جمهورية العراق
وزارة التعليم العالي
والبحث العلمي
جامعة بابل
كلية الطب
فرع الامراض

مستوى إرثروبويتين المصل في مرضى فقر الدم وغير المصابين بفقر الدم للمصابين بسرطان الدم المزمن وسرطان الغدد الليمفاوية اللاهودجكيني

رسالة

مقدمة إلى مجلس كلية الطب في جامعة بابل كجزء من متطلبات نيل

درجة الماجستير في الطب / الأمراض

من قبل

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١٤٤٤هـ

٢٠٢٣م

الخلاصة :

الخلفية :

فقر الدم هو مشكلة متكررة لدى مرضى اللوكيميا المزمنة و مرضى سرطان الغدد الليمفاوية اللاهودجكيني مما يؤدي إلى نتائج أسوء وفترة حياة أقصر. وفقًا لباحثين آخرين ، فإن التسبب في فقر الدم الناجم عن الأمراض المزمنة للمرضى المصابين بأورام الدم الخبيثة هو متعدد العوامل : قد يلعب الإنتاج غير الكافي لـلارثروبويتين مقارنة بدرجة فقر الدم دورًا مهمًا في هذا النوع وقد يؤدي توضيح دور الإرثروبويتين في فقر الدم الناجم عن الأمراض المزمنة للمرضى المصابين بأورام الدم الخبيثة إلى توفير فهم أفضل لمسبباته ، وبالتالي علاجا أكثر ملاءمة وتوجيهًا للمرضى

أهداف الدراسة:

تهدف هذه الدراسة إلى تقييم مستويات هرمون الإرثروبويتين في مصل مرضى اللوكيميا المزمنة وسرطان الغدد الليمفاوية اللاهودجكيني المصابين وغير المصابين بفقر الدم لعينة من المرضى العراقيين في منطقة الفرات الأوسط وللتحقق من أي علاقة محتملة بين مستوى الإرثروبويتين وسبب فقرالدم .

العينات وطرق العمل:

هذه دراسة مقطعية شملت ٦٠ مريضًا كانوا يحضرون للعيادة الخارجية لأمراض الدم في مستشفى مرجان التعليمي ومستشفى الإمام الصادق التعليمي و مستشفى بغداد التعليمي من ايلول ٢٠٢٢ إلى اذار ٢٠٢٣ ، مع ٣٠ من غير المصابين بمرض كمجموعة ضابطة للمقارنة مع مجموعة المرضى. تم تشخيص جميع المرضى المعنيين على أنهم مصابون بمرض بناءاً على الفحص البدني للأخصائي ، والتقييم الشكلي المجهري لعينات الدم ونخاع العظام ، والفحص النسيجي ، والتصوير المقطعي بالإصدار البوزيتروني ، والمظهر الخلوي المناعي للتدفق الخلوي والكيمياء المناعية في هذه الدراسة. تم جمع عينات الدم من كل شخص ، وتم إجراء الفحوصات التالية: صورة الدم الكاملة ، فحص وظائف الكلى ، قياس مستوى هرمون الارثروبويتين في المصل

النتائج : كان متوسط عمر مريض سرطان الدم النخاعي المزمن (٥٠,١ ± ١٢,٨٥) سنة ويتراوح من ٢٤-٧٢ ، كان متوسط عمر مرضى سرطان الدم الليمفاوي المزمن(٥٨,٢ ± ١٠,٤) سنة ويتراوح من ٣٨-٧٥ ومتوسط عمر مرضى وسرطان الغدد الليمفاوية اللاهودجكيني (٦١,١±٨,٨٦) سنة ويتراوح من ٤٢-٧٣. غالبية المرضى كانوا من الذكور (العدد = ٣٦ ، ٦٠%). تم تقسيم مجموعة المرضى إلى ٣١ (٥١,٧٪) من المرضى مصابين بفقر الدم و ٢٩ (٤٨,٣٪) من المرضى غير مصابين بفقر الدم. أظهرت الفروق المتوسطة في تركيز الارثروبويتين (U / L) بين مجموعات الدراسة مستوى أعلى بين مجموعة المرضى (٥٣,٩٣ ± ٥٢,٥٧) من المجموعة الضابطة (١٢,٧٣ ± ٤,٧٢) بدلالة احصائية(P <٠,٠٠٠١). تم العثور على مستوى الارثروبويتين في المصل لمرضى فقر الدم (٩٠,٣٨ ± ٥٠,٦٩) ليكون أعلى من المرضى غير المصابين بفقر الدم (١٤,٩٦ ± ٥,٠٣) وبدلالة إحصائية (P <٠,٠٠٠١). وجد أن إنتاج الارثروبويتين الداخلي معيب في ١٠٪ من مرضى سرطان الدم النخاعي المزمن ، و ٥٠٪ من مرضى سرطان الدم الليمفاوي المزمن و ٣٨,٥٪ من مرضى سرطان الغدد الليمفاوية اللاهودجكيني كما تم الحكم عليه من خلال قيمة نسبة مستويات ارثروبويتين المصل المرصودة إلى المتوقعة (نسبة O / P) اقل او مساوي ل ٠,٩.

الاستنتاج : تشير هذه النتائج إلى أن فقر الدم المرتبط بأورام الدم الخبيثة قد ينتج عن استجابة منخفضة بشكل غير مناسب للأرثروبويتين. قد يستفيد المرضى من العلاج بالارثروبويتين.

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REPUPLIC OF IRAQ
MINISTRY OF HIGHER
EDUCATION
AND SCIENTIFIC RESEARCH
UNIVERSITY OF BABYLON
COLLEGE OF MEDICINE
DEPARTMENT OF PATHOLOGY

Serum Erythropoietin Levels in Anemic and Non-Anemic Patients with Chronic Leukemia and Non-Hodgkin Lymphoma

A thesis

Submitted to the Council of the College of Medicine at the University of Babylon in partial fulfillment of the requirement for the degree of Master in Medicine / Pathology

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بِسم اللهِ الرَّحمنِ الرَّحِيم

﴿ فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُلْ رَبِّ زِدْنِي عِلْمًا ﴾

صَدَقَ اللهُ العَظِيم

سورة طة الآية ﴿١١٤﴾

Certification of Supervision

We certify that this thesis entitled " **Serum Erythropoietin Levels in Anemic and Non-Anemic Patients with Chronic Leukemia and Non-Hodgkin Lymphoma**" was prepared under our supervision at the **College of Medicine / Babylon University** as a partial requirement for the master degree in pathology.

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In view of the available recommendations, we forward this thesis for the debate by the examining committee.

Professor
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Date: / / 2023

Dedication

To my amazing wife and lovely children, whose affection, love, encouragement makes me able to get such Success and honor

To the memories of my late father, mother and my siblings who provided inspiration, patience and support to make this thesis a reality

To my supervisors
Dr.Najlaa Al-Awadi & Dr.RAwaa Ghalib
For great help and continuous support

To all Iraqi patients who are suffering wish them an Immediate Cure

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My deepest appreciation extend to the patients and volunteers who expressed their assistance and made this work possible and to every person who, in one way or another, had contributed in the completion of this study.

Ali Hasan

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List of Abbreviations

Abbreviation	Key
2G	Second generation
ABL	Abelson murine leukemia
ACD	Anemia of Chronic Disease
ACTH	Adrenocorticotropic hormone
AID	Activation induce cytidine deaminase
ALL	Acute lymphoid leukemia
ALP	Alkaline phosphatase
AML	Acute myloid leukemia
AP	Accelerated Phase
Bcl-2	B-cell lymphoma 2
BCR	Breakpoint cluster region
BFU-E	Burst forming unit erythroid
BHK	Baby hamster kidney
BP	Blastic Phase
CBA	Chromosome binding analysis
CBC	Complete blood count
CCyR	Complete Cytogenetic response
CD	Cluster of Differentiation
CERA	Continuous erythropoietin receptor activator
CFU-E	Colony forming unit erythroid
CFU-GEMM	colony forming unit that generates myeloid cells
CHO	Chinese hamster ovaries
CHR	Complete hematological response
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myeloid Leukemia
COPD	Chronic obstructive pulmonary disease
CP	Chronic Phase
CyR	Cytogenetic response
del	Deletion
DNA	Deoxy-ribonucleic acid
EDTA	Ethylene-diamine-tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
ELTS	EUTOS Long term survival
EMA	European Medicines Agency
EPO	Erythropoietin
EpoR	Erythropoietin receptor
ESA	Erythropoietin stimulating agent

EUTOS	European treatment and outcome study
FDA	Food and drug administration
FISH	Florescent in situ hybridization
fL	Femtoliter
g/dL	Gram per deciliter
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
Hb	Hemoglobin
HCV	Hepatitis C virus
HIF	Hypoxia-inducible factor
HIV	Human immunodeficiency virus
HRE	Hypoxia-responsive element
IAP	inhibitors of apoptosis
IL	Interleukin
IPI	International prognostic index
JAK2	Janus-associated kinase 2
kDa	Kilo Dalton
LAP	Leukocyte alkaline phosphatase
LRD	Leukemia related death
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
mg/dL	Milligram per deciliter
ml	Milliliter
mRNA	Messenger RNA
ng/ml	Nanogram per milliliter
NHL	Non-Hodgkin Lymphoma
NK	Natural killer
nm	Nanometer
OD	Optical density
PCR	Polymerase chain reaction
PCV	Packed cell volume
pg	Picogram
Ph	Philadelphia
PHD	Prolyl hydroxylase
PHI	Prolyl hydroxylase inhibitors
RAG	Recombination activating gene
RBC	Red blood cell
RFT	Renal function test
RNA	Ribonucleic acid
RQ-PCR	Quantitative reverse PCR

SOCS	Suppressor of Cytokine Signaling
STAT5	Signal transducer and activator of transcription 5
TC1	Trans cobalamin 1
TKI	Tyrosine Kinase Inhibitors
Tpo	Thrombopoietin
U/L	Unit per liter
VHL	Von Hippel-Lindau
WBC	White Blood Cells
WHO	World Health Organization

Abstract

Background:

Anemia is a frequent problem in Chronic Leukemia and Non-Hodgkin Lymphoma's patients leading to poorer outcome and shorter survival. According to other investigators, the pathogenesis of anemia of chronic disease (ACD) is multifactoria1.Insufficient production of EPO as compared to the degree of anemia may play important role in ACD for patients with hematological malignancies. Clarifying the role of erythropoietin in ACD for patients with hematological malignancies may provide a better understanding of its pathogenesis, and thereby more proper and directed management of patients.

The Aim of The Study:

To determine serum erythropoietin levels in anemic and non-anemic patients with chronic leukemia and non-Hodgkin lymphoma in a sample of Iraqi's patients in Middle Euphrates region and to investigate any possible correlation between erythropoietin and the degree of anemia in these patients.

Subjects and Methods:

This is a cross sectional study included 60 patients (19 CML ,19 CLL , 22 NHL)who were attending the outpatient clinic of hematology in Baghdad teaching hospital , Marjan teaching hospital and Imam Alsadiq teaching hospital from September 2022 to March 2023,all on treatment together with 30 adult participants without disease as a control group were involved for comparison with patients group. All patients involved were

diagnosed as having disease based on a specialist's physical examination, morphological evaluation of peripheral blood films and bone marrow, and histological examination, PET scan and flow cytometric immunophenotypic profile and immunohistochemistry were included in this study. Blood samples were collected from each subject, and the following investigations were done: CBC, blood urea, blood film examination and ELISA assay for serum Epo.

The Results:

The mean age of CML patient was (50.1 ± 12.85) range from 24-72 , CLL patients (58.2 ± 10.4) range from 38-75 and NHL patients (61.1 ± 8.86) range from 42-73. The majority of patients were males (N=36, 60 %). The patient group was subdivided into 31 (51.7%) patients with anemia and 29 (48.3%) patients without anemia. The mean differences of Epo concentration (U/L) between study groups demonstrated significant higher level among patient group (53.93 ± 52.57) than control group (12.73 ± 4.72) with P value < 0.0001. The level of serum Epo in anemic patients (90.38± 50.69) have been found to be higher than non-anemic patients (14.96 ± 5.03) and its statistically significant (P<0.0001). Endogenous Epo production was found to be defective in 10% of CML patients, 50% of CLL patient and 38.5% of NHL patients as judged by the value for the ratio of observed-to-predicted serum Epo levels (O/P ratio) of ≤ 0.9.

Conclusions:

These findings indicate that anemia associated with hematological malignancies (Chronic Leukemia and Non-Hodgkin Lymphoma) may result from an inappropriately low Epo response to the degree of anemia . Epo treatment should benefit in this group of patients.

CHAPTER ONE

INTRODUCTION

1.1: Introduction

The leukemias are a group of diseases characterized by the increase of cancerous white cells in the blood and bone marrow. They are four forms of leukemia include acute or chronic leukemia, which are further split into lymphoid or myeloid leukemia .Because they progress more slowly than acute leukemia, chronic leukemia can be recognized from them.(1)

Chronic myeloid leukemia (CML) is a myeloproliferative tumor in which granulocytes and precursors are the primary proliferative components. Nearly half of newly diagnosed CML patients are asymptomatic and are discovered when a white blood cell (WBC) count is high during a routine medical evaluation. (2) Untreated CML has a biphasic or triphasic natural history, with an initial indolent chronic phase (CP) followed by an accelerated phase (AP), blastic phase (BP), or both. Since the introduction of tyrosine kinase inhibitors (TKI) as standard therapy for CML patients, the incidence of progression from CML-CP to CML-BP has been decreased. Detection of t(9;22)(q34;q11.2) or its variations resulting in a derived chromosome 22, also known as the Philadelphia chromosome (Ph), and/or detection of BCR-ABL1 fusion is necessary for diagnosis.(1)(2)

Chronic Lymphocytic leukemia (CLL) is the most frequent adult leukemia in Western countries, with a male predominance and a diagnosis age of 72 years. The condition is distinguished by an overabundance of monoclonal, mature CD5+ B lymphocytes in the peripheral blood, bone marrow, and secondary lymphoid organs. CLL is 10- 20 times more

common in Western countries than in Asia, implying that genetic, environmental, or combined factors influence susceptibility to the disease. CLL is frequently discovered following a workup for incidental lymphocytosis. The clinical course and presentation are highly variable, ranging from asymptomatic, indolent disease that may never require treatment (30% of patients) to active disease that can result in progressive lymphocytosis, B symptoms (i.e., weight loss, night sweats, and fever), fatigue, recurrent infections, cytopenias (anemia , thrombocytopenia), lymphadenopathy, hepatosplenomegaly, or autoimmune complications.(3)

Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of cancers that develop from B-lymphocytes, T-lymphocytes, or natural killer (NK) cells. In the western world, B-cell lymphomas account for the majority of NHL (approximately 85%), but T-cell lymphomas are far less prevalent (about 15%).While there are over 40 major subtypes, DLBCL (aggressive diffuse large B-cell lymphoma) and indolent follicular lymphoma are the most prevalent kinds . Depending on the site of the disease and the subtype of lymphoma, NHL patients can present with a wide range of clinical manifestations. Obtaining a complete medical history, doing a good physical examination, and carrying out laboratory and radiographic studies are the fundamental steps in the diagnosis of NHL.(4)

Anemia is a common clinical characteristic associated with a poor prognosis in individuals with hematological malignancies. It can aggravate cancer at any stage of the disease's progression. Anemia is caused by a variety of reasons, including leukemic bone marrow infiltration, the myelosuppressive action of chemotherapy and inhibitory cytokines, autoimmune disorders, hypersplenism, and a low nutritional condition, which results in folic acid, vitamin B12, and iron deficiency. Most cancer patients have low serum erythropoietin levels, which contributes to the anemia of chronic illness produced by cytokine-mediated inhibition of erythropoiesis.(5)A shortened red blood cell (RBC) lifespan and failure of the bone marrow to increase RBC production to compensate are believed to be key contributors to the pathogenesis of ACD.

Erythropoietin (Epo) is a glycoprotein hormone that regulates the synthesis of red blood cells in the body. It stimulates red cell progenitors in the bone marrow by acting on particular receptors, resulting in increased proliferation and differentiation of these cells.(6)

Erythropoietin may have a significant role in the development of anemia, either directly or indirectly. Clarifying this role could lead to a more accurate diagnosis of the etiology of anemia and, as a result, more appropriate management. (7)

1.2: The aims of study

1. To determine serum erythropoietin levels in anemic and non-anemic patients with chronic leukemia and non-Hodgkin lymphoma in a sample of Iraqi patients in Middle Euphrates region.

2. To investigate any possible correlation between erythropoietin and the degree of anemia in anemic patients with chronic leukemia and non-Hodgkin lymphoma.

CHAPTER TWO

LITERATURE REVIEW

2.1: Erythropoietin (Epo)

2.1.1: Definition and Production

Erythropoietin (EPO) is a hormone that considers the physiologic regulator of red cell production and protects them against destruction. About 90% of all erythropoietin is formed in the kidneys and the remainder is formed mainly is produced by hepatocytes in the liver. In kidney, it secreted by fibroblast-like interstitial cells surrounding the tubules in the cortex and outer medulla, where much of the kidney's oxygen consumption occurs. (8) It is likely that other cells, including the renal epithelial cells, also secrete erythropoietin in response to hypoxia (Fig 2.1). Renal tissue hypoxia leads to increased tissue levels of hypoxia--inducible factor-1 (HIF-1), which serves as a transcription factor for a large number of hypoxia-inducible genes, including the erythropoietin gene. HIF-1 binds to a hypoxia response element (HRE) in the erythropoietin gene, inducing transcription of messenger RNA and, ultimately, increased erythropoietin synthesis. (9) The half-life of HIF-1 inside the cell is about several minutes because it is rapidly removed by the action of von Hippel-Lindau (VHL) protein, which cause it to undergo degradation by the ubiquitin-proteasome pathway.(10) Two additional homologs of HIF-1 were recognized and named HIF-2 and HIF-3.(11) HIF-2 is the main regulator of EPO gene transcription in the kidney and other tissues, such as the liver and brain, whereas HIF-1 is expressed only in renal tissue.(12)

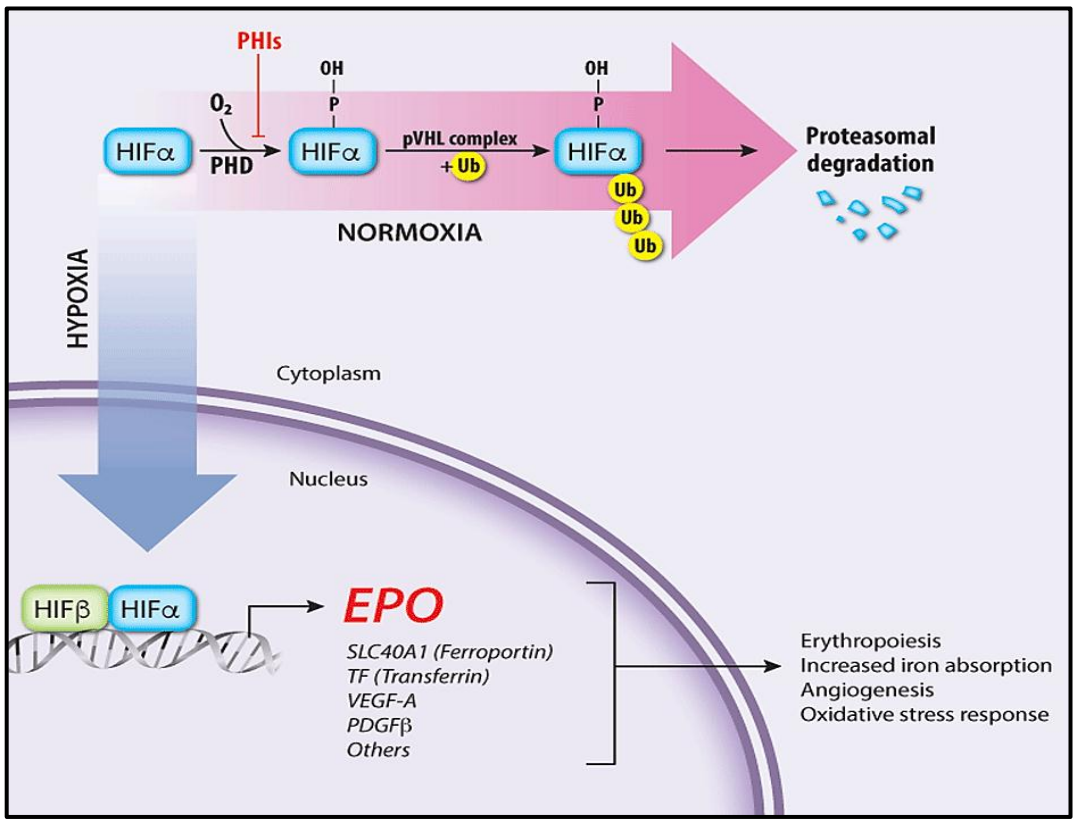

Fig. 2.1: Regulation of erythropoietin production by hypoxia. At high oxygen concentrations, prolyl hydroxylase (PHD) enzymes hydroxylate the HIFα subunit, targeting it for ubiquitination by the von Hippel–Lindau protein (pVHL). Under hypoxia, PHD enzymes are inactive, thereby stabilizing HIF, which activates transcription of *EPO* and other target genes involved in tissue oxygen delivery. PHD inhibitors (PHIs) stabilize HIFα and are under investigation for treating anemia associated with chronic renal failure.(13)

At times, hypoxia in other parts of the body, but not in the kidneys, stimulates kidney erythropoietin secretion, which suggests that there might be some non-renal sensor that sends an additional signal to the kidneys to produce this hormone. In particular, norepinephrine and epinephrine and several of the prostaglandins stimulate erythropoietin production.(9)

2.1.2: Structure

EPO gene is located on the short arm of chromosome 7q22.1 and is 3 kilo base long. The expression of the gene results in the production of a 193 amino acid prohormone that gets modified to 165 or 166 amino acid hormone. It is glycosylated at 3 N sites and one O site.(14)34.4-kDa glycoprotein, found in serum at baseline levels of 5–25 U/L that can be elevated 1000- fold by severe anemia. It contains about 40% carbohydrate, is rich in sialic acid residues, and has a half-life of 7–8 hours in plasma, whereas non-glycosylated Epo is rapidly cleared from the circulation. Under normoxic conditions, little or no Epo mRNA is detectable in the kidneys; hypoxia results in the rapid induction of its transcription such that levels may increase up to 200-fold over baseline within 30 minutes.(1)

2.1.3: Erythropoietin Receptors (EpoR)

Erythropoietin receptors (EpoR) is part of a large family of type I cytokine receptors, which includes receptors for IL-2 through IL-7, and the growth factors GM-CSF, G-CSF and thrombopoitein (Tpo) . Type I cytokine receptors share basic structural features and are characterized by four conserved cysteine residues and a tryptophan-serine-xserine-tryptophan (WSXSW) motif in the extracellular domain, and by conserved box1/box2 regions in the intracytoplasmic domain adjacent to the membrane. While some type I cytokine receptors share common subunits and thus are heterodimeric, the EpoR, Tpo and G-CSF consist of homodimers.(15) EpoR are expressed on human BFU-E(20–50 EpoR); expression increases as BFU-E mature to CFU-E(~1000 EpoR), erythroid cells at a stage between CFUE and proerythroblast have the highest expression of EpoR (Figure 2.2), which decreases as the proerythroblast

matures and eventually disappears at the stage of orthochromatic erythroblast.(1)

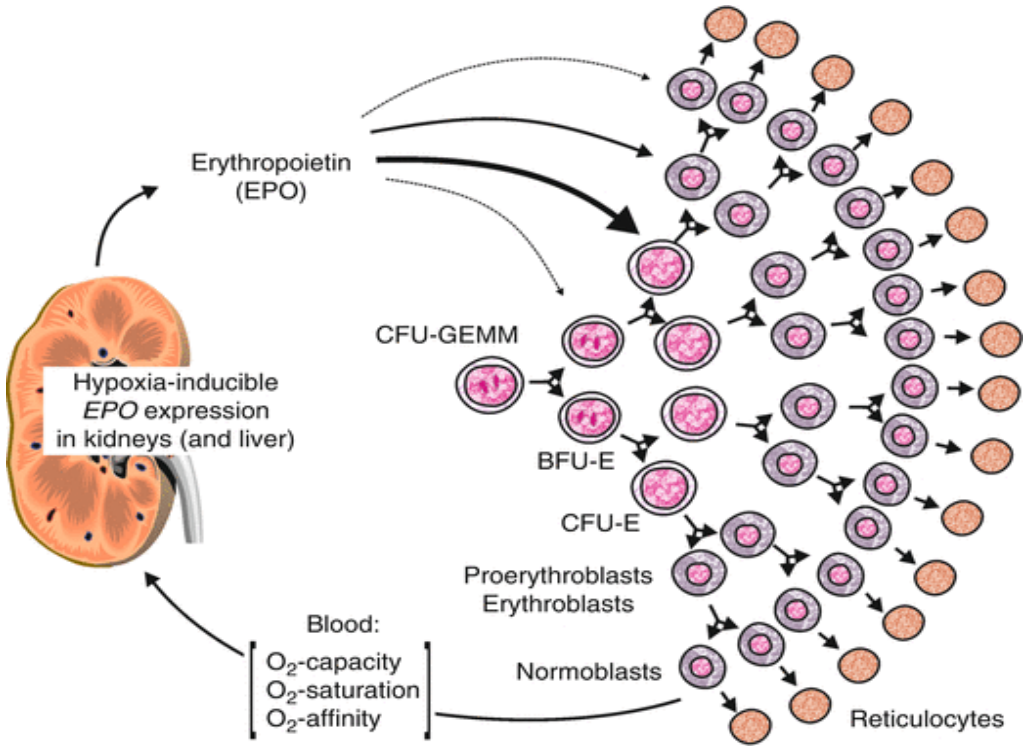

Fig. 2.2: Frequency EpoR expressions on RBC progenitor cells(15)

