* 2020].

Galectins were initially discovered in 1976 by isolating them from the lungs and hearts of small calves, as well as from the muscles of chicks [Hisrich *et al.,* 2020; Ebrahim *et al.,* 2014]. In addition, galectins have been identified in some other invertebrates such as; oysters and amphioxus [Chen *et al.,* 2016]. Galectins are made up of one or more subunits called carbohydrate-recognition domains (CRDs) with consent about 130 amino acid sequences [Varki *et al.,* 2015].

In mammals, about 15 members of galectins have been isolated and recognized as well as classified into three categories depending on the number composition and organization of CRD subunits in which as follows into [Saccon *et al.,* 2017]: **(1) Dimeric galectins** have two identical CRD domains that comprise (1, 2, 5, 7, 10, 11, 13, 14, and 15) galectins. **(2) Tandem galectins** have two different CRD domains that include (4, 6, 8, 9, and 12) galectins.**(3) Chimeric type galectins** which have either one or multiple flexible subunits of CRDs to increase their linkers that include just now Galectin-3, as shown in **Figure 1-2**.

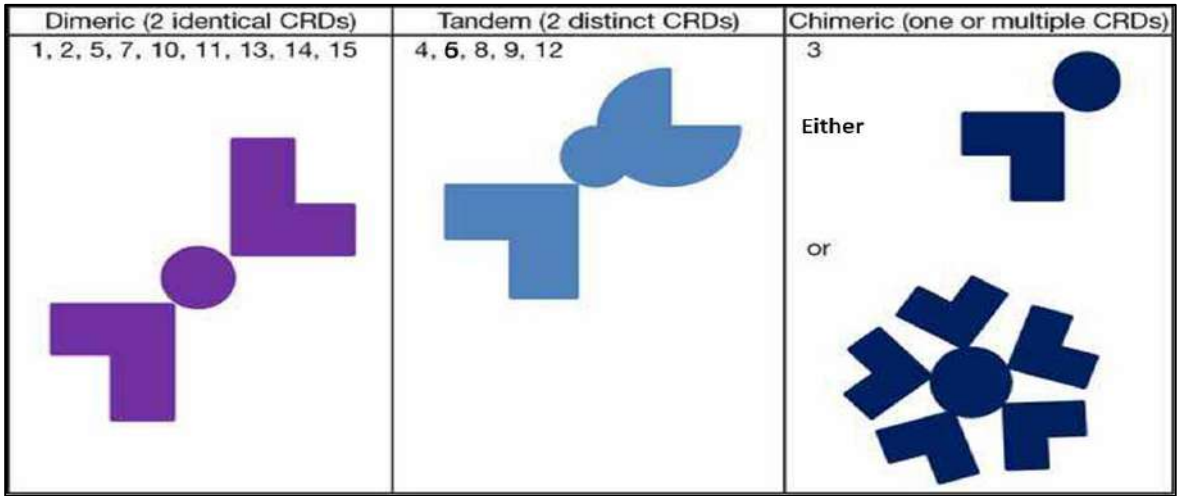


Figure 1-2: Classification and Structure of Different Galectins Categories [Ebrahim *et al.*, 2014]

The function of galectins varies with their tissue-specific and sub-cellular location, with their binding to carbohydrates makes them important fundamentals in several intra- and extracellular pathways [Modenutti *et al.*,2019; Pasmatzi *et al.*, 2019], thus galectins become more complicated in the study [Modenutti *et al.*, 2019; Thijssen *et al.*, 2015]. On the other hand, galectins are known to involve in various biological functions such as; cell adhesion, cell surface signalling, angiogenesis, proliferation, migration, invasion, inflammation and regulation of apoptosis [Hisrich *et al.*, 2020; Thijssen *et al.*, 2015]. Therefore; the role of galectins has been broadly studied in different types of cancers, especially with the most frequently observed galectin-1, 3 and 4, followed by 7 and 9 in which could be noted that galectins might have different functions in different malignancies, as well as it's possible to use galectins as effective targets for therapeutic approaches and/or diagnostic biomarkers [Chou *et al.*, 2018].

1.2.4: Galectin-9

Galectin-9 (Gal-9) was primarily isolated from '' mouse embryonic kidney in 1997 as a 36 kDa beta-galactoside lectin protein [Thijssen *et al.*, 2015; Chou *et al.*, 2018]. Human Gal-9 is distinct from other galectins

because it has multi-functions with strong immune modification impacts [Chou *et al.,* 2018]. Gal-9 is one of the utmost studied ligands for T-Immunoglobulin and mucin-domain containing protein-3 (TIM-3) associated with various tumour cells either stimulate or dampen tumour activity according to its links with different ligands on the cell surface of T-cells [Chetry *et al.,* 2018].

In addition, Gal-9 also has essential cytoplasmic intra and extra-cellular functions, as well as, it was involved in tumorigenesis by cell adhesion, proliferation, tumour cell transformation angiogenesis and T-lymphocytes apoptosis [Taghiloo *et al.*, 2017]. Furthermore, malignant cells have the ability to release Gal-9 through the process of autocrine due to the efficacy of immunoglobulin by T-cells and TIM-3.The TIM-3/Gal-9 complex is formed via Gal-9 ligand, as a result of this association through the induction mechanism of Gal-9, and the ability of Gal-9 to link with the TIM-3 receptors, and then the initiating to suppress T-lymphocytes and Natural Killer cells (NK), finally dysfunction is occurring [Taghiloo *et al.*, 2017; Kursunel and Esendagli, 2017], as clarified in **Figure 1-3**.

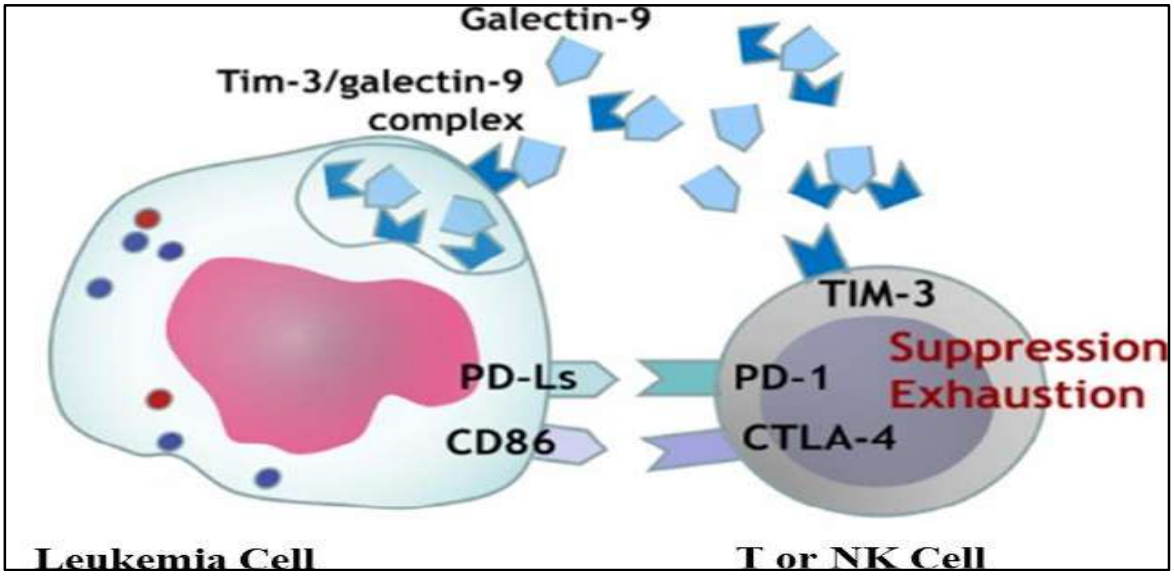


Figure 1-3: Immune Evasion of Leukemia Cells Through Co-Inhibitory Molecules [Kursunel; Esendagli, 2017]

Gal-9 was studied being in a wide range of solid malignancies such as gastric, breast cancer, prostate, bladder, and lung cancers; as well as hematological malignancies including; AML, CLL, Hodgkin and Non-Hodgkin lymphomas [Wang *et al.*, 2018; Pang *et al.*, 2018].

Gal-9 has exhibited higher levels in sera of patients with newly diagnosed CLL compared to healthy individuals that may be associated with the development and progression of the disease [Taghiloo *et al.*, 2017; Wdowiak *et al.*, 2019]. Other studies are suggested to reactivate of T-cells and secretion of cytokines by a variety of galectin inhibitors in the Gal-9/TIM-3 complex pathway as a result of choosing an optional range of therapies for patients with various malignancies and designing different protocols to activate immunotherapy and chemotherapy [Goncalves *et al.*, 2017].

1.2.5: Mannose Binding Lectin

Mannose-binding lectin (MBL) is an oligomeric protein molecule which is a C-type lectin made in the liver then secreted into the blood in response to infection [Auriti *et al.*, 2017]. MBL is formed of 96-kDa organizational units, which in turn are contained of three matchings 32-kDa main subunits [Gupta and Gupta, 2021]. It is characterized by fucose residues and N-acetyl glucosamine as molecular forms domains which are distinctive on the microbial surfaces in viruses, bacteria, fungi and parasites through identification the recognition of carbohydrates and stimulates the complement system for opsonisation of pathogens via a process of phagocytosis by polymorphonuclear (PMN) cells in which leading to the decomposition of the microorganisms [Gupta, 2020].

MBL participates in the immune system response against pathogens because it is one of the acute phase proteins, hence MBL is considered one of the most important components of the first line of defence in various immune

responses, especially in early childhood [Wahlund *et al.,* 2020]. MBL requires to be linked with serine proteases enzymes to form the Mannose associated serine proteases (MASPs)to motivate the complementary pathway of lectin. it's possible that MBL has a major role as anti-inflammatory due to the presence of an association between serum levels of MBL and levels of IL-6 which respond to release during the acute stage of disease [Cedzynski and Swierzk, 2020].

The probability of multiple genetic patterns of the low-levels in MBL is associated with a significant increase in the risk of developing ALL, especially at the early age of children in comparison with a healthy group which it's possible that the interactions between the immune system and early childhood infectious diseases could be particularly induced to cause ALL [Li *et al.,* 2019b]. Although it has been exposed that there is little data on the possible potential role of MBL in the mechanisms involved in the carcinogenicity pathway or through protection against cancer, while MBL is possible to be considered an important biochemical indicator that contributes to cancer risks in humans [Li *et al.,* 2019a; Sokolowska *et al.,* 2020].

1.3: Normal Cellular Oxidation and Oxidative Stress

Generally, oxygen is one of the most important molecules in various biological activities and energy production, it's approximately 1-3% amount of inhaled oxygen, as naturally converted into reactive species (RS) molecules which known as oxidants such as reactive oxygen species (ROS) [Quijano *et al.,* 2016].

Reactive molecules are divided according to their nature into two types:

- Free radical molecules like superoxide anion (O^{2-}) and nitric oxide (NO).
- Non-radical molecules such as malondialdehyde (MDA) and hydrogen peroxide (H_2O_2).

Free radicals are highly reactive molecules by unpaired electrons in an atomic or molecular orbit. Meanwhile, free radicals are produced under specific physiological conditions during aerobic metabolism [Ling *et al.*, 2018]. RS molecules have a pivotal role in phagocytosis processes, as well as free radicals are involved in the biosynthesis of prostaglandins by oxidized polyunsaturated fatty acids through the lipid peroxidation process as well as, RS molecules have a significant role in the ‘’intracellular signalling’’ pathway and play a central role in the therapeutic action of drugs [Herb and Schramm, 2021].

The main intracellular production site of ROS in living organisms is mitochondria via oxidative phosphorylation [Qazi and Khurshid, 2018; Zhao *et al.*, 2019]. Under normal physiological conditions, the cellular defence mechanism is principal to protect the vital cellular components from the effect of cellular damage in which produced by ROS, and that’s through a group of anti-oxidant defence systems by different protective enzymes including; glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and peroxiredoxins (Prxs) [Zhang *et al.*, 2018; Namrata *et al.*, 2019], while another group of anti-oxidants molecules which named the non-enzymatic endogenous and exogenous antioxidants that consist of glutathione, α-tocopherol, beta-carotene, ascorbic acid and plant flavonoids which act as scavengers of free radicals and thereby prevent the formation of new free radical molecules [Hangauer *et al.*, 2017].Therefore; the antioxidant defence system has a key role in the suppression of various diseases, and their clinical manifestations which is working against the production of further ROS [Balasaheb and Pal, 2015].

On the other hand, ROS has related to a wide range of tumour and non-tumour diseases [Rezaieg and Musleh, 2019]. In pathological conditions, the increasing of RS and/or reduction of antioxidants leads to enhance oxidative

stress (OS), which results in tissue damage due to a state of imbalance between oxidants and antioxidant activity [Matlab and Jasim, 2017].

According to the roles of free radicals in the living cells and potentially of their harmful effects with the destruction of vital cellular molecules, the free radicals have the possibility, in turn, to convert the normal cells into cancerous cells [Rasool *et al.,* 2015; Matlab and Jasim, 2017].

Leukemic patients have more OS and inflammatory responses, then it's increased the extent of ROS which causes cell death via necrosis, thus OS could lead to excessive production of ROS in which have quite a role in many hematopoietic leukemias that including acute and chronic leukemia [Dong *et al.,* 2021]. As far as, the normal activity of anti-oxidants levels would become insufficient to suppress the excessive production of free radicals [Matlab and Jasim, 2017], which may lead to increasing the activity of cancer-stimulating genes (oncogenes) and causing carcinogenicity [Klaunig and Wang, 2018].

Alternatively, many studies have been pointed to the essential role of non-enzymatic antioxidants such as; glutathione, vitamins C, D, E and some natural compounds that intake nutrients which consider as natural antioxidants have vital roles in the treatment of some cancerous tumours, as these compounds either inhibit or eliminate the growth of cancer cells [Kabeel *et al.,* 2018]. other studies have focused on the use of antioxidants in many different types of cancer therapies as alternative treatments to prevent the occurrence of carcinogenic conditions and reduce the likelihood of spreading the cancerous cells to other regions of the body [Singh *et al.,* 2018].

1.3.1: Nitric Oxide

Nitric Oxide (NO) is a simple structure molecule and quite unstable with a very lipophilic free radical with a little molecular weight to be allowed it in diffusion through cell membranes to achieve a wide range of functions in

many biological systems [Picón-Pagès *et al.*, 2019]. NO produces by transforming the amino acid L-arginine to L- citrulline, with releasing of the oxidized nitrogen via means of the nitric oxide synthases (NOs).

$$\textbf{L-Arginine} \xrightarrow{\textbf{NO synthases}} \textbf{L-Citrulline + NO}$$

NO bioavailability is structurally simple in common which has a very short half-life of 6-10 seconds in the blood, and its end products are nitrate or nitrite which are considered as an indicator for its production [Levine *et al.*, 2015]. NOs has four isoforms formed of NO, in which is synthesized stable and non-volatile molecules that are known for the NOs enzyme: neuronal NOs (nNOs), inducible NOs (iNOs), endothelial NOs (eNOs), and mitochondrial NOs (mtNOs) [Clementi and Nisoli, 2015; Lundberg, 2016].

NO has various actions at the physiologic and pathologic levels which associated with neurotransmission, cardiovascular homeostasis, vasodilatation and the metabolism of cells, besides, it is also related to numerous inflammation and immune responses, particularly in cancer growth and wound healing process, as well as NO, could be an intermediary in various mutations, carcinogenicity, and inflammatory [Lundberg, 2016; Hu *et al.*, 2020].

Hematopoietic cells could produce NO and influence the processes of growth and differentiation [Cheng *et al.*, 2015]. It's possible that NO and its derivatives become cytostatic and cytotoxic factors, as well as it could promote cancer growth by which the controlling on cancer by several steps of carcinogenesis through up-regulating transmuted of tumour suppression protein (p53) in the cells which could be modulated tumour cell DNA and contribute to tumour angiogenesis via regulating vascular endothelial growth factor (VEGF) [Levine *et al.*, 2015; Cheng *et al.*, 2015].

Several studies had shown that the three isoforms of NO may contribute to enhancing the aetiology of cancer and NO may associate with various carcinogenesis stages [Cheng *et al.,* 2015; Ghaffaria *et al.,* 2015; Khan *et al.,* 2020]. The levels of NO raised up in different types of leukemias, including CML and AML, while iNO expression is elevated normally in cancer cells [Khan *et al.,* 2020]. Conversely; other studies have indicated that the high levels of NO have cytotoxic properties in cell lines of patients with different malignancies; such as lymphoma or leukemia [Umbrello *et al.,* 2015; Sangwan *et al.,* 2016].

Various studies suggested that remained a significant elevation in the levels of NO in CLL patients after delivered chemotherapy which could be remarked a possible to be mediated mechanisms of cancer growth, progression and metastasis [Cheng *et al.,* 2015; Sangwan *et al.,* 2016]. The possibility of increased levels of NO could be mediated to the chemotherapeutic treatment, especially for patients with ALL and CLL Leukemias, and then NO could be considered a potential chemotherapeutic indicator in the treatment of patients with different types of cancers especially in ALL, particularly a certain value of treating leukemia by using NOs inhibitors [John *et al.,* 2011; Cheng *et al.,* 2015; Sangwan *et al.,* 2016]. Also, it could be a diagnostic and prognostic tool during the treatment of leukemic patients [John *et al.,* 2011; Sangwan *et al.,* 2016].

1.3.2: Glutathione and Oxidized Glutathione Disulfide

Glutathione (GSH) is a non-enzymatic antioxidant, exists in most organisms, ranging from bacteria to humans and is composed of three important amino acids γ-L-glutamyl, L-cysteinyl, and glycine [Giovanna *et al.,* 2017; Miess *et al.,* 2018]. GSH is found in the blood and could be isolated from the liver and muscles and there are two different forms of glutathione,

Active form which is known as reduced glutathione (GSH), it represents the majority of intracellular GSH about 90-95% in the cytosol, nucleus, and mitochondria [Bajic *et al.*, 2019]. **Inactive form**, it called oxidized glutathione (GSSG) that represents approximately 10% in the mitochondria and endoplasmic reticulum [Kennedy *et al.*, 2020; Mari *et al.*, 2020]. GSSG is converted into GSH by glutathione reductase (GR) in presence of nicotinamide adenine dinucleotide phosphate (NADPH) [Couto *et al.*, 2016; Salbitani *et al.*, 2017].

GSH has normally antioxidants features to protect cells against damage by oxidants, as well as it plays a key role in the control of many cellular processes such as; DNA and protein synthesis, gene expression, signalling pathways, cell growth, differentiation, apoptosis, and inhibits cell destruction produced by ROS particularly that relevant with carcinogenic mechanisms [Teskey *et al.*, 2018].

In humans, GSH is considered an essential indication for various diseases and its concentrations could be correlated with different pathogenesis include; respiratory diseases, alcoholic liver disease, rheumatoid arthritis, Alzheimer disease, and leukemia [Simpson *et al.*, 2015; Silvagno *et al.*, 2020].

The increase of ROS levels and reduction of GSH levels are associated with over expression of mucin-1 (MUC1-C) oncoprotein which is frequently related to AML. Several studies have been indicated to impaired the antioxidant system due to the increase of free radicals in childhood leukemia with a negative correlation between GSH with MDA, vitamins (A, E, and C) respectively, and then it is possible causing of the increase in liver enzymes, but a decline in the liver glutathione that could be as a target response of toxic effects by free radicals in leukemia patients [Rezaieg and Musleh, 2019].

1.4: Serotonin

Serotonin (ST) is a biogenic amine, formed by alteration of an essential amino acid (L-tryptophan) which is gained mainly from the nutrition into 5-hydroxytryptamine using tryptophan hydroxylase and 5-hydroxy tryptophan decarboxylase enzymes [Sarrouilhe and Mesnil, 2019; Yabut *et al.,* 2019]. It has a number of physiological and behavioural functions, it acts as a neurotransmitter in the brain, and is produced within serotonergic neurons of the central nervous system (CNS) where it regulates the behaviour, sensory functions, mood, appetite, sleep, cognition, endocrine action, and gastrointestinal functions [Szabo *et al.,* 2018; Julian *et al.,* 2019]. Moreover, it has a vital metabolic effect to enhance nutrient absorption and storage of glucose and fatty acids, though increasing lipogenesis and insulin secretion and decreasing lipolysis [Yabut *et al.,* 2019; Julian *et al.,* 2019].

ST is synthesized and released by enterochromaffin cells, which coat the lumen of the gastrointestinal tract (GIT).it has a fundamental role in the gut regulation, motility of the intestine, and secretion [Elshayeb *et al.,* 2016; Periayah *et al.,* 2017]. ST stored within granules in blood platelet cells and involved in the platelets aggregation as a local facilitator in vasoconstriction during blood clotting that leads to regulate of homeostasis [Periayah *et al.,* 2017]. Furthermore, ST is appeared to be a growth factor for tumour cell lines in several types of malignancies [Sarrouilhe and Mesnil, 2019; Liu *et al.,* 2017].

ST exhibits a complex mechanism to evoke signalling pathways that stimulate tumour development in some cancerous types, it was represented as a pattern of mitogen as a specific receptor pathway [Liu *et al.,* 2017; Zweckstetter *et al.,* 2021]. Moreover, the information available on ST participation in cancer cells migration, metastatic processes, as well as it might be elaborated in one or more essential phases of cancer progression

and/or growth of the primary tumour, invasion up to metastasis [Rasha and Matlab, 2017]. ST can be used as a specific tumour marker in particular types of cancers such as hepatic, pancreatic and GIT cancers [Elshayeb *et al.,* 2016; Rasha and Matlab, 2017]. ST levels have significant correlations to the incidence of various types of cancers, as lymphoma and colorectal cancer, whereas it seems that ST levels may be affected by the type of cancer and the cytotoxic effects of chemotherapy in different cancer patients, therefore, it's possible that ST levels were associated with the stimulation of ST receptors [Rasha and Matlab, 2017; Ballou1y *et al.,* 2018; Zmudzka *et al.,* 2018].

1.5: Erythropoietin Hormone

Human erythropoietin (EPO) is a glycoprotein hormone that has a molecular weight of 30.4 kDa, the main sites of its production are peritubular capillary endothelial cells in the renal cortex in adults [Suresh *et al.,* 2020]. Essentially, it is produced in the liver in small amounts during fetal stages [Gaine *et al.,* 2017]. The production of erythrocytes in the bone marrow is normally organized by EPO in response to the cellular decreased amounts of oxygen, thus the declinein oxygen levels is a major factor to stimulate EPO production, and then increasing number of RBCs which deliver oxygen to the tissues, so the disturbance of EPO levels is generating interruption of RBCs production in the bone marrow [Denka, 2016].

The elevationof EPO levels are contributed to the occurrence of numerous cancer especially leukemia, as well as, anemia which accompanied by different leukemias, and it seems that EPO could be one of the therapeutic factors that are used in the treatment of anemia caused by chemotherapy and radiotherapy in patients with malignancy, particularly in ALL and CLL patients [Bhoopalan *et al.,* 2020; Khalife *et al.,* 2021].

1.6: Parathyroid Hormone and Calcium

Parathyroid hormone (PTH) is one of the important protein hormones, which it has consisted of 84-amino acids that are regulated as a single-chain polypeptide. As well as, PTH is made and secreted through chief cells of the parathyroid glands which are situated on the posterior surface of the thyroid gland [Bhattarai *et al.*,2020].

Both PTH and calcitonin hormones have a central role in the control of calcium ion (Ca^{2+}) balance and regulation of the movement of Ca^{2+} among intestine, bone, and kidney [Inaguma *et al.*, 2017; Hannan *et al.*, 2018; Dhivyasree *et al.*, 2018], as well as it has an important regulator role in binding amino acids to their receptors on the cell membrane by cellular pathways [Randolph *et al.*, 2016].

Ca^{2+} is an important element in the body for various vital functions in the intracellular and extracellular, as well as it's associated with contractions muscles cycle as a cross-bridge binding with actin and myosin proteins [Bhattarai *et al.*, 2020]. Serum $Ca^{2+}$is existing to be approximately 50% as free Ca^{2+}, and/or nearly 40% bound to plasma proteins with closely 10% chelated to serum anions in complex of total circulating calcium [Kaku *et al.*, 2015]. Abnormal concentrations of serum Ca^{2+} (hypercalcemia or hypocalcemia) are contributed to different pathological disorders [Randolph *et al.*, 2016; Kaku *et al.*, 2015].

Secretion of PTH is stimulated by decreasing Ca^{2+} levels in the blood (hypocalcemia) in combination with calcitonin secreted by the C cells in the thyroid gland to provide some complementary mechanisms to sustain blood Ca^{2+} levels within optimal limits. PTH passes through the blood to act in bone, intestine, and kidney, then commencing responses that increase the concentration of Ca^{2+} in the blood [Kaku *et al.*, 2015; Young *et al.*, 2018]. Consequently, the raise of blood Ca^{2+} concentration is a negative feedback

index in which turn off the reaction of the decreasing Ca^{2+} in patients, and then finally the releasing of PTH, as the elevated PTH levels would be enhanced serum Ca^{2+} levels by acting on major target organs include; intestine, bone, and kidney by normal homeostatic mechanisms through three ways as following: [Young *et al.*, 2018; Silverthorn, 2018].

(1) Direct action on bone by increasing the rate of bone resorption via osteoclasts which are responsible for dissolving and breakdown the calcified bone matrix. **(2)** Direct action on the kidney by increasing the rate of renal tubular reabsorption of Ca^{2+}especially, in the renal distal nephron and inhibiting the reabsorption of phosphate ions from the glomerular filtrate.

(3) PTH indirectly action on intestinal by the promotion of absorption Ca^{2+} from the small intestine through its involvement and influence on vitamin D, as clarified in **Figure 1-4.**

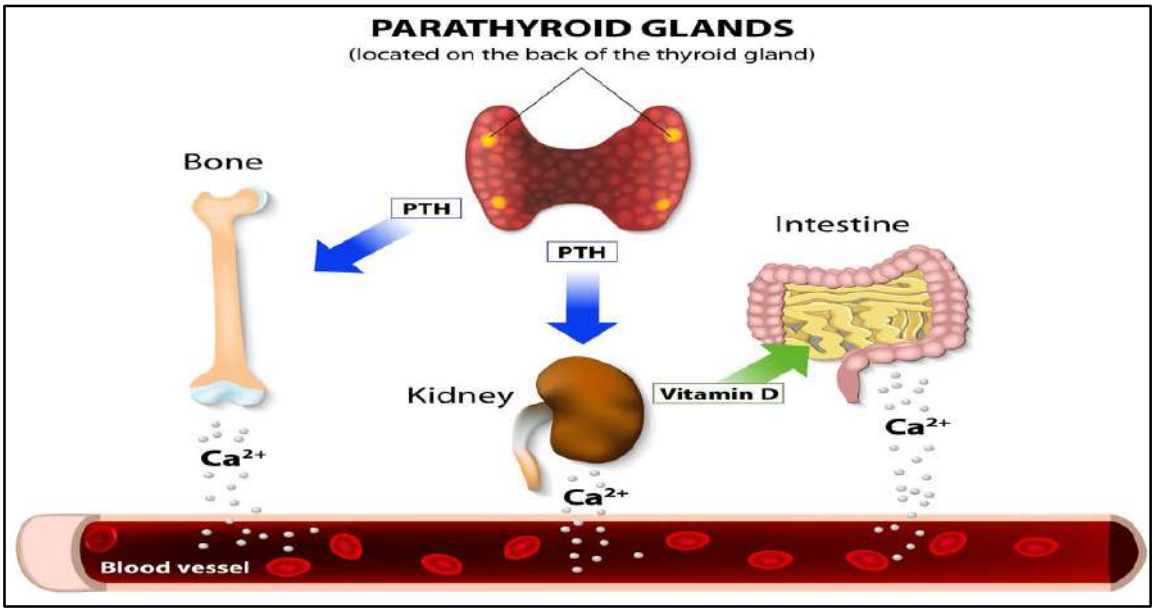


Figure 1-4: The Major Target Organs Affected by Calcium Regulatory Hormones [Young *et al.*, 2018]

PTH is mostly mediated with hypercalcemia in patients with leukemia, likewise, according to Dhivyasree's study hypercalcemia is relatively one of the important complications in childhood ALL [Dhivyasree *et al.*, 2018], as

well as, it has predictable between 5-20% as common complications in various malignancies of adults [Dhivyasree *et al.*, 2018; Bechir *et al.*, 2017].

1.7: Vitamin D_3

Vitamin D_3 (Vit D_3) is a progenitor of steroid hormones, it is one of the lipid-soluble vitamins, therefore; Vit D_3 could be deposited in adipose tissues [Seyedalipour *et al.*, 2017]. Moreover, it has a significant role in regulating the metabolism of calcium, phosphate, and magnesium in the intestine and kidneys, as well as in bone mineralization to conserve mineral balance by a wide range of biological effects on bone cells, along with it is involved in numerous cellular functions in the body such as; proliferation, differentiation, immune regulation and apoptosis [Casan *et al.*, 2017; Pilz *et al.*, 2019].

In circulation, Vit D_3 is associated with Vit D_3 binding protein (DBP), and then it should be passed through a number of hydroxylation stages to be an effective vitamin, the first hydroxylation mostly comes to pass within the liver, leading to the formation of 25(OH)D or calcidiol, and the second hydroxylation occurs in the kidneys, epithelial cells of the intestine and immune cells by using various enzymes to form the most active of Vit D_3 which called 1,25 Dihydroxy vitamin D_3 ($DHVD_3$), or calcitriol, as far as the active form of Vit D_3 is contributed in many regulatory mechanisms to protect target tissues or organs from different pathological disorders [Damoiseaux; Smolders, 2018; Krishna, 2019].

Vit D_3 activities are mediated through intracellular Vit D_3 receptors, these receptors of Vit D_3 are found in almost all cells in the body as well as, in different target organs such as; intestine, bone, kidney [Young *et al.*, 2018]. Moreover, Vit D_3 plays an important regulator role of immune system components, especially T-lymphocytes in development, differentiation and activity functions [Martens *et al.*, 2020].

Vit D_3 is recognized in cancer by which its cytotoxic properties on cancer cells thus, it acts as ‘’as anticancer’’ through suppression of carcinogenesis by the promotion of apoptosis, anti-proliferation of cells, and induction of differentiation, besides, it could be inhibited or suppressed tumours angiogenesis, invasion and metastasis [Jeon and Shin, 2018; Wadhwa *et al.,* 2018]. The main steps of Vit D_3 synthesis are illustrated in **Figure 1-5.**

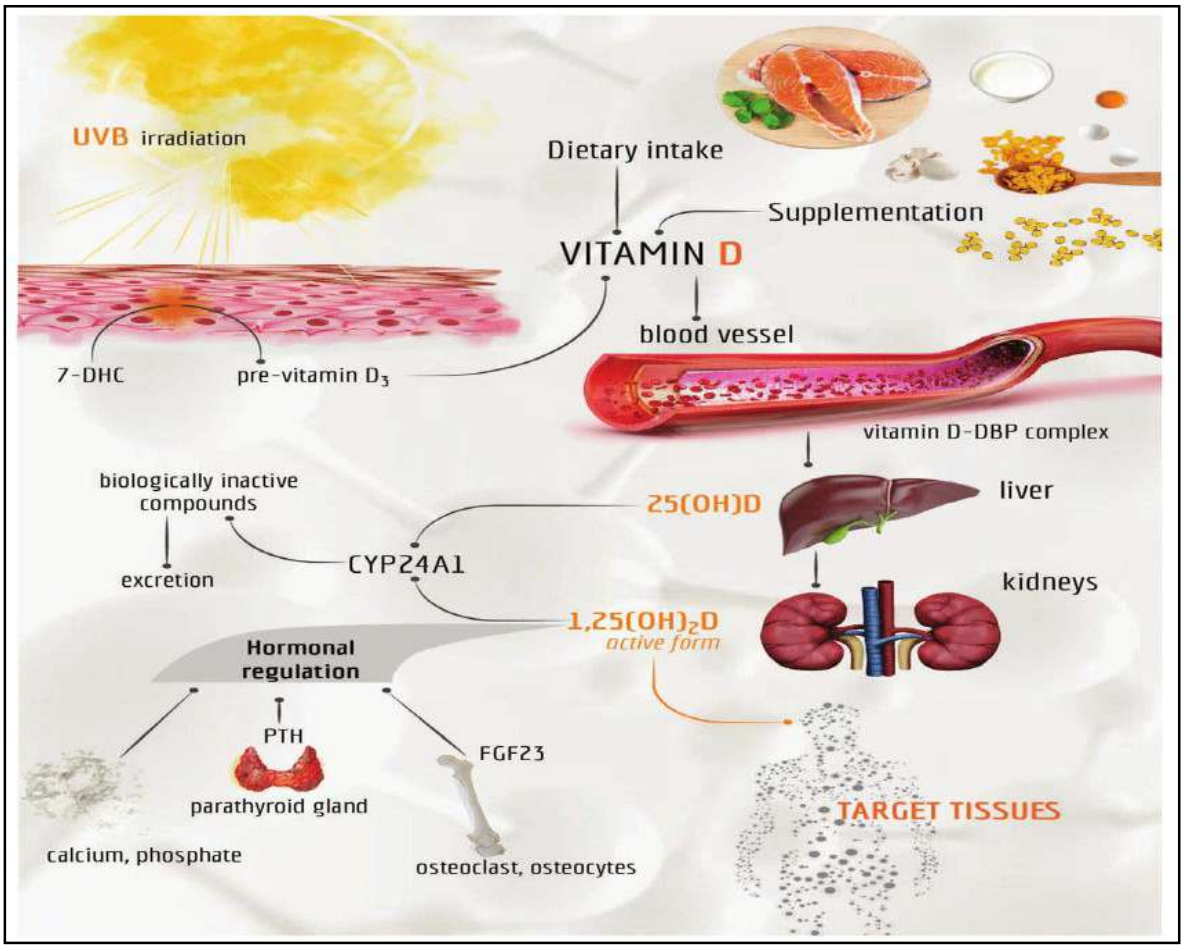


Figure 1-5: The Essential Steps of Vitamin D_3 Synthesis [Krishna, 2019]

1.8: Vitamin B_{12}

Vitamin B_{12} (Vit B_{12}) or it is called cobalamin is a water-soluble vitamin, it has a vital role as micro nutrients involved in metabolism, cell division and maintains body health with the efficient quantity of absorption and

transportation, as well as, it is important for the nervous system, synthesis of DNA, and erythropoiesis process [Castellanos-Sinco *et al.,* 2015].

The lack of vit B_{12} is a major public health condition that is common in hematological malignancies as compared to non-hematological diseases [Horie *et al.,* 2017]. Furthermore, the reduction in levels of Vit B_{12} is recognized considerably in CML, ALL and Non-Hodgkin's lymphoma (NHL) patients who are receiving therapy and accompanied with macrocytic anemia [Horie *et al.,* 2017; Bordbar *et al.,* 2018]. Besides, it has been pointed to decreased levels of Vit B_{12} and folic acid in dietary supplements of childhood ALL may actually be a causative factor for tumor-initiating, as well as the development of acute leukemia, as the decreasing levels of Vit B_{12} causes enormous changes in the bone marrow of patients with cancers, then causes severe pancytopenia, macrocytic anemia, and megaloblastic anemia [Obaid *et al.*, 2018; Konda *et al.,* 2019; Anjana *et al.*, 2020].

Thus, the incidence of Vit B_{12} deficiency is varied among specific cancer types [Singh *et al.,* 2015]. Hence, it could be caused developing a type of anemia known as pernicious anemia due to a failure of parietal cells in the gastric glands to produce an important factor called intrinsic factor to form a complex with Vit B_{12}, and then it is required for Vit B_{12} absorption [Andres *et al.,* 2016; Hannibal *et al.,* 2016]. Therefore; the deficient of Vit B_{12} may be specifically caused by the absence of an intrinsic factor and then failure of Vit B_{12} to absorb by the intestine, also, it may directly cause a severe decline in the erythropoiesis process which depends upon Vit B_{12} [Green *et al.,* 2017; Sezer *et al.,* 2018].

Alternatively, some studies have been shown that the high levels of vit B_{12} could be a potential biomarker for pathogenesis, diagnosis and prognosis of malignant (solid or hematological) tumors, however, the rise of Vit B_{12}

levels is not yet completely understood [Arendt *et al.,* 2016; Arendt *et al.,* 2019].

Recently, it is noticed that the raised of Vit B_{12} levels are associated with poor survival with mortality among patients with specific malignancies, which indicates more progressive and aggressive cancers and thus it reflects the metabolic changes of vit B_{12} that may cause cancer [Arendt *et al.,* 2019; Hoogstraten *et al.,* 2019]. Vit B_{12} levels are significantly high values in patients suffering from CML and AML patients, while ALL and CLL patients have variables between normal serum levels to moderate elevations of Vit B_{12} [Hoogstraten *et al.,* 2019].

1.9: Total Protein

Proteins are important construction masses for the formation of all cells and tissues, as the proteins represent the basic part of most organs, as well as in various enzymes and hormones, thus proteins regulate numerous functions of the body such as growth and development, as the total serum protein is measured two types of serum proteins which comprising of albumin (Alb) and immunoglobulins (Igs) [Kornblau *et al.,* 2018].

Alb is mostly made by the liver and forms approximately 60% of the total protein (TP). It has a main role in carrying vitamins, hormones, medications, and some elements like Ca^{2+} throughout the body, as well as it's essential for tissue growth and healing. Alb is considered as an indicator for chronic inflammation in various human cancers as one of the acute-phase proteins [Bozkaya *et al.,* 2019]. Igs are primarily made up the residual 40% of proteins in the blood, they consist of diverse subtypes of proteins are known: alpha, beta, and gamma, some of them are formed by the liver, whereas others are produced via immune system cells, as the Igs carrying iron metal which

necessary for binding with hemoglobin in the blood [Pagana *et al.,* 2016; Chernecky and Berger, 2017].

TP levels could be increased or decreased according to several disorders as liver diseases, renal nephrotic syndrome, congestive heart failure, dehydration, malabsorption, inflammatory conditions, multiple myeloma and some type of leukemia [Bozkaya *et al.,* 2019; Chernecky and Berger, 2017].

Decreasing of total protein levels and hypoalbuminemia in ALL patients at diagnosis period and then begin to increase throughout the induction therapy, it's possibly due to the catalytic state in ALL patients is associated with a widespread suppression of nutrients absorption during, as the growth of cancerous cells in leukemia patients is accompanied by the synthesis of glutamine synthesis and ammonium ion (NH^{4+}) scavenging [Chung *et al.,* 2020]. Besides, the TP and Alb levels could be an independent prognostic factor for survival in several cancer diseases, especially ALL and AML to be considered either before beginning treatment or at the same time of starting induction therapy particularly patients with hypoalbuminemia [Dyczynski *et al.,* 2018].

1.10: Ferritin

Ferritin (FT) is one of the most important medical indices proteins which is indicated by the amount of iron content stored in the humans' body [Murphree *et al.,* 2020]. It could be considered as an assistant diagnostic marker in patients with leukemia, although high levels of ferritin have an impact on the onset of many diseases; such as cardiovascular diseases and some other malignant tumours [Cullis *et al.,* 2018].

Previous studies on serum FT concentrations have shown variable levels of FT in patients with different forms of leukemia and during various stages of therapy [Hamad *et al.,* 2019; Ihlow *et al.,* 2019; Wang *et al.,* 2019a].

Additionally, several epidemiologic studies have shown that the higher levels of FT associated with several human cancers during different age groups with a medical historic hereditary of cancer in combination with the period before and after chemotherapy [Bertoli *et al.,* 2019; Lee *et al.,* 2019; Nair *et al.,* 2018].

It's published that the patients with different hematologic malignancies have higher FT levels at the time of diagnosis than in the course of remission induction stage in multiple myeloma and malignant lymphomas, however; FT concentrations are still lower than patients who have acute leukemia, whereas the causes somewhat unknown [Hamad *et al.,* 2019; Senjoa *et al.,* 2018].

Conversely, the decreasing level of FT concentrations was recorded in patient's survivors of ALL those underwent chemotherapy for a long-term period of treatment that would be useful prognostic and prediction markers for relapse in advance of programs therapy, and it's similar to be in AML patients [Hamad *et al.,* 2019; Ihlow *et al.,* 2019]. Eventually, the revealing monitoring of any alteration in serum ferritin concentrations can be a helpful indicator for evaluating conditions and predictive signs in patients with various malignancies to improve of the therapeutic regimens [Ihlow *et al.,* 2019; Senjoa *et al.,* 2018].

1.11: Trace Elements

In general, the human body requires a wide-ranging of nutrient elements to complete normal physiological processes and the majority of these elements play pivotal roles in the continuity of many vital cellular functions [Mohammad and Fezea, 2016]. Numerous trace elements are required as enzymatic cofactors in physiological and metabolic processes and protect cells against oxidative stress [Elshaygi, 2018]. Recently, numerous studies have been prepared on the trace elements and their effects on the aetiology of

neoplastic diseases [Elshaygi, 2018; Zekavat *et al.*, 2020]. The variations of trace elements levels that might be used as markers in prognosis and diagnosis of different pathological symptoms, fatal diseases, and several types of malignancies include colorectal, bladder cancer, lymphomas, and leukemia [Nawi *et al.*, 2019; Qayyum *et al.*, 2019; Saleh *et al.*, 2020; Valadbeigi *et al.*, 2019].

Zinc (Zn) is an important mineral in living organisms that spreads widely through the body. It's about 2g of Zn in the adult human body, which is distributed by 25% in the muscles and 65% in the bones [Lu *et al.*, 2021]. Zn is found in the vital cellular components, especially the nucleus, hence it plays an important role in the synthesis of DNA which controls the level of production of proteins in the cell, as well as its significance in the proliferation and differentiation of cells [Valadbeigi *et al.*, 2019; Lu *et al.*, 2021].

Zn acts together with copper (Cu)and manganese (Mn) as cofactors in holoenzyme for more than 300 enzymes to perform their certain functions perfectly [Asif, 2017; Rifai, 2018]. Acute cases of sickle cell anemia and imbalance in the immune functions of T-lymphocytes are associated with the acute deficiency of Zn [Molina-Lopez *et al.*, 2015].

Copper (Cu) is one of the main constituents in various biological functions by its contribution to energy release through involvement in oxidative and reduction processes [Abolbashari *et al.*, 2019]. Furthermore, Cu plays vital roles in metabolism processes, production of RBCs via ''erythropoiesis'', control of hemoglobin construction, regulation of blood pressure, and osteoporosis [Kardos *et al.*, 2018].

Cu is collaborated with iron (Fe) in inhibition the production of free radicals, other than in regulation catalytic agent of many common biological processes and antioxidant enzymes of superoxide dismutase (Cu/Zn-SOD) to

detoxify free radicals [Kardos *et al.,* 2018; Azeez *et al.,* 2015]. Cu levels have been shown a slight decline in sera of patients with different malignancies such as CLL, AML, CML, and ALL at the time of diagnosis [Elshaygi, 2018; Akhgarjand *et al.,* 2017], while higher concentrations of Cu levels were recorded in patients with ALL and AML, before received chemotherapeutic treatment [Asfour *et al.,* 2017; Kim *et al.,* 2019].

Cobalt (Co) is one of the essential minerals in mammals, it's found in the form of corrin ring in Vit.B_{12} [Prashanth *et al.,* 2015]. Co binds to four pyrrole rings in Vit B_{12} which is important in the active production of erythrocytes, as the enzyme-mediated cobalt reduction is either caused by cytoplasmic methylation of methyl cobalamin or through mitochondrial adenosylation to product adenosylcobalamin [Akiibinu *et al.,* 2019].

Most studies have been focused on the evaluation of Co levels in diverse groups of leukemia which illustrated a significant decrease in the levels of Co in patients with AML, CLL, CML, and ALL [Elshaygi, 2018; Akiibinu *et al.,* 2019]. Additionally, the decrease in Co levels which is combined with the deficient of other elements such as Cu, Zn, Mn, and Fe could be the main influences in the emergence of lack Vit.B_{12} and pernicious anemia accompanied by lymphatic diseases and leukemia [Prashanth *et al.,* 2015; Akiibinu *et al.,* 2019].

Manganese (Mn) is a necessary element for the cellular biological processes and consider an assistant metal for many important enzymes involved in various diseases, specifically in leukemia [Nancy *et al.,* 2016]. It plays a vital role with additional minerals in sustaining the integrity of DNA of the intra-membrane transfer, neural conduction, and then muscle contraction as well as in the functions of mitochondria in the cells [Nancy *et al.,* 2016; Muzolf-Panek *et al.,* 2017].

Iron (Fe) is one of the most copious intermediate minerals in the body that is one of some essential nutritional elements in the performance of many physiological processes in the body [Wang *et al.,* 2019b]. The body content of Fe is usually determined a proximate 5 g and is concentrated in the blood proteins, hemoglobin, bone marrow, liver, and kidneys [Wang *et al.,* 2019a; Yang, 2015]. Fe exists in two forms: the oxidized form (ferrous "Fe^{2+}") which is soluble; and reduced form (ferric "Fe^{3+}") which is insoluble form, as well as overload Fe may act as a stimulator for generation of ROS through Fenton's reaction in many pathological conditions leads to oxidative stress that causes DNA damage [Sun *et al.,* 2018a; Tahir and Obed, 2019] and development of variable types of leukemia [Tahir and Obed, 2019].

Fe also plays the main role as an enzymatic cofactor for a large number of oxido-reductases and it is stored by iron reservoir protein (ferritin) [Tahir and Obed, 2019; Wang *et al.,* 2019a], while it carries in the blood by transferrin which is naturally saturated with Fe, so total iron-binding capacity (TIBC) ratio is turnd to reflect the amount of iron entirely saturated with ferritin [Pfeiffer and Looker, 2017]. A moderate decrease in the Fe levels was recorded in patients with AML incidence synchronization with lowering levels of Zn and Cu [Prashanth *et al.*, 2015; Pfeiffer and Looker, 2017], while a slight increase in the Fe levels was noted in sera of patients with ALL that would go along with the rise in Se, Zn, and SOD levels, besides; the alterations in the Fe levels in various types of leukemia were registered during pathogenesis and prognosis especially in acute leukemias [Pfeiffer and Looker, 2017; Zekavat *et al.*, 2021].

1.12: Electrolytes

Electrolytes have an important role in metabolic and biological pathways, mainly in the production of some proteins and nuclear acids, as

well as, defence in opposing cellular oxidative stress [Tahir; Obed, 2019]. Electrolyte disorders are associated with diseases of the liver and renal failure, in addition to other disturbances which are specifically related to malignant diseases and chemotherapeutic treatment, as well the use of diuretic surge to decrease sodium and potassium levels [Shirali, 2016].

The low levels of Na^+, K^+, and Ca^{2+} are usually in most electrolyte complications which come across in ALL and AML patients with more morbidity and mortality [Bowman, 2017]. In particular, Na^+ and K^+ are significant reductions in ALL patients which combined with an increase of Ca^{2+} levels in patients with acute leukemia, so it is possible one of the main reasons to lead renal complications in acute leukemia patients [Shirali, 2016; Koumpis *et al.*, 2020].

Besides, electrolytes could be reflected as one of the mutual abnormalities of electrolytes that are caused by cancer and/or particularly related to the chemotherapy system protocols which are used [Shirali, 2016; Yang, 2020]. It could cause a serious of life-threatening with dangerous difficulties in patients with cancer, which is required expansion in therapeutic programmes [Bowman, 2017; Yang, 2020]. Accordingly, further studies are necessary to approve the possibility of using such elements disorders as diagnostic and prognostic tools for cancers [Ahmadi *et al.*,2018; Elshaygi, 2018].

Aims of The Study

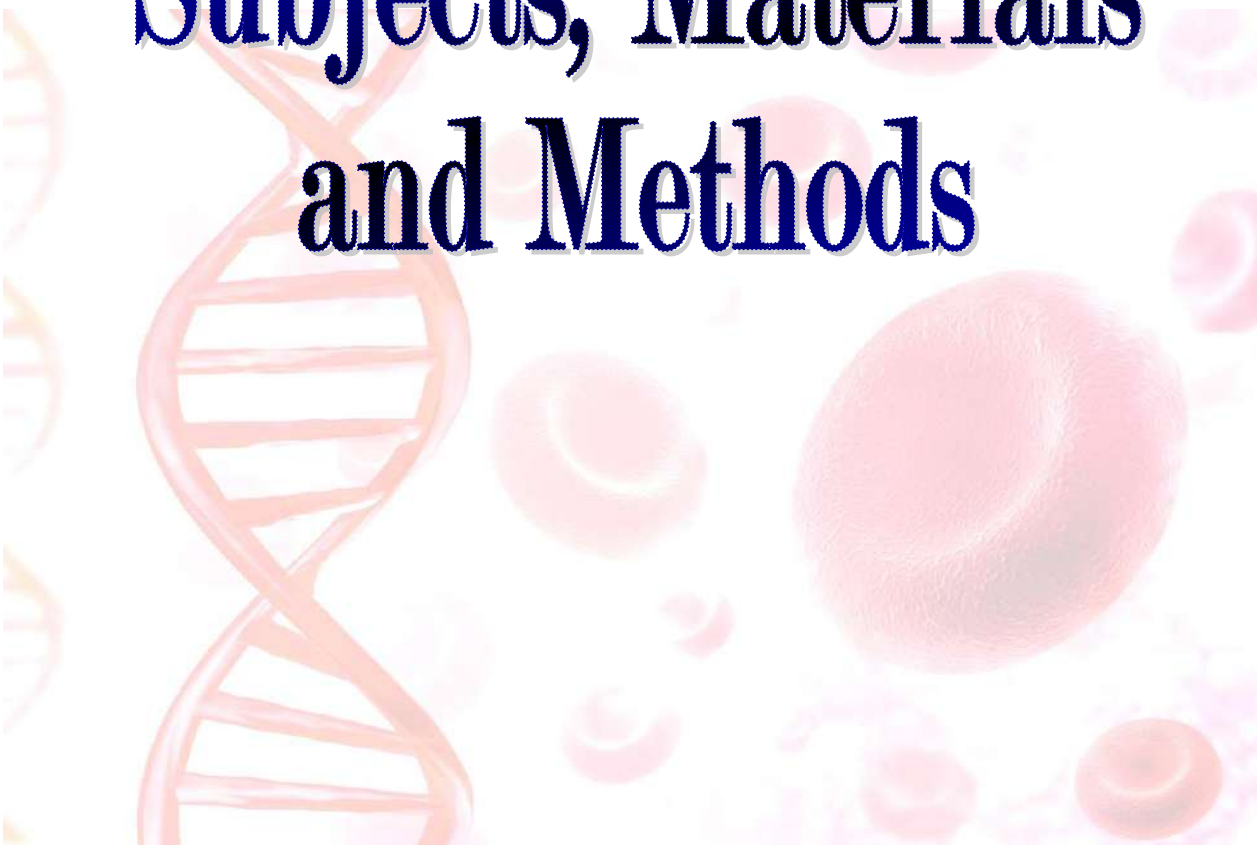
Leukemia refers to a wide range of malignant disorders in hematopoietic stem cells (HSCs) of the bone marrow due to the accumulation of abnormal white blood cells (WBCs), especially lymphocytes, which causes leukemia. Leukemia is considered one of the most aggressive, dangerous hematologic disorder types of cancers all over the world. The prevalence of all types of leukemia appears to be various according to age, gender, race and geographical distribution worldwide.

