Assessment of *P53* gene mutations and Immunophenotyping patterns in Acute Leukemia Patients in Nanakali Hospital- Erbil

A Thesis
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DECLARATION

I declare that the Master Thesis entitled: **Assessment of *P53* gene mutations and Immunophenotyping patterns in Acute Leukemia Patients in Nanakali Hospital- Erbil**, is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

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Dedication

This thesis is dedicated to:

My mother and memory of my father…

My lovely wife…..

My always encouraging, ever faithful brothers and sisters…

Ashqi Mohammed Kareem

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SUMMARY

Hematological malignancies are among the many diseases that exhibit *P53* mutations. The main aim of the present study to assess the prevalence of *P53* gene mutations among Acute Myeloid Leukemia and Acute Lymphoblastic Leukemia cases. We conducted a comprehensive evaluation using *P53* mutational screening through hematological changes, bone marrow aspiration reports, PCR, and gel electrophoresis in the current research to assess the *P53* mutation frequencies in AML and ALL patients.

This study evaluated 61 patients of Acute Leukemia referred from Nanakaly Hospital for Blood Diseases and Cancer in Erbil-City, from July 1, 2021, to March 11, 2022. For a total of 61 patients (29 patients with Acute Myeloid Leukemia and 32 patients with Acute Lymphoblastic Leukemia), which had been selected for the study depending on the Complete Remission/Partial Remission association, we compared the CBC parameters, immunophenotyping CDs, and bone marrow reports. Of these, 40 samples (20 from AML and 20 from ALL) were followed up for DNA extraction, PCR amplification and visualized by Gel Electrophoresis.

Overall 61 patients (29 AML patients achieved CR 24(82.7%) and PR 5(17.3%) and (32 ALL patients achieved CR 25(79.3%) and PR 7(20.7%). One of the important findings of our study, the *P53* gene was mutated in all of AML and ALL patients. The most frequent positive CDs in AML patients, includes (CD13, CD33, MPO, HLADR, CD64, CD117, CD34), and the mean of them are (75%, 70%, 60%, 60%, 55%, 55% and 50% respectively) , and according to CR/PR association those CDs were statistically showed significant (CD64, CD117, CD13, CD33, CD34, MPO, TdT and CD38,), p-values (<0.0001, <0.0001, 0.0012, 0.0012, 0.0067, 0.0103, 0.0209 and 0.0235 respectively). The most frequent CDs in ALL patients, includes (CD19, CD79a, TdT, HLADR, CD10, CD22 and CD34), the mean of them are (95.24%, 95.24%, 95%, 90%,

85.71%, 80% and 50% respectively), and according to CR/PR association, those CDs markers(CD2, CD10, CD19, CD22, CD34, CD79A and TdT) were statistically showed significant, with p-values (<0.0001, <0.0001, <0.0001, <0.0001, <0.0001, 0.0003 and 0.0124 respectively). The majority of bone marrow aspirates in AML cases during the post-induction stage were primarily hypercellular(100%) in CR group and hypercellular (85%) in PR group, and the p-value depending on CR/PR ratio showed mildly significant for Cellular fragments (0.0068), also the blast percentages were significant. For instances of ALL, the bone marrow aspiration reports were primarily hypercellular(100%) in CR group and hypercellular (45%) in PR group, and the p-value of CR/PR ratio showed strongly significant(<0.0001). The present study identified by Sanger sequencing, 28 mutations from 17 mutated samples(from 20 samples of AML and 20 samples of ALL), which includes, 17 mutations from 10 samples of AML and 11 mutations from 7 samples of ALL.

We conclude that *P53* were highly mutated in AML and ALL cases and immunophenotyping CD markers significantly expressed in acute leukemias, also the reports showed the hypercellular bone marrow.

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List of Abbreviations

The following table explains the meaning of several acronyms used in the thesis. The abbreviations that are used for one time are not mentioned in this list.

Abbreviations	Meaning
ABC	avidin–biotin complex phosphatase
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloblastic Leukemia
APAAP	Alkaline Phosphatase Anti Alkaline
ATM	ataxia telangiectasia-mutated
ATR	(ATM Rad3-related)
B-ALL	B Lymphocyte Acute Lymphoblastic Leukemia
BMA	Bone marrow aspiration
BP	Base Pairs
BRCA1	Breast Cancer Gene 1
CALLA	Common acute lymphoblastic leukemia antigen
CGA	Cystine Guanine Cytosine
Chk1	Checkpoint Kinase I
Chk2	Checkpoint Kinase II

C>T	Cystine Thymine
C terminal	Carboxy terminal
CBC	Complete blood count
CR	Complete remission
CD	Clusters of differentiation
CLL	Chronic lymphoid leukemia
CK-AML	Complex karyotype- Acute myloid leukemia
DBD	DNA-binding domain
DL	Deciliter
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EDTA	Ethylenediamine tetraacetic acid
FAB	French America British
FCM	flowcytometry
FCI	Flow cytometric immunophenotyping
g/dl	Gram per Decilitre
G0	Growth phase zero
G1	Growth phase one
G-C	Guanine-Cytosine
Hb	Hemoglobin
HLADR	Human leukocyte antigen –DR type
ICC	Immunocytochemistry
IHC	immunohistochemistry
LDH	Lactate Dehydrogenase
MPO	Myeloperoxidase
MoAb	Monoclonal antibody
MgCl2	Dichloride megniseum
MDM2	Mouse double minute-2-homolog
MDM4	Mouse double minute-4-homolog

MDS	myelodysplastic syndrome
Mmol	Millimole
MRD	minimal residual disease
µM	Micrometre
µL	Microlitere
NGS	Next generation sequencing
NHL	nonHodgkin's lymphoma
N-terminal	Amino terminal
nsSNVs	nonsynonymous single-nucleotide variants
OD	oligomerization domain
OD_{260}	Optical Density at 260 nanometer of wavelengths
PCR	Polymerase chain reaction
PR	Parial remission
PRD	proline rich domain
PPM1D	Protein phosphate, Mg2+/Mn2+ dependent 1D
Phe19	Phenylalanine 19
P53	Tumour Protein 53
P53RE	Tumour Protein 53 Responive Element
PIKKs	phosphoidylinositol-3-kinase-related kinases
RB	Retionblastoma
RNA	Riboxyribonucleic acid
ROS	reactive oxygen species
RT-PCR	Real time-Polymerase chain reaction
RNase	Ribonuclease
S phase	Synthesis phase
SSCP	single-stranded conformation polymorphism
t-AML	Therapy related Acute Myeloid Leukemia
TP53/RB	Tumour protein 53/retinoblastoma
TA	transactivation domain

Ta	Annealing temperature
TBE Buffer	Ttis-borate EDTA buffer
TdT	Terminal deoxynucleotidyl transferase
Tm	Melting temperature
TP53	Tumour protein 53 gene
T-Cell	T lymphocyte
TP53 CN	Tumour protein 53 gene copy number
Taq	Thermus aquaticus
UK	United Kingdom
UV	Ultraviolet
VAF	variant allele frequency
WT-P53	wild-type
WHO	World Health Organization
≥	Greater than or equal to
>	Greater than
<	Less than
~	Approximately
%	Percentage

INTRODUCTION

1. INTRODUCTION

The P53, is a gene located on the short arm of chr-17 codes for the nucleus phosphoprotein(17p13 band)(Gruszka-Westwood, 2001). After being discovered to be the common denominator in over fifty percent of human malignancies, the TP53 gene and its protein initially became known in 1979 and have since gained prominence as a result of an upsurge in study(George et al., 2021). The P53-tumor suppressor gene is a crucial pace in the developing of human tumours, the loss of P53 function is related to a number of tumor traits, including cell cycle dysregulation, genome instability, and chemotherapy resistance(Joruiz and Bourdon, 2016). Solid cancers have the highest rate of P53 gene mutations(Li et al., 2019a). P53 mutations are less communal in hematological tumours(Cheung et al., 2009, Szymczyk et al., 2018).

P53 is one of members of the tumor suppressor family genes (Sanaei and Kavoosi, 2021), which includes genes with oncogenic loss-of-function variants. Numerous different types of human tumors have been discovered to have the P53 gene inactivated by point mutations, deletions, or rearrangements(Bougeard et al., 2008, Di Fiore et al., 2013). Lately, it was confirmed that point mutations in the other P53 allele causes loss of the protein's normally growth-inhibitory action in a series of human malignancies(Marei et al., 2021). One of the genes with the highest frequency of mutations found in human tumors is P53(Bouaoun et al., 2016). Additionally, P53 alterations are common in AML that develops as a result of prior chemotherapy(McNerney et al., 2017, Welch, 2018). These findings have given rise to the hypothesis that P53 alterations in acute leukemia are less significant than those in other tumor types(Merino et al., 2018, Arber, 2019).

Acute Myeloid Leukemia is a malignancy that is aggressive and has an annual age-adjusted prevalence of 3.5 cases per 100,000 people. Above the lifetime of sixty, the occurrence rises to 15-20 cases for each 100,000 (Kukreja, 2005). The typical hematopoietic cells that make up AML have genetic abnormalities. These impacted genes are associated with transcription factors, cell communication molecules, or proteins that control the cell cycle. They typically function as proto-oncogenes or tumor suppressors(Kumar, 2011). In a study that was released in the Asian Public Journal of Cancer, researchers in Erbil (4.59 vs. 11.55 cases/100,000 people) and Duhok (3.52 vs. 9.25 cases/100,000 people) discovered that haematological cancers were the most prevalent type of cancer among children (0–14 years) and adolescents (15–19 years)(M-Amen et al., 2021).

Acute Lymphoblastic Leukemia is a malignant disorder that originates from hemopoietic precursors of B-cell (80-85%) or T-cell (20-25%) derivation; the acquisition of a series of genetic aberrations leads to impaired maturation, with arrest of the differentiation process and abnormal proliferation. As a consequence, the accumulation of leukemic cells occurs in both the bone marrow, where it suppresses the physiologic hemopoiesis, and at extramedullary sites(Chiaretti et al., 2014a). The most recurrent form of malignancy in kids is acute lymphoid leukemia, which also makes up about 25% of cases of acute leukemia in adults(Bhojwani et al., 2015). Adults are more prone than children to develop high-risk P53 disease, and despite aggressive chemotherapy intake &/or allogeneic-stem cell transplantation therapies, adult long-term disease-free survival rates are only around 40%. In stark comparison, pediatric ALL has improved treatment protocols that have led to cure rates that are close to 80%(Stanchina et al., 2020, Makimoto, 2022).

Several symptoms like fatigue are caused by anemia, a frequent clinical complication accompanying acute leukemia. A typical textbook account of ALL involves normocytic normochromic anemia, increased or decreased WBC count, neutropenia and the presence of blasts. Inclusive of thrombocytopenia. Fast treatment follows opportune diagnosis. The presence of specific physical findings, accurate interpretation of the CBC and recognizing symptoms early are extremely important. No recent information focused on detailed analysis of CBC abnormalities, signs and symptoms, and physical findings in children with newly diagnosed ALL was found. Consequently, this report looked into the data from patients that were treated at an academic institution for 10 years(Jaime-Pérez et al., 2019).