The presence of EpoR on megakaryocytes explains why Epo at physiologic concentrations promotes megakaryocyte differentiation and can thus affect platelet levels. EpoR are also observed on non-hematopoietic tissues(Figure 2.3), including neurons and cardiac myocytes , endothelial cells, the kidneys, and embryonic muscle, myoblasts and skeletal muscle, adipocytes and bone.(15)

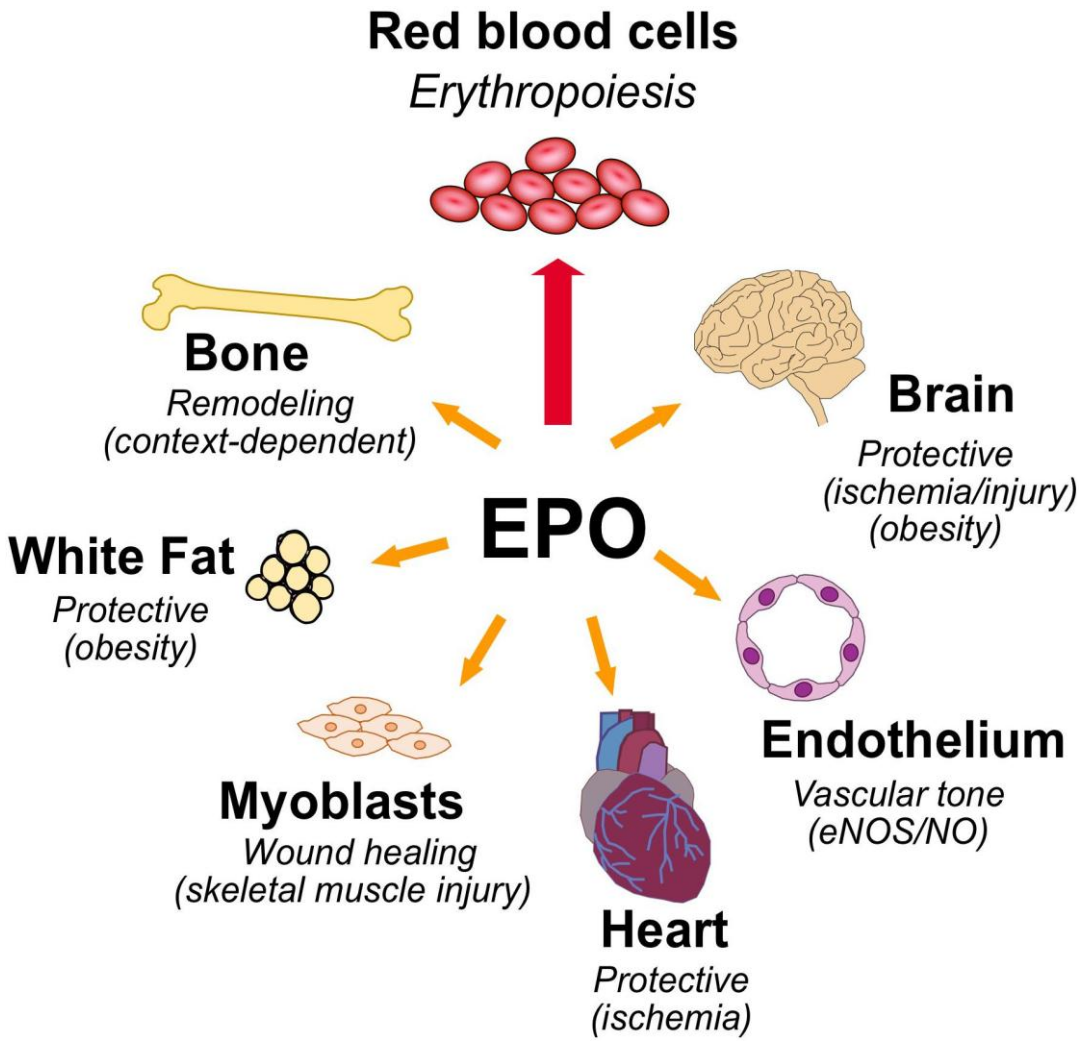

Fig. 2.3: Pleiotropic effects of erythropoietin. High level of EpoR on erythroid progenitor cells accounts for the sensitive erythropoietic response in the bone marrow to hypoxic induction of Epo. EpoR expression determines Epo response and expression beyond erythroid tissue provides for Epo response in non-hematopoietic tissues that include the following: brain for a neuroprotective and metabolic response; cardiovascular system for regulating vascular tone and oxygen delivery in endothelium and protection in heart against ischemic injury; skeletal muscle for muscle maintenance and repair; white adipose tissue for protection for inflammation associated with diet-induced obesity and fat mass accumulation, particularly in males; and bone remodeling.(16)

2.1.4: Signaling Pathways

One Epo molecule binds two EpoR molecules lead to activating JAK2 kinases of each receptor by physically bringing the inactive (or low-activity) JAK2 kinases into close proximity and these kinases cross phosphorylate each other, gaining full activity. Jak2 kinase activation results in phosphorylation of several tyrosines in the EpoR cytoplasmic tail that then serve as docking sites for signaling or adaptor proteins. The signaling proteins become phosphorylated and function in numerous downstream signaling cascades as in following (figure 2.4). (17)

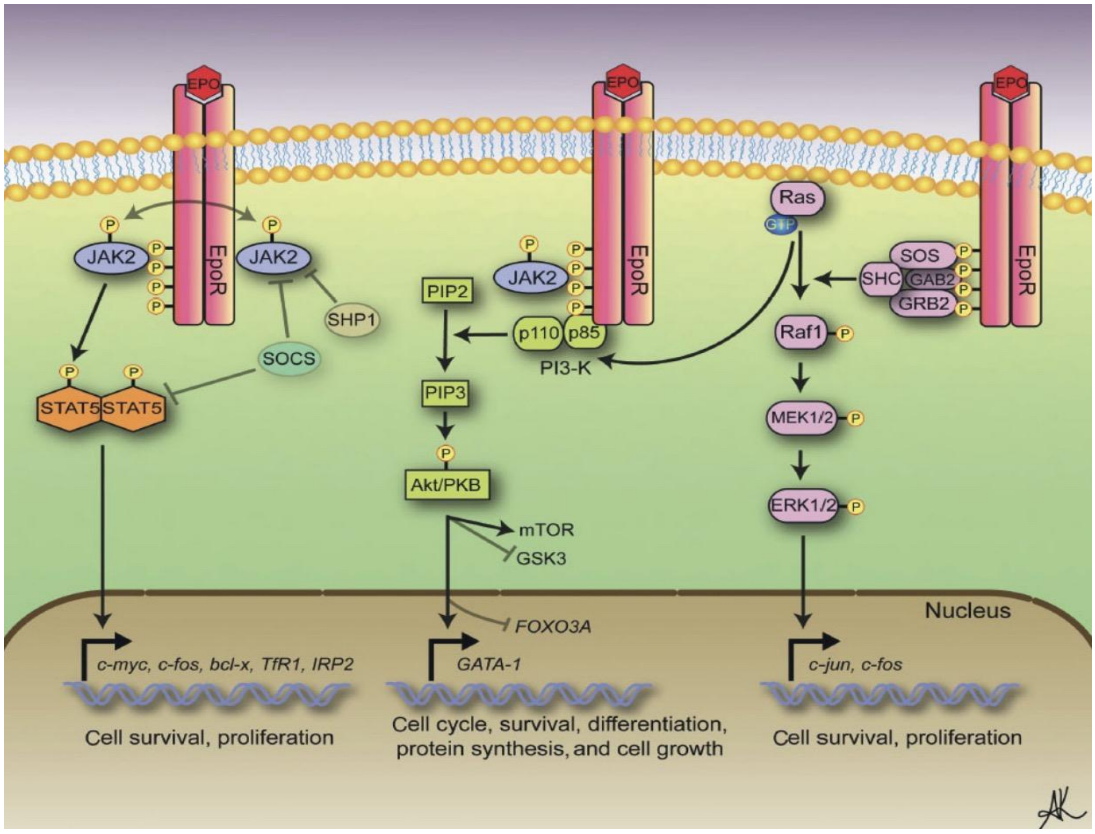

Fig. 2.4: Epo signaling pathway(15)

Negative regulatory proteins are necessary to dampen EpoR signaling. Suppressor of Cytokine Signalling(SOCS) proteins inhibit Jak2 and Stat5

activation, whereas phosphatases such as SHP1 and SHIP inhibit other phosphorylation-dependent pathways.(18)

The overall effect is that, secondary to a hypoxia-driven rise in Epo, a population of erythroid progenitors normally destined for apoptosis at steady-state receive antiapoptotic signals, survive and expand the erythroid precursor pool,(19) which rapidly respond by proliferating and differentiating into viable pronormoblasts , it has also been suggested that Epo is able to speed up the rate of terminal differentiation by shortening the cell cycle and maturation times of erythroblasts.(20)

2.1.5: Abnormalities in Serum Erythropoietin

Causes of increased serum EPO include:

1. Anemias: most anemias, such as iron deficiency anemia, megaloblastic anemia and hemolytic anemias, stimulate EPO secretion due to the subsequent tissue hypoxia.(13) Exceptions to this rule include anemias associated with chronic renal failure, chronic inflammatory diseases (e.g., rheumatoid arthritis),
2. Acute bleeding: loss of red cells stimulates EPO production.
3. Conditions associated with systemic hypoxia, such as chronic obstructive pulmonary disease (COPD), carbon monoxide poisoning, heavy cigarette smoking, and living in high altitudes.
4. Tumors such as renal cell carcinoma, hepatoma, nephroblastoma, pheochromocytoma, and polycystic kidney disease present with high serum EPO level due to ectopic EPO production by the tumor cells. (21)

5. Pregnancy: dilutional anemia of pregnancy (due to plasma volume overload) induces EPO secretion.(22)

6. Drugs like ACTH, oral contraceptive pills, and anabolic steroids: may cause slight elevations in serum EPO by a complex hormonal effect on erythropoiesis and erythroid progenitors response to EPO.(23)

Causes of decreased serum EPO include:

1. Chronic renal failure: reduced EPO production by renal cells (anemia of renal disease).(24)

2. Primary polycythemia (Polycythemia Vera): caused by overproduction of red blood cells as a result of marked erythroid hyperplasia that is characteristic of this disorder.(25)

3. Anemias of chronic inflammatory diseases (such as rheumatoid arthritis) and some cancers. These anemias, termed anemia of chronic disorders, tend to present with inappropriately low serum EPO levels, either due to the action of proinflammatory cytokines (such as IL-6 that suppresses EPO production), or renal impairment that accompanies many of these diseases (and/or their treatment regimens).(26)

4. Some chemotherapeutic agents: thought to directly affect the renal secretion of EPO.

5. After blood transfusion: due to the rapid rise in red cell mass.

6. Autonomic neuropathy: diabetic patients with autonomic neuropathy have shown inappropriately low serum EPO levels in case of anemia. It was thought to be caused by impaired sympathetic innervation.(27)

2.1.6: Therapeutic Uses

Advances in recombinant DNA technology lead to the successful production of recombinant erythropoietin analogues which are also referred to as Epoetins. The introduction of Epoetins revolutionized the way renal patients with anemia are managed. Its introduction to clinical use significantly decreased the need of blood transfusion which was previously the mainstay of the management. There are three generations of Erythropoietin Stimulating Agents (ESAs)(Table 2.1). ESAs are named according to the location of glycosylation site by using Greek letters. The first commercially available and FDA approved ESA was epoetin alpha. ESAs are largely produced in Chinese Hamster Ovaries (CHO). Baby Hamster kidneys (BHK) cell line is another cell line that is used to produce ESA .A human cell line was used to produce EPO, but manufacturing stopped due to marketing issues.(14)

The main indications of ESA are : (1)

1. Anemia of chronic renal disease
2. Myelodysplastic syndrome
3. Anemia associated with malignancy and chemotherapy
4. Anemia of chronic disease e.g. rheumatoid arthritis
5. Anemia of prematurity
6. Preoperative uses

Side-effects include a rise in blood pressure, thrombosis and local injection site reactions. The marrow requires many other precursors for effective erythropoiesis. These include metals such as iron and cobalt, vitamins (especially vitamin B12, folate, vitamin C, vitamin E, vitamin B6, thiamine

and riboflavin) and hormones such as androgens and thyroxine. Deficiency in any of these may be associated with anemia.

Table 2.1: ESA generations. Note that the darbepoetin has two additional glycosylation sites, which causes an increased molecular weight and half-life of the molecule. Polyethylene glycol is added to the continuous erythropoietin receptor activator (CERA) making it the longest acting currently available ESA(28)

ESA generation	Examples	Recommended dosing interval
First generation	Epoetin alfa Epoetin beta Epoetin omega	One to three times per week
Second generation	Darbepoetin alpha	Once weekly or every two weeks
Third generation	Methoxy polyethylene glycol epoetin beta	Every two weeks or monthly dose

2.2: Leukemia

The leukemias are a group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood. In Iraq According to International agency for research on cancer/the Global Cancer Observatory (Globocan2020),the number of new cases of leukemia in 2020 for both sexes, all ages is 2027 which account 6% of all cancer, Leukemia is rank 3 of all type of cancer (after breast and lung) with mortality rate 7.8%.(29)

The main classification is into four types (Table 2.2): acute or chronic leukaemias, which are further subdivided into lymphoid or myeloid leukaemias. (1)

Table 2.2: Leukemia Classification(1)

Cell type	Acute	Chronic
Lymphocytic Leukemia	Acute Lymphoblastic Leukemia (ALL)	Chronic Lymphocytic Leukemia (CLL)
Myelogenous Leukemia	Acute Myeloid Leukemia (AML)	Chronic Myeloid Leukemia (CML)

2.2.1: Chronic Myeloid Leukemia (CML)

It's a clonal hematopoietic stem cell malignancy that is characterized by the Philadelphia (Ph) chromosome, which is caused by a translocation between chromosome 9 and 22 forming the oncogenic fusion gene *BCR-ABL1* on chromosome 22.(30)

2.2.1.1: Incidence

The annual incidence of CML varies from 0.4/100,000 - 1.75/100,000 persons in different countries. CML is more common in males than in females with the male/female ratio of 1.2–1.7. (31)(32) The incidence of CML increases with age and is rare in children less than 14 years of age (0.7/million children/year).(33) The disease is most commonly diagnosed in the fifth and sixth decades of life in developed countries. In the developing world, presentations in the third and fourth decades are more common, possibly reflecting younger population demographics. (34)

According to The American Cancer Society's estimates for chronic myeloid leukemia (CML) in the United States for 2022 are:

- About 8,860 new cases will be diagnosed with CML (5,120 in men and 3,740 in women), which account 0.5% of all new cancer cases.
- About 1,220 people will die of CML (670 men and 550 women), which account 0.2% of all cancer deaths.
- About 15% of all new cases of leukemia are chronic myeloid leukemia.
- About 1 person in 526 will get CML in their lifetime in the United States.(35)

In a local study in Karbala province in Iraq 2019, CML was the second common type of leukemia which represented only 24.1% of all leukemia types.(36)Also in another local study in Sulaymaniyah Province, Kurdistan, Iraq 2016, CML was the second common type of leukemia which represented only 20% of all leukemia types.(37)

2.2.1.2: Etiology and Risk Factors

The only risk factors for chronic myeloid leukemia (CML) are: (38)

1. **Radiation exposure**: Being exposed to high-dose radiation (such as being a survivor of an atomic bomb blast or nuclear reactor accident) increases the risk of getting CML.
2. **Age**: The risk of getting CML goes up with age.
3. **Gender**: This disease is slightly more common in males than females, but it's not known why.

There are no other proven risk factors for CML. The risk of getting CML does not seem to be affected by smoking, diet, exposure to chemicals, or infections. And CML does not run in families.

2.2.1.3: Pathogenesis

CML is a hematopoietic stem cell disease, 90% to 95% of patients with CML have a shortened chromosome 22 because reciprocal translocation between chromosomes 9 and 22, resulting in the formation of the Philadelphia chromosome (Ph chromosome). This translocation t(9;22) results in the head-to-tail fusion of the breakpoint cluster region (BCR) gene on chromosome 22 at band q11 and the Abelson murine leukemia (ABL) gene located on chromosome 9 at band q34.2. (39) The product of the fusion gene (BCR-ABL) is believed to play a central role in the initial development of CML. The BCR-ABL gene encodes a protein (p210 BCR-ABL), with deregulated tyrosine kinase activity. The mechanisms by which p210BCR-ABL promote the transition from a benign to a malignant state are not entirely understood. However, attachment of the BCR sequences to ABL results in 3 critical functional changes: **1)** the ABL protein becomes constitutively active as a protein tyrosine kinase enzyme, **2)** the DNA protein-binding activity of ABL is attenuated, and **3)** the binding of ABL to cytoskeletal actin microfilaments is enhanced. The downstream pathways affected include JAK/STAT, PI3K/AKT, and RAS/MEK, these effects increase proliferation, affect differentiation, and block apoptosis. The bulk of the genetic changes in progression occur during transition from chronic to accelerated phase.(40)

The remainder of patients 5-10 %n has variant or complex translocations involving additional chromosomes detected by routine cytogenetics or a cryptic BCR-ABL1 translocation detected with fluorescent in situ hybridization (FISH) or reverse transcriptase-polymerase chain reaction (PCR).(41)

2.2.1.4: Clinical Features

In up to 50% of cases the diagnosis is made incidentally from a routine blood count. In those cases where the disease presents clinically, the following features may be seen: (1)

1. Symptoms related to hyper metabolism (e.g. weight loss, lassitude, anorexia or night sweats).
2. Splenomegaly is nearly always present and may be massive. In some patients splenic enlargement is associated with considerable abdominal discomfort, pain or indigestion.
3. Features of anemia may include pallor, dyspnea and tachycardia.
4. Bruising, epistaxis, menorrhagia or hemorrhage from other sites because of abnormal platelet function.
5. Gout or renal impairment caused by hyperuricaemia from excessive purine breakdown may be a problem.

2.2.1.5: Diagnosis

Diagnosis of CML is generally straightforward. In most cases, the diagnosis can be made on the basis of a characteristic blood count and differential (excessive granulocytosis with typical left shift of granulopoiesis). Confirmation of diagnosis is obtained by the identification of the Philadelphia chromosome(Ph+), in peripheral blood or bone marrow (BM) cells.(42)

2.2.1.5.1: CBC and Morphology

Which show leukocytosis with basophilia and immature granulocytes, mainly metamyelocytes, myelocytes and promyelocytes, and few or occasional myeloblasts. Severe anemia is rare which is usually normochromic normocytic anemia(Figure2.5). Blood counts and differential are very important for the calculation of a prognostic risk and for the distinction between chronic, accelerated and blast phases. The mature granulocytes, have decreased apoptosis (programmed cell death), resulting in accumulation of long-lived cells with low or absent enzymes, such as alkaline phosphatase (ALP). Consequently, the leukocyte alkaline phosphatase stains very low to absent in most cells. (43)

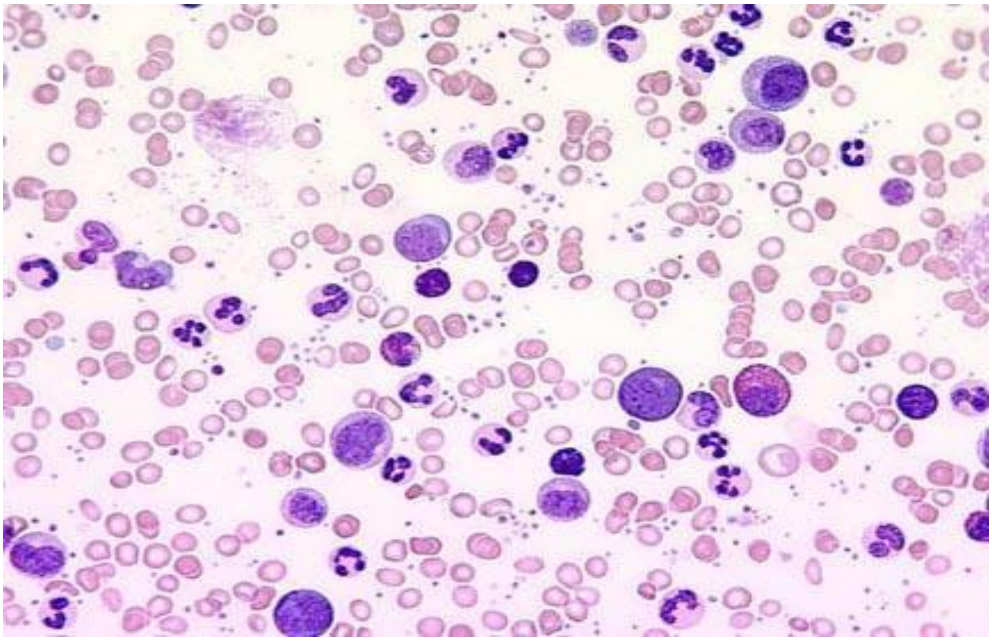

Fig. 2.5: Blood Film of CML Patient. At 400X magnification demonstrates leukocytosis with the presence of precursor cells of the myeloid lineage. In addition, basophilia, eosinophilia, and thrombocytosis can be seen. (43)

2.2.1.5.2: Bone Marrow

Its characteristically hyper cellular, with expansion of the myeloid cell line (e.g., neutrophils, eosinophils, basophils) and its progenitor cells. Megakaryocytes are prominent and may be increased. Mild fibrosis is often seen in the reticulin stain (Figure 2.6)

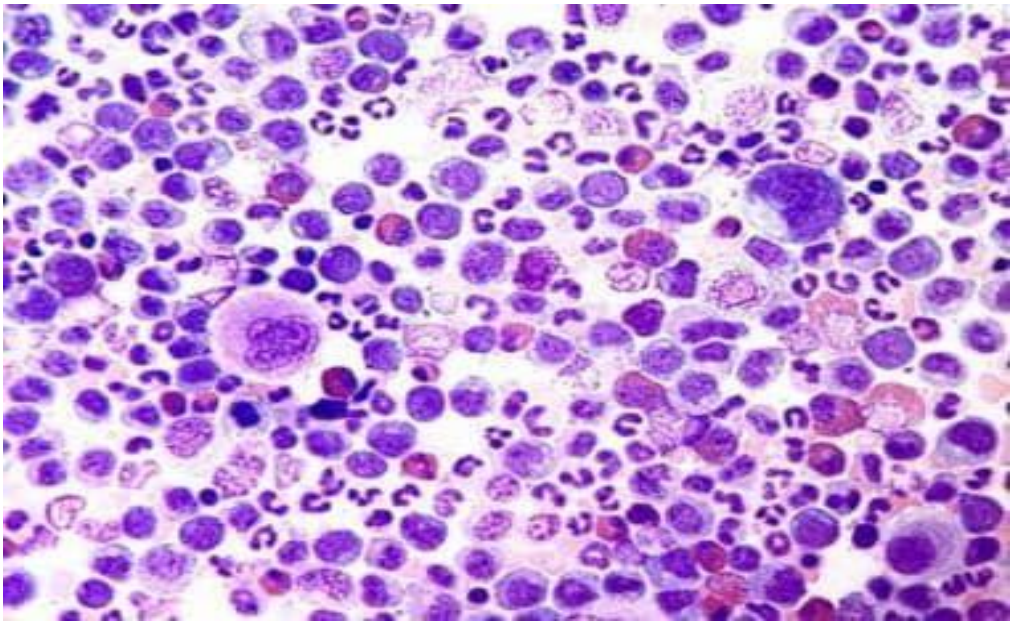

Fig. 2.6: Bone marrow film of CML Patient. 400 X magnifications demonstrate clear dominance of granulopoiesis. The number of eosinophils and megakaryocytes is increased.