Thus the present study aims to accomplish set of tasks that can be summarized as following:

- Measuring the levels of two types of lectins (Galectin-9 and Mannose Binding Lectin) and number of hormones such as; (Serotonin, Erythropoietin and Parathyroid) in serum samples of children with ALL (before and during receiving chemotherapy) to evaluate them as indices in the diagnosis of this type of blood cancer and follow-up of response chemotherapy to increase survival rates and predict relapse.
- Assessment of the potential cellular damage caused by ALL and evaluate number of oxidative stress criteria, as well as antioxidants and number of coenzymes of the redox enzymes selected in the current study, then comparing their levels at diagnosis and after chemotherapeutic treatment.
- Follow-up of hematological and biochemical variables in children with ALL before and after chemotherapy.
- A Study of the potential correlations among the new parameters in the current work and the routine hematological along with biochemical parameters.

Chapter Two

Subjects, Materials and Methods



2.1: The Ethical Committee Approval

Approvals of the scientific committee in the Biology Department/College of Education for Pure Sciences/University of Karbala, as well as the Ministry of Health-Karbala Health Directorate (Department of Laboratories) of the current study were taken. Oral approval for the study procedure had been taken from all study participants who were enrolled in the study.

2.2: Chemicals

Kits used in the current work were supplied from different companies, as shown in the **Table 2-1**

Table 2-1: Kits with Manufactured Companies

Kit & Chemical	Company & Country
Human Erythropoietin ELISA Kit	Bioassay Technology Laboratory, China
Human Serotonin ELISA Kit	Bioassay Technology Laboratory, China
Human Nitric Oxide ELISA Kit	Bioassay Technology Laboratory, China
Human Mannose Binding Protein Lectin (MBL) ELISA Kit	Bioassay Technology Laboratory, China
Human Galectin 9 ELISA Kit	Bioassay Technology Laboratory, China
Human Ferritin Kit ELISA Kit	Bioassay Technology Laboratory, China
Total Glutathione/ Oxidized Glutathione Assay Kit	Elabscience, USA
Human Parathormone (PTH) ELISA Kit	Elabscience, USA
1,25- Dihydroxy vitamin D_3 ($DHVD_3$) ELISA Kit	Elabscience, USA
Vitamin B_{12} (Vit B_{12}) ELISA Kit	Accu-Bind, ELISA Microwells-Monobind Inc. USA
Calcium Method CPC Kit	Biolab SAS, France
Total Protein & Albumin Kit	Roche, Germany

2.3: Work Location and Equipment

The main measurements and evaluations of the current study parameters were done in the Central Laboratory of Imam Ali the Holy Shrine and Baniqia Specialized Medical Laboratories for Pathological Analysis in Al-Najaf Al-Ashraf Province, as well as in the Central Laboratory of Hematology and Oncology in Medical City of Imam Al-Hussain Hospital in Holy Karbala Province. The used equipment and their manufacture details are shown in **Table 2-2.**

Table 2-2: Equipment, Manufacturers, Countries and Models

Equipment	Model	Manufactured Company & Country
Blood Roller Mixer Machine	TYMR-ZA	Jiangsu Kangjie Medical Devices, China
Hematological Automatic Analyzer	Micros ES 60	Horiba Medical-ABX, China
Centrifuge	Z 200 A	Hermle, Germany
Frozen	Craft	Craft, China
Water Bath	LWB-111D	Labtech, Korea
Spectrophotometer	V-1100D Spect.	Emclab, Germany
Incubator	854-Schwabach	Memmert, Germany
ELISA (Microplate Washer)	MW-100A	Genex Laboratories, USA
ELISA (Microplate Reader)	MR-100	Genex Laboratories, USA
Vortex mixer	Digi-system	Taiwan
General Laboratory Equipment ‘’Cobas’’	Cobas c111	Roche, Germany
Atomic Absorption Spectrometry (AAS) (Scientific Instrument)	AA-6300	Shimadzu, Columbia
Medical-Electrolyte Analyzer Device	LW E60	Medical Landwind, Germany
FUJI DRI-CHEM Automated Clinical Chemistry Analyzer	NX500	Nishiazabu 2-Chome- Minato-KU, Japan

2.4: Patients and Healthy Controls

2.4.1: The Study Population

During the extended period between the beginning of March 2019 to the end of February 2020, 71 participants were enrolled in the current study. Thirty-one children residents of the Medical City of Imam Al-Hussain Hospital/Central Laboratory of Hematology and Oncology in Holy Karbala Governorate were included to participate in the current study as a basic group in the study. Moreover, based on several critical criteria, 40 healthy individuals were selected to participate in the current work as a control group.

2.4.2: Description of The Study Groups

Seventy-one participators in the current work were classified into two main groups and as follows:

Patients group: 31 children who were diagnosed with Acute Lymphoblastic Leukemia (ALL), their ages ranged between 2 and 12 years, who had no medical family history of cancer (18 males and 13 females).

Control group: 40 children who appeared to be healthy, their ages ranged between 2 and 12 years (20 males and 20 females). Full detailed information about the study participants were summarized in **Figure 2-1** and **2-2**.

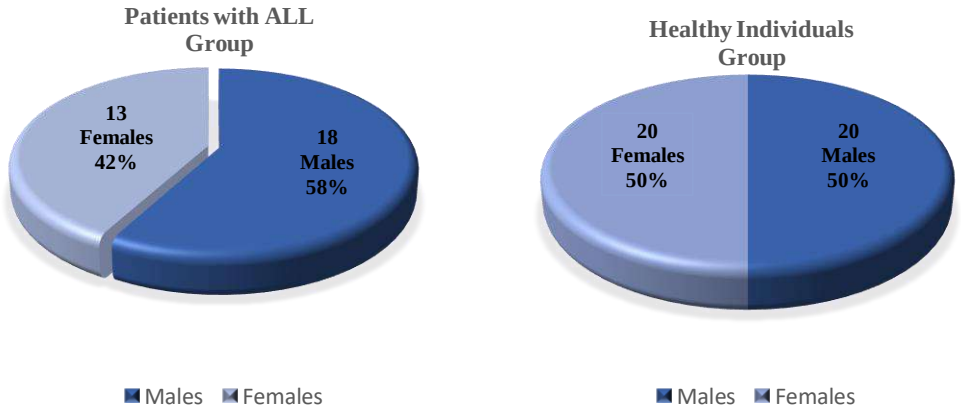


Figure 2-1: Distribution of Numbers and Percentages of Individuals Participating According to Their Genders

2.4.2.1: Inclusion Criteria of ALL Patients

Thirty-one of children with ALL in between the ages of 2 and 12 years were included in the present work. An initial diagnosis of ALL patients was completely performed by specialist physicians in Central Laboratory of Hematology and Oncology/Medical City of Imam Al-Hussain Hospital in Holy Karbala Governorate and through several clinical and laboratory tests, as well as by means of cytochemical tests and bone marrow biopsy smears. In addition, the patients were selected at this stage without received any treatment.

2.4.2.2: Exclusion Criteria of ALL Patients

The current study required the exclusion of group of children patients with ALL, include the following:

- Patients who have symptoms of other types of hematological malignancies such as; AML, CLL, CML, as well as of lymphomas, and other solid tumors.
- Patients who have previously undergone different types of cancer treatments having been treated and cured
- Patients who underwent splenectomy and hepatectomy.
- Diabetic children Patients (Type I) with diabetes complications added to cardiomyopathy and renal dysfunction
- Patients with thyroid and liver diseases

2.4.2.3: Treatment Protocol for The Study Patients

The patients with ALL groups in the current study were followed-up depending on the treatment protocol including at least 4 doses of chemotherapy to assess their physiological and biochemical responses, as in the following:

A) The children with ALL patients during Induction-Remission phase regularly received drugs of chemotherapy for 4-5 weeks of treatment, these comprised; L-asparaginase, vincristine, methotrexate and 6-mercaptopurine, as well as a steroid medication such as; dexamethasone. As usually, methotrexate is given to the

patients intrathecally (ALL children had gotten therapy into the cerebrospinal fluid (CSF).

B) ALL patients during the Consolidation-Intensification phase for 14-16 weeks, the children with ALL were treated with drugs such as methotrexate, 6-mercaptopurine (6-MP), vincristine, L-asparaginase, and prednisone or dexamethasone. Intrathecal therapy (as prescribed above) is continued at this stage.

As regards the questionnaire, it has been approved in the current work and designed according to the opinion of specialists, which includes information on the following: age, gender, place of residence, the period of onset symptoms of disease, any medications used by patients or any other diseases experienced by patients and family medical history. Full information was provided on the patients who enrolled in the present study through oral interviews with patients' parents and with their accompanying relatives, as well as the physicians supervising patients.

2.4.2.4: Criteria of Controls Selection

The selection of healthy individuals as a control group based on several criteria included: they didn't have a family medical history to any types of leukemia or other types of cancers, no current medication, they were not subjected to major surgical intervention. Besides, they should show a subjective perception of a good health as determined by health questionnaire. Moreover, the control group might be at an approximate age range with the patients group. Finally; they were appeared to be in similar to the patients group in the terms of food style.

Full data on participants involved in the current study is summarized in the **Figure 2-2**.

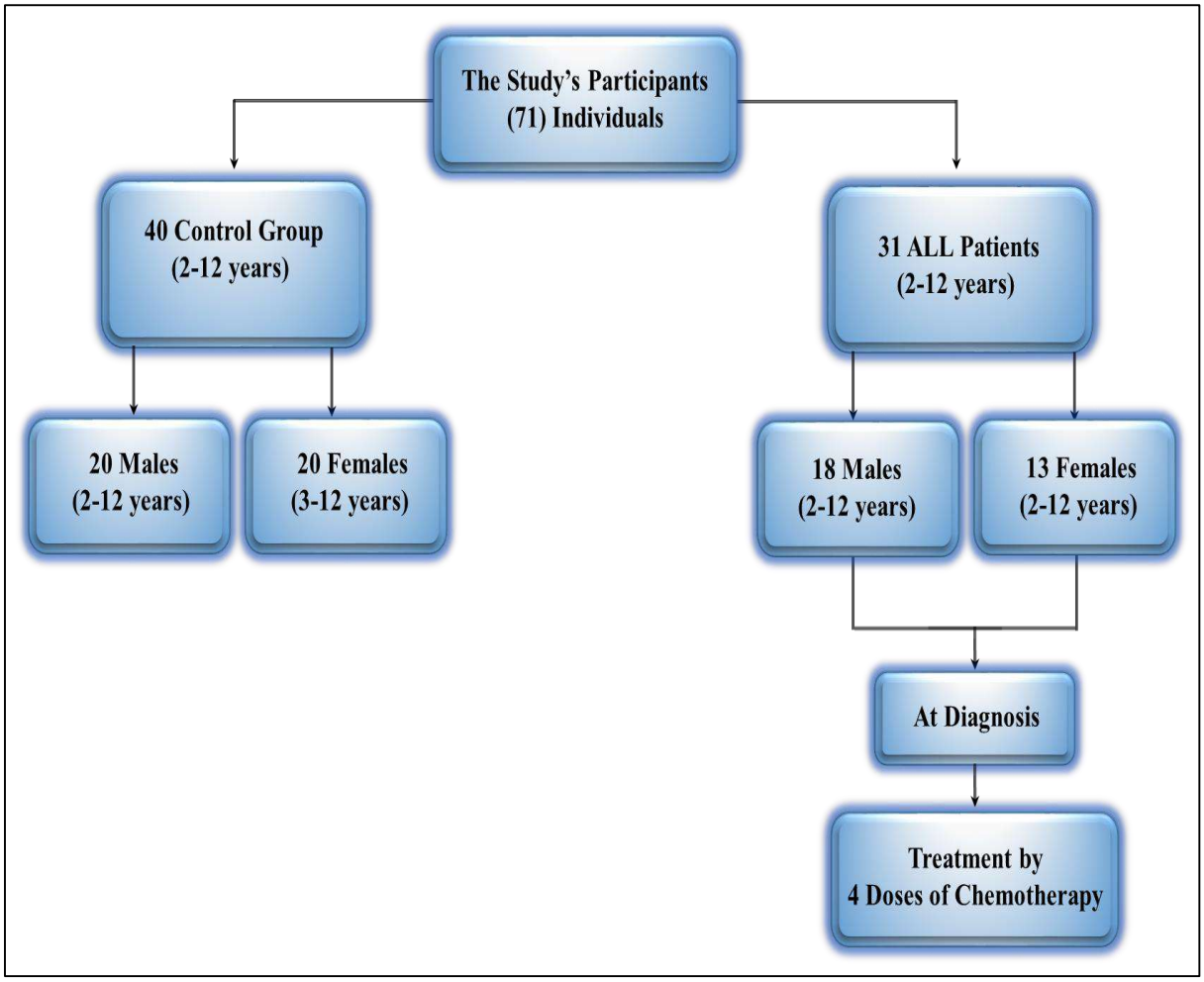


Figure 2-2: Distribution of Individuals Participating in The Study

2.5: Sample Collection

In the morning, approximately at 9 a.m. and after, at least 8 hours of fasting, A quantity of five millilitres of intravenous blood samples were collected from the patients and healthy subjects (Pre-treatment with chemotherapy), while the participants were fasting at least 8 hours, the blood samples were divided into the following parts:

- One millilitre of whole blood was added to EDTA- anticoagulant tube to use for Complete Blood Count (CBC).

- Four millilitres of blood samples were allowed to coagulate at laboratory temperature, and then centrifuged at 5,000xg for 10 minutes.

Isolated sera samples were collected and stored by Eppendorf tubes utilised after divided into the following parts:

The first part was used to evaluate EPO, PTH, ST, FTP, vit D and vit B_{12}, as well as, TP and Alb.

The second part was used to evaluate the concentrations of NO, GSH and GSSG.

The third part was used to measure Galectin-9 (Gal-9), Mannose Binding Lectin (MBL), and selected Trace Elements (Zn, Co, Fe, Mn, and Cu) and Electrolytes (Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, and K^{+}). These parts were kept at -18°C after separation until used.

2.6: Clinical Assays of Lectins

2.6.1: Calculation of Galectin-9 (Gal-9) Levels

- **The Principle**

Sandwich-Enzyme-Linked Immune Sorbent Assay (Sandwich-ELISA) method was applied to evaluate Gal-9 concentrations. Sandwich-ELISA technique is based on the reaction between the microplate pre-coated with human Gal-9 antibody (Capture Ab) and Gal-9 in the serum sample (Gal-9 Ag), then a biotinylated detection antibody specific Gal-9 and Streptavidin-Horseradish Peroxidase (HRP) conjugated, then the microplate wells that contain Gal-9, biotinylated detection antibody (primary Ab), and streptavidin-HRP (enzyme-labelled secondary Ab) conjugate will reacting, blue color will appearing. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution (stop solution) and the color turns yellow quickly. The absorbance (Abs) was measured spectrophotometrically at the wavelength of 450 nm. Gal-9 concentration in the sample was calculated by comparing the absorbance of the sample to the supplied standard curve.

Reagents

- Standard Solution
- Biotinylated human Gal-9 antibody
- Streptavidin-HRP
- Wash Solution
- Substrate Solution A
- Substrate Solution B
- Stop Solution

The Procedure

Additives	Standard (S)	Sample (T)
Standard Human Gal-9	**50 µl**	-
Serum Sample	-	**40 µl**
Anti-Gal-9 antibody	-	**10 µl**
Streptavidin-HRP	**50 µl**	**50 µl**
Mix well and cover the microplate with a sealer, then it was incubated for 60 minutes at 37 ºC		
Remove the sealer and wash the microplate 5 times with wash buffer		
Substrate Solution A	**50 µl**	**50 µl**
Substrate Solution B	**50 µl**	**50 µl**
The microplate covered with a new sealer & incubated for 10 minutes at 37º C in the dark		
Stop Solution	**50 µl**	**50 µl**
Determine the optical density (OD value) for each well immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution which performed with computer-based fitting software		

Calculation

$$\textbf{Gal} - \textbf{9 Concentration (pg/mL)} = \frac{\textbf{Abs. of T}}{\textbf{Abs. of S}} \textbf{ x S Concentration}$$

2.6.2: Calculation of Mannose Binding Lectin Levels

The applied procedure for the estimation of Mannose Binding Lectin (MBL) concentration is similar to that mentioned in section **2.6.1**.

2.7: Measurement of Complete Blood Count in The Blood Samples of The Study Groups

Complete blood count (CBC) includes: Hemoglobin (Hgb), Hematocrit (HCT), Red Blood Cells (RBCs) count, White Blood Cells (WBCs) count, Platelets count (Plts), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). All these parameters were measured automatically by an automated hematology analyzer machine called ‘’**HORIBA Medical-ABX Micros ES 60 Hematology Automatic Analyzer**’’.

- **The Principle**

The measurements are performed by an electronic variation principle by which two electrodes that are placed on each side of a micro-aperture attached to the device and a constant electronic current passes between them. The electronic field is generated around the micro-aperture and the diluted blood sample will pass through the calibrated micro-aperture, it will create a resistance in the electronic field, then it in turn causes an electronic pulse, that is amplified, measured, and then mathematically calculated to create numerical values with accurate cellular identification (the device holds automatic reagents to dilute samples and these reagents are in the case of daily maintenance).

Full automated procedure, as following:
Set the specific Using Barcode Reader
Use micro-sampling only 10µL of whole blood per analysis (the sample is auto-analyzed) and it will process by automatic reagent to count the values of parameters
The sample is auto-analyzed and the result of analysis will be appeared directly on an automatic system monitoring within 5 minutes

2.8: Determination of Nitric Oxide concentrations in The Sera Samples of The Study Groups

The applied procedure for the estimation of Nitric Oxide (NO) concentration is similar to that mentioned in section **2.6.1**.

2.9: Estimation of Total Glutathione and Oxidized Glutathione Levels in The Sera Samples of The Study Groups

The Principle

The total glutathione (T-GSH) and oxidized glutathione (GSSG) were measured by the cyclic reaction of Ellman's Reagent (5,5-dithio-bis- [2-nitrobenzoic acid]) also known as (DTNB).

Reagents

- Reagent 1 working solution
- Reagent 2 working solution
- Reagent 3 working solution
- Reagent 4 working solution
- Reagent 5 working solution
- Reagent 6 working solution
- Standard reduced glutathione
- Standard oxidized glutathione

Procedure Steps

Detection of T-GSH concentration

Additives	Standard (S)	Sample (T)
50µmol/L GSH standard	**10 µl**	-
Sample	-	**10 µl**
Reagent 1 working solution	**100 µl**	**100 µl**
Reagent 2 working solution	**10 µl**	**10 µl**
Microplate mixed fully & stand for 2 min at 25°C		
Reagent 3 working solution	**50 µl**	**50 µl**
Immediately measure the Absorbance (A) at 405 nm with microplate reader at 30 second (A_1) & 360 second (A_2), respectively. **$\Delta A = A_2 - A_1$**		

Calculations

$$\textbf{T-GSH Concentration (µmol/L)} = \frac{\Delta A \text{ of (T)}}{\Delta A \text{ of (S)}} \times \textbf{Concentration of S (50µmol/L)} \times \textbf{D.F.}$$

Detection of GSSG concentration

The procedure included two steps, the first is pre-treatment and the second is the determination step, as follows:

Pre-treatment Step

Additives	Standard (S)	Sample (T)
50µmol/L GSSG standard	**100 µl**	-
Sample	-	**100 µl**
Reagent 5 working solution	**2 µl**	**2 µl**
Reagent 6 working solution	**5 µl**	**5 µl**
The tubes mixed entirely for 1 min & left to react for 30 min at 37°C, then take 10 µl for test		

Determination Step

Additives	Standard (S)	Sample (T)
Pre-treatment solutions of GSSG standard	**10 µl**	-
Pre-treatment solutions of Sample	-	**10 µl**
Reagent 1 working solution	**100 µl**	**100 µl**
Reagent 2 working solution	**10 µl**	**10 µl**
Mix fully & stand for 2 min at room temperature		
Reagent 3 working solution	**50 µl**	**50 µl**
Immediately measure the Absorbance (A) at 405 nm with microplate reader at 30 second (A_1) & 360 second (A_2), respectively. **$\Delta A = A_2 - A_1$**		

Calculations

$$\textbf{GSSG Concentration (µmol/L)} = \frac{\Delta A \text{ of (T)}}{\Delta A \text{ of (S)}} \textbf{ X Concentration of S (50µmol/L) X D.F.}$$

2.10: Calculation of Erythropoietin Hormone Levels in The Sera Samples of The Study Groups

The applied procedure for the estimation of EPO concentration is similar to that mentioned in section **2.6.1**.

2.11: Evaluation of Parathyroid Hormone Levels in The Sera Samples of The Study Groups

The applied procedure for the estimation of Parathyroid (PTH) concentration is similar to that mentioned in section **2.6.1**.

2.12: Evaluation of Serotonin Hormone Levels in The Sera Samples of The Study Groups

The applied procedure for the estimation of Serotonin (ST) concentration is similar to that mentioned in section **2.6.1**.

2.13: Clinical Assay of Vitamins

2.13.1: Determination 1,25 Dihydroxy Vitamin D_3 Concentrations in The Sera Samples of The Study Groups

The Principle

Competitive Enzyme Linked Immune Sorbent Assay (Competitive ELISA) technique is based on the use of inhibitor antigen (the microplate is pre-coated with antigen), in the competitive ELISA, the inhibitor antigen and the antigen of serum compete for binding to the primary antibody. The procedure of competitive ELISA, as the following:

Firstly, the serum sample containing human 1,25 Dihydroxy vitamin D_3 (Vit D_3) is incubated with the biotinylated detection specific antibody, leading to the formation of antigen-antibody complex. **Secondly**, the Ag-Ab mixture is added to the supplied microplate which is pre-coated with human 1,25 Dihydroxy vitamin D_3. The free primary antibody in the mixture binds to the inhibitor antigen on the microplate, while the excess conjugated and unbound sample are washed from the plate.

Thirdly, HRP conjugated is added to the microplate wells and binds to the primary antibody on the plate. **Finally**, a substrate solution is added to each well and incubated. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at the wavelength of 450 nm.

Reagents

- Standard Working Solution
- Biotinylated Detection Antibody
- Wash Buffer
- Concentrated HRP Conjugate
- Substrate Solution
- Stop Solution

Procedure

Additives	Standard (S)	Sample (T)
Standard working solution	**50 µl**	-
Sample	-	**50 µl**
Biotinylated Detection Ab working solution	**50 µl**	**50 µl**
The microplate was incubated for 45 minutes at 37° C		
Aspiration and wash for 5 times	**350 µl**	**350 µl**
HRP Conjugate working solution	**100 µl**	**100 µl**
The microplate was incubated for 30 minutes at 37° C		
Aspiration and wash for 5 times	**350 µl**	**350 µl**
Substrate Reagent	**90 µl**	**90 µl**
Incubation for 15 minutes at 37° C		
Stop Solution	**50 µl**	**50 µl**
Read OD values for each well immediately at 450nm within 15 minutes after adding the stop solution by which utilizing with computer data -based on curve-fitting software designed		

Calculation

$$\textbf{1,25 Dihydroxy vitamin D3 (pg/mL)} = \frac{\textbf{Abs. of T}}{\textbf{Abs. of S}} \textbf{ x S Concentration}$$

2.13.2: Evaluated Vitamin B_{12} Levels in The Sera Samples of The Study Groups

- **The Principle**

The applied procedure for the estimation of Vitamin B_{12} (Vit B_{12}) concentration is similar to that mentioned in section **2.13.1**.

2.14: Calculation of Ferritin Protein Levels in The Sera Samples of The Study Groups

The applied procedure for the estimation of Ferritin Protein **(**FT) concentration is similar to that mentioned in section **2.6.1**.

2.15: Determination of Total Protein Levels in The Sera Samples of The Study Groups

Principle

Total protein concentration in sera samples of study groups was determined by colorimetric assay (Biuret method). Divalent copper salt reacts in alkaline solution with protein peptide bonds to form the characteristic purple-coloured biuret complex and sodium potassium tartrate prevents the precipitation of copper hydroxide, then potassium iodide prevents autoreduction of copper.

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{At pH 13.4}} \text{Cu-protein complex}$$

The color intensity is directly proportional to the protein concentration which can be determined photometrically at wavelength 552nm [Henok *et al.*, 2020].

Reagents

All reagents are ready for use as following:

Reagent 1 (R1): which consists of 400mmol/L of sodium hydroxide and 89mmol/L of sodium tartrate; at pH 13.4

Reagent (R2): that reagent consists of 400mmol/L of sodium hydroxide and 89mmol/L potassium sodium tartrate, in addition 61mmol/L potassium iodide and 24.3mmol/L copper sulfate.

Procedure

Additives	Sample (T)	Diluent (H_2O)
R1	**90µL**	-
Sample	**2µL**	**28µL**
R2	**32µL**	-
Total Volume	**152µL**	-

Calculation

The cobas c111analyzer automatically calculates the analyte concentration of each sample.

$$\textbf{Total Protein Concentration } (\textbf{g/dL}) = \frac{\textbf{Abs. of T x S Conc.}}{\textbf{Abs. of S}}$$

2.16: Determination of Albumin Levels in The Sera Samples of The Study Groups

Principle

Albumin (Alb) concentration in sera samples of study groups was measured by colorimetric assay (bromocresol green (BCG)). At a pH value of 4.1, Alb displays a sufficiently cationic character to be able to bind with BCG, an anionic dye, to form a blue-green complex.

$$\text{Alb} + \text{BCG} \xrightarrow{\text{At pH 4.1}} \text{Alb-BCG complex}$$

The color intensity of blue-green color is directly proportional to the albumin concentration in the sample and is measured photometrically at wavelength 583nm [D'Silva *et al.*, 2017, Henok *et al.*, 2020].

Reagents

All reagents are ready for use as following:

Reagent 1 (R1): which consists of 95mmol/L of citrate buffer, pH 4.1; preservatives; stabilizers.

Reagent (R2): that reagent consists of 95mmol/L of citrate buffer, pH 4.1; (0.66mmol/L) bromcresol green; preservatives; stabilizers.

Procedure

Additives	Sample (T)	Diluent (H_2O)
R1	**100µL**	-
Sample	**2µL**	**20µL**
R2	**20µL**	**10µL**
Total Volume	**152 µL**	-

Calculation

The cobas c111analyzer automatically calculates the analyte concentration of each sample.

$$\textbf{Albumin Concentration (g/dl)} = \frac{\textbf{Abs. of T x S Conc.}}{\textbf{Abs. of S}}$$

2.17: Assessment of Trace Elements in The Sera Samples of The Study Groups

Concentrations of serum trace elements (Zn, Co, Fe, Mn, and Cu) in the study groups were determined using atomic absorption spectrophotometry (AAS) technique.

The Principle

AAS quantitatively measures concentrations of the trace elements present in liquid samples. It utilises the elements in the gas phase which absorb light at specific wavelengths (after the liquid is drawn in to a flame where it is ionised in

the gas phase) and the absorption of element is proportional to the concentration of the element. Determined the quantification is achieved by preparing standards for each element. Elemental analysis enables to be at parts per million (ppm) levels.

Calculation

$$\textbf{Trace Element Concentration (ppm)} = \frac{\textbf{Abs. of T x S Conc. x D. F.}}{\textbf{Abs. of S}}$$

2.18: Assessment of Electrolytes in The Sera Samples of The Study Groups

2.18.1: Estimation Levels of Sodium, Chloride, and Potassium in The Sera Samples of TheStudy Groups

Sera samples of children with ALL and healthy children groups were analyzed by medical electrolyte analyzer (LW E60) to evaluate electrolytes levels which include; Na^+, Cl^-, and K^+ within a maximum of 5 minutes by ion selectivity electrode (ISE), and then the results of analyses were appeared directly on the front monitor screen of the device.

2.18.2: Estimation of Total Calcium Levels in The Sera Samples of The Study Groups

- **The Principle**

Total calcium concentration in serum was determined by Moorehead and Briggs derived cresol phtalein complexone (CPC) method by spectrophotometer instrument. In alkaline solution CPC reacts with calcium to form a dark-red coloured complex which absorbance measured at 570nm, which is proportional to the amount of calcium in the specimens [Wu, 2020].

Reagents

- Reagent 1 Calcium Buffer (200ml)
- Reagent 2 Calcium Dye (Chromogen) (200ml)
- Reagent 3 Calcium Standard (10ml)
- Working Reagent

Procedure

Additives	Blank	Standard (S)	Sample (T)
Working Reagent (R1+R2)	**1 ml**	**1 ml**	**1 ml**
Distilled Water	**25 µl**	-	-
Standard	-	**25 µl**	-
Specimen	-	-	**25 µl**
Mix well and incubate for 5 minutes at room temperature, then the absorbance (Abs) was read at 570nm against reagent blank and standard			

Calculation

$$\mathbf{T - Calcium\ Concentration (mg/dL)} = \frac{\mathbf{Abs.\ of\ T}}{\mathbf{Abs.\ of\ S}}\ \mathbf{x\ S\ Concentration\ (10mg/dL)}$$

2.18.3: Estimation of Total Magnesium Levels in The Sera Samples of The Study Groups

The Principle

Concentration of magnesium (Mg) is measured by an automated clinical chemistry analyzer ‘’DRI-CHEM’’ which can accomplish numerous biochemical tests. It has a built-in auto-pipetting system by touch screen of key board requirement for only one sample of each run of operation through the following:

- **Barcode Reader:** It is available as selection item to read sample ID on sample tube.

Dry Slide Reagent: It is used for serum samples analyses. This slide has high dependability and stability brought by acceptable chemical technology through high precision of the photographic film.

QC Card System: It has a magnetic card called QC card which regulate the proportion variability in the slide reagents. A QC card comes with every reagent box and use the lot number of adjustment information once of the QC card is swiped.

Multi-Layered Slide: It is comprising of chemical constituents required for the reaction.

Full automated procedure by three steps
Set the specific dry slide reagent for Mg^{2+} test
Set 10µL of the serum sample on dry slide reagent
Press start on the touch screen (the sample is auto-analyzed and the result of analysis will be appeared directly on the monitor screen within 5 minutes and the slide reagent will be automatically discarded after each measurement

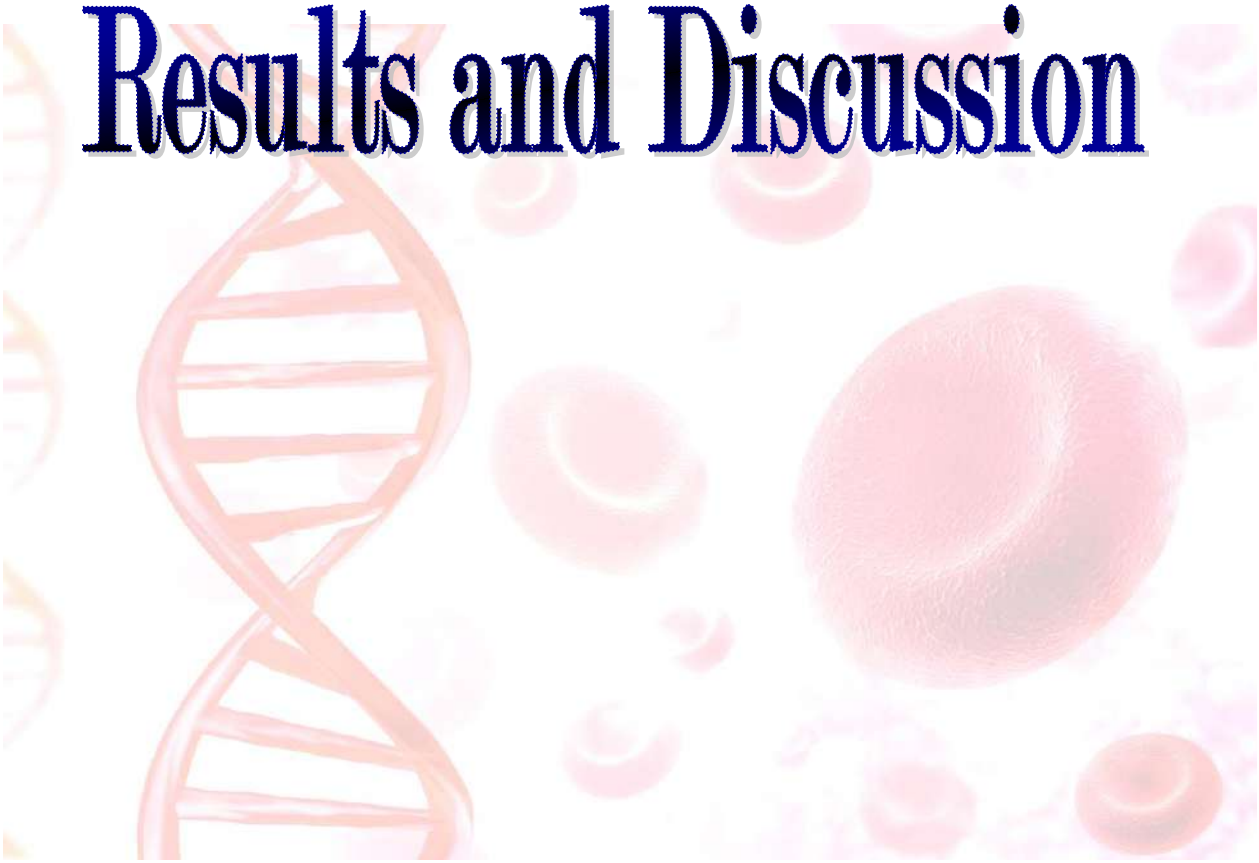
2.19: Statistical Analysis

The statistical analysis was achieved by the Statistical Package for the Social Science (**SPSS**) software for windows, version 20.0. The results were expressed as Mean ± Standard Deviation (**Mean ± S.D.**), maximum, minimum and range. **Independent Student's *t*-test** was used to analyse the data of studied parameters. One- way Analysis of Variance **(ANOVA)** was used to compare variables in different studied subgroups.

Pearson's correlation was applied to determine the relation among the measurable factors of the present study. ***p-values*** less than 5% ($p<0.05$) were considered statistically significant. **Sensitivity percentage** was calculated according to biomedical statistical.

Chapter Three

Results and Discussion



3.1: Study Groups Individuals

The current study involved 71 participants who were divided into two groups, the first included 31 children their ages ranged between 2-12 years, they suffered ALL diseases, this group comprised 18 males (58%) and 13 females (42%). The second group included 40 individuals with age range 2-12 years, who were distributed into two subgroups 20 males (50%) and 20 females (50%) seemed to be healthy. This group was subjected to a set of criteria to involve in the study as a control group. **Table 3-1** shows the average age of the current study groups and their age range.

Table 3-1: Age (Year) of The ALL Patients and Healthy Individuals

Study Groups (n)	*Age (year) Mean ± S.D.*	*Min-Max Age (Year)*	*Range*	*p-value*
ALL Patients 31	5.420±2.711	2 – 12	10	***0.413***
Healthy Individuals 40	8.725±2.416	2 – 12	10	

The Mean Difference is Significant at 0.05 Level

While the **Table 3-2** displays the distribution of children with ALL and healthy individuals according to their gender.

Table 3-2: Levels (Mean±S.D.) of Age (Year) in The Study Individuals according to their Gender

Study Groups (n)	*Gender (n)*	*Age (Year) Mean ± S.D.*	*Min-Max Age (Year)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	5.611±3.051	2 – 12	10	***0.683 For1vs2 0.497 For3vs4 0.001 For1vs3 0.000 For2vs4***
	Female 13	5.230±2.166	2 – 8	6	
Healthy Individuals **40**	**Male 20**	8.450±2.645	2 – 12	10	
	Female 20	9.000±2.127	3 – 12	9	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

As the results achieved from the current study based on sex, it indicated that the incidence ratio of male children with ALL was higher than that in the ALL females children, these results were similar to that reported for local and international statistics on the epidemiology of ALL disease and documented in the recommendations of WHO [WHO, 2020] which confirmed the high incidence proportion of ALL males compared to females and it is probably due to the possible effect of hormones, as well as the influence of gender factor on patients at which the progression of ALL disease in children because males have more physiological tendencies and affinities for the occurrence of ALL [Al-Asadi and Ibrahim, 2018; Hade, 2018].

In addition, based on the information obtained from the questionnaire form designed with the current study, it is possible that several factors have contributed to the incidence of ALL such as; passively using cigarettes, exposure to chemicals, radioactive contamination, oil and its derivatives, as well as other undiagnosed pollutants [Al-Asadi and Ibrahim, 2018]. The results were consistent with studies that attributed to the causes of ALL to various environmental and chemical factors, along with exposure to the ionizing radiation [Al-Asadi and Ibrahim, 2018; Serbanica *et al.,* 2018], as well as, many variances among genders could be due to ecological factors, whereas others appear to be accompanied with genetic influences [Fathi *et al.,* 2015].

Besides, the possibility of another partial explanation that may be associated with stimulating immune functions as a result of responding to changes in gene expression [Jin *et al.*, 2016; Klein and Flanagan, 2016]. As the radiation and chemical contamination have been dramatically risen, especially in the middle regions of Iraq at the period after the year 1993, which mainly could lead to an increase in the incidence of genetic mutations in most patients causing leukemogenesis rates, and thus increased prevalence rates of leukemia in between boys and girls [Hade, 2018; Sung *et al.*, 2020].

Other than that, numerous participating patients in the present study were from residents of rural areas and outskirts of cities according to the information of data form for individuals' study group which recorded most cases among patients group. Thus, it may be explained in several ways and as follows:

- It is possible that the leukemic cases came as a result of the infection by some types of viruses such as; Epstein-Barr Virus (EBV), as Guan's study [Guan *et al.,* 2017] was stated that the degree incidence of EBV among ALL and AML patients, and then the rate of relapse and mortality in these patients were higher than in patients with leukemia resulting from other causes than this virus. On the other hand; the retrovirus which is called Bovine Leukemia Virus (BLV) in ''livestock'' has been considered as one of the main causes in the occurrence of ALL diseases among the people who deal with direct contact of livestock infected with this virus, as the pathogenicity of this virus has already been studied in some Asian countries [Mui *et al.,* 2017].
- The effect of chemicals, pesticides and insecticides are used in these rural areas and direct contact by individuals, which may cause several genetic modifications and cellular changes which have a clear effect in raising of the rates patients with ALL [Chang *et al.,* 2021]. Additionally, the middle and southern regions of Iraq are the most exposed areas to radiation through the use of Uranium-containing weapons during the Gulf War and the subsequent battles, as this element has a long-term of radioactivity, as its atoms begin slowly disintegrate with absolute energy in the form of radiation, which makes these contaminated areas for a long period of time, and then it was well-known according to most studies that Uranium element causes many genetic mutations that lead to the occurrence of cancer; such as leukemia and especially acute leukemia which is the focus of the current study, this is one of the most common types of leukemia [Hade, 2018; Parka *et al.,* 2020].

3.2: Evaluation of Galectin-9 Levels in The Sera Samples of ALL Patients and Healthy Control Groups

Galectin-9 (Gal-9) concentration was assessed in the sera samples of ALL patients and healthy subjects. Gal-9 levels were evaluated prior-chemotherapy as well as during treatment (approximately four following doses of chemotherapeutic treatment). Gal-9 concentration was elevated in the patients' group (at diagnosis) when its concentrations were tested in the sera samples of the study individuals. Statistically, a highly significant difference at (**p=0.000**) was detected between the two study's groups when compared together, as shown in **Table 3-3**.

Table 3-3: Levels of Galectin-9 Concentrations (ng/mL) in The Sera Sample of ALL Patients and Healthy Control

Study Groups (n)	*Gal-9 Conc. (ng/mL) Mean ± S.D.*	*Min-Max Gal-9 (ng/mL)*	*Range*	*p-value*
ALL Patients 31	721.667±303.273	240.14 - 1245.87	1005.73	***0.000***
Healthy Individuals 40	280.014±26.035	241.38 - 372.49	131.11	

The Mean Difference is Significant at 0.05 Level

These results were consistent with several studies that performed by Taghiloo *et al.,* [2017] and Wdowiak *et al.,* [2019] which had exhibited high levels of Gal-9 in sera of patients with CLL and AML at the period of diagnosis compared to healthy individuals by which it may be associated with growth and progression of the disease [Wdowiak *et al.*, 2019].

Results of the present study did not record statistical variations between both sexes within study groups (either patients or controls). On the other side, the outcomes have been shown a significant increase (**p=0.000**) of Gal-9 levels in males and females with ALL when compared with their counterparts in the control of subgroups (**p=0.000**), as clarified in **Table 3-4**. The applied

observations indicated that the highest concentration of Gal-9 (**1245.87 ng/mL**) was recorded in the ALL female patients, while the lowest concentration of this protein (**240.140 ng/mL**) was noticed in the sample of healthy males.

Table 3-4: Levels (Mean±S.D.) of Galectin-9 Concentration (ng/mL) in The Study Individuals

Study Groups (n)	*Gender (n)*	*Gal-9 Conc. (ng/mL) Mean ± S.D.*	*Min-Max Gal-9 (ng/mL)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	731.521±302.463	380.19 - 1218.67	838.48	***0.786 For1vs2***
	Female 13	711.813±298.181	375.93 - 1245.87	869.94	***0.984 For3vs4***
***Healthy Individuals* 40**	**Male 20**	279.391±35.090	240.140 - 372.49	132.35	***0.000 For1vs3***
	Female 20	280.637±14.470	250.55 - 301.60	51.05	***0.000 For2vs4***

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

Galectins are β-galactoside binding lectin which well-known as a main member of lectin families and the galectin family has received great attention caused by their close relation to many hematological and cancerous diseases specifically which have been documented as neoplastic functions in the number of cancers [Chou *et al.,* 2018]. Numerous previous studies indicated that the levels of many galectins were detected in many pathological conditions, and there was a change in many levels of galectin types [Colomb *et al.,* 2017; Taghiloo *et al.,* 2017; Song *et al.,* 2020]. It was considered that the changes in galectins levels were corresponding with disease progression, patient age, and injury severity of disease [Cousin and Cloninger, 2016; Pang *et al.,* 2018].

The present study is the first in the investigation to follow Gal-9 levels in sera samples of children with ALL during treatment with chemotherapy, thus

these observations raise the hypothesis that suggests the Gal-9 may perhaps contributed to the development of the body's anti-inflammatory immune defence mechanisms in children with ALL in similar to the role of MBL levels as a direct reason for reviewing the body's protection ability against various diseases [Cedzynski and Swierzko, 2020; Gupta, 2020]. However; many previous kinds of literature had not been found to support this hypothesis, and particularly with regard to the role of increasing levels of Gal-9 in pediatric ALL [Plummer *et al.*, 2016; Gordon-Alonso *et al.*, 2018] and the role of Gal-9 in regulating cell signals by binding to its receptors in many physiological functions such as; cell growth, adhesion, cell surface signalling, angiogenesis, proliferation, migration, invasion, inflammation and apoptosis [Gordon-Alonso et al., 2018].

Consequently, galectins have essential roles in several intra-and extracellular pathways, as well as the malignant cells have the ability to release Gal-9 in the duration of disease development through the ''autocrine'' process due to the effectiveness of immunoglobulin by T-cells and TIM-3, thus the TIM-3/Gal-9 complex is produced by Gal-9 ligand, as a result of this association through the induction mechanism of Gal-9 [Kikushige *et al.*, 2015; Chou *et al.*, 2018] and the possibility of Gal-9 to bind TIM-3 receptors which causes inhibition both of T-lymphocytes and Natural Killer cells (NK), then these cells become unable to perform their immune functions properly [Kikushige *et al.*, 2015; Kursunel and Esendagli, 2017].

Another hypothesis to explain the observed increase of Gal-9 levels in the patients' group which could be clarified by the association of stimulating the synthesis of Gal-9 when the transformation of normal cells into the cancerous cells occurs during the carcinogenesis process [Kursunel and Esendagli, 2017]. Outcomes of the present study were in line with other studies [Cousin and Cloninger, 2016; Tseng *et al.*, 2016; Gordon-Alonso *et al.*, 2017], whereas

these studies have been carried out to evaluate other different galectins levels in patients with other various types of cancers.

Figure 3-1 shows the apparent gradual decrease of Gal-9 concentrations in ALL patients after getting approximately four consecutive doses of chemotherapy in comparison with its levels at diagnosis (pre-treatment).

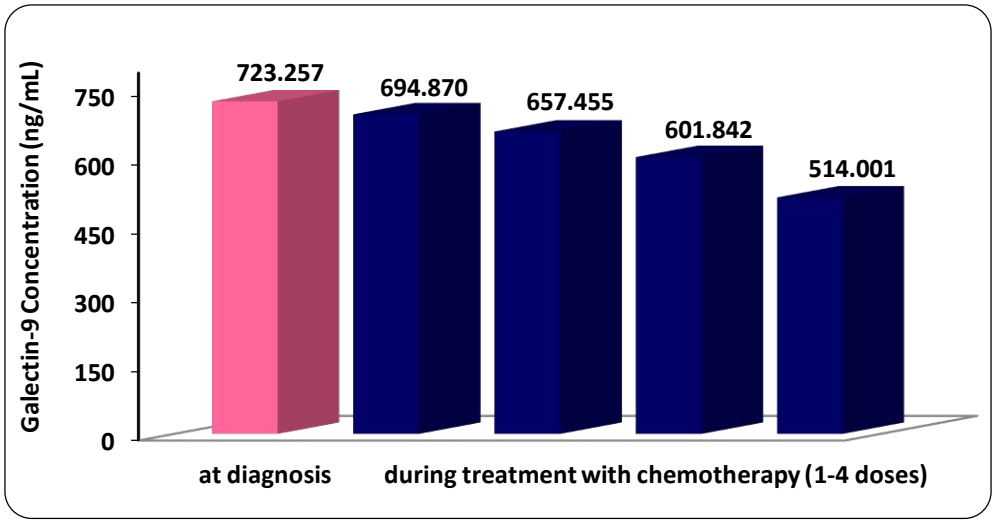


Figure 3-1: Gal-9 Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

The results of the current study showed an obvious reduction in Gal-9 levels in ALL children after receiving consecutive doses of chemotherapy, which could be referred to as the patients' response to the chemotherapy protocol due to the effect and ability of treatment to control the cancer cells proliferation, so it's essential that the decrease in Gal-9 levels be a reaction to the destruction and/or elimination in both healthy and cancerous cells after the use of chemotherapy [Hade, 2018; Niki *et al.*, 2018]. In contrast, Tadokoro's study [Tadokoro *et al.*, 2017] indicated that it is possible that Gal-9 may play an important role in the apoptotic pathway through inhibited leukemic cell proliferation, but the mechanisms that support this pathway are yet unclear [Tadokoro *et al.*, 2017].

Although; TIM-3/Gal-9 complex which has an associated mechanism to suppress and impaire the responses of immune cells, cancer cells can benefit

from these immunosuppressive pathways via increasing their growth, proliferation, and spread to other tissues [Kursunel and Esendagli, 2017].

On the other hand, some studies may have attributed continued high levels of Gal-9 in some patients with ALL even after received doses of chemotherapy, which explained that based on the Gal-9 is one of the types of lectins formed and produced by tumour cells due to the cancer incidence, and some of the patients have advanced stages of leukemia, then causing malignant cells resistance to the protocol treatment of chemotherapy [Pena *et al.*, 2015].

Unfortunately, the action mechanisms and the prospective beneficial effects of galectins in cancer have not yet been fully supposed, moreover; the difficulty of studying its precise details of vital functions and their roles in the occurrence of different types of cancers [Chen *et al.*, 2017; Swierzko *et al.*, 2018]. So, the detailed mechanisms of Gal-9 are still unclear and have not yet been well studied, hence in order to be able to well-assessment in which is essential advance investigations, as it is potential to study Gal-9 in collaboration with other types of galectins family that affect the growth and development of malignant cells [Tadokoro *et al.*, 2017].