Both hematological and non-hematological illnesses can be diagnosed with the help of a bone marrow examination. Bone marrow aspiration and trephine biopsy are the two most crucial procedures used to diagnose hematological diseases. Through a needle aspiration, spongy bone marrow is taken through the invasive BMA method for diagnostic evaluations, including cytology and stem cell harvest(Reshma et al., 2022). The majority of acute leukemias and nutritional anemias can be diagnosed with BMA alone. BMA samples can be used in additional diagnostic procedures such as cytochemical/special stainings, immunophenotyping, microbiological examinations, cytogenetic analysis, and molecular research. The objective was to research the range of hematological conditions identified by bone marrow aspiration(Khan et al., 2018).

One of the newly emerging importance of immunophenotypical aberrancies using FCM is the detection and quantification of minimal residual disease (MRD) for providing prognostic information, and make use of such aberrancies in routine management of patients to guide therapy. The clinical

characteristics and relevance of the co-expression of two or more aberrant lineage leukemia markers are still being thoroughly analyzed(Abdulateef et al., 2014). A panel of monoclonal antibodies which consisted of B cell lineage markers (such as: cd10 cd19 cd20 cd22 cyt cd79a kappa lambda light chains surface igm) t-cell lineage markers (CD2 surface cytoplasmic CD3 CD5 CD7 CD4 CD8 and CD1 a) myeloid markers (CD13 CD14 CD15 CD33 CD64 CD117 CD11c CytoMPO) miscellaneoussmarkers (CD34 CD45 CD56 CD38 HLA-DR and Tdt), was utilized in order to draw conclusions regarding this technique(Abdulateef et al., 2014).

This study's objectives are:

To assess the prevalence of P53 gene mutations acute leukemia cases and to find out allele frequencies of P53 mutations to determine the association of P53 mutations with clinical and hematological profiles. The objectives of this study was to determination the following parameters:

1- Evaluation and comparing of complete blood count results before starting chemotherapy.
2- Evaluation and comparing CD markers depending on complete remission and partial remission.
3- Evaluation and comparing bone marrow aspiration (BMA) reports.
4- Measuring and determination the frequency of P53 mutation in in AML and ALL patients.

LITERATURE REVIEW
AND
THEORETICAL BACKGROUND

2. LITERATURE REVIEW AND THEORETICAL BACKGROUND

2.1 ACUTE LEUKEMIAS

2.1.1 Acute Myeloid Leukemia

Acute myeloid leukemia develops when the healthy hematopoietic cells have chromosomal anomalies. The expression patterns of abundant important genes elaborate in the control of cellular activity are impacted by these genetic abnormalities. These impacted genes are associated with transcription factors, cell communication molecules, or proteins that control the cell cycle. Typically, they serve as tumor suppressors or potential oncogenes (Preudhomme et al., 2002). The primary way that tumor suppressors carry out their function is by taking part in cell differentiation and healing. One of the more important genes that suppresses tumor growth is P53. P53 activates various substances that play a role in cell cycle checkpoints, autophagocytosis, cell differentiation, cell senescence, as well as regulates the reaction to DNA degradation as a result(Goudarzipour et al., 2017).

Numerous P53 gene abnormalities, such as deletions, insertions, point mutations, and epigenetic alterations, occur frequently in the wide range of human malignances (Hou et al., 2015). This high rate of anomalies in cancer may be attributed to P53's crucial function in conjunction with a number of other cell regulatory proteins. despite the fact that the TP53 mutation in AML has stood extensively studied and is known to be a bad prognosis factor, the consequences of cases with low P53-frequency mutations (variant allele frequency (VAF) 20%) have not yet been thoroughly studied. Two databases were recently examined for patients with low-frequency TP53 mutations, and the incidence rate is described in (figure 2.1). It's significant that both

researches demonstrated that low TP53-frequency mutations require similarly bad consequences to high TP53-frequency mutations (Prochazka et al., 2019, Yan et al., 2020)

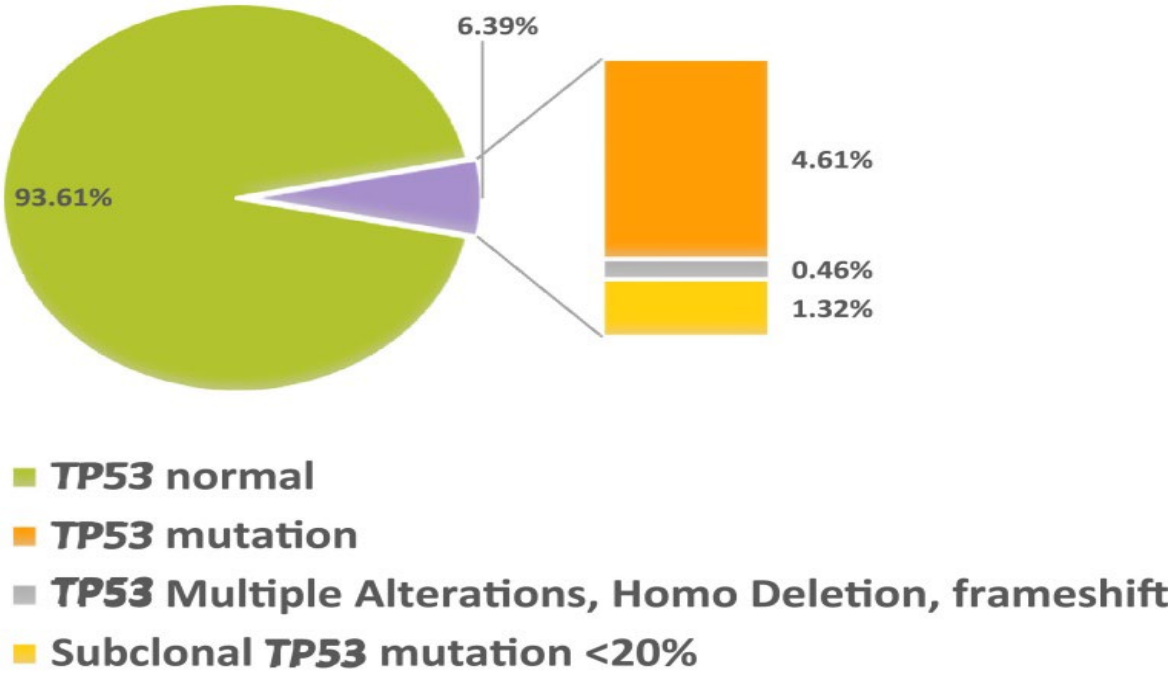


Figure 2.1: TP53 state in people with AML. TP53 gene variant analysis using patient information from the European Genome-Phenome Archive (www.ebi.ac.uk/ega), Accession No. EGAS00001000275) and the Cancer Genome Atlas (TCGA) data portal (https://tcga-data.nci.nih.gov/tcga).

2.1.2 Acute Lymphoblastic Leukaemia

Acute lymphoblastic leukemia is the type of childhood cancer that occurs most frequently. which also makes up approximately 25% of cases of acute leukemia in adolescence (Redaelli et al., 2005). Adults are more prone than children to develop high-risk B-ALL disease, and despite aggressive chemotherapeutic &/or allogeneic stem cell transplant procedures, adults long term malady rates of survival are only 40%. In stark comparison, pediatric ALL has improved treatment protocols that have led to cure rates that are close to

80%(Bhojwani et al., 2015). Nevertheless, despite this high cure rate, certain childhood with ALL experience with a bad prognosis; 15% of them pass away from ALL relapses(Gowda and Dovat, 2013).

One of the genes with the great incidence of mutations in human tumors has been identified as P53(Baugh et al., 2018). The investigation into TP53 mutations has been lacking in all. The increase in incidence occurs during relapse and affects mainly children who have a poor outcome. The frequency of TP53 mutated ALL seems to be marginally greater in adults compared to children: while the reported prevalence is 2% in childhood B-ALL.(Chiaretti et al., 2013). T-lineage ALL was shown to have higher rates of TP53 mutations than B-lineage ALL, and these mutations were discovered in 14% of cases without recurring fusion genes. (Stengel et al., 2014). In all, three involved exon 4, nine exon 5, six exon 6, nine exon 7, nine exon 8, and none exon 9. The majority of TP53 mutations were discovered in exons 5-8(Hou et al., 2015) (figure 2.2).

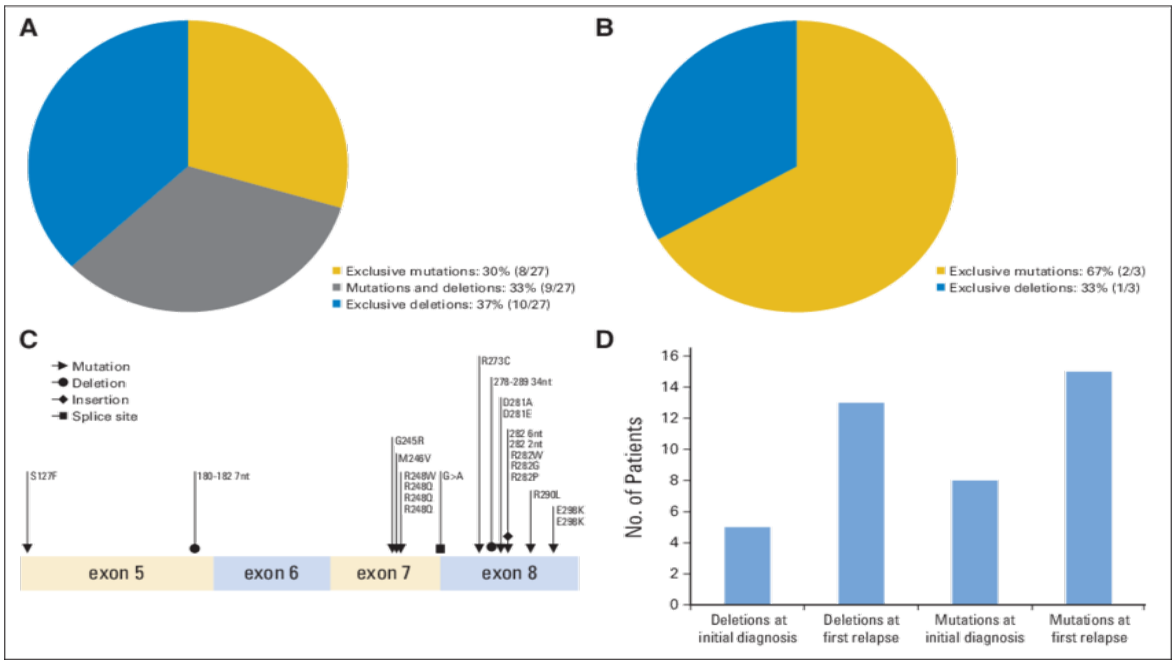


Figure 2.2: P53 mutations in the first pediatric ALL relapse. (A) The incidence of P53 mutations in B-ALL cases with relapse. (B) The incidence of P53

mutations in individuals with T-ALL that has relapsed. (C) Dissemination of P53 exons and codons affected by TP53 changes found by directly sequencing in relapsed B-lymphocyte ALL and T-lymphocyte ALL. (D) Retrospective copy number and sequencing study of 23 patients' matched relapse samples from their original ALL and initial samples with P53 alterations(Hof et al., 2011).