Also bone marrow aspirate is required for morphology, since the proportion of blast cells and of basophils is important to distinguish chronic phase(CP) from accelerated phase (AP) and blastic phase (BP), and for cytogenetic.(44)

2.2.1.5.3: Cytogenetic Studies

Cytogenetic study of the bone marrow cells, and even peripheral blood, performed by Chromosome banding analysis (CBA) of Giemsa-stained metaphases, should reveal the typical Ph chromosome. This is the hallmark of CML, found in almost all patients(95%) with the disease and present throughout the entire clinical course of CML (Ph+ CML)(Figure 2.7).(45)

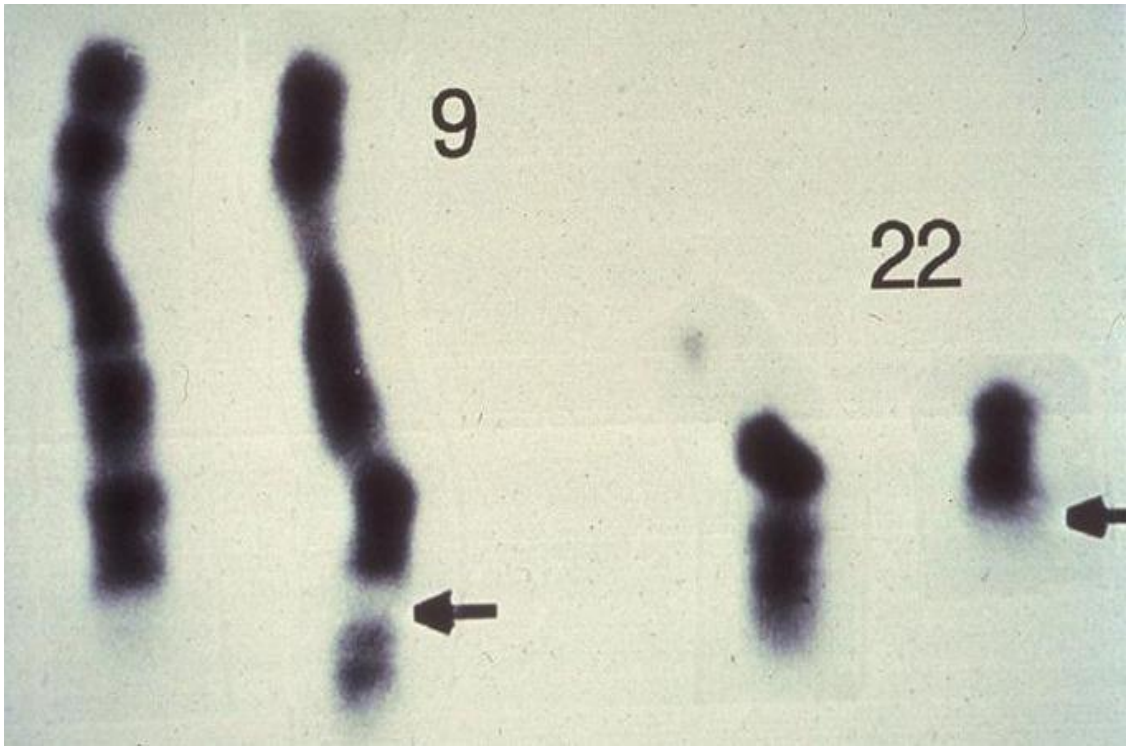

Fig. 2.7: Cytogenetic Study of CML Patient .The Philadelphia chromosome, which is a diagnostic karyotypic abnormality for chronic myelogenous leukemia, is shown in this picture of the banded chromosomes 9 and 22. Shown is the result of the reciprocal translocation of 22q to the lower arm of 9 and 9q (c-abl to a specific breakpoint cluster region [bcr] of chromosome 22 indicated by the arrows)

In ~5% of cases the Philadelphia chromosome cannot be detected (Ph-CML)and confirmation of diagnosis depends on the confirmation of the BCR–ABL1 fusion by fluorescent in situ hybridization (FISH)(Figure2.8).(46)

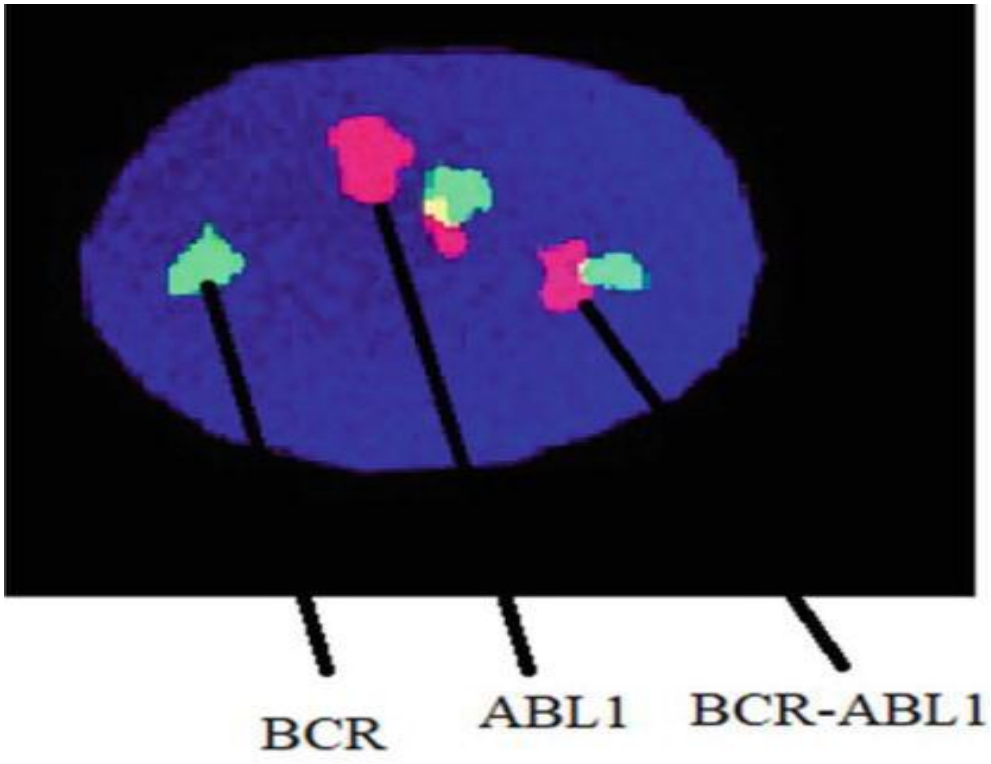

Fig.2.8: FISH image depicting the fusion of red (ABL1) – green (BCR) signal representing BCR-ABL fusion gene.

A qualitative reverse transcriptase PCR (RQPCR)on peripheral blood cells is mandatory to identify the type of BCR-ABL1 transcripts that can be appropriately followed when assessing response to TKI therapy. Cytogenetics and RQ-PCR offer complementary information and both tests should be performed routinely at diagnosis. (47)

2.2.1.6: Staging

Unlike most cancers, chronic myelogenous leukemia (CML) is classified into phases rather than stages, based partly on the percentage of immature white blood cells (blasts) in peripheral blood and bone marrow. A complete blood count with differential, peripheral blood smear and bone marrow analysis are used to determine the phase. The 3 phases of CML, as defined by the World Health Organization (WHO), are listed below (Table 2.3).(40)

Table 2.3: Phases of Chronic Myelogenous Leukemia

CML phase	WHO Definition
Chronic Stable Phase	• Blasts < 10% in peripheral blood and bone marrow
Accelerated Phase	• Blasts comprising 10-19% of white blood cells (WBCs) in peripheral blood and/or nucleated bone marrow cells • Peripheral blood myeloblasts and promyelocytes combined ≥30% • Peripheral blood basophils ≥20% • Persistent thrombocytopenia (< 100×10^{9}/L) unrelated to therapy or persistent thrombocytosis (> 1000×10^{9}/L) unresponsive to therapy • Increasing WBC counts and spleen size unresponsive to therapy • Additional clonal cytogenetic abnormalities in Philadelphia chromosome–positive cells
Blast Crisis Phase	• Blasts ≥ 20% of peripheral blood WBCs or nucleated bone marrow cells • Extramedullary blast proliferation • Large foci or clusters of blasts on bone marrow biopsy

2.2.1.7: Treatment

The life expectancy of a newly diagnosed patient with Philadelphia chromosome-positive (Ph+), BCR-ABL1+ chronic myeloid leukemia (CML) in chronic phase (CP) is now very close to that of age matched individuals in the general population, at least in western countries. Treatment involve:- (48)

1. Tyrosine kinase inhibitors(TKI)

With the exception of cases of CML newly diagnosed during pregnancy, first-line treatment is a TKI. A short course of hydroxyurea may be given in symptomatic patients with high white blood cell or platelet counts while molecular and cytogenetic confirmation of the CML diagnosis is pending. Currently, four TKIs have been approved for first-line treatment by the FDA and EMA: imatinib ,dasatinib, nilotinib , and bosutinib. These are available almost everywhere, though with some differences in indications, dosing, and reimbursement. A fifth TKI, radotinib has been approved in South Korea only.

Treatment is often changed from the first-line TKI for several reasons. In cases of failure/resistance, the change is mandatory and must be accompanied by investigation of BCR-ABL1 mutations. In case of intolerance and treatment related complications, the decision to change is in part subjective, depending upon the patient, physician, options for supportive care, and also upon the level of response.(49)

2. Chemotherapy:

Hydroxycarbamide (hydroxyurea) treatment can control and maintain the white cell count in the chronic phase, but does not reduce the percentage of BCR-ABL1-positive cells.(1)

3. Interferon α (IFNα):

In the pre-TKI era, IFNα was the treatment of choice. With the advent of pegylated (PEG) formulations that require less frequent administration and have improved efficacy and tolerability, IFNα may re-emerge as a therapeutic option in CP-CML.(50)

4. Allogeneic stem cell transplantation (alloSCT)

Still has a place in managing the small number of patients with disease resistant or intolerant to multiple TKIs, and for the very rare patient with inadequate recovery of normal hematopoiesis.(47)

2.2.1.8: Response Definitions

Dynamic response assessment is essential to identify patients at high risk of disease progression, who may benefit from a change of therapy. A complete hematologic response (CHR) is defined by clinical and peripheral blood criteria. Cytogenetic response (CyR)is classified according to the percentage of Ph-positive metaphases by routine karyotype on bone marrow aspiration. A complete cytogenetic response (CCyR) has also been defined in some instances by interphase FISH on peripheral blood as the absence of detectable BCR-ABL fusion in at least 200 examined nuclei. Molecular testing for BCR-ABL transcripts using QRT-PCR is more sensitive for low-level residual disease than cytogenetics or FISH (sensitivity of 104 to 105). (51)

Table 2.4: Definition of Response(52)

TABLE Definitions of Response	
Response	**Definition**
CHR	Leukocyte count <10 109/L, basophils <5%, platelets <450 109/L,the absence of immature granulocytes, impalpable spleen
Minor CyR	36%-95% Ph+ metaphases in bone marrow
Major CyR	1%-35% Ph+ metaphases in bone marrow
CCyR	0% Ph+ metaphases in bone marrow
MMR	BCR-ABL International Scale ≤ 0.1%
CMR	Undetectable BCR-ABL with assay sensitivity ≥4.5 or 5.0 logs
CHR = complete hematologic response; CMR = complete molecular response; CyR = cytogenetic response; MMR = major molecular response; Ph = Philadelphia chromosome.	

2.2.1.9: Prognosis

The prognosis of CML has markedly improved since the development of TKIs. Moreover, the development and certification of second-generation TKIs (2G-TKIs), with greater potency and more tolerable adverse effects, as first-line therapy for the chronic phase of CML (CML-CP) have further increased disease control rates. Several prognostic scoring systems have been developed to assess the risk of poor outcome at presentation: the Sokal score and Hasford score were developed in 1984 and 1998 respectively in the pre imatinib era but retain prognostic significance in imatinib-treated patients. After the introduction of imatinib, the European Treatment and Outcome Study (EUTOS) score, published in 2011, another simpler system based on basophil percentage in peripheral

blood and spleen size, was developed to predict a complete cytogenetic response (CCyR) at 18 months after treatment initiation.(53) Since most patients die from causes other than leukemia while still in remission, a fourth risk score has been developed to predict the probability of dying from CML (leukemia-related death, LRD) in patients treated with imatinib: the new EUTOS Long Term Survival (ELTS) score was established in 2016 .(54)

Among four risk scores, including the Sokal, Hasford, EUTOS, and ELTS scores, risk stratification by the ELTS score had the highest predictive value in assessing patient prognosis, and also in treatment responses(Table 2.5).(55)

Table 2.5: CML Scoring Systems(56)

Scoring System	Calculation	Risk Definition
Sokal score	Exp 0.0116 × (age - 43.4) + 0.0345 × (spleen - 7.51) + 0.1880 × [(platelet count/700)^2-0.563] + 0.0887 × (blasts - 2.10)	Low risk: <0.8 Intermediate risk: 0.8–1.2 High risk: >1.2
Hasford score	(0.6666 × age [0 when age <50 years; 1, otherwise] + 0.0420 × spleen + 0.0584 × blasts + 0.0413 × eosinophils + 0.2039 × basophils [0 when basophils <3%; 1, otherwise] + 1.0956 × platelet count [0 when platelets <1500 × 10^9/L; 1, otherwise]) × 1000	Low risk: ≤780 Intermediate risk: 781–1480 High risk: >1480
EUTOS score	(Basophils × 7) + (spleen × 4)	Low risk: ≤87 High risk: >87
ELTS score	0.0025 × (age/10)^3 + 0.0615 × spleen + 0.1052 × blasts + 0.4104 × (platelet count/1000)$^{-0.5}$	Low risk: ≤1.5680 Intermediate risk: 1.5680–2.2185 High risk: >2.2185

Note: Exp, exponential function; Age is in years; Spleen is in cm below the costal margin; Platelet count is in × 10^9/L; Blasts, eosinophils and basophils are in percent of peripheral blood.

Abbreviations: EUTOS, European Treatment and Outcome Study; ELTS, EUTOS long-term survival.

2.2.2: Chronic Lymphocytic Leukemia (CLL)

It's a lymphoproliferative disorder recognized with accumulated small lymphocytes at lymph nodes, bone marrow, blood, liver, spleen, or often at other organs. These lymphocytes are marked with mature morphology and immature biology.(57)

2.2.2.1: Incidence

CLL accounts for 25–30% of all the leukemia in Western Countries with over 100,000 incidence cases and over 40,000 death cases globally reported in 2019. The incidence of CLL is approximately 2 times higher in males than that in females. (58)(59)Epidemiological studies found that the incidence of CLL rises exponentially with age and reaches a peak in elderly populations. The median age at diagnosis is 72 years. About 10% of CLL patients are reported to be younger than 55 years. (60)(61)

Additionally, markable geographical imbalances were found in CLL-related incidence cases. While CLL is the most prevalent adult leukemia in Western countries, it is relatively rare in Asia, even in Asian immigrants moving to the Western hemisphere.(62)

According to The American Cancer Society's estimates for leukemia in the United States for 2022 are:

- About 20,160 new cases of chronic lymphocytic leukemia (CLL) which about 1.1% of all new cancer cases.
- About 4,410 deaths from CLL which about 0.7% of all cancer death

CLL accounts for about one-quarter of the new cases of leukemia. The average person's lifetime risk of getting CLL is about 1 in 175 (0.57%).(35)

Another specific depiction of CLL epidemiology comes from the Arab world. It is reported that CLL incidence of the Jews in Israel is significantly higher than the Arabs in Israel and the Arabs in the surrounding Middle Eastern countries, which suggested that ethnic factors rather than geographical factors may be a more critical contributor .(63)

In a local study in Karbala province in Iraq2019, CLL was the least common type of leukaemia which represented only 15.7% of all leukaemia types.(36)Also in another local study in Sulaymaniyah Province, Kurdistan,Iraq 2016, CLL was the third common type of leukaemia which represented only 18% of all leukaemia types.(37)

2.2.2.2: Etiology and Risk Factors

There are very few known risk factors for chronic lymphocytic leukemia (CLL). These include: (64)

1. Age: The risk of CLL goes up as you get older. About 9 out of 10 people with CLL are over age 50.
2. Exposure to certain chemicals: Some studies have linked exposure to Agent Orange, an herbicide used during the Vietnam War, to an increased risk of CLL. Some other studies have suggested that farming and long-term exposure to certain pesticides may be linked to an increased risk of CLL.
3. Radon exposure at home has been linked to an increased risk.
4. Family history: First-degree relatives (parents, siblings, or children) of people with CLL have more than twice the risk for this cancer.
5. Gender: CLL is more common in males than females. The reasons for this are not known.

6. Race/ethnicity: CLL is more common in North America and Europe than in Asia. Asian people who live in the United States do not have a higher risk than those living in Asia. This is why experts think the differences in risk are related to genetics rather than environmental factors.
7. Infection :-HIV and hepatitis C virus infections.

2.2.2.3: Pathogenesis

Chronic lymphocytic leukemia results from the clonal expansion of a CD5-positive subpopulation of B lymphocytes which progressively accumulate in the bone marrow, lymph nodes and peripheral blood. Although proliferative pools in the bone marrow and lymph nodes probably feed the blood compartment, the leukemic cells in the blood are quiescent but are unable to initiate their apoptotic program. This situation is due to several factors including defects in the CLL cells apoptotic machinery and excessive survival signals delivered by the microenvironment .(65)Micro environment (Bone marrow stroma cells, nurse-like cells and T cells) produce chemokines and cytokines that activate survival pathways such as NF-kB or PI3K/AKT. Indeed, these pathways are constitutively activated in CLL cells and this leads to the transcription and overexpression of key anti apoptotic proteins (notably several members of the Bcl-2 and IAP families).(66)Other signaling pathways are also involved in the overexpression of anti-apoptotic proteins in CLL cells: B cell receptor (BCR) signals reportedly upregulate Mcl-1 expression through the PI3K/AKT pathway .(67)

Lastly, alterations in apoptosis regulators such as p53 (which are frequently observed in CLL) may be implicated in the defective apoptosis (Figure 2.9).

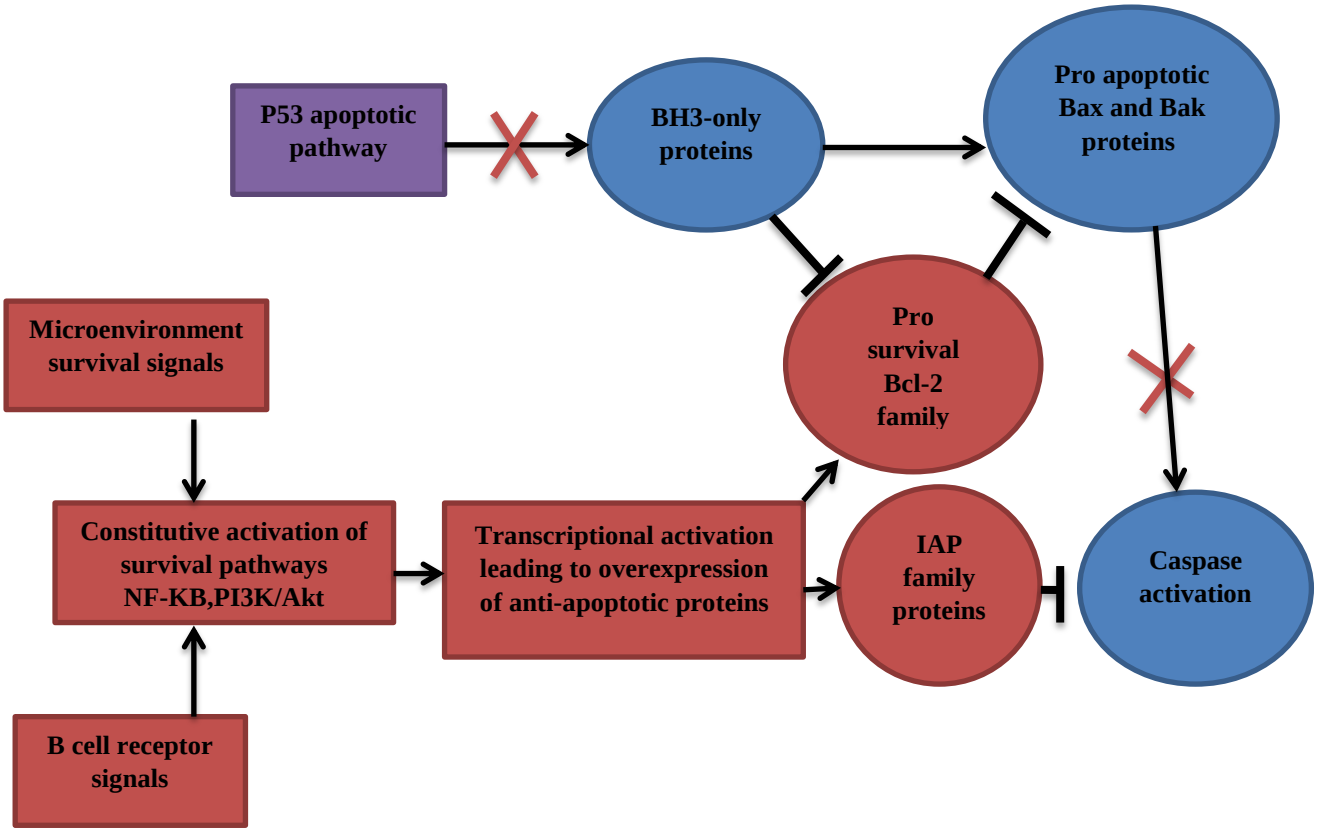

Fig. 2.9: Schematic representation of the impaired mitochondrial caspase-dependent apoptosis in CLL cells

Anti-apoptotic factors are colored in red and proapoptotic components are colored in blue. Constitutive activation of survival pathways by microenvironment signals and B cell receptor signaling leads to the transcriptional activation of prosurvival factors from the Bcl-2 and IAP families which are thus overexpressed in CLL cells. The increased antiapoptotic activity exerted by these factors results in **(i)** sequestration of the proapoptotic proteins Bax and Bak (which thus prevents the mitochondrial membrane permeabilization and the subsequent cascade of caspase activation) and (ii) direct inhibition of caspase activities by IAP proteins. Moreover, deficiencies in the p53 apoptotic pathway (which are frequently observed in CLL) reduce the expression of BH3-only proteins like Puma and Noxa (known to block the antiapoptotic activity of prosurvival Bcl-2 family members and promote Bax and Bak activation). IAP, inhibitor of apoptosis protein; NF-kB, nuclear factor-kappaB; PI3K, phosphoinositol-3 kinase.(65)

Most, if not all cases of CLL precedes by monoclonal B cell lymphocytosis (MBL), a very indolent cell expansion defined by less than 5,000 monoclonal B cells in the peripheral blood. MBL is found in about 5% of the elderly people and has a risk of developing into CLL of about 1% / year.(68)

2.2.2.4: Clinical Features

70% to 80% of patients are diagnosed incidentally when they have a routine blood count and will have early-stage (Rai 0 or I) disease. The most frequent complaint is fatigue or a vague sense of being unwell. Less frequently, enlarged nodes or infections are the initial complaint. Fever and weight loss are uncommon at presentation but may occur with advanced and drug-resistant disease. (69)

Most symptomatic patients have enlarged lymph nodes and/or splenomegaly. Enlargement of the cervical and supraclavicular nodes occurs more frequently than axillary or inguinal lymphadenopathy. The lymph nodes are usually discrete, freely movable, and non-tender. Painful enlarged nodes usually indicate a superimposed bacterial or viral infection, or possibly a Richter transformation.