As yet, according to the tracing changes in the levels of Gal-9 at diagnosis and during the period of chemotherapeutic treatment in ALL patients, as well as, its usage as a biological marker in clinical diagnosis will be an important tool for monitoring and determining the activity and development of leukemia, so it is possible to make appropriate treatment decisions for the patients by the competent physician, and it is possible to be considered Galectin-9 a valuable factor for prediction and evaluation of disease, as well as patients' response to the strategies of chemotherapeutic treatment.

3.3: Assessment of Mannose Binding Lectin Levels in The Sera Samples of ALL Patients and Healthy Control Groups

The outcomes demonstrated a highly significant elevation (**p=0.000**) of MBL concentrations in the sera samples of ALL patients group compared to healthy individuals, as shown in **Table 3-5.**

Table 3-5: Levels of Mannose Binding Lectin Concentration (ng/mL) in The ALL Patients and Healthy Control

Study Groups (n)	*MBL Conc. (ng/mL) Mean ± S.D.*	*Min-Max MBL (ng/mL)*	*Range*	*p-value*
ALL Patients 31	767.187±335.857	358.85 - 1260.38	901.53	***0.000***
Healthy Individuals 40	347.078±38.228	279.80 - 427.19	147.39	

The Mean Difference is Significant at 0.05 Level

According to the ANOVA test, the current study established that no statistical differences between male and female subgroups in the MBL concentrations were observed, neither in ALL patients nor control groups, while significant statistically variations (**p=0.000**) were recorded when compared ALL males and females with their healthy peers in the control subgroups, as illustrated in **Table 3-6**.

The results of the present study noted the highest value (**1260.38 ng/mL**) of MBL were recorded in males' samples and the lowest value (**279.80 ng/mL**) of MBL in females' samples, as illustrated in **Table 3-6**, by which indicating that males have a physiological tendency to increase this type of lectins, in the case of cancer in general, and in ALL cases especially; this finding confirms and supports the incidence of ALL in males is higher than in females [Al-Asadi and Ibrahim, 2018].

Table 3-6: Levels (Mean±S.D.) of Mannose Binding Lectin Concentration (ng/mL) in The Study Individuals

Study Groups (n)	*Gender (n)*	*MBL Conc. (ng/mL) Mean ± S.D.*	*Min-Max MBL (ng/mL)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	804.294±337.736	390.28 - 1260.38	870.10	***0.462 For1vs2 0.962 For3vs4 0.000 For1vs3 0.000 For2vs4***
	Female 13	743.746±340.815	358.85 - 1250.57	891.72	
***Healthy Individuals* 40**	**Male 20**	348.778±35.695	285.08 - 427.19	142.11	
	Female 20	345.379±42.631	279.80 - 417.36	137.56	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

In order to investigate the effect of chemotherapy in the production of serum MBL levels was followed in patients after receiving at least four successive doses of chemotherapy.

Figure 3-2 indicated to the continuous significant increasing of MBL level after delivered the first dose of chemotherapy compared to the baseline (pre-chemotherapy), and then its concentrations begin gradually decline in sera samples of ALL patients down to beyond the fourth dose of treatment in comparison toat diagnosis (before starting the treatment), however; its concentrations did not reach the levels of normal values.

Results of the present study were agreed with previous works performance by Riwes *et al.*, [2016]; Auriti *et al.*, [2017]; Peterslund *et al.*, [2017] when these studies mentioned that low serum levels of MBL could be predictable for pediatric malignant patients at the levels of increased risk of infection.

Conversely, various studies have shown that the deficient of MBL could become a severe risk factor for the development of infectious diseases in ALL patients and dampen their immunity [Frakking *et al.*, 2015; Ghazi *et al.*, 2015], as well as during receiving treatment [Riwes *et al.*, 2016].

The results of the present study showed that MBL levels were elevated when the incidence of ALL and it noted a rise in MBL levels in 29 out of the 31 patients' samples at 94% even after the first dose, in addition, the outcomes of the study indicated to decrease in the serum levels of MBL by 68% of 21 out of 31 patients with ALL after getting the second and third doses of chemotherapy, and then MBL levels returned to be higher again in children with ALL after the fourth dose in 15 out of 31 at 48% than its levels after the third dose, but it did not reach to high levels as observed at pre-chemotherapeutic treatment.

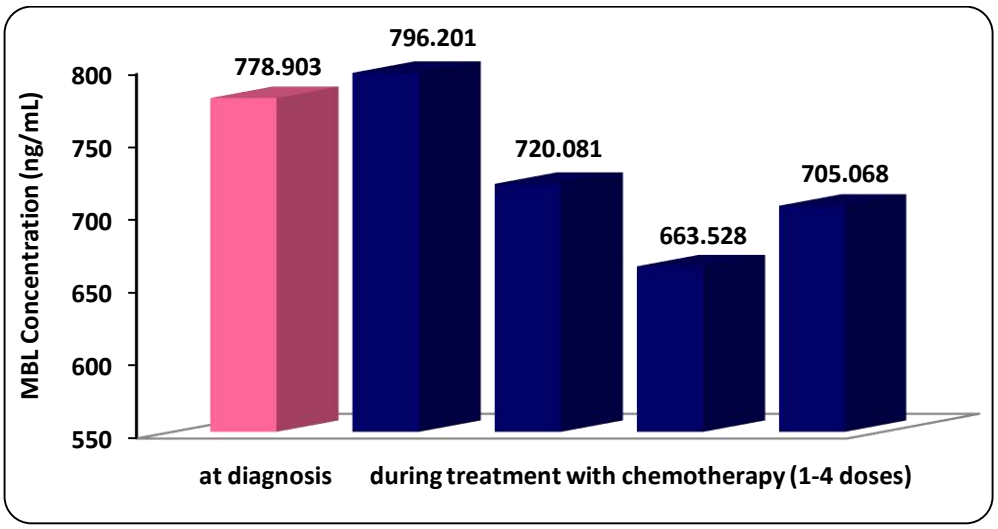


Figure 3-2: MBL Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

Mannose-Binding Lectin (MBL) is one of the collagenous C-type lectins, and it has important roles in specific ''innate immune'' protection, especially with an early age of childhood at the onset when the ''adaptive immune response'' which is either undeveloped to identify microorganisms and control infection or it is at risk from the use of immunosuppressive drugs [Varki *et al.*, 2015; Coulibaly and Youan, 2017]. As well as, MBL works in combination with different leukocytes as the first line of defence against infectious agents,

foreign invaders and cancer cells through immune responses [Wahlund *et al.*, 2020].

MBL level is double-edged for its functions and their effects in different cancers, especially in leukemia which means that the variations in its levels could be pointed to an increased risk of infections and so on cancer [Frakking *et al.*, 2015; Wahlund *et al.*, 2020]. Besides, the levels of MBL also play a role in modifying different cytokines levels in a way that depends on the dose of treatment given to the patient [Roland *et al.*, 2015]. Therefore; several expectations that could be explained the current outcomes about MBL levels as the following:

- It appears that MBL levels associated with different hematological malignancies which are very complex and indistinct, thus these levels could be influenced by the range of risk factors that including, age, type of disease, and routine programme of therapy [Hao *et al.*, 2020]. Consequently, it is suggesting that the MBL has a protective role in the complement system (also known as complement cascade) which is a part of the immune system, even to stimulate the lectin complement pathway in infection, or by advantage of opsonization with the receptors on monocytes/macrophages [Cedzyński and Świerzko, 2020], in which the activation of MBL during the infection due to its role as an effective regulator of inflammation during an acute-phase response, and then induce recruitment ‘’proinflammatory of several cytokines such as; IL-6’’ to stimulate the releasing of C-reactive protein (CRP) [Dhimana, 2019]. Hence, it is possible to remain MBL in the rise levels in patients with ALL after getting the first chemotherapeutic dose which it could be described by a continuing acute phase response [Kalia *et al.*, 2021].
- The increasing levels of MBL in ALL patients before treatment could be related to the disease itself, as well as, the MBL levels in sera patients with ALL are higher than in the healthy children group which are probably reflected

by the cytogenetically of ALL patients with some modifications of cells during carcinogenesis process [Wahlund *et al.,* 2020], as these findings were consistent with a study was done by Coulibaly and Youan, [2017]. In contrast, Gupta's study [Gupta *et al.,* [2015], who had shown that the high concentrations of MBL in childhood ALL patients may be expected caused by the emergence of severe bacterial, viral or even fungal infections and after receiving doses of chemotherapy which was stated in similar findings perspective by Riwes's study [Riwes *et al.,* 2016] and Peterslund's study [Peterslund *et al.,* 2017].

However, the study was exposed by Frakking *et al.,* [2015] had revealed that most ALL patients have increased levels of MBL and/or presently at diagnosis because of the increased synthesis of MBL via the liver, with paralleled to healthy individuals. In addition, the high levels of MBL that could consequence without apparent signs of infection as being to **''re-activation''** of MBL levels in ALL patients after association with further ''immunosuppression or immunodeficiency'' when the patients received chemotherapy [Papaiakovou *et al.,* 2017; Wahlund *et al.,* 2020].

Moreover, Maestri's study [Maestri *et al.,* 2018] showed that the possibility of the existence of some mechanisms which express high levels of MBL, and these mechanisms may work in particular to stimulate or inhibit the development of cancers, then it has been proven that the elevation of MBL and MBL-Associated Serine Protease-2 (MASP-2) performance cause the increasing of complement factor (C5A) with the rise of the malignant cells' growth. Moreover, the fact that MBL is an important component in the acute stage during various infections, therefore; it may enhance during different infection cases, especially leukemia [Cedzyński *et al.,* 2018]. Otherwise; it can become part of the immune responses requirements of the cancerous tumors in

patients and/or consequently it is possible to express MBL levels effectively as a result of the activity of cancer cells at this stage [Kalia *et al.,* 2021].

On the other hand, a study achieved by Keizer *et al.,* [2018] has identified that the clinical indicators of deficient MBL when the immune system is still immature, especially during early childhood and maybe due to an immunodeficiency which is associated with a deficiency in the WBCs number, specifically neutrophils (neutropenia) and other complications of diseases associated with chemotherapy in ALL patients [Puente *et al.,* 2019]. On the other hand, MBL levels were increased slightly and/ or with a normal range in some cases of ALL patients when admitted to the pediatric intensive care unit (PICU) during their seizures of the febrile neutrophils due to complications and inflammation [Roland *et al.*, 2015].

Besides, some other studies have stated that the decrease in MBL concentrations after treatment could be associated with the genetic patterns and polymorphisms in the immune system (as MBL plays a critical role in innate immunity), which has contributed to an increased risk of infection, inflammation and hence the risk of acute leukemia during early childhood [Kalia *et al.*, 2021]. Thus, either the genetic mutations in the collagen-like domain (R32C, G34D, or G37E) of MBL which caused a serum MBL deficiency [Speletas *et al.,* 2015; Auriti *et al.*, 2017], or it may lead to critical damage of DNA, that could be more obvious in children with MBL low-level of genetic patterns compared to those with high-level patterns, although the related molecular mechanisms are still unclear and basically, the destruction of DNA may increase the risk of harmful mutations and cytogenetic abnormalities that could comprise in an increasing ‘’T- and B-lineage lymphoblasts’’ which could be accompanied by the relapse of ALL [Holanda *et al.,* 2015; Wahlund *et al.*, 2020].

Many other assumptions focused on the association between deficiency of MBL levels and severe infections after chemotherapy; i.e. ‘’hygiene hypothesis’’ which assumes that the exposure of the body during a childhood stage to certain pathogenesis and infection may stimulate the child’s immune system and lead to the body’s discrimination of harmful and non-harmful substances and vice versa with more specifically, ALL could be caused by exposure of the to infection or inflammation at an early stage of childhood, and cause an abnormal response to infection as a ‘’hypothesis of delayed infection’’ [Szaka´cs *et al.,* 2019] and even if these types of infections might affect the risk of developing ALL, but ‘’biological mechanisms’’ contributing to onset leukemia are not entirely associated with this infection and eventually, that can affect the emergence of only a specific type of leukemias [Peterslund *et al.,* 2017; Szaka´cs *et al.,* 2019].

Whereas the study conducted by Wahlund *et al.,* [2020] has estimated that the use of ‘’L-asparaginase’’ as a type of chemotherapy in treatment ALL is probably the cause of an evident reduction in the concentrations of MBL, which was assumed in the involvement of higher potential risk of febrile risk caused by the lack of neutrophils ‘’neutropenia’’ by the infections.

The data around MBL and its influences have expanded on cancer, particularly leukemia refers to the fact that MBL levels are associated with diagnosis and prognosis of these cancers or not, even its concentrations may be altered due to the common complications; such as infection and inflammation which resulting from the severity of the effects of chemotherapy, nevertheless MBL have a vague role in the human immune system.

3.4: Evaluation of Some Hematological Parameters and Indices in Blood Samples of ALL Patients and Healthy Groups

The results of the current study exhibited statistical significant decreases in some blood parameters and indices; RBCs count, Hgb, HCT, Plts and MCHC (**p=0.009**), (**p=0.001**), (**p=0.000**), (**p=0.008**), and (**p=0.000**) respectively; in children with ALL. However; the levels of MCH have not changed, while there was an increase in MCV level, but it wasn't a significant difference in blood samples of patients with ALL at diagnosis than that observed in healthy individuals, as shown in **Table 3-7**.

Table 3-7: Mean ± S.D. of Some Hematological Parameters and Indices Levels in Blood Samples of ALL Patients and Healthy Control

Blood Parameters and Indices	***Subject Groups (n)***		***p-value***
	ALL Patients (31)	***Healthy Individuals (40)***	
	Mean ± S.D. Min-Max (Range)	**Mean ± S.D. Min-Max (Range)**	
RBCs ($10^6/mm^3$)	3.522±0.506 2.500-4.150 (1.65)	4.569±0.329 4.020-5.380 (1.360)	***0.009***
Hgb Conc. (g/dL)	9.451±1.379 7.450-10.800 (3.350)	12.125±0.620 11.100-13.400 (2.300)	***0.001***
HCT (%)	29.674±4.367 22.150-34.950 (12.800)	34.565±2.067 30.200-38.400 (8.200)	***0.000***
Plts ($10^3/\mu L$)	82.373±45.327 43.50-144.00 (100.500)	268.275±35.175 192.000-353.000 (161.000)	***0.008***
MCV (FL)	85.500±6.238 74.700-107.100 (32.400)	75.900±5.192 58.500-85.500 (27.000)	***0.418***
MCH (pg/cell)	26.503±2.261 22.000-32.500 (10.500)	26.828±1.931 20.800-32.200 (11.400)	***0.237***
MCHC (g/dL)	30.950±2.346 24.800-34.700 (9.900)	34.751±1.066 32.0 00-36.200 (4.200)	***0.000***

The Mean Difference is Significant at 0.05 Level

On the other hand, the present work recorded a significant difference (***p<0.05***) in the levels of RBCs, Hgb, HCT, Plts, MCV and MCHC between the

males and females in the group of ALL patients when compared with their healthy individuals, as illustrated in **Table 3-8** and **Table 3-9**.

Table 3-8: Mean ± S.D. of Some Blood Parameters Levels in Males and Females of Study Groups

Blood Parameters	*Subject Groups (n)*				*p-value*
	ALL Patients (31)		*Healthy Individuals (40)*		
	Male 23 Mean ± S.D. Min-Max Range	**Female 18 Mean ± S.D. Min-Max Range**	**Male 20 Mean ± S.D. Min-Max Range**	**Female 20 Mean ± S.D. Min-Max Range**	
RBCs ***($10^6/mm^3$)***	3.480±0.488 2.620-4.100 1.480	3.516±0.512 2.500-4.060 1.560	4.609±0.356 4.020-5.380 1.360	4.519±0.296 4.070-5.160 1.090	***0.806*** For1vs2 ***0.493*** For3vs4 ***0.000*** For1vs3 ***0.000*** For2vs4
Hgb Conc. (g/dL)	8.711±1.462 5.500-10.600 5.100	10.192±0.407 9.400-11.000 1.600	12.170±0.67 11.10-13.100 2.000	12.080±0.62 11.20-13.400 2.200	***0.000*** For1vs2 ***0.751*** For3vs4 ***0.000*** For1vs3 ***0.000*** For2vs4
HCT (%)	28.588±3.974 22.20-33.900 11.700	30.761±4.800 22.10-36.000 13.900	34.945±1.93 30.30-38.100 7.800	34.185±2.17 30.20-38.400 8.200	***0.000*** For1vs2 ***0.461*** For3vs4 ***0.000*** For1vs3 ***0.004*** For2vs4
Plts ***($10^3/\mu L$)***	69.055±29.204 40.00-128.00 88.00	95.692±38.913 47.00-160.00 113.00	273.20±31.85 203.0-322.00 119.00	263.35±37.98 192.0-353.00 161.00	***0.002*** For1vs2 ***0.266*** For3vs4 ***0.031*** For1vs3 ***0.021*** For2vs4

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

Table 3-9: Mean ± S.D. of Some Blood Indices Values in Males and Females of Study Groups

Blood Parameters and Indices	*Subject Groups (n)*				*p-value*
	ALL Patients (31)		*Healthy Individuals (40)*		
	Male (23) Mean ± S.D. Min-Max Range	**Female (18) Mean ± S.D. Min-Max Range**	**Male (20) Mean ± S.D. Min-Max Range**	**Female (20) Mean ± S.D. Min-Max Range**	
MCV (FL)	83.327±4.114 74.70-90.40 15.70	87.992±7.393 79.0-107.1 28.10	76.075±4.870 63.90-85.50 21.60	75.890±5.496 58.50-81.50 23.00	***0.021*** For1vs2 ***0.914*** For3vs4 ***0.000*** For1vs3 ***0.000*** For2vs4
MCH (pg/cell)	26.283±2.248 2.00-31.00 9.00	26.838±2.417 24.20-32.50 8.30	26.710±2.412 20.80-32.20 11.40	26.910±1.281 23.90-28.50 4.60	***0.472*** For1vs2 ***0.765*** For3vs4 ***0.536*** For1vs3 ***0.924*** For2vs4
MCHC (g/dL)	31.483±2.279 24.80-34.20 9.40	30.507±2.279 26.00-33.00 7.00	34.725±0.868 32.50-36.10 3.60	34.775±1.232 32.00-36.20 4.20	***0.120*** For1vs2 ***0.926*** For3vs4 ***0.000*** For1vs3 ***0.000*** For2vs4

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

In addition, a fluctuating decrease in the number of RBCs count was recorded during treatment with chemotherapy in comparison to it's levels at diagnosis period, as illustrated in **Figure 3-3**.

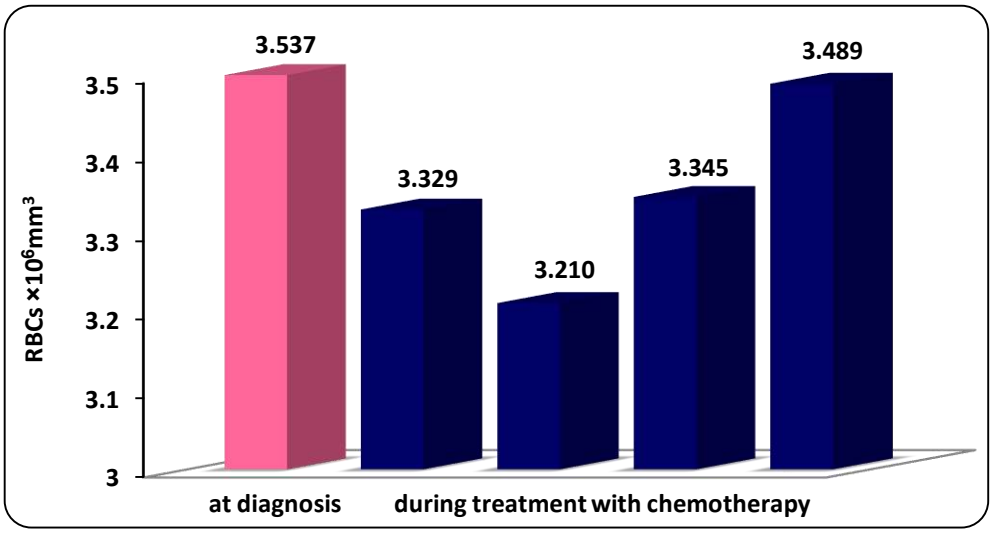


Figure 3-3: RBCs Count of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

However; Hgb levels in blood samples of ALL patients showed a slight rise after receiving more than three of chemotherapeutic doses, while Hgb levels have increased after getting the fourth dose of treatment than what is noted at diagnosis (pre-treatment), as clarified in **Figure 3-4.**

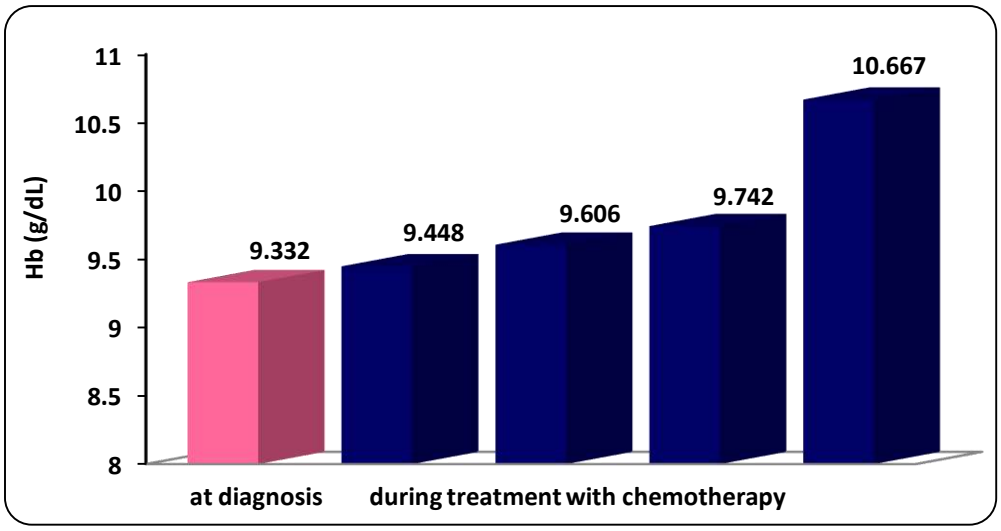


Figure 3-4: Hgb Concentrations of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

Results of present work indicated a decrease in the ratios of the HCT in combination with the reduction in RBCs count and Hgb levels, then began to rise after the fourth dose (**Figure 3-5**).

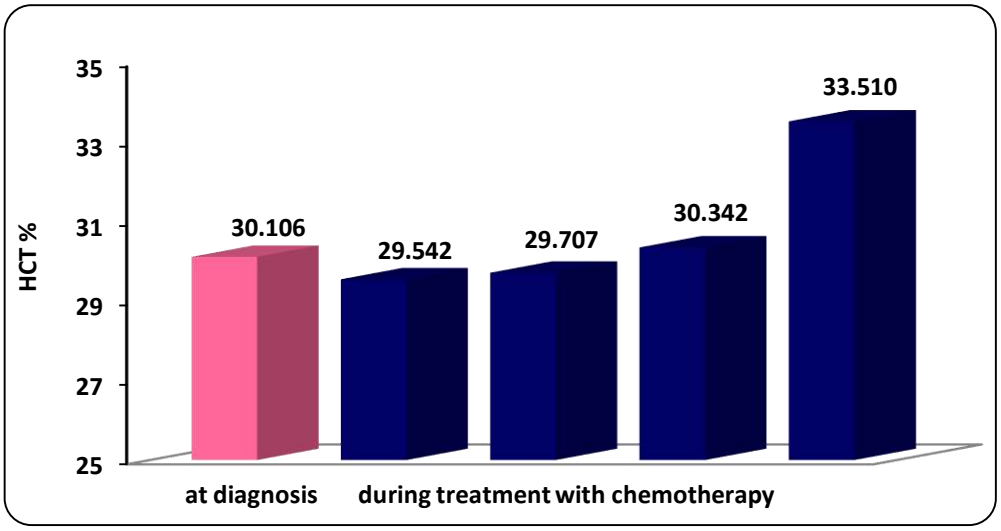


Figure 3-5: HCT Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

Figure 3-6 shows a rise of the total number of Plts in the blood samples of ALL patients after receiving at least four doses of chemotherapy.

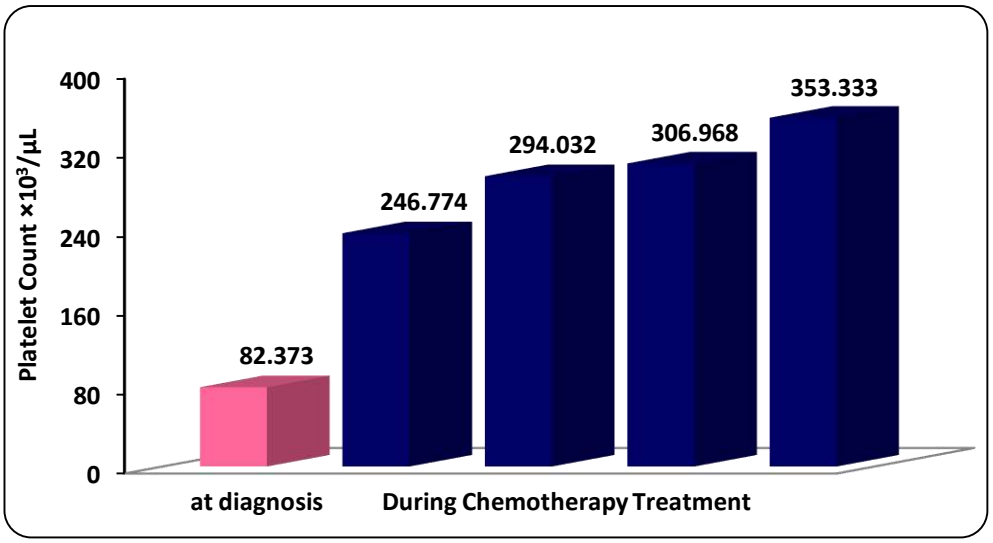


Figure 3-6: Plts Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

According to the Plts, the current results were consistent with [Ross *et al.*, 2017; Samra *et al.*, 2020] studies. Although the results of the current study were inconsistent with that indicated the absence of difference in numbers of Plts count among patients with ALL and the group of healthy individuals either at

the diagnosis of leukemia or after receiving several doses of chemotherapy [Dewan and Agarwal, 2015; Bhushan *et al.*, 2018].

In addition, Noronha's study [Noronha *et al.*, 2016] pointed to the fact that patients with ALL disease have a reduction of the numbers of Plts after chemotherapy, as the results of this study that are not consistent with the results of current study. In similar to Rauch's study [Rauch's *et al.*, 2017] who specified a decreasing number of Plts in blood samples of ALL patients after getting more than three doses of chemotherapy, as well as, by ALL patients had frequent bleeding appearances due to thrombocytopenia with severe anemia.

Thus, the number of Plts is an important parameter for investigating acute leukemia status in children and the risk of not responding for treatment or relapse leukemia which is reduced when the number of Plts would be increased after received therapy [Terwilliger and Abdul-Hay, 2017].

Figure 3-7 shows increased levels of MCV of blood samples in patients with ALL after delivered consecutive doses of chemotherapy in compared to its level that noted at diagnosis.

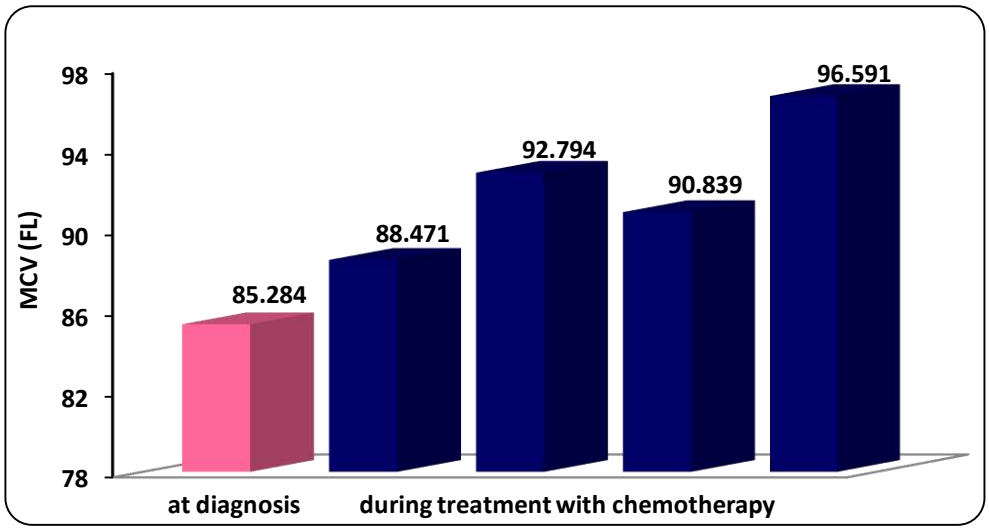


Figure 3-7: MCV Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

In addition, the values of MCH showed uneven rise during successive doses of treatment compared to its level that recorded at diagnosis (**Figure 3-8**).

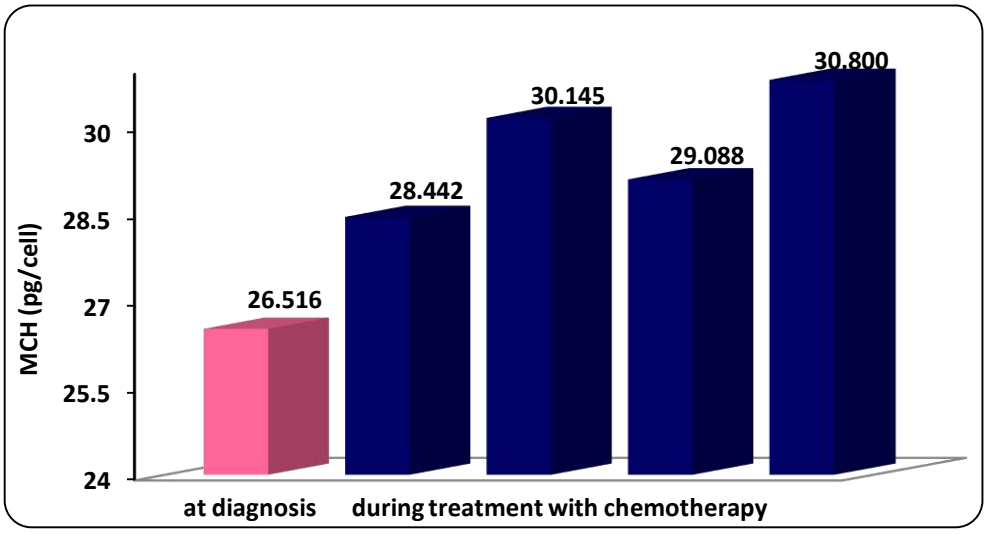


Figure 3-8: MCH Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

Whereas there was a gradual increase in concentrations of MCHC in conjunction with levels of Hgb and HCT values, then it began to decrease after the fourth dose, as elucidated in **Figure 3-9**.

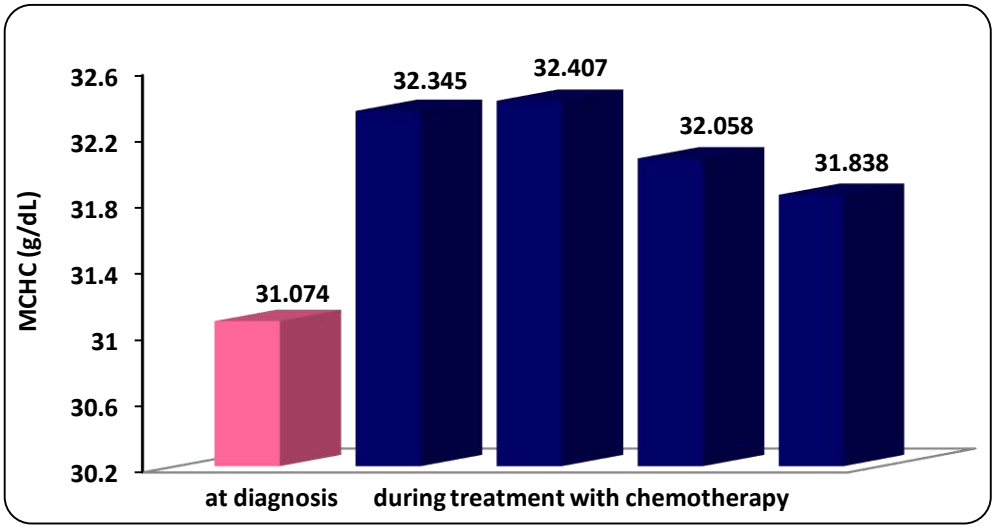


Figure 3-9: MCHC Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

Results of the present study were in agreement with Hade's study, [2018], as the majority of ALL patients suffer from severe anemia, especially macrocytic anemia, because of lack numbers of mature erythroblasts with a low amount of hemoglobin, and then decrease in the number of mature red cells which causing a high growth with an accumulation of abnormal erythroblasts in the bone marrow at the expense of other cellular lines, consequently leading to failure of bone marrow functions by which could be explained the decrease in the levels and concentrations of many blood values and indicators in most leukemia patients [Hoffbrand and Moss, 2016].

The results of the current study were consistent with [Pandey *et al.,* 2017; Terwilliger and Abdul-Hay, 2017] studies which confirmed that the decline in most hematological parameters and indices of leukemia patients before receiving doses of chemotherapy, as well as, the results showed that the significant increase in some blood standards and indicators in the group of ALL patients who delivered doses of chemotherapy compared with individuals in the control group in which indicating the response of patients to chemotherapeutic treatment [Wei *et al.,* 2020].

It could be explained the result by two hypotheses as follows:

The First: it because of the insufficiency of vit B_{12} which is a major public health condition with more common in some types of anemia and hematological malignancies as compare to non-hematological diseases [Horie *et al.,* 2017]. Although, vit B_{12} deficiency causes vast '' megaloblastic changes'' in the bone marrow of patients who have various malignancies [Horie *et al.,* 2017; Konda *et al.,* 2019] who confirmed to diagnose of severe **pancytopenia** and **macrocytic** anemia [Jalaeikhoo *et al.,* 2017], as well as **megaloblastic** anemia as a result of decreasing levels of vit B_{12} [Konda *et al.,* 2019]. However, Kavanagh's study [Kavanagh *et al.,* 2018] have indicated that the decrease of vit B_{12} levels and accompanied with macrocytic anemia was

recognized in leukemia patients with CML who were receiving therapy and accompanied with macrocytic anemia. According to the recent study conducted by Tandon *et al.*, [2015] who had stated considerably lower levels of vit B_{12} in ALL, AML and NHL patients, and followed by the lowest levels of folic acid at the time of diagnosis.

The Second: includes the occurrence of aplastic bone marrow anemia in patients with ALL , therefore; the aplastic bone marrow may be possible as a result of ‘’immunological reactions’’ which activated by the increase of proliferation and widespread of leukemic cells, especially T-lymphocytes, and these cells could be produced the ‘’lymphokines’’ to attract other immune cells; such as B-lymphocytes to produce antibodies, and thus attack the normal blood cells, especially RBCs and work to reduce their numbers and/or to be in larger than normal size [Scheurera *et al.*, 2018; Filbin and Monje, 2019].

However; several previous studies achieved by [Bryer and Henry, 2018; Tebbi, 2021] have shown a reduction in the levels of Hgb, RBCs count, HCT and MCHC after chemotherapy, the decrease has attributed to the effect of chemotherapeutic management on the bone marrow, which leads to a decrease in the values of some blood indicators and may lead to the failure of the bone marrow to perform its functions normally and properly.

Qingkai’s study [Qingkai *et al.*, 2021] was agreed with current results which indicated that children with ALL suffered from a decrease in blood indicators, which it’s possible referred to an increased risk for patients not responding to treatment, as well as increased the toxic side effects of chemotherapy. On the other hand, Robert’s study [Robert *et al.*, 2015] and Pereira’s study [Pereira *et al.*, 2017] came incompatible with present data.

Thus it is likely such blood indicators could be used as good factors for predicting prognosis, as well as the response for treatment.

3.4.1: Evaluation of White Blood Cells Count in the Samples of the Study Groups

The statistical analysis using **Student's *t-test*** shows that there was a significant increase (***p<0.043***) in the number of WBCs in the samples of patients with ALL at diagnosis in comparison to healthy individuals, as shown in **Table 3-10**.

Table 3-10: White Blood Cells Count in The ALL Patients and Healthy Individuals

Study Groups (n)	*WBCs (10^3/µL) Mean ± S.D.*	*Min-Max WBCs (10^3/µL)*	*Range*	*p-value*
ALL Patients 31	13.4811±1.814	8.600 - 16.400	7.800	***0.043***
Healthy Individuals 40	8.538±1.192	6.500 - 10.500	4.000	

The Mean Difference is Significant at 0.05 Level

However; ANOVA test demonstrated a significant increase (**p=0.000**) in the total number of WBCs as proportional for males and females ALL patients when compared to their peers in the group control. Statistically, the results show there are no variations among the genders within the same group of patients and healthy individuals, as shown in **Table 3-11**.

Table 3-11: Levels (Mean±S.D.) of White Blood Cells Count in the Study Individuals

Study Groups (n)	*Gender (n)*	*WBCs ($10^3/\mu L$) Mean ± S.D.*	*Min-Max WBCs ($10^3/\mu L$)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	13.500±1.491	11.400 - 16.300	4.900	***0.678 For1vs2***
	Female 13	13.723±2.068	8.600 - 16.400	7.800	***0.797 For3vs4***
***Healthy Individuals* 40**	**Male 20**	8.515±1.316	6.500 - 10.500	4.000	***0.000 For1vs3***
	Female 20	8.635±1.101	6.500 - 10.200	3.700	***0.000 For2vs4***

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

The numbers of WBCs are determined as an essential indicator of the overall health, therefore; abnormal WBCs production is an important sign of the occurrence of leukocytosis, especially during inflammation, hematological and solid tumours, while the decrease in the number of WBCs which considered as a medical condition known as leukopenia [Iraqi Cancer Board, 2017; Scheurera *et al.*, 2018].

Therefore, a high WBCs count in the blood samples of ALL patient is belonged to the huge number of lymphoblasts due to the defect in the growth and development of lymphoid progenitors in the bone marrow and mostly the increase in productivity of leukocytes is frequently a result of increased numbers of neutrophils and lymphocytes in the whole blood [Iraqi Cancer Board, 2017].

The results of the current study were in agreement with Hade's, [2018] study which referred to decrease count of leukocytes numbers in the blood sample of patients' group after receiving more than three doses of

chemotherapy which specified by the specialists in comparison to the account of WBCs at diagnosis, as shown in **Figure 3-10**.

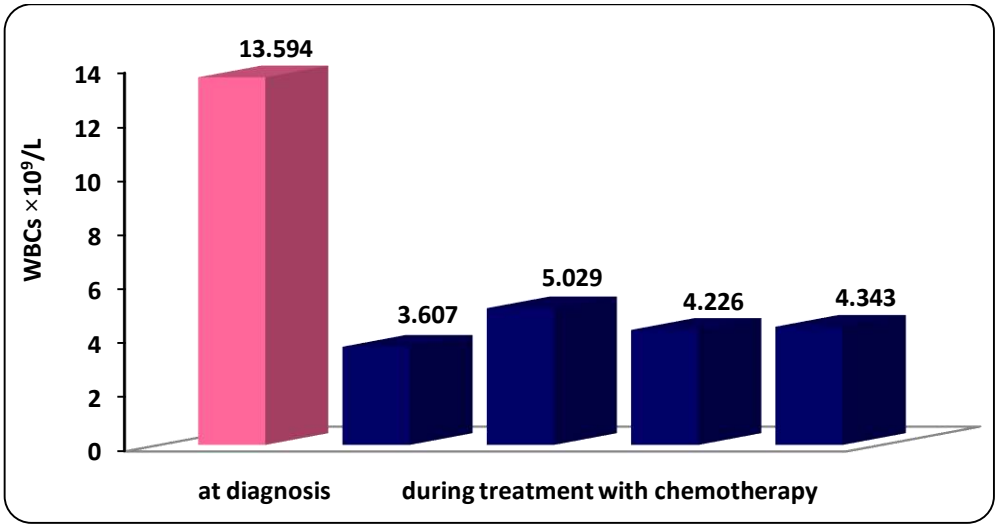


Figure 3-10: WBCs Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

It's possible that there was a clear decrease in WBCs numbers after periods of chemotherapy and may return gradually to normal levels after treatment if there is a strong response for treatment [Chen *et al.*, 2015].

The routine use of chemotherapy drugs in the treatment of different types of malignancies is the most effective on WBCs count, particularly its effects on the number of lymphocytes, neutrophils, and monocytes as these cells are considered the main target and the first line of defence in the body with a basic database of physicians to choose the appropriate chemotherapeutic protocol for each case, as well as to follow up the progression of the disease and patients' response among children with ALL during different stages of chemotherapeutic treatment [Fieg, 2016].

3.5: Evaluation Levels of Nitric Oxide in The Sera Samples of Patients with ALL (at diagnosis) and Healthy Controls

Level of Nitric Oxide (NO) concentration was estimated in the sera samples of the participant individuals. Outcomes of the present work recorded a statistical significant increase (**p=0.008**) of NO concentrations of ALL patients in comparison to the healthy controls, as illustrated in **Tables 3-12**.

Table 3-12: Levels of Nitric Oxide Concentration (µmol/L) in The ALL Patients and Healthy Individuals

Study Groups (n)	*NO Conc. (µmol/L) Mean ± S.D.*	*Min-Max NO (µmol/L)*	*Range*	*p-value*
ALL Patients 31	52.059±9.263	40.310 - 73.350	33.040	***0.008***
Healthy Individuals 40	36.096±10.025	24.100 - 55.300	31.200	

The Mean Difference is Significant at 0.05 Level

Applied ANOVA test showed a respectable significant increase (**p=0.000**) of NO levels in male and female patients with ALL when were compared with their analogues individuals in the healthy group. On the other hand, no significant variations were shown among males and females within controls subgroups, as well as within both genders of ALL patients, as summarized in **Table 3-13**.

Table 3-13: Levels (Mean±S.D.) of Nitric Oxide Concentration*(µmol/L)* in The Study Individuals

Study Groups (n)	*Gender (n)*	*NO Conc. (µmol/L) Mean ± S.D.*	*Min-Max NO (µmol/L)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	52.826±9.774	40.310 - 72.900	32.590	***0.664 For1vs2 0.360 For3vs4 0.000 For1vs3 0.000 For2vs4***
	Female 13	51.262±9.151	40.310 - 73.350	33.040	
***Healthy Individuals* 40**	**Male 20**	37.846±11.901	24.300 - 55.300	31.000	
	Female 20	34.972±7.941	24.100 - 53.400	29.300	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

The present outcomes were in agreement with Levine's [Levine *et al.*, 2014] study which demonstrated that serum NO concentration was significantly higher in subjects with ALL. Despite the simple structural molecule of NO, it plays a key role in many biological functions [Levine *et al.*, 2014; Vahora *et al.*, 2016].

In addition, NO is a main metabolic product of the ''oxidation process'' which due to the interaction of NO with ''reactive oxygen and nitrogen species'', leading to the production of peroxynitrite anion, tyrosine-protein nitrogen, and then the formation of hydroxyl radical, as well as, it is a highly reactive free radical, thus the excessive production of NO could be considered as a toxic agent which responsible for many complication conditions [Picón-Pagès *et al.*, 2019; Chen *et al.*, 2021].

Hence the increased levels of NO concentration which could be characterized as a general mechanism contributed to many diseases [Clementi and Nisoli, 2015; Gureev *et al.*, 2019], then as a result of either excessive or lacking production of NO that could lead to pathological changes in various physiological systems caused by the disturbances of NO [Gureev *et al.*, 2019].

Explanations of results of the current study are based on a set of hypotheses:

- As the significant increase levels of NO which consequently with the high activity of enzymes responsible for the regulation of the NO synthesis [Keshet and Erez, 2018].
- NO levels may be associated with high levels of Malondialdehyde (MDA) as a cellular oxidation criterion which has a high concentration and activity in ALL children pre-chemotherapeutic treatment [Hade, 2018]. Thus, the levels of NO production increase concurrently with the occurrence of cellular changes of the natural cell and its transformation into a cancerous cell during the phase of carcinogenicity, which parallels to the level of production and the severity of progression, as well as metastasis of the disease [Cheng *et al.,* 2021], which it could be caused by the high levels of oxidative stress due to depleting endogenous antioxidants [Hade *et al.,* 2018; Elham, 2020].
- It seems that an increase of ROS production caused to be generated oxygen radicals on the vascular endothelial surface, and then it acts in response to NO and reduces its bioavailability, thus it causes an increase in oxidative stress (An imbalance of NO and ROS) [Elham, 2020]. In addition, the activity of gene expression is responsible for the enzymatic forms which induce the biosynthesis of NO in diverse tissues, thus the NO metabolites may play a crucial role in mediating many genotoxic/carcinogenic effects such as; the alterations of protein, lipid, and DNA [Luanpitpong and Chanvorachote, 2015].
- It's possible that NO and its derivatives at higher concentrations become cytostatic and cytotoxic [Levine *et al.,* 2014; Cheng *et al.,* 2015], while at the lower levels of NO that could promote cancer growth by several steps of carcinogenesis through suppression and/or changes of protein p53 in the cells which could modulate tumor cell DNA and cause tumor angiogenesis via regulating vascular endothelial growth factor (VEGF) [Keshet and Erez, 2018;

Porporato *et al.*, 2018]. The results of the present study agree with several studies that emphasized the evaluation of NO levels as a parameter of oxidative stress in patients with ALL, and other various malignancies [Matlab and Jasim, 2017; Pasha *et al.*, 2017].

Furthermore, data analysis of the current work indicated a highly significant decrease in the NO after the first dose of treatment in a group of ALL children, then the reduction became moderate after receiving the fourth dose of chemotherapy which was applied in the management plan of treatment when compared its levels with the pre-treatment stage according to the planned design of therapy, as demonstrated in **Figure 3-11**.

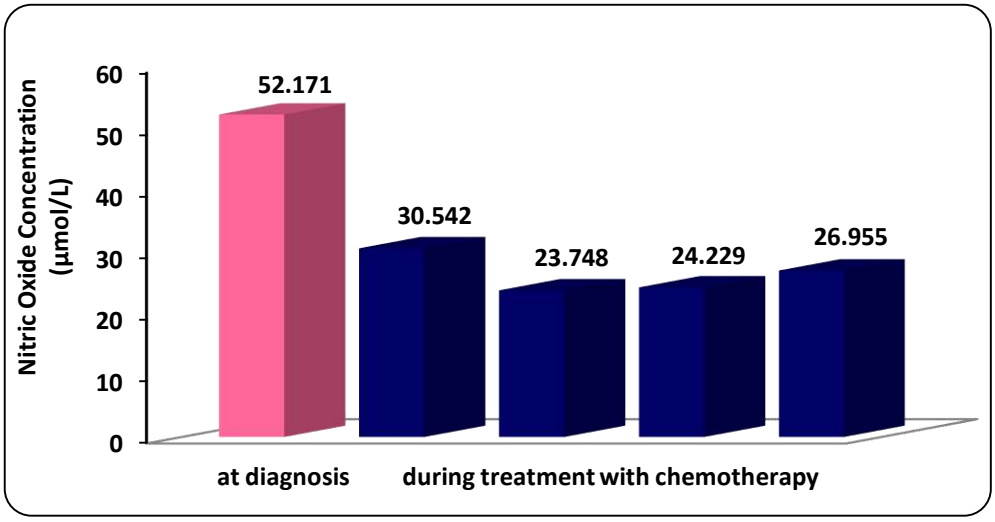


Figure 3-11: NO Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

It may probably indicate to the low levels of cellular damage caused by oxidative stress in ALL patients at the diagnostic stage, then the levels of excessive cellular oxidation decline as the protocol of applied therapy progressing, which may give this criterion importance in tracking it down in the success of treatment management strategy [Caplin and Leiper, 2014; Zhang *et al.*, 2018]. In addition, the results of the current study were in the same line with numerous studies that investigated the oxidative stress levels before and

after getting chemotherapy in some types of leukemia, as well as other cancers [Balasaheb and Pal, 2015; Matlab and Jasim, 2017; Zhang *et al.*, 2018].

3.6: Assessment of Total Glutathione Levels in The Sera Samples of ALL Patients and Healthy Control Groups

The levels of Total Glutathione (T-GSH) concentration were evaluated in the sera samples of the present study participants. The results showed a significant decrease (**p=0.031**) in the T-GSH concentrations in the sera samples of ALL patients group at diagnosis in comparison to the control, as shown in **Table 3-14**.

Table 3-14: Levels of Total Glutathione Concentration (µmol/L) in The ALL Patients and Healthy Individuals

Study Groups (n)	*T-GSH Conc. (µmol/L) Mean ± S.D.*	*Min-Max T-GSH (µmol/L)*	*Range*	*p-value*
ALL Patients 31	10.9647±3.249	3.520 - 18.020	14.500	***0.031***
Healthy Individuals 40	17.294±3.623	10.800 - 23.430	12.630	

The Mean Difference is Significant at 0.05 Level

In order to study the effect of gender on the T-GSH levels, an ANOVA test was applied. The statistical results showed that no significant changes of T-GSH concentrations among male and female patients within ALL group, so the two genders in the controls group, while there were significant variations (**p=0.000**) between ALL males as well as females, in comparison to their healthy subject peers, as illustrated in **Table 3-15**.