2.2 DIAGNOSIS IN ACUTE LEUKEMIAS

2.2.1 Bone Marrow Aspiration in Acute Leukemias

Both hematological and non-hematological ailments can be diagnosed through bone marrow examination. Bone marrow aspiration and trephine biopsy rank as the two most critical diagnostic techniques for hematalogical disorders. Bone marrow aspiration (BMA) involves obtaining spongy bone marrow through needle aspiration for diagnostic evaluations, specifically cytology and stem cell harvest; it is an invasive procedure. One can conduct diagnostic evaluations with it(Reshma et al., 2022). Mosler performed the first bone marrow examination in 1876 by aspirating bone marrow from a leukemia patient using a standard wood drill. Nutritional anemias and the majority of acute leukemias may typically be diagnosed solely by bone marrow aspiration (BMA). BMA is being used for purposes other than evaluating hematopoietic and non-hematopoietic bone marrow cells. BMA specimens are helpful in further diagnostic assays such cytochemical/special stainings, immunophenotyping, microbiologic tests, cytogenetic analysis, and molecular research. Studying the range of hematological conditions identified by bone marrow aspiration was the goal(Khan et al., 2018).

Morphologic assessment of the bone marrow for the evaluation of acute leukemias was initially standardized through pathologic review by the French-

American-British (FAB) cooperative group in 1976, and revised a decade later.19,20 While the small study mentioned previously showed good correlation between morphology in the peripheral blood and bone marrow, the question remains whether morphology is necessary at all for the diagnosis of AML. In fact, at times, the bone marrow may be inaspirable, making morphology moot. In limited subtypes, including acute megakaryoblastic leukemia, acute panmyelosis with myelofibrosis, and acute myeloid leukemia with myelodysplasia-related changes, some have argued that immunohistochemistry performed on a bone marrow biopsy is a crucial adjunct to peripheral blood analysis in order to make the final diagnosis(Percival et al., 2017). It should be noted that one way for the diagnosis of AML with myelodysplasia-related changes to be made in the 2016 WHO classification is with multilineage dysplasia (defined as >50% of cells with dysplasia in at least two cell lines), which can only be assessed on bone marrow sampling(Merino et al., 2018). Although the pathogenesis of the hypocellularity is unknown, specimens of serial biopsy typically show a consistent hypocellularity patterns, demonstrating that this disease is a genuine variant of AML (Horny et al., 2020).

To diagnose or classify ALL, it is typical to perform morphological, along with immunophenotypic analysis, on leukemia-afflicted cells from both peripheral-blood(PB) & aspirated-bone-marrow(BM). A BM core biopsy's histological examination is usually only done in dry tap aspiration cases. It is also done in instances where the smears have insufficient cell numbers. Still, BM biopsy has proven its worth in forecasting patient prognosis for numerous hematologic conditions. The poor investigation conducted so far on the prognostic value of BM histological patterns in ALL needs improvement.

Nonetheless, a gradual elevation in marrow reticulin levels has been connected to an upcoming recurrence. In this setting, any patient presenting with fibrosis features was placed within the high-risk category resulting in receiving enhanced therapeutic measures(Thomas et al., 2002). Acute Lymphoblastic Leukemia would be diagnosed if there are ≥ 20 % Blast Cells originated from Lymphocyte which forms Marrow's Nucleate Cell Count and similarly Non-Erythrocyte Cells Count. When the erythroid component exceeds 50%. Diagnosis can be made using the same criteria with a peripheral blood sample if marrow cells are not enough or unavailable(Bates and Bain, 2012).

2.2.2 Immunobiology of Acute Leukemias

The B-cell ancestry is responsible for about 80% of ALL cases. The majority of these instances express CALLA, which was recently given the name CD10. Additionally, these cells express the B-lymphocyte differentiation antigens CD19 and CD20 as well as have experienced gene rearrangements for the immunoglobulins of heavy and/or light chains. For about 80% of ALL instances, the B-cell ancestry is to blame. Most of these cases express CALLA, recently named CD10, which is now expressed in many different circumstances. These cells also exhibit CD19 and CD20, which are B-lymphocyte differentiation markers, as well as gene rearrangements for the heavy and/or light chains of the immunoglobulin molecule.(Devine and Larson, 1994, Rayyan et al., 2010).

Between 15 and 20 percent of ALL instances have a T-cell ancestor. T-cell markers like CD5 and CD7 are expressed by these cells, and CD10 may also be present. The genes of T-cell receptors are typically rearranged in at least some instances. Depending on the expression of distinct T-lymphocyte differentiation Ags, T-lymphocyte ALL is further divided into early, intermediate, and mature thymocyte kinds. Null-cell ALL refers to a tiny part

of ALL cases that are devoid of either B- or T-cell characteristics. Malignant lymphoblasts of both B-lymphocye or T-lymphocyte origin can express some antigens that are typically only detected on myeloid cells Myeloid antigen positive ALL is the term used for these patients(Juárez-Velázquez et al., 2013).

There are currently no known surface proteins that are unique to AML. Instead, AML cells frequently coexpress antigens that are not typically coexpressed through their normal myeloblastic peers on the cell surface. Generally speaking, it has been challenging to appropriately relate immunologic phenotyping to the FAB categorization of AML or to clinical prognosis. However, the presence of some antigens, which include the earliest stem-cell markers CD34, might provide valuable prognostic data(Stock and Larson, 2003, Rayyan et al., 2010).

2.2.3 Cytogenetics in Acute Leukemias

The role of cytogenetics in determining the biologic basis of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) is now widely recognized. By identifying acquired chromosome aberrations that recur in AML and ALL, and providing precise chromosomal location of breakpoints in leukemia-associated translocations and inversions, cytogenetics aided in cloning of many genes whose activation or fusion with other genes contributes to the neoplastic process(Vardiman et al., 2002). Further characterization of these genes revealed that they are often involved directly or indirectly in the development and homeostasis of normal blood cells, and that abnormal protein products of fusion genes created by specific translocations and inversions can dysregulate proliferation, differentiation or programmed cell death (apoptosis) of blood cell precursors.[1] This has paved the way to designing novel therapeutic agents targeting specific genetic abnormalities in leukemic blasts, such as

imatinib mesylate, the Bcr–Abl tyrosine kinase inhibitor that suppresses proliferation of cells harboring the *BCR–ABL* fusion gene created by t(9;22)(q34;q11.2), a recurrent chromosome aberration in chronic myelogenous leukemia (CML) and ALL(Mrozek et al., 2004).

2.3 P53 and TP53

Since the finding of P53 protein was in 1979, Numerous studies have been conducted on P53, confirming its crucial function in tumor control(Levine and Oren, 2009, Aubrey et al., 2016). By inducing cell cycle arrest, repairing damaged genetic material or promoting programmed cellular death, the P53 molecule serves to prevent tumorigenesis and is considered a tumor suppressor. P53 isn't easily noticed in healthy cells; however, its accumulation is observed in most tumor ones. Accumulation of tumor-associated P53 protein could potentially lead to P53-autoimmune response; hence identifying tumors through detection of anti-P- 5 3 antibodies is possible Conversely, even in non-cancerous cells exposed to different types of stressors can lead them towards accumulating P53 protein. This clearly indicates that presence or absence P- 53 cannot be used for diagnosing Cancer or other tumours. It can also manifest in numerous autoimmune conditions. In addition, autoantibodies to DNA seem to be linked with P53 antibodies found in autoimmune disease. The significance of P53 autoantibodies in causing disease is presently uncertain(Herkel and Cohen, 2007). As a transcription factor, P53 primarily controls the expression of its target genes by attaching to the P53 DNA-binding elements in those genes. Apoptosis cell death, cell cycle arrest, cell senescence, repairing DNA, metabolism of cells, and antioxidant defense are just a few of the crucial biological processes that P53 regulates through transcriptional regulation. (Haupt et al., 2019, Li et al., 2019b, Bieging-Rolett et al., 2020). Although more

than 50% of all malignanced have P53 mutations, the P53 gene is the one that is most commonly altered in human cancers. Missense mutations, which produce fully mutant P53 proteins with only a single amino acid changed, account for the majority of P53 mutations in malignancies(Schlereth et al., 2013, Chen et al., 2020, Rozenberg et al., 2021).

Through direct DNA binding and inducing reversible cell-cycle arrest, the tumor protein TP53, also known as 53-P53, regulates the cell cycle. If the damaged DNA can be repaired, the protein activates the genes necessary for DNA repair; otherwise, it triggers apoptosis. As a result, by enabling DNA repair, the so-called "guardian of the genome," P53, supports cell survival. But in half of all human malignancies, P53's tumor-suppressor activity is altered—either lost or gained. This study describes how the P53 pathway is functionally perturbed at breast, bladder, liver, brain, lung, and osteosarcoma cancers. In addition to reducing tumor suppressor efficacy, P53 mutations also change the protein's structure so that it becomes an oncogenic one. Additionally, TP53 dysfunction causes the cell genome to accumulate more oncogenic mutations. Therefore, altering TP53-dependent survival pathways encourages the spread of cancer. This oncogenic TP53 encourages cell survival, inhibits apoptosis to prevent cell death, and aids in the growth and spread of tumor cells. This chapter's goal is to explore how different cancer types are affected by mutant P53(Coskun et al., 2022). Hundreds of novel cancer-related genes were identified, but none were able to dethrone TP53 from its infamous throne. Even after sequencing the entire genomes of thousands of tumor genomes, no new contenders for the throne have emerged, and TP53 mutations are still commonly among the top 5 most frequently modified genes in the mainstream of human malignancies (Stratton, 2011, Kandoth et al., 2013). But based on the type of malignancy, the incidence of TP53 mutations be different

greatly(Hainaut and Pfeifer, 2016). The incidence of TP53 alterations seems to ranging from below five percent in cervical carcinoma to ninety percent in ovarian carcinoma, but these figures need to be interpreted with attention because of a number of variables(figure 2.3) (Leroy et al., 2014).

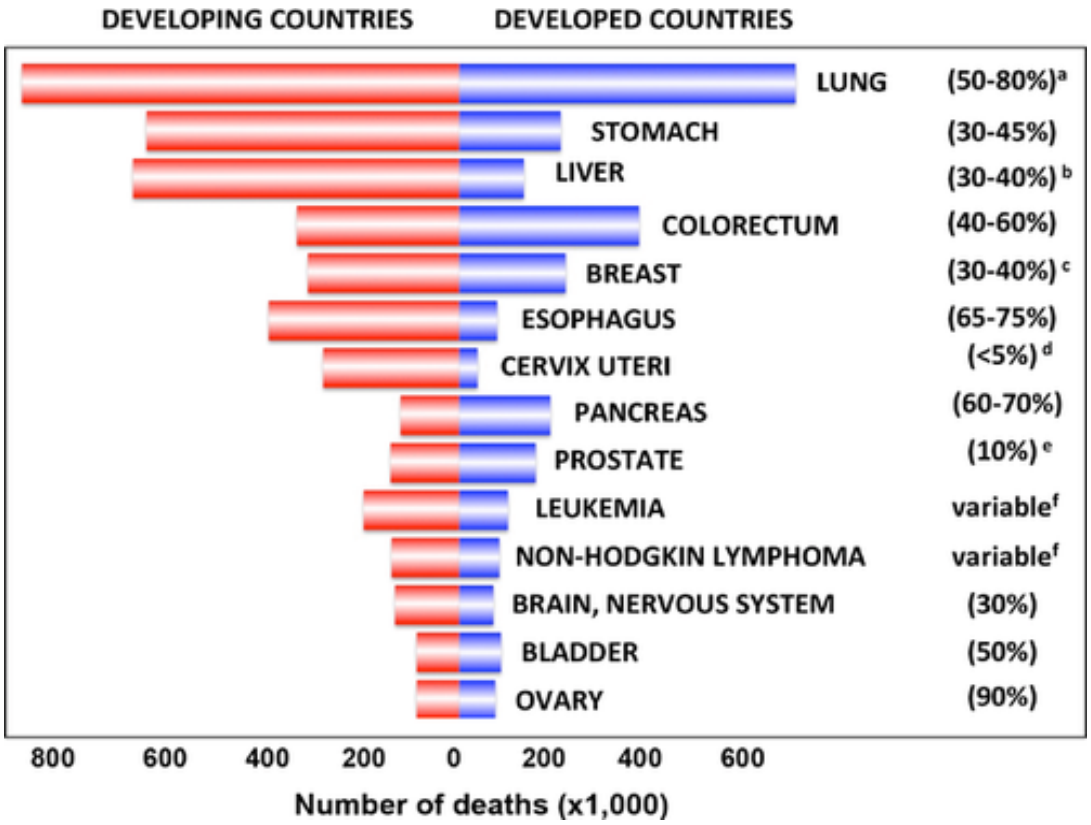


Figure 2.3: Frequency of cancer deaths worldwide and relationship to the frequency of TP53 mutations. Cancer death numbers from GLOBOCAN 2008 (http://globocan.iarc.fr: TP53 mutation data from the UMD TP53 database 2017.