There is usually only mild to moderate enlargement of the spleen, and splenic infarction is uncommon. Hepatomegaly is common in later staged. Less common manifestations are enlargement of the tonsils, abdominal masses because of mesenteric or retroperitoneal lymphadenopathy, and skin infiltration.(70)

Symptomatic anemia can also be a presenting feature, which may be related to marrow replacement or, more rarely, to autoimmune hemolysis or red cell aplasia. Alternatively, patients may have bruising or bleeding, most commonly related to thrombocytopenia and infrequently to acquired von Willebrand disease. Rarely, patients present with a paraneoplastic syndrome, such as nephrotic syndrome, paraneoplastic pemphigus, or angioedema.(15)

Immunosuppression is often a significant problem resulting from hypogammaglobulinaemia and cellular immune dysfunction. Early in the

disease course bacterial infections, such as sinus and chest infections, predominate but with advanced disease viral infections, especially herpes zoster, and fungal infections are also seen.(71)

2.2.2.5: Diagnosis

The diagnosis of CLL requires the presence of $\geq 5 \times 10^9$/L B lymphocytes in the peripheral blood, sustained for at least 3 months. The clonality of these B lymphocytes needs to be confirmed by demonstrating immunoglobulin light chain restriction using flow cytometry.(61)

2.2.2.5.1: CBC and Morphology

The leukemia cells found in the blood smear are characteristically small, mature lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernable nucleoli and partially aggregated chromatin. Gumprecht nuclear shadows, or smudge cells, found as cellular debris, are additional morphologic features commonly associated with CLL (Figure 2.10). A small percentage of larger or atypical cells or prolymphocytes can be found admixed with morphologically typical CLL cells. Finding ≥55% prolymphocytes would favor a diagnosis of prolymphocytic leukemia; however. A significant proportion of circulating prolymphocytes (≥10%) seems to indicate a more aggressive form of CLL (with *NOTCH1* or genetic *TP53* aberrations).(72)

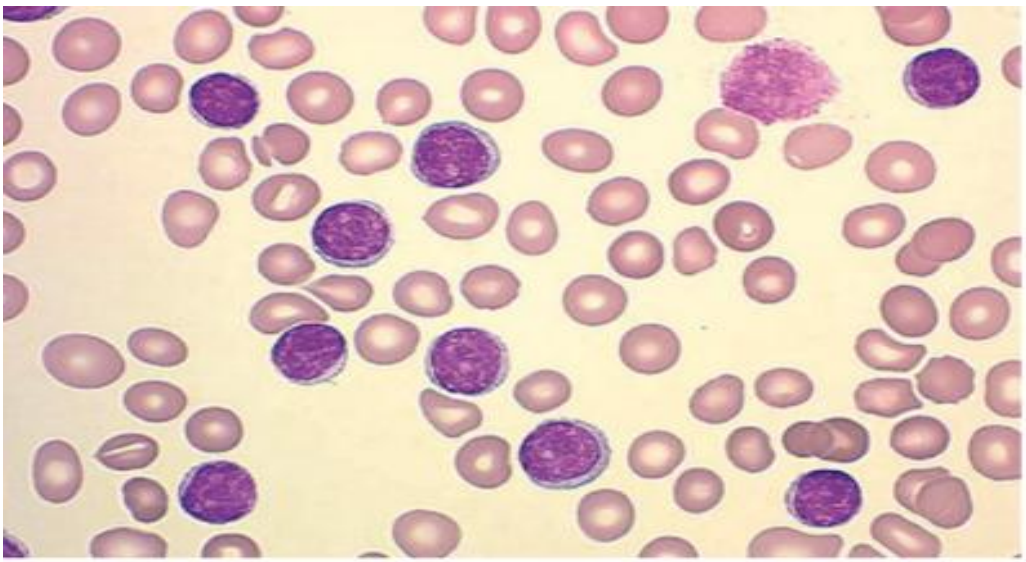

Fig. 2.10: Blood film of CLL patient. A picture of anemia (which may be due to the infiltration of the bone marrow by the disease, autoimmunity, iron, folate or vitamin B12 deficiency particularly in the elderly or poorly nourished people) can be present. Likewise, thrombocytopenia is infrequent at diagnosis. (1)

2.2.2.5.2: Immunophenotyping

CLL cells coexpress the surface antigen CD5 together with the B-cell antigens CD19, CD20, and CD23. The levels of surface immunoglobulin, CD20, CD79b and FMC7 are characteristically low compared with those found on normal B cells. Each clone of leukemia cells is restricted to expression of either κ or λ immunoglobulin light chains.(73)

Scoring system was defined for diagnosis of CLL depending on immunophenotyping profile which composed of five parameters that include CD5, CD22, CD23, SIG and FMC7 (Table 2.6). Thus, a score of 4-5 indicates typical CLL and a score of 3 suggest atypical CLL whereas 0 - 2 excludes CLL. (74)

Table 2.6: Scoring system for diagnosis of (CLL)

Marker	**point**	
	1	**0**
CD5	Positive	Negative
CD23	Positive	Negative
FMC7	Negative	Positive
SIG	Low	Medium/high
CD22/CD7	Low/negative	Medium/high

.

2.2.2.5.3: Molecular Genetics

Interphase fluorescence in situ hybridization (FISH) can be performed with peripheral blood lymphocytes and identifies cytogenetic lesions in >80% of all CLL cases. The most common deletions are in the long arm of chromosome 13 (del (13q)). Additional, frequent chromosomal aberrations comprise trisomy of chromosome 12 and deletions in the long arm of chromosomes 11 (del(11q)) and in the short arm of chromosome 17 (del(17p)).(75)

2.2.2.5.4: Pathological Features

- **Bone marrow biopsy:** Bone marrow aspiration and biopsy are not advised for routine diagnosis. It is indicated when there is diagnostic doubt or clinical suspicion of lymphomatous transformation, and it is particularly beneficial in detecting the source of pre-treatment cytopenias and prolonged post-treatment cytopenias. The presence of CLL cells in the bone marrow smear should be distinguished, with a

percentage of more than 30%. The infiltration can have a nodular, interstitial, or diffuse growth pattern in a bone marrow biopsy.(76)

- **Lymph nodes biopsy:** LN biopsy is not essential for the diagnosis of CLL, but is indicated when there is diagnostic uncertainty or clinical suspicion of lymphomatous transformation into Richter syndrome.(77)

2.2.2.6: Staging

In the 1970, two clinical staging systems (Rai and Binet) were established and are still commonly used. They are easy to use in clinical practice, rely on clinical data, and consider lymph node, spleen and liver involvement, as well as the existence of cytopenias (anemia and thrombocytopenia). In recent times, the modified Rai and Binet clinical staging systems are routinely applied to divide the patients into different prognostic groups.(78)

The survival of CLL patients is varied according to the staging of the illness as for patients with Rai 0 or Binet A, it was with average of >10 years, for CLL patients with stage I and II or B, the average survival reached >8 years, while for CLL patients with stage III and IV or C, the average survival reached to about 6.5 years.(79)

Clinical staging is still used in routine clinical practice and it can reliably predict the outcomes of the patients at the time of initial diagnosis(Table 2,7).(80)

Table 2.7: staging systems for CLL

Stage		Definition
Binet- system		
A		Hb ≥ 10 *g/dl* platelets ≥100× 10^9/L <3 involved lymphoid sites [a]
B		Hb ≥10 *g/dl* Platelets ≥100× /10^9/L ≥3 involved lymphoid sites [a]
C		Hb <10 *g/dl* platelets <100× 10^9/L
Modified Rai staging system		
Low risk	0	Lymphocytosis> 5x10^9/L
Intermediate risk	I II	Lymphocytosis and lymphadenopathy Lymphocytosis and hepatomegaly and/or splenomegaly with/without lymphadenopathy
High risk	III IV	Lymphocytosis and Hb <11 g/dl with/without lymphadenopathy/organomegaly Lymphocytosis and platelets <100× **10^9**/l with/without lymphadenopathy/organomegaly

Hb, haemoglobin ; a Binet's system considers five possible sites of involvement: cervical, axillary, inguinal lymphadenopathy (either uni or bilateral), spleen and liver.

2.2.2.7: Treatment

It is very difficult to cure CLL and so the approach to therapy is generally conservative, aiming for symptom control rather than a normal blood count. Indeed, chemotherapy given too early in the disease can shorten rather than prolong life expectancy. Another important fact is that many patients never need treatment.(1)

Based on their physical condition, comorbidities, and predicted life expectancy, there are three groups of elderly patients can be identified. First, physically well patients with no or minor comorbidities that reduce their life expectancy should be treated with standard therapies. Second, patients with relevant comorbidities that affect life expectancy who should have modified disease- control therapy. Third, patients with a multiple and/or severe comorbidities that significantly reduce life expectancy should receive the best supportive care possible .(81) Different therapeutic goals should be followed for these three patient groups .The goal for the first group is to achieve long-term remission and prolong survival, whereas the second and third groups should seek disease control and symptom palliation.(82)

The vast majority of CLL patients have early-stage asymptomatic disease at diagnosis. Only those patients who meet the 2018 IWCLL criteria for initiation of therapy (Table2.8) should be offered treatment.(73)

Table 2.8: Updated 2018 International Workshop on CLL (IWCLL) guidelines to initiate CLL therapy

Any **one** of the following criteria should be met to initiate CLL therapy:
1. Progressive marrow failure, hemoglobin <10 gm/dL or platelet count of <100 × 109/L **2.** Massive (≥6 cm below the left costal margin) or progressive or symptomatic splenomegaly **3.** Massive (≥10 cm in longest diameter) or progressive or symptomatic lymphadenopathy **4.** Progressive lymphocytosis with an increase of ≥50% over a 2-month period or lymphocyte doubling time of <6 months **5.** Autoimmune complications of CLL, that are poorly responsive to corticosteroids **6.** Symptomatic extra nodal involvement (e.g., skin, kidney, lung, spine) **7.** Disease-related symptoms, including: ❖ Unintentional weight loss of ≥10% within the previous 6 months ❖ Significant fatigue ❖ Fever ≥38 °C for 2 or more weeks without evidence of infection ❖ Night sweats for ≥1 month without evidence of infection

2.2.2.8: Prognosis

The natural history of CLL varies, with a wide spectrum ranging from a slow, indolent course to rapid disease progression. Several decades ago, staging systems (Rai , Binet et) were developed incorporating simple clinical parameters like complete blood counts and findings from physical examination. These systems are widely accepted as validated prognostic tools, but limitations were identified on their usefulness when dealing with individual patients. Furthermore, cytogenetic and molecular profiles have been identified that provide additional information to predict the clinical outcome of patients with CLL.(73)

To overcome this challenge, an international consortium of study groups created a new prognostic score in 2016, which incorporates clinical, biochemical, and genetic parameters: the CLL-International Prognostic Index (CLL-IPI). The CLL-IPI score uses 5 prognostic markers that have been identified as independent predictors of overall survival (OS): age, clinical stage, TP53 mutation status, IGHV mutation status, and serum β2-microglobulin level (Table 2.9). (83)

Table 2.9: CLL-IPI scoring system

Prognostic factor	Points
Del17p on FISH or TP53 mutation	4
Unmutated IGHV genes	2
Serum β2 microglobulin >3.5 mg/L	2
Rai stage I–IV /Binet B-C	1
Age >65 years	1

This tool also provides a practical approach to treatment recommendation (Table 2.10). (84)

Table 2.10: CLL-IPI treatment recommendation

CLL-IPI Category (Score)	OS at 5 Years, %	Treatment Recommendation
Low risk (0-1)	93.2	Do not treat (wait-and-watch approach)
Intermediate risk (2-3)	79.4	Do not treat except when the patient is symptomatic
High risk (4-6)	63.6	Treat except when the patient is asymptomatic
Very high risk (7-10)	23.3	Treat in clinical trial or with noncytotoxic drugs (no chemotherapy or chemo immunotherapy)

2.2.3: Non Hodgkin Lymphoma

Its malignant neoplasms of B, T, and natural killer (NK) cells that typically infiltrate both lymphoid and hematopoietic tissues but can also extend to other organs.(85)

2.2.3.1: Incidence

Non-Hodgins's lymphoma (NHL), the most common hematological malignancy worldwide.(86)According to the latest GLOBOCAN data 2020, NHL is rank 12th of all cancer cases, comprising 2.8% of worldwide cancer diagnoses and approximately comprising 2.6% of worldwide cancer death. Incidence rates were higher in men than in women, with similar geographical patterns. (87) (88) They are more common in adults than in children and have a steady increase in incidence from childhood through age 80 years, The mean age at diagnosis ranges between 45 and 55 years and the median age is 66 years.(15)

In the United States Non-Hodgkin lymphoma (NHL) is one of the most common cancers, accounting for about 4 % of all cancers. The American Cancer Society's estimates for non-Hodgkin lymphoma in 2022 are:

- About 80,470 people (44,120 males and 36,350 females) will be diagnosed with NHL. This includes both adults and children.
- About 20,250 people will die from this cancer (11,700males and 8,550 females).

Overall, the chance that a man will develop NHL in his lifetime is about 1 in 42; for a woman, the risk is about 1 in 52. But each person's risk can be affected by a number of risk factors.

The risk of developing NHL increases throughout life, and more than half of patients are 65 or older at the time of diagnosis.(35)

Non-Hodgkin Lymphoma in the Middle East Is Characterized by Low Incidence Rates With Advancing Age.(89)

In Iraq According to International agency for research on cancer/the Global Cancer Observatory (Globocan2020), number of new cases of NHL in 2020, both sexes, all ages is 1744 which account 5.1% of all cancer, NHL rank 4 of all type of cancer (breast ,lung ,leukemia)with Mortality rate 5.1% . (29)

2.2.3.2: Etiology and Risk Factors

1. Age

Getting older is a strong risk factor for lymphoma overall, but some types of lymphoma are more common in younger people. (90)

2. Gender

Overall, the risk of NHL is higher in men than in women, the reasons for this are not known. (90)

3. Race, Ethnicity, and Geography

In the United States, whites are more likely than African Americans and Asian Americans to develop NHL. (90)

4. Family History

Having a first degree relative (parent, child, and sibling) with NHL increases risk of developing NHL.(90)

5. Exposure to Certain Chemicals and Drugs(91)

-Certain chemicals such as benzene and herbicides and insecticides may be linked to an increased risk of NHL.

-Some chemotherapy drugs used to treat other cancers, drugs used to treat rheumatoid arthritis (RA), may increase the risk of developing NHL many years later.

6. Radiation Exposure

Exposure to radiation such as exposure to atomic bombs and nuclear reactor accidents or treated with radiotherapy for other cancer (e.g. Hodgkin lymphoma) shown they have an increased risk of developing several types of cancer, including NHL.(92)

2.2.3.3: Pathogenesis

NHLs arise by a multistep accumulation of genetic aberrations that induce a selective growth advantage of the malignant clone. Recurrent translocations, which occur during different steps of B-cell differentiation, are often an initial step in the malignant transformation .These translocations lead to deregulated expression of oncogenes that often control cell proliferation, survival, and differentiation. Interestingly, these translocations alone are often insufficient for lymphoma development. Accordingly, secondary genetic alterations are required for the full malignant transformation .(93)

B-cell development encompasses different stages and is initiated in the primary lymphoid organs with subsequent differentiation in secondary lymphoid tissues, during these stages of development, several DNA modifications occur that are essential for a normal immune response. However, these modifications might predispose to genetic abnormalities leading to lymphoma evolution. (94)

The development of B cells in the bone marrow is initiated by random recombination of genes that encode the variable regions of the heavy and light antibody chains to form the B-cell receptor (BCR).

This process is referred as V(D)J recombination and involves double-stranded DNA breaks by recombination activating gene 1 (RAG1) and recombination activating gene 2 (RAG2), which are resolved by nonhomologous end-joining repair processes.(93)(94)The immunoglobulin heavy chain genes (IgH) are assembled from various V (variable), D (diversity) and J (joining) elements, whereas the light chain is recombined from V and J elements.(95)

Once the BCR is expressed, the lymphocytes leave the bone marrow and become mature, naive B cells.

On antigen-induced B-cell activation, the germinal center reaction in secondary lymphoid tissues is initiated. During the germinal center reaction at least two distinct DNA modifications—somatic hypermutation (SHM) and class switch recombination (CSR).Both reactions are mediated by the B-cell specific enzyme activation-induced cytidine deaminase (AID). After the germinal center reaction, B cells develop into memory B cells or plasma cells.(96)

The tightly controlled steps in B-cell development, however, can go awry and lymphomas may arise. V(D)J recombination, SHM, and CSR especially represent critical processes that might predispose to these malignancies. B-cell lymphomas arise at different stages of differentiation, and accordingly, pregerminal and postgerminal center lymphomas can be distinguished (Figure 2.11). (93)

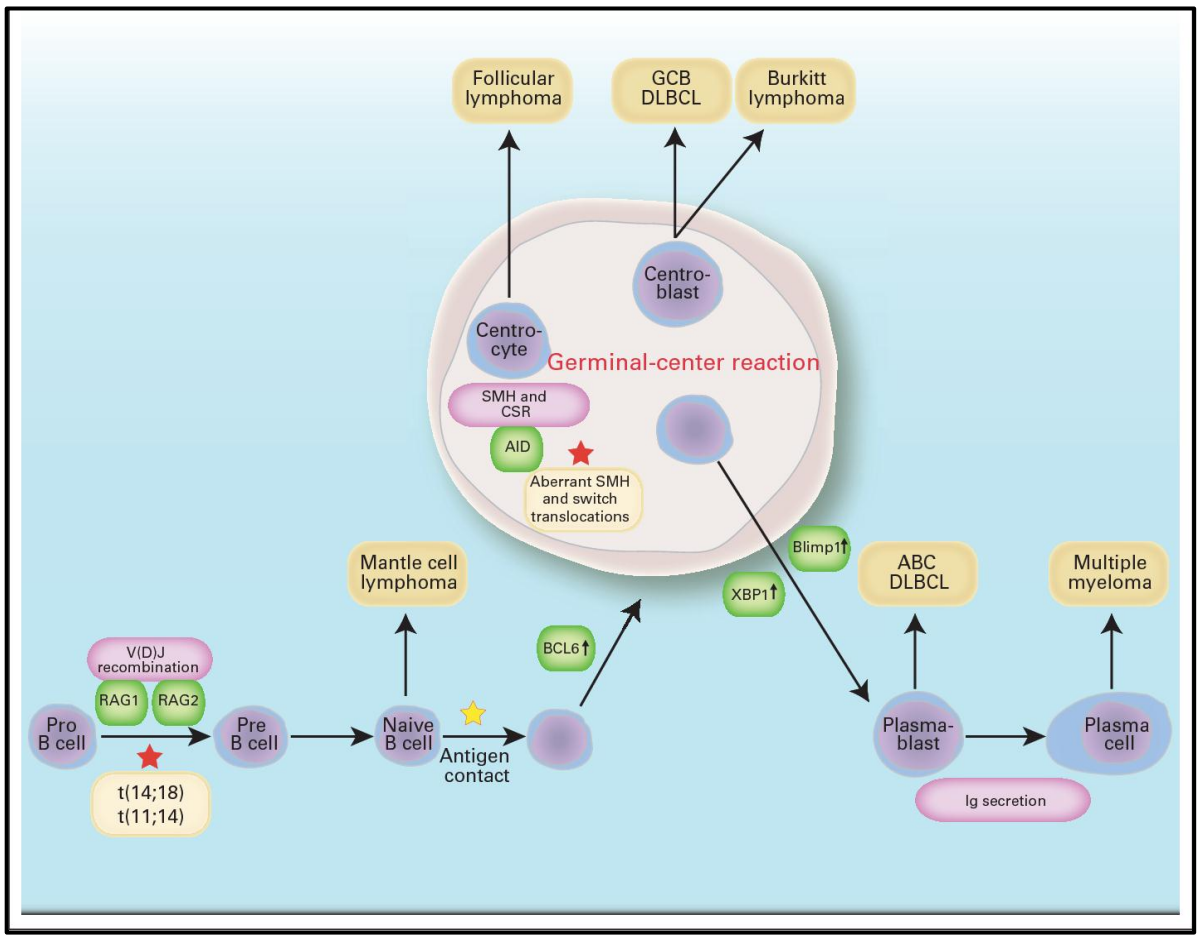

Fig.2.11: Lymphomas arise at different stages of B-cell differentiation. Specific recombination events are prone to the development of chromosomal aberrations. Recombination activating gene 1 (RAG1)-dependent and RAG2-dependent V(D)J recombination takes place in the bone marrow. The potentially resulting t(14;18) and t(11;14) represent critical first steps in lymphomagenesis of different lymphoma subtypes. After antigen contact, the stimulated B cells migrate to the lymph node and form the germinal center after upregulation of BCL6. The events during the germinal center reaction include activation-induced cytidine deaminase (AID) –mediated somatic hypermutation and class-switch recombination, which are critical events for lymphoma evolution. The germinal center reaction is terminated by the differentiation of B cells into plasma cells. XBP1 and Blimp-1 are key regulators for plasmacytic differentiation. GCB DLBCL, germinal center B-cell–like diffuse large B-cell lymphoma; SMH, somatic hypermutation; CSR, class-switch recombination; ABC DLBCL, activated B-cell–like diffuse large B-cell lymphoma; Ig, immunoglobulin.(93)

2.2.3.4: Classification

- NHL can be classified according to: (97)
    - Pathological grade (high grade or low grade)
    - Cell of origin (B cell or T cell or NK cell)
    - Histological features

Table 2.11: Classification of Non-Hodgkin Lymphoma subtypes.

Mature B-cell neoplasms	Mature T-cell and natural killer (NK)-cell neoplasms
• Chronic lymphocytic leukaemia and small lymphocytic lymphoma • Monoclonal B-cell lymphocytosis • B-cell prolymphocytic leukaemia • Splenic marginal zone lymphoma • Hairy cell leukaemia • Unclassifiable splenic B-cell lymphoma or leukaemia† • Splenic diffuse red pulp small B-cell lymphoma† • Hairy cell leukaemia variant • Lymphoplasmacytic lymphoma • Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue • Nodal marginal zone lymphoma • Paediatric nodal marginal zone lymphoma† • Follicular lymphoma • In-situ follicular neoplasia • Paediatric-type follicular lymphoma • Large B-cell lymphoma with rearrangement of IRF4† • Primary cutaneous follicle centre lymphoma • Mantle cell lymphoma • In-situ mantle cell neoplasia • Diffuse large B-cell lymphoma (DLBCL), not otherwise	• T-cell prolymphocytic leukaemia • T-cell large granular lymphocytic leukaemia • Chronic lymphoproliferative disorder of NK cells† • Aggressive NK-cell leukaemia† • EBV-positive T-cell lymphoproliferative diseases of childhood, including cutaneous chronic active EBV infection, hydroa vacciniforme-like lymphoma, severe mosquito-bite hypersensitivity, systemic chronic active EBV infection, and systemic EBV-positive T-cell lymphoma of childhood • Adult T-cell leukaemia or lymphoma • Nasal-type extranodal NK–T-cell lymphoma • Enteropathy-associated T-cell lymphoma • Monomorphic epitheliotropic intestinal T-cell lymphoma • Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract† • Hepatosplenic T-cell lymphoma • Subcutaneous panniculitis-like T-cell lymphoma

specified • T-cell-rich or histiocyte-rich large B-cell lymphoma • Primary DLBCL of the CNS • Leg-type primary cutaneous DLBCL • Epstein-Barr virus (EBV)-positive DLBCL, not otherwise specified • EBV-positive mucocutaneous ulcer • DLBCL associated with chronic inflammation • Lymphomatoid granulomatosis • Primary mediastinal (thymic) large B-cell lymphoma • Intravascular large B-cell lymphoma • ALK-positive large B-cell lymphoma • Plasmablastic lymphoma • Primary effusion lymphoma • Human herpesvirus 8-positive DLBCL, not otherwise specifi ed† • Burkitt lymphoma • Burkitt-like lymphoma with chromosome 11q aberrations • High-grade B-cell lymphoma with rearrangements of BCL2 and MYC or of BCL6 and MYC • High-grade B-cell lymphoma, not otherwise specified† • Unclassifiable B-cell lymphoma with features that are intermediate between DLBCL and classic Hodgkin's lymphoma	• Mycosis fungoides • Sézary syndrome • Primary cutaneous CD30-positive T-cell lymphoproliferative disorders • Lymphomatoid papulosis • Primary cutaneous anaplastic large cell lymphoma • Primary cutaneous γδ T-cell lymphoma • Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma† • Primary cutaneous acral CD8-positive T-cell lymphoma† • Primary cutaneous CD4-positive small or medium T-cell lymphoproliferative disorder† • Peripheral T-cell lymphoma, not otherwise specified • Angioimmunoblastic T-cell lymphoma • Follicular T-cell lymphoma† • ALK-positive anaplastic large cell lymphoma • ALK-negative anaplastic large cell lymphoma • Breast implant-associated anaplastic large cell lymphoma

2.2.3.5: Clinical Features

Most patients with non-Hodgkin lymphoma present with painless lymphadenopathy, and might or might not have systemic symptoms such as fevers, drenching night sweats, weight loss, pruritus, and fatigue. Significant cytopenias are rare unless marrow involvement is extensive or there are associated immune-mediated cytopenias, hypersplenism, or rarely, hemophagocytosis.

Oropharyngeal involvement in 5–10 % (Waldeyer's ring), which may cause complaints of a ‘sore throat’ or noisy or obstructed breathing.(97)

However, extra nodal disease can be detected at presentation in up to 40% of patients and varies depending on immune status and geographic differences.