Table 3-15: Levels (Mean±S.D.) of Total Glutathione Concentration (µmol/L) in The Study Individuals

Study Groups (n)	*Gender (n)*	*T-GSH Conc. (µmol/L) Mean ± S.D.*	*Min-Max T-GSH (µmol/L)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	11.103±3.018	3.520 - 14.600	11.080	***0.446 For1vs2 0.328 For3vs4 0.000 For1vs3 0.000 For2vs4***
	Female 13	10.192±3.089	4.190 - 15.510	11.320	
***Healthy Individuals* 40**	**Male 20**	16.134±3.768	10.800 - 23.430	12.63	
	Female 20	18.454±3.043	11.640 - 22.320	10.680	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

In order to verify the changes of T-GSH concentration during chemotherapy progress, the estimation of T-GSH was carried out after each dose of chemotherapy. **Figure 3-12** shows a significant rise in the levels of T-GSH after the doses of chemotherapy planned within the current study design, but these concentrations remain higher than T-GSH levels at the baseline stage.

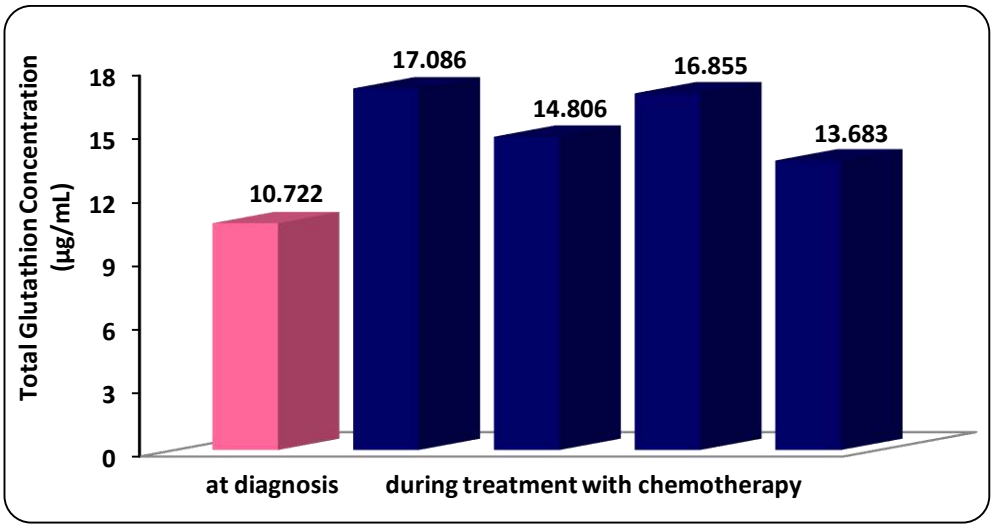


Figure 3-12: T-GSH Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

3.7: Assessment of Oxidized Glutathione Disulfide Levels in The Sera Samples of Patients and Control Groups

Oxidized Glutathione Disulfide (GSSG) concentrations were evaluated in sera samples of the current study participants. According to the levels of GSSG, the data were exhibited a significant decrease (**p=0.044**) in GSSG levels of ALL patients group as compared with the healthy control (**Table 3-16**).

Table 3-16: Levels of Oxidized Glutathione Disulfide Concentration (µmol/L) in The ALL Patients and Healthy Individuals

Study Groups (n)	*GSSG Conc. (µmol/L) Mean ± S.D.*	*Min-Max GSSG (µmol/L)*	*Range*	*p-value*
ALL Patients 31	0.344±0.157	0.100 - 0.630	0.530	***0.044***
Healthy Individuals 40	0.360±0.224	0.112 - 0.860	0.748	

The Mean Difference is Significant at 0.05 Level

Table 3-17 shows there are no statistical variations in the levels of GSSG between males and females in the patients' group when compared with their counterparts in the control group. Despite the absence of significant differences in the levels of GSSG between genders within the study groups (either ALL patients or controls groups), but the results illustrated an elevation of this parameter in the females' subgroups compared to males.

Table 3-17: Levels (Mean±S.D.) of Glutathione Disulfide Concentration (µmol/L) in The Study Individuals

Study Groups (n)	*Gender (n)*	*GSSG Conc. (µmol/L) Mean ± S.D.*	*Min-Max GSSG (µmol/L)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	0.312±0.179	0.100 - 0.600	0.500	***0.323 For1vs2 0.152 For3vs4 0.941 For1vs3 0.738 For2vs4***
	Female 13	0.383±0.121	0.160 - 0.630	0.470	
***Healthy Individuals* 40**	**Male 20**	0.316±0.203	0.118 - 0.860	0.742	
	Female 20	0.406±0.234	0.112 - 0.792	0.680	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

The outcomes of the current study recorded a statistical variation in the GSSG levels of ALL patients after they received four consecutive doses of chemotherapy, but GSSG concentration remain higher than its levels at the diagnostic stage (**Figure 3-13**). The present results agreed with [Rasool *et al.*,2015; Kaweme *et al.*,2020; Chen *et al.*,2021] studies which had reported that glutathione levels were decreased in ALL and AML patients who in turn showed impairment of antioxidant system, then the levels of glutathione increased gradually after the course of chemotherapeutic regimen.

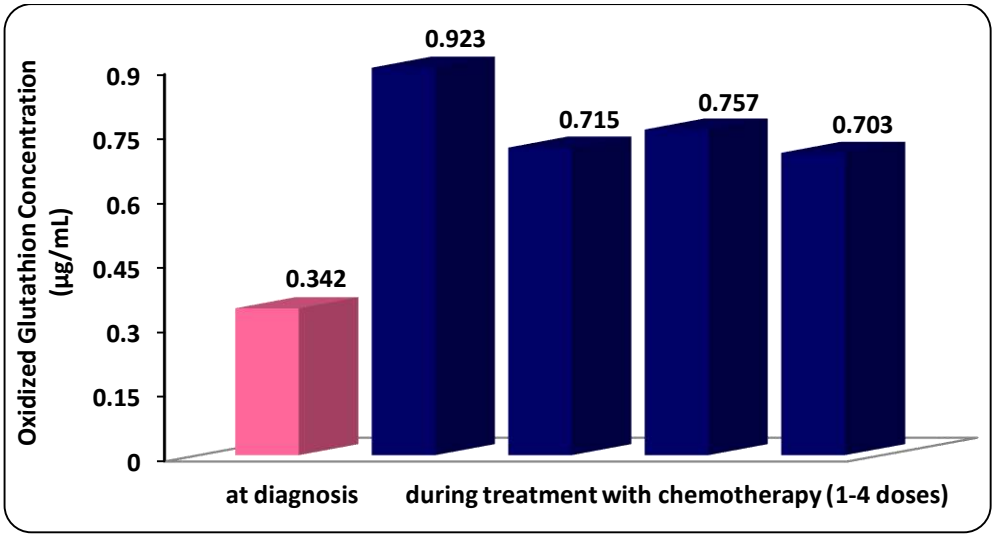


Figure 3-13: GSSG Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

According to the present study findings, the decreased glutathione levels in patients with ALL could be explained as a result of declining production of glutathione levels caused by reduced and/or insufficient enzymes which formed glutathione in the liver that could play an important role in the production of glutathione as a ‘’target response’’ of toxic effects by free radicals in leukemic patients [Rezaieg and Musleh, 2019].

In addition, it could be related directly to unusual modifications in proteins produced by leukemic cells, and its increased consumption in the case of suppression oxidative stress, so it has indicated that the impairment of the antioxidant system due to the increase of free radicals in ALL cases which are based on an increase of the oxidative damage and prevent motivation of cells apoptosis pathway, thus stimulating cancer progression [Bispo *et al.,* 2020; Tebbi, 2021].

On the other hand, many cancer types including; head, neck, lung, breast, ovarian and colon may show high levels of GSH, while its levels have been decreased in brain and liver tumors, in addition to some types of leukemia, that is possible due to the abnormal regulation of genes involved in GSH metabolism which correlated to many cancer types [Desideri *et al.,* 2019; Luke *et al.,* 2020].

Moreover, an increase in GSH concentrations in the sera of ALL patients after a delivered course of chemotherapy may be explained according to the fact that chemotherapy treatment could be effective and beneficial to patients at this stage of management when it is likely to inhibit the growth of leukemic cells (blasts), thus increasing the levels of antioxidants represented at this stage of treatment on the opposite side of oxidative agents and free radicals, therefore the regulation of GSH metabolism could be a targeted treatment for cancer patients, and so the possibility of regulating cellular responses to various cancer treatment agents [Bansal and Simon 2018; Desideri *et al.,* 2019].

However, the outlooks of the anticancer treatment established by the inclusion of concentration levels of GSH that achieved to be inadequate because of the potentially damaging properties to the normal cells [Rafieemehr *et al.,* 2019; Desideri *et al.,* 2019]. Hence, the GSH system has drawn the considerations for many researchers to deal with some strategies which aimed for developing attempts to disrupt the growth of cancerous cells, and to increase the effectiveness of anticancer managements that can be obtainable for cancer patients [Desideri *et al.,* 2019].

Furthermore, GSH is produced through many anti-cancer treatments, which leads to the formation of GSSG by Glutathione Peroxidase (GPx), then the product (GSSG) is promptly converted and returned to GSH by Glutathione Reductase (GR) to inhibit the accumulation of GSSG that could be caused cells toxicity, then further activation of cells death [Couto *et al.,* 2016; Desideri *et al.,* 2019].

Additionally, increasing of GSSG concentration within cells has assumed to be an important therapeutic strategy for inducing the death of cancerous cells [Couto *et al.,* 2016; Desideri *et al.,* 2019]. In this direction, there is another approach that should be kept in the respects of strategies to use the GSH therapy, by which in the case of resistance to consumption and depletion of GSH, as it's the possibility of a crossing between the GSH metabolism and different cell death pathways; such as '' autophagy, apoptosis, and necrosis'' [Miess *et al.,* 2018; Sun *et al.,* 2018].

This would be indicated that the excessive levels of cellular oxidation prior to the delivery of the chemotherapeutic drug are declining directly with the use of treatment protocol and this requires to be further followed-up to these criteria, which are seemed to be important in the tracking of the successful therapeutic plan that has been applied.

3.8: Assessment of Erythropoietin Hormone Levels in The Sera Samples of Patients and Healthy Groups

Evaluation of Erythropoietin (EPO) concentrations indicated to high EPO levels in the samples of ALL patients than its levels in the healthy children. **Table 3-18** shows that there are statistically significant differences (**p=0.000**) of the EPO in the sera of ALL patients compared to their corresponding in the control group.

Table 3-18: Levels of Erythropoietin Concentration (mIU/mL) in The Sera of ALL Patients and Healthy Individuals

Study Groups (n)	*EPO Conc. (mlU/mL) Mean ± S.D.*	*Min-Max EPO (mIU/mL)*	*Range*	*p-value*
ALL Patients 31	3551.232±53.341	171.530 - 408.230	236.700	***0.000***
Healthy Individuals 40	147.692±18.889	123.840 - 193.730	69.890	

The Mean Difference is Significant at 0.05 Level

The highest level of EPO was recorded in the sample of child females recorded **(408.23 mlU/mL)**, while the lowest level (**123.84 mlU/mL**) was recorded in a healthy female. Statistical analysis showed no significant differences between males and females patients, as well as in the healthy individuals when compared two sexes together. On the other hand, the study succeeded in recording high significant differences (**p=0.000**) of EPO levels among males' and females' patients with ALL and their healthy counterparts in the control group, as shown in **Table 3-19**.

Table 3-19: Levels (Mean±S.D.) of Erythropoietin Concentration (mIU/mL) in The Study Individuals

Study Groups (n)	*Gender (n)*	*EPO Conc. (mIU/mL) Mean ± S.D.*	*Min-Max EPO (mIU/mL)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	360.781±46.195	264.69 - 407.00	142.31	***0.938 For1vs2 0.592 For3vs4 0.000 For1vs3 0.000 For2vs4***
	Female 13	361.679±37.735	303.10 - 408.23	105.13	
***Healthy Individuals* 40**	**Male 20**	150.986±22.313	125.90 - 193.73	67.83	
	Female 20	145.590±15.151	123.84 - 176.55	52.71	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

It was observed that EPO levels increased in the patients' group after receiving chemotherapeutic doses as compared to its levels at diagnosis (**Figure 3-14**). These results were matching with the results of Hade's study, [2018], which indicated an increase EPO level in children with ALL compared to its levels before treatment beginning. EPO hormone is secreted by the kidney and liver in response to the low oxygen levels in the body when this hormone works to increase the production of RBCs in the bone marrow that leads to elevate rates of oxygen delivery to the different tissues [Aapro *et al.,* 2018]. Several previous studies have shown that the increased levels of EPO hormone might be considered as an indicator of the incidence of cancers, in general; and particularly acute lymphoblastic leukemia [Pandey *et al.,* 2017].

The growth of malignant cells at the transformation stage causes a decrease in the production of erythrocytes due to the defect that affects the bone marrow and causing pernicious or malignant anemia, as this stage is characterized by the deficiency of vit B_{12}, as well as a decrease in processed oxygen rates. The body works at this stage to have great efforts by alerting to increase the amount of oxygen incoming to the cells through the raising of EPO productivity [Gavars *et al.,* 2019; Kesbeha and Pakbaza, 2019].

The results of the current study were in agreement with previous studies in which confirmed that leukemic patients suffered from different types of anemia such as; pernicious and macrocytic anemia which coinciding with the elevation of EPO levels [Tanyildiz *et al.,* 2016; Konda *et al.,* 2019].

Figure 3-14 shows that hormone levels during the period of received chemotherapy agents witnessed a significant increase (almost constant during getting the four doses of treatment) compared to the levels of this hormone at diagnosis.

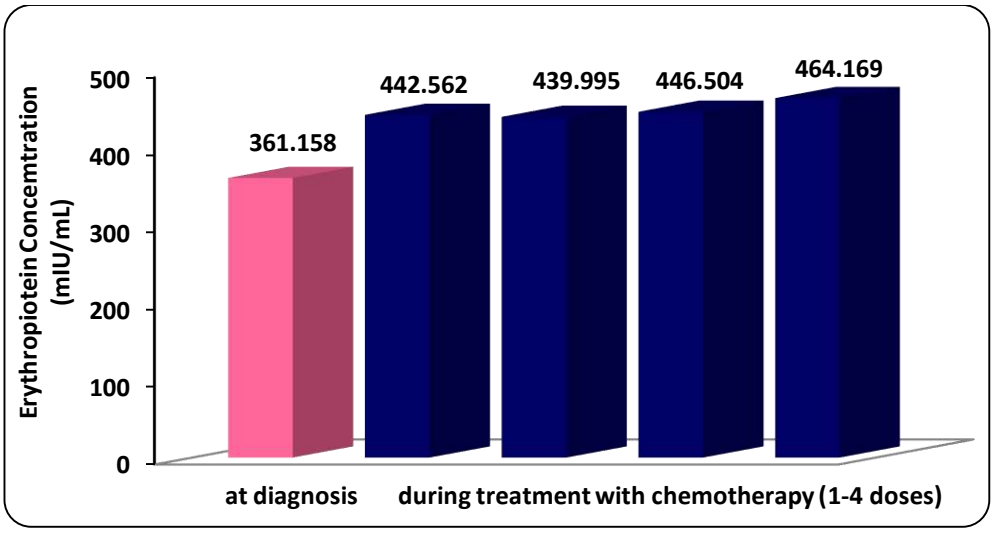


Figure 3-14: EPO values of patients with ALL at diagnosis (pre-treatment) and during treatment with chemotherapy

It's possible that outcomes of the present study indicated that patients may not be able to respond to chemotherapeutic treatment, especially for four doses, they may have the possibility of either relapse after the period of induction or the spread of the disease to other areas of the body, which may cause liver and kidney diseases, as well as bone marrow disorders, this also supports the continued presence of anemia in patients even after four doses of chemotherapy [Gaine *et al.,* 2017; Terwilliger and Abdul- Hay, 2017; Silverthorn, 2018].

3.9: Assessment of Parathyroid Hormone Levels in The Sera Samples of ALL Patients and Control Groups

Parathyroid hormone (PTH) has an important regulator role in binding preserved amino acids to their receptors on the cell membrane by cellular pathways [Silverthorn, 2018]. Both PTH and calcitonin hormones have a central role in the involvement of the control of calcium ions (Ca^{2+}) balance and regulation of the movement of Ca^{2+} among intestine, bone, and kidney [Kaku *et al.,* 2015; Randolph *et al.,* 2016].

The important results of PTH in the current study revealed a significant decrease in PTH concentration in the sera samples of children with ALL in comparison to the healthy children (**p=0.016**), as shown in **Table 3-20**.

Table 3-20: Levels of Parathyroid Hormone Concentration (pg/mL) in The ALL Patients and Healthy Individuals

Study Groups (n)	*PTH Conc. (pg/mL) Mean ± S.D.*	*Min-Max PTH (pg/mL)*	*Range*	*p-value*
ALL Patients 31	53.482±22.293	27.400 - 92.910	65.510	***0.016***
Healthy Individuals 40	54.189±15.915	25.700 - 75.040	49.340	

The Mean Difference is Significant at 0.05 Level

Moreover; significant differences (**p=0.002**) were observed in ALL males and females when compared together in the same group. A statistically significant (**p=0.031**) increase in PTH concentrations recorded in ALL males' patients' subgroup comparison to those in healthy males' controls. On the other hand, PTH showed a significant decrease (**p=0.021**) when ALL females patients were compared to healthy females, as demonstrated in **Table 3-21**.

Table 3-21: Levels (Mean±S.D.) of Parathyroid Hormone Concentration (pg/mL) in The Study Individuals

Study Groups (n)	*Gender (n)*	*PTH Conc. (pg/mL) Mean ± S.D.*	*Min-Max PTH (pg/mL)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	63.178±20.638	33.100 - 92.910	59.810	***0.002 For1vs2***
	Female 13	41.808±18.670	27.400 - 82.900	55.500	***0.266 For3vs4***
Healthy Individuals 40	**Male 20**	50.449±15.958	29.800 - 75.040	45.24	***0.031 For1vs3***
	Female 20	56.755±16.104	25.700 - 74.200	48.500	***0.021 For2vs4***

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

Figure 3-15 indicates to decrease of PTH levels in the patients' samples after four consecutive doses of chemotherapy in comparison to its levels at diagnosis.

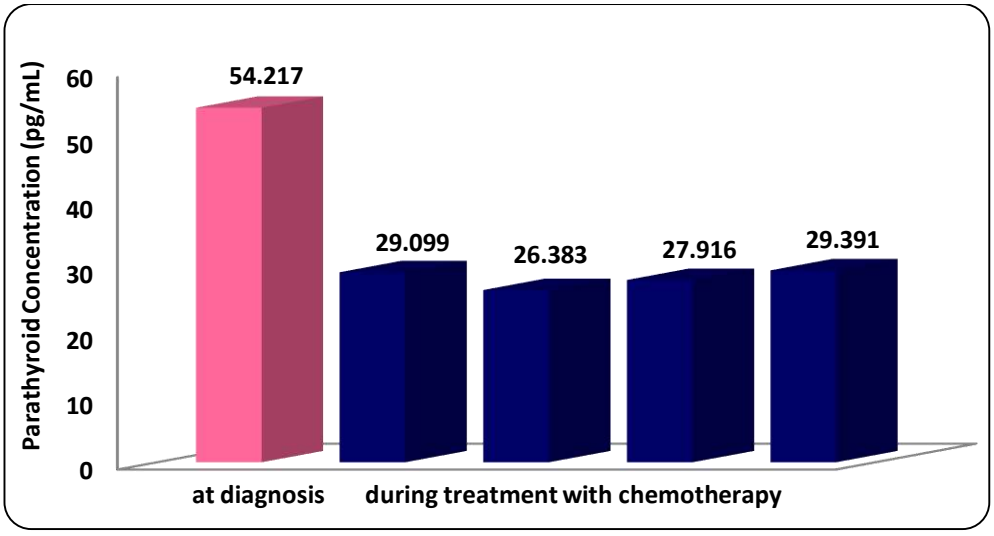


Figure 3-15: PTH Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

The secretion of PTH is stimulated by decreasing Ca^{2+} levels in the blood (hypocalcemia) in combination with calcitonin secreted by the C cells of the thyroid gland to provide some complementary mechanisms to sustain blood Ca^{2+} levels within optimal limits, hence; PTH passing through the blood to act

on bone, intestine, and kidney then commencing responses that increase the concentration of Ca^{2+} ions in the blood [Wang *et al.*, 2017; İlarslan *et al.*, 2017] because many metabolic abnormalities were observed in ALL patients, the concentrations of Ca^{2+} in the blood become in very high levels and the parathyroid glands may sometimes produce too much PTH, therefore; this leads to the highest levels of Ca^{2+}, and then it could be caused hypercalcemia and primary hyperparathyroidism which have detected in many conditions of hematological malignancies and lymphoma, thus it's possible that high levels of PTH were associated with higher probabilities of ALL occurrence [Karaköse *et al.*, 2021].

The regulated functions of many organs such as; thyroid gland (especially C cells), parathyroid gland, and kidney are required to extracellular fluid (ECF) Ca^{2+} by the binding of PTH to cell membrane calcium-sensing receptors to maintain normal homeostatic control on the serum Ca^{2+} levels within the adequate range of intracellular Ca^{2+}, and then regulate any change of abnormal concentrations of serum Ca^{2+} for hypercalcemia or hypocalcemia which contributed to different pathological disorders such as; different types of leukemia [Randolph *et al.*, 2016; Hannan *et al.*, 2018]. Therefore, the rise of Ca^{2+} in the blood is a negative feedback index in which turn off the reaction of severe decreasing of Ca^{2+} ions, especially in bones of patients, and then finally the releasing of PTH [Goldner, 2016; Dhivyasree *et al.*, 2018].

In addition, PTH is mostly mediated with hypercalcemia in patients with leukemia, specifically childhood ALL patients [Tagiyev *et al.*, 2016; Dhivyasree *et al.*, 2018]. Hoyoux's study [Hoyoux *et al.*, 2017] stated that hypercalcemia is relatively one of the most aggressive complications in childhood ALL, as well as, it has predictable between 5-20% as common complications in various types of malignancies, as well as the development of hypercalcemia as an outcome of ALL accompanied by elevated levels of PTH

by which associated with patients of ALL during childhood stage, which has considered as a strong diagnostic factor in patients [Walker and Silverberg, 2018].

3.10: Measurement of Serotonin Levels in The Sera Samples of Patients with Acute Lymphoblastic Leukemia and Healthy

Levels of serum serotonin (ST) were studied in the two study groups (ALL patients and healthy subjects). **Table 3-22** illustrated a statistically significant increase (**p=0.001**) of serum ST levels in ALL patients group when compared to those of healthy individuals.

Table 3-22: Levels of Serotonin Concentration (ng/mL) in The ALL Patients and Healthy Individuals

Study Groups (n)	*Serotonin Conc. (ng/mL) Mean ± S.D.*	*Min-Max Serotonin (ng/mL)*	*Range*	*p-value*
ALL Patients 31	218.938±74.488	136.500 - 383.910	247.410	***0.001***
Healthy Individuals 40	197.182±49.331	101.780 - 276.94	175.160	

The Mean Difference is Significant at 0.05 Level

The present study demonstrated a significant difference (**p=0.038**) in the ST levels when the comparison between male and female patients with ALL together was done, while there was no statistical difference in ST levels between males and females in the healthy group, as presented in **Table 3-23**.

In the same manner, the statistical results were significant (**p=0.020**) when performing the implicit comparison between ALL male patients and their counterparts in the healthy group, whereas there were no statistically significant differences observed when the comparison was made by the ANOVA test among ALL female patients with their peers in the healthy control group (**Table 3-23**).

Table 3-23: Levels (Mean±S.D.) of Serotonin Concentration (ng/mL) in The Study Individuals

Study Groups (n)	*Gender (n)*	*Serotonin Conc. (ng/mL) Mean ± S.D.*	*Min-Max Serotonin (ng/mL)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	238.975±75.599	140.990 - 383.910	242.920	***0.038 For1vs2 0.071 For3vs4 0.020 For1vs3 0.524 For2vs4***
	Female 13	193.331±69.715	136.500 - 330.820	194.320	
***Healthy Individuals* 40**	**Male 20**	214.274±42.377	125.030 - 275.540	150.510	
	Female 20	179.790±49.470	101.780 - 276.940	175.160	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

The existent results agreed with other studies conducted by [Elshayeb *et al.*, 2016; Rasha and Matlab, 2017, Vahid-Ansari *et al.*, 2019], in which elevation ST levels in several other cancer types was published.

ST is structured by two distinct systems, the first in the central nervous system (CNS) and the other in the periphery, the Blood-Brain Barrier (BBB) obstructs the passage of peripheral ST into (CNS) [Vahid-Ansari *et al.*, 2019]. Nearly 2% of ST in the body is located in CNS [Lv and Liu, 2017; Hadeer, 2020].

A neuroserotonin has a vital role to modulate many sensory, motor and behavioural routes, as well as, it has stated that neurotransmitter ST is involved in the control of nutrition behavior and obesity [Liu *et al.*, 2017], besides a large amount of ST plays an essential role with respect to the consumption, absorption and metabolism of fat and glucose in the body [Julian *et al.*, 2019].

According to the chemical structure of ST, it is transported by using a carrier, as the transporter of ST ‘’SERT or 5-hydroxytryptamine; 5-HT transport protein) as well identified as sodium-dependent ST transporter’’, it is responsible for transferring ST from the ‘’synaptic neurons’’ in the nervous endings either within the nervous system or non-neural areas of gut,

lymphocytes, monocytes, and platelets [Jolanta *et al.,* 2017]. Furthermore, ST has more appeared to be as a ‘’growth factor’’ for tumor cell lines in several types of malignancies [Elshayeb *et al.*, 2016; Sarrouilhe and Mesnil, 2019].

As previous studies were published by Liu *et al.,* [2017] and Vahid-Ansari *et al.,* [2019], which have indicated to intensely elevation, in particular ST sites, in specifically, ‘’SERT’’ in the membrane of tumor cells of the samples patients with various malignancies, thus the expression of SERT is regulated by the activities of ‘’protein-kinases’’ enzymes and gene transcription by which regulatory the basic cellular requirements and the formal modifications of the plasma membrane in which the carrier SERT is associated with the cell membrane through its receptors [Szabo *et al.,* 2018; Balakrishna *et al.,* 2021].

However; a complex mechanism to evoke signaling pathways that stimulate tumor development in some cancer types was demonstrated, whether it was represented as a pattern of ‘’mitogen’’ as a specific receptor pathway which regulated through the molecular mechanism of substrate transport pathways [Ballou *et al.,* 2018], so it’s possible that enhanced ST levels could be associated with the stimulation of these sensitive ‘’serotonergic receptors’’ [Vahid-Anasri *et al.,* 2019; Balakrishna *et al.,* 2021].

In addition, another proposed hypothesis is based on either it’s possibly caused by the high ST in specific cells which depend upon the expression of serotonergic mechanisms in immune cells [Herr *et al.,* 2017]. Hence, ST may contribute to the modification of several inflammatory and immunological responses, for instance; leukocyte activation, proliferation, cytokine secretion, chemotaxsis, and apoptosis [Szabo *et al.,* 2018]. Moreover, it may be caused by the high expression of SERT, as a result of abnormal activity that interferes with the processes of cancer conversions, as it’s noted for the behaviors of

neurons when the changes of SERT receptors, and then ST in the case of cancer incidence [Luc *et al.,* 2017; Anne *et al.,* 2019].

The comparison among ALL children patients during pre and post chemotherapeutic protocol revealed high levels of ST in the samples at diagnosis in comparison to the hormone levels during sequencing treatment stages which may be attributed to the effectiveness of enzymatic mechanisms which responsible for the synthesis of ST hormone even it might have resulted from abnormal cellular transformations during carcinogenicity [Elshayeb *et al.,* 2016; Liu *et al.,* 2017].

When the production of ST during treatment was tracking, results showed a notable decrease after four sequence dosages of chemotherapy until ST levels have reached approximate to this hormone levels in the healthy group, as shown in **Figure 3-16**.

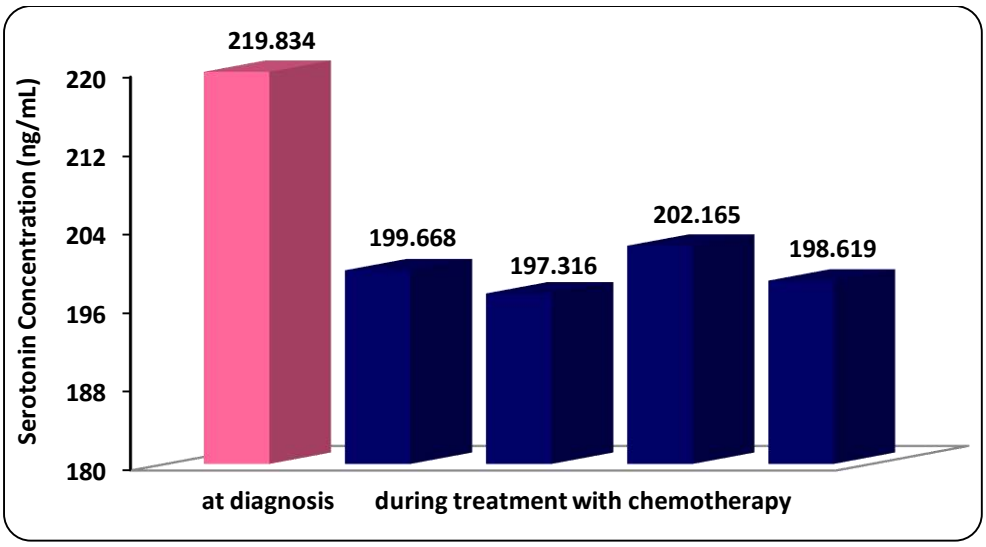


Figure 3-16: Serotonin Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

It is possible that enhanced ST levels that could be associated with the stimulation of sensitive ‘’serotonergic receptors’’ [Luc *et al.,* 2017; Rasha and Matlab, 2017; Szabo *et al.,* 2018]. The effect of chemotherapy in undermining cancerous cells could be explained either by inhibition of ST receptors (serotonin-1 and serotonin-2 receptors) which are extensively over-expressed

in various types of cancer and/or through the inhibition of ST transporter via suppressing matrix metalloproteinases (MMP-9 or collagenase) and (MMP-12 or macrophages metalloelastase), and then it blocks the growth of new blood vessels generation by angiostatin [Zaminpira and Niknamian , 2018; Montilla *et al.*, 2019].

A study was accomplished by Etxabe *et al.*, [2017] has shown that AML cells are characterized by an increase of the expression of ST receptor type 1 [5-hydroxytryptamine receptor 1] (HTR1) compared to the normal hematopoietic progenitor cells in the bone marrow, so it is possible improved that ST levels could be associated with the stimulation of sensitive ‘’serotonergic receptors’’ [Luc *et al.*, 2017; Szabo *et al.*, 2018], therefore; the inhibition of expression ST receptors which cause to stimulate the apoptosis, and reduced the ability of cancer cells proliferation [Szabo *et al.*, 2018].

Conversely; the results of a study performed by Sarrouilhe and Mesnil, [2019] were indicated that ST cause to motivate apoptosis in various malignant cells after its entering through an active transport mechanism, in which it is acting as a potential cytotoxic therapeutic treatment method in most patients with different types of cancers such as; Burkitt lymphoma and acute leukemia by its ability to reduce the tumor burden.

3.11: Assessment of 1, 25 Dihydroxy Vitamin D_3 Concentrations in The Sera Samples of Acute Lymphoblastic Leukemia Patients and Healthy Control Groups

1, 25 Dihydroxy vitamin D_3 concentrations were evaluated in the sera samples of the participants in the present study. Results of the statistical analysis by the **Student's *t-test*** show that there was a significant difference (**p=0.000**) between patients with ALL at diagnosis and healthy subjects group, as clarified in **Table 3-24**.

Table 3-24: Levels of 1, 25 Dihydroxy Vitamin D_3 (pg/mL) in The ALL Patients and Healthy Individuals

Study Groups (n)	*Vit D_3 Conc. (pg/mL) Mean ± S.D.*	*Min-Max Vit D_3 (pg/mL)*	*Range*	*p-value*
ALL Patients 31	21.038±5.996	11.200 - 35.600	24.400	***0.000***
Healthy Individuals 40	18.825±1.935	16.530 - 25.300	8.770	

The Mean Difference is Significant at 0.05 Level

1, 25 Dihydroxy vitamin D_3 data of the four subgroups were analyzed by ANOVA test, outcomes showed there are no significant variation between two genders when the comparison was done in the same group (neither patients nor controls).

Furthermore, statistical significant differences (**p=0.006**) were recorded when comparing the males in the subgroups together, as well as ALL females (**p=0.017**) with their peers in the healthy group (**Table 3-25**).

Table 3-25: Levels (Mean±S.D.) of 1, 25 Dihydroxy Vitamin D_3 (pg/mL) in The Study Individuals

Study Groups (n)	*Gender (n)*	*Vit D_3 Conc. (pg/mL) Mean ± S.D.*	*Min-Max Vit D_3 (pg/mL)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	20.888±5.886	12.900 - 32.600	19.700	***0.698 For1vs2***
	Female 13	21.500±6.530	11.200 - 35.600	24.400	***0.695 For3vs4***
Healthy Individuals 40	**Male 20**	18.530±2.078	16.530 - 25.300	8.770	***0.006 For1vs3***
	Female 20	19.066±1.755	17.160 - 24.000	6.840	***0.017 For2vs4***

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

Although 1,25-DIHO-D_3 level was under normal value in both study groups; the levels of this vitamin were clearly decreased in ALL patients after received two consecutive doses of chemotherapy compared to their levels before treatment, and then its concentrations start to rise after almost the fourth

dose of therapy, but it remains lower than its levels at diagnosis and before receiving the doses of the chemotherapeutic regime, as illustrated in **Figure 3-17**.

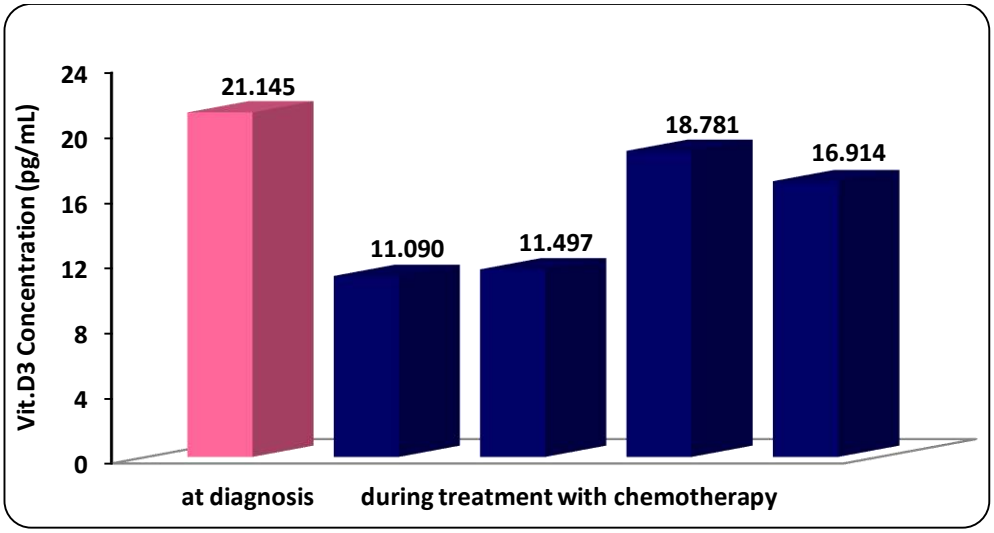


Figure 3-17: 1, 25 Dihydroxy Vitamin D_3 Values of Patients with ALL at Diagnosis (Pre-treatment) and During Treatment with Chemotherapy

Vit D_3 is a major regulator of Ca^{2+} metabolism and responsible for the availability of Ca^{2+} for bone mineralization [Pilz *et al.*, 2019], as well as its actions are mediated through intracellular vit D_3 receptors (VDR) [Zhou *et al.*, 2017]. It has well recognized that the genetic expression of VDR is not only existent in the target organs (bones, kidneys, intestine, and parathyroid glands) but in the most types of immune cells [Zhou *et al.*, 2017].

However, the metabolic disorders of vit D_3 metabolism could be a key linkage between primary hyperparathyroidism and the risk of different malignant diseases [Wang *et al.*, 2017], therefore; the defects in the gene alleles of vit D_3 receptors may cause abnormal growth of the parathyroid gland (hyperparathyroidism) and then the parathyroid gland begins to secretion abnormal amounts of PTH, which may inhibit the genetic transcription of PTH [Chatterjee *et al.*, 2015; Wang *et al.*, 2017].

The fact that there may be impaired in the process of apoptosis in other cell lines in which it expresses vit D_3 receptors, as the majority of ALL patients have found further low levels of vit D_3, especially after the remission-induction period [Chatterjee *et al.,* 2015; Maher *et al.,* 2017].

Likewise, deficiency of vit D_3 is most predominant among patients diagnosed with ALL and AML before and after treated with chemotherapy [Seyedalipour *et al.,* 2017; Icacan *et al.,* 2018], therefore; insufficient of vit D_3 status is a common health problem that accompanying with increases risks of other severe diseases, including; types of diabetes, cardiovascular disease, autoimmune diseases, osteoporosis, and many types of leukemia [Icacan *et al.,* 2018; Krishna, 2019].

It's possible that hypovitaminosis of vit D_3 is associated with the decreased number of specific receptors of vit D_3 with subsequent deficient of vit D_3, then, it acts as a ''negative prognostic indicator'' in many types of cancers, and other certain hematological malignancy condition such as; ALL, which has series of modifications that categorized by abnormal production and altered in metabolism process [Icacan *et al.,* 2018; Horie *et al.,* 2017; Wadhwa *et al.,* 2018].

3.12: Evaluation of Vitamin B_{12} Concentration in The Sera Samples of Acute Lymphoblastic Leukemia Patients and Healthy Control Groups

Vitamin B_{12} (vit B_{12}) concentration was evaluated in the sera samples of the participants in the current study, and the results of the statistical analysis by the **Student's *t-test*** show that there was significant variation (**p=0.001**) when comparing the levels of vit B_{12} between the two main study groups, as shown in **Table 3-26**.

Table 3-26: Levels of Vitamin B_{12} Concentration (pg/µL) in The ALL Patients and Healthy Individuals

Study Groups (n)	*Vit B_{12} Conc. (pg/µL) Mean ± S.D.*	*Min-Max Vit B_{12} (pg/µL)*	*Range*	*p-value*
ALL Patients 31	395.882±112.540	218.000 - 690.750	472.750	***0.001***
Healthy Individuals 40	207.944±34.566	141.020 - 256.100	115.080	

The Mean Difference is Significant at 0.05 Level

The highest value of vit B_{12} (**690.75 pg/mL**) has recorded in the case of male patient, while the lowest concentration of this vitamin (**141.02 pg/mL**) was found in the sample of healthy male. The statistical analysis of vit B_{12} data for the study subgroups by ANOVA test established that no significant differences were noticed within the same group, whether between ALL patients or healthy children.

Moreover, the statistically significant difference (**p=0.000**) was recorded when comparing patients ALL males and females with their healthy peers (**Table 3-27**).

Table 3-27: Levels (Mean±S.D.) of Vitamin B_{12} Concentration (pg/µL) in The Study Individuals

Study Groups (n)	*Gender (n)*	*Vit B_{12} Conc. (pg/µL) Mean ± S.D.*	*Min-Max B_{12} (pg/µL)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	394.902±111.984	307.50 - 690.75	383.250	***0.571 For1vs2 0.533 For3vs4 0.000 For1vs3 0.000 For2vs4***
	Female 13	410.923±109.861	310.88 - 646.50	335.620	
Healthy Individuals 40	**Male 20**	200.522±35.048	141.02 - 256.10	115.08	
	Female 20	215.869±32.288	149.51 - 252.02	102.51	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

Vit B_{12} has an important biological role as micronutrients involved in variety cellular functions in humans; such as metabolism, cell division, erythropoiesis process, and in the synthesis of DNA, as well as, it maintains the major public health condition with efficient quantity of absorption [Delvinc *et al.*, 2021].

The current results were consistent with Gavars's study [Gavars *et al.*, 2019] and Urbanski's study [Urbanski *et al.*, 2020], as regards that the high levels of vit B_{12} in sera patients with cancer by which association with macrocytic [Kesbeha and Pakbaza, 2019].

As the **Figure 3-18** illustrates the continuation of the gradual rise of vit B_{12} levels, even though after the four doses of chemotherapeutic treatment. Otherwise, limited studies have shown that the high levels of vit B_{12} could be a potential biomarker among individuals who have occurrence with several types of cancer either in solid or hematological malignancies in the status of cancer pathogenesis and prognosis [Arendt *et al.*, 2016].

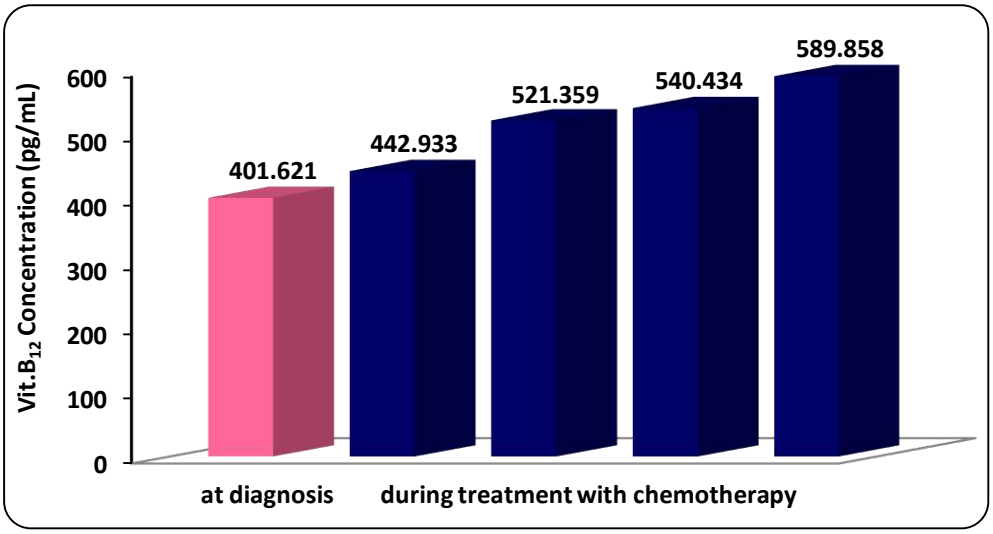


Figure 3-18: Vitamin B_{12} Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

The present data referred to possible hypothesis that the raised of vit B_{12} levels may associate with poor survival among patients with specific malignancies, in which it's indicating to more progressive and aggressive cancers and thus it reflects the metabolic changes of vit B_{12} that may cause cancer, however; the rise of vit B_{12} levels is not yet completely understood [Arendt *et al.,* 2016; Urbanski *et al.,* 2020].

3.13: Assessment of Ferritin levels in The Sera Samples of Acute Lymphoblastic Leukemia Patients and Healthy Groups

The results of present study recorded high significant differences (**p=0.000**) in the levels of FT concentrations in ALL patients (**500.796 ng/mL**) when compared to the healthy children in the control group (**129.245 ng/mL**) using **Student's *t-test***, as shown in **Table 3-28**.

Table 3-28: Levels of Ferritin Concentration (ng/mL) in The ALL Patients and Healthy Individuals

Study Groups (n)	*Ferritin Conc. (ng/mL) Mean ± S.D.*	*Min-Max Ferritin (ng/mL)*	*Range*	*p-value*
ALL Patients 31	500.796±131.743	129.020 - 850.010	720.990	***0.000***
Healthy Individuals 40	129.245±8.186	117.710 - 150.150	32.440	

The Mean Difference is Significant at 0.05 Level

The results of ANOVA test of the study groups were recorded high significant differences (**p=0.000**) in the present work when the (two) patients' subgroups compared to their corresponding genders in the control subgroups, while no such results were noticed when the two genders in same group (patients or healthy) were compared together, as shown in the **Table 3-29**.

Table 3-29: Levels (Mean±S.D.) of Ferritin Concentration (ng/mL) in The Study Individuals

Study Groups (n)	*Gender (n)*	*Ferritin Conc. (ng/mL) Mean ± S.D.*	*Min-Max Ferritin (ng/dL)*	*Range*	*p-value*
ALL Patients 31	**Male 18**	531.416±130.461	378.620-850.010	471.390	***0.111 For1vs2 0.849 For3vs4 0.000 For1vs3 0.000 For2vs4***
	Female 13	486.997±87.145	378.620-651.110	272.490	
***Healthy Individuals* 40**	**Male 20**	131.522±10.193	117.710-150.150	32.440	
	Female 20	126.957±4.375	121.490-140.480	18.990	

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

Figure 3-19 shows the levels of FT in the sera of ALL patients during receiving chemotherapy, as there was a decrease in ferritin levels in patients, especially after getting at least two doses, and then it went back up to elevation after the fourth doses of treatment, as the present results were corresponded to the results of Hade's study [2018] in decreasing ferritin levels in ALL patients after receiving at least two doses of chemotherapy, and so on the results of the current study also correspond to Shah's study [2017] that showed the relationship of high levels ferritin in many certain diseases; such as cardiovascular diseases and some types of cancer.

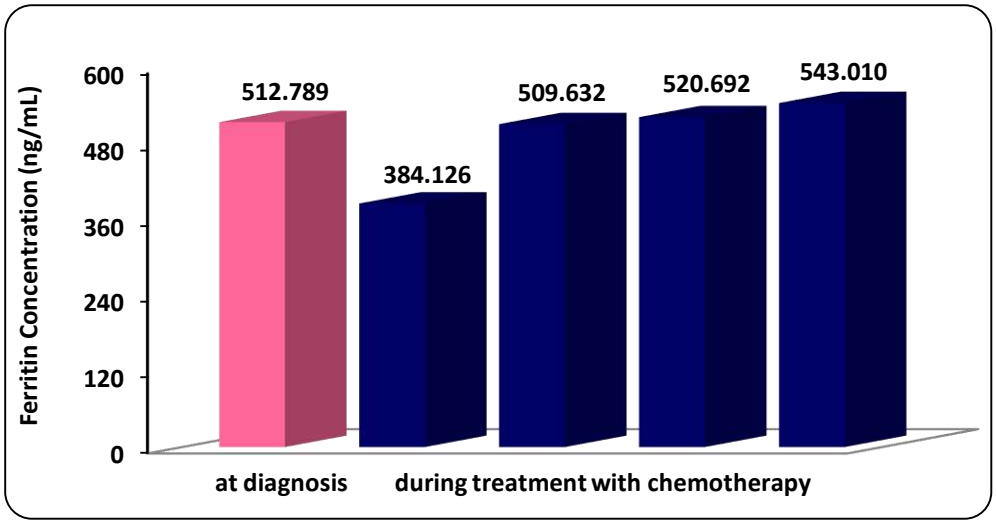


Figure 3-19: Ferritin Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

Moreover; various studies were conducted by Moafi *et al.*, [2017]; Lyle and Hirose, [2018] who showed that a higher level of FT in different age groups of ALL patients than its level in normal individuals and it has attributed to the production of large quantities of free iron, which acts as free radical molecules and in turn it acts a carcinogenic factor like other free radicals, thus it works to destroy the DNA of the cells.

Other preivous studies were done by Jain *et al.*, [2016]; Sirvent *et al.*, [2017]; Tachibana *et al.*, [2018] that published high FT levels in ALL patients before chemotherapy comparison to the normal individuals, however; its levels during treatment with chemotherapy still higher than its levels at diagnosis.

Thus, FT is one of the important medical markers which shows the amount of iron stored in the humans and it is considered as an essential signs of diagnosis many diseases, including ALL, as it maybe a prognostic factor that associated with elevation baseline at initial diagnosi as a risk of relapse of mortality [Tachibana et al., 2018; Ihlow et al., 2019].

3.14: Evaluation Levels of Total Protein and Albumin in The Sera Samples of Patients with Acute Lymphoblastic Leukemia (at diagnosis) in Comparison to The Healthy Individuals Group

At diagnosis of ALL patients, TP and Alb concentrations were determined in the sera of the two study groups. Results of statistical analyses of these criteria showed decreasing in the levels of TP and Alb in the patients group when compared to the control group, but not in significant levels by using **Student's *t-test***, as clarified in the **Table 3-30**.

These results were come in similar with Hade's study [2018], as these outcomes might be referred to the continuity of hepatic cells to perform their vital functions for proteins production but almost less than normally rates, since these patients have diagnosed as newly detected with ALL.

Table 3-30: Levels of Total Protein and Albumin Concentrations (g/dL) in The ALL Patients and Healthy Individuals

Serum Parameters	*Subject Groups(n)*		*p-value*
	ALL Patients (31)	*Healthy Individuals (40)*	
	Mean ± S.D. Min-Max (Range)	**Mean ± S.D. Min-Max (Range)**	
TP Conc. (g/dL)	5.859±0.564 5.100 - 7.200 (2.100)	6.617±0.830 4.400 - 8.500 (4.100)	***0.188***
Albumin Conc. (g/dL)	3.250±0.555 1.800 - 4.400 (2.600)	4.261±0.266 3.500 - 4.600 (1.100)	***0.105***

The Mean Difference is Significant at 0.05 Level

As the most blood proteins are mainly produced in the liver, lymphatic system, plasma cells, and in the bone marrow, however; in many pathological conditions, the total protein concentrations can be varied considerably from normal standard values [Chernecky and Berger, 2017; Kornblau *et al.*, 2018].