2.3.1. P53 Structure and Cellular Functions

The TP53 gene, which produces the normal P53 protein, is found on chromosome 17p13.1. The 393 amino acids long phosphoprotein known as the P53 protein product has five critical signature domains: the amino N-terminal

transactivation domain, the central DNA-binding domain (DBD), the carboxy-terminal oligomerization domain, & the regulation domain (Husaini, 2014, George et al., 2021)(figure 2.4). P53 is referred to as the "guardian of the genome" because it regulates cell development and arrest in responding to genomic stress(Békhazi, 2015, Telfer, 2022). There are numerous methods for P53 to become inactive, which leads to a drop in P53 ranks and subsequently cellular growth. The TP53-gene mutation in one of TP53 alleles, which occurs 50% of the time, can ultimately cause lossing or partially inactivation of the additional WT alleles over time or throughout sickness progression due to lossing of heterozygosity(Zhou et al., 2016, Sabapathy and Lane, 2018, Baslan et al., 2022).

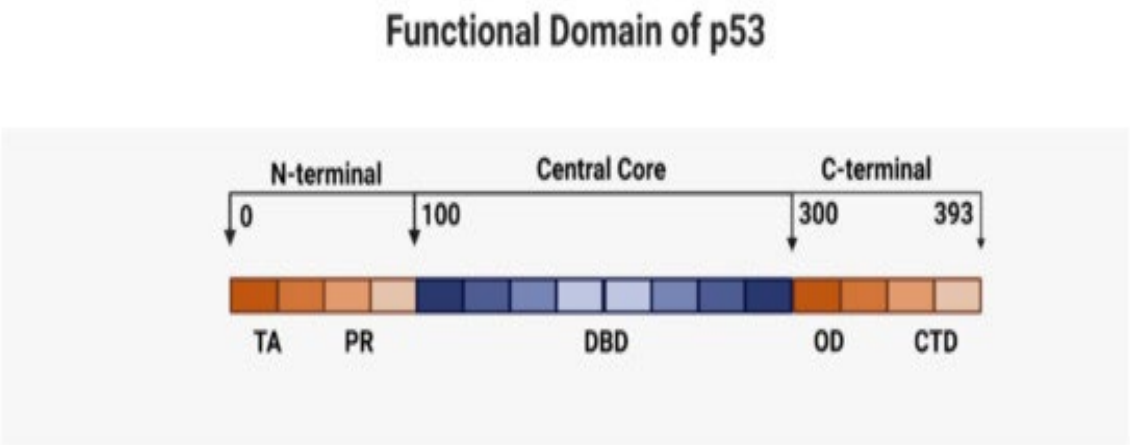


Figure 2.4: The P53's functional areas include: The transactivation domain (TA) and the proline-rich domain(PR) make up the N-terminal region. TA is necessary for the association of MDM2 ubiquitin ligase and the transactivation of numerous transcription factors. The majority of TP53's exons have been mapped, and the DNA binding domain (DBD) makes up the majority of the central core. The oligomerization domain (OD) and the carboxy-terminal regulatory region make up the C terminal(Laptenko et al., 2016).

P53 is typically "off" and has a brief half-life. It is neatly controlled at the protein levels by post-translational alterations like ubiquitination, phosphorylation, acetylation, as well as methylation(McClure, 2017, George et al., 2021). It is activated when cells are hard stressing (Iurlaro and Muñoz-Pinedo, 2016) or damaging avoiding of more proliferation of stressed cells over the G1-phase/S-phase of the human cell cycle(Heldt et al., 2018). The cell can continue to have a low amount of WT P53 due to continuous ubiquitylation and subsequent degradation when it is not under stress. While under hypoxic circumstances, oncogene activation, and destruction of DNA, P53 ubiquitylation is suppressed, resulting in its accumulation. Tetrameric complexes of stabilized P53 are then formed in the nucleus, where it is ultimately activated.(figure 2.5). For P53 to be completely active and function as a transcriptional activator of over 150 different targeted genes, tetramers must form (Ranaweera, 2013, Kamada et al., 2016).

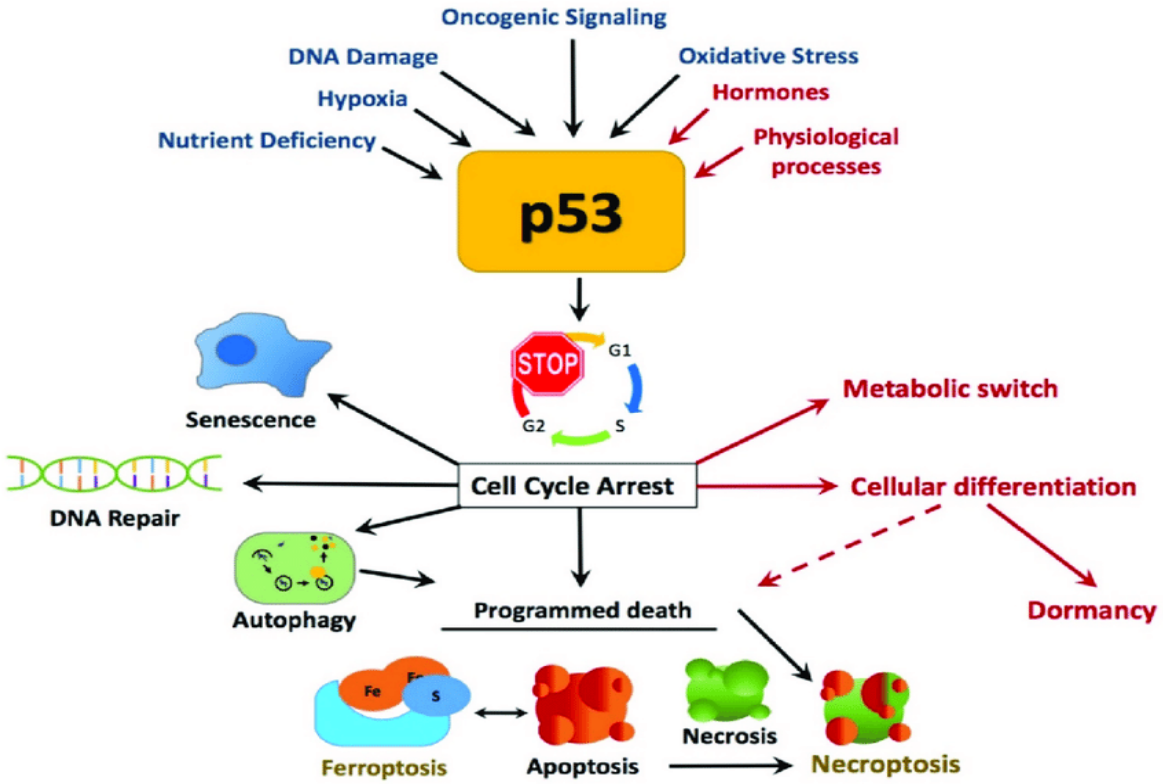


Figure 2.5: P53 plays both canonical and non-canonical roles in tumor suppression. It activates through various stress cues, including nutrient stress, hypoxia, oncogene activation, DNA damage, and ROS. Non-canonical mechanisms include autophagy pathways, necrosis, necroptosis, and ferroptosis. (Hager and Gu, 2014, Olivos III and Mayo, 2016, Moulder et al., 2018).

Rather than the rate of production of cells, the destiny of the P53 protein in the cell is primarily governed by the degree of degradation. The association of P53 with MDM2 is the main cause of P53 degradation. It's interesting to note that mainly. AML with WT TP53 can bond to the AT domain of P53 to overexpress highly amounts of MDM4 (also identified as MDMX), a crucial regulator of P53. This discovery shows the negative effects of impaired P53 pathways coupled with decreased survival. A number of important DNA based protein kinases, such as ATM, Chk1, & Chk2, detecting DNA damages,

phosphorylate-P53 at the AT (amino terminal domain) sites adjacent to the MDM2 attaching area, and subsequently blocking MDM2 collaboration with P53. The stabilization of P53 is the final step in this chain.(Bursac et al., 2014, George et al., 2021). Leukemia TP53 mutations can be related with somatic and germline mutations; the second type is linked to Li-Fraumeni syndrome and causes the developing of particular solid tumors and is fewer expected to manifest in a variety of tissues.(Guha and Malkin, 2017). However, in the sporadic context, TP53 mutations appear to be brought on by exposure to genotoxic abuse, oncogenic agents, or carcinogens (environmental variables). This finding clarifies the healthy functionality of P53 in preserving genomic stability and stopping the growth of tumors(George et al., 2021).

2.3.2. Distribution of P53 Mutations

The majority of P53 mutations are missense mutations, generally located in the central domain of the P53 coding region (exons 5–8) (Piaskowski et al., 2010). Though partiality may result from the systematic examination of those particular exons, sequencing the entire genome has only lowered the dissemination of that bias. (Sharma et al., 2019) (Figure 2.6, A and B).

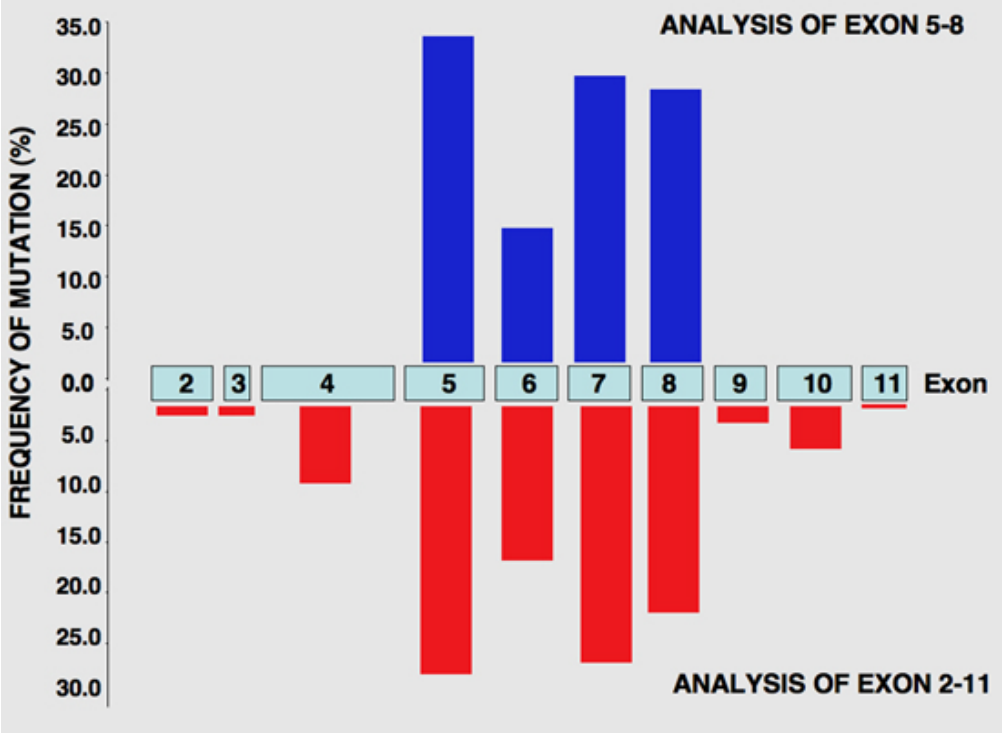


Figure 2.6: Distribution of mutational events in each exon of the TP53 gene. Studies focusing on the central region (exons 5–8,blue bars, top) are compared with those analyzing all coding regions (exons 2–11, red bar, bottom)(http://P53.free.fr/Database/P53).