The gastrointestinal tract is the most common extra nodal site at presentation and is involved in 5% to 20% of adults with NHL. Gastrointestinal symptoms are often nonspecific with vague abdominal pain is the most common presenting symptom. The stomach is most frequently involved followed by the small intestine, the colon, and esophagus. Epigastric pain, dyspepsia, nausea and, less often, early satiety, suggest stomach involvement.(98)

Hepatosplenomegaly is a common feature of advanced indolent B-cell lymphoma, including SLL, and splenic marginal zone lymphoma (SMZL).(99)

The skin is another common site of extra nodal NHL, and the most common primary cutaneous type is the cerebriform T cell of mycosis fungoides/Sezary.(100)

Neurologic symptoms and signs, including headache, confusion, lethargy, dysphasia, hemiparesis, seizures, and cranial nerve palsies, and rarely, multifocal leukoencephalopathy, may be presenting features of CNS involvement.(101)

Other symptoms and signs depend on unusual extra nodal presentations, such as bone, lung, heart, genitourinary tract and breast.

2.2.3.6: Diagnosis

2.2.3.6.1: Medical History and Physical exam

All lymphoma patients should have a thorough medical history (including information about symptoms, possible risk factors, and other medical conditions) and physical examination (the lymph nodes and other areas of the body that might be affected, including the spleen and liver), to determine the extent of lymphadenopathy, extra nodal illness, and functional abnormalities in the affected organ systems.

2.2.3.6.2: Blood Tests

No blood tests are specific for a diagnosis of non-Hodgkin lymphoma. In many patients routine blood tests are normal. Renal or liver function tests may be abnormal if the respective organs are affected by lymphoma. Bone marrow involvement may cause anemia, thrombocytopenia, and neutropenia. Lactate dehydrogenase is often elevated in high grade lymphomas, but the test is not specific.(85)

2.2.3.6.3: Biopsy

A biopsy is required to confirm the diagnosis of non-Hodgkin lymphoma. An excision lymph node biopsy is best, but where the anatomical location of the lymph node makes this technically challenging, a radiologically guided core biopsy may suffice. Fine needle aspiration is not usually sufficient for diagnosis.

Bone marrow biopsy is sometimes performed for staging but is rarely the diagnostic investigation. A normal bone marrow biopsy does not exclude lymphoma.(102)

Lab tests on biopsy samples: (103)

-Flow cytometry and immunohistochemistry: These tests can help determine whether a lymph node is swollen because of lymphoma, some other cancer, or a non-cancerous disease. The tests can also be used for *immunophenotyping* – determining which type of lymphoma a person has, based on certain proteins in or on the cells.

-Chromosome tests:-

Diagnosis may require molecular analysis of chromosomal aberrations by fluorescent in situ hybridization(FISH)

2.2.3.6.4: Imaging Tests(104)

2.2.3.7: Staging

The Ann Arbor staging system and its modification called the Lugano Classification are used for staging Hodgkin lymphoma (HL) and non-Hodgkin lymphomas (NHL).

2.2.3.7.1: Ann Arbor Staging System

The Ann Arbor staging system originally applies to Hodgkin's lymphoma developed in 1971 but has been used for staging NHL. Unlike Hodgkin's lymphoma in which spread of disease occurs through the involvement of contiguous sites, disease involvement in NHL is more random, limiting the utility of the Ann Arbor system (Table 2.12). (105)

Table 2.12: Ann Arbor staging system

Stage	Defining status
Stage I	Restricted to single lymph node region (I) or a single extranodal site (I-E).
Stage II	Two or more areas of nodal involvement on the same side of the diaphragm (II) or one or more lymph node regions with an extranodal site (II-E).
Stage III	Lymphatic involvement on both sides of the diaphragm (III), possibly with an extranodal site (III-E), the spleen* (III-S), or both (III-SE).
Stage IV	Liver, marrow, or other extensive extranodal disease.
Substage	
Substage E	Localized, extranodal disease.
Substage A	Absence of systemic signs.
Substage B	Presence of unexplained weight loss (10% in 6 months), and/or unexplained fever, and/or night sweat.

2.2.3.7.2: The Lugano Staging System

In the Lugano classification, a modification of the Ann Arbor classification was recommended for anatomic description of disease extent (Table 2.13)(Figure 2.) .The designation (E) for extranodal disease is applicable only for patients with limited extranodal disease in the absence of nodal involvement (stage IE) or limited nodal disease directly extended to a non-nodal site (stage IIE). On the contrary, E suffix does not apply to patients with stage III nodal disease; any patient with nodal disease above and below the diaphragm with concurrent contiguous extralymphatic involvement is diagnosed with stage IV disease (previously classified into stage IIIE). (106)

Table 2.13: Staging system depend on Lugano classification

Stage	Description
Limit stages	
Stage I	single lymphatic site involved
Stage IE	Single extra-lymphatic site involved in the absence of nodal involvement
Stage II	Two or more lymph node regions involved on the same side of the diaphragm
Stage IIE	Extra-lymphatic extension(Contiguous) from a nodal site with or without involvement of other lymph node regions on the same side of the diaphragm
Stage II bulky*	Stage II with bulky disease
Advance stages	
Stage III	Lymph node involvement on both sides of the diaphragm or above the diaphragm nodes with spleen involvement
Stage IV**	Diffuse or widespread involvement of one or more extra-lymphatic organs, with or without accompanying lymph node involvement; or extra-lymphatic organ involvement(non-contiguous) in association with nodal stage II disease, or any extra-lymphatic organ involvement in nodal stage III disease.

*****Stage II bulky considers either early or advance stage based on histology of lymphoma and prognostic factors.

******Stage IV includes any involvement of the bone marrow, liver or lung lesions ,cerebrospinal fluid and Centeral nervous system

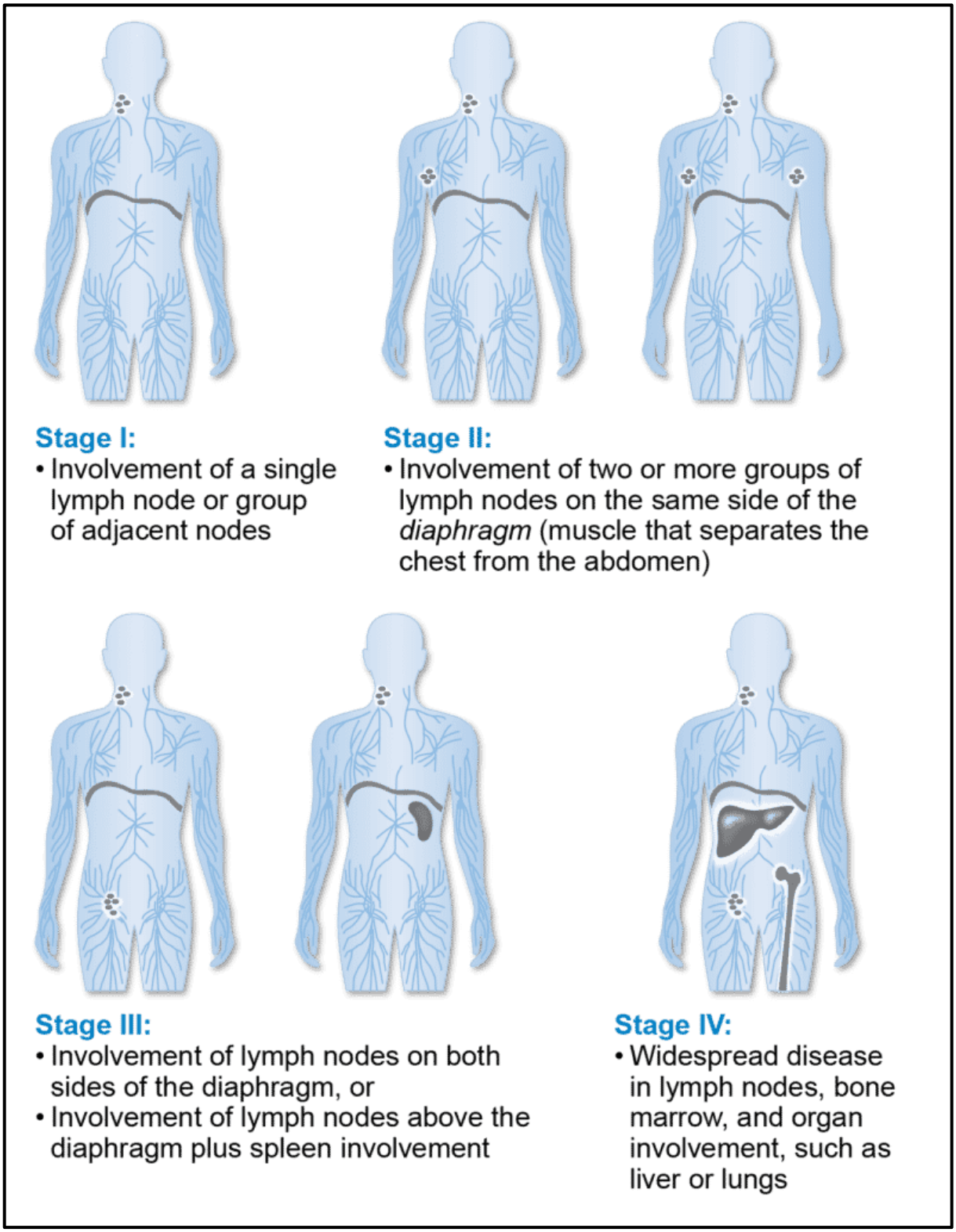

Fig. 2.12: The Lugano Staging System(106)

2.2.3.8: Treatment

Based on the type, stage, histological characteristics, and symptoms, Non-Hodgkin lymphoma is treated. Chemotherapy, radiation, immunotherapy, stem cell transplant, and, in rare circumstances, surgery are among the most often used forms of treatment. The most popular form of therapy is chemoimmunotherapy. Early-stage (I, II) are primarily treated with radiation. (107)

The majorities of patients with indolent, asymptomatic diseases are handled with a "wait and watch" strategy and may never need therapy. Systemic symptoms, bulky lymphadenopathy, increasing nodal enlargement, and impending compromise of vital organ function are indications to begin treatment..(85)

2.2.3.9: Prognosis

Non-Hodgkin lymphoma prognosis is mostly determined by histology, the involvement's extent and patient characteristics. In patients with NHL, the International Prognostic Index (IPI) and its variation are the primary prognostic tools. It aids in determining overall survival following standard care treatment**.**

Parameters

- Age is 60 years or more
- Advanced stage (stage III or stage IV)
- Extra-nodal involvement more than one site
- Performance status =2 or more
- Raised serum lactate dehydrogenase level (more than normal).

Each element is assigned one point, resulting in a total score ranging from (0 to 5), which determines the level of risk. Categorization of risk groups

0–1 → Low risk

2 → Low intermediate risk

3 → High intermediate risk

4– 5 → High risk

The expected 5-year survival rates for these four groups were 73%, 51%, 43%, and 26%, respectively. When predicting long-term survival, it was discovered that this approach was noticeably more accurate than the Ann Arbor categorization**.** (105)

For most NHL, IPI has been adjusted to improve prognosis, e.g., FLIPI for follicular lymphoma, MIPI for mantle cell lymphoma, and so on.

Patients suffering from T- or NK cell lymphomas (aggressive type) typically have a poor prognosis. Patients with low-grade lymphomas have a higher survival rate, which ranges from 6 to 10 years. However, they have the potential to develop into high-grade lymphomas.(91)

CHAPTER THREE

MATERIALS & METHODS

3.1: Materials

3.1.1: Chemicals

The chemicals and materials used in this study and their manufacturers are shown in (Table 3.1).

Table 3.1: Chemicals & materials used in the study and their origin.

Chemical/material	Manufacturer (country)
Human Erythropoietin ELISA Kit	Bioassay Technology Laboratory (Shanghai, China)
Leishman stain	SYRBIO (SAR)
Methylene blue stain	J&K Scientific (China)
Oil	SYRBIO (SAR)
deionized water	Baghdad/ Iraq

3.1.2: Instruments

There is a list of all the equipment and tools utilized in this research in (Table 3.2).

Table 3.2: Instruments used in this study

Instrument	Manufacturer (country)
Deep Freeze	Naïve (Turkey)
Hematology Analyzer XP-300	Sysmex (Japan)
Hematology Analyzer Ruby	Abbott (Germany)
Biochemistry Respons 920	DiaSys (Germany)
ELISA (Washer and Reader)	Paramedical (Italy)
Incubator	Fisher Scientific (Germany)

Centrifuge H-19f	Kokusan (Japan)
Microcentrifuge	Prism-R (USA)
Micropipette 10-1000	Slammed (Germany)
Micropipette 2- 20	Gilson (France)
Light microscope	Olympus (Japan)
EDTA tube (5 ml)	Plasmatic Laboratory (UK)
Eppendorf tube (1.5 ml)	IkEME (China)
Slides	SAIL BRAND (China)
Gloves	Kleinhans (Malaysia)

3.1.3: Study Subjects

90 Iraqi adult from three Iraqi centers: Baghdad Teaching Hospital , Marjan Teaching Hospital and Imam Alsadiq teaching hospital were enrolled in this study, 60 patients who attended the outpatient clinic in these hospitals during the period from September 2022 to march 2023, they were diagnosed as having Chronic Leukemia (CML & CLL) and Non-Hodgkin Lymphoma (NHL),and another 30 adult participants without disease were included and served as a normal control for comparison with the patients' study group; all control members had normal CBC measured at the time of blood sampling. The informed consent was acquired from both patients and control groups.

3.1.3.1: Patients

Sixty adult patients diagnosed with chronic leukemia (CML=19 patients ,CLL=19patients) and Noh Hodgkin Lymphoma (22 patients) based on a specialist's physical examination, morphological evaluation of peripheral blood films and bone marrow, and histological examination,

PET scan and flow cytometric immunophenotypic profile and immunohistochemistry were included in this study. The patient group included 36(60%) male and 24(40%) female, their age range from 24 to 75 years. All patients received treatment on regular schedule.

Patients who met one of the following criteria were disqualified from the study to ensure accurate measurement of erythropoietin (Epo):

1. Blood loss (Acute).
2. Concurrent renal illness or insufficiency.
3. Hemolytic anemias, either inherited (thalassemia and sickle cell disease) or acquired.
4. Cancer of any type.(solid tumors)
5. The presence of concurrent chronic pulmonary illnesses.
6. Smoking (currently).
7. Blood transfusion (within 3 months).
8. Living in high altitude (north Iraq)
9. Patients with iron deficiency anemia or megaloblastic anemia.
10. Autonomic neuropathy: diabetic patient.

All patients have been physically examined and investigated for complete blood count, blood film , retic count ,blood urea and all having flow cytometry reports, PET scan reports and histopathological reports taken from their data or hospital archives where they were done and the information was obtained using a questionnaire form.

We used the WHO definition of anemia(108) (Hemoglobin ≤ 13 g/dL in male and ≤ 12 g/dL in female) to classification of patients into two groups:

1) **Anemic Group:** 31 patients (CML= 10, CLL= 8, NHL= 13) with mean Hb 10.67 ± 1.51 g/dL.
2) **Non-anemic Group:** 29 patients(CML= 9 , CLL= 11 ,NHL= 9)with mean Hb 13.86 ± 1.03 g/Dl

3.1.3.2: Control

Presence of control group in cross sectional study has advantageous at least on two aspects: 1) control of confounding factors. 2) Increasing the power of the study as the sample size is increased.(109)

Thirty healthy subjects were taken included 18 (60%) male and 12 (40%) female as a controls group. They were all non-anemic adults who attended Merjan Teaching Hospital, Imam Al Sadiq Teaching hospital for checking. We selected those who were most closely matched for the age and sex of patients. For all controls, clinical history were taken, physical examination and CBC, retic count, blood film exam and biochemical tests were done.

3.2: Methods

3.2.1: Study Design

This is a cross sectional study. 30 age and sex healthy controls and 60 patients, made totaled 90 participants. ELISA assays were used to quantify serum erythropoietin levels and CBC, blood urea tests were done for both the patient and control groups, and reticulocyte count, blood film examination done for patients group.

3.2.2: Evaluate Adequacy of Serum Epo Level

Serum Epo levels should not be quantitated in absolute terms in anemic patients but should be evaluated in relation to the degree of anemia. In fact, if the Epo-generating apparatus in the kidney is efficient, levels should increase exponentially as the Hb level decreases; consequently, serum Epo levels must be expressed in relation to Hb levels.(110) The definition of "inadequate Epo response to anemia" relies primarily on documentation of a downregulated dependence of serum Epo levels on Hb levels in comparison with reference patients.(111) The adequacy of endogenous EPO in investigated anemic patients was evaluated using the observed/predicted (O/P) ratio in each patient, EPO O/P ratio was calculated as follows: $\log_{10}$(EPO observed)/$\log_{10}$(EPO predicted).

Log_{10}(EPO predicted)was calculated with the following formula: For Hb values <13 g/dL, the regression equation was log(Epo)=4.746−(0.275×Hb). O/P ratio values ≤O.9 indicate an inadequate endogenous erythropoietin concentration with respect to the degree of anemia."(112)(113)

3.2.3: Collection of Blood Sample:

All participants were told about the study's objectives and their agreement was obtained prior to blood sampling. Via a disposable syringe, blood samples were drawn from patients and controls using venipuncture. Blood samples totaling four milliliters were taken from each patient and control. 2 ml of blood were taken and dispensed in E.D.T.A. tubes and sent to the laboratory for complete blood count and blood film evaluation. Another 2 mL was administered in plain tubes and left to clot at room temperature for around 20 minutes. After that, it was centrifuged at 2000 RPM for 20 minutes to get serum. Sera were aspirated and divided into two Eppendorf tubes. These tubes were labeled and stored at deep freeze (–50°C) until the time of test. EDTA tube was used to perform CBC, blood film and reticulocyte count.

3.2.4: Biochemical Tests

3.2.4.1: Erythropoietin ELISA Assays

3.2.4.1.1: Principle

It is an Enzyme-Linked Immunosorbent Assay kit (ELISA). Human EPO antibody has been pre-coated on the plate. Sample's EPO binds to antibodies coated on the wells. The biotinylated human EPO Antibody is then added to the sample and binds to the EPO. Streptavidin-HRP is added then which binds to the Biotinylated EPO antibody and incubate. During the washing stage after incubation, unbound Streptavidin-HRP is rinsed away. After that, the substrate solution is added, and the color develops in accordance to the amount of human EPO. The process is stopped by adding an acidic stop solution, and the absorbance at 450 nm is measured

3.2.4.1.2: Preparation of Standard

Before use, all reagents were initially brought to room temperature. To create a 400mIU/ml standard stock solution, reconstitute 120 µl of the standard (800mIU/ml) with 120 µl of the standard diluent. Before producing dilutions, let the standard sit for 15 minutes with mild agitation. Create duplicate standard points by sequentially diluting the standard stock solution (400mIU/ml) 1:2 with a standard diluent to create solutions with concentrations 200mIU/ml, 100mIU/ml, 50mIU/ml and 25mIU/ml respectively. The zero standard (0 mIU/ml) is standard diluent**.**

3.2.4.1.3: Wash Buffer Preparation

We diluted 20ml of Wash Buffer Concentration (25x) into distill water to produce 500 ml of (1x) Wash buffer.

3.2.4.1.4: Assay Procedure

The assay was carried out according to the directions in the kit's manual, which are given in the stages below:

1. We followed the instructions for preparing all reagents, standard solutions, and samples. Before using any reagents, bring them to room temperature. The assay was carried out at room temperature..

2. We determined the number of strips needed for the assay and placed them in the appropriate frames. Unused strips were kept at 2-8°C.

3. We added a 50µl standard to the standard well. Because the standard solution contains biotinylated antibody, we did not add antibody to the standard wells.

4. We added 40µl sample to sample wells, followed by 10µl anti-EPO antibody, and finally 50µl streptavidin-HRP to sample and standard wells (except the blank control well). We combined thoroughly before sealing the plate. The plate was incubated at 37°C for 60 minutes.

5. After removing the sealer, we washed the plate 5 times with the automated washer with the wash buffer. We blotted the plate several times onto filter papers to ensure that it was completely dry.

6. We added 50µl of substrate solution A to each well, followed by 50µl of substrate solution B. We incubated the plate covered with a new sealer at 37°C in the dark for 10 minutes.

7. We added 50µl Stop Solution to wells, the blue color immediately changed into yellow.

8. Using a microplate reader set to 450 nm, we instantly calculated the optical density (OD value) of each well.

*Detection Limit: minimum detection limit (Sensitvity) of this assay is defined as the smallest single value, which can be distinguished from zero at the 95% confidence limit. The EPO ELISA has a calculated sensitivity of 1.02 mIU/mL .

*Standard Curve Range: 2mIU/ml - 600mIU/ml

3.2.4.1.5: Standard Curve

A standard curve should be generated with each assay. Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

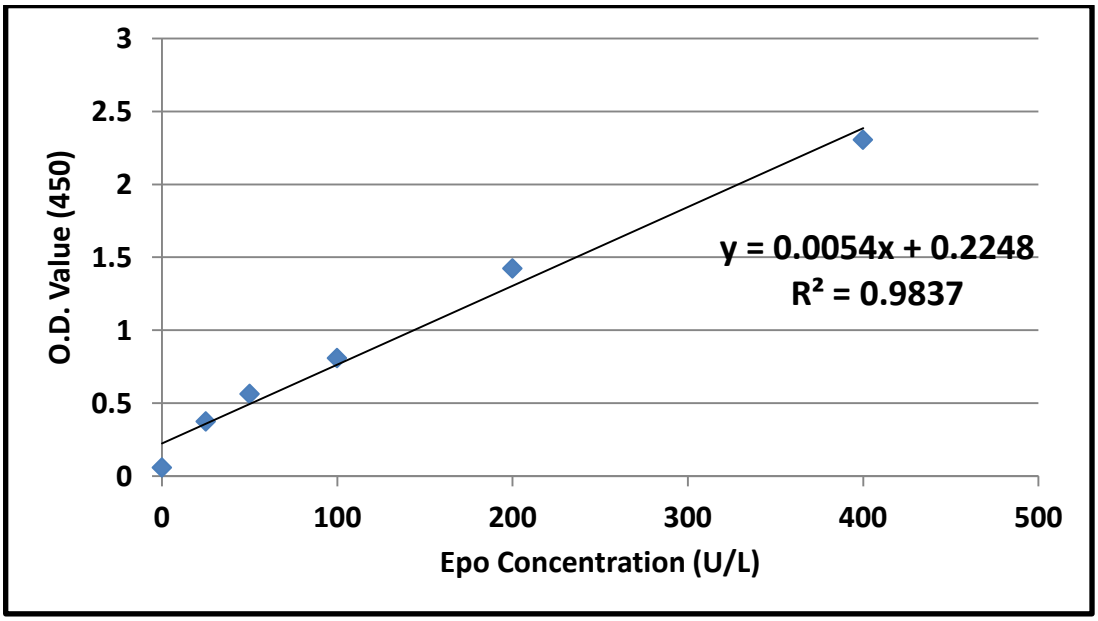

Fig. 3.1: EPO standard curve.

3.2.4.2: Renal Function Tests

Renal function tests include measurement of serum creatinine,urea and blood urea nitrogen. Urea and creatinine are normally filtered and cleared by the kidneys, and high blood urea and/or serum creatinine mostly suggest renal dysfunction. (114)

All biochemical tests in our study were done by an automated biochemistry analyzer based on specific enzymatic reactions and spectro-photometric analysis of the result.

3.2.5: Hematological Studies

3.2.5.1: Automated Blood Count

Blood samples were drawn and placed in EDTA tubes before being sent to the laboratory for a CBC using an Auto Analyzer Hematology (five parts).

The blood is mixed inside the tube (without vigorous shaking) and then transferred into the analyzer, which assesses the number and types of different blood cells. The Auto analyzer system obtains a standard volume of blood using narrow tubing and provides measured data using a mix of procedures:

- Photometric light absorbance measurements are used to detect hemoglobin content, which measures the amount of hemoglobin in the total sample as well as within each red cell..(115)
- Volumetric impedance is used to calculate WBC, RBC, and platelet volume distributions. An automatic cell analyzer has a very accurate calculation since it samples and records too many cells. However, for some abnormal cells in the blood, the findings can be inaccurate and not as precise as a hematologist's manual count.
- Optical light scattering and diffraction measurement are utilized to evaluate leukocyte differential parameters (lymphocyte, monocyte, neutrophil, eosinophil, and basophil percentages)

3.2.5.2: Blood Smear Preparation:

Blood film made on clean glass slide by using EDTA anticoagulated blood sample. The slide measured 75 x 25mm and approximately 1mm thick. One centimeter from one end of the slide (the frosted end), a tiny drop of blood is inserted in the slide's center line. A second slide used as a spreader was then positioned in front of the drop at an angle of roughly 30 degrees to spread the drop and create a thin coating of blood over the slide. The slide was spread, labeled, and left to dry in the air before being stained with Leishman dye and examined.