In addition, proteins are important structural molecules to form all cells and tissues in the body, moreover; they represent the essential part of the most body organs, as well as have an important role in the effectiveness of various enzymes, hormones, and regulation of many functions in the body; such as growth, development and responsibility toward foreigns bodies attacks [Bozkaya *et al.*, 2019], in order to investigate possible differences in the levels of TP and Alb among the study subgroups (that classified according to their genders), as showed in the **Table 3-31**.

Table 3-31 shows significant statistical decreases in the levels of TP (**p=0.001**) and Alb (**p=0.000**), separately between children males with ALL and their counterparts in the healthy group, as well as, same findings was observed when the comparison was done between females in the two study subgroups.

On the other hand, results of the current study indicated to the absence of statistical variations (**p>0.05**) for the serum TP and Alb, when the levels of these criteria were compared to the same genders in the two study groups, as observed in the **Table 3-31**.

Table 3-31: Levels (Mean±S.D.) of Total Protein and Albumin Concentrations (g/dL) in The Study Individuals

Serum Parameters	*Subject Groups (n)*				*p-value*
	ALL Patients (31)		*Healthy Individuals (40)*		
	Male (23) Mean ± S.D. Min-Max Range	**Female (18) Mean ± S.D. Min-Max Range**	**Male (20) Mean ± S.D. Min-Max Range**	**Female (20) Mean ± S.D. Min-Max Range**	
TP Conc. (g/dL)	5.711±0.466 5.100 - 6.500 1.400	5.984±0.612 5.100 - 7.200 2.100	6.500±0.612 4.900 - 7.500 2.600	6.750±0.987 4.400 - 8.500 4.100	***0.294 For1vs2 0.270 For3vs4 0.001 For1vs3 0.004 For2vs4***
Albumin Conc. (g/dL)	3.316±0.474 2.100 - 4.000 1.900	3.069±0.570 1.800 - 3.900 2.100	4.245±0.274 3.500 - 4.500 1.000	4.285±0.258 3.500 - 4.600 1.100	***0.089 For1vs2 0.749 For3vs4 0.000 For1vs3 0.000 For2vs4***

1: Male Patients with ALL, 2: Female Patients with ALL, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

However; the condition of hypoproteinemia may be caused by some disorders such as; severe anemia, nephrotic syndrome, salt retention syndrome, acute protein deficiency, and some types of leukemia such as; acute myeloid leukemia, whereas hyperproteinemia can be detected in some cases of multiple myeloma and severe dehydration [Kornblau *et al.,* 2018; Wei *et al.,* 2021].

Alb is mostly made by the liver and forms approximately 60-65% of total blood proteins [Pagana and Pagana, 2016], it maintains colloidal osmotic pressure of blood, and it has a main role in the carrying and storage of wide-ranging of different compounds such as; hormones, vitamins, medications, bilirubin, calcium, and fatty acids, as well as, it's essential for tissue growth and wound healing, as well as Alb is considered as one of the important acute stage proteins which its concentration decrease rapidly after the initiation of

infection, inflammation and various malignant conditions [Bozkaya *et al.*, 2019; Wei *et al.*, 2021].

Besides, hypoalbuminemia occurs during many diseases including liver, kidney diseases and various types of cancers which either caused by low protein absorption with some certain of amino acids in the body or as a result of a high catabolism process due to tissue damage and severe inflammation [Lv *et al.*, 2018; Artigas *et al.*, 2016]. Furthermore, in severe conditions of hypoalbuminemia, which causes low osmosis pressure in the blood, and water infiltrates into the capillaries and then enters the tissues causes edema [Chung *et al.*, 2020].

The tracking levels of TP and Alb were specified to the decrease concentrations in the sera of ALL patients nearly after received at least three consecutive doses of chemotherapy than at diagnosis, as presented in **Figure 3-20** and **Figure 3-21**. On the other hand, there was a noticeable decrease in the levels of TP and Alb in sera patients with ALL after the third dose, while the levels become to increase until received the fourth dose of treatment in comparison with the pre-treatment period (at the initial diagnosis), as shown in **Figure 3-21**.

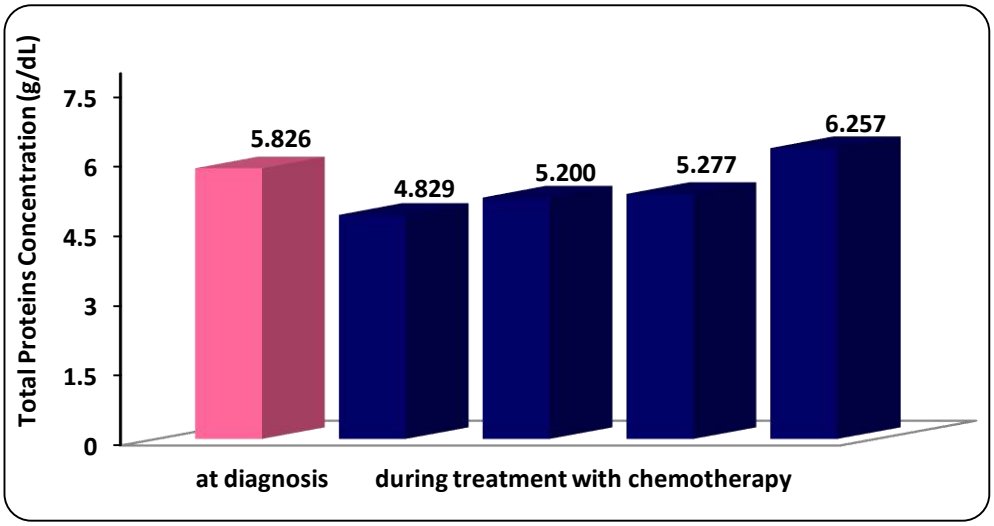


Figure 3-20: Total Protein Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

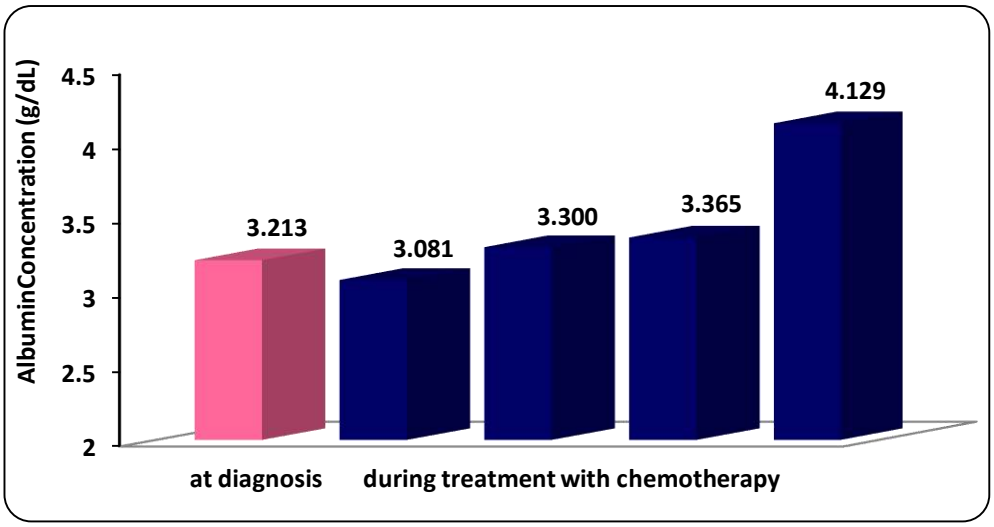


Figure 3-21: Albumin Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

These results may be explained by several assumptions, which including: It's possible to confirm the role of proteins, especially Alb within the system of protection against diseases status in which the body may be exposed, and the important role of Alb as one of acute phase proteins which may be attributed to the range of alterations occurring during the process of transformation of a normal cell into a cancer cell in the bone marrow in associated with the

consumption of many vital components of cell which important in cellular functions [Gradel *et al.*, 2020]. It may be explanation the decreasing by the initiation of patient's response to the protocol of chemotherapy provided by the specialists, as a result of protein consumption via inhibition the growth and proliferation of blasts cells, and thus leads to depletion in their concentrations [Hade, 2018].

Decrease of TP and Alb ''hypoalbuminemia''in sera samples of ALL patients which it's possible due to the catalyst proteins status in ALL patients that could be associated with a widespread of consuming and absorption of nutrients during the growth of cancerous cells in leukemia patients and accompanied with the glutamine metabolism [Cluntun *et al.*, 2017; Dyczynski *et al.*, 2018]. On the other hand, there is an obvious steady increase after received the fourth dose of treatment may indicate that patients may not respond to the applied therapy which provided by the physicians or may be due to the possibility of progressing stages of leukemia in some children which makes the cancer cells to be resistance against to the treatment that equipped for patients, and then the applied chemotherapeutic treatment become useless and unsuccessful in suppression the advanced carcinogecity condition or preventing of its metastasis [Dyczynski *et al.*, 2018; Chung et al., 2020].

Therefore; serum TP and Alb levels could be an independent prognostic factor for survival patients in several cancer diseases, especially in patients with ALL to be considered either before beginning treatment or in the same time of starting induction chemotherapy.

3.15: Evaluation of Trace Elements in The Sera Samples of The Study Groups

In the present study, levels of five trace elements (Zn, Co, Fe, Mn, and Cu) were assessed in the sera samples of ALL patients in Pre and Post-treatment with chemotherapy in addition to the healthy control individuals.

The results of statistical analysis by **Student's *t-test*** of the current study was shown a significant decrease (**p=0.003**) of Co in the sera samples of ALL patients compared to healthy control. Outcomes exposed a statistical increase (**p=0.000**) of Mn level in patients' samples as compared with control individuals, while non-significant differences (**p>0.05**) in the levels of Zn, Fe, and Cu between sera samples of study groups, as illustrated in **Table 3-32**.

Table 3-32: Comparison The Levels of Some Evaluated Trace Elements (µg/mL) in The Sera Samples of ALL patients and Controls Individuals at diagnosis

Subjects (n)	*Zn Conc. (µg/mL) Mean ± S.D. Min-Max Range*	*Co Conc. (µg/mL) Mean ± S.D. Min-Max Range*	*Fe Conc. (µg/mL) Mean ± S.D. Min-Max Range*	*Mn Conc. (µg/mL) Mean ± S.D. Min-Max Range*	*Cu Conc. (µg/mL) Mean ± S.D. Min-Max Range*
ALL Patients (31)	4.194±0.786 3.115 - 6.981 3.866	3.596±1.703 1.517 - 9.442 7.925	7.518±2.638 2.300 - 11.200 8.900	1.769±1.009 0.622 - 3.452 2.830	7.826±0.273 7.414 - 8.755 1.341
Controls (40)	4.475±1.017 3.005 - 7.158 4.153	9.064±0.996 6.510 - 10.285 3.775	8.118±2.449 4.040 - 13.190 9.150	0.545±0.232 0.126 - 0.971 0.845	7.596±0.195 7.287 - 8.100 0.813
p-value	***0.434***	***0.003***	***0.494***	***0.000***	***0.121***

The Mean Difference is Significant at 0.05 Level

In order to investigate the possible relationship between the evaluated levels of trace elements of participators sex in the study groups, comparison between these elements levels in different study subgroups were evaluated using **ANOVA**.

Although levels of Fe and Zn in the patients' samples were less than those recorded in the healthy children, but results showed non-significant values.

Table 3-33 showed a significant difference (**p=0.001**) and (**p=0.029**), respectively in the assessed levels of Zn and Fe between males' patients with ALL and healthy males, as well as, there was a statistical variance (**p=0.001**) in both evaluated levels of Zn and Fe among male and female in healthy individuals. In addition, there was a high significance (**p=0.000**) in the levels of (Co, Mn, and Cu) in between patients' males and females with ALL and with their peers in the control group, as presented in **Table 3-33**.

The results of current work did not record statistical differences in Zn, Co, Fe, Mn, and Cu levels when males and females compared together within a group of ALL patients. On the other side, the comparison between ALL female patients and healthy females was statistically consistent with what was observed when associated the patients' males with their healthy peers' controls according to the elements levels of Co, Mn, and Cu; whereas the outcomes of the study did not succeed in finding a statistically significant difference according to the levels of Zn and Fe in the case of comparing between ALL females with their healthy counterparts controls, this results were agreed with Hade's study [2018] and Valadbeigi *et al.*, [2019].

Table 3-33: Evaluated Trace Elements Levels of Zn, Co, Fe, Mn, and Cu (µg/mL) in The Sera Samples of males and females in The Study Groups

Trace Elements (ppm)	*Subject Groups (n)*				*p-value*
	ALL Patients (31)		*Healthy Individuals (40)*		
	Male (23) Mean ± S.D. Min-Max Range	**Female (18) Mean ± S.D. Min-Max Range**	**Male (20) Mean ± S.D. Min-Max Range**	**Female (20) Mean ± S.D. Min-Max Range**	
Zn Conc. (µg/mL)	3.990±0.656 3.115 - 5.407 2.292	4.263±0.521 3.489 - 5.223 1.734	4.991±1.116 3.005 - 7.140 4.135	4.085±0.844 3.230 - 7.158 3.928	***0.379 For1vs2 0.001 For3vs4 0.001 For1vs3 0.556 For2vs4***
Co Conc. (µg/mL)	3.651±1.485 1.517 - 6.465 4.948	3.071±1.106 1.530 - 5.778 4.248	9.504±0.155 9.195 - 9.831 0.636	8.643±1.256 6.510-10.285 3.775	***0.156 For1vs2 0.117 For3vs4 0.000 For1vs3 0.000 For2vs4***
Fe Conc. (µg/mL)	8.057±2.665 2.530 - 11.200 8.670	6.711±2.592 2.300 -10.100 7.800	9.671±1.649 6.740 - 13.190 6.450	6.575±2.057 4.040 -12.280 8.240	***0.102 For1vs2 0.001 For3vs4 0.029 For1vs3 0.865 For2vs4***
Mn Conc. (µg/mL)	1.863±1.123 0.848 - 3.452 2.604	1.728±0.849 0.847 - 3.452 2.605	0.601±0.133 0.126 - 0.732 0.606	0.494±0.291 0.126 - 0.971 0.845	***0.593 For1vs2 0.626 For3vs4 0.000 For1vs3 0.000 For2vs4***
Cu Conc. (µg/mL)	7.794±0.234 7.535 - 8.139 0.604	7.901±0.305 7.599 - 8.755 1.156	7.620±0.210 7.334 - 8.100 0.766	7.562±0.179 7.287 - 7.820 0.533	***0.202 For1vs2 0.425 For3vs4 0.012 For1vs3 0.000 For2vs4***

1: ALL Male Patients, 2: ALL Female Patients, 3: Healthy Males, and 4: Healthy Females. The Mean Difference is Significant at 0.05 Level

During chemotherapeutic treatment, Zn, Co, and Mn with except of Fe and Cu the evaluated trace elements were elevated comparison to their levels at diagnosis, as shown in **Figures 3-22**, **3-23**, and **3-24**, consequently. The outcomes of the study indicated to observable decrease in the levels of Fe and Cu which were recorded in the ALL patients' samples during receiving chemotherapy dosages, as illustrated in **Figures 3-25** and **3-26**, respectively.

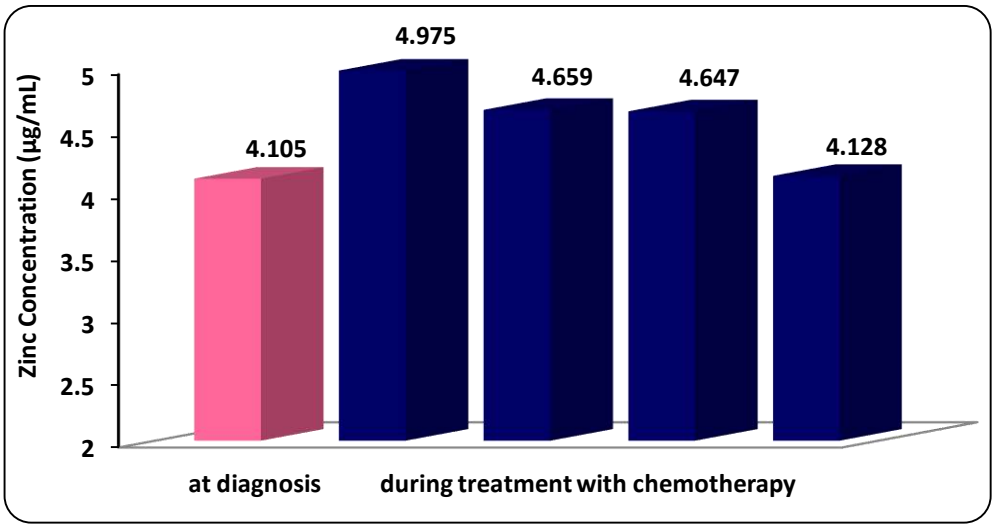


Figure 3-22: Zn Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

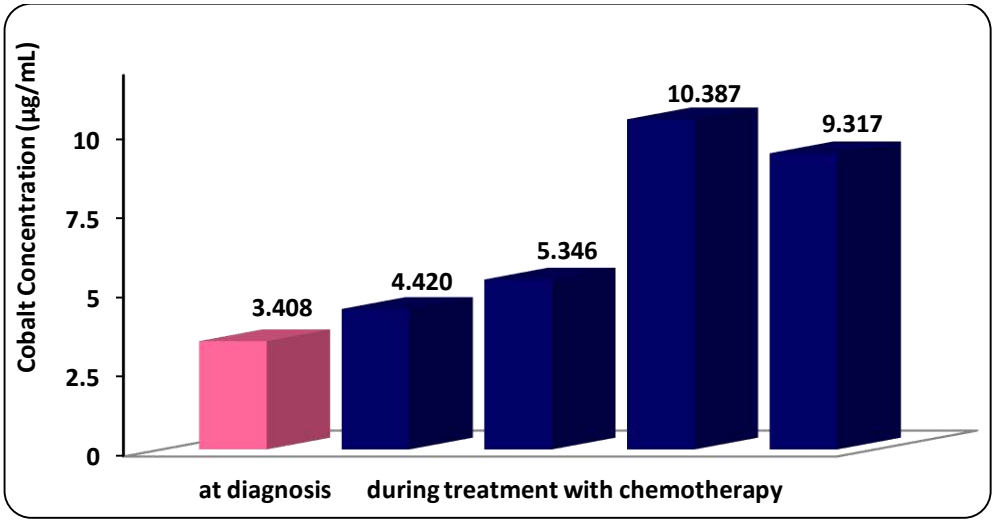


Figure 3-23: Co Values of Patients with ALL at Diagnosis (Pre-treatment) and During Treatment with Chemotherapy

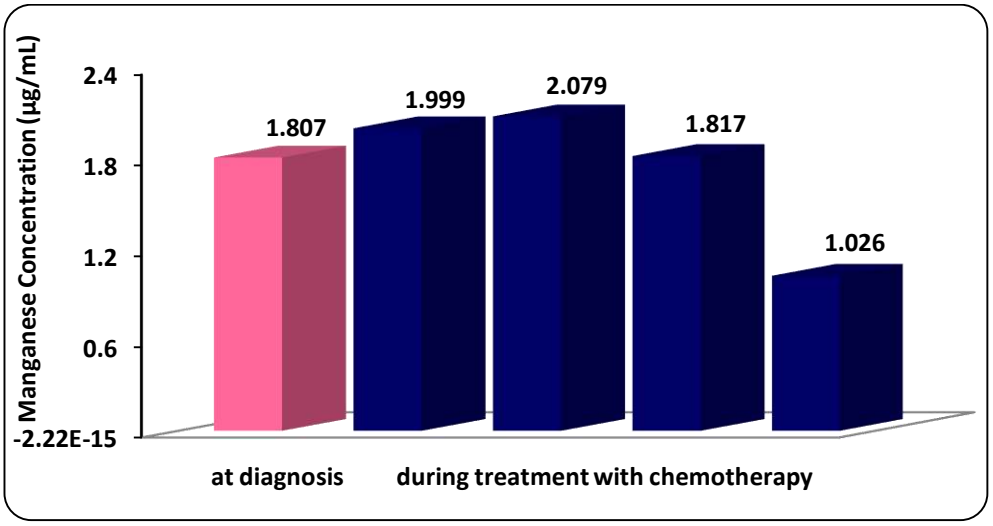


Figure 3-24: Mn Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

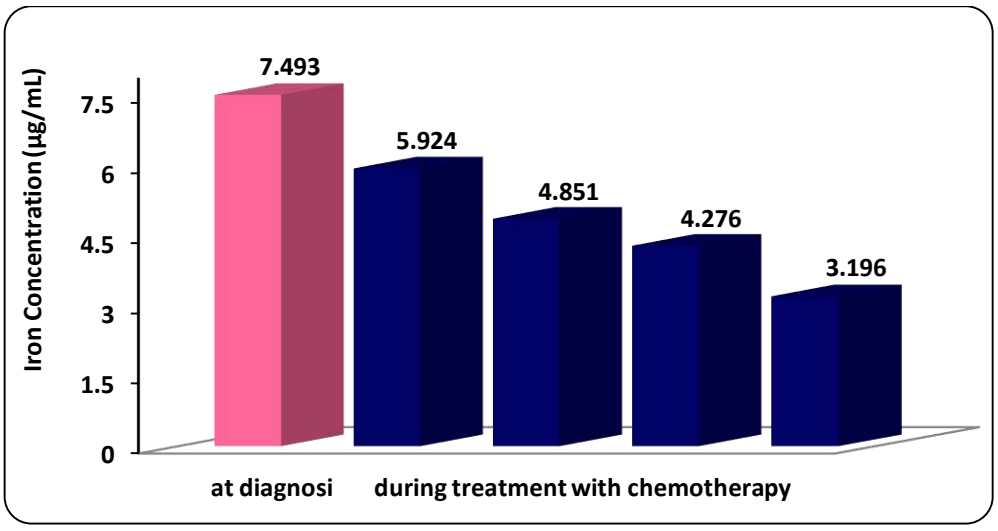


Figure 3-25: Fe Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

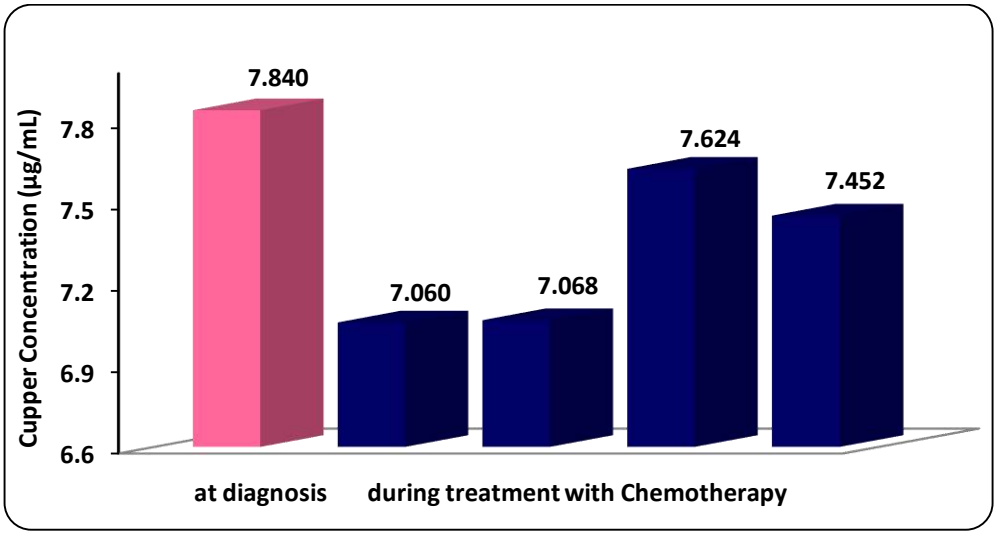


Figure 3-26: Cu Values of Patients with ALL at Diagnosis (Pre-treatment) and During Treatment with Chemotherapy

As the human body requires an extensive of nutrient elements, so these elements are essential in various physiological processes in the body and the majority of them play central roles in the continuity of many vital events in the cell [Mohammad and Fezea, 2016], thus the alteration in their levels could be related to many pathological symptoms such as diabetes, obesity, hypertension, renal failure, and heart diseases, along with the variation in their levels are involved in several malignancies include, lymphoma and leukemia [Prashanth *et al.*, 2015; Elshaygi, 2018].

Although, the low levels of some trace elements were described in the results of current study as related with ALL patients after the patients getting the fourth dose of chemotherapeutic treatment, which agreed with other studies were achieved by [Liamis *et al.*, 2016; Tahir and Obed, 2019], as an incidence of other hematopoietic and lymphatic tumors; such as AML, CLL, and CML [Elshaygi, 2018].

Several hypotheses were assumed to explain the alterations in trace elements levels as follow:

- Estimation of vital trace element roles is so important in the diagnosis and development of diseases [Mohammad and Fezea, 2016]. Recently, several studies have done on the trace elements and their effects in the ‘’etiology of neoplastic diseases’’ and in their roles as antioxidants by facilitating many enzymatic antioxidants such as an endogenous antioxidant enzyme, which is important in preventing cellular damage by the destruction of free radicals in stimulating ‘’ dismutation of superoxide radicals and producing hydrogen peroxide and oxygen’’ [Mohammad and Fezea, 2016; Muzolf-Panek *et al.*, 2017; Elshaygi, 2018].
- Cancer affects adversely the natural balance of nutrients in the body, particularly trace elements due to range of effects which mostly, loss of appetite, vomiting, malnutrition, poor digestion and absorption, which is also associated with the toxic effects of the use doses of chemotherapy [Lison, 2015; Tariq *et al.*, 2016], as well as it causes the development of many pathological symptoms and problems such as; neurological complications, disorders of synapses and bone marrow disruption [Prashanth *et al.*, 2015; Valadbeigi *et al.*, 2019]. Moreover, one of the abnormal characteristics for transformations that occurs to the cell is the decline in the ability of cancer cells to synthesis many essential constituents in which including; proteins and these proteins may act as carriers for a number of trace elements, and this hypothesis has reinforced by Khoshdel’s study [Khoshdel *et al.*, 2016] and Zengin’s study [Zengin, 2019] which showed decreasing in the levels of carrier proteins such as albumin.
- Most evaluated trace elements in the current work are involved in the fact that they are as ‘’enzymatic co-factors’’ to the large number of stimulated enzymes for oxidation-reduction processes which directly related to the status of cellular-based oxidative stress that occurs in the event of cancer, and as since

of the progression stages of cancer leads to defect, disruption, and reduction the activity of the oxidizing enzymes system with decrease levels of these accompanying elements [Akhgarjand *et al.,* 2017; Asfour *et al.,* 2017]. It is considered as an essential element in some enzymes for the stability of combination between substrate and enzyme, however; in others, it acts as a fundamental ion for the activity of these enzymes, and frequently it does both important functions [Hade *et al.,* 2018].

Alterations in the trace elements during treatment by chemotherapy may caused by the nature and influences of chemotherapy and its toxic properties which affects both infected and healthy cells and tissues, which in turn may inhibit and delay many cellular processes in the body, including the production of several proteins that directly affect the levels of trace elements and the mechanism of their functions in the cells [Molina-Lopez *et al.,* 2015; Hade *et al.,* 2018], as it's possible to clarify that the elevated levels of trace elements in the sera of leukemic patients may be associated with the release of large amounts of most elements from both destroyed and normal cells in patients after received toxic drugs of chemotherapy, thus their concentrations become in high levels [Luciano and Brewste, 2015; Afridi *et al.,* 2018].

To certain extent, no previous studies carried out to evaluate Mn levels, specially in the sera of children with ALL, but there are former studies focused on other different types of cancers i.e., in the samples of breast cancer, adult AML, CM, lung, gastric and prostate cancers [Hassan *et al.,* 2017; Saleh *et al.,* 2020; Zekavat *et al.,* 2021].

As a result, the outcomes of the current study may represent an initial point for many types of research and studies which are viewing the impacts of the alteration in levels of trace elements that assessment in the present work as assistant markers in the diagnosis and follow up and it might use as indicators in clinical medicine, prognosis, and diagnosis of ALL diseases.

3.16: Assessment Levels of The Electrolytes in The Sera Samples of Control Subjects and Children with ALL at Diagnosis

This part of the present study was designed to measure some necessary electrolytes which be demonstrated in the sera samples of patients with ALL (at diagnosis and during the treatment period), as well as the control individuals which included; Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, and K^{+}.

When **Student's *t-test*** was applied, significant alterations were observed when the levels of Ca^{2+} and K^{+} (**p=0.000**) and (**p=0.003**) in the two study groups were compared, while no such statistical variations were recorded when all of Mg^{2+}, Na^{+}, and Cl^{-} were evaluated in both study groups (**Table 3-34**).

Table 3-34: Comparison The Levels of Evaluated Electrolytes in The Sera Samples of ALL patients and Controls Individuals at diagnosis

Subjects (n)	*Ca^{2+} Conc. (mg/dL) Mean ± S.D. Min-Max Range*	*Mg^{2+} Conc. (mg/dL) Mean ± S.D. Min-Max Range*	*Na^{+} Conc. (mmol/L) Mean ± S.D. Min-Max Range*	*CL^{-} Conc. (mmol/L) Mean ± S.D. Min-Max Range*	*K^{+} Conc. (mmol/L) Mean ± S.D. Min-Max Range*
ALL Patients (31)	8.850±1.118 7.100-11.900 4.800	2.021±0.226 1.500-2.500 1.000	130.612±5.398 110.600-136.700 26.100	104.956±5.088 85.300-110.000 24.700	2.953±0.474 3.060-4.810 1.750
Controls (40)	7.920±0.322 7.100-8.400 1.300	2.169±0.259 1.70 -2.800 1.100	130.520±5.581 115.500-140.300 24.800	105.900±3.115 100.000-11.500 11.500	4.016±0.271 3.530-4.780 1.250
p-value	***0.000***	***0.694***	***0.533***	***0.146***	***0.003***

The Mean Difference is Significant at 0.05 Level

Thus, to investigate the possible differences in the levels of the evaluated electrolytes in the two genders of the study, the results were compared among the same group and with other subgroups (patients and healthy individuals) by applying **ANOVA**. The results of the present work indicated to significant differences (**p=0.002**) and (**p=0.000**), respectively in the levels of Ca^{2+} and Na^{+} when male and female patients with ALL compared together.

Besides, there was also a statistical difference (**p=0.009**) when females with ALL and their counterparts in controls were compared.

In addition, the results recoded a statistical difference (**p=0.000**) between males with ALL and their peers in controls, as concerning to the Ca^{2+} levels. The significant differences (**p=0.038**) and (**p=0.008**); separately were noted when the levels of Na^{+} and Cl^{-} in the males and females within control group individuals were compared. Furthermore, the results concerning to the K^{+} levels did not show a statistical significance among males and females within the same subgroups (patients and control), as presented in **Table 3- 35**.

Table 3-35: Evaluated Electrolytes Levels of Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, and K^{+} in The Sera Samples of males and females in The Study Groups

Electrolytes	*Subject Groups (n)*				*p-value*
	ALL Patients (31)		*Healthy Individuals (40)*		
	Male (23) Mean ± S.D. Min-Max Range	**Female (18) Mean ± S.D. Min-Max Range**	**Male (20) Mean ± S.D. Min-Max Range**	**Female (20) Mean ± S.D. Min-Max Range**	
Ca^{2+} Conc. (mg/dL)	9.238±1.307 7.100 - 11.900 4.800	8.376±0.529 7.800 - 9.900 2.100	7.850±0.337 7.100 - 8.300 1.200	7.995±0.287 7.500 - 8.400 0.900	***0.002 For1vs2 0.535 For3vs4 0.000 For1vs3 0.149 For2vs4***
Mg^{2+} Conc. (mg/dL)	2.027±0.249 1.500 - 2.500 1.000	1.992±0.193 1.600 - 2.300 0.700	2.120±0.221 1.700 - 2.600 0.900	2.225±0.284 1.900 - 2.800 0.900	***0.690 For1vs2 0.177 For3vs4 0.248 For1vs3 0.009 For2vs4***
Na^{+} Conc. (mmol/L)	129.272±6.375 110.60-136.50 25.90	132.800±2.879 126.80-136.70 9.90	127.090±5.889 115.50-140.30 24.80	133.74±2.291 129.20-136.90 7.70	***0.000 For1vs2 0.038 For3vs4 0.167 For1vs3 0.585 For2vs4***
CL^{-} Conc. (mmol/L)	103.711±6.324 85.30-110.00 24.70	105.046±2.830 100.10-109.40 9.30	104.055±3.275 100.00-111.50 11.50	107.480±1.986 103.00-111.10 8.10	***0.359 For1vs2 0.008 For3vs4 0.790 For1vs3 0.090 For2vs4***
K^{+} Conc. (mmol/L)	3.908±0.497 3.180 - 4.720 1.540	4.022±0.471 3.060 - 4.810 1.750	3.956±0.209 3.560 - 4.340 0.780	4.069±0.313 3.530 - 4.780 1.250	***0.410 For1vs2 0.350 For3vs4 0.696 For1vs3 0.730 For2vs4***

1: ALL Male Patients, 2: ALL Female Patients, 3: Healthy Males, and 4: Healthy Females. The Mean Difference is Significant at 0.05 Level

It is clear that there were variations increased in the overall concentrations of evaluated electrolytes Ca^{2+}, Mg^{2+}, Na^{+}, CL^{-}, and K^{+} in ALL patients after delivered the three doses of chemotherapy than before treatment (when diagnosed with the ALL), as illustrated in the **Figure 3-27**, **Figure 3-28**, **Figure 3-29**, **Figure 3-30**, **Figure 3-31;** respectively.

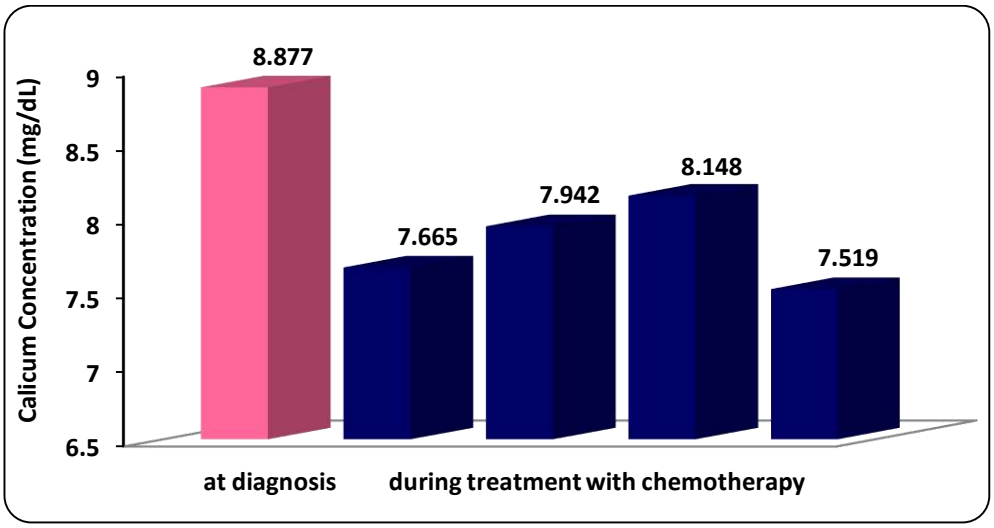


Figure 3-27: Ca^{2+} Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

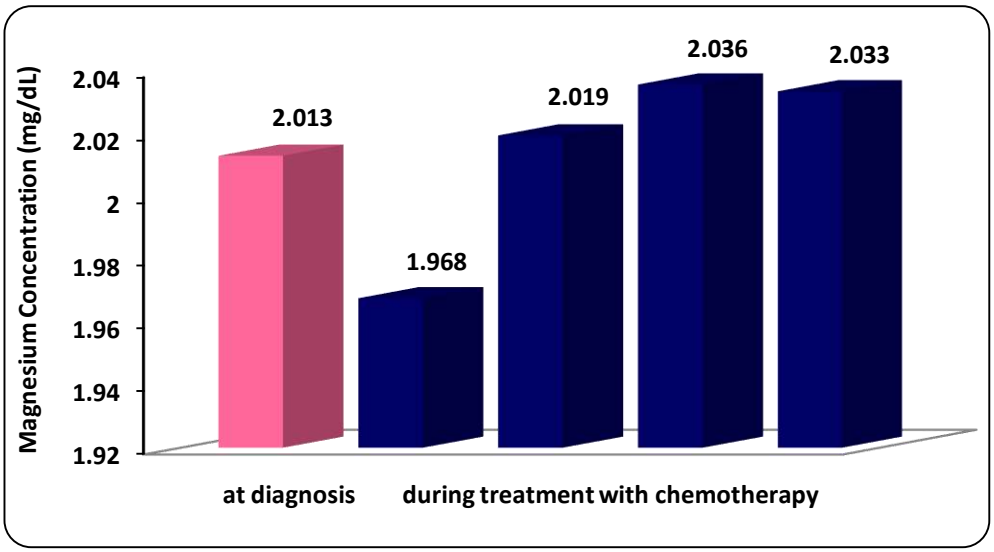


Figure 3-28: Mg^{2+} Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

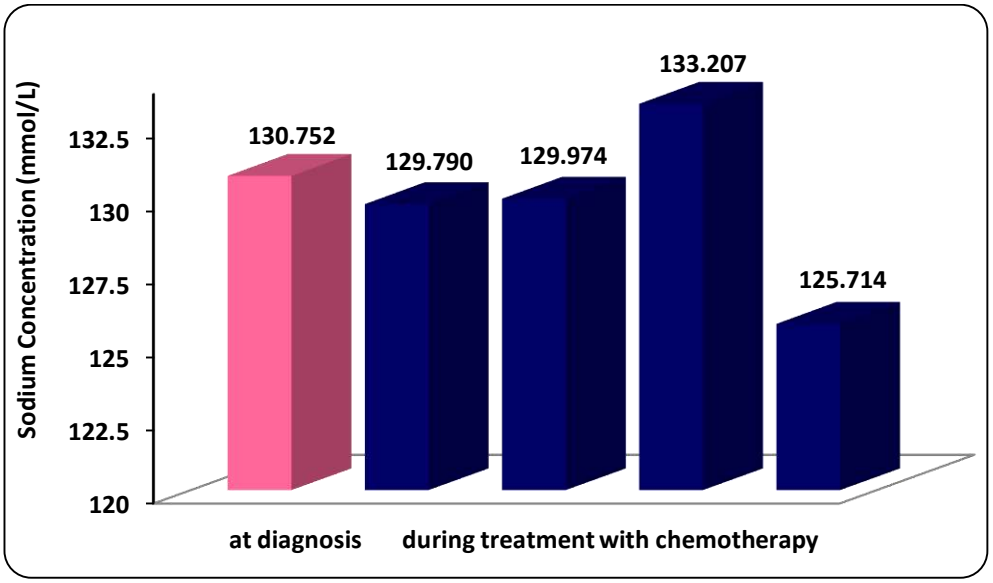


Figure 3-29: Na^+ Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

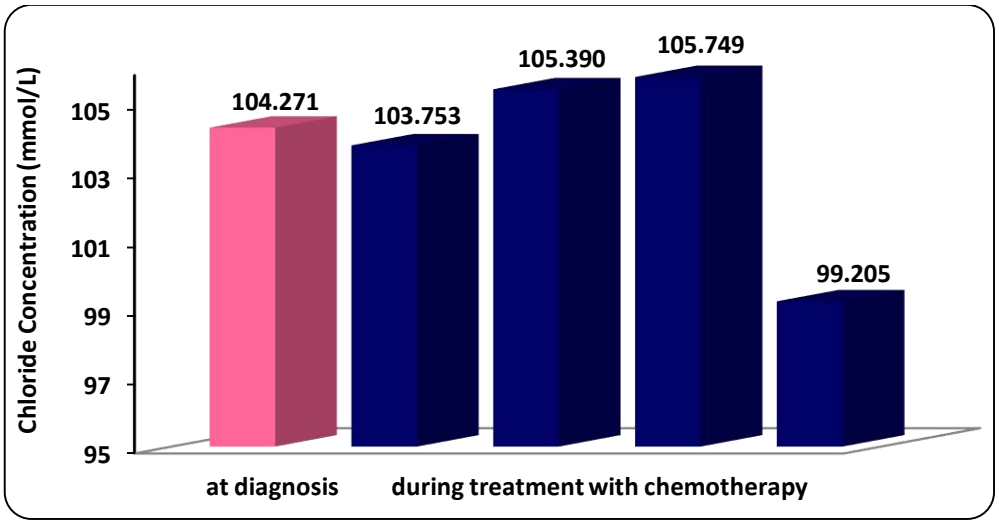


Figure 3-30: Cl^- Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

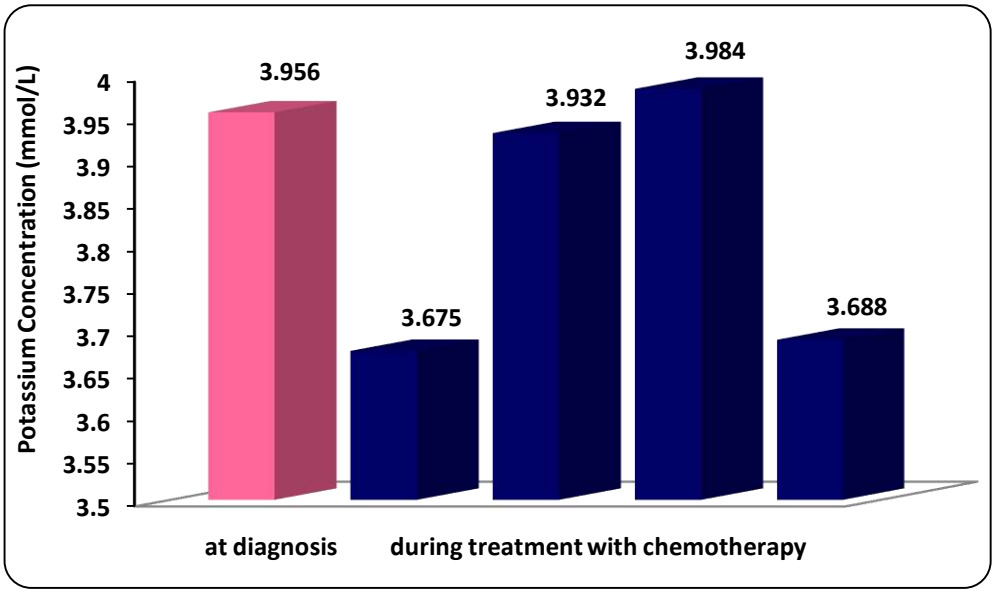


Figure 3-31: K^+ Values of Patients with ALL at Diagnosis (Pre-Treatment) and During Treatment with Chemotherapy

Afterwards, their concentrations returned to be at low levels after the fourth dose of treatment in Ca^{2+}, Mg^{2+}, Na^+, Cl^-, and K^+, as shown in **Figure 3-27**, **Figure 3-28, Figure 3-29**, **Figure 3-30**, and **Figure 3-31**, respectively.

Electrolytes have important roles in different biological pathways [Tahir and Obed, 2019] and the disorders of electrolyte levels are usually associated with pathological causes in various diseases [Shirali, 2016].

As a result, distinct assumptions have suggested which are likely to lead to disturbances in electrolyte levels as follows:

Disorders in electrolytes maybe rather caused by either to several complications such as; ‘’acute renal injury, rhabdomyolysis, or tumor lysis syndrome’’, and adrenal deficiency, as well as in accompanied with the use of some medications such as anti-inflammatory (nonsteroidal) agents and/or in the case of metastatic disease [Goldner, 2016; Shirali, 2016], in which causes electrolytes imbalance due to renal dysfunctions, essentially in patients with different types of cancers as it’s supported by [Hade, 2018; Torki, 2020], and then it’s enhanced electrolytes elimination [Shirali, 2016].

The reduction levels of some electrolytes such as Na^+ after the fourth dose of therapy may explain severe electrolyte disturbances related to hazardous neurological conditions, this is one of the most severe complications in electrolytes associated with the status of malignant tumors [Elshaygi, 2018]. On the other hand, it is possible the decrease levels of electrolytes could be linked to the inappropriate releasing of ‘’antidiuretic hormone (ADH)’’ [Elshaygi, 2018; Tahir and Obed, 2019].

Moreover; it’s possible that electrolyte variations in children with ALL could be attributed to the multi- pathological factors causes as described according to either the stage of disease or response patients to the protocol of treatment which was supplied, in particular, it seems that the variables of electrolytes are more obvious in malignant diseases, therefore; electrolyte conditions could lead to serious life-threatening and dangerous problems in patients with cancers, as well as, it’s a result of being associated with apparent kidney and liver dysfunctions, and then it’s required development therapy [Hade, 2018; Tahir and Obed, 2019].

On the other hand, it seems that the low levels of hypocalcemia were occasionally described in patients with acute leukemia which could be attributed essentially to hypoalbuminemia, even if the acute acid-base (metabolic acidosis and/or alkalosis) caused by modifications in the binding of Ca^{2+} to Alb or HCO3-Ca complex, as a portion of bounded with Alb [Goldner, 2016; Smith, 2019], thus it forms a rapid deficient in Ca^{2+} levels [Verzicco *et al.*, 2020; Zekavat *et al.*, 2021].

As a result, it is essential for further studies to approve the possibility of using such electrolyte derangements as prognostic and diagnostic tools for the cancer patients.

3.17: Study The Correlations among The Evaluated criteria

In order to shed light on the possibility of using the studied parameters in the current work as a predictive and diagnostic tumor tools for children with ALL, thus the study has assessed the relationships between these criteria in children who underwent of ALL, and then the results compared with what is recorded in group of healthy individuals.

Pearson's Correlation Coefficient was applied to study the relations among parameters of the present study, and to follow the mutual effects of changes in the evaluated factors in patients' samples and then the comparison with the healthy counterparts was done.

3.17.1: Assessment The Relationship of Galectin-9 to The Routine Biochemical Parameters in The Study Groups

Table 3-36 shows a high significant positively correlation (**r=0.940** at **p<0.000**) between Gal-9 and MBL. Furthermore, there was a moderate significant positive correlation (**r=0.326** at **p<0.037**) and (**r=0.458** at **p<0.005**) between the Gal-9 to NO and ST levels, respectively. The correlation between Gal-9 and EPO was positive significant (**r=0.740** at **p<0.000**) in patients with ALL.

On the other hand, there were significant negatively correlations (**r=-0.369** at **p<0.020**) and (**r=-0.466** at **p<0.004**) in concerning to Gal-9 with T-GSH and GSSG in ALL patients group, while no such correlations were noted when the correlations were estimated intended for biochemical parameters in the healthy control group, as presented in **Table 3-36**.

Table 3-36: Correlation of Galectin-9 to The Evaluated Biochemical Parameters

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	***r***	***P***
Galectin-9 To MBL	0.940**	*0.000*	-0.245	***0.127***
Galectin-9 To Total Glutathione	-0.369*	*0.020*	-0.065	***0.690***
Galectin-9 To Oxidized Glutathione	-0.466**	*0.004*	0.174	***0.284***
Galectin-9 To Nitric Oxide	0.326*	*0.037*	-0.039	***0.811***
Galectin-9 To Serotonin	0.458**	*0.005*	0.118	***0.468***
Galectin-9 To Total Proteins	-0.107	*0.283*	-0.043	***0.791***
Galectin-9 To Albumin	-0.017	*0.464*	-0.021	***0.899***
Galectin-9 To Ferritin	0.250	*0.175*	-0.193	***0.232***
Galectin-9 To Vitamin D_3	-0.039	*0.835*	-0.127	***0.434***
Galectin-9 To Parathyroid	-0.290	*0.114*	0.144	***0.375***
Galectin-9 To Erythropoietin	0.740**	*0.000*	0.192	***0.234***
Galectin-9 To Vitamin B_{12}	0.324	*0.075*	-0.160	***0.324***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

Galectins are known to involve in various biological functions such as; cell adhesion, cell surface signalling, angiogenesis, proliferation, migration, invasion, inflammation and regulation of apoptosis [Thijssen *et al.*, 2015; Hisrich *et al.*, 2020]. Therefore; the role of galectins has been broadly studied in different types of cancers, especially with the most frequently observed galectin-1, 3, 4, and 7 in which could be noted that galectins might have different functions in different malignancies [Ebrahim *et al.*, 2014; Hisrich *et al.*, 2020].

During inflammation, both chemokines and galectin are increased and mediate leukocyte recruitment molecules are thought to function separately. The current work is based on the theory of the possibility of galectin crosses based on several interactions between Gal-9 and MBL [Chou *et al.*, 2018;

Eckardt *et al.,* 2020]. MBL was considered one of the most important lines of protecting the body against diseases, as a part of other factors called ‘’acute phase proteins’’, particularly in early childhood [Sokotowska *et al.,* 2020; Wahlund *et al.,* 2020].

Moreover, Gal-9 is one of the most studied ligands for T-Immunoglobulin and Mucin- domain containing protein-3 (TIM-3) associated with various tumour cells either stimulate or dampen tumour activity according to its links with different ligands on the cell surface of T-cells [Chou *et al.,* 2018; Pasmatzi *et al.,* 2019]. Similarly, Taghiloo’s study [Taghiloo *et al.,* 2017] has indicated that Gal-9 has essential cytoplasmic intra and extra-cellular functions, as well as, it was involved in inflammation, tumorigenesis by cell adhesion, proliferation, tumour cell transformation angiogenesis and T-cell apoptosis.

Furthermore, malignant cells have the ability to release Gal-9 through the process of autocrine due to the efficacy of immunoglobulin by T-cells and TIM-3, thus the TIM-3/galectin-9 complex is formed via Gal-9 ligand, as a result of this association through the induction mechanism of Gal-9 and the ability of Gal-9 to link with the TIM-3 receptors [Taghiloo *et al.,* 2017].