Among all cancer genes, including tumor suppressors and oncogenes, the spread of TP53 protein mutations is distinct. TP53 is a 393 aminoacid long proteins, and all but five of its residues were found to be the subject of at least only one-mutation in human malignances. Additionally, every residue in the protein's core domain has been identified as having undergone a minimum of five mutations. Due to the core region's high fragility and extreme sensitivity to substitutions, TP53 mutations have been widely dispersed. In fact, large-scale mutational study of each of its residues supports this (Levine et al., 1991, Soussi and Wiman, 2015).

It is crucial to remember that the current study concentrates on the main TP53 protein isoforms. A significant quantity of mutations do not targeting all TP53-isoforms, as thoroughly discussed in an accompanying paper(Leroy et al., 2014). Additionally, just a small quantity of mutations have been stated, and neither beta nor gamma exons located in intron 9 have ever undergone a thorough analysis for mutations. It is necessary to conduct a more thorough study of this area in human tumors. Any possible mutation-targeting discoveries will help to clarify the putative tumor-suppressive properties of its isoforms(Leroy et al., 2014).

2.3.3. P53 Signaling Pathway

In healthy cells, the levels of gene transcription, translation, post-translational modifications, and intracellular subcellular localization all play a significant role in controlling the intracellular content of wild-type P53 protein. Its amount is kept very low, and the P53 signal network is not active(Marinho et al., 2014). P53 is stabilized and accumulates in cells that are stressed or damaged, acting as a "brake" on cell growth, removing damaged DNA and mutant cells, and averting cancer. UV and ionizing radiation damaging, aberrant proto-oncogene activation, and hypoxia are examples of upstream cues that cause the P53 signaling network to become active(Mazi and Gania, 2013)(figure 2.7).

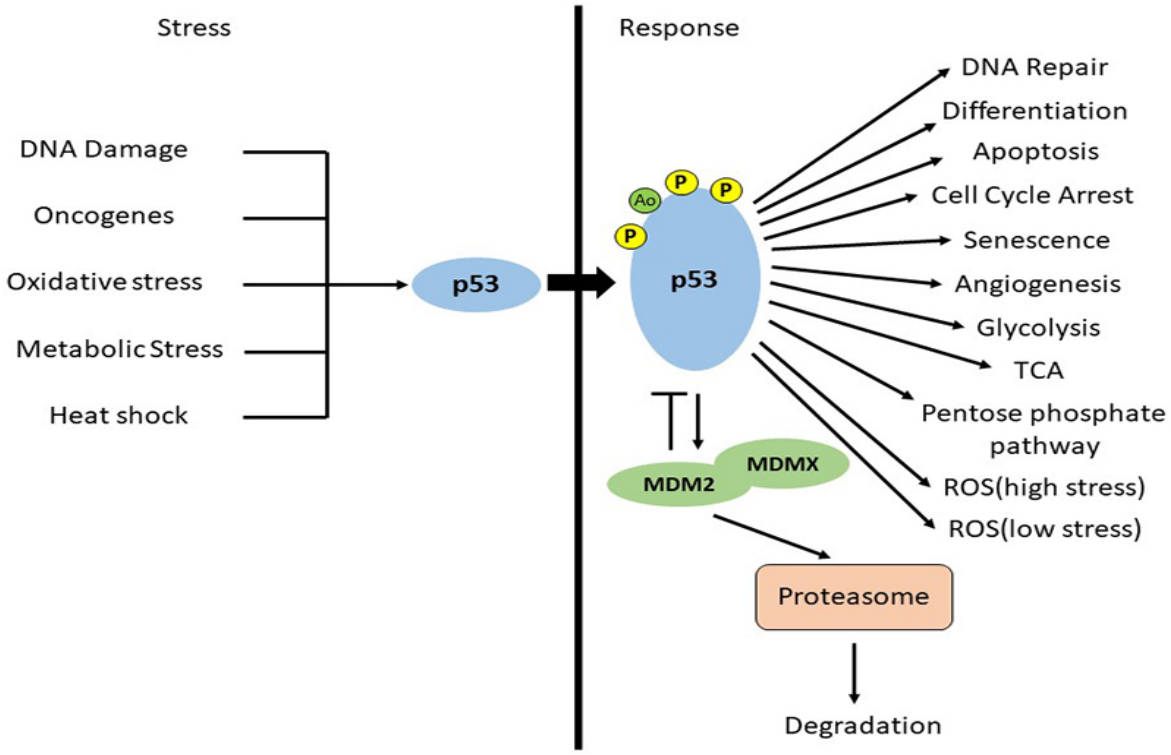


Figure 2.7: Pathway for P53 signaling(Huang, 2021)

The majority of the aforementioned activation signals are brought on by cell mutation and damage, and the P53 signaling network is engaged to stop further mutation or cell damage. There are numerous causes of DNA damage, including ionizing radiation, treatment such as chemotherapy, and UV rays. Different DNA damage causes trigger various downstream signals. For instance, phosphoidylinositol-3-kinase-related kinases (PIKKs), which start a number of phosphorylation reactions, eventually trigger P53 to stabilize and develop intracellularly in respond to ionize radiations and UV irradiation, respectively. These kinases includes the ATM (ataxia telangiectasia-mutated) and ATR (ATM and Rad3-related)(Chen, 2016).

2.3.4. P53 Mutation in Leukemias

Compared to other tumors, leukemias have a comparatively low prevalence of P53 mutations. However, it was discovered that the incidence

increased in some instances as the disease progressed and was linked to a poorly prognosis(Stengel et al., 2014). Because the investigations were limited to these exons, the widely held of research found mutations in exons 5-8 of the P53 gene. AML, one of the acute leukemias, was discovered to have a low frequency of mutations (4.5%–9%), mostly occurring in individuals with a bad prognosis and a complex karyotype. There is a possibility that P53 mutations, which are more common in elderly individuals with unfavorable karyotypes, may be a separate cause for short survival(Piaskowski et al., 2010). However, the prevalence of mutations rose to 27% in individuals with AML or MDS who had previously received alkylating agents(Bhatia, 2013). Additionally, a great frequency of mutations in AML individuals has stayed linked to poor DNA mismatch repairing and a complicated karyotype(Gaymes et al., 2013).

P53 mutations were reported to be infrequent (5%) in cases of newborn ALL in numerous investigations on this condition. During relapse t(4;11), a translocation mutation was only present in one patient. Three additional patients with mutations had poor clinical outcomes(Peller and Rotter, 2003). A large percentage of mutations are existing in acute phase individuals with chronic myeloid leukemia(Müller et al., 2009).

In another research, P53 exon 5 and exon 7 mutations were found in two out of nine cases with unusual CLL. Both had an illness that was aggressive and has a translocation(11;14) (Midena et al., 2000, Peller and Rotter, 2003). The outcomes of a different studies indicated that 47% of CLL patients had mutations, which had abnormal cytogenetics but were unrelated to the course of the disease (Barnabas et al., 2001). A study by (Lazaridou et al., 2000), three out of the 19 CLL patients reported a decreased incidence of mutations (15%). P53 mutations were present in two out of eight (25%) Richter's transformation of CLL cases(Kanagal-Shamanna et al., 2019)(figure 2.8).

These had a large S-phase fraction, a subpar therapeutic response, and a brief survival. Despite being an indolent low-grade lymphoma, splenic lymphoma with villous lymphocytes showed signs of a more aggressive illness in individuals with P53 abnormalities and reduced survival(Gruszka-Westwood et al., 2001, Peller and Rotter, 2003). In addition, individuals with diffuse large B-cell lymphoma, the NHL subtype with the highest death rates, had mutations in 22% of cases(Flowers et al., 2010).

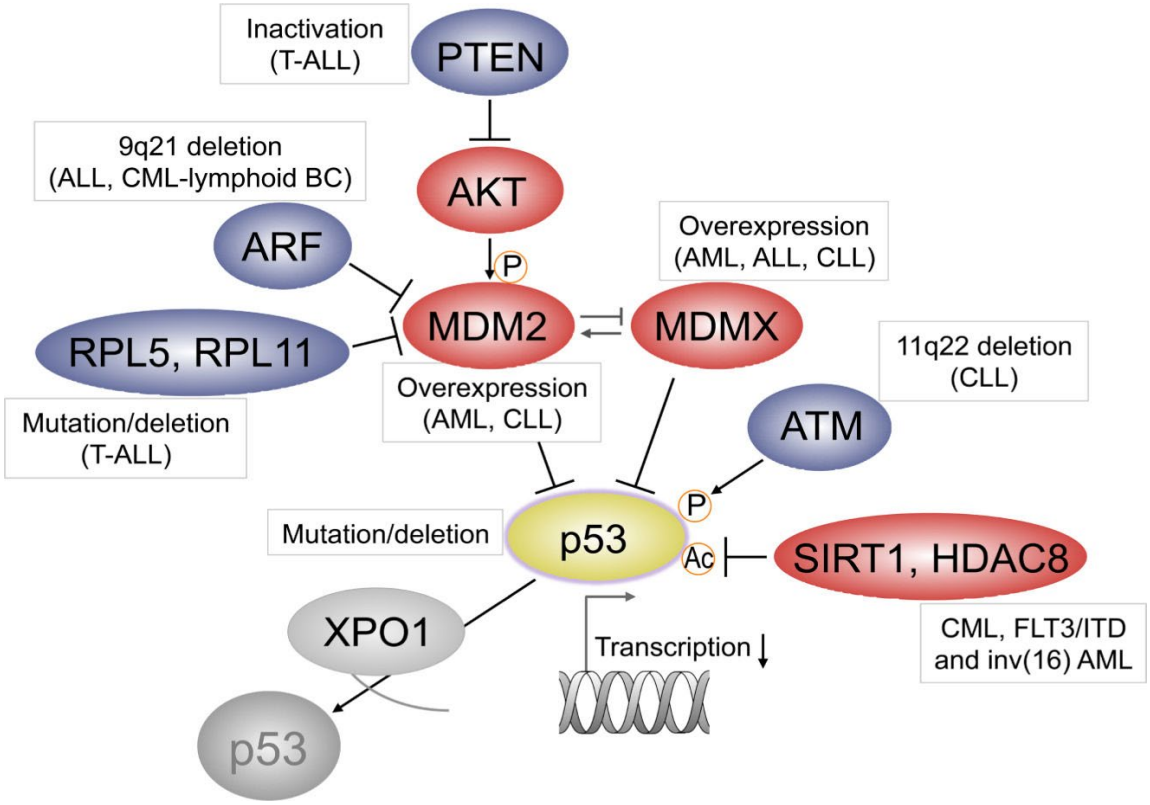


Figure 2.8: Impaired P53 response in leukemia. P53 transcriptional activity is suppressed by P53-regulatory proteins upstream of P53. Red ovals indicate overexpressed or activated proteins and blue ovals indicate inactivated proteins in leukemia(Kojima et al., 2016).