3.2.5.3: Blood Film Examination

Blood film systematically inspected, starting with a macroscopic examination of the stained film to determine whether the spreading method was effective and whether any aberrant particles are present, then moving on to low power and high power examination by microscopic.

Examination of a blood film under microscopic aims to determine the morphological features of cells in blood and hematopoietic organs. The results obtained are reliable when the smear was well made and stained.

3.2.5.4: Manual Reticulocyte Count:

Reticulocytes are immature RBCs that retain parts of cytoplasmic RNA and ribosomes. They circulate in peripheral blood for 1-2 days before full maturation. Their name was derived from their microscopic appearance when stained with supravitally dyes. The most common supravitally dye used in our practice is methylene blue, which stains the RNA and ribosomes in deep blue color. Reticulocytes are then counted under 100X and reported as a percentage of red cells. The absolute reticulocyte count can be calculated by multiplying retic percentage by the RBC count.(116)

3.3: Data Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 27. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. ANOVA test was used to compare means between three groups or more. Correlation coefficient was used to assess the relationship between two continuous variables. Pearson chi-square was used to find the association between categorical variables. A *p*-value of ≤ 0.05 was considered as significant.

CHAPTER FOUR

THE RESULTS

4.1: The Study Groups

The study involved two groups; the first group consisted of 60 patients with specific diagnosis (CML, CLL, NHL) and the second control group of 30 healthy subjects. The patient group was subdivided into 31 (51.7%) patients with anemia and 29 (48.3%) patients without anemia.

4.1.1: Age Distribution in the Patient and Control Groups:

All patients and controls in this study were divided into 6 age groups (20-30, 30-39, 40-49, 50-59, 60-69 and 70-75 years). (Table 4.1), (Fig. 4.1)

Table 4.1: Age distribution in patients and control groups.

Age	Patients	Controls
20 _ 29	1(1.7%)	1(3.3%)
30 – 39	4(6.7%)	1(3.3%)
40 – 49	8(13.3%)	4(13.3%)
50 – 59	19(31.7%)	11(36.7%)
60 – 69	19(31.7%)	10(33.3%)
70 – 79	9(15%)	3(10%)
Sum	**60**	**30**

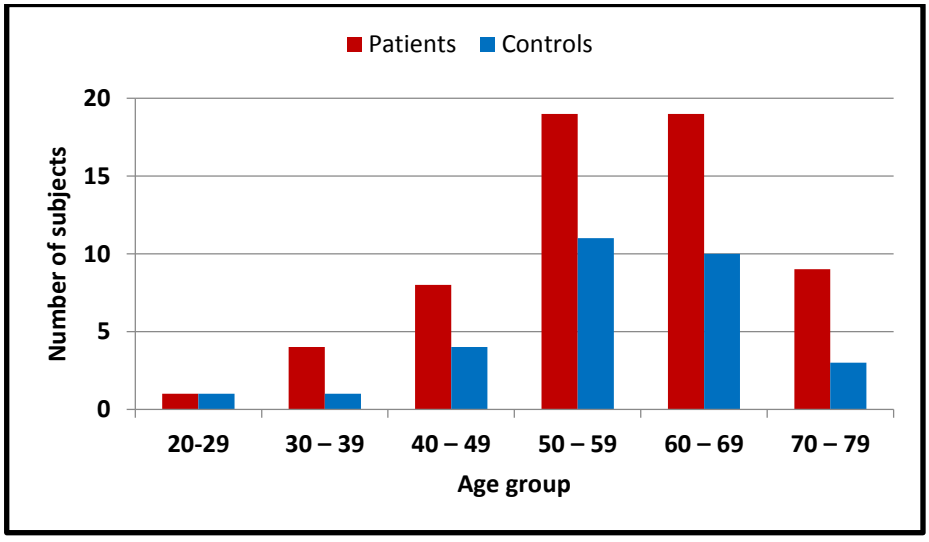

Fig. 4.1: Age distribution in patients and control groups

The Mean age of patients ± SD was (56.7 ± 11.5) years with older patient was 75 years and younger patient was 24 years.

For control group, the mean age ± SD was (55. 8 ± 10.6) years with older subject was 70 and younger subject was 29 years.

The difference between the mean age of the patient group and the control group was statistically insignificant (P=0.73, Fig. 4.2).

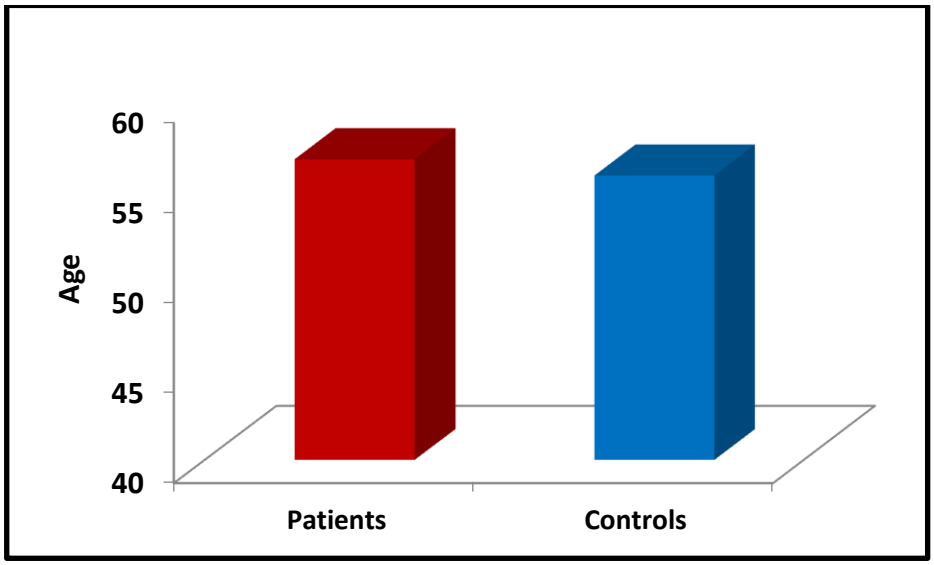

Fig. 4.2: Mean age (Years) of patients and control groups

For patient group: The difference in the mean age between anemic patients (57.8± 10.4) and non-anemic patients (55.5 ± 12.6) was also not significant (P=0.44, Fig. 4.3).

The mean age of CML patient was (50.1 ± 12.85) , CLL patients (58.2 ± 10.4) and NHL patients (61.1 ± 8.86).

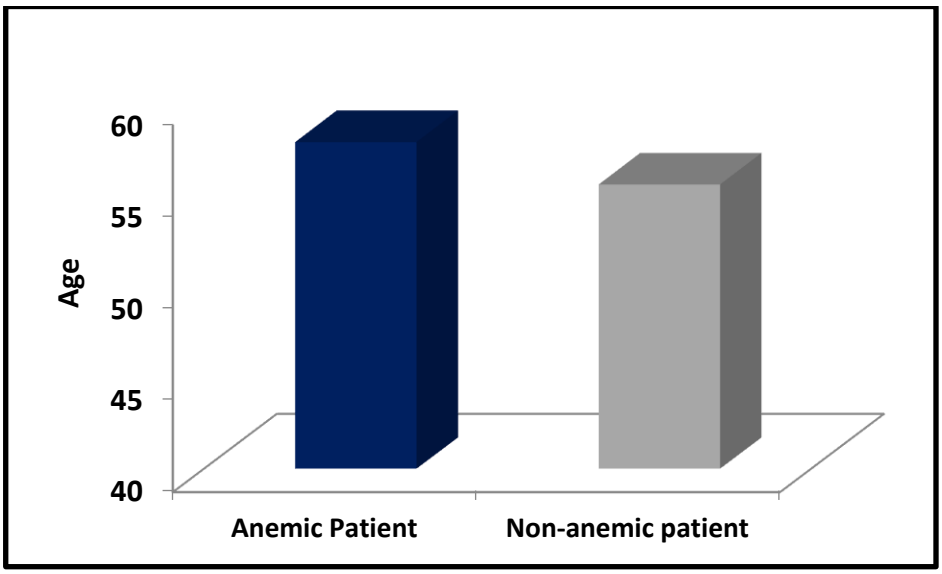

Fig. 4.3: Mean age (Years) of anemic and non-anemic patients

4.1.2: Sex Distribution in the Patient and Control Groups:

In this study, 36 of 60 patients (60%) and 18 of 30 controls (60%) were males, There were no significant differences in percentage of gender. (Fig. 4.4)

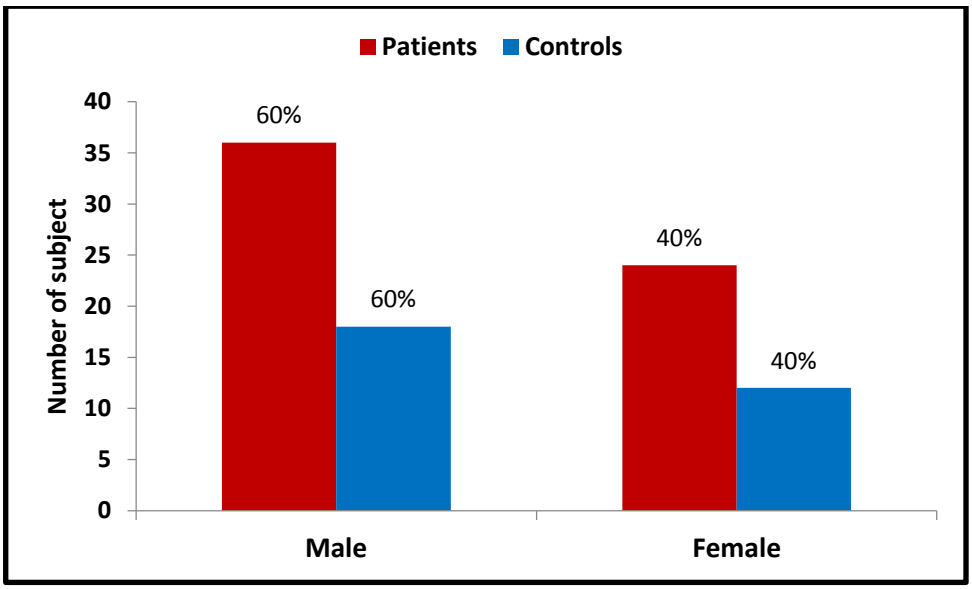

Fig. 4.4: Distribution of patient and control by sex.

For patient group: The anemic group of patients, 16 of 31 cases (51.6%) were males, whereas in the non-anemic group, 20 of 29 cases

(69 %) were males. There were no significant differences in percentage of gender among patient group (p-value =0.17, Fig. 4.5).

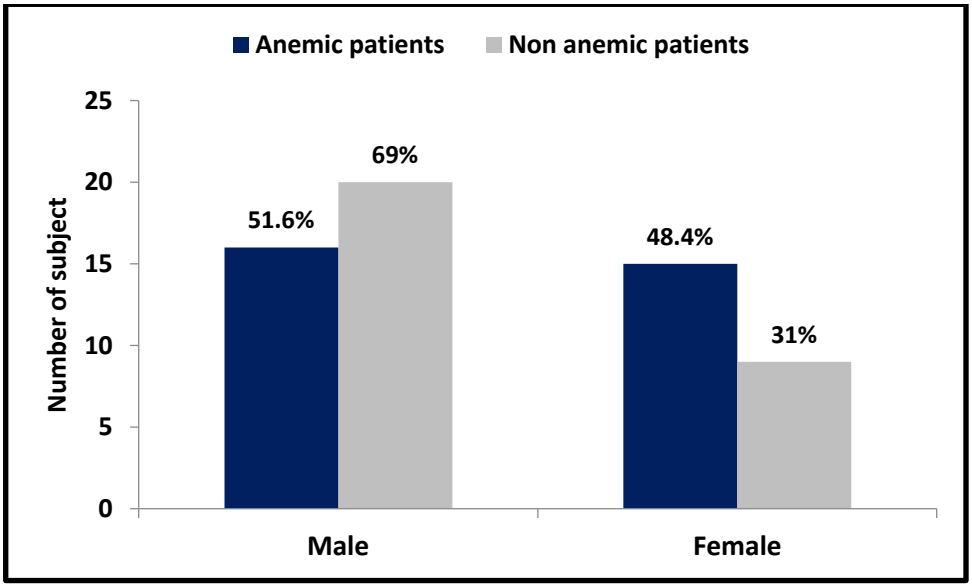

Fig. 4.5: Sex distribution in anemic and non-anemic groups of patients.

CML, CLL and NHL (Male/Female) were found to be more in male than female, (13/6) (10/9) (13/9) respectively

4.1.3: Frequency of Specific Diagnoses Among Patients Group:

Specific diagnoses of patients are shown in(Table 4.2).

Table 4.2: Patient distribution according to diagnosis.

Specific Diagnosis	Number (% of total)
Chronic Myeloid Leukemia (CML)	19 (31.7%)
Chronic Lymphocytic Leukemia(CLL)	19 (31.7%)
Non-Hodgkin Lymphoma(NHL)	22 (36.6%)
Sum	**60 (100%)**

Distribution of anemic and non-anemic patient among specific diagnosis show in (Table 4.3)

Table 4.3: Distribution of anemic and non-anemic patient among specific diagnosis

Specific Diagnosis	No. of Anemic patient (%)	No. of Non-Anemic Patient (%)	Sum of Specific Diagnosis
CML	10 (16.7%)	9 (15%)	19 (31.7%)
CLL	8 (13.35%)	11 (18.35%)	19(31.7%)
NHL	13(21.6%)	9 (15%)	22(36.6%)
Total	**31 (51.7%)**	**29(48.3%)**	**60(100%)**

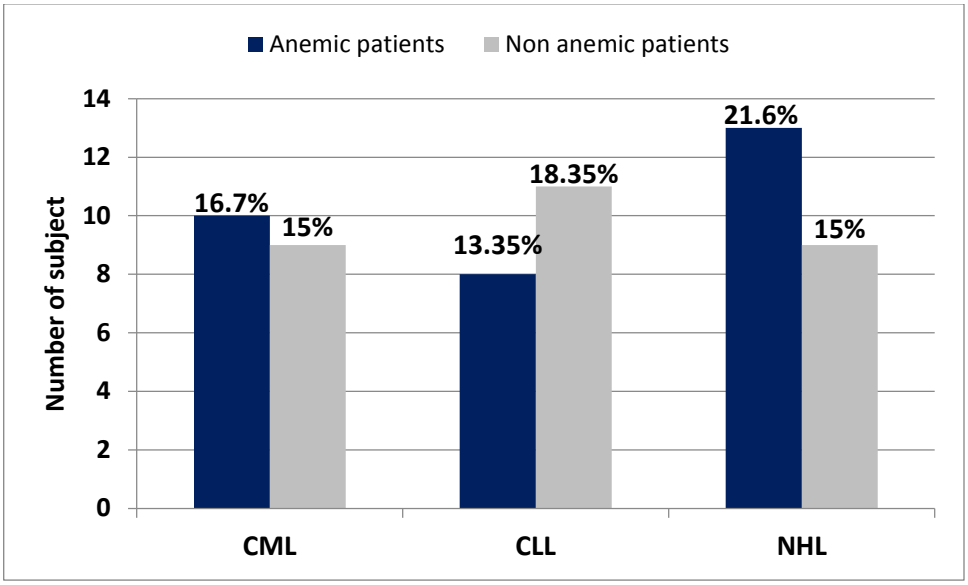

Fig. 4.6: Patients distribution according to specific diagnosis in anemic and non-anemic groups.

4.2: Staging of Specific Diagnosis in Patients Group

4.2.1: Staging of CML

Chronic myelogenous leukemia (CML) is classified into phases rather than stages. By applying the WHO classification system, (40) the studied patients were categorized into the following:

1. 18 (94.7%) patients were in chronic phase.
2. 1 (5.3%) patient was in blastic phase

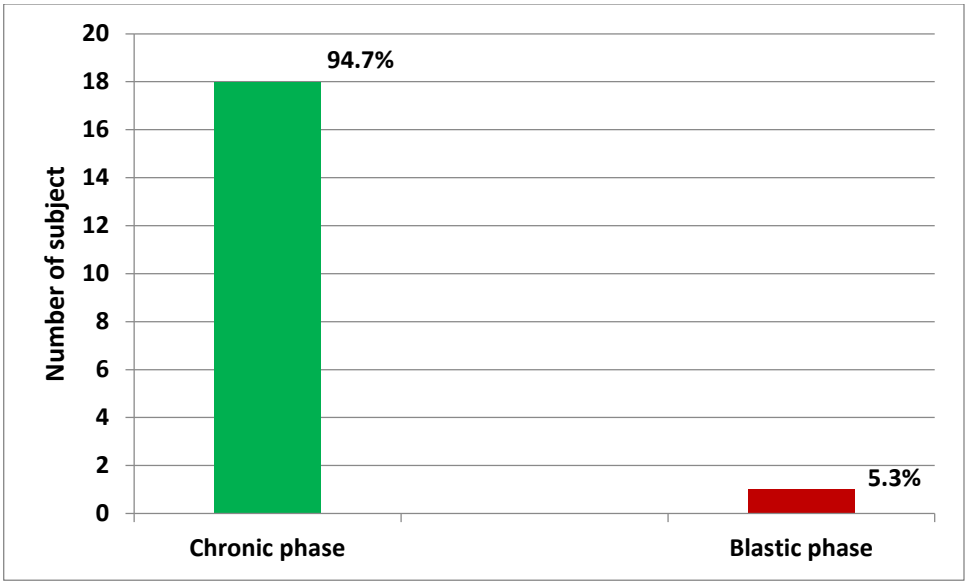

Figure 4.7: Distribution of patients with chronic myeloid leukemia (CML) according to WHO classification system (N=19)

4.2.2: Staging of CLL

Staging according to Modified Rai Staging System(80), by applying the Modified Rai Staging System, the studied patients were categorized into the following:

1. 14 (73.7%) patients were in low risk.
2. 2 (10.5%) patients were in moderate risk.
3. 3 (15.8%) patients were in high risk, as shown in (Figure 4.8).

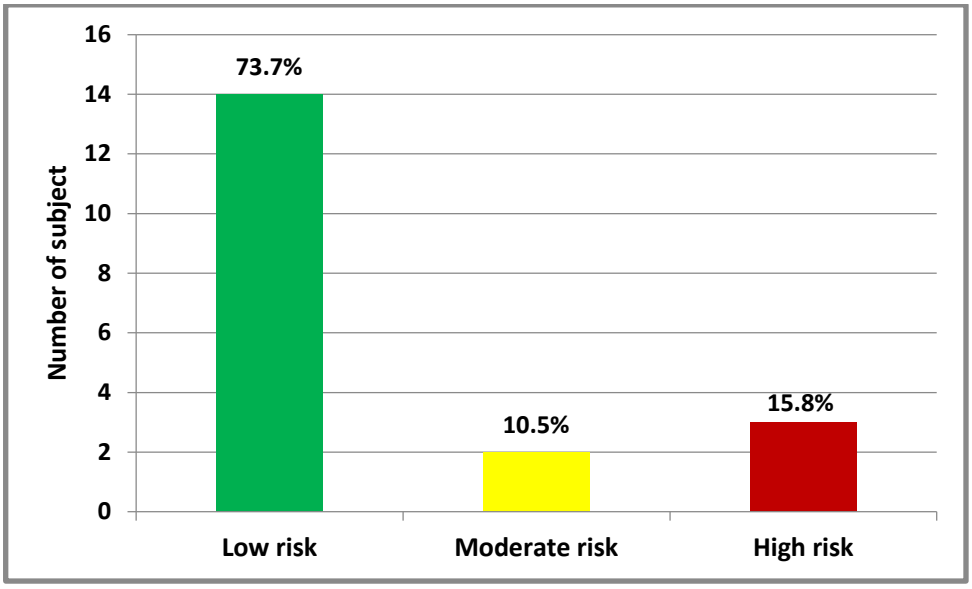

Figure 4.8: Distribution of patients with chronic lymphocytic leukemia (CLL) according to Modified Rai stage (N=19)

4.2.3: Staging of NHL

Staging according to the Lugano classification, a modification of the Ann Arbor classification, the studied patients were categorized into the following:

1. 4 (18.2%) patients were in Stage I.
2. 5 (22.7%) patients were in Stage II.
3. 8 (36.4%) patients were in Stage III.
4. 5 (22.7%) patients were in Stage IV.

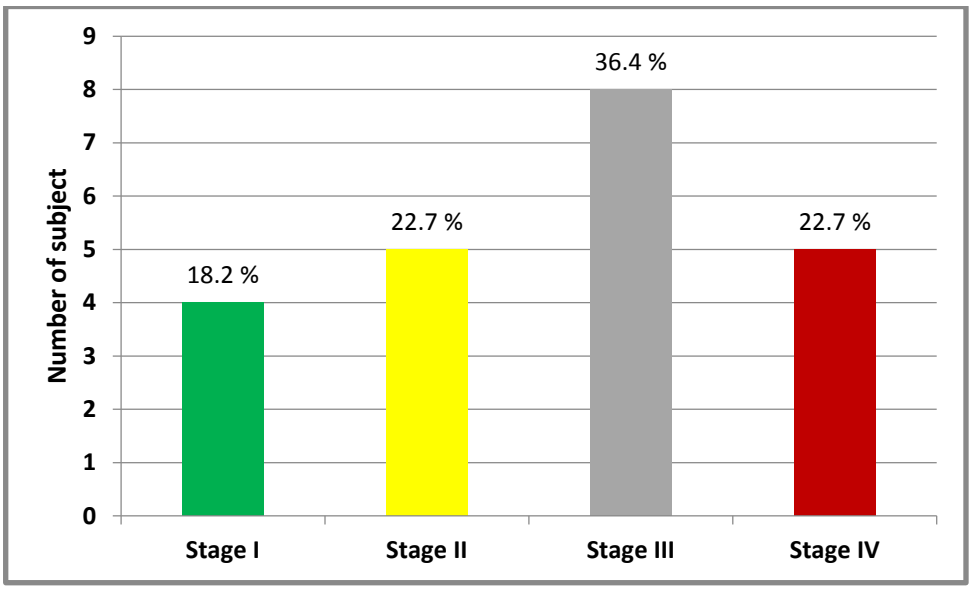

Figure 4.9: Distribution of patients with Non-Hodgkin Lymphoma (NHL) according to Lugano classification system (N=22)

4.3: Laboratory Investigations

The comparison in haematological and biochemical parameters including (total WBC count ($\times 10^9$/L), absolute lymphocyte count($\times 10^9$/L) ,Hb level (g/dl), RBC count($\times 10^9$/L)**,** platelet count ($\times 10^9$/L), B.Urea(mg/dL)), according to study groups (patients and control group) revealed a statistically significant differences between the study groups in terms of absolute lymphocyte count(P-value = 0.03), Hb level and RBC count (P-value <0.0001). The normal individuals in control group showed a significant higher means of Hb level, RBC count and lower means of absolute lymphocyte count (Table 4.4).

Table 4.4: The mean differences of hematological and biochemical parameters in study groups

Hematological variable	Study group	N	Mean ± SD	t-test	P-value
Total WBC count ($\times10^9$/L)	Patient	60	9.63 ± 11.13	**1.539**	**0.12**
	Control	30	7.36 ± 1.84		
Absolute lymphocyte count($\times10^9$/L)	Patient	60	4.71 ± 10.09	**2.115**	**0.03***
	Control	30	1.95 ± 0.53		
Hb level (g/dl)	Patient	60	12.26 ± 2.03	**-5.910**	**<0.0001***
	Control	30	14.37 ± 1.32		
RBC count($\times10^9$/L)	Patient	60	4.13 ± 0.71	**-5.620**	**<0.0001***
	Control	30	4.96 ± 0.54		
Platelet count ($\times10^9$/L)	Patient	60	213.76 ± 80.95	**-1.752**	**0.083**
	Control	30	243.90± 68.00		
B.Urea mmol/L)	Patient	60	4.45 ± 1.32	**-0.922**	**0.35**
	Control	30	4.72 ± 1.19		

*P ≤ 0.05 was significant.

The comparison in haematological and biochemical parameters including (total WBC count ($\times 10^9$/L), absolute lymphocyte count ($\times 10^9$/L) , Hb level (g/dl), RBC count($\times 10^9$/L)**,** platelet count ($\times 10^9$/L), B.Urea(mg/Dl)), according to patient groups (Anemic patients and Non-anemic patient) revealed a statistically significant differences between the patients groups in terms of Hb level and RBC count (P-value <0.0001). The anemic patient showed a significant lower means of Hb level and RBC count (Table 4.5).