Thus, the probability of multiple genetic patterns of MBL which could be associated with a significant increase the risk of developing of ALL, especially at early age of children in comparison with a healthy group which it’s possible that the interactions between the immune system and early childhood infectious diseases could be particularly induced to cause ALL [Świerzko *et al.,* 2020].

In the current study, it is possible to explain a good positive relationship between Gal-9 and MBL of the patients participating in the current study according to the potential role of Gal-9 and MBL in the mechanisms involved in the ‘’carcinogenicity’’ pathway against cancer [Ebrahim *et al.,* 2015; Świerzko *et al.,* 2020], in which it’s possible to be considered one of the most

important biochemical indicators that contribute in cancer risks in humans [Cedzyński and Świerzko, 2020].

However, Gal-9 and MBL may require a longer time to return normal which is dependant on the doses that were delivered, and this will be done until after the completion of protocol chemotherapeutic treatment.

Although it has been exposed that there is little data on the possible potential role of Gal-9 and MBL in the mechanisms involved in the ‘’carcinogenicity’’.

Accordingly, the current study is the first of its kind in the field of studying the relationship between Gal-9 and MBL in patients with ALL at diagnosis before receiving chemotherapeutic treatment.

3.17.2: Assessment The Relationship of Mannose Binding Lectin to The Routine Biochemical Parameters in The Study Groups

In the same manner, the evaluation results of the correlation between the MBL levels with other estimated biochemical influences in the existing work came to be very remarkable when the outcome results have demonstrated the presence of a positive correlation (**r=0.314** at **p<0.043**) between MBL and NO. A high significant positive correlation (**r=0.523** at **p<0.001**) was noticed between MBL and ST in the affected group (patients with ALL), as well as the correlation relation between MBL and EPO was highly significant positive (**r=0.693** at **p<0.000**) in patients with ALL, as shown in **Table 3-37**.

Data of the current study showed negative significant correlations (**r=-0.310** at **p<0.045)** and **(r=-0.467** at **p<0.004**) between MBL to T-GSH and GSSG. In addition, the results of the study didn’t record any correlations between MBL and other biochemical factors in regard to the control group, as demonstrated in **Table-3-37**.

Table 3-37: Correlation of Mannose Binding Lectin to The Evaluated Biochemical Parameters

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	***r***	***P***
MBL To Total Glutathione	-0.310**	*0.045*	-0.089	***0.583***
MBL To Oxidized Glutathione	-0.467**	*0.004*	0.083	***0.609***
MBL To Nitric Oxide	0.314*	*0.043*	-0.035	***0.828***
MBL To Serotonin	0.523**	*0.001*	0.139	***0.392***
MBL To Total Proteins	-0.008	*0.482*	0.024	***0.884***
MBL To Albumin	0.065	*0.365*	0.231	***0.151***
MBL To Ferritin	0.342	*0.060*	-0.084	***0.608***
MBL To Vitamin D_3	-0.078	*0.675*	-0.057	***0.728***
MBL To Parathyroid	-0.288	*0.116*	-0.027	***0.869***
MBL To Erythropoietin	0.693**	*0.000*	-0.054	***0.740***
MBL To Vitamin B_{12}	0.282	*0.124*	0.081	***0.620***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

Under normal physiological conditions, the cellular defence mechanism is principally to protect the vital cellular components from the effect of cellular damage in which produced by ROS, and that's through a group of anti-oxidant defence systems by different protective enzymes including; Glutathione Peroxidase (GPx), Catalase (CAT), Superoxide Dismutase (SOD) and Peroxiredoxins (Prxs) [Zhang *et al.*, 2018; Namrata *et al.*, 2019]. In addition, the antioxidant defence system has a key role in the suppression of various diseases and their clinical manifestations which is working against the production of further ROS [Balasaheb and Pal, 2015; Namrata *et al.*, 2019].

Thus, it is possible to explain the inverse relationship among Gal-9, MBL and antioxidants molecules (T-GSH and GSSG), as the ROS has related to a wide range of pathological conditions [Rezaieg and Musleh, 2019]. As well as, it's clearly associated with many other hematopoietic malignancies that

accompanied with deficiency of the antioxidant system and caused by low and/or insufficient enzymes responsible for its production in the liver in leukemia patients [Matlab and Jasim, 2017; Rezaieg and Musleh, 2019].

Additionally, the oxidant molecule of NO that belong to the various pathological conditions, involving the increasing of RS and/or reduction of antioxidant defence system which leads to excessive production of ROS and causing to enhance oxidative stress (OS), resulting in tissue damage due to a state of imbalance between oxidants and antioxidant activity [Rasool *et al.*, 2015; Singh *et al.*, 2018; Elham, 2020].

Thus, the results of the present work may likely be expected the NO which is one of the toxic factors that responsible for many of the complications associated with leukemic patients [Cheng *et al.*, 2015; Picón-Pagès *et al.*, 2019] due to high increase in NO production coupled with Gal-9 and MBL levels, in which possibly because of the extreme production of free radicals based on increased oxidative damage and prevention of apoptosis, thereby stimulating the development of cancer, as well as the activity of enzymes in control for the production of NO [Keshet and Erez, 2018].

The current study is the first that aimed to investigate the interrelationship between Gal-9, MBL and oxidant and antioxidant molecules levels in children with leukemia type ALL.

On the other hand, it is probably caused by abnormal cellular transformations that occur to cells when the normal converted into malignant cells in the carcinogenic process, which likely influence on the accumulative of effectiveness enzymes responsible for the production of ST and EPO, as well as its accompanying with increased Gal-9 and MBL levels [Szabo *et al.*, 2018; Hadeer, 2020].

ST hormone may act to be as a ‘’growth factor’’ for cancerous cells in numerous types of malignant tumours [Elshayeb *et al.,* 2016; Liu *et al.,* 2017; Sarrouilhe and Mesnil, 2019] which is a complex molecular mechanism that stimulates cellular routes to rely on special and organized receptors that may lead to alteration in many cellular immune responses in the case of cells proliferation, secretion of cytokines and chemokines, and then during the development stages of carcinogenicity with a dramatically significant increase of blast cells in the bone marrow and peripheral blood of ALL patients, which in turn faces reduced production of various blood cells and causes the occurrence different types of anemia, as well as a decrease in the amount of oxygen carried in the body, which is accompanied by body work to increase the production of EPO [Gaine *et al.,* 2017; Suresh *et al.,* 2020].

In turn, it’s could be directly associated with the deficiency of the antioxidant system and increased of oxidant molecules in patients [Rezaieg and Musleh, 2019] and in the same certain context, the outcomes of Kikushige’s study [Kikushige *et al.,* 2015] were consistent with the present results of current work, but in sera samples of AML mice and patients with CLL [Wdowiak *et al.,* 2019].

Based on the literature review, the existing study is the first of its respectful to suggest that children with ALL showed high serum levels of Gal-9 and MBL, which would be associated with the development of leukemia in patients during childhood.

Actually, yet no references have been found to indicate the relationship of Gal-9, MBL, especially with ALL children, although there were some difficulties and limitations faced by this study, especially that associated with the number of children with ALL enrolled in the current study and the limited number of chemotherapy doses which delivered to the patients. Thus, it’s possible to use Gal-9 and MBL as a novel biomarker for ALL patients at

diagnosis that would be an important tool for monitoring and determining the progression of ALL during the childhood period. As well as, it has a likelihood a role to make appropriate treatment protocol decisions for the patients in the future by the competent physicians in clinical and therapeutic applications of leukemia patients. The novelty of the current work backs to the detailed comparison of the Gal-9 levels and corresponds with the new diagnosis ALL at the early childhood stage. Thus the selection of this criterion used to prognosis and follow ALL patients at diagnosis period (pre-treatment), which correlated with the Gal-9 levels, are not shown in previous studies, nor human neither animals.

3.17.3: Evaluation The Relationship of Total Glutathione to The Routine Biochemical Parameters in The Study Groups

Table 3-38 showed a positively significant correlation (**r=0.372** at **p<0.020**) between T-GSH and GSSG levels in the patients' group with ALL. As well as the outcomes of the present study were recorded a significantly negative correlation (**r=-0.531** at **p<0.002**) between the T-GSH and vit D in patients with ALL group. However, the results of the current study did not indicate the certain significant correlations between T-GSH with the other chemical measurements in respect to the control group, as illustrated in **Table 3-38.**

Table 3-38: Correlation of Total Glutathione to The Evaluated Biochemical Parameters

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***P***
Total Glutathione To Oxidized Glutathione	0.372*	*0.020*	-0.011	***0.945***
Total Glutathione To Nitric Oxide	-0.014	*0.471*	0.152	***0.349***
Total Glutathione To Serotonin	-0.262	*0.077*	-0.104	***0.525***
Total Glutathione To Total Proteins	0.110	*0.277*	0.206	***0.201***
Total Glutathione To Albumin	0.190	*0.153*	0.145	***0.371***
Total Glutathione To Ferritin	-0.247	*0.181*	-0.094	***0.562***
Total Glutathione To Vitamin D_3	-0.531**	*0.002*	-0.033	***0.840***
Total Glutathione To Parathyroid	0.348	*0.055*	0.236	***0.143***
Total Glutathione To Erythropoietin	-0.323	*0.076*	-0.059	***0.719***
Total Glutathione To Vitamin B_{12}	0.010	*0.956*	0.068	***0.677***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

The oxidative agents and free radicals could be able to adjust antioxidants, especially T-GSH concentrations in cancer cells, and then it's possible to regulate the cellular responses to therapy for various cancers, therefore, T-GSH levels may be low or insufficient due to the potentially harmful properties of natural cells when the cells developed to cancerous cells [Desideri *et al.,* 2019].

Consequently, the production of GSH leads to the assembly of GSSG, and then the latter turns immediately into the GSH by GR, and the exhaustion of the formation of GSH and GSSG which could be caused to advance motivation of cells death by oxidation and cell toxicity [Couto *et al.,* 2016; Kennedy *et al.,* 2020]. Perhaps, it will be the resistance of exhaustion and depletion of GSH as well as GSSG, as it's the possibility of a connection between the GSH metabolism and different biological pathways [Kennedy *et al.,* 2020].

The GSH system has taken into the attention of many scientists to arrange with some approaches intended to raise their efforts to interrupt the progression of cancer cells in patients.

In contrast, the outcomes of the current study recorded a high significant negatively correlation (**r=-0.531** at **p<0.002**) between the T-GSH and vit D_3 in patients ALL group, as this succession of modifications, may attribute to metabolic changes associated with increased vit D_3 production in leukemia patients [Wadhwa *et al.,* 2018]. It's likely associated with the active form of vit D_3 which is contributed in regulatory mechanisms to protect target tissues or organs from different pathological disorders [Tasian and Hunger, 2018; Krishna, 2019]. The activities of vit D_3 are mediated through intracellular vit D_3 receptors, these receptors of vit D_3 are found in almost all cells in the body as well as, in different target organs such as; intestine, bone, kidney [Christakos *et al.,* 2016; Krishna, 2019].

Moreover, vit D_3 plays an important regulator factor and maybe to compensate for the shortage in the antioxidant molecules in ALL patients, in addition, it has an important role in regulating a wide range of biological effects on bone cells, along with it is involved in numerous cellular functions in the body such as; proliferation, differentiation, immunoregulation and apoptosis, as well as its effects on immune system components [Casan *et al.,* 2017; Pilz *et al.,* 2019; Gonzalo *et al.,* 2020].

Additionally, vit D_3 is recognized by which its high levels in various types of cancer because of its cytotoxic properties on cancer cells thus, it acts as ''as anticancer'' through suppression of carcinogenesis by the promotion of apoptosis, antiproliferation of cells, and induction of differentiation, besides, it could be inhibited or suppressed tumours angiogenesis, invasion and metastasis [Grant, 2018; Chandler *et al.,* 2020].

3.17.4: Evaluation The Relationship of Oxidized Glutathione Disulfide to The Routine Biochemical Parameters in The Study Groups

The results of the existing work indicated a low significant negatively correlation (**r=0.329** at **p < 0.036**) between GSSG and TP levels in the group of ALL patients. However, the correlation links for other factors were seemed to be non-significant according to the children with ALL and group of healthy individuals, as demonstrated in **Table 3-39**.

Table 3-39: Correlation of Oxidized Glutathione Disulfide with The Evaluated Biochemical Parameters

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***P***
Oxidized Glutathione To Nitric Oxide	-0.185	*0.160*	0.068	***0.675***
Oxidized Glutathione To Serotonin	-0.174	*0.174*	0.024	***0.884***
Oxidized Glutathione To Total Proteins	-0.329*	*0.036*	-0.021	***0.900***
Oxidized Glutathione To Albumin	0.132	*0.239*	-0.083	***0.612***
Oxidized Glutathione To Ferritin	-0.298	*0.104*	-0.119	***0.463***
Oxidized Glutathione To Vitamin D_3	-0.137	*0.463*	-0.126	***0.440***
Oxidized Glutathione To Parathyroid	0.101	*0.588*	0.039	***0.812***
Oxidized Glutathione To Erythropoietin	-0.198	*0.286*	-0.033	***0.842***
Oxidized Glutathione To Vitamin B_{12}	-0.180	*0.334*	-0.090	***0.581***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

It's might due to the many changes in the normal cells when shifted to the cancer cells in the bone marrow in which attributed to the depletion of many important vital constituents in the cell that accompanied with reduction of antioxidants system, in particular, related to the elevation of proteins consumption due to tissue damage and inflammation when cancer progression and increased the growth of blast cells in leukemic patients [Cluntun *et al.*, 2017; Dyczynski *et al.*, 2018; Zengin, 2019].

Therefore; there is the consumption of numerous proteins in order to inhibit the growth and reduce the spread of cancerous blast cells, thus it's a principle to an exhausted of proteins concentrations [Hade, 2018].

3.17.5: Assessment The Relationship of Nitric Oxide to The Routine Biochemical Parameters in The Study Groups

In the present work, ALL patients were correlated to the levels of NO for the purpose of monitoring the probable cellular changes concurrent with the progression of childhood leukemia.

Table 3-40 presented, the results of the existing study were recorded high positive significant correlations (**r=0.544** at **p<0.001**), (**r=0.412** at **p<0.011**), and (**r=0.478** at **p<0.006**), respectively among NO with both of ST, Alb, and FT in sera samples of children with ALL, while the group of healthy children exhibited a negative correlation relationship (**r=-0.314** at **p<0.0**49) between NO and FT, whereas the resident measurements, whether in the patients or in the healthy groups, did not show reliable significant statistical variations.

Table 3-40: Correlation of Nitric Oxide with Some Biochemical Evaluated Parameters

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***p***
Nitric Oxide To Serotonin	0.544**	*0.001*	0.029	***0.861***
Nitric Oxide To Total Proteins	0.173	*0.176*	0.230	***0.154***
Nitric Oxide To Albumin	0.412*	*0.011*	0.256	***0.111***
Nitric Oxide To Ferritin	0.478**	*0.006*	-0.314*	***0.049***
Nitric Oxide To Vitamin D_3	-0.240	*0.194*	0.219	***0.174***
Nitric Oxide To Parathyroid	-0.187	*0.313*	0.187	***0.249***
Nitric Oxide To Erythropoietin	0.136	*0.467*	0.122	***0.455***
Nitric Oxide To Vitamin B_{12}	0.107	*0.566*	0.175	***0.280***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

NO has various actions at the physiologic and pathologic levels which associated with neurotransmission, cardiovascular homeostasis, vasodilatation and in the metabolism of cells, besides, it is also related to numerous inflammation, immune responses, and particularly in cancer growth, as well as NO, could be an intermediary in inflammation, various mutations, and various stages of carcinogenicity, as well as its levels, was raised up in different types of leukemias; such as CML and AML [Cheng *et al.,* 2015; Sangwan *et al.,* 2015]. Besides, NO has an important role in tumour angiogenesis via stimulation of the release of a signal protein which is essentially known as ‘’Vascular endothelial growth factor (VEGF)’’ [Cheng *et al.,* 2015; Levine *et al.,* 2015].

Whereas other studies disagreed with current outcomes which have indicated that the high levels of NO have cytotoxic properties on human cell lines of carcinogenesis in patients with different malignancies in such lymphoma or leukemia [Umbrello *et al.,* 2014; Levine *et al.,* 2015]. However; Cheng’s study [Cheng *et al.,* 2015] and Jain’s study [Jain *et al.,* 2017] were remarked that a possibility of NO to be mediated mechanism of cancer growth and metastasis.

On the other hand, ST corresponds to the NO that appeared to be as a ‘’growth factor’’ for malignant cell lines in various types of cancer [Herr *et al.,* 2017; Sarrouilhe and Mesnil, 2019], as ST exhibits a complex mechanism to evoke signalling pathways that stimulate tumour development in some cancerous types, whether it was represented as a pattern of ‘’mitogen’’ as a specific receptor pathway [Liu *et al.,* 2017].

In addition, ST acts as a specific tumour marker in particular types of cancers; such as hepatic cancer, pancreatic islet cells, and gastrointestinal tract tumours [Elshayeb *et al.,* 2016; Rasha and Matlab, 2017]. Moreover, there is some information available on ST participation in cancer cells migration and

metastatic processes and it might be elaborated in one or more essential phases of cancer progression and/or growth of the primary tumour, invasion up to metastasis, and then it's possible that enhancement ST levels could be associated with the stimulation of sensitive ''serotonergic receptors'' [Rasha and Matlab, 2017]. Thus, it's feasibly that ST levels be able associated with stimulating sensitive ST receptors, which in turn rely on encoded genetic expression, as it's widely expressed in different types of cancer, as well as ST carriers represented by Na^+-dependent serotonin (5-HTT or SERT) [Herr *et al.*, 2017; Vahid-Ansari *et al.*, 2019].

The current results afford the first evidence that NO and ST are positively related at peripheral levels, in general, and a special correlation was noted in children with ALL. The obtained outcome results enrich the scientific knowledge with new information about the relationship between the levels of oxidant molecule (NO) with ST hormone in abnormal conditions (ALL) that have not been touched upon in this field and detail previously.

In the same context, a positive correlation between NO and Alb in ALL patients, Alb is considered as one of the main acute stage proteins [Bozkaya *et al.*, 2019], as its levels decline promptly with the onset of infection disorders, as well as its levels play a crucial role during infection and inflammation in different types of cancer conditions [Bozkaya *et al.*, 2019; Zengin, 2019], and then it's potentially correlated with the catalytic protein disorders in ALL patients [Dyczynski *et al.*, 2018].

Therefore; increased consumption of proteins by cells during the carcinogenic process as a result of cellular changes that occur during this phase, which are accompanied by high levels of oxidative stress, i.e. an increase in NO molecules and/or enzymes consumption with deficiency of antioxidant molecules [Zengin,2019].

The outcomes of the present study recorded a positive significant correlation (**r=0.371** at **p=0.040**) between ST and FT, in regards to the children's group with ALL patients **Table 3-41**. On the other hand, the observed results of the current study did not notice appropriate statistical associations in relative to ST with other evaluated biochemical aspects for patients and healthy participants, as elucidated in **Table 3-41**.

Table 3-41: Correlation of Serotonin to The Evaluated Biochemical Parameters

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***p***
Serotonin To Total Proteins	-0.042	*0.412*	-0.221	***0.171***
Serotonin To Albumin	0.259	*0.080*	0.002	***0.989***
Serotonin To Ferritin	0.371*	*0.040*	0.021	***0.897***
Serotonin To Vitamin D_3	-0.066	*0.724*	0.193	***0.232***
Serotonin To Parathyroid	-0.069	*0.714*	0.009	***0.958***
Serotonin To Erythropoietin	0.286	*0.118*	0.232	***0.150***
Serotonin To Vitamin B_{12}	-0.018	*0.922*	-0.335	***0.035***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

FT is pointed to the amount of iron content stored in humans' body [Zhang *et al.*, 2015]. Hence; FT could be considered as an assistant diagnostic marker in patients with different diseases, in cardiovascular diseases as well as in various types of hematologic malignancies; such as leukemia [Zhang *et al.*, 2015; Wang *et al.*, 2019], which may be due to the production of large amounts of free iron, as in turn to their actions as carcinogenic free radical agents, and hence damage the DNA of cells [Hoffbrand and Moss, 2016; Baliakas *et al.*, 2019].

Besides, it perhaps caused by increased the activity of the enzymatic system that supported the production of NO [Keshet and Erez, 2018], and thus causes high levels of oxidative stress associated with the lack and/or depletion of the activity of the antioxidant system [Elham, 2020].

As the results of current work were agreed with the study of [Mejía-Aranguré, 2016; Bartenhang *et al.*, 2017; Terwilliger and Abdul-Hay, 2017].

3.17.6: Assessment The Relationship of Total Protein and Albumin to The Routine Biochemical Parameters in The Study Groups

The statistical analysis of the current study failed to find significant correlations between TP and other biochemical factors in concern to the children patients with ALL, as well as in a healthy group, but with one exception of a positive statistical correlation (**r=0.552** at **p<0.000**) between TP and Alb in the control group, as illustrated in **Table 3-42**.

Table 3-42: Correlation of Total Protein to The Evaluated Biochemical Parameters

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***p***
Total Proteins To Albumin	0.126	*0.249*	0.552**	***0.000***
Total Proteins To Ferritin	0.351	*0.053*	-0.194	***0.232***
Total Proteins To Vitamin D_3	-0.041	*0.825*	0.157	***0.332***
Total Proteins To Parathyroid	-0.227	*0.220*	0.160	***0.323***
Total Proteins To Erythropoietin	-0.140	*0.451*	-0.105	***0.520***
Total Proteins To Vitamin B_{12}	-0.209	*0.260*	0.110	***0.498***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

Similarly, as in the total protein, the outcomes of existing work did not succeed in finding statistically significant correlations amongst measured biochemical factors for the group of ALL patients and for the healthy children group as well. The details are shown in **Table 3-43**.

Table 3-43: Correlation of Albumin to The Evaluated Biochemical Parameters

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***p***
Albumin To Ferritin	0.285	*0.120*	-0.280	***0.080***
Albumin To Vitamin D_3	-0.209	*0.260*	0.216	***0.182***
Albumin To Parathyroid	-0.047	*0.800*	0.235	***0.145***
Albumin To Erythropoietin	-0.219	*0.236*	-0.060	***0.715***
Albumin To Vitamin B_{12}	0.080	*0.669*	0.152	***0.350***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

Proteins are important construction masses for the formation of all cells and tissues in the body, as the proteins represent the basic part of most organs, as well as in various enzymes and hormones, thus proteins regulate numerous functions of the body such as; body growth, development and health [Kornblau *et al.*, 2018].

Total proteins involve albumin and globulin which are mostly made by the liver, thus TP levels in the blood could be increased or decreased, according to several disorders; such as liver diseases, renal nephrotic syndrome, congestive heart failure, dehydration, malabsorption, inflammatory conditions, multiple myeloma and some type of leukemia, as well as the use of some certain drugs; such as insulin and steroid hormones [Pagana and Pagana, 2016; Chernecky and Berger, 2017; Bozkaya *et al.*, 2019].

3.17.7: Assessment The Relationship of Ferritin to The Evaluated Vitamins and Hormones in The Study Groups

In the same way, the current outcomes of the present study were unsuccessful to find statistical correlation among FT with vit D_3, PTH, EPO, and vit B_{12} in the samples of patients with ALL, as indicated in **Table 3-44**, however; there were positive statistical correlations (**r=0.333** at **p<0.036**) and

(**r=0.313** at **p<0.049**), respectively observed between FT with EPO and vit B_{12} in healthy control, as exposed in **Table 3-44**.

Table 3-44: Correlation of Ferritin to The Evaluated Vitamins and Hormones

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***p***
Ferritin To Vitamin D_3	0.096	*0.607*	-0.116	***0.476***
Ferritin To Parathyroid	-0.288	*0.116*	-0.019	***0.909***
Ferritin To Erythropoietin	0.126	*0.498*	0.333*	***0.036***
Ferritin To Vitamin B_{12}	0.170	*0.362*	0.313*	***0.049***

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

FT is one of the most important medical indices proteins, around 20% of Fe is storage in FT, mainly in the liver, as typically 70% of Fe has found in the circulating RBCs, therefore; the distribution of Fe stores in the body demonstrates the importance of Fe to stimulate the production of erythrocytes [Zhang *et al.*, 2015].

Production of erythrocytes, which are usually regulated by EPO hormone secretion, in response to cellular oxygen deficiencies, thus low oxygen levels are the main factor motivating EPO production, consequently, EPO hormone stimulates growth, development of precursor RBCs and transformation into mature erythrocytes, therefore; the disruption of EPO levels is causing interruption of RBCs production in the bone marrow [Denka, 2016; Hussain *et al.*, 2017]. On the other hand, vit B_{12} has involved in many biological processes as in metabolism, cell division and maintains body health with the efficient quantity of absorption and transportation, besides, its importance in the nervous system, erythropoiesis process, and in the synthesis of DNA [Arendt *et al.*, 2016; Hoogstraten *et al.*, 2019].

Furthermore, the deficiency of vit B_{12} may be specifically caused by the lack of an essential factor called ‘’intrinsic factor’’ which is important in the composing of a complex with vit B_{12}, and the failure of vit B_{12} to absorb by the intestine due to anemia, then it may directly cause a severe decline in the of erythropoiesis [Tandon *et al.,* 2015; Tanyildiz *et al.,* 2016; Horie *et al.,* 2017].

3.17.8: Assessment The Relationship of Vitamin D_3 to The Evaluated Parathyroid, Erythropoietin, and Vitamin B_{12} in The Study Groups

The observed results in the current study revealed that there was no correlation relationship that appeared to be statistically in significant levels on the topic of the measurement among vit D_3 with PTH, EPO, and vit B_{12}, even for ALL patients and group of the healthy children, as showed in **Table 3-45**.

Table 3-45: Correlation of Vitamin D_3 to The Evaluated Parathyroid, Erythropoietin, and Vitamin B_{12}

Subjects	***ALL Patients***		***Healthy Individuals***	
	r	***p***	**r**	***p***
Vitamin D_3 To Parathyroid	-0.266	*0.148*	0.008	***0.959***
Vitamin D_3 To Erythropoietin	0.033	*0.862*	0.136	***0.403***
Vitamin D_3 To Vitamin B_{12}	-0.185	*0.318*	-0.156	***0.337***

3.17.9: Assessment The Relationship of Parathyroid to The Evaluated Erythropoietin and Vitamin B_{12} in The Study Groups

Likewise, **Table 3-46** was shown that no significant statistical correlations were recorded in observed results of the participants regarding to PTH, EPO and vit B_{12} measurements of ALL patients and control group, as presented in **Table 3-46**.

Table 3-46: Correlation of Parathyroid to The Evaluated Erythropoietin and Vitamin B_{12}

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***p***
Parathyroid To Erythropoietin	-0.197	*0.289*	0.017	***0.918***
Parathyroid To Vitamin B_{12}	-0.019	*0.919*	0.017	***0.918***

3.17.10: Assessment The Relationship of Erythropoietin to The Evaluated Vitamin B_{12} in The Study Groups

According to **Table 3-47**, the results obtained from the current study indicated that there was a non-significant correlation between EPO and vit B_{12} when linked together in patients with ALL and healthy control.

Table 3-47: Correlation of Erythropoietin and Vitamin B_{12}

Subjects	*ALL Patients*		*Healthy Individuals*	
	r	***p***	**r**	***p***
Erythropoietin To Vitamin B_{12}	**0.003**	***0.986***	**0.286**	***0.074***

3.18: Study The Correlations Between Routine Blood Factors and Specific Biochemical Parameters in The Study Groups

The current study assessed the relationships between the routine hematological and measured biochemical parameters by applying **Person's Correlation** in a group of ALL patients (at diagnosis) and a control group.

The outcomes of the study recorded positive correlations with statistical significance (**r=0.369** at **p<0.041**) and (**r=0.373** at **p<0.039**) between TP with RBCs and HCT, respectively.

As well as, the positive correlation relations with significant level (**r=0.401** at **p<0.025**) and (**r=0.406** at **p<0.023**), correspondingly between Alb with RBCs and HCT were noted in ALL patients (at diagnosis), while the

correlation relationships did not reach to the significant levels among the rest of evaluated criteria, as described in **Table 3-48**.

Table 3-48: Correlation of Specific Hematological and Biochemical Parameters in The Patients Group

	Parameters	RBCs	HCT	Hgb	MCV	MCH	MCHC	WBCs	Plts
r	Gal-9	-0.197	-0.231	-0.241	-0.128	-0.150	-0.031	0.208	-0.047
p		0.288	0.212	0.192	0.493	0.420	0.867	0.260	0.801
r	MBL	-0.254	-0.286	-0.233	-0.118	-0.036	0.094	0.349	-0.106
p		0.169	0.119	0.207	0.526	0.849	0.616	0.176	0.569
r	T-GSH	0.008	-0.082	0.013	-0.164	0.036	0.164	-0.200	-0.063
p		0.968	0.661	0.946	0.378	0.849	0.377	0.282	0.738
r	GSSG	0.117	0.121	0.288	0.069	0.340	0.324	-0.271	0.189
p		0.532	0.518	0.116	0.711	0.061	0.076	0.140	0.309
r	NO	-0.291	-0.176	-0.221	0.205	0.105	-0.063	0.339	-0.021
p		0.113	0.343	0.232	0.269	0.575	0.735	0.062	0.912
r	ST	-0.230	-0.207	-0.276	0.016	-0.104	-0.120	0.082	-0.037
p		0.214	0.265	0.133	0.931	0.579	0.522	0.662	0.844
r	TP	0.369*	0.373*	0.293	0.011	-0.137	-0.162	0.280	0.105
p		0.041	0.039	0.110	0.955	0.462	0.383	0.126	0.574
r	Alb	0.401*	0.406*	-0.285	-0.044	0.177	0.240	0.331	-0.115
p		0.025	0.023	0.120	0.815	0.340	0.193	0.069	0.539
r	Ferritin	-0.261	-0.200	-0.171	0.071	0.119	0.095	0.001	-0.134
p		0.156	0.280	0.357	0.703	0.525	0.611	0.999	0.473
r	EPO	-0.040	-0.060	-0.093	-0.070	-0.150	-0.068	0.126	0.179
p		0.830	0.747	0.620	0.709	0.421	0.714	0.501	0.336
r	Vit B_{12}	-0.071	-0.027	0.065	0.092	0.231	0.144	0.251	-0.032
p		0.706	0.884	0.728	0.624	0.212	0.440	0.174	0.866
r	PTH	0.157	0.012	0.160	-0.244	0.070	0.273	-0.171	-0.081
p		0.398	0.947	0.389	0.185	0.708	0.137	0.357	0.666
r	Vit D_3	-.014-	-0.017	-0.080	-0.052	-0.109	-0.064	-0.198	-0.097
p		.939	0.927	0.669	0.781	0.558	0.732	0.286	0.605

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

On the other hand, **Table 3-49** was shown that there were noticed low positive significant correlations (**r=0.331** at **p<0.037**), and (**r=0.336** at **p<0.034**), separately between GSSG and HCT, as well as TP and Hgb. In addition, low negative correlations with statistical significance were observed (**r=-0.390 at p<0.001**) and (**r=-0.364 at p<0.021**), respectively between MBL and Plts, as well as between vit B_{12} and HCT in the control group (**Table 3-49**).

Table 3-49: Correlation of Specific Hematological and Biochemical Parameters in The Control Group

	Parameters	RBCs	HCT	Hgb	MCV	MCH	MCHC	WBCs	Plts
r	Gal-9	-0.244	-0.030	0.091	0.252	0.291	0.094	-0.280	0.087
p		0.129	0.854	0.576	0.116	0.069	0.565	0.080	0.594
r	MBL	0.070	0.188	-0.027	0.057	-0.104	-0.073	-0.073	-0.390*
p		0.666	0.245	0.869	0.726	0.521	0.655	0.654	0.001
r	T-GSH	0.031	-0.008	-0.041	-0.042	-0.097	-0.209	0.192	0.003
p		0.849	0.961	0.802	0.797	0.551	0.196	0.236	0.986
r	GSSG	0.034	0.331*	0.085	0.248	-0.024	-0.288	0.094	-0.085
p		0.834	0.037	0.600	0.123	0.881	0.072	0.564	0.601
r	NO	0.232	0.141	0.209	-0.086	-0.097	-0.280	0.376	-0.011
p		0.149	0.387	0.196	0.600	0.552	0.081	0.017	0.948
r	ST	-0.166	0.239	0.094	0.372	0.114	0.143	-0.145	0.056
p		0.307	0.138	0.563	0.018	0.483	0.379	0.371	0.732
r	TP	0.110	0.086	0.336*	-0.310	-0.168	-0.016	0.221	-0.123
p		0.501	0.596	0.034	0.052	0.301	0.922	0.171	0.451
r	Alb	0.111	0.107	0.138	-0.026	-0.070	0.063	0.077	-0.241
p		0.495	0.510	0.396	0.871	0.667	0.700	0.635	0.135
r	Ferritin	0.135	-0.118	-0.289	-0.230	-0.294	-0.140	-0.260	-0.022
p		0.407	0.467	0.070	0.153	0.066	0.388	0.105	0.893
r	EPO	0.133	0.133	0.128	0.009	0.076	0.080	0.236	-0.223
p		0.412	0.415	0.432	0.958	0.643	0.624	0.143	0.166
r	Vit B_{12}	0.136	-0.364*	0.082	0.174	0.138	0.213	0.114	-0.196
p		0.402	0.021	0.614	0.284	0.396	0.187	0.485	0.225
r	PTH	0.054	-0.059	0.006	-0.087	0.005	-0.099	0.024	0.096
p		0.741	0.718	0.973	0.594	0.975	0.543	0.882	0.556
r	Vit D_3	0.054	0.102	0.169	0.019	0.029	0.062	-0.272	0.013
p		0.742	0.531	0.298	0.909	0.858	0.705	0.090	0.936

**Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level*

As it's known that the deficient proteins may be due to some disorders such as; acute anemia, kidney syndrome, acute protein deficiency syndrome, and some types of leukemia; such as ALL and AML which makes it difficult to maintain the balance of substances in the body, especially in the case of reduction the metabolism process, and then it becomes a source of iron loss from the body that is accompanying with abnormal disorders and causes various types of anemia with shed a light on low levels of many blood constituents, including erythrocytes, the amount of hemoglobin and many other blood indices [Vogelaar *et al.,* 2015; Pagana and Pagana, 2016; Chernecky and Berger, 2017].

In addition, the condition of leukocytosis and thrombocytosis can be potentially contributed to iron-deficiency anemia (IDA) because it is associated with ''hepatomegaly and splenomegaly'', which in turn reflected the main severe complications that occur in leukemic patients because of low proteins and Alb levels [Chernecky and Berger, 2017; Kornblau *et al.,* 2018].

The current outcomes were consistent with other studies in regards to Alb levels [Pagana and Pagana, 2016; Bozkaya *et al.,* 2019]. As well as, in respect to the status of Alb that is usually synthesized by the liver [Jones and Koetsier, 2017] which plays a key role in the extensive carrying and storage for many elements [Chernecky and Berger, 2017; Bozkaya *et al.,* 2019]. Moreover, Alb is reflected as one of the important proteins during the acute stage of inflammation, its levels promptly drop at the beginning of infection, and various malignant conditions [Bozkaya *et al.,* 2019; Zengin, 2019].

Besides, Alb deficiency which occurs either due to the low absorption of protein with some certain amino acids in the body or accordingly of a high undermining process due to tissue damage, muscle atrophy, weakness and/or inability to physical movement, that it's possible to be existent through a decrease in the extent of available proteins delivered nutritionally due to

malnutrition and loss of appetite in ALL patients coinciding with cancer progression and/or the continuous use of chemotherapy [Kornblau *et al.,* 2018; Zengin, 2019].

3.19: Study The Correlations Among Trace Elements in The Study Groups

The current study assessed the relationship between measured trace elements which was applied by **Person's Correlation Analysis** in patients with ALL at the time of diagnosis (pre-treatment with chemotherapy), in addition to the control individuals.

The data of the current study showed the existence of a positive correlation with statistical levels (**r=0.659 at p=0.000**), (**r=0.629 at p=0.000**), and (**r=0.481 at p=0.003**), respectively between Zn and Co, Mn, Cu as well. In addition, there was a strong significant positive correlation (**r=0.895 at p=0.000**) between Co and Mn, while the correlation was moderate (**r=0.523 at p=0.001**) between Co and Cu. Moreover, a positive correlation (**r=0.606 at p=0.000**) was noted in Mn and Cu levels when compared together in ALL patients group. However; the relationship of the levels of Fe with other trace elements in the patients' group were uncorrelated, as shown in **Table 3-50**.

Table 3-50: Correlation of Estimated Trace Elements in The Patients Group (at diagnosis)

	Trace Elements (ppm)	Zn	Co	Fe	Mn	Cu
r	Zn	1	0.659**	0.280	0.629**	0.481**
p			0.000	0.063	0.000	0.003
r	Co	0.659**	1	0.012	0.895**	0.523**
p		0.000		0.474	0.000	0.001
r	Fe	0.280	0.012	1	0.043	0.155
p		0.063	0.474		0.409	0.203
r	Mn	0.629**	0.895**	0.043	1	0.606**
p		0.000	0.000	0.409		0.000
r	Cu	0.481**	0.523**	0.155	0.606**	1
p		0.003	0.001	0.203	0.000	

As exposed in **Table 3-51**, it was noticed that the absence of such correlation relations among the trace elements members in the control individuals.

Table 3-51: Correlation of Estimated Trace Elements in The Control Group

	Trace Elements (ppm)	Zn	Co	Fe	Mn	Cu
r	Zn	1	0.157	0.159	0.173	-0.030
p			0.332	0.328	0.285	0.856
r	Co	0.157	1	0.129	0.001	-0.037
p		0.332		0.427	0.996	0.821
r	Fe	0.159	0.129	1	0.176	0.155
p		0.328	0.427		0.277	0.338
r	Mn	0.173	0.001	0.176	1	-0.066
p		0.285	0.996	0.277		0.686
r	Cu	-0.030	-0.037	0.155	-0.066	1
p		0.856	0.821	0.338	0.686	

3.19.1: Correlations of Specific Biochemical Parameters and Trace Elements in The Study Groups

In order to assess the correlations between the selected biochemical criteria in the current work and the levels of trace elements with the alterations that result from the cancerous condition. **Person's Correlation** was adopted to find the synchronization of these changes in the levels of standards tested together.

The outcomes of the present study recorded high negative statistical correlations (**r=-0.556** at **p<0.001**), (**r=-0.639** at **p<0.000**), (**r=-0.623** at **p<0.000**), and (**r=-0.540** at **p<0.002**), individually for Gal-9 according to the Zn, Co, Mn, and Cu in respect to the group of children with ALL (pre-treatment), as together in a similar manner for MBL levels of significant negative correlation interactions (**r=-0.617** at **p<0.000**), (**r=-0.618** at **p<0.000**), (**r=-0.615** at **p<0.000**), and (**r=-0.664** at **p<0.000**), in respect for the Zn, Co, Mn, and Cu.

In the same way, according to the EPO in patients with ALL had been recorded negative correlation relationships (**r=-0.605** at **p<0.000**), (**r=-0.618** at **p<0.000**), (**r=-0.518** at **p<0.003**), and (**r=-0.395** at **p<0.028**), respectively with Zn, Co, Mn, and Cu. Moreover, the results also recorded low negatively correlation which was statistically significant (**r=-0.399** at **p<0.026**) in regards to ST and Zn levels in ALL patients, while the correlation relation between ST and the rest of the trace elements were non-significant, as clarified in **Table 3-52**.

On the contrary, the data of the study noted highly positive correlations with the statistical significance levels (**r=0.452** at **p<0.015**), (**r=0.631** at **p<0.000**), (**r=0.591** at **p<0.000**), and (**r=0.459** at **p<0.089**), exclusively of T-GSH with each selected trace elements; Zn, Co, Mn, and Cu levels in concerning to the ALL patients (before delivered treatment). As well as with

the same way for the GSSG levels, the finding outcomes indicated positively significant correlation relationships (**r=0.497** at **p<0.027**), (**r=0.573** at **p<0.001**), (**r=0.583** at **p<0.001**), and (**r=0.480** at **p<0.035**) with particular trace elements; Zn, Co, Mn, and Cu.

Besides, there were somewhat moderate positively correlations (**r=0.466** at **p<0.008**) and (**r=0.396** at **p<0.027**), respectively in between PTH with both Co and Mn, although the correlations didn't reach the levels of significance among the rest of specific biochemical factors and trace elements, as elucidated in **Table 3-52**.

Table 3-52: Correlation of Evaluated Biochemical Parameters to Trace Elements in The Patients Group

	Parameters	Zn	Co	Fe	Mn	Cu
r p	Gal-9	-0.556** 0.001	-0.639** 0.000	-0.037 0.844	-0.623** 0.000	-0.540** 0.002
	MBL	-0.617** 0.000	-0.618** 0.000	-0.002 0.993	-0.615** 0.000	-0.664** 0.000
	T-GSH	0.452* 0.015	0.631** 0.000	0.011 0.951	0.591** 0.000	0.459* 0.089
	GSSG	0.497* 0.027	0.573** 0.001	0.075 0.687	0.583** 0.001	0.480* 0.035
	NO	-0.010 0.957	-0.053 0.778	-0.164 0.378	-0.100 0.594	-0.239 0.195
	ST	-0.399* 0.026	-0.126 0.500	0.073 0.698	-0.145 0.436	-0.334 0.066
	TP	0.132 0.480	-0.130 0.485	-0.233 0.208	-0.053 0.778	-0.199 0.283
	Alb	0.192 0.302	0.273 0.137	-0.256 0.165	0.284 0.121	-0.206 0.265
	Ferritin	-0.240 0.193	-0.271 0.140	0.217 0.241	-0.322 0.077	-0.330 0.069
	EPO	-0.605** 0.000	-0.618** 0.000	0.208 0.262	-0.518** 0.003	-0.395* 0.028
	Vit B_{12}	-0.160 0.389	-0.177 0.342	-0.191 0.302	-0.267 0.146	-0.164 0.379
	PTH	-0.034 0.854	0.466** 0.008	0.191 0.304	0.396* 0.027	0.018 0.923
	Vit D_3	-0.150 0.419	-0.199 0.282	0.200 0.281	-0.126 0.500	0.275 0.135

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

However, the existing work stated that the results of the study did not record correlations up to the level of significance among these parameters in children of the control group, as **Table 3-53** illstrates.

Table 3-53: Correlation of Evaluated Biochemical Parameters to Trace Elements in The Control Group

	Parameters	Zn	Co	Fe	Mn	Cu
r	Gal-9	0.069	0.074	0.087	0.139	-0.196
p		0.671	0.651	0.594	0.394	0.225
r	MBL	-0.051	-0.016	0.122	-0.137	-0.074
p		0.755	0.921	0.454	0.399	0.652
r	T-GSH	-0.297	-0.266	-0.071	-0.013	-0.060
p		0.062	0.097	0.662	0.936	0.711
r	GSSG	-0.122	-0.194	-0.038	-0.169	0.038
p		0.455	0.231	0.817	0.298	0.818
r	NO	0.272	0.177	0.108	-0.033	0.191
p		0.089	0.274	0.507	0.839	0.237
r	ST	0.105	0.142	0.241	0.219	0.043
p		0.518	0.383	0.134	0.174	0.793
r	TP	-0.014	-0.104	0.001	0.218	0.005
p		0.930	0.523	0.996	0.177	0.973
r	Alb	0.166	-0.133	0.151	0.076	0.130
p		0.305	0.413	0.352	0.641	0.424
r	Ferritin	-0.214	0.006	0.248	0.043	0.054
p		0.186	0.971	0.123	0.792	0.741
r	EPO	-0.126	0.183	0.011	0.012	-0.056
p		0.437	0.259	0.948	0.941	0.731
r	Vit B_{12}	-0.055	0.151	-0.229	-0.170	0.111
p		0.734	0.351	0.155	0.294	0.497
r	PTH	-0.292	-0.041	0.032	-0.040	0.148
p		0.067	0.801	0.843	0.805	0.364
r	Vit D_3	-0.068	-0.191	-0.088	-0.128	-0.177
p		0.679	0.239	0.588	0.431	0.273

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

Number of trace elements have important and multiple functions based on their structure and chemical compositions, as well as their existence in different tissues and fluids in the body, which include playing a very important role in the effectiveness of the immune system in the body; therefore any defect or disorder in the levels of these elements might be impaired or deficient in many

immune functions and growth of immune cells with a lack of immune responses for leukemic patients [Skrajnowska and Bobrowska-Korczak, 2019; Zekavat *et al.*, 2020].

Moreover, these elements play a vital and influential role in the stability of the composition, organization and efficiency of numerous enzymes to perform the various necessary cellular processes and biological functions that have basically actual effects, even with little concentrations or small quantities [Hade *et al.*, 2018; Valadbeigi *et al.*, 2019].

In addition, the central roles of trace elements as contributor factors for many enzymes formation and production of their effective forms, especially in relation to the cellular metabolism and the preservation of DNA entity and the organization of gene expression [Akhgarjand *et al.*, 2017; Valadbeigi *et al.*, 2019]. In particular, the trace elements have a main physiological role in muscle contraction, neurotransmitters and nerve generation, as the transfer of trace minerals to the brain is severely regulated through the blood-brain barrier system and cerebrospinal fluids, and it's possibly by occupied neurotransmitter receptors and ion transport channels [DeBenedictis *et al.*, 2020].

On the other hand, some acute cases of anemia may be associated with a decreased number of trace elements due to a deficiency in some of these metals that go together with the occurrence of lacking vit B_{12} due to different types of anemia [Elshaygi, 2018; Akiibinu *et al.*, 2019; Tahir and Obed, 2019].

Furthermore, the participation of trace elements in the mechanisms of defences against oxidative stress processes through the stimulation of the antioxidant system in the cell [Mohammad and Fezea, 2016]. Hence the trace minerals have highly active importance in inhibiting the free radicals' production from H_2O_2 units, as the fat peroxides collapse into the alloxyl and peroxyl free radicals which in turn is based on the oxidation of fat particles [Mohammed and Fezea, 2019; Nawi *et al.*, 2019]. Somehow, it seems that these

minerals act as catalysts for antioxidants by preventing cells damage and breaking down free radicals [Muzolf-Panek *et al.*, 2017].

Even though the obtainable data regarding the levels of trace elements and their association with cancer diseases are not yet sufficient, thus it's necessary for more wide-ranging vital studies which are focused to clarify the relationship of trace elements levels with the occurrence of cancers, particularly with childhood acute leukemia.

3.20: Study The Correlations Among Electrolytes in The Study Groups

The existing study evaluated the relationship between measured different Electrolytes which applied by **Person's Correlation Analysis** in patients' children with ALL at the time of diagnosis (before receiving chemotherapeutic treatment), as well as in the control individuals.

Although there was a highly significant correlation (**r=0.653** at **p<0.000**) between Na^+ and Cl^- in the patients' subjects of the present study, while such results were not recorded significant correlations among other electrolytes of the patients' group, as noted in **Table 3-54**.

Table 3-54: Correlation of Evaluated Electrolytes in The Patients Group (Pre-Chemotherapy)

	Electrolytes	Ca^{2+}	Mg^{2+}	Na^+	Cl^-	K^+
	Ca^{2+}	1	-0.098 0.301	-0.227 0.110	-0.081 0.332	0.190 0.153
	Mg^{2+}	-0.098 0.301	1	0.138 0.229	0.176 0.172	0.180 0.166
r **p**	**Na^+**	-0.227 0.110	0.138 0.229	1	0.653** 0.000	0.250 0.087
	Cl^-	-0.081 0.332	0.176 0.172	0.653** 0.000	1	0.123 0.254
	K^+	0.190 0.153	0.180 0.166	0.250 0.087	0.123 0.254	1

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

Similarly, the current study indicated that there was a high positive correlation (**r=0.801** at **p<0.000**), between Na^+ and Cl^- among control individuals, as clarified in **Table 3-55**. While the correlation relationships between other measured elements were devoid of significance even in children with ALL before receiving chemotherapeutic doses or in healthy individuals of the control group.

Table 3-55: Correlation of Evaluated Electrolytes in The Control Group

	Electrolytes	Ca^{2+}	Mg^{2+}	Na^+	Cl^-	K^+
r p	Ca^{2+}	1	0.033 0.841	0.226 0.162	0.127 0.434	0.074 0.648
	Mg^{2+}	0.033 0.841	1	0.142 0.383	0.111 0.495	0.201 0.214
	Na^+	0.226 0.162	0.142 0.383	1	0.801** 0.000	0.271 0.090
	Cl^-	0.127 0.434	0.111 0.495	0.801** 0.000	1	0.068 0.676
	K^+	0.074 0.648	0.201 0.214	0.271 0.090	0.068 0.676	1

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

3.20.1: Correlations of Specific Biochemical Parameters and Electrolytes in The Study Groups

The abnormalities of electrolytes are usually known in cancer patients, especially in leukemic patients, which could be associated with leukemia conditions, organ infiltration, or cells death from the harmful effects of toxic chemotherapeutic treatments for cancerous and normal cells as well. **Person's Correlation Coefficient** has been applied to follow up the electrolyte changes in the childhood ALL condition.

Table 3-56 was demonstrated moderate negatively significant correlation (**r=-0.364** at **p<0.044**); (**r=-0.496** at **p<0.005**) and (**r=-0.360** at **p<0.047**), individually among NO and Na^+, Alb and Na^+, as well as FT and Mg^{2+} in ALL patients group. On the other hand, there were positive correlations (**r=0.440** at **p<0.013**) and (**r=0.437** at **p<0.014**), respectively between EPO with Na^+ and

Cl^-, as shown in **Table 3-56**. Whereas the rest of the measured criteria did not record acceptable significant correlations (**Table 3-56**).