2.3.5. Methods of detection P53 mutation in Acute Leukemias

Bone marrow aspiration (BMA) is the most frequent and safe invasive procedures done routinely in the hospitals for the diagnosis and management of

hematological disorder. There is very little or no risk of bleeding and can be safely done in case of severe thrombocytopenia(Pudasaini et al., 2012). Leukemia is a class of hematological neoplasia that often affects lymph nodes, bone marrow, and blood. It is characterized by an aberrant white blood cell proliferation (leukocyte proliferation) in the bone marrow that does not respond to cell growth inhibitors. Anemia, thrombocytopenia, and neutropenia follow the suppression of hematopoiesis as a result of this. Additionally, immature WBC can build up in a number of extramedullary locations, particularly the meninges, gonads, thymus, liver, spleen, and lymph nodes. As a result, they also leak into the peripheral blood stream as a result of an overabundance of lymphoid or myeloid blasts in the bone marrow. Leukemia is diagnosed based on an elevated white blood cell count with immature blast (lymphoid or myeloid) cells and a decreased neutrophil and platelet count. A significant indicator of leukemia is the presence of an excessive number of blast cells in peripheral blood. Therefore, hematologists regularly look at blood smears under a microscope to properly identify and classify blast cells. Acute and chronic leukemia can be pathologically classified into two groups in a more general sense(Mohapatra et al., 2010).

Although molecular and genetic characteristics are becoming more significant in the subclassification of acute leukemias, morphologic and immunophenotypic analyses continue to be the principal methods used to assess these diseases at the outset. Because there are so many different genes involved, it is currently impractical to perform a thorough, unguided molecular analysis of hematolymphoid cancers, but a quick initial interpretation of morphologic and immunophenotypic data can effectively direct a narrow and specific search for molecular lesions. In order to prioritize instances for repeated molecular and genetic testing, flow cytometry is used. Additionally,

molecular assays could call for a high level of technical know-how that is only available in specialist reference laboratories, requiring lengthy processing and analysis times for specimens that might conflict with quick clinical therapy of acute leukemia. Contrarily, flow cytometric analysis can be finished in a matter of hours on almost any case and is frequently sufficient, in conjunction with traditional morphologic assessment, to reach a limited differential or even a conclusive diagnosis(Peters and Ansari, 2011).

A panel of monoclonal antibodies to myeloid-associated antigens, including CD13, CD33, CD11b, CD15, CD14 and CD41a, as well as lymphoid-associated antigens, including CD2, CD5, CD7, CD19, CD10 and CD20, and lineage nonspecific antigens HLA-DR, CD34 and CD56 were used to characterize the phenotypes of the leukemia cells as previously described(Hou et al., 2015). Antigens normally expressed on myeloid blasts are frequently present including CD34, CD117, and HLA-DR; TdT expression is also not uncommon. Pan-myeloid antigens such as CD13 and CD33 are present, but markers normally restricted to maturing myeloid cells such as CD15, CD36, and CD64 are typically absent. Although cytochemical stain results for myeloperoxidase are negative, limited expression may be detected by flow cytometry. B- and T-lineage markers are absent, but CD7 may be seen in the absence of CD3. Acute myeloid leukemia without maturation, acute myeloid leukemia with maturation, and the blasts of acute panmyelosis with myelofibrosis show nonspecific features, with frequent expression of markers associated with myeloid immaturity, such as CD34 and CD117, combined with variable expression of more general markers of myeloid lineage, including CD13, CD15, CD33, and CD64. The rare cases of acute basophilic leukemia(Peters and Ansari, 2011).

The immunodetection of proteins, especially those with low quantity, is accomplished in large part using the Western blotting technique. Protein patterns are transferred from gel to a microporous membrane during this process. Protein transfer to membranes via electrophoretic and non-electrophoretic means was first described in 1979. Since the introduction of this approach, protein blotting has developed significantly, enabling protein transfer to be carried out in a variety of methods(Kurien and Scofield, 2015).

Molecular and cell biology employs the Western blotting technique. A complicated combination of proteins that have been isolated from cells can be used to create a western blot, which allows researchers to isolate particular proteins. The method accomplishes this objective by combining three components: size separation, transfer to a solid substrate, and seeing the target protein by labeling it with the appropriate primary and secondary antibodies(Mahmood and Yang, 2012).

PCR has become a mainstay of molecular pathology due to its high analytical sensitivity. Because each DNA or cDNA target sequence has been replicated 230 times during the course of 30 cycles of amplification, resulting in a billion amplicons, PCR can detect "a needle in a haystack." These amplicons can be measured precisely in real time utilizing instruments, or they can be further assessed using a variety of analytical techniques like sequencing, melt curve analysis, or electrophoresis. In uncommon samples like cerebrospinal fluid or biopsied myeloid sarcoma, paucicellular specimens and partially degraded nucleic acid can frequently be accommodated. Commercial primers and probes are available for certain fusion transcripts, mutations, and controls, and molecular procedures have been described(Gulley et al., 2010).

Real-time PCR is frequently regarded as the gold standard for measuring gene expression because of its high specificity, highly detection sensitivity and

wide linear dynamic range(Yan-Fang et al., 2012). Using one or more fluorescent internal probes to track product accumulation throughout each cycle, a variation known as quantitative PCR (Q-PCR) can be used to measure the amount of target DNA. The time course of product accumulation is then compared to a number of standards with known concentrations(Gulley et al., 2010). Sequence variations within the amplicon are found using a fluorescent internal probe and "melt curve analysis". Half of all real-time PCR users use the SYBR® Green PCR test, which is another frequently used real-time PCR technology in addition to the TaqMan assay. Because of how simple it is to develop the assays and how inexpensive it is to set up and run, SYBR Green PCR is widely employed. Careful primer design and fast validation of the PCR results using dissociation curve analysis can solve those issues(Yan-Fang et al., 2012).

One DNA strand is replicated, and the order in which the tagged nucleotides are added is watched to identify the nucleotide sequence. Dideoxynucleotide incorporation-based traditional techniques can spot a variant that accounts for at least 20% of the specimen's alleles (or 40% of the cells). Potentially more accurate and quantitative is pyrophosphate release detection by pyrosequencing. Now, high throughput sequencing techniques are being approved to increase coverage and, in certain cases, boost test sensitivity(Gulley et al., 2010). High throughput and multiplexed sequencing of certain gene panels or entire exomes/genomes is now possible because to the massively parallel sequencing capabilities of NGS technologies. These technologies are currently being used widely to characterize genomic changes in cancer and are very pertinent for diagnostic applications as alternatives to first-generation sequencing techniques. However, because to a lack of

sufficient guidelines and thorough validation, the introduction of NGS technologies into the diagnostic arena has been delayed(Chemegni et al., 2016).

Sanger sequencing is frequently employed for TP53 mutational investigation, but when variations with allelic frequencies below the Sanger detection limit are present, it may misclassify cases of TP53 mutations as wildtype. Recent research employing next-generation sequencing (NGS) have demonstrated that TP53 mutations can be present in tumor cell populations at low clonal abundance, or low-burden, and have the same negative impact on disease course in some studies(Catherwood et al., 2022).

METHODOLOGY AND RESEARCH DESIGN

3. METHODOLOGY AND RESEARCH DESIGN

3.1. MATERIALS

3.1.1 Equipments

Table 3.1: Equipments were used in this research, as well as its manufacturers.

No.	Instruments	Suppliers	Origin
1	Analytical balance	Floria	Turkey
2	Microcentrifuge	Sozhou Bioselec Biotechnology	China
3	Centrifuge	MMN	Sweden
4	Tube Roller Mixer	KJMR-II	China
5	Digital Deep freezer	Hisense	China
6	Nanodrop UV Spectrophotometer	Thermo Fisher Sciebtific	USA
7	DNA/RNA Gel Electrophoresis	UVP	USA
8	Micropipettes	Bibby Sterilin	UK
9	Mini Vortexer	Neuation iSwix Jr. VT	India
10	Water bath	Memmert	Germany
11	Verti 96-Well Fast Thermo Cycler	Thermo Fisher Sciebtific	USA
12	Microwave Oven	Hisense	China
13	Agarose Gel Electrophoresis Unit	NOGEN	Iran
14	Laboratory Hood	Pars Azma	Iran
15	Herather Compact Incubator	Thermo Fisher Sciebtific	USA

3.1.2 Kits

Table 3.2: Kits were used in the current study.

No.	Kits	Suppliers	Origin
1	Blood DNAPreparation-Solution Kit	Jena Bioscience	Germany
2	Primers	Macrogen	Korea
3	DNA Ladder	NORGEN	Canada
4	10X TBE Buffer	Invitrogen	UK
5	Go Taq G2 Green Master mix	Amplicon	Denmark
6	Agarose Powder	Biobasic	USA
7	DNA Gel Loading Dye	Norgen Biotek	Canada
8	Nuclease Free Water	Norgen Biotek	Canada
9	99.8+% Isopropanol	Thermo Fisher Scientific	USA
10	99.9% Ethanol	SLC-Delhi Chemicals	India
11	Microcentrifuge Tubes (0.2, 1.5 ml)	Biotech Concern	Bangladish

3.2 METHODS

3.2.1 Study design

The present retrospective study was managed into two groups, complete remission group(CR) and partial remission(PR) group. These two groups ordered based on the blast% of the second bone marrow aspiration reports after short term of chemotherapy induction. CR was well-defined as decreasing of blast cells from bone marrow to (lower than 5%) in peripheral blood samples. PR was defined as no reporting of decreasing in the percentages of blasts from bone marrow, and its blast% equal or more than (>5%). After that all our study data were conducted on the CR/PR association.

3.2.2 Participants

Whole blood samples used in this study taken from 61 cases with acute leukemias, was directed of the present study collected from July 1, 2021, to March 11, 2022, including 29 cases with AML (median age 49 years; range 21-80 years) & 32 cases with ALL (median age 29 years; range 3-54 years) individuals. Patients underwent a thorough physical examination, CBC, bone marrow aspiration for flow cytometric immunophenotyping, and detailed history taking, which included questions on their ages, gender, medical conditions, and family history with leukemia. The genomic extracted DNA samples of all patients were analyzed and detected by performing the PCR and Gel Electrophoresis techniques. After receiving extensive chemotherapy for their condition, all patients have attained a full remission, based on Nanakali Hospital for Blood diseases and Cancer guidelines.

3.3 Laboratory Analysis

In order to extract genomic DNA, samples of blood were taken from EDTA tubes. For other diagnostic tests like, flow cytometery, bone marrow reported, and complete blood count and parameters was collected from Nanakaly hospital saved data reports. The purified DNA were obtained by using DNA extraction kit and measured the running the purity of DNA samples by utilizing Nanodrop instrument. Then the specific target P53 gene sequence was amplified by achieving PCR technique and followed by gel electrophoresis technique to visualize the mutated bands of AML and ALL genomic DNA samples.