Table 4.5: The mean differences of hematological and biochemical parameters in Patient groups

Hematological variable	Patient group	N	Mean ± SD	t-test	P-value
Total WBC count ($\times 10^9$/L)	Anemic Patient	31	7.36 ± 5.56	**-1.616**	**0.11**
	Non-anemic patient	29	12.06 ± 14.71		
Absolute lymphocyte count($\times 10^9$/L)	Anemic Patient	31	2.63 ± 2.82	**-1.631**	**0.11**
	Non-anemic patient	29	6.95± 14		
Hb level (g/dl)	Anemic Patient	31	10.76 ± 1.51	**-9.298**	**<0.0001***
	Non-anemic patient	29	13.86 ± 1.03		
RBC count($\times 10^9$/L)	Anemic Patient	31	3.76 ± 0.60	**-4.731**	**<0.0001***
	Non-anemic patient	29	4.51± 0.62		
Platelet count ($\times 10^9$/L),	Anemic Patient	31	202.29 ± 97.37	**-1.157**	**0.2**
	Non-anemic patient	29	226.03± 57.88		

Urea mmol/L)	Anemic Patient	31	4.18 ± 1.35	**-1.681**	**0.9**
	Non-anemic patient	29	4.74 ± 1.25		
Retic count %	Anemic Patient	31	1.3 ± 0.55	1.117	0.17
	Non-anemic patient	29	1.1 ± 0.32		

*P ≤ 0.05 was significant.

The mean WBC count in CML patients (7.47 ± 5.95) which was not statically significant different from control group (p=0.9), CLL (16.49 ± 16.97) and NHL (5.58 ± 2.68), which were statically significant from control group. (p=0.03)(p=0.007) respectively.

The mean hemoglobin level of CML, CLL, NHL patients was (12.47± 1.76) (12.44 ± 2.2) (11.93 ± 2.13) mg / dl respectively which were statically significant lower then control group. (p<0.005)

The comparison in haematological parameters among specific groups:

- There was significance difference (p value = 0.01) in mean of WBC And absolute lymphocyte count among specific diagnosis (CLL and NHL only) in patient group.

- The difference between the mean Hb of the control group and non-anemic patient group was statistically insignificant (p-value =0.10).

- The difference between the mean RBC of the control group (4.96 ±0.54) and the non-anemic patient group (4.51 ± 0.62) was statistically significant (p-value =0.005).(Table 4.6)

Table 4.6: The mean differences of hematological parameters in Specific groups

Hematological variable	Specific group	N	Mean ± SD	t-test	P-value
Total WBC count ($\times 10^9$/L)	CLL	19	16.49 ± 16.97	**2.770**	**0.01***
	NHL	22	5.58 ± 2.68		
Absolute lymphocyte count($\times 10^9$/L)	CLL	19	11.24 ± 16.31	**2.623**	**0.01***
	NHL	22	1.41± 0.70		
Hb level (g/dl)	Non-anemic patient	29	13.86 ± 1.03	**-1.628**	**0.10**
	Control	30	14.37 ± 1.32		
RBC count($\times 10^9$/L)	Non-anemic patient	29	4.51± 0.62	**-2.941**	**0.005***
	Control	30	4.96± 0.54		

4.4: Erythropoietin (Epo)

4.4.1: Comparison Between Study Groups

The mean differences of Epo concentration (U/L) according to ELISA technique demonstrate statically significant($p<0.0001$) higher level among patient group (53.93 ± 52.57) than control group (12.73 ± 4.72), as shown in (Fig. 4.10)

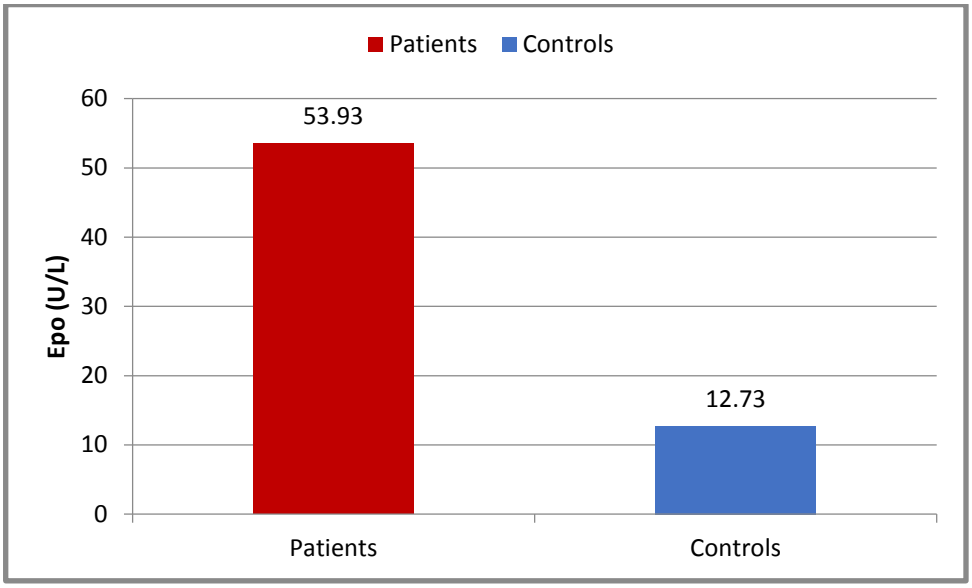

Figure 4.10: The mean differences of Epo (U/L) in patients and control group (N=90, P<0.001*)

The difference in the mean serum erythropoietin levels between the anemic and non-anemic patients was statistically significant (P<0.0001). Furthermore, non-anemic patients had insignificantly higher mean serum erythropoietin than the control group (P=0.085). (Fig. 4.11)

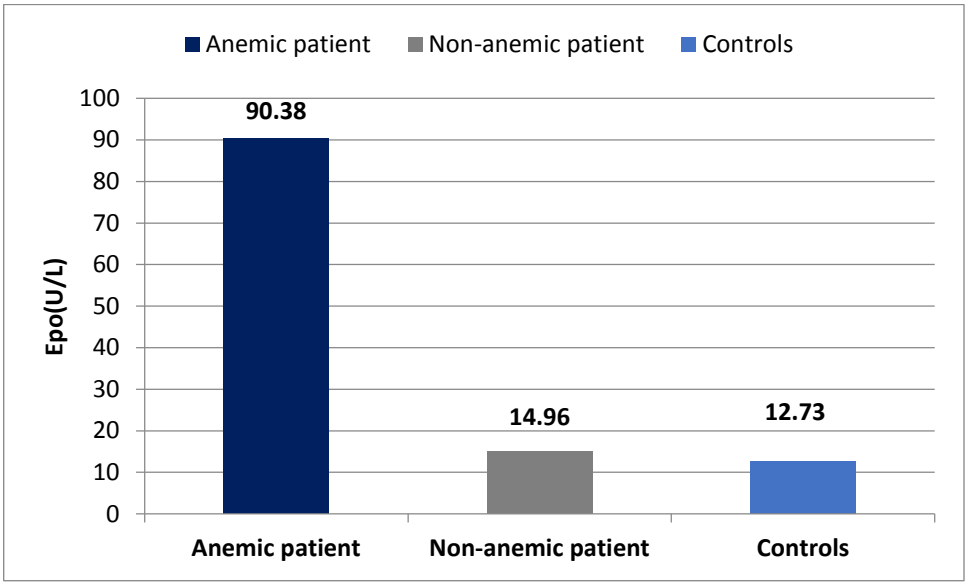

Fig.4.11: Mean serum erythropoietin (U/L) in the control group, anemic and non-anemic patients groups.

4.4.2: Epo Distribution with Gender and Age

4.4.2.1: Epo Distribution with Gender

Females in the study groups had higher mean serum erythropoietin (46.14 ± 57.77 U/L) than males (35.73 ± 38.78 U/L). However, this difference was statistically not significant (P-values =0.3). (Table 4.7), (Fig.4.12)

Table 4.7: Mean serum erythropoietin level (U/L) in the study groups according to sex

Investigated variable	Gender	N	Mean ± SD	t-test	P-value
Epo (U/L) Control group	Female	12	11.79 ± 4.45	0.888	0.38
	Male	18	13.36 ± 4.92		
Epo (U/L) Patient group	Female	24	63.32 ± 64.38	-1.132	0.26
	Male	36	47.67 ± 42.82		

*P ≤ 0.05 was significant

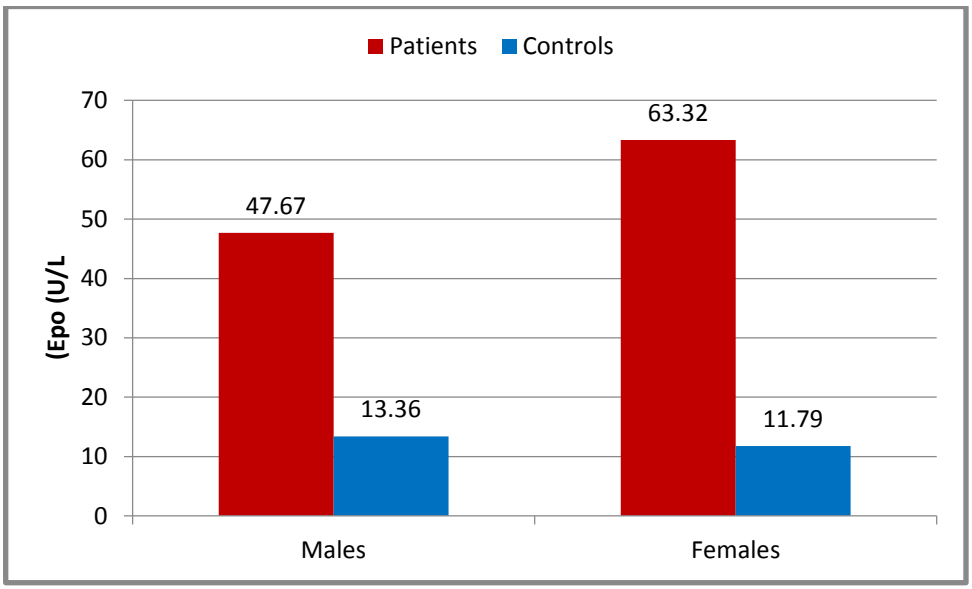

Fig.4.12: Mean serum erythropoietin level (U/L) in the study groups according to sex

4.4.2.2: Epo Distribution with Age

The mean serum erythropoietin level showed no significant difference across study age groups (P=0.51). Tables(4.8_4.10) ,(Fig. 4.13).

Table 4.8: Mean serum erythropoietin (U/L) distribution across the age groups of the controls

Age Group	Number of subjects	Mean Serum Erythropoietin ±SD
20 – 29	1	18.36
30 – 39	1	7.39
40 – 49	4	13.56 ± 1.96
50 – 59	11	13.39 ± 5.47
60 – 69	10	11.60 ± 4.83
70 – 79	3	12.88 ± 4.8
Total	30	12.73 ± 4.72

Table 4.9: Mean serum erythropoietin (U/L) distribution across the age groups of anemic patients.

Age Group	Number of subjects	Mean Serum Erythropoietin ±SD
30-39	3	72.09 ± 38.33
40-49	1	97.23
50-59	12	82.96 ± 29.55
60-69	11	110.54 ± 73.87
70-79	4	69.25 ± 26.08
Total	31	90.38 ± 50.69

Table 4.10: Mean serum erythropoietin (U/L) distribution across the age groups of non-anemic patients

Age Group	Number of subjects	Mean Serum Erythropoietin ±SD
20 – 29	1	12.02
30 – 39	1	10.82
40 – 49	7	14.27 ± 4.19
50 – 59	7	12.88 ± 3.77
60 – 69	8	15.72 ± 5.46
70 – 79	5	19.04 ± 6.37
Total	29	14.96 ± 5.03

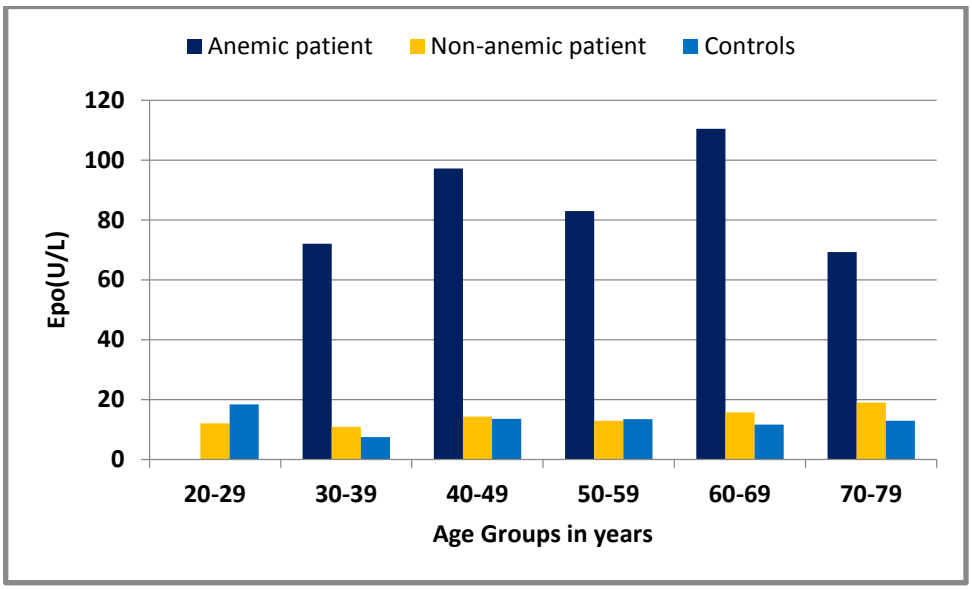

Fig. 4.13: Mean erythropoietin level (U/L) in age groups.

4.4.3: Mean Arythropoietin Level and Specific Diagnoses

Mean serum EPO level show a significant difference between CLL patients and NHL patients only (P=0.03). (Fig. 4.14)

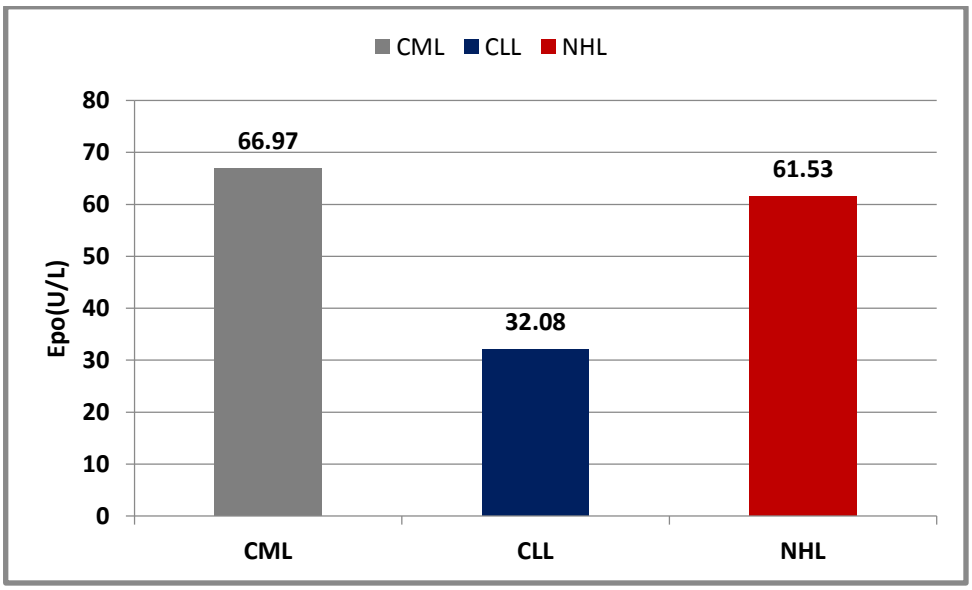

Fig.4.14: Mean erythropoietin level and specific diagnoses

4.4.3.1: Mean Erythropoietin Level and CML

There were statistically significant differences in means of Epo level (U/L) between control group and CML patients, as well as between anemic and non-anemic CML patients (P<0.05). Table (4.11)

Table 4.11: Mean serum erythropoietin level (U/L) in the study groups with CML

Investigated variable	Gender	N	Mean ± SD	t-test	P-value
Epo (U/L) Study Group	CML	19	66.97 ± 73.48	3.213	0.005*
	Control	30	12.73 ± 4.72		
Epo (U/L) CML patients	Anemic	10	115.92 ± 71.9	4.539	0.001*
	Non-Anemic	9	12.58 ± 3.24		

*P ≤ 0.05 was significant

4.4.3.1.1: Correlation Between Mean of Epo(U/L) and Hemoglobin(g/dl) in Anemic CML Patients

Serum erythropoietin levels in Anemic CML patients showed negative correlation with Hb (r=- 0.492), but statically not significant (P=0.14).(Fig.4.15)

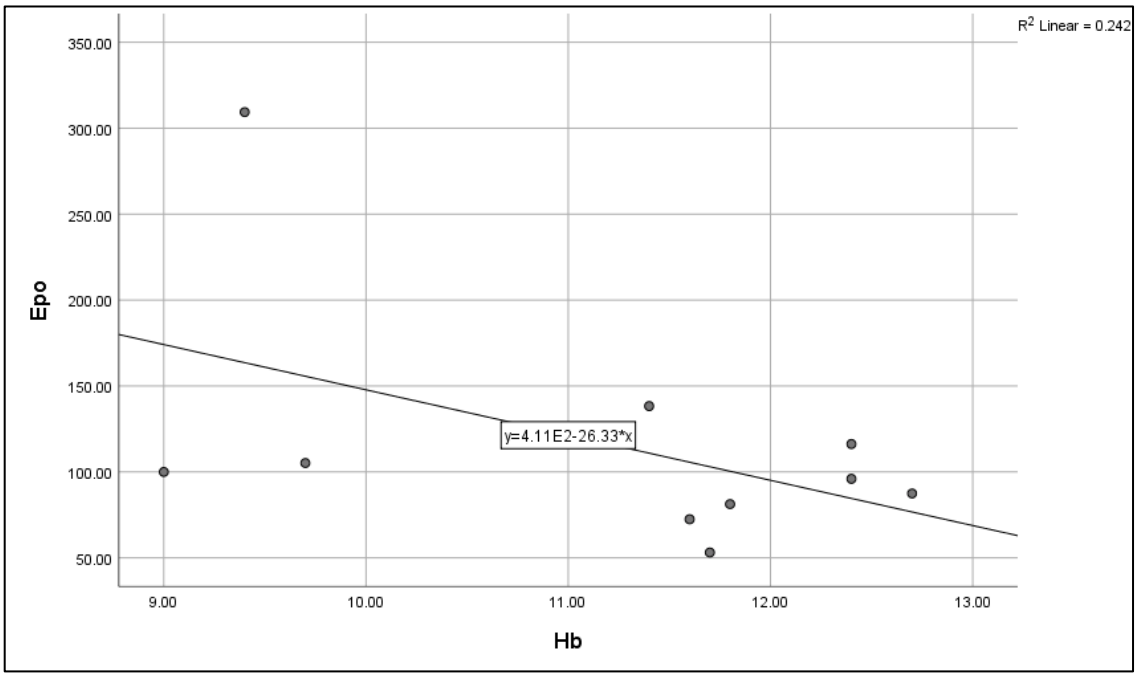

Fig.4.15: Show correlation between Epo(U/L) and Hb(g/dl) in anemic CML patients

4.4.3.1.2: Evaluation of Adequacy of Serum Epo Level in Anemic CML Patients

We calculated adequacy of serum Epo using O/P ratio= $\log_{10}$(EPO observed)/$\log_{10}$(EPO predicted) for each anemic CML patient ,O/P Ratio ≤0.9 indicate inadequate Epo For degree of anemia and we found that only 10% (one patient) had inadequate Epo and 90% (9 patients) had adequate Epo concentration for degree of anemia. (Figure 4.16)

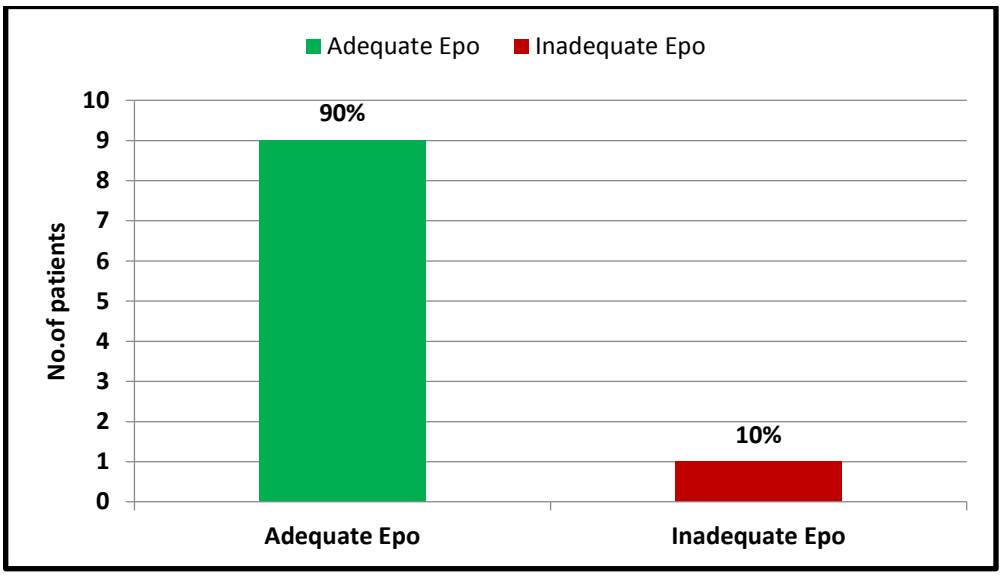

Fig.4.16: Adequacy of serum Epo in anemic CML patients

4.4.3.2: Mean Erythropoietin Level and CLL

There were statistically significant differences in means of Epo level (U/L) between control group and CLL patients, as well as between anemic and non-anemic CLL patients (P<0.05). Table (4.12**)**

Table 4.12: Mean serum erythropoietin level (U/L) in the study groups with CLL

Investigated variable	Gender	N	Mean ± SD	t-test	P-value
Epo (U/L) Study Group	CLL	19	32.08 ± 24.38	-3.418	0.003*
	Control	30	12.73 ± 4.72		
Epo (U/L) CLL patients	Anemic	8	55.81 ± 19.5	5.797	< 0.0001*
	Non-Anemic	11	14.83 ± 5.14		

*P ≤ 0.05 was significant

4.4.3.2.1: Correlation Between Mean of Epo(U/L) and Hemoglobin(g/dl) in Anemic CLL Patients

Serum erythropoietin levels in Anemic CLL patients showed negative correlation with Hb (r= - 0.505) but statically not significant (P=0.2). (Fig.4.17)

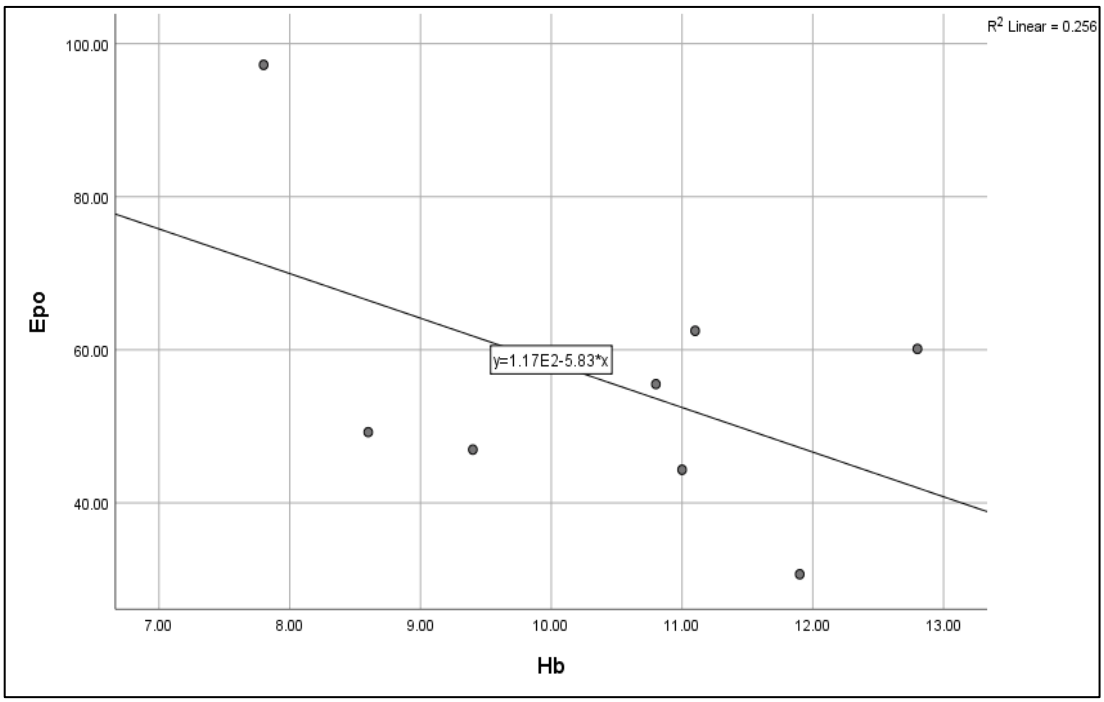

Fig.4.17: Correlation between Epo(U/L) and Hb(g/dl) in anemic CLL patients

4.4.3.2.2: Evaluation of Adequacy of Serum Epo Level in Anemic CLL Patients

We calculated adequacy of serum Epo using O/P ratio= $\log_{10}$(EPO observed)/$\log_{10}$(EPO predicted) for each anemic CLL patient ,O/P Ratio ≤0.9 indicate inadequate Epo For degree of anemia and we found that 50% (4 patients) had inadequate Epo and 50% (4 patients) had adequate Epo concentration for degree of anemia. (Figure 4.18)

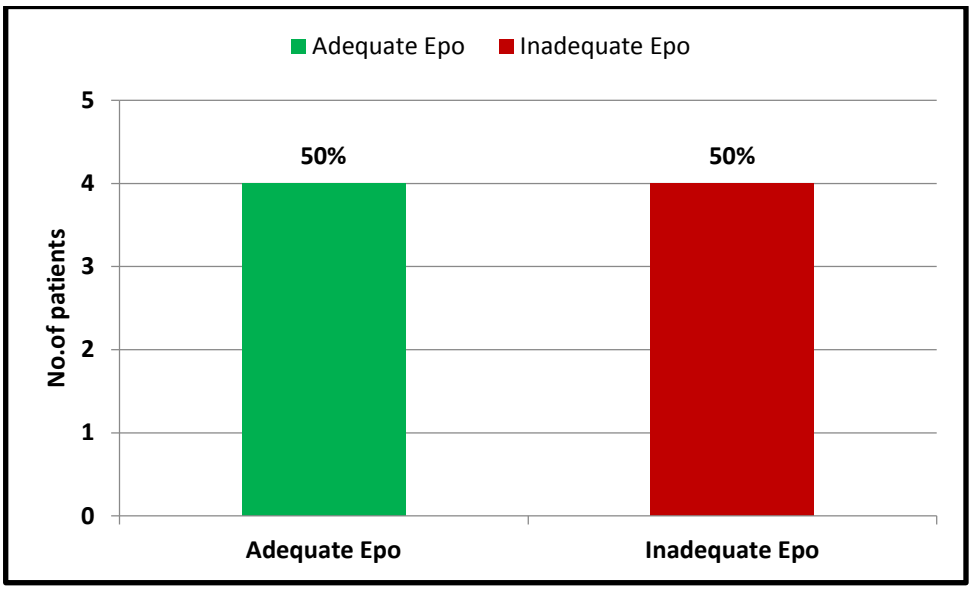

Fig.4.18: Adequacy of serum Epo in anemic CLL patients

4.4.3.2.3: Comparison with the Modified Rai Staging System

In anemic CLL patients there were increase in serum Epo(U/L) and decrease in Hb(g/dl) in relation to increase staging but there were statically not significance (p>0.05).(Table 4.13), (Fig.4.19)

Table 4.13: The mean differences of Epo(U/L) according to Modified Rai Staging System in anemic CLL patients

Study variables	Modified Rai stage	N	Mean ± SD	F	P-value
Epo (U/L)	Low risk	4	48.24 ± 13.9	**0.539**	**0.614**
	Moderate risk	1	60.11		
	High risk	3	64.47 ± 28.39		

*P ≤ 0.05 was significant

-However all high risk CLL patients in the study had low serum Epo in relation to degree of anemia.