Table 3-56: Correlation of Evaluated Biochemical Parameters to Electrolytes in The Patients Group (at diagnosis)

	Parameters	Ca^{2+}	Mg^{2+}	Na^+	Cl^-	K^+
r p	Gal-9	-0.172 0.355	0.067 0.721	0.220 0.233	0.240 0.194	-0.158 0.396
	MBL	-0.148 0.427	0.153 0.412	0.165 0.374	0.238 0.198	-0.086 0.645
	T-GSH	0.006 0.975	-0.171 0.358	-0.216 0.244	-0.264 0.151	-0.147 0.429
	GSSG	0.104 0.577	0.045 0.810	-0.085 0.649	-0.215 0.245	0.269 0.143
	NO	-0.010 0.957	0.031 0.867	-0.364* 0.044	-0.153 0.412	-0.062 0.741
	ST	0.109 0.561	0.271 0.141	-0.117 0.530	0.230 0.213	-0.039 0.834
	TP	-0.196 0.290	-0.170 0.360	0.129 0.489	0.091 0.625	0.117 0.530
	Alb	0.285 0.120	-0.007 0.970	-0.496** 0.005	-0.354 0.051	0.115 0.537
	Ferritin	0.032 0.866	-0.360* 0.047	-0.277 0.131	-0.154 0.408	-0.004 0.982
	EPO	-0.138 0.460	0.084 0.654	0.440* 0.013	0.437* 0.014	0.017 0.927
	Vit B_{12}	-0.222 0.230	-0.033 0.860	0.040 0.829	-0.270 0.142	-0.259 0.160
	PTH	0.187 0.315	0.143 0.442	0.063 0.735	-0.041 0.828	0.088 0.638
	Vit D_3	0.131 0.483	-0.069 0.713	-0.066 0.723	-0.019 0.918	0.076 0.683

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

As shown in **Table 3-57**, positive significant correlations (**r=0.414** at **p<0.008**) and (**r=0.463** at **p<0.003**), respectively were recorded between Gal-9 with Na^+ and Cl^- in the healthy group. Furthermore, the finding results indicated positive correlations with significant level**s** (**r=0.409** at **p<0.009**) and (**r=0.328** at **p<0.039**), respectively among TP, Alb and K^+, as well as the data of the current study pointed to a positive correlation (**r=0.440** at **p<0.005**) between vit B_{12} and K^+ in control individuals, as specified in **Table 3-57**.

Table 3-57: Correlation of Evaluated Biochemical Parameters to Electrolytes in The Control Group

	Parameters	Ca^{2+}	Mg^{2+}	Na^{+}	Cl^{-}	K^{+}
r	Gal-9	-0.210	-0.146	0.414**	0.463**	0.137
p		0.193	0.369	0.008	0.003	0.398
r	MBL	-0.039	-0.054	-0.183	-0.206	0.051
p		0.812	0.741	0.257	0.202	0.755
r	T-GSH	0.002	0.136	0.078	0.032	0.260
p		0.989	0.401	0.634	0.845	0.105
r	GSSG	-0.037	-0.099	0.093	0.122	-0.122
p		0.823	0.544	0.568	0.454	0.454
r	NO	-0.048	-0.200	-0.106	-0.175	0.198
p		0.768	0.215	0.515	0.279	0.220
r	ST	-0.147	0.032	-0.183	-0.208	-0.077
p		0.366	0.843	0.258	0.197	0.635
r	TP	-0.147	-0.247	0.089	-0.038	0.409**
p		0.364	0.125	0.583	0.815	0.009
r	Alb	-0.009	-0.071	0.048	-0.003	0.328*
p		0.957	0.662	0.768	0.986	0.039
r	Ferritin	-0.094	0.206	0.009	0.078	-0.288
p		0.565	0.201	0.955	0.632	0.071
r	EPO	-0.296	0.040	0.147	0.100	0.010
p		0.064	0.806	0.365	0.540	0.951
r	Vit B_{12}	0.310	-0.107	0.160	0.099	0.440**
p		0.051	0.512	0.323	0.542	0.005
r	PTH	0.109	0.002	0.152	0.201	0.133
p		0.505	0.990	0.349	0.213	0.413
r	Vit D_3	0.243	0.216	0.153	0.055	0.003
p		0.131	0.182	0.347	0.738	0.988

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

With respect to severe disorders in electrolyte levels that may be described as the basic ‘’pathophysiological mechanisms’’ of electrolytes with disturbances of acid-base, it is one of the main indicators that cause ‘’kidney complications’’ in cancer patients, particularly in acute leukemia patients [Liamis *et al.*, 2016].

Therefore, electrolyte variables are probably to be due to imbalances in renal functions, mostly in patients with different types of cancers [Hade, 2018; Torki, 2020] that may be caused by inappropriate levels of some electrolytes

caused by renal glomerular-tubular damage caused by leukemia, thereby further enhancing the elimination of electrolytes with urine [Shirali, 2016].

In particular, it's necessary to study the common electrolyte abnormalities due to cancer and/or principally associated with the type of chemotherapeutic system protocols, the number of doses and the period of use, which followed in different patients with their capabilities and responses, as well as the complications, thus treat critical cases as a matter of urgency [Shirali, 2016; Verzicco *et al.*, 2020].

Therefore; focusing on the electrolyte disorders in children patients with ALL can be considered as important complications associated with leukemia patients that are basic towards follow-up of these disorders, exclusively with regard to impaired kidney functions and/or ''renal syndrome'', especially during the childhood stage.

3.21: Sensitivity of the Evaluated Parameters

Sensitivity is known as the true positive rate or the probability of detection, it is measuring the proportion of positives that are correctly identified. Calculation of sensitivity used for assessing the efficiency of the tested parameters to be classified as disease markers. Percentages of sensitivity were calculated according to biomedical statistical.

The individual and combined sensitivity of the nine parameters were evaluated in the group of children with ALL.

3.21.1: Sensitivity of the Evaluated Parameters in the Detection ALL in Children

The individual and combined sensitivity of the nine parameters (FT, EPO, PTH, Gal-9, MBL, T-GSH, GSSG, NO, and ST) were evaluated in the group of children with ALL, as summarized in **Table 3-58**.

Table 3-58: The Individual and Combined Sensitivity of The Evaluated Parameters in Group of Children Patients with ALL

Parameters	Ferritin	Epo	PTH	Gal-9	MBL	T-GSH	GSSG	NO	Serotonin
Ferritin	100	100	100	100	100	100	100	100	100
Epo	100	100	100	100	100	100	100	100	100
PTH	100	100	29	100	84	61	39	52	52
Gal-9	100	100	100	100	100	100	100	100	100
MBL	100	100	84	100	77	87	90	94	87
T-GSH	100	100	61	100	87	39	48	87	61
GSSG	100	100	39	100	90	48	10	65	35
NO	100	100	52	100	94	87	65	39	65
Serotonin	100	100	52	100	87	61	35	65	29

Both **FT**, **Epo**, and **Gal-9** showed the highest (**100%**) single sensitivity when the levels of these parameters in all of the cases diagnosed with ALL included in the present study were significantly higher than those of healthy peers. Moreover, **MBL** was the second in its susceptibility to ALL **77%**, when **24** out of **31** children with ALL showed a rise in MBL levels compared to the healthy individuals. Furthermore, **NO** and **ST** have evaluated in the current work came in the third place, where the lowest patients' sensitivity **39%** and **29%**, respectively of children with ALL in compared to the control group.

Finally, **T-GSH**, **PTH**, and **GSSG** had the least individuals' sensitivity to ALL with a sensitivity of **39%**, **29%**, and **10%**, separately when children were diagnosed with ALL had significantly lower levels of these criteria than their control group counterparts. These findings reinforced the study's objectives of the current work to investigate the possibility of using parameters as diagnostic tools for acute lymphoblastic leukemia in children.

It was noted that the differential capacity of the evaluated criteria increases in general when these standards are linked with each other. As a maximum sensitivity, **100%** was observed when **FT**, **EPO**, and **Gal-9** were combined to **PTH**, **MBL**, **T-GSH**, **GSSG**, **NO**, and **ST**, respectively.

In the same way, the results of the current study indicated that the sensitivity of diagnostic efficiency to **MBL** has increased to **94%** and **90%**, simultaneously when assessing its levels with both **NO** and **GSSG**. Besides, the diagnostic sensitivity of **T-GSH** was reached **87%** when evaluating its levels with both **MBL** and **NO**, while the sensitivity rate reached its lowest levels **39%** and **35%** of **PTH** with both **GSSG** and **ST**.

Conclusions

Based on the results obtained from the current work, a number of conclusions can be reached, the most important of which are:

- **Galectin-9 is an excellent new tool for diagnosing childhood ALL and predicting patients' response to chemotherapy.**
- **MBL is a supportive indicator in the diagnosis of children with ALL when measured with other neoplastic markers, and a good sensitizer to the extent of chemotherapy-related damage.**
- **It is possible to adopt the change in the complete peripheral blood count concurrent with the changes in the biochemical parameters evaluated to infer the incidence of acute lymphocytic leukemia in children and to assess the extent of the response to chemotherapy.**
- **The defect caused by acute lymphocytic leukemia affects all components and sites of blood production and is not limited to lymphocytes.**
- **It is possible that the incidence of acute lymphocytic leukemia in children affects the gene expression of EPO and parathyroid hormones, despite the response to chemotherapy, the levels of EPO hormone remain high in patients with acute lymphocytic leukemia, while the levels of the parathyroid hormone remain below the normal values.**
- **Trace element levels are good indicators for assessing cellular homeostasis and organ efficiency, as the change in trace element levels in acute leukemia patients is directly related to the disruption of the oxidative-antioxidant system.**

Recommendations

Based on the promising results of the study, we propose adopting the following recommendations in order to start with future studies integrating with the current work.

- **Study of the *LGALS9* gene polymorphism (the gene responsible for encoding Galectin-9) in samples of children with ALL.**
- **Evaluation of Galectin-9 levels as a follow-up tool during the complete recovery phase.**
- **Evaluation of Galectin-9 levels in the sera of patients with other types of leukemia in addition to solid cancers to determine the extent of the specificity of this tool for ALL in children.**
- **Measurement of high-sensitivity CRP (hs-CRP) in pediatric samples with ALL to assess the inflammatory process concurrent with carcinogenesis and treatment with chemotherapy.**
- **Evaluation of leukemic specific cytokines (interleukin-7 receptor α, CXCL12/CXCR4, and CCL25/CCR9) and combine results to the present study parameters.**

References

🕮 Aapro M, Beguin Y, Bokemeyer C, Dicato M, Gasco P, Glaspy J & Herrstedt J. (2018): Management of anaemia and iron deficiency in patients with cancer: ESMO Clinical Practice Guidelines. ***Annals of Oncology,*** 29 (Supplement 4): iv96–iv110

🕮 Abdelmabood S, Fouda AE, Boujettif F & Mansour A. (2020): Treatment outcomes of children with acute lymphoblastic leukemia in a middle-income developing country: high mortalities, early relapses, and poor survival. ***J Pediatr (Rio J),*** 96(1): 1-10

🕮 Abdualameer HH. (2020): A Biochemical Study for Evaluation of CLEC4A, Serotonin and Some Cellular Oxidation Parameters in Sera Samples of GIT Tumors. MSc. Thesis, Department of Chemistry/Faculty of Education for Girls/University of Kufa

🕮 Abolbashari S, Darroudi S, Tayefi M, Khashyarmaneh Z & Mobarhan MG. (2019): Association between serum zinc and copper levels and antioxidant defense in subjects infected with human T-lymphotropic virus type1. ***Journal of Blood Medicine,*** 10: 29-35

🕮 Afridi HI, Kazi TG & Talpur FN. (2018): Correlation of Calcium and Magnesium levels in the biological samples of different types of acute leukemia children. ***Biol Trace Elem Res,*** 86: 395-406

🕮 Ahmadi N, Mahjoub S, Hosseini RH, TaherKhani M & Moslemi D. (2018): Alterations in serum levels of trace element in patients with breast cancer before and after chemotherapy. ***Caspian J Intern Med,*** 9(2):134-139

🕮 Akhgarjand C, Djafarian K, Rezvani H, Azargashb E & Vafa M. (2017): Effect of Chemotherapy on Zinc, Copper, Vitamin D Levels and Inflammatory Marker in Adult Acute Lymphoblastic Leukemia. ***JNFS,*** 2(2): 179-184

🕮 Akiibinu MO, Oseni BS, Adekunle A Adesiyan AA, Akiibinu SO & Anetor JI. (2019): Inflammation, oxidative stress and cobalt deficiency in acute childhood leukemia. ***Clinics in Oncology - General Oncology,*** 4(1663): 1-4

🕮 Al-Asadi JN & Ibrahim SJ. (2018): Childhood Cancer in Basrah, Iraq During 2012-2016: Incidence and Mortality. ***Asian Pac J Cancer Prev,*** 19 (8): 2337-2341

🕮 Al-Mafragy HAS. (2019): Evaluation of the predictive efficiency of flow cytometry for some immunophenotyping in patients with chronic myeloid

leukemia in Karbala province. ***PhD. Thesis***, Dept., of Biology/College of Education for Pure Sciences/University of Karbala

🕮 Amarullah A, Hasmono D, Ugrasena I, Yulistiani M. (2018): Analysis of adrenal suppression after high dose prednisone therapy on children with Acute Lymphoblastic Leukemia in induction and consolidation phase. ***Folia Medica Indonesiana,*** 54(1): 59-63

🕮 Andres E, Vogel T, Kaltenbach G & Lang PO. (2016): Food-cobalamin malabsorption and vitamin B_{12} deficiency in adults and in elderly patients: what is the problem? ***Rev Med Interne,*** 37: 511-513

🕮 Anjana MK, Malladad A & Kariyappa M. (2020): Estimation of serum folate and vitamin B_{12} levels in children with severe acute malnutrition. ***International Journal of Contemporary Pediatrics,*** 7(5): 1013-1016

🕮 Anne B, Pietri M, Launay JM, Kellermann O & Schneider B. (2019): Multifaceted Regulations of the Serotonin Transporter: Impact on Antidepressant Response.***Frontiers in Neuroscience,*** 13: 91

🕮 Arellano-Galindo J, Barrera AP, Jimenez-Hernandez E & Mejia-Arangure JM. (2017): Infectious Agents in Childhood Leukemia. ***Arch Med Res,*** 48(4): 305-313

🕮 Arendt JFH, Farkas DK, Pedersen L, Nexo E & Sorensen HT. (2016): Elevated plasma vitamin B_{12} levels and cancer prognosis: A population-based cohort study. ***Cancer Epidemiology,*** 40: 158-165

🕮 Arendt JFH, Sorensen HT, Horsfall LJ & Petersen I. (2019): Elevated Vitamin B_{12} Levels and Cancer Risk in UK Primary Care: A thin Database Cohort Study. ***Cancer Epidemiol Biomarkers Prev,*** 28(4): 814-821

🕮 Artigas A, Wernerman J, Arroyo V, Vincent JL, Levy M. (2016): Role of albumin in diseases associated with severe systemic inflammation: Pathophysiologic and clinical evidence in sepsis and in decompensated cirrhosis. ***J Crit Care,*** 33:62-70

🕮 Asfour IA, Hegab HM, Mohammed RM & Sheeba MS. (2017): Assessment of copper, zinc and nitric oxide status in patients with chronic lymphocytic leukemia. ***Can Res Metastasis,*** 1(1): 3-8

🕮 Asif M. (2017): Role of heavy metals in human health and particularly in respect to diabetic patients. ***TANG*** / (***by Association of Humanitas Medicine***), 7(1): 1-10

🕮 Auriti C, Prencipe G, Moriondo M, Bersani I, Bertaina C, Mondi V & Inglese R. (2017): Mannose-Binding Lectin: Biologic Characteristics and

Role in the Susceptibility to Infections and Ischemia-Reperfusion Related Injury in Critically Ill Neonates. ***Journal of Immunology Research,*** 17: 1-11

🕮 Azeez L, Oyedeji AO, Adewuyi SO & Tijani KO. (2015): Syntheses, characterizations and antioxidant activities of copper complexes of quercetin as influenced by redox states. ***Int J Biol Chem Sci,*** 9(5): 2712-2718

🕮 Bajic VP, Neste CV, Obradovic M, Zafirovic S, Radak D, Essack M & Isenovic ER. (2019): Glutathione 'Redox Homeostasis' and its Relation to Cardiovascular Disease. ***Oxidative MedCellular Longevity,*** Hindawi: Article ID: 5028181, 1-14

🕮 Balakrishna P, George S, Hatoum H & Mukherjee S. (2021): Serotonin Pathway in Cancer. ***Int J Mol Sci,*** 22(3): 1268

🕮 Balasaheb NS & Pal D. (2015): Free Radicals, Natural Antioxidants, and their reaction mechanisms. ***Rsc Advances,*** 5(35): 27986-27996

🕮 Baliakas P, Jeromin S, Iskas M, Puiggros A, Plevova K & Stamatopoulos S. (2019): Blood Cytogentictogenetic complexity in chronic lymphocytic leukemia: definitions, associations, and clinical impact. ***Blood,*** 133(11): 1205-1216

🕮 Ballou Y, Rivas A, Belmont A, Pate L, Amaya CN, Lipson S & Bryan BA. (2018): 5-HT serotonin receptors modulate mitogenic signaling and impact tumor cell viability. ***Molecular and Clinical Oncology,*** 9(3): 243-254

🕮 Bansal A & Simon MC. (2018): Glutathione metabolism in cancer progression and treatment resistance***. J Cell Biol,*** 217 (7): 2291-2298

🕮 Bartenhang C, Fischer U, Korn K, Pfister, SM & Borkhardt A. (2017): Infection as a cause of childhood leukemia: virus detection employing whole genome sequencing. ***Haematologica,*** 102(5): e179

🕮 Bechir A, Haifa R, Atef BA, Emna B, Asma A, Nesrine BS, Yosra BU, Abdrrahim K. (2017): Osteolytic bone lesions, severe hypercalcemia without circulating blasts: unusual presentation of childhood acute lymphoblastic leukemia. ***Pan African Medical Journal,*** 26: 1-4

🕮 Bertoli S, Paubelle E, Berard E, Saland E & Huguet F. (2019): Ferritin heavy/light chain (FTH1/FTL) expression, serum ferritin levels, and their functional as well as prognostic roles in acute myeloid leukemia. ***European Journal of Haematology,*** 102(2): 131-142

🕮 Bhatia S, Landier W, Hageman L, Chen Y, Kim H & Sun CL. (2015): Systemic exposure to thiopurines and risk of relapse in children with acute

lymphoblastic leukemia: a children's oncology group study. ***JAMA Oncol,*** 1: 287-295

🕮 Bhattarai HK, Shrestha S, Rokka K & Shakya R. (2020): Vitamin D, Calcium, Parathyroid Hormone, and Sex Steroids in Bone Health and Effects of Aging. ***Journal of Osteoporosis,*** Volume 2020, Article ID 9324505: 1-10

🕮 Bhoopalan SV, Huang LJ & Weiss MJ. (2020): Erythropoietin regulation of red blood cell production: from bench to bedside and back. ***F1000Research (Faculty Rev),*** 9:1153 1-17

🕮 Bhushan R, Agarwal K and Garg J. (2018): Acute Lymphoblastic Leukemia with Normal Platelet Count. ***Oncology Journal of India,*** 1(2): 43-45

🕮 Bispo JAB, Pinheiro PS & Kobetz EK. (2020): Epidemiology and Etiology of Leukemia and Lymphoma. ***Cold Spring Harb Perspect Med,*** 10(6): a034819

🕮 Bordbar MR, Haghpanah S, Shakibazad N & Zarei T. (2018): Serum Folate and Vitamin B_{12} Levels in Survivors of Childhood Malignancy in Southern Iran. ***Middle East Journal of Cancer,*** 9(3): 202-207

🕮 Bowman BT. (2017): Electrolyte Disorders Associated with Cancer. ***Journal of Onco-Nephrology,*** 1(1): 30-35

🕮 Bozkaya Y, Erdem GU, Demirci NS, Yazici O, Ozdemir NY, Kostek O & Zengin N. (2019): Prognostic importance of albumin to globulin ratio in metastatic gastric cancer patients. ***Curr Med Res Opin,*** 35(2): 275-282

🕮 Bryer E & Henry D. (2018): Chemotherapy-induced anemia: Etiology, Pathophysiology, and Implications for Contemporary Practice. ***International Journal of Clinical Transfusion Medicine,*** 6: 21-31

🕮 Caplin B & Leiper J. (2014): Endogenous Nitric Oxide Synthase Inhibitors in the Biology of Disease Markers, Mediators, and Regulators. ***Arteriosclerosis, Thrombosis, and Vascular Biology,*** 32(6): 1-19

🕮 Casan JML, Ghotb S, Morgan S, Wei AH & Ting SB. (2017): Up-Regulation of the Vitamin D Pathway in Acute Myeloid Leukemia: A Novel Cause of Hypercalcemia. ***Ann Hematol Oncol,*** 4: 1-3

🕮 Cassandra L, Coelho BB, Silva PMS, Lima VLM, Pontual EV & Correia MTS. (2017): Lectins, Interconnecting Proteins with Biotechnological/Pharmacological and Therapeutic Applications. ***Evidence-Based Complementary and Alternative Medicine,*** 20: 1-23

🕮 Castellanos-Sinco HB, Ramos-Penafiel CO, Santoyo-Sánchez, Collazo-Jaloma & Sinco-Ángeles A. (2015): Megaloblastic anaemia: Folic acid and vitamin B_{12} metabolism. ***Rev Med Hosp Mex,*** 78(3): 135-143

🕮 Cedzyński M & Świerzko AS. (2020): Components of the Lectin Pathway of Complement in Haematologic Malignancies. ***Cancers,*** 12(7): 1-18

🕮 Cedzyński M, Kilpatrick DC & Świerzko AS. (2018): Mannose-Binding Lectin.Chapter4, 2nd Ed., ***The complement Facts Book,*** 33-43

🕮 Chandler PD, Chen WY, Ajala ON, Hazra A, Cook N & Manson JE. (2020): Effect of Vitamin D_3 Supplements on Development of Advanced Cancer a Secondary Analysis of the VITAL Randomized Clinical Trial. ***JAMA,*** 3(11): 1-13

🕮 Chang Y, Yu C, Jou S, Lin C, Lin K, Lu M, Wu K, Chen H & Yang Y. (2021): Targeted sequencing to identify genetic alterations and prognostic markers in pediatric T-cell acute lymphoblastic leukemia. ***Nature, Scientific Reports,*** 11(769): 809

🕮 Chatterjee K, Sen C & Ghosh GC. (2015): Primary hyperparathyroidism and malignancy: Forgotten friends or new acquaintances. ***Indian J Endocrinol Metab,*** 18(3): 436-446

🕮 Chen TC, Chen CH, Wang CP, Lin PH & Chang YL. (2017): The immunologic advantage of recurrent nasopharyngeal carcinoma from the viewpoint of Galectin-9/Tim-3-related changes in the tumour microenvironment. ***Sci Rep,*** 7: 10349

🕮 Chen X, Wei J, Meng X & Yang M & Qin Q. (2016): Molecular cloning and characterization of a galectin-1 homolog in orange-spotted grouper, Epinephelus coioides. ***Fish & Shellfish Immunology,*** 54: 333-341

🕮 Chen Y, Li J & Zhao Z. (2021): Redox Control in Acute Lymphoblastic Leukemia: From Physiology to Pathology and Therapeutic Opportunities. ***Cells,*** 10(5): 1218

🕮 Cheng H, Wang L, Mollica M, Re T, Wu S & Zuo L. (2015): Nitric oxide in cancer metastasis. ***Cancer Lett,*** 353(1): 1-16

🕮 Chen W, Zheng R, Zeng H & Zhang S. (2015): The updated incidences and mortalities of major cancers in China. ***Chin J Cancer,*** 34(3): 1-6

🕮 Chernecky CC & Berger BJ. (2017): Laboratory Tests and Diagnostic Procedures, 6th ed. ***St. Louis: Saunders***

🕮 Chetry M, Thapa S, Hu X, Song Y, Zhang J, Zhu H & Zhu X. (2018): The Role of Galectins in Tumor Progression, Treatment and Prognosis of Gynecological Cancers. ***Journal of Cancer,*** 9: 4742-4755

🕮 Chettri D, Boro M, Sarkar L& Verma AK. (2021): Lectins: Biological significance to biotechnological application. ***ScienceDirect: Elsevier,*** 506: e 108367

🕮 Chou FC, Chen HY, Kuo CC & Sytwu HK. (2018): Role of galectins in tumors and in clinical immunotherapy. ***Int J Mol Sci,***19: 1-11

🕮 Christakos S, Dhawan P, Verstuyf A, Verlinden L & Carmeliet G. (2016): Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. ***Physiol Rev,*** 96: 365-408

🕮 Chung JW, Park DJ, Chun SY, Choi SH & Kwon TG. (2020): The prognostic role of preoperative serum albumin/globulin ratio in patients with non-metastatic renal cell carcinoma undergoing partial or radical nephrectomy. ***Nature (Scientific Reports),*** 10: 1-10

🕮 Clementi E & Nisoli E. (2015): Nitric oxide and mitochondrial biogenesis: a key to long-term regulation of cellular metabolism. ***Comp Biochem Physiol-A Mol & Integr Physiol,*** 142(2): 102-110

🕮 Cluntun AA, Lukey MJ, Cerione RA & Locasale JW. (2017): Glutamine Metabolism in Cancer: Understanding the Heterogeneity. ***Trends Cancer,*** 3(3): 169-180

🕮 Colomb F, Wang W, Simpson D, Zafar M, Beynon R, Rhodes JM & Yu LG. (2017): Galectin-3 interacts with the cell-surface glycoprotein CD146 (MCAM, MUC18) and induces secretion of metastasis-promoting cytokines from vascular endothelial cells. ***J Biol Chem,*** 292: 8381-8389

🕮 Coulibaly FS & Youan BC. (2017): Current status of lectin-based cancer diagnosis and therapy. ***AIMS Molecular Science,*** 4(1): 1-27

🕮 Cousin JM & Cloninger MJ. (2016): The role of galectin-1 in cancer progression, and synthetic multivalent systems for the study of Galectin-1. ***Int J Mol Sci,*** 17: E1566

🕮 Couto N, Wood J & Barber J. (2016): The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. ***Free Radic Biol Med,*** 95: 27-42

🕮 Cullis JO, Fitzsimons EJ, Griffiths WJH, Tsochatzis E & Thomas DW. (2018): Investigation and management of a raised serum ferritin. ***British Journal of Haematology,*** 181(3): 331-340

🕮 Damoiseaux J & Smolders J. (2018): The Engagement Between Vitamin D and the Immune System: Is Consolidation to Be Expected? ***EBioMedicine (published by Lancet),*** 31: 1-10

🕮 Daniel A. Arber DA, Borowitz MJ, Cessna M, Etzell J & Vardiman JW. (2017): Initial Diagnostic Workup of Acute Leukemia Guideline from the College of American Pathologists and the American Society of Hematology. ***Arch Pathol Lab Med,*** 141: 1342-1393

🕮 DeBenedictis CA, Raab A, Ducie E, Howley S, Feldmann J & Grabrucker AM. (2020): Concentrations of Essential Trace Metals in the Brain of Animal Species-A Comparative Study. ***Brain Sci,*** 10(460): 1-16

🕮 Delvinc E, McCaddone A, Ahmadif KR & Harrington DJ. (2021): Vitamin B_{12} status in health and disease: a critical review. Diagnosis of deficiency and insufficiency-clinical and laboratory pitfalls. ***Critical Reviews in Clinical Laboratory Sciences,*** 58(6): 399-429

🕮 Denka A. (2016): Differentiating Chronic Lymphocytic Leukemia from small Lymphocytic Lymphoma: A Manitoba Cancer Registry Perspective. ***J Registry Manag,*** 43(2): 90-91

🕮 Desideri E, Ciccarone F & Ciriolo MR. (2019): Targeting Glutathione Metabolism: Partner in Crime in Anticancer Therapy. ***Nutrients,*** 11(8): 1-12

🕮 Dessie G, Molla D, Shibabaw T & Ayelign B. (2020): Role of Stem-Cell Transplantation in Leukemia Treatment. ***Stem Cells and Cloning: Advances and Applications,*** 13: 67-77

🕮 Dewan K and Agarwal K. (2015): Acute lymphoblastic leukemia with normal platelet count. ***Cancer Transl Med,*** 1: 181-183

🕮 Dhimana R. (2019): Structure-function and application of plant lectins in disease biology and immunity. ***Food & Chemical Toxicology,*** 134: 1-18

🕮 Dhivyasree S, Dhivyalakshmi J, Sankaranarayanan S & Scott JX. (2018): Severe hypercalcemia: A rare and unusual presentation of acute lymphoblastic leukemia. ***J Can Res Ther,*** 14(12): S1244-1246

🕮 Dong C, Zhang N & Zhang LJ. (2021): Oxidative stress in leukemia and antioxidant treatment. ***Chin Med J (Engl),*** 134(16): 1897-1907

🕮 Dyczynski M, Vesterlund M, Björklund AC, Zachariadis V & Nilsson R. (2018): Metabolic reprogramming of acute lymphoblastic leukemia cells in response to glucocorticoid treatment. ***Cell Death & Disease,*** 9(846): 1-13

🕮 Ebrahim AH, Alalawi Z, Mirandola L, Rakhshanda R, Dahlbeck S, Nguyen D & Chiriva-Internati M. (2014): Galectins in cancer: Carcinogenesis, Diagnosis and Therapy. ***Ann Transl Med,*** 2(9): 1-7

🕮 Eckardt V, Miller MC, Blanchet X, Duan R, Leberzammer J, Duchene J, Soehnlein O, Weber C & Mayo KH. (2020): Chemokines and galectins form heterodimers to modulate inflammation. ***EMBO Reports,*** 21(4): 1-17

🕮 Elham A M. (2020): A Biochemical and Molecular Study of Oxytocin Hormone and its receptor in patients with Metabolic Syndrome. ***PhD. Thesis,*** Department of Chemistry/Faculty of Education for Girls/University of Kufa

🕮 Eloranta S, Smedby KE, Dickman PW & Andersson TM. (2021): Cancer survival statistics for patients and healthcare professionals – a tutorial of real-world data analysis. ***Journal of Internal Medicine,*** 289:12-28

🕮 Elshayeb EI, Korani MAR, Elmaidany NF, Helwa MA & Abd-Elatty EA. (2016): Serum Serotonin as a Novel Marker for Hepatocellular Carcinoma. ***Adv Res Gastroentero Hepatol,*** 1: 1-7

🕮 Elshaygi EAA. (2018): Assessment of Serum Levels of Electrolytes and Trace Elements in Leukaemia Patients in Sudan. ***Pharmaceutical Sciences,*** 2(10): 136-141

🕮 Estey EH. (2018): Acute myeloid leukemia; 2019 update on risk-stratification and management. ***Am J Hematol,*** 93:1267-1291

🕮 Etxabe A, Lara-Castillo MC, Cornet-Masana JM, Banus-Mulet A, Nomdedeu M & Risueno RM. (2017): Inhibition of serotonin receptor type 1 in acute myeloid leukemia impairs leukemia stem cell functionality: a promising novel therapeutic target. ***Leukemia,*** 31: 2288-2302

🕮 Fathi A, Bahadoram M, Amani A (2015). Epidemiology of childhood cancer in Northwest Iran. ***Asian Pac J Cancer Prev,*** 16: 5459-5462

🕮 Fieg M. (2016): Epidemiology, pathogenesis, and etiology of acute leukemia. In: Hiddemann (eds.), Handbook of Acute Leukemia: Hematologic Malignancies.***Springer International Publishing Switzerland,*** Pp: 3-13

🕮 Filbin M & Monje M. (2019): Developmental origins and emerging therapeutic opportunities for childhood cancer. ***Nat Med,*** 25(3): 367-376

🕮 Frakking FNJ, Brouwer N, Dolman KM, van Woensel JBM, Caron HN, T W Kuijpers, TW & van de Wetering MD. (2015): Mannose-binding lectin (MBL) as prognostic factor in paediatric oncology patients. ***Clin Exp Immunol,*** 65(1): 51-59

🕮 Gaine ME, Sharpe DJ, Smith JS & Ken IM. (2017): Gata2 regulates the erythropoietin receptor in t (12;21) ALL. ***Oncotarget,*** 8(39): 66061-66074

🕮 Gavars D, Perminov D, Tauckels E, Lindenberga I, Auce A, Lejniece S. (2019): association of elevated vitamin B_{12} with Onco hematological diseases in a cohort of 79,524 patients from Latvia. ***Exp Oncol,*** 41(4): 357-362

🕮 Ghaffaria MA, Elyaderania MK, Saffarib MR & Pedram M. (2015): Monitoring of serum nitric oxide in patients with Acute Leukemia. ***Iranian Journal of Pharmaceutical Research,*** 4: 233-237

🕮 Ghazi M, Isadyar M, Gachkar L, Mahmoudi S, Goudarzi H, Eslami G, Pourakbari B & Fallah F. (2015): Serum levels of mannose-binding lectin and the risk of infection in pediatric oncology patients with chemotherapy. ***Journal of Pediatric Hematology/oncology,*** 34(2):128-130

🕮 Goldner W. (2016): Cancer-Related Hypercalcemia. ***Journal of Oncology Practice,*** 12(5): 433-435

🕮 Goncalves SI, Yasinska IM, Sakhnevych SS, Fiedler W, Wellbrock J & Bardelli M. (2017): The tim-3-galectin-9 secretory pathway is involved in the immune escape of human acute myeloid leukemia cells. ***EBioMedicine,*** 22: 44–57

🕮 Gonzalo Fernandez Lahorea GF, Raposoa B, Lagerquistb M, Ohlssonb C, Sabatierc P, Xua B & Holmdah R. (2020): Vitamin D_3 receptor polymorphisms regulate T cells and T cell-dependent inflammatory diseases. ***PNAS,*** 117(40): 1-12

🕮 Gordon-Alonso M, Bruger AM & Bruggen PD. (2018): Extracellular galectins as controllers of cytokines in hematological cancer. ***Blood,*** 132(5): 484-491

🕮 Gordon-Alonso M, Hirsch T, Wildmann C & Van der Bruggen P. (2017): Galectin-3 captures interferon-gamma in the tumor matrix reducing chemokine gradient production and T-cell tumor infiltration. ***Nat Commun,***8: 793

🕮 Gradel KO, Póvoa P, Garvik OS, Vinholt PJ& Frederiksen H. (2020): Longitudinal trajectory patterns of plasma albumin and C-reactive protein levels around diagnosis, relapse, bacteraemia, and death of acute myeloid leukaemia patients. ***BMC Cancer,*** 20(249): 1-13

🕮 Grant WB. (2018): A Review of the Evidence Supporting the Vitamin D-Cancer Prevention Hypothesis in 2017. ***Anticancer Research,*** 38: 1121-1136

🕮 Green R, Allen LH & Bjorke-Monsen AL. (2017): Vitamin B_{12} deficiency. ***Nat Rev Dis Primers,*** 3(17040): 217-227

🕮 Guan H, Miao H, Ma N, Lu W & Luo B. (2017): Correlations between Epstein-Barr virus and acute leukemia. ***Medical Virology,*** 89(8):1453-1460

🕮 Gupta A & Gupta GS. (2021): Status of mannose-binding lectin (MBL) and complement system in COVID-19 patients and therapeutic applications of antiviral plant MBLs. ***Mol Cell Biochem,*** 21: 1-26

🕮 Gupta A. (2020): Emerging applications of lectins in cancer detection and biomedicine. ***Materials Today: Proceedings,*** 31: 651-661

🕮 Gupta K, Gupta RK & Hajela K. (2015): Disease Associations of Mannose-Binding Lectin & Potential of Replacement Therapy. ***Indian J Med Res,*** 127(5):431-440

🕮 Gureev AP, Shaforostova EA & Popov VN. (2019): Regulation of Mitochondrial Biogenesis as a Way for Active Longevity: Interaction Between the Nrf2 and PGC-1α Signaling Pathways. ***Frontiers in Genetics,*** 10: 1-12

🕮 Hade HA, Jasim RH & Hatrosh SJ. (2018): CLEC4E as Novel Tumor Marker. A Biochemical Study for Prediction Acute Lymphocytic Leukemia at Iraqi Children. ***J Pharm Sci & Res,*** 10(3): 556-561

🕮 Hade HA. (2018): A physiological, biochemical, and histological study to non-genetic Acute Lymphocytic Leukemia for patients in Karbala city. ***PhD. Thesis,*** Dept., of Biology/College of Education for Pure Sciences/University of Karbala

🕮 Hallek M. (2019): Chronic lymphocytic leukemia:2020 update on diagnosis, risk stratification and treatment. ***Am J Hematol,*** 94:1266-1287

🕮 Hamad MNM, Kamal M, Saeed MA & Suliman MA. (2019): Assessment of serum ferritin levels in Sudanese patients with Acute Lymphoblastic Leukemia. ***Inter J Med Res & Health Sci,*** 8(7): 92-96

🕮 Hangauer MJ, Viswanathan VS, Ryan MJ, Bole D, Eaton JK, Matov A, Galeas J, Dhruv HD, Berens ME, Schreiber, SL, Mccormick F & Mcmanus MT. (2017): Drug-Tolerant persister cancer cells are vulnerable to GPX4 inhibition. ***Nature,*** 551**:** 247-250

🕮 Hannan FM, Kallay E, Chang W, Brandi ML & Thakker RV. (2018): Calcium-sensing receptor in physiology and in calcitropic and non-calcitropic diseases. ***Nat Rev Endocrinol,*** 15(1): 33-51

🕮 Hannibal L, Lysne V & Bjorke-Monsen AL. (2016): Biomarkers and algorithms for the diagnosis of vitamin B_{12} deficiency. ***Front Mol Biosci,*** 3(27): 1-16

🕮 Hao TK, Hiep PN, Hoa NTK & Ha CV. (2020): Causes of Death in Childhood Acute Lymphoblastic Leukemia at Hue Central Hospital for 10 Years (2008-2018). ***Global Pediatric Health,*** 7: 1-8

🕮 Hassan T, Qureshi W & Bhat SA. (2017): Study of serum levels of trace elements (selenium, copper, zinc, and iron) in breast cancer patients. ***Int J Clin Oncol Cancer Res,*** 2: 82-85

🕮 Henok JN, Okeleye BI, Omodanisi EI, Ntwampe SKO & Aboua UG. (2020): Analysis of Reference Ranges of Total Serum Protein in Namibia: Clinical Implications. ***Proteomes,*** 8(7): 1-10

🕮 Herb M & Schramm M. (2021): Functions of ROS in Macrophages and Antimicrobial Immunity. ***Antioxidants,*** 10(313): 1-39

🕮 Herr N, Bode C & Duerschmied D. (2017): The Effects of Serotonin in Immune Cells. ***Front Cardiovasc Med,*** 4: 1-11

🕮 Hisrich BV, Young RB, Sansone AM, Bowens Z, Green LJ, Lessey BA & Blenda AV. (2020): Role of Human Galectins in Inflammation and Cancers Associated with Endometriosis. ***Biomolecules,*** 10(230): 1-12

🕮 Hochhaus A, Saussele S, Rosti G, Mahon FX, Richter J & Buske C. (2017): Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. ***Annals of Oncology,*** 28 (Supplement 4): iv41–iv51

🕮 Hoffbrand AV & Moss PAH. (2016): Hoffbrand's Essential Hematology. 7th ed., ***Hoboken, NJ: Wiley-Blackwell Publishing Ltd.,*** p:159

🕮 Hoffbrand AV & Steensma DP. (2020): Hoffbrand's Essential Hematology. 8th ed., ***Hoboken, NJ: Wiley-Blackwell Publishing Ltd.***

🕮 Holanda K, Lucena-Araujo AR, Quintas A, Mendonça T, Lima A & Bezerra MA. (2015): Mannose-binding lectin 2 (MBL2) gene polymorphisms do not influence frequency of infections in chronic lymphocytic leukemia patients. ***Rev Bras Hematol Hemoter,*** 36(1): 29-34

🕮 Hoogstraten B, Baker H & Gilbert HS. (2019): Serum folate and serum Vitamin B_{12} patients with malignant hematologic diseases. ***Cancer Research,*** 25: 1933-1938

🕮 Horie M, Kaori Ito1 K, Hayashi T, Ando M, Tokuda M & Yamada S. (2017): Investigation of blood levels of zinc, vitamin B_{12} and folate in patients with haematological malignancy. ***Fujita Medical Journal,*** 3(4): 1-5

🕮 Hoyoux C, Lombet J, Nicolescu CR (2017): Malignancy-Induced Hypercalcemia-Diagnostic Challenges. ***Front Pediatr,*** 5(233): 1-5

🕮 Hu Y, Xiang J, Su L & Tang X. (2020): The regulation of nitric oxide in tumor progression and therapy. ***Journal of International Medical Research,*** 48(2):1-9

🕮 Hussain MA, Hassan BB, Masoud RE & Al Tamany D. (2017): Curcumin attenuates erythropoiesis in recombinant human erythropoietin induced polycythemia in rats. ***National Journal of Physiology, Pharmacy and Pharmacology,*** 7(7): 766-770

🕮 Hussein HAH & Alkhayat ZAY. (2021): Seroprevalence of EBV antibodies in children with acute lymphoblastic leukemia. ***Mosul Journal of Nursing,*** 9(1): 58-61

🕮 Iacobucci I & Mullighan CG. (2017): Genetic Basis of Acute Lymphoblastic Leukemia. ***Journal of Clinical Oncology,*** 35(9): 975-983

🕮 Icacan OC, Yokus O, Sametoglu F & Gedik H. (2018): Serum Vitamin D values of acute leukemia cases. ***Acta Medica Mediterranea,*** 34: 1883-1887

🕮 Ihlow J, Gross S, Sick A, Schneider T, Flörcken A & Westermann J. (2019): AML: high serum ferritin at initial diagnosis has a negative impact on long-term survival. ***Leukemia & Lymphoma,*** 60(1): 69-77

🕮 İlarslan CNE, Şıklar Z & Berberoğlu M. (2017): Childhood Sustained Hypercalcemia: A Diagnostic Challenge. ***J Clin Res Pediatr Endocrinol,*** 9(4): 315-322

🕮 Inaba H & Mullighan CG. (2020): Pediatric Acute Lymphoblastic Leukemia. ***Haematologica,*** 105(11): 2524-2539

🕮 Inaguma D, Koide S, Takahashi K, Hayashi H, Hasegawa M & Yuzawa Y. (2017): Relationship between serum calcium level at dialysis initiation and subsequent prognosis. ***Renal Replacement Therapy,*** 3(2): 1-9

🕮 International Agency for Research on Cancer (IARC) in WHO (2020): Latest global cancer data, Cancer burden rises in 2020. The GLOBOCAN 2020 database, accessible online as part of the ***IARC*** Global Cancer Observatory

🕮 Itzykson R, Fenaux P, Bowen D, Cross NCP & Malcovati L. (2018): Diagnosis and Treatment of Chronic Myelomonocytic Leukemias in Adults

Recommendations from the European Hematology Association and the European LeukemiaNet. ***HemaSphere,*** 2(6): 1-16

🕮 Jain P, Casteel K, Allen CE & McClain KL. (2016): Elevated Ferritin Predicts for Inferior Survival in Patients with Acute Leukemia and May be an Early Marker of an Underlying Systemic Pathologic Inflammation. ***Blood,*** 128(22): 2791-2791

🕮 Jalaeikhoo H, Kashfi SMH, Azimzadeh P, Narimani A & Keyhani M. (2017): Acute Myeloid Leukemia as the Main Cause of Pancytopenia in Iranian Population. ***Iran J Pathol,*** 12(3): 265-271

🕮 Jenna B Bhattacharya JB, Richa Gupta R & Samadhiya A. (2017): Acute megakaryoblastic blast crisis as a presentation manifestation of chronic myelogenous leukemia. ***Blood Res,*** 52(2): 137-139

🕮 Jeon SM & Shin EA. (2018): Exploring vitamin D metabolism and function in cancer. ***Experimental & Molecular Medicine,*** 50(20): 1-14

🕮 Jin MW, Xu SM, An Q, Wang P. (2016): A review of risk factors for childhood leukemia. ***Eur Rev Med Pharmacol Sci,*** 20: 3760-3764

🕮 John OA, Agnes A, Oluyemi A, Emmanuel AO, Sheu RK & Ganiyu AO. (2011): Antioxidant levels of Acute Leukemia patients in Nigeria. ***Sierra Leone J Biomed Res,*** 3(3): 133-137

🕮 Jolanta D, Florczak-Wyspianska J, Kowalska M, Stanski M, Kowalewska A & Kozubski W.(2017): Serotonin in Neurological Diseases.Serotonin: A chemical messenger between all types of living cells. ***IntechOpen Limited in England and Wales,*** 5Princes Gate Court, London, SW7 2QJ, UK

🕮 Jones GRD & Koetsier S. (2017): Uptake of recommended common reference intervals for chemical pathology in Australia. ***Annals of Clinical Biochemistry,*** 54(3): 395-397

🕮 Julian M, Yabut JM, Crane JD, Green AE, Keating DG, Khan WI & Steinberg GR. (2019): Emerging roles for serotonin in regulating metabolism: New implications for an ancient molecule. ***Endocrine Reviews,*** 40(4):1092-1107

🕮 Kabeel MM, Ghoneim AM & Mansy SE. (2018): Anti-leukemic activity of a four-plant mixture in a leukemic rat model. ***The Journal of Basic and Applied Zoology,*** 79(7): 1-12

🕮 Kaku Y, Ookawara S, Miyazawa H, Ito K, Ueda Y & Tabei K. (2015): Approximation of corrected calcium concentrations in advanced chronic kidney disease patients with or without dialysis therapy. ***Nephron Extra,*** 5: 39-49

🕮 Kalia N, Singh J & Kaur M. (2021): The ambiguous role of mannose-binding lectin (MBL) in human immunity. (Review Article), ***Open Medicine,*** 16: 299-310

🕮 Kaplan JA. (2019): Leukemia in Children, ***Pediatrics in Review,*** 40 (7):319-331

🕮 Karaköse M, Kocabaş M, Can M, Burgucu HC, Çordan I, Kulaksizoğlu M & Karakurt F. (2021): Increased incidence of malignancy in patients with primary hyperparathyroidism. ***Turk J Med Sci,*** 51: 2023-2028

🕮 Kardos J, Héja L, Simon A, Jablonkai I, Kovács R & Jemnitz K. (2018): Copper signalling: causes and consequences. ***Cell Communication and Signaling,*** 16(71): 1-21

🕮 Kavanagh S, Bril V, Lipton JH. (2018): Peripheral neuropathy associated with imatinib therapy for chronic myeloid leukemia. ***Blood Research,*** 53(2): 172-174

🕮 Kaweme NM, Zhou S, Changwe GJ & Zhou F. (2020): The significant role of redox system in myeloid leukemia: from pathogenesis to therapeutic applications. ***Biomark Res,*** 8(63): 1-12

🕮 Keizer MP, Kamp A, Mierlo GV, Kuijpers TW & Wouters D. (2018): Substitution of Mannan-Binding Lectin (MBL)-Deficient Serum with Recombinant MBL Results in the Formation of New MBL/MBL-Associated Serine Protease Complexes. ***Front Immunol,*** 9: 1-8

🕮 Kennedy L, Sandhu JK, Harper ME & Cuperlovic-Culf M. (2020): Role of Glutathione in Cancer: From Mechanisms to Therapies. ***Biomolecules,*** 10(1429): 1-28

🕮 Kesbeha Y & Pakbaza Z. (2019): Pernicious anemia: a myelodysplastic syndrome look-alike. ***Journal of Community Hospital Internal Medicine Perspectives,*** 9(30): 240-243

🕮 Keshet R & Erez A. (2018): Arginine and the metabolic regulation of nitric oxide synthesis in cancer. ***Dis Model Mech,*** 11(8): 1-11

🕮 Khalife M, Aziz MB, Balestra C, Valsamis J & Sosnowski M. (2021): Physiological and Clinical Impact of Repeated Inhaled Oxygen Variation on Erythropoietin Levels in Patients After Surgery. ***Front Physiol,*** 27: 1-21

🕮 Khan FH, Dervan E, Dibyangana D. Bhattacharyya DD, McAuliffe JD, Miranda KM & Glynn SA. (2020): The Role of Nitric Oxide in Cancer: Master Regulator or not? ***Int J Mol Sci,*** 21: 1-30

🕮 Khan MI, Naseem L, Manzoor R & Yasmeen N. (2017): Mortality Analysis in Children during Induction Therapy for Acute Lymphoblastic Leukemia. ***JIMDC,*** 6(2): 69-72

🕮 Khoshdel Z, Naghibalhossaini F, Abdollahi K, Shojaei S, Moradi M & Malekzadeh M. (2016): Serum Copper & Zinc levels among Iranian colorectal cancer patients. ***Biol Trace Elem Res,*** 170(2): 294-299

🕮 Kikushige Y, Miyamoto T, Yuda J, Jabbarzadeh-Tabrizi S, Shima T, Takayanagi S, Niiro H, Yurino A, Miyawaki K & Takenaka K. (2015): A TIM-3/Gal-9 autocrine stimulatory loop drives self-renewal of human myeloid leukemia stem cells and leukemic progression. ***Cell Stem Cell,*** 17: 341-352

🕮 Kim S, Freeland-Graves JH, Babaei M, Sachdev PK & Beretvas SN. (2019): Quantifying the association between acute leukemia and serum zinc, copper, and selenium: a meta-analysis. ***Leukemia & Lymphoma,*** 60(6): 1548-1556