3.3.1 Flow Cytometric Immunophenotyping

Following the manufacturer's directions, specific directly conjugated flourochrome-labeled monoclonal mouse anti-human antibodies were used to perform direct immune fluorescence staining on the bone marrow aspirates. Acute leukemia was identified using immunofluorescence using a Becton Dickinson FACS Caliber flow cytometer and BD Cell Quest pro software (BD Biosciences, San Jose, CA, USA)(Sadek et al., 2020). This panel includes:

- To differentiate between AML and ALL, a main panel was used: CD13, CD33, CD117, anti-MPO, CD19, and CD79a.

- Additionally, CD34 was examined because it is a non-lineage specific marker that is produced by hematopoietic progenitor cells.

- If monocytic AML was predicted, CD64 tests were conducted.

The percent of gated blast cells expressing CDs was measured, and the expression was deemed positive when $\geq 20\%$ of the gated cells did so at the moment of the diagnosis.

3.3.2 Molecular Characterization

P53 gene was performed in the current study using a molecular approach(DNA Extraction, PCR Amplification, Gel Electrophoresis and Sequencing) to evaluate and asses the frequency of P53 mutation in AML and ALL cases, in Nanakali Hospital of Erbil City.

3.3.2.1. Genomic DNA Extraction

Genomic DNA was extracted from whole blood samples, collected from 20 AML individuals & 20 ALL patients, Blood DNA Preparation-solution kit were used, from (Jena Bioscience, Germany), (Catalogue No.PP-205S). DNA was extracted in the Nobel Medical Laboratory in 40 meter road-Arbil city.This tool is made to make it easy and quick to separate genomic DNA from whole blood samples. About (30-50µg) purified genomic DNA was expected per preparation of (300µL) whole blood sample. DNA fragmentation, which can be a problem in spin-column / filtration-based methods, is decreased by the solution-based method(used Solution Based Chemistry Method). Since neither phenol nor chloroform were used, it was also secure and created no hazardous waste. The following parts will go over the procedures for extracting DNA.

3.3.2.1.1. Samples Preparation and DNA Extraction

. Depending on the factories instructions, the frozen EDTA blood samples are gently mixed to guarantee uniformity while defrosting at room temperature prior to the extraction. Also included in the addition was (48ml) of 99.9% ethanol for the washing buffer container.

Cell Lysis:

Nine hundred µL of RBC lysis solution and 300 µL of whole blood were combined into a 1.5 ml microtubes, which was then reversed 10 times and incubated for 3 minutes at at room temperature before being centrifuged for 3 minutes at 14,000 rpm. The visible cell pellet was left behind after a portion of the supernatant was extracted with a pipette. The tube was then violently

vortexed for about 10 seconds to make sure it was completely re-suspended. The re-suspended cells were then pipette up and down with (300 µL) of cell lysis solution until no clusters were observed. The samples were then warmed in a thermo-shaker incubator (Thermo Fisher Scientific, USA) for 10 minutes at 65°C to aid in lysis.

Protein Precipitation:

The cell lysate was mixed with 100 microliters of protein precipitation solution and rapidly vortexing for 20 seconds to thoroughly mix and remove any clumps. After centrifuging the precipitated proteins at 14,000 rpm for three minutes, they form a compact, dark pellet.

DNA Precipitation:

The supernatant was added to a clean (1.5 ml) microtube along with 300 µL of 99.8% isopropanol, which was then carefully inverted for 1 minute before being centrifuged at 14,000 rpm for 3 minutes. Importantly, DNA appeared to the unaided eye as a tiny white pellet. The tube was quickly drained on fresh absorbent paper after the supernatant was thrown away. After adding 500 µL of washing buffer and repeatedly inverting the container, the DNA pellet was washed. The ethanol was carefully removed after centrifugation at 14,000 rpm for 3 mins, and the DNA pellet was allowed to dry for 10 to 15 mins at room temperature.

DNA Hydration:

This stage involved adding 100 µL of DNA hydration solution and mixing it with a vortex for 5 seconds. The sample was then refrigerated at (-20°C) until the qualitative and quantitative analysis of genomic DNA, after which it was incubated at 65 celisius degree for 30 minutes to expedite rehydration.

3.3.2.2. Quantification of Genomic DNA Concentration and Purity

The goal of the Nanodrop spectrometer is to quantify the quantity of nucleic acids present in a 1 microliter sample. The revolutionary sample retention technique used by this spectrophotometer eliminates the need for cuvettes when getting readings. Using a NanoDrop UV spectrophotometer from Thermo Fisher Scientific, USA, the quantity and purity of the extracted DNA were assessed in this study. The concentration of DNA in the solution was determined for DNA using the DNA conc. (ug/µl) = (OD260 *100 (dilution factor) * 50µg/ml)/1000.

Theoretically, an OD_{260} of one corresponds to a double strand DNA content of 50 ug/ml. The optical density, on the other hand, was calculated by calculating the absorbance at (OD260nm and OD280nm), and the (A260/A280) ratio indicated the DNA's purity(Russell and Sambrook, 2001). Exceptionally pure DNA had an absorption ratio between 1.8 and 2.0. An indication of protein contamination is a ratio of less than 1.6, and an indication of RNA contamination is a ratio of greater than 2.0(Ghatak et al., 2013, Lucena-Aguilar et al., 2016).

The Nanodrop's software and nucleic acid switch were activated to start the quantitative measurement process. The measuring pedestal's surface was first cleaned with deionized pure water or water that hasn't been treated with nuclease. The blank button was pressed to read the one microliter DNA hydration solution (TE Buffer)-containing blank. The surface of the NanoDrop spectrophotometer was first carefully cleaned with deionized purified water before adding (1 ul) of the first DNA sample. The reading was then completed by clicking the measurement icon, which also recorded the DNA concentration and purity. The same procedure was followed for every DNA sample, which entailed wiping the sample off the measurement base once the spectrometer process was finished. The DNA was stored at -20°C until it was used in the PCR.

3.3.2.3. Analysis of P53 mutation

A PCR-based method was used to detect the P53 gene mutation, and particular sequence primers enabled for accurate, sensitive, repeatable genotyping of that allele with improved flexibility and resolution(Law, 2019).

3.3.2.3.1. Region of Interest

The sequenced portion of the TP53 gene should at least contain exons 4–10, which correlate to the DNA-binding domain (codons 100–300) and the oligomerization domain. (codons 323–356). Exon 10 should be sequenced because new studies have shown that the incidence of mutations in exons 9 and 10 is comparable to or even higher in exon 10(Leroy et al., 2017)(figure 2.7). The complete coding region should ideally be covered by analysis of exons 2 to 11(Leroy et al., 2017). Four exons situated in these conserved regions contain the widely held of the P53 mutations found in human tumors. (exons 5-8). Exons 2, 3, and 11 are frequently included in TP53 gene profile researchs by NGS, and these studies have demonstrated the presence of variations in these exons despite their low frequency(Soussi, 2011)(figure 3.1). Sequencing of +2/2 intronic nucleotides is necessary to identify variants that may impede splicing and result in inactive proteins because every one of those exons is surrounded by a splice of donor and a splice of acceptoring area (Malcikova et al., 2018).

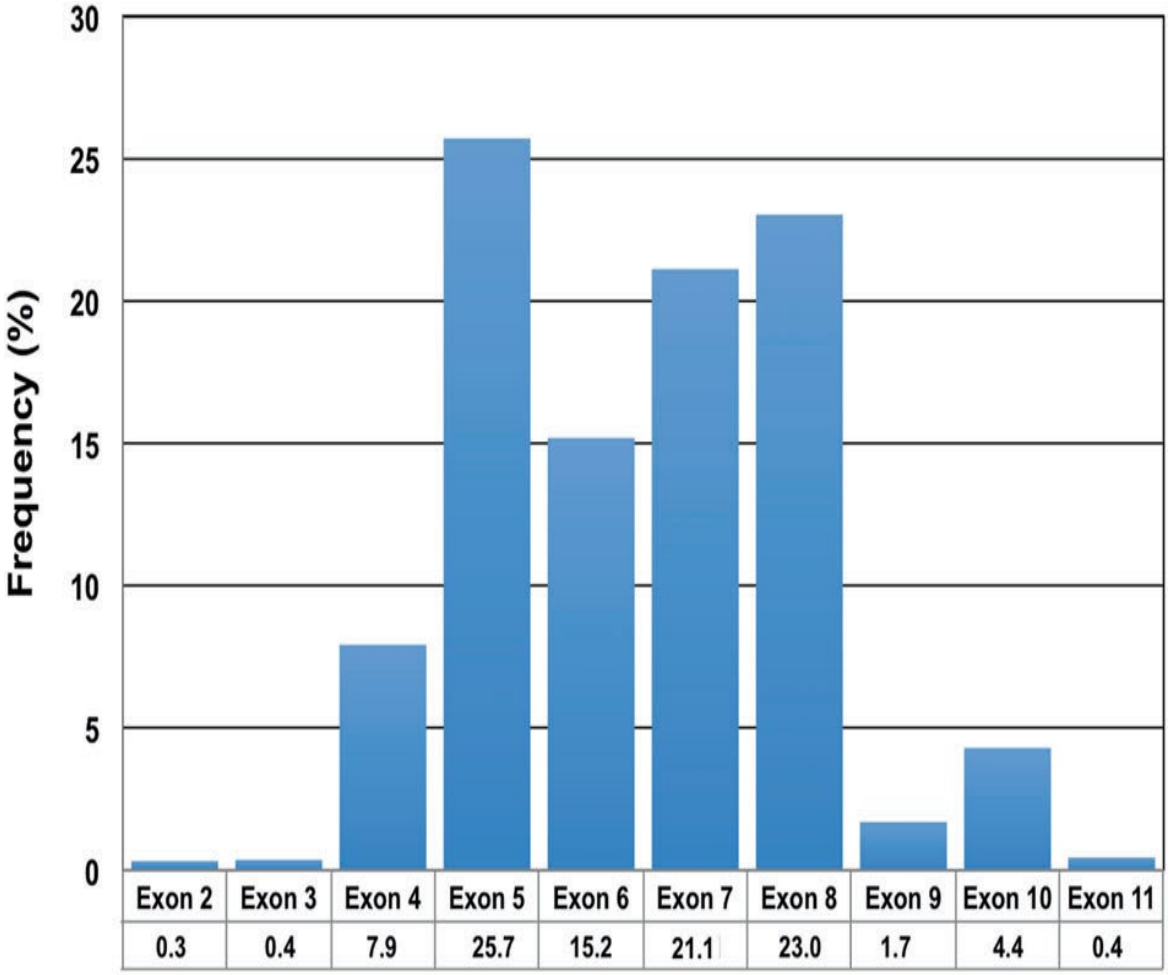


Figure 3.1: The presence of P53-gne variants in each exon was identified. The most recent UMD P53-gene database edits have taken this material (http://P53.fr) & Exons 2–11 of the next-generation sequencing of the gene were used to find the somatic and germline variant.

3.3.2.3.2. Primers

For the Forward and Reverse primers, respectively, the primers were provided in lyophilized form by (Macrogen company, Korea). The primers were dissolved in nuclease-free water (163 microliters for the forward primer and 168 microliters for the reverse primer, respectively) in accordance with the assembly requirements to achieve (100 µM) concentration. There is an inventory of the primers used in this research in Table (3-4).