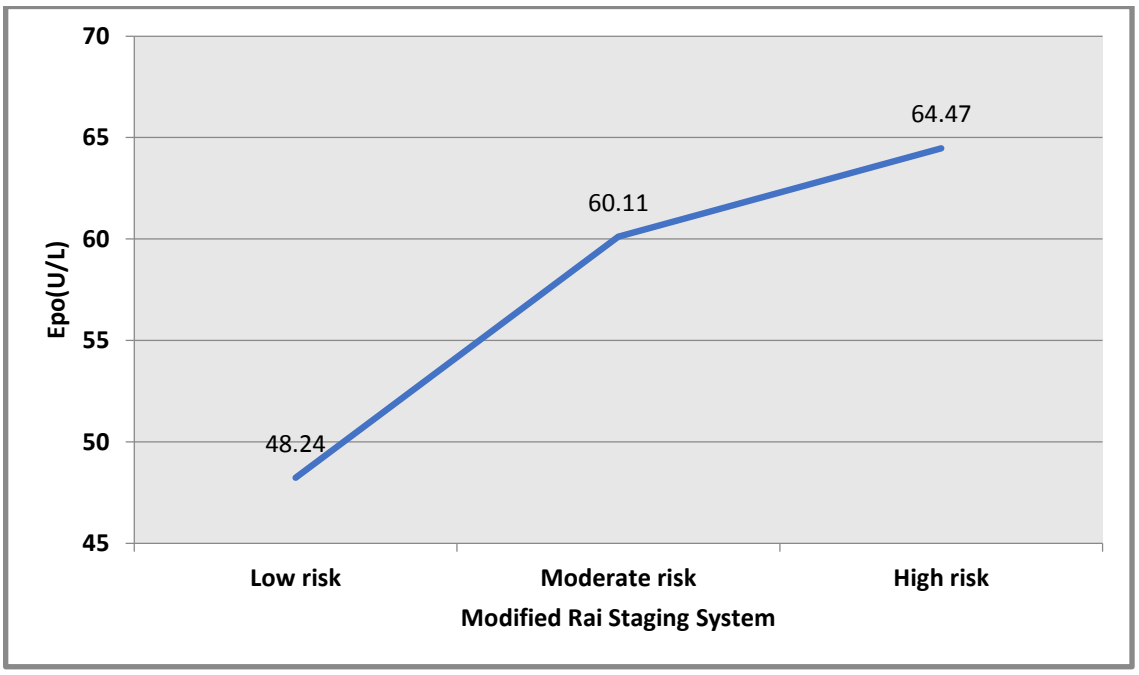

Figure 4.19: The mean differences of Epo(u/l) according to Modifiied Rai Staging System in anemic CLL patients (N=8, P=0.614)

4.4.3.3: Mean Erythropoietin Level and NHL

There were statistically significant differences between means of Epo level (U/L) between control and NHL patients, as well as between anemic and non-anemic NHL patients (P<0.05). (Table 4.14**)**

Table 4.14: Mean serum erythropoietin level (U/L) in the study groups with NHL

Investigated variable	Gender	N	Mean ± SD	t-test	P-value
Epo (U/L) Study Group	NHL	22	61.53 ± 44.49	-5.142	<0.0001 *
	Control	30	12.73 ± 4.72		
Epo (U/L) NHL patients	Anemic	13	90.02 ± 31.34	8.379	< 0.0001*
	Non-Anemic	9	17.5 ± 5.61		

*P ≤ 0.05 was significant

4.4.3.3.1: Correlation Between Mean of Epo(U/L) and Hemoglobin(g/dl) in Anemic NHL Patients

Serum erythropoietin levels in Anemic NHL patients showed negative correlation with Hb (r= - 0.229) but statically not significant(P=0.45). (Fig.4.20)

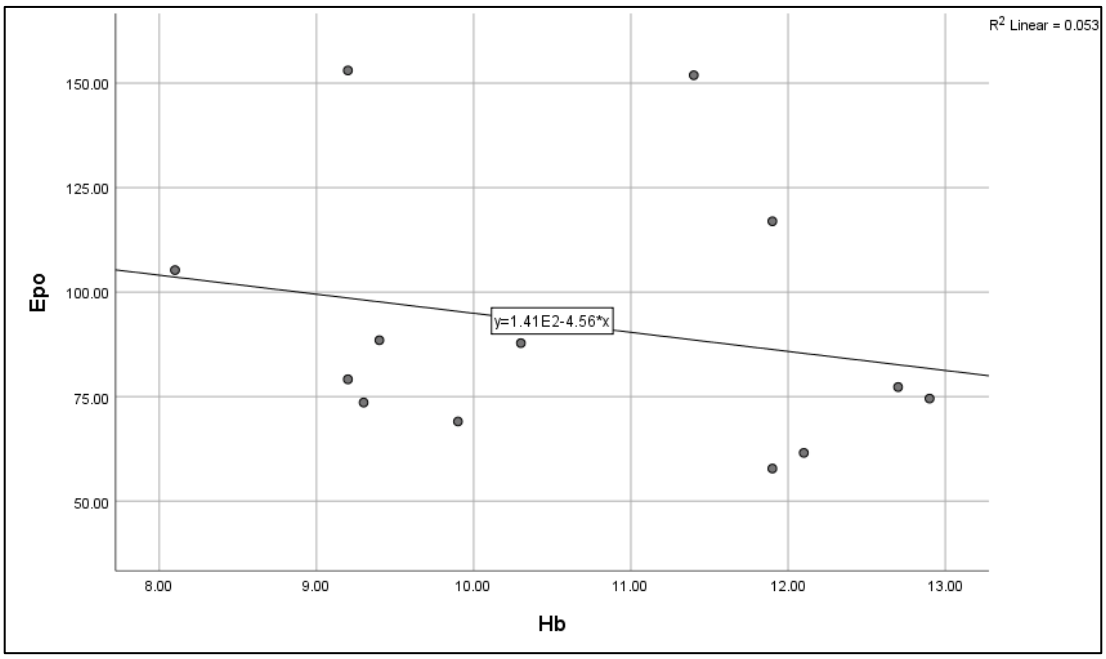

Fig.4.20: Correlation between Epo(U/L) and Hb(g/dl) in anemic NHL patients

4.4.3.3.2: Evaluation of Adequacy of Serum Epo Level in Anemic NHL Patients

We calculated adequacy of serum Epo using O/P ratio= $\log_{10}$(EPO observed)/$\log_{10}$(EPO predicted) for each anemic NHL patient ,O/P Ratio ≤0.9 indicate inadequate Epo For degree of anemia and we found that 38.5 % (5 patients) had inadequate Epo and 61.5% (8 patients) had adequate Epo concentration for degree of anemia. (Figure 4.21)

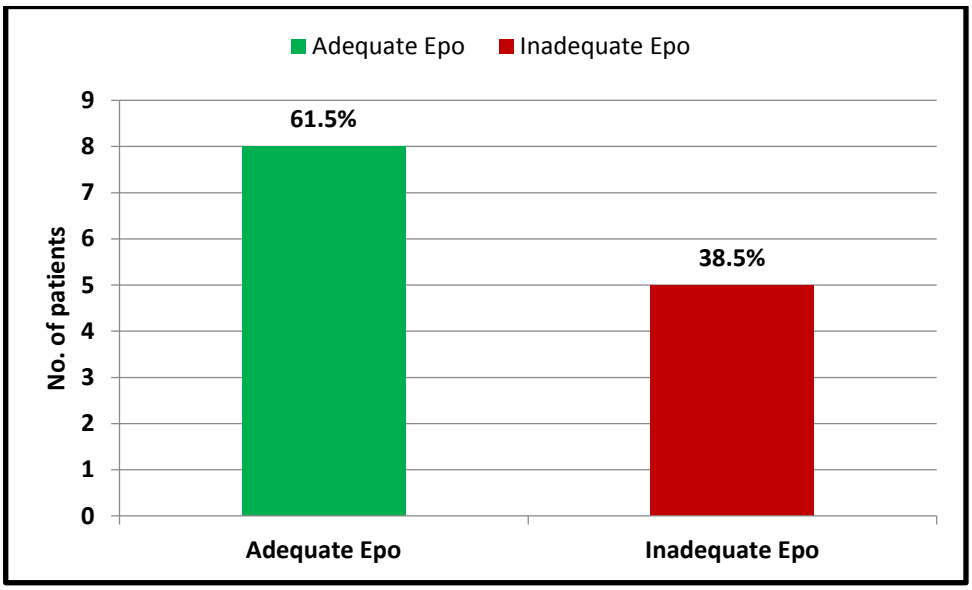

Fig.4.21: Adequacy of serum Epo in anemic NHL patients

4.4.3.3.3: Comparison with the Lugano Staging System

In anemic NHL patients there were increase in serum Epo and decrease in Hb in relation to increase staging but there were statically not significance (p>0.05).(Table4.15), (Fig.4.22)

Table 4.15: The mean differences of Epo(U/L) according to Lugano Staging System in anemic NHL patients

Study variables	Lugano Staging System	N	Mean ± SD	F	P-value
Epo (U/L)	Stage I	2	81.51 ± 9..86	**0.405**	**0.753**
	Stage II	2	75.43 ± 2.61		
	Stage III	5	92.85 ± 38.32		
	Stage IV	4	104.53 ± 38.34		

*P value ≤ 0.05 was significant

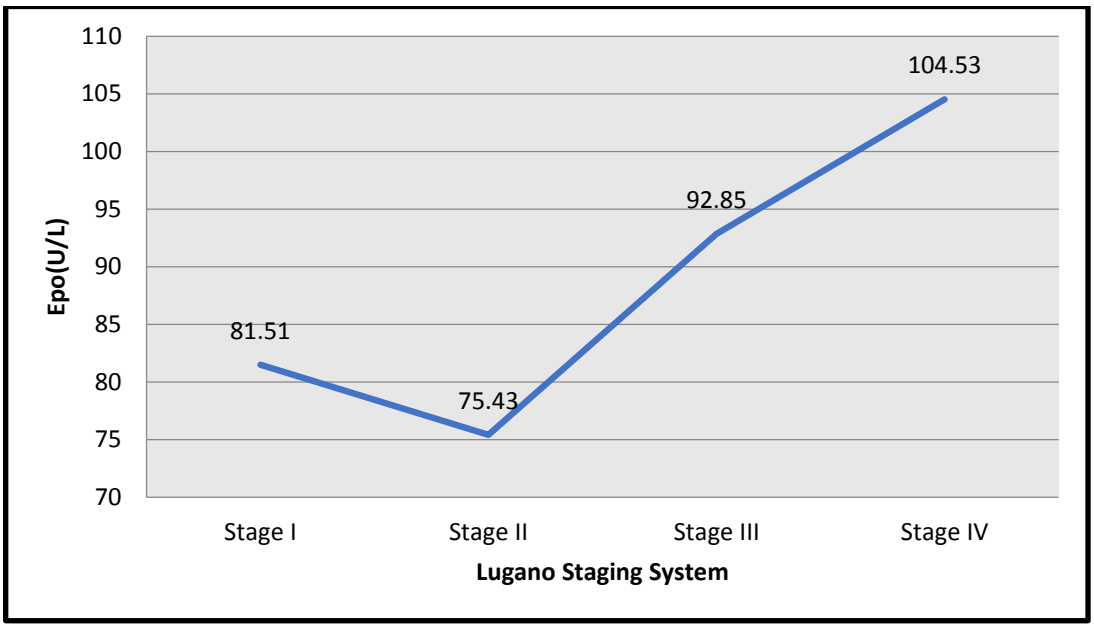

Figure 4.22: The mean differences of Epo(u/l) according to Lugano Staging System in anemic NHL patients (N=13, P=0.753)

CHAPTER FIVE

DISCUSSION

DISCUSSION

Anemia is a frequent problem in CML, CLL and NHL patients leading to poorer outcome and shorter survival.

In hematological malignancies, multiple factors may contribute to development of ACD such as poor utilization of apparently adequate iron stores, excessive cytokine production(such as the tumor necrosis factor-(TNF-), interleukin-1 (IL- 1), tumor growth factor-(TGF-) and -interferon (-IFN)) and a blunted Epo synthesis , BM infiltration ,splenomegaly, radiotherapy and chemotherapy .(113)

In our patients, we found evidence of inadequate endogenous Epo production, as expressed by a reduced O/P ratio. This was particularly evident in anemic patients. In renal failure, anemia is characterized by defective Epo production in the kidney .In all patient of our study, RFT were normal, indicating that renal damage was not the cause of defective Epo production.

5.1: Clinicopathological Assessment

5.1.1: Age Distribution

This study found the mean age of CML patients was 50.1 years range from(24-72 years) which is comparable to that reported by other Iraqi study at 2019 which the mean age in their study 51.7 years (117),and higher than the mean age found in Turkey and Iran (41,44 respectively) this disparity in the main age group is probably related to the genetic background.(118)(119)

The mean age of CLL patients was 58.2 years range from (38-75) years which is comparable to that reported by other Iraqi studies 2019, 2018 which the mean age in their studies were 57.18 and 59.24 years

respectively.(120)(121) . While it was lower than that reported in western countries. (122)(123)These differences may be related to the effect of geographical, environmental factors and population structure between Iraq and Western countries that made life expectancy lower among Iraqi population.

While the mean age of NHL patient was 61.1 years range from (42-73) which is comparable to other studies ,in Iraq 2020 which the mean age in their study was 57.5 years(124) and in USA 2019 which the mean age in their study was 61 years, and its higher than other study in Iraq (125)which the mean age was 53 years ,this might result from delay in seeking medical advice.

5.1.2: Gender Distribution

CML, CLL and NHL (Male/Female) were found to be more in male than female, (13/6) (10/9) (13/9) respectively. This finding is consistent with a studies conducted in Karbala 2019, Baghdad 2021 respectively. (36) (126) The real cause of male predominance is unknown(127).

5.1.3: Staging System Distribution

According to WHO classification system most CML patient were in chronic phase (90 %) while the remaining in blastic phase (10 %) (Figure 4.7),these result consistent with study conduct in Karbala 2021 .(128) This can explained by most patients were diagnosed for long time on treatment.

According to Modified Rai Staging System, most CLL patients were in low risk stage (73.3%) followed by high risk stage (15.8%) while the

lowest percent in moderate risk stage (10.5%) (Figure 4.8),these result consistent with a study in Barcelona 2017 which found that the majority of patients at low risk stage.(129) This may be attributed to the early in seeking medical advice and control of disease progression.

According to Lugano classification system, Most NHL patient were in stage III (36.4%) followed by stage II, IV (22.7%) while the lowest percent in stage I (18.2%) (Figure 4.9) these result consistent with study conduct in Misan 2019,Pakistan 2019.(130)(131) This may be attributed to the delay in seeking medical advice.

5.1.4: Hematological and Clinical Assessment

In the assessment of hematological parameters (Table 4.4-6) . The mean WBC count in CML patients (7.47 ± 5.95) which was not statically significant different from control group, these result consistent with study conduct in Baghdad 2020 which the mean WBC count was (7.9±3.17).(132) Mostly due to most CML patients (90%) in our study were in chronic stage on treatment.

The mean WBC count in CLL patients (16.49 ± 16.97), absolute lymphocyte count (11.24± 16.31) were statically significant higher from control group respectively which were a diagnostic criteria for CLL. This result consistent with study conduct in Erbil 2018.(133)

The mean WBC count in NHL patient (5.58 ± 2.68) which is statically significant lower than control group but still within normal WBC range and this result consistent within study conduct in Baghdad 2007 and Italy 2017. (134)(135) This can be explain by the white blood cells restricted to the lymph nodes , bones marrow and most patient in advanced stage on treatment.

The mean platelet count in patients group was (213.76 ± 80.95) it was with nearly similar result as with other studies.(132)(135)

The mean hemoglobin level was (12.26 ± 2.03) g/dl which was statically significance lower than control group.

The mean hemoglobin level for CML patient was (12.47± 1.76) mg / dl and this result consistent with study conduct in Baghdad 2020.(132) While the number of anemic patients was 10(52.6%) which was comparable with other study conduct in USA 2004.(136)

The mean hemoglobin level for CLL patient was (12.44± 2.2) mg / dl and this result consistent with study conduct in Al Mosul 2022.(137)While number of anemic patients was 8 (42.1%) and this result consistent with study conduct in Croatia 2011.(138) Which is higher than other study, this difference could probably be because of the small sample size of CLL patients in our study. This parameter (anemia) representing a determinant for high risk stage in Binet and modified Rai staging Systems(139)(78).

The mean hemoglobin level for NHL patient was (11.93± 2.13) mg / dl while number of anemic patients was 13 (59.1%) and this result consistent with study conduct in Mosul 2021,Pakistan 2019 .(140)(131) This can explain by most patients in developing country diagnosed in advance stage of disease and this can be one factor for high prevalence of anemia.

5.2: Erythropoietin (Epo)

5.2.1: Level of Epo in patients and control group

In this study, we used quantitative ELISA kit to assess the level of serum Epo in patient and control groups. Serum EPO level is useful in investigating whether defective EPO production contributes to anemia. We discovered that Epo level was significantly higher in patient group compared to control group (p-value <0.0001) (Figure 4.10). This result was in agreement with other workers in many previous studies which reported higher mean of Epo in CML, CLL, NHL patients.(141)(142)(143)

Furthermore, anemic patients (51.7%) had statically significant higher (p<0.0001) EPO levels compared to non-anemic patients. Similar findings have been reported by other studies.(144)(145)(146)(143) This is most likely due to EPO response to anemia in anemic patients.

Instead of only quantitating serum EPO levels in absolute terms, we evaluated them in relation to the degree of anemia. Serum EPO levels were inappropriately low for the degree of anemia in (10%) of CML, (50%) of CLL and (38.5%) of NHL patients as indicated by the low O/P ratios ≤0.9 (Fig. 4.16, 4.18, 4.21).

5.2.2: Correlation Epo Level with Gender and Age

In the present study, the level of Epo concentration was higher in females than males (Table 4.7); however, the difference is not significant, these finding may explained by females more prone for iron depletion than males. In addition there was no significant correlation between Epo level and the age groups of patients (Table 4.9-10).These finding in agreement with other study(147)(148),however Epo high in higher age group patients , probably as a compensatory mechanism for peripheral tissue hypoxia, subclinical blood loss, increased red blood cell turnover, or increased erythropoietin resistance of red cell precursors.(149)

5.2.3: Correlation of EPO Level with Specific Diagnosis

Serum EPO levels were inappropriately low for the degree of anemia in (10%) of CML patients while (90%) of patients have adequate Epo for degree of anemia (O/P >0.9) and these finding in agreement with other study.(141)This might be explained based on fact that multiple factors contribute in development of anemia and myelosuppression occurs in up to 50% of patients with chronic myeloid leukemia (CML) who are treated with imatinib and ≥ Grade 3 myelosuppression is reported in approximately 10% of these patients which may lead to higher serum Epo .(136)

Serum EPO levels were inappropriately low for the degree of anemia in (50%) of CLL patients and (38.5%) of NHL patients (O/P≤ 0.9) and these finding in comparable with other studies.(150) (151)(113)Many factors responsible for blunt erythropoietin production include cytotoxic drugs, excessive release of cytokines such as IL-1 and TNF. The blunted EPO response, however, is unlikely to be the only factor responsible for anemia in the above conditions. Erythroid marrow can be suppressed directly by inhibitory cytokines and by chemotherapy or radiotherapy themselves.(152)The percent of defective Epo production for degree of anemia lower than in other studies (153) might result from small sample size.

All high risk CLL patients had higher serum Epo level but still low for degree of anemia and this indicate that blunt Epo response was the major role in developing anemia. (Table 4.13)

CHAPTER SIX

CONCLUSIONS

&

RECOMMENDATIONS

6.1: Conclusions:

1. The mean difference of Epo concentration(U/L) is higher in patients than controls group with significant correlation between two groups .(Fig. 4.10)
2. The level of Epo (U/L) in anemic patients was higher than non-anemic patients with significant correlation .(Fig. 4.11)
3. There was defect in Epo production in CML ,CLL , NHL as 10%, 50%, 38.5% respectively.

These findings indicate that anemia associated with hematologic malignancy may result from an inappropriately low Epo response. Epo treatment should benefit in this group of patients.

6.2: Recommendations:

1. Further studies that include larger numbers of patients with longer time of follow up.
2. Give recombinant Erythropoietin as a therapeutic drug in treatment of anemia and improve quality of life in patients with hematological malignancy.

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APPENDIX

The questioner used in this study:

Flow No.	Coding No.			Tel. No.
Name:				
Age:				
Gender:	▪ male			▪ female
Complications:				
Lymphadenopathy:	▪ cervical	▪ axillary		▪ inguinal
Organomegally:	▪ splenomegaly			▪ hepatomegaly
CBC:	Total WBC	Absolute lymphocyte count	Hb level	Platelet count
PCV	MCV	MCH	MCHC	RBC
Retic % if done:				
Coombs test if done:				
Diagnosis by:	flowcytometry			Bone marrow
Stage:				
urea				
Received Erythropoietin ?				
On Teatment?				