🕮 Klaunig JE & Wang Z. (2018): Oxidative stress in carcinogenesis. ***Current Opinion in Toxicology,***7: 116-121

🕮 Klein SL & Flanagan KL. (2016): Sex differences in immune responses. ***Nature Reviews Immunology,*** 16: 626-638

🕮 Konda M, Godbole A, Pandey S & Sasapu A. (2019): Vitamin B_{12} deficiency mimicking Acute leukemia. Baylor University Medical Center Proceedings ***(Taylor & Francis Group),*** 32(4): 589-592

🕮 Kornblau SM, Ruvolo PP, Wang RY, Battula VL & Andreeff M. (2018): Distinct protein signatures of acute myeloid leukemia bone marrow-derived stromal cells are prognostic for patient survival. ***Haematologica,*** 103(5): 810-821

🕮 Koumpis E, Florentin M, Hatzimichael E & Liamis G. (2020): Hyponatremia in Patients with Hematologic Diseases. ***J Clin Med,*** 9(3721): 1-24

🕮 Krishna SM. (2019): Vitamin D as a protector of arterial health: Potential role in peripheral arterial disease formation. ***Int J Mol Sci,*** 20(19): 1-25

🕮 Kumar KK, Chandra KLP, Sumanthi J, Reddy GS, Shekar PC & Reddy BVR. (2012): Biological role of lectins. ***J Orofacial Sci,*** 4: 20-25

🕮 Kursunel MA & Esendagli G. (2017): A Co-Inhibitory Alliance in Myeloid Leukemia: TIM-3/Galectin-9 Complex as a New Target for Checkpoint Blockade Therapy. ***EBioMedicine,*** 23: 6-7

🕮 Lee JW & Cho B. (2017): Prognostic factors and treatment of pediatric acute lymphoblastic leukemia. ***Korean J Pediatr,*** 60(5): 129-137

🕮 Lee S, Jeon H & Shim B. (2019): Prognostic Value of Ferritin-to-Hemoglobin Ratio in Patients with Advanced Non-Small-Cell Lung Cancer. ***Journal of Cancer,*** 10(7): 1717-1725

🕮 Levine AB, Punihaole D & Levine TB. (2015): Characterization of the Role of Nitric Oxide and its Clinical Applications. ***Cardiology,*** 122: 55-68

🕮 Li J, Li H, Yua Y, Liua Y, Mab Q, Zhanga L & Zhou J. (2019a): Mannan-binding lectin suppresses growth of hepatocellular carcinoma by regulating hepatic stellate cell activation via the ERK/COX-2/PGE2 pathway. ***Oncoimmunology,*** 8(2): 1-14

🕮 Li Q, Xie Y, Xu G & Lebrilla CB. (2019b): Identification of potential sialic acid binding proteins on cell membranes by proximity chemical labelling. ***Chem Sci,*** 10: 6199-6209

🕮 Liamis G, Theodosios D. Moses F & Elisaf S. (2016): Electrolyte disorders associated with the use of anticancer drugs. ***European Journal of Pharmacology,*** 777: 78-87

🕮 Ling TB, Norhaizan ME, Liew WPP & Rahman HS. (2018). Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. ***Frontiers in Pharmacology,*** 9: 1162

🕮 Lison D. (2015): Handbook on the Toxicology of Metals,4thed., Academic Press, ***Elsevier,*** Pp:743-763

🕮 Liu S, Miao R, Zhai M, Pang Q, Deng Y, Liu S, Qu K, Liu C & Zhang J. (2017): Effects and related mechanisms of serotonin on malignant biological behavior of hepatocellular carcinoma via regulation of Yap. ***Oncotarget,*** 8(29): 47412-47424

🕮 Lu CW, Lee UC, Kuo CS, Chiang CH, Chang HH & Huang KC. (2021): Association of Serum Levels of Zinc, Copper, and Iron with Risk of Metabolic Syndrome. ***Nutrients,*** 13(548): 1-9

🕮 Luanpitpong S & Chanvorachote P. (2015): Nitric Oxide and Aggressive Behavior of Lung Cancer Cells (Review). ***Anticancer Research,*** 35: 4585-4592

🕮 Luc M, Ayme-Dietrich E, Aubertin-Kirch G, Banas S & Monassier L. (2017): New Therapeutic Opportunities for 5-HT2 Receptor Ligands.***Pharmacology & Therapeutics,*** 170: 14-36

🕮 Luciano R & Brewste UC. (2015): Kidney involvement in Leukemia and Lymphoma. ***Advanced in Chronic Kidney Disease,*** 21(1): 27-35

🕮 Luke Kennedy L, Sandhu JK, Harper ME & Culf MC. (2020): Role of Glutathione in Cancer: From Mechanisms to Therapies. ***Biomolecules,*** 10(1429): 1-28

🕮 Lundberg JO. (2016): Nitric oxide metabolites and cardiovascular disease. Markers, mediators, or both ***J Am Coll Cardiol,*** 47: 580-581

🕮 Lv GY, An L, Sun XD, Hu YL, Sun DW. (2018): Pre-treatment albumin to globulin ratio can serve as a prognostic marker in human cancers: a meta-analysis. ***Clin Chim Acta,*** 476:81-91

🕮 Lv J & Liu F. (2017): The Role of serotonin beyond the central nervous system during embryogenesis. ***Front Cell Neurosci,*** 11: 1-7

🕮 Lyle L & Hirose A. (2018): Iron Overload in Myelodysplastic Syndromes: Pathophysiology, Consequences, Diagnosis, and Treatment. ***J Adv Pract Oncol,*** 9(4): 392-405

🕮 Maestri CA, Nisihara R, Mendes HW, Jensenius J NS &Carvalho NS. (2018): MASP-1 and MASP-2 Serum Levels Are Associated with Worse Prognostic in Cervical Cancer Progression. ***Front Immunol,*** 9: 1-5

🕮 Maher FT, Majed HM & Mohammed OM. (2017): Relationship between Parathyroid hormone and some electrolytes in patients with End-Stage Renal Disease. ***Tikrit Journal for Pure Science,***4(22):106-112

🕮 Majbel KH. (2020): Assessment of C- Type Lectin Domain Family 4 Recombinant Protein in Diabetics during Renal Dialysis. ***MSc. Thesis,*** Department of Chemistry/Faculty of Education for Girls/University of Kufa

🕮 Manikandan SB, Manikandan R, Arumugam M & Mullainadhan P. (2020): An overview on human serum lectins (Review article). ***ScienceDirect: Heliyon,*** 6(8): e04623

🕮 Mari M, Gregorio E, Dios C, Roca-Agujetas V, Cucarull B, Tutusaus A, Morales A & Colell A. (2020): Mitochondrial Glutathione: Recent Insights and Role in Disease. ***Antioxidants,*** 9(909): 1-21

🕮 Martens PJ, Gysemans C, Verstuyf A & Mathieu C. (2020): Vitamin D's Effect on Immune Function. ***Nutrients,*** 12: 1-22

🕮 Matlab N & Jasim R. (2017): Assessment of the cellular balance for production of oxidants-antioxidants in serum samples of patients with advanced stages of cancer tumors. ***International Peer Reviewed Journal,*** 6(3): 433-447

🕮 McReynolds LJ & Savage SA. (2017): Pediatric leukemia susceptibility disorders: manifestations and management. ***Hematology Am Soc Hematol Educ Program,*** 1: 242-250

🕮 Mehranfar S, Zeinali S, Hosseini R, Mohammadian M, Akbarzadeh A, Hosein A & Feizi P. (2017): History of Leukemia: Diagnosis and Treatment from Beginning to Now. ***Galen Med J,*** 6(1): 1-23

🕮 Mejía-Aranguré JM. (2016): Molecular epidemiology of acute leukemia in children: causal model, interaction of three factors-susceptibility, environmental exposure and vulnerability period. ***Bol Med Hosp Infant Mex,*** 73(1): 55-63

🕮 Miess H, Dankworth B, Gouw AM, Rosenfeldt M, Schmitz W & Schulze A. (2018): The glutathione redox system is essential to prevent ferroptosis caused by impaired lipid metabolism in clear cell renal cell carcinoma. ***Oncogene,*** 37: 5435-5450

🕮 Ministry of health, Iraqi Cancer Board. (2008): ***Results of Iraqi Cancer Registry 1993***. (Ministry of health, 1993)

🕮 Ministry of health, Iraqi Cancer Board. (2017): ***Results of Iraqi Cancer Registry 2017***

🕮 Mishraa A, Behuraa A, Mawatwala S, Kumara A & Dhiman R. (2019): Structure-function and application of plant lectins in disease biology and immunity. ***Food and Chemical Toxicology,*** 134: 1-17

🕮 Mo XD, Wang YU, Zhang XH & Huang XJ. (2018): Interferon-α Is Effective for Treatment of Minimal Residual Disease in Patients with t (8;21) Acute Myeloid Leukemia After Allogeneic Hematopoietic Stem Cell Transplantation: Results of a Prospective Registry Study. ***The Oncologist,*** 23: 1349-1357

🕮 Moafi A, Ziaie M, Abedi M, Rahgozar S, Reisi N & Moafi H. (2017): The relationship between iron bone marrow stores and response to treatment in pediatric acute lymphoblastic leukemia. ***Medicine (Baltimore),*** 96(44): e8511

🕮 Modenutti CP, Capurro JIB, Di Lella S & Marti MA. (2019): The Structural Biology of Galectin-Ligand Recognition: Current Advances in

Modelling Tools, Protein Engineering, and Inhibitor Design. ***Front. Chem,*** 7: 1-14

🕮 Mohammad RK & Fezea SM. (2016): Determination of some trace element levels in Iraqi male patients with colorectal cancer. ***Ibn Al-Haitham J Pure & Appl Sci,*** 29(2): 254-261

🕮 Molina-Lopez J, Florea D, Herrera-Quintana L, Adam V, Kizek R, Quintero B & Planells E. (2015): Biomarkers of Zn status associated to colorectal cancer pathogenesis. ***Journal of Metallomics & Nanotechnologies,*** 2: 11-18

🕮 Montilla NA, Bailon E, Uceda-Castro R, Ugarte-Berzal E & Pardo-Garcia A. (2019): MMP-9 affects gene expression in chronic lymphocytic leukemia revealing CD99 as an MMP-9 target and a novel partner in malignant cell migration/arrest. ***Oncogene,*** 38: 4605-4619

🕮 Moorman AV. (2016): New and emerging prognostic and predictive genetic biomarkers in B-cell precursor acute lymphoblastic leukemia. ***Haematologica,*** 101(4): 407-416

🕮 Mui UN, Haley CT & Tyring SK. (2017): Review Viral Oncology: Molecular Biology and Pathogenesis. J Clin Med, 6(111): 1-58

🕮 Murphree CR, Nguyen NN, Raghunathan V, Olson SR, Deloughery T & Shatzel JJ. (2020): Diagnosis and management of hereditary haemochromatosis. ***Vox Sanguinis (International Society of Blood Transfusion),*** 115: 255-262

🕮 Muzolf-Panek M, Kleiber T & Kaczmarek A. (2017): Effect of increasing manganese concentration in nutrient solution on the antioxidant activity, vitamin C, lycopene and polyphenol contents of tomato fruit. ***Food Additives & Contaminants: Part A,*** 34(3): 379-389

🕮 Nair M, Kuttath V, Nair AR, Rajeswari B & Parukkutty K. (2018): Iron Overload in Children with Leukemia Receiving Multiple Blood Transfusions. ***Indian Pediatrics,*** 55(15): 962-965

🕮 Namrata S, Savanur MA, Srivastava S, D'Silva P & Mugesh G. (2019): A Manganese Oxide Nanozyme Prevents the Oxidative Damage of Biomolecules without Affecting the Endogenous Antioxidant System. ***Nanoscale,*** 11(9): 3855-3863

🕮 Nancy L, Parmalee ML & Aschner M. (2016): Manganese and aging. ***Neuro Toxicology,*** 56: 262-268

🕮 Narang V, Sachdeva MUS, Bose P, Varma N, Malhotra P & Varma S. (2016): Immunophenotyping in chronic myeloid leukemia blast crisis: looking beyond morphology. ***J Postgrad Med Edu Res,*** 50(4): 181-184

🕮 Nawi AM, Chin SF, Shah SA & Jamal R. (2019): Tissue and Serum Trace Elements Concentration among Colorectal Patients: A Systematic Review of Case-Control Studies. ***Iran J Public Health,*** 48(4): 632-643

🕮 Niki T, Fujitac K, Rosend H, Hirashimab M, Masakic, T, Hattorie H & Hoshinoa K. (2018): Plasma Galectin-9 Concentrations in Normal and Diseased Condition. ***Cell Physiol Biochem,*** 50: 1856-1868

🕮 Noronha EP, Marinho HT, Bárbara E, Fonseca, AT and Oliveira RAG. (2016): Chemotherapy for children with ALL. ***Sao Paulo Med J,*** 129(6): 1-12

🕮 Obaid J, Gracia W, Ali A & Barakat A. (2018): Could Vitamin B_{12} Deficiency Mimic Erythroleukemia? ***Bahrain Med Bull,*** 40(3): 184-186

🕮 Pagana KD & Pagana TJ. (2016): Mosby's Manual of Diagnostic and Laboratory Tests, 6th ed., ***St. Louis: Mosby***

🕮 Pandey V, Kumar M & Indira K. (2017): Early diagnosis of childhood Acute lymphoblastic leukemia. ***Perspectives in Medical Research,***5:1-3

🕮 Pang X, Li H, Guan F & Li X. (2018): Multiple Roles of Glycans in Hematological Malignancies. ***Front Oncol,*** 8(36): 1-12

🕮 Papaiakovou EE, Dimopoulos MA, Kastritis E, Christoulas D, Roussou M, Migkou M & Terpos E. (2017): Low circulating mannan-binding lectin levels correlate with increased frequency and severity of febrile episodes in myeloma patients who undergo ASCT and do not receive antibiotic prophylaxis. ***Bone Marrow Transplantation,*** 52: 1537-1542

🕮 Parka AS, Ritza B, Yub F, Cockburnc M & Heck JE. (2020): Prenatal Pesticide Exposure and Childhood Leukemia-a California state-wide case control study. ***Int J Hyg Environ Health,*** 226: 1-22

🕮 Pasha K, Reddy DM, Kumar RB, Ayesha Q& Srinivasulu M. (2017): Study of oxidative stress & antioxidant status in ascitic patients with ovarian cancer in comparison to liver cirrhosis patients. ***MOJ Proteomics Bioinform,*** 6(1): 186

🕮 Pasmatzi E, Papadionysiou C, Monastirli A, Badavanis G & Tsambaos D. (2019): Galectin 1 in dermatology: Current knowledge and perspectives. ***Acta Dermatovenerologica Alpina, Pannonica Adriat,*** 28(1): 27-31

🕮 Pena C, Mirandola L, Figueroa JA, Hosiriluck N, Suvorava N, Trotter K, Reidy A, Rakhshanda R, Payne D &Jenkins M. (2015): Galectins as

therapeutic targets for hematological malignancies. ***Ann Transl Med,*** 2: 87-90

🕮 Pereira FAC, Mirra AP, Latorre MRO & Assuncao JV. (2017): Environmental Risk Factors and Acute Lymphoblastic Leukaemia in Childhood. ***Rev Cienc Salud,*** 15(1):129-144

🕮 Pereira FAC, Mirra AP, Rosário MD, Latorre DO & Assunção JV. (2017): Environmental Risk Factors and Acute Lymphoblastic Leukaemia in Childhood. ***Rev Cienc Salud,*** 15(1):129-144

🕮 Periayah MH, Halim AS & Saad AZM. (2017): Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. ***IJHOSCR,*** 11(4): 1-10

🕮 Peterslund NA, Koch C, Jensenius JC & Thiel S. (2017): Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. ***Lancet,*** 358(282): 637-638

🕮 Pfeiffer CM& Looker AC. (2017): Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges. ***Am J Clin Nutr,*** 106(Suppl):1606S-1614S

🕮 Picón-Pagès P, Garcia-Buendia j & Muñoz FJ. (2019): Functions and dysfunctions of nitric oxide in brain. ***Review Biochim Biophys Acta Mol Basis Dis,*** 1865(8):1949-1967

🕮 Pierro J, Hogan LE, Bhatla T & Carroll WL. (2017): New targeted therapies for relapsed pediatric acute lymphoblastic leukemia. ***Expert Rev Anticancer Ther,*** 17(8): 725-736

🕮 Pilz S, Zittermann A, Trummer C, Theiler-Schwetz V & Pandis M. (2019): Vitamin D testing and treatment: a narrative review of current evidence. ***Endocr Connect,*** 8(2): R27-R43

🕮 Plummer M, De Martel C, Vignat J, Ferlay J, Bray F & Franceschi S. (2016): Global burden of cancers attributable to infections: A synthetic analysis. ***Lancet Glob Health,*** 4(9): E609-616

🕮 Porporato PE, Filigheddu N, Pedro GM, Kroemer G & Galluzzi L. (2018): Mitochondrial Metabolism & Cancer. ***Cell Res,*** 28(3): 265-280

🕮 Prado JCM, Monezi TA, Amorim AT & Boccardo E. (2018): Human polyomaviruses and cancer: an overview. ***Clinics (Sao Paulo)***, 73(Suppl 1): e558s

🕮 Prashanth L, Kattapagari KK, Chitturi RT, Baddam VRR & Prasad LK. (2015): A review on role of essential trace elements in health and disease. ***J Dr NTR University of Health Sciences,*** 4(2): 75-85

🕮 Puente M, Fariñas-Alvarez C, Moreto A, Sánchez-Velasco P, Ocejo-Vinyals JG & Fariñas MC. (2019): Low pre-transplant levels of mannose binding lectin are associated with viral infections and mortality after haematopoietic allogeneic stem cell transplantation. ***BMC Immunology,*** 20(40): 1-9

🕮 Qayyum MA & Shah MH. (2019): Disparities in the Concentrations of Essential/Toxic Elements in the Blood and Scalp Hair of Lymphoma Patients and Healthy Subjects. ***Scientific Reports (NatureResearch),*** 9: 1-15

🕮 Qazi M & Khurshid M. (2018): Free Radicals and Their Management. ***Am J Pharm Heal Res,*** 6(4): 487-504

🕮 Qingkai D, Rui S, Zhang G, Yang D & Yongmei J. (2021): Combined use of peripheral blood blast count and platelet count during and after induction therapy to predict prognosis in children with acute lymphoblastic leukemia. ***Medicine,*** 100(15): p e25548

🕮 Quah SR. (2017): Leukemia. 2nd ed. International Encyclopaedia of Public Health (Reference Work). ***Academic Press. Elsevier Inc. Direct Science,*** p:4470

🕮 Quesada J, Cuccuini W, Saultier P, Loosveld M, Harrison CJ & Lafage-Pochitaloff M. (2021): Cytogenetics of Pediatric Acute Myeloid Leukemia: A Review of the Current Knowledge. ***Genes,*** 12: 924

🕮 Quijano C, Trujillo M, Castro L & Trostchansky A. (2016): Interplay between oxidant species and energy metabolism. ***Redox Biology,*** 8: 28-42

🕮 Rafieemehr H, Calhor F, Esfahani H & Gholiabad SG. (2019): Risk of Acute Lymphoblastic Leukemia: Results of a Case-Control Study. ***Asian Pac J Cancer Prev,*** 20 (8), 2477-2483

🕮 Randolph A, Chen RA & Goodman WG. (2016): Role of the calcium-sensing receptor in parathyroid gland physiology. ***Am J Physio Ren Physio,*** 286(6):1-11

🕮 Rasha H & Matlab NS. (2017): Serotonin as a marker to the response of patients with advanced stages of cancer during treatment with chemotherapy and radiotherapy. ***Clin Med Biochem,*** 3(2): 1-4

🕮 Rasool M, Farooq S, Malik A, Shaukat A, Manan A, Asif M, Sani S & Hussain A. (2015): Assessment of circulating biochemical markers and

antioxidative status in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) patients. ***Saudi Journal of Biological Sciences,*** 22: 106-111

🕮 Rauch PJ, Ellegast JM, Widmer CC, Fritsch K, Goede JS, Valk PJ, Lowenberg B, Takizawa H, Manz MG :(2017). MPL expression on AML blasts predicts peripheral blood neutropenia and thrombocytopenia. ***Blood,***128: 2253-2257

🕮 Rezaieg NS & Musleh MH. (2019): Assessment of the role of oxidative stress and circulating biochemical markers in childhood leukemia. ***J Phys: Conf. Series,*** 1294: 1-9

🕮 Rifai N. (2018): Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 8th ed., ***Published: St. Louis, Mo: Saunders/Elsevier,*** P:1088

🕮 Riwes MM, Leather H, Neal D & Wingard JR. (2016): Association of mannose-binding lectin levels and invasive fungal disease in hematologic malignancy patients receiving myelosuppressive chemotherapy or allogeneic hematopoietic stem cell transplantation. ***Bone Marrow Transplantation,*** 51: 1228-1232

🕮 Robert A. Johnson RA, Kantarjian HM, Klisovic RB, Kupfer G, Litzow M & Smith C. (2015): Acute Lymphoblastic Leukemia, Version 2.2015. © ***JNCCN-Journal of the National Comprehensive Cancer Network,*** 13(10): 1-40

🕮 Robert BM, Dakshinamoorthy M, Ramamoorthy BG, Dhandapani M & Prasad NR. (2018): Predicting Tumor Sensitivity to Chemotherapeutic Drugs in Oral Squamous Cell Carcinoma Patients. ***Scientific Reports,*** 8: 1-10

🕮 Roland A. Ammann RA, Bodmer N, Simon A, Agyeman P, Leibundgut K, Schlapbach LJ & Niggli FK. (2015): Serum Concentrations of Mannan-Binding Lectin (MBL) and MBL-Associated Serine Protease-2 and the risk of adverse events in pediatric patients with cancer and fever in Neutropenia. ***Journal of the Pediatric Infectious Diseases Society,*** 2(2): 155-161

🕮 Ross JA, Spector LG & Davies SM. (2017): Biological Basis of cancer & blood disorder. Etiology of childhood cancer: Recent Reports. ***Pediatric Blood & Cancer,*** 45(3): 239-241

🕮 Saccon F, Gatto M, Ghirardello A, Iaccarino L, Punzi L & Doria A. (2017): Role of galectin-3 in autoimmune and non-autoimmune nephropathies. ***Autoimmun Rev,*** 16(1): 34-47

🕮 Salbitani G, BottoneC &Carfagna S. (2017): Determination of Reduced and Total Glutathione Content in Extremophilic Microalga Galdieria Phlegrea.***Bio Protocol,*** 7(13): e2372

🕮 Saleh SAK, Adly HM, Abdelkhaliq AA & Nassir AM. (2020): Serum Levels of Selenium, Zinc, Copper, Manganese, and Iron in Prostate Cancer Patients. ***Curr Urol,*** 14: 44-49

🕮 Samra B, Jabbour E, Ravandi F, Kantarjian H & Short NJ. (2020): Evolving therapy of adult acute lymphoblastic leukemia: state-of-the-art treatment and future directions. ***Journal of Hematology & Oncology,*** 13(70): 1-17

🕮 Sangwan L, Kumar R, Peter R & Arun P. (2016): Evaluation of nitric oxide levels in chronic myeloid leukemia. ***Inter J Innov Res Rev,*** 2(2): 1-5

🕮 Sarrouilhe D & Mesnil M. (2019): Serotonin and human cancer: a critical view (Mini-review). ***Biochem,*** 161: 46-50

🕮 Sas V, Moisoiu V, Teodorescu P, Tranca S & Kitano S. (2019): Approach to the Adult Acute Lymphoblastic Leukemia Patient. ***J. Clin. Med,*** 8: 1175

🕮 Scheurera ME, Lupoa PJ, Schüzc J, Logan G. Spectord LG, Russella HV, David G. Poplacka DG. (2018): An overview of disparities in childhood cancer: Report on the Inaugural Symposium on Childhood Cancer Health Disparities, Houston, Texas, 2016. ***Pediatr Hematol Oncol,*** 35(2): 95-110

🕮 Senjoa H, Higuchib T, Okadab S & Takahashi O. (2018): Hyperferritinemia: causes and significance in a general hospital. ***Hematology,*** 23(10): 817-822

🕮 Serbanica A, Radu L, Jercan C & Beldiman A. (2018): 2014-2017 Retrospective study on the analysis of causes of death, other than disease progression, in children diagnosed with acute lymphoblastic leukaemia-experience of the paediatric department of Fundeni clinical institute. ***Romanian Journal of Pediatrics,*** 67(4): 177-184

🕮 Seyedalipour F, Mansouri A, Vaezi M, Gholami K, Heidari K, Hadjibabaie M & Ardeshir Ghavamzadeh A. (2017): High prevalence of Vitamin D deficiency in newly diagnosed Acute Myeloid Leukemia patients and its adverse outcome. ***IJHOSCR,*** 11(3): 1-8

🕮 Sezer RG, Akoglu HA, Bozaykut A & Ozdemir GN. (2018): Comparison of the efficacy of parenteral and oral ¨ treatment for nutritional vitamin B_{12} deficiency in children. ***Hematology,*** 23(9): 653-657

🕮 Shah N. (2017): Advances in Iron chelation therapy: Transitioning to a new oral formulation. Drugs in Context, 6:1-10

🕮 Shirali AC. (2016): Electrolyte and Acid–Base Disorders in Malignancy. Chapter 5: ***American Society of Nephrology Onco-Nephrology Curriculum,*** 1-6

🕮 Silvagno F, Vernone A & Pescarmona GP. (2020): The Role of Glutathione in Protecting against the Severe Inflammatory Response Triggered by COVID-19. ***Antioxidants,*** 9(624): 1-16

🕮 Silverthorn DU. (2018): Human Physiology ''An integrated Approach''. 8th ed., ***Pearson Benjamin Cummings, international edition.*** USA

🕮 Simioni C, Martelli AM, Zauli G, Melloni E & Neri LM. (2019): Targeting mTOR in Acute Lymphoblastic Leukemia. ***Cells,*** 8(190):1-26

🕮 Simpson T, Pase M & Stough C. (2015): Bacopa monnieri as an antioxidant therapy to reduce oxidative stress in the aging brain. ***Evidence-Based Comple Altern Med,*** 10: 1-10

🕮 Singh K, Bhori M, Kasu AY, Bhat G & Marar T. (2018): Antioxidants as precision weapons in war against cancer chemotherapy induced Toxicity-Exploring the armoury of obscurity. ***Saudi Pharmaceutical Journal,*** 26: 177-190

🕮 Singh N, Qayyum S, Wasik MA & Luger SM. (2015): Combined B_{12} and folate deficiency presenting as an aggressive hematologic malignancy. ***Am J Hematol,*** 90: 964-965

🕮 Sirvent A, Auquier P, Oudin C, Bertrand Y, Bohrer S, Chastagner P & G Michel G. (2017): Prevalence and risk factors of iron overload after hematopoietic stem cell transplantation for childhood acute leukemia: a LEA study. Bone Marrow ***Transplantation,*** 52, 80-87

🕮 Skrajnowska D & Bobrowska-Korczak B. (2019): Role of Zinc in Immune System and Anti-Cancer Defense Mechanisms. ***Nutrients,*** 11(10): 2273

🕮 Smith J. (2019): Classification of Bone Mineral Density and Cognitive Health among Children with Acute Lymphoblastic Leukemia in a Health and Wellness Program. ***Thesis in Department of Nutritional Sciences***/Health Sciences Center/University of Oklahoma

🕮 Sokotowska A, Swierzko AS, Gajek G, Gotos A, Jamroziak K, Kowalski M & Cedzynski M. (2020): Associations of ficolins and mannose-binding

lectin with acute myeloid leukaemia in adults. ***Scientific Reports, NatureResearch,*** 10: 1-14

🕮 Song M, Pan O, Yang J, He J, Zeng J, Cheng S & Xia JC. (2020): Molecular Diagnostics Galectin-3 favours tumour metastasis via the activation of β-catenin signalling in hepatocellular carcinoma. ***British Journal of Cancer,*** 123: 1521-1534

🕮 Speletas NM, Gounaris A, Sevdali E, Kompoti M & Germenis AE. (2015): MBL2 Genotypes and Their Associations with MBL Levels and NICU Morbidity in a Cohort of Greek. ***Journal of Immunology Research,*** 10: 1-11

🕮 Sun H, Zhang C, Cao S, Sheng T, Dong N & Xu Y. (2018): Fenton reactions drive nucleotide and ATP syntheses in cancer. ***Journal of Molecular Cell Biology,*** 10(5): 448-459

🕮 Sun Y, Zheng Y, Wang C & Liu Y. (2018): Glutathione depletion induces ferroptosis, autophagy, and premature cell senescence in retinal pigment epithelial cells. ***Cell Death and Disease,*** 9(753): 1-15

🕮 Sung H, Ferlay J, Siegel R, Laversanne M & Bray F. (2021): Global Cancer Statistics 2020: Globocan Estimate of Incidence and Mortality Worldwide***. CA Cancer H Clin,*** 71(3): 209-249

🕮 Suresh S, Rajvanshi PK & Noguchi CT. (2020): The Many Facets of Erythropoietin Physiologic and Metabolic Response. ***Front Physiol,*** 10: 1-20

🕮 Świerzko A, Michalski M, Sokołowska A, Nowicki M & Cedzyński M. (2020): Associations of fcolins with haematological malignancies in patients receiving high-dose chemotherapy and autologous haematopoietic stem cell transplantations (auto-HSCT). ***Front Immunol,*** 10(3097): 1-11

🕮 Swierzko AS, Michalski M, Sokotowska A, Nowicki M & Cedzynski M. (2018): The Role of Complement Activating Collectins and Associated Serine Proteases in Patients with Hematological Malignancies, Receiving High-Dose Chemotherapy, and Autologous Hematopoietic Stem Cell Transplantations (Auto-HSCT). ***Front Immunol,*** 20: 1-26

🕮 Szabo A, Gogolak P, Koncz G, Foldvari Z, Pazmandi K, Miltner N & Rajnavolgyi E. (2018): Immunomodulatory capacity of the serotonin receptor 5-HT2B in a subset of human dendritic cells. ***Scientific Reports,*** 8: 1-12

🕮 Szaka´cs D, Kocsis A, Sza´sz R, Ga´l P & Pal G. (2019): Novel MASP-2 inhibitors developed via directed evolution of human TFPI1 are potent lectin pathway inhibitors. ***J Biol Chem,*** 294(20): 8227-8237

🕮 Tachibana T, Andou T, Tanaka M, Ito S, Miyazki T, Ishii Y, Ogusa E, Nakajima H & Kanamori H. (2018): Clinical Significance of Serum Ferritin at Diagnosis in Patients with Acute Myeloid Leukemia: A YACHT Multicentre Retrospective Study. ***Clin Lymphoma Myeloma Leuk,*** 18(6): 415-421

🕮 Tadokoro T, Fujihara S, Chiyo T, Oura K, Hirashima M & Masaki T. (2017): Induction of apoptosis by Galectin-9 in liver metastatic cancer cells: In vitro study. ***Journal of Oncology,*** 51(2): 607-614

🕮 Taghiloo S, Allahmoradi E, Ebadi R, Tehrani M, Hosseini-Khah Z, Janbabaei G, Shekarriz R & Asgarian-Omran H. (2017): Upregulation of galectin-9 and PD-L1 immune checkpoints moles in patients with Chronic Lymphocytic Leukemia. ***Asian Pacific Journal of Cancer Prevention,*** 18 (8): 2269-2274

🕮 Tagiyev A, Demirbilek H, Tavil B, Buyukyilmaz G, Gumruk F & Cetin M. (2016): Severe Hypercalcemia in a Child with Acute Lymphoblastic Leukemia Relapse: Successful Management with Combination of Calcitonin and Bisphosphonate. ***J Pediatr Hematol Oncol,*** 38(3): 232-234

🕮 Tahir NT & Obed FA. (2019): Hematological and analytical study among Iraqi patients with Acute Myeloid Leukemia. ***East African Scholars J Med Sci,*** 2(7): 381-386

🕮 Tandon S, Singh K, Ruban A, Singh B, Mahdi AA & Kumar A. (2015): Estimation of serum folate and vitamin B_{12} levels in children with hematologic malignancies. ***Gomal J Med Sci,*** 12(2): 89-92

🕮 Tanyildiz HG, Malbora B, Yesil S, Tekgunduz SA, Candir MO, Bozkurt C & Sahin G. (2016): Vitamin B_{12} deficiency mimicking Acute Leukemia in a child. ***J Clin Case Rep,*** 4: 1-2

🕮 Tariq SR, Ejaz A, Mahmud T & Tariq AR. (2016): Distributive variability of Leukemia patients. ***Journal of Heavy Metal Toxicity & Diseases,*** 1(15): 1-10

🕮 Tasian SK & Hunger SK. (2018): Genomic Characterization of Paediatric Acute Lymphoblastic Leukaemia: An Opportunity for Precision Medicine Therapeutics. ***Br J Haematol,*** 176(6): 867-882

🕮 Tebbi CK. (2021): Etiology of Acute Leukemia: A Review. ***Cancers,*** 13: 1-19

🕮 Terwilliger T & Abdul- Hay M. (2017): Acute lymphoblastic leukemia: a comprehensive review and 2017 update. ***Blood Cancer J,*** 7(e577): 1-12

🕮 Teskey G, Abrahem R, Gyurjian K & Cao R. (2018): Glutathione as a Marker for Human Disease. ***Advances in Clinical Chemistry,*** 87: 141-159

🕮 Thijssen VL, Heusschen R, Caers J & Griffioen AW. (2015): Galectin expression in cancer diagnosis and prognosis: A systematic review. ***Biochim Biophys Acta,*** 1855: 235-247

🕮 Thomas X. (2019): Acute Promyelocytic Leukemia: A History over 60 Years-from the most malignant to the most curable form of Acute Leukemia. ***Oncol Ther,*** 7: 33-65

🕮 Torki WM. (2020): Evaluation of Osteoponetin, Galectin, and Some Oxidative Stress parameters in patients with Renal Failure. MSc. ***Thesis in Biochemistry,*** Faculty of Education for Girls/University of Kufa

🕮 Tseng PC, Chen CL, Shan YS & Lin C.F. (2016): An increase in galectin-3 causes cellular unresponsiveness to IFN-gamma-induced signal transduction and growth inhibition in gastric cancer cells. ***Oncotarget,*** 7: 15150-15160

🕮 Umbrello M, Dyson A, Feelisch M & Singer M. (2015): The Key Role of Nitric Oxide in Hypoxia: Hypoxic Vasodilation and Energy Supply-Demand Matching. (Review Article). ***Antioxidants & Redox Signaling,*** 19(14): 1607-1618

🕮 Urbanski G, Hamel JF, Prouveur B, Annweiler C, Ghali A & Lacombe V. (2020): Strength of the Association of Elevated Vitamin B_{12} and Solid Cancers: An Adjusted Case-Control Study. ***J Clin Med,*** 9(474): 1-11

🕮 Vahid-Ansari F, Zhang M, Zahrai A & Albert PR. (2019): Overcoming resistance to selective serotonin reuptake inhibitors: Targeting Serotonin, Serotonin-1A Receptors and Adult Neuroplasticity. ***Front Neurosci,*** 13: 404

🕮 Vahora H, Khan MA, Alalami U & Hussain A. (2016): The Potential Role of Nitric Oxide in Halting Cancer Progression Through Chemoprevention. ***Journal of Cancer Prevention,*** 21(1): 1-12

🕮 Valadbeigi S, Javadian S, Ebrahimi-Rad M, Khatami S & Saghiri R. (2019): Assessment of trace elements in serum of acute lymphoblastic and myeloid leukemia patients. ***Experimental Oncology,*** 41(1): 69-71

🕮 Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M & Seeberger PH. (2015): Essentials of Glycobiology. 3rd ed., Cold Spring Harbor (NY): ***Cold Spring Harbor Laboratory Press***

🕮 Verzicco I, Regolisti G, Quaini F, Bocchi P & Cabassi A. (2020): Electrolyte Disorders Induced by Antineoplastic Drugs. ***Front Oncol,*** 10: 1-16

🕮 Vogelaar JL, Loar RW & Bram RJ. (2015): Anasarca, Hypoalbuminemia, and Anemia: What Is the Correlation. ***Clinical Pediatrics,*** 53(7): 710-712

🕮 Wadhwa S, Mehta M, Singh SK & Satija S. (2018): Vitamin D deficiency, skin, and sunshine: A review. ***International Journal of Green Pharmacy,*** (Suppl) 12 (2): S345

🕮 Wahlund M, Appell ML, Myrberg IH, Berggren A & Nilsson A. (2020): Genetic Sequence Variants in TLR4, MBL or IL-1 Receptor Antagonist is not Associated to Increased Risk for Febrile Neutropenia in Children with ALL. ***Children,*** 7(296): 1-11

🕮 Walker MD & Silverberg SJ. (2018): Primary hyperparathyroidism. ***Nature Reviews Endocrinology,*** 14 (2): 115-125

🕮 Wang F, Lv H, Zhao B, Zhou L, Wang S, Luo J, Liu J & Shang P. (2019a): Iron and leukemia: new insights for future treatments. ***J Exp & Clin Cancer Res,*** 38: 1-17

🕮 Wang K, Chen Z, Wu R, Yin J, Fan M & Xu X. (2018): Prognostic Role of High Gal-9 Expression in Solid Tumours: A meta-analysis. ***Cell Physiol Biochem,*** 45: 993-1002

🕮 Wang WH, Chen LW, Lee CC, Sun CY, Shyu YC, Hsu HR, Chien RN, Wu IW. (2017a): Association between Parathyroid Hormone, 25 (OH) Vitamin D, and Chronic Kidney Disease: A Population-Based Study. ***BioMed Research International,*** Article ID 7435657: 1-10

🕮 Wang Y, Yu L, Ding J & Chen Y. (2019b): Iron Metabolism in Cancer. ***Int J Mol Sci,*** 20(95): 1-22

🕮 Wdowiak K, Gallego-Colon E, Francuz T, Czajka-Francuz P, Ruiz-Agamez N & Wojnar J. (2019): Increased serum levels of Galectin-9 in patients with Chronic Lymphocytic Leukemia. ***Oncol Lett,*** 17(1): 1019-1029

🕮 Wei AH, Ribera JM, Larson RA & Kantarjian H. (2020): Biomarkers associated with blinatumomab outcomes in acute lymphoblastic leukemia. ***Leukemia,*** 8(31): 1-12

🕮 Wei C, Yu Z, Wang G, Zhou Y & Tian L. (2021): Low Pre-treatment Albumin-to-Globulin Ratio Predicts Poor Prognosis in Gastric Cancer: Insight from a Meta-Analysis. ***Frontiers in Oncology,***10: 1-10

🕮 Wohlfahrt AB, Hannel L, Oliverira LZ, Soares PB & Silva JEP. (2015): The importance of immunophenotyping by flow cytometry in distinction between hematogones and B-lymphoblasts. ***J Bras Patol Med Lab,*** 51(1): 7-12

🕮 Wu AHB. (2020): A General clinical chemistry (Section A): Antiquated and novel clinical laboratory tests. Self-assessment Q & A in Clinical Laboratory Science, ***Elsevier,*** p:75

🕮 World Health Organization (WHO) (2016): Acute Lymphoblastic Leukemia (ALL) Staging. Cancer Stat Facts: Leukemia

🕮 World Health Organization (WHO) (2020): Acute leukemia forum. 26th Annual, Cancer Stat Facts: Leukemia

🕮 Yabut JM, Crane JD, Green AE, Keating DJ & Steinberg GR. (2019): Emerging Roles for Serotonin in Regulating Metabolism: New Implications for an Ancient Molecule. ***Endocrine Reviews,*** 40: 1092-1107

🕮 Yang Li Y, Chen X, Shen Z, Wang Y, Hu J, Xu J, Shen B & Ding X. (2020): Electrolyte and acid-base disorders in cancer patients and its impact on clinical outcomes: evidence from a real-world study in China. ***Renal Failure,*** 42(1): 234-243

🕮 Yang WC. (2015): Iron metabolism and leukemia. ***Adv Tech Biol Med,*** 3(1): 1-4

🕮 Yoong J & Poon P. (2018): Principles of cancer pain management: An overview and focus on pharmacological and interventional strategies. ***AJGP,*** 47(11): 1-10

🕮 Young B, Woodford P & O'dowd G. (2018): Wheater's functional histology: A text and colour atlas. 5th ed., ***Churchill Livingstone, an imprint of Elsevier Ltd.*** USA

🕮 Zaminpira S & Niknamian S. (2018): The impact of the Serotonin on the cause and treatment of cancer. ***International Journal of Cancer & Oncology,*** 5(1): 1-7

🕮 Zekavat OR, Karimi M, Majidi F, Bordbar M, Haghpanah S, Parand S & Bozorgi H. (2020): Trace Elements in Children with Acute Lymphoblastic Leukemia. ***Asian Pacific Journal of Cancer Prevention,*** 22: 43-47

🕮 Zengin N. (2019): Prognostic importance of albumin to globulin ratio in metastatic gastric cancer patients. ***Curr Med Res Opin,*** 35(2): 275-282

🕮 Zhang JIN, Lei WEN, Chen X, Wang S & Qian W. (2018): Oxidative stress response induced by chemotherapy in leukemia treatment (Review). ***Mol Clin Oncol,*** 8(1): 391-399

🕮 Zhang XZ, Su AL, Hu MQ, Zhang XQ & Xu YL. (2015): Elevated serum ferritin levels in patients with hematologic malignancies. ***Asian Pacific Journal of Cancer Prevention,*** 15: 6099-6101

🕮 Zhao R, Jiang S, Zhang L & Yu Z. (2019): Mitochondrial electron transport chain, ROS generation and uncoupling (Review). ***Int J Mol Med,*** 44(1): 3-15

🕮 Zhou Q, Qin S, Zhang J, Zhon L, Pen Z & Xing T. (2017): 1,25(OH)2D3 induces regulatory T cell differentiation by influencing the VDR/PLC-γ1/TGF-β1/pathway. ***Molecular Immunology,*** 91: 156-164

🕮 Zmudzka E, Salaciak K, Sapa J & Pytka K. (2018): Serotonin receptors in depression and anxiety: Insights from animal studies. ***Life Sci,*** 1(210) :106-124

🕮 Zweckstetter M, Dityatev A & Ponimaskin E. (2021): Structure of serotonin receptors: molecular underpinning of receptor activation and modulation. ***Signal Transduction and Targeted Therapy,*** 6(243): 1-3

الخلاصـــة

خلال الفترة الممتدة من بداية اذار 2019 وحتى نهاية شباط 2020 وفي مركز الأورام وأمراض الدم في مدينة الإمام الحسين الطبية في كربلاء المقدسة، تم جمع31 طفلا ممن شخصوا بالاصابة بسرطان الدم الحاد وقبل تلقيهم العلاج الكيمياوي، وقد تراوحت أعمارهم من 2-12 سنة (18 من الذكور و 13 الإناث) وبدون تاريخ عائلي طبي للإصابة بالسرطان، وتم متابعة المرضى خلال فترة تلقيهم العلاج الكيمياوي (حتى تلقوا أربع جرعات من العلاج). وقد شملت الدراسة أيضا 40 فردا من الاطفال الاصحاء قد تراوحت أعمارهم ما بين2-12 سنة (20 ذكرا و20 أنثى).

وقد صممت هذه الدراسة لتقييم مستويات (Galectin-9)، mannose-binding lectin (MBL)، فضلا عن العديد من المعلمات والمؤشرات الدموية الروتينية؛ مثل RBCs ، WBCs، Plts، Hgb، HCT، MCV، MCH، وMCHC. بالإضافة إلى ذلك، تم تقييم مستويات مؤشرالإجهاد التأكسدي الذي ينطوي على أكسيد النيتريك (NO) الى جانب ذلك شملت الدراسة تحديد المعلمات المضادة للأكسدة .(T-GSH and ,GSSG) وشملت بيانات هذه الدراسة أيضا رصد مستويات هرمونات معينة؛ مثل Erythropoietin (EPO), Serotonin (ST), Parathyroid (PTH), وكذلك البروتين الكلي ,(TP) والألبومين (Alb), والفيريتين (FT) اضافة الى تقييم مستويات بعض الفيتامينات بما في ذلك vit D_3 and vit B_{12} وكذلك تقييم بعض العناصر النزرة مثل الزنك والكوبالت والحديد والمنغنيز والنحاس، فضلا عن بعض الشوارد الأخرى مثل الكالسيوم والمغنيسيوم والصوديوم والكلور والبوتاسيوم.

لقد أظهرت نتائج الدراسة الحالية زيادة احصائية كبيرة (**$p<0.05$**) على مستويات WBCs، Gal-9، MBL، NO، EPO، ST، vit D_3 ، vit B_{12}، FT، Mn، Ca^{2+}، في حين أظهرت النتائج انخفاضا كبيرا إحصائيا (**$p<0.05$**) على مستويات RBCs ، Hgb، HCT، Plts، MCHC، T-GSH، GSSG، PTH، TP، Alb، Co، K^+ في عينات مصول المرضى الذين يعانون من ALL قبل تلقيهم العلاج الكيمياوي) بالمقارنة مع مجموعة التحكم، في حين أن مستويات MCH ، Zn، Fe، Cu، Mg^{2+}، Na^+، CL^- لم تختلف بشكل كبير، ولكن كانت هناك زيادة غير كبيرة في مستوى MCV في عينات دم الاطفال المرضى. ومن ناحية أخرى، أظهرت بيانات الدراسة انخفاض المستويات في كل من المعلمات التالية في المرضى بعد تلقي العلاج الكيميائي (WBCs, Gal-9, MBL, NO, ST, vit D, FT, Fe, Cu, Ca^{2+}, Mg^{2+}, Na^+, CL^-, and K^+)

ومع ذلك؛ فقد اشارت النتائج إلى انخفاضا متقلبا في اعداد كريات الدم الحمراء، في حين أن المقاييس الدموية الأخرى تمثلت بارتفاع طفيف وتدريجي عن مستوياتها عند التشخيص؛ مثلHgb،

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HCT، Plts، MCV، MCH، MCHC، T-GSH، GSSG، TP، Alb، Zn، Co، في حين أن كل من EPO الـ$vit B_{12}$، ومستويات الـ Mn لا تزال ترتفع باستمرار بعد العلاج الكيميائي، بينما بقيت مستويات الـ PTH منخفضة بعد العلاج، كما كانت عليه في وقت التشخيص (قبل العلاج).

وقد أظهرت نتائج هذا العمل وجود ارتباطات إحصائية مقبولة عند المستويين ($p<0.05$),($p<0.01$) (إيجابي أو سلبي) بين المعايير التي تم تقييمها في الدراسة. فقد لوحظ وجود ارتباط كبير إيجابي بين Gal-9 and MBL في مصول المرضى ومجموعة التحكم مع NO, ST, ومستويات الـ EPO, فضلا عن ارتباطات إيجابية عالية سجلت بين جزيئات مضادات الأكسدة T-GSH, GSSGوالعناصر النزرة Zn, Co, Mn, Cu وكذلك بين Zn مع Cu, Mn, Co من ناحية فان هناك علاقة ارتباط ايجابي للـCo مع Mn وCu، في حين سجلت نتائج الدراسة علاقة ارتباط سلبية عند مقارنة Gal-9 و MBL مع مستويات مضادات الأكسدة T-GSH و GSSG، وكذلك مع العناصر النزرة Zn، Co، Mn، Cu، بينما لوحظ ارتباط سلبي كبير بين EPO مع كل من العناصر النزرة Zn و Co و Mn و Cu.

وقد اظهر كل من FT, EPO, Gal-9 اعلى حساسية مفردة (100%) بين المعايير التي تم تقييمها متبوعة بالـــMBL (77%) في حين كان ادنى مستوى حساسية للمرضى 39% و 29%، 10% للـT-GSH, PTH, GSSG على التوالي في الأطفال المصابين باللوكيميا الحادة مقارنة مع اطفال مجموعة التحكم.

وقد لوحظ ايضا أن القدرة التفاضلية للمعايير المقيمة تزداد عموما عندما ترتبط هذه المعايير ببعضها البعض. فقد وجد أن أقصى معدل للحساسية (100%) عندما تم الجمع بين الـFT، EPO، Gal-9 من جهة وكل من الـPTH، MBL، T-GSH، GSSG، NO، ST من جهة اخرى.

وقد أشارت نتائج الدراسة الحالية إلى أن حساسية الكفاءة التشخيصية لـــ MBL قد ازدادت إلى 94%, 90% عند تقييم مستوياتها مع كل من الـــ NO والـــ GSSG على التوالي, الى جانب ذلك تم الوصول إلى الحساسية التشخيصية (87%) لـ T-GSH عند تقييم مستوياتها مع كل من الـــ MBLوالـــ NO ، في حين وصل ادنى معدل للحساسية 39%, 35% لـــ PTH مع كل من الـــ GSSG والـــ ST.

الكلمات المفتاحية: بسرطان الدم الحاد، الكالاكتين-9، المانوز المرتبط بالاكتين، المعلمات والمؤشرات الدموية، الاكسدة ومضادات الاكسدة، فيتامينات D_3 و B_{12}، الهورمونات والبروتينات، العناصر النزرة، جرعة الكيمياوي

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أطــروحة مقدمـــة إلى
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وهي جزء من متطلبات نيـل درجة الدكتوراه في علم الحيوان (فسلجة حيــوان)

كُــتِبَت بواســـطة:

زيــنب توفيـــق شناوه الجبــوري

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