Table(3.3): P53 primer sequences

Exons	Primer recognition sequence (5'- 3')	Product size(bp)	Source Ann. Temp.	Opt. Ann. Temp.	Ref.
Exon 5	F:TCTGTTCACTTGTGCCCTGACTTTC	282 bp	55	55	(Wada et al., 1993)
	R:ACCCTGGGCAACCAGCCCTGTCGTC				
Exon 6	F:CAGGGCTGGTTGCCCAGGGTCCCA	221 bp	55	62	
	R:ACTGACAACCACCCTTAACCCCTCC				
Exon 7	F:AAGGCGCACTGGCCTCATCTTGGGC	180 bp	55	62	
	R:AGTGTGCAGGGTGGCAAGTGGCTCC				
Exon 8	F:TAGGACCTGATTTCCTTACTGCCTC	236 bp	55	59	
	R:AACTGCACCCTTGGTCTCCTCCACC				

F: Forward primer; R: Reverse primer; bp: Base Pair; Ann.: Annealing; Temp.: Temperature; Ref: References

3.3.2.3.3. PCR Amplification

PCR-amplification of DNA was carried out employing the GoTaq®G2 Green Master Mix reagent from (Amplicon, Denmark). DNA was amplified in the Nobel Medical Laboratory in 40 meter road-Arbil city. This package contained the right amounts of GoTaq® G2 DNA Polymerase, μM μl dNTPs, MgCl2, and reaction buffers for efficient PCR amplification. In addition, GoTaq® G2 Green Master Mix included 2 colors (blue & yellow) that made it possible to track development during electrophoresis. Its reactions appeared sufficiently dense for direct loading onto agarose gels, and the GoTaq® G2 DNA Polymerase exhibited 5′→3′ exonuclease activity. According to the manufacturer's directions, the PCR must be run with the ingredients listed in Table (3-4a) for the master mix reaction of one sample in paired primers (25 μL as a final volume).

Table (3-4a): Reaction mixes for a (25µL) volume for PCR per/sample

Components of PCR reaction mixture	**Volume**	**Conc. Final**
***GoTaq*® G2 Green Master Mix, 2X**	12.5µL	1X
Upstream primer, 10µM	0.25-2.5µL	0.1-1.0µM
Downstream primer, 10µM	0.25-2.5µL	0.1-1.0µM
DNA template	1- 5µL	< 250ng
***Nuclease* free water**	To a final volume of 25µL	N.A

The final concentration of the DNA and (upstream and downstream) primers in a 25-µL final volume PCR run, in particular, were common PCR problems that needed to be addressed to achieve the best amplification results. Table (3-4b) lists the PCR reaction master mixtures necessary for one sample in paired primers (25µL as a final volume) for PCR to run after optimization.

Table (3-4b): Reaction mixes for a (25µL) volume for PCR/one sample after optimization

Components of PCR reaction mixture	**Volume**	**Final Concentration**
GoTaq® G2 Green Master Mix, 2X	12.5µL	1X
Upstream primer, 10µM	0.75µL	0.3µM
Downstream primer, 10µM	0.75µL	0.3Mm
DNA template	2µL	< 250ng
Nuclease free water	9µL	N.A

The full automated PCR cycle process took close to an hour and 45 minutes. It was managed by a VeritiTM 96-Well Fast Thermal Cycler (Thermo Fisher Scientific, USA), which was set up to alter the reaction temperature every few minutes to enable efficient amplification of DNA by denaturing and synthesizing it. The PCR cycler's procedure includes the following steps:

1. Initial DNA Denaturation: The ds-DNA was transformed into single-stranded DNA after being heated to 95°C for five minutes.
2. DNA Denaturation: 30 seconds were needed to complete the denaturation.
3. Primer Annealing: The temperature was then lowered to 63°C for 30 seconds to enable the primers to be annealed to the complementary regions of the template after the strands had been separated. The temperature necessary for primer annealing was correlated with the melting temperature (Tm) of the primers, and the base composition (G-C) concentration also had an impact on the time and temperature needed.
4. Extension: The DNA polymerase added dNTPs to produce a new DNA strand that is complimentary to the DNA template strand. The thermo-stable DNA polymerase was incubated at 72°C for 1 min.
5. Final Extension: To ensure that any single-stranded DNA was fully extended, this procedure was carried out five minutes after the last PCR cycle at 72°C. The PCR procedures and conditions applied in this research are presented in Table (3.5).

Table (3.5): Setting up thermo-cycling conditions(Lorenz, 2012).

PCR process steps	Temperature	Required time	Cycles Number
Initial DNA Denaturation	95°C	5 min	1

Denaturation	95°C	30 sec	35
Primer Annealing	63°C	30 sec	
Extension	72°C	1 min	
Final Extension	72°C	5 min	1

The thermal cycler was set to 4°C after the PCR was complete in order to preserve the PCR products until the containers could be removed from the machine and the agarose gel electrophoresis procedure could begin.

3.3.2.3.4. Optimization of Primer Annealing Temperature in Acute Leukemia:

In order to standardize microsatellite amplification, PCR was conducted in this work using the following conditions: initial denaturation at 94°C for five minutes, followed by 35 cycles of denaturation (94°C for 30 seconds), annealing (55°C for 30 sec), extension (72°C for 1 min), and final extension (72°C for 4 mins). The current investigation exhibited outstanding cross amplification performance using the conventional PCR settings and was visualized, despite the standard DNA sequencing gel electrophoresis being the preferred technique. According to earlier research, the ideal annealing temperature was between 58 and 60 degrees Celsius (Ta 56 degrees Celsius) (Rosenblum et al., 2007, Narina et al., 2011). However, 20 out of the 40 AML and ALL samples that were tried to be amplified by the PCR of chosen markers under these circumstances failed. These primer sets were used in a series of PCRs at various temperatures, (54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C 62°C, and 63°C), as presented from 58°C to 63°C in (Figure 4.7).

On 1 to 1.5% agarose gels, the PCR results could not be seen clearly as solid bands. Instead of a solid single band, a collection of fragments or a shadow of the band was seen. In order to get rid of the smaller pieces, it was discovered that the samples needed to be electrophoresed on a 2% of agarose gel. All of

the loci could be successfully amplified under the ideal conditions at the lower annealing temperature of 55°C (Figure 3.2), and visualized on 2percent of agarose gel. The pictures taken at annealing temperatures of 55°C for exon 5, 56°C for exon 6 and 7, and 59°C for exon 8 were also included, as presented in (figure 3.3). to demonstrate and contrast the development at 55°C and other optimized temperatures, as shown in (figure 3.2 and 3.3).

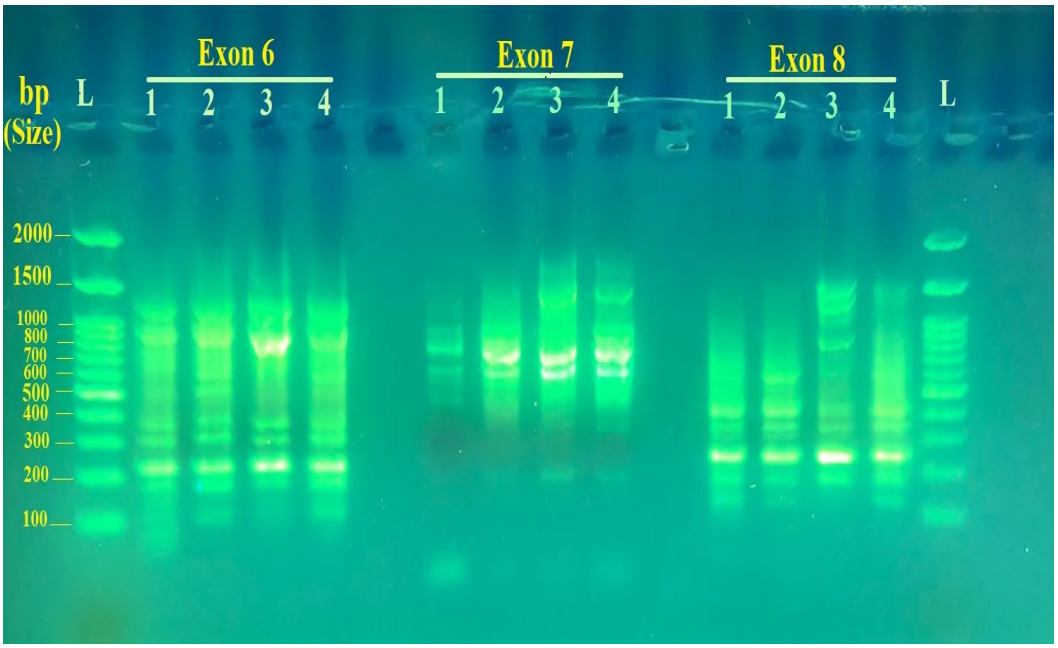


Figure 3.2: Illustrated before optimization of three samples of AML and ALL were tested for P53 mutated band in exons 6, 7, and 8 at annealing temperatures (55°C), and a chains of multiple basnds or a shadow of the band was detected in its place of the solid single bands and non-targeted mutated band were identified.

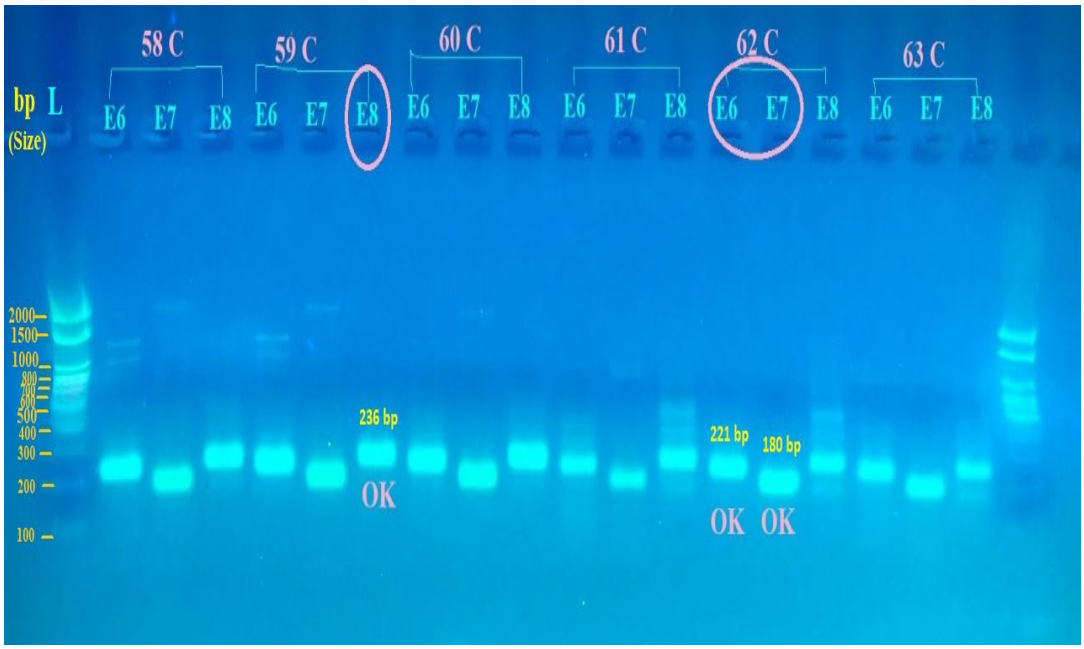


Figure 3.3: Illustrated the optimization of three samples of AML and ALL were tested for P53 mutated band in exons 6, 7, and 8 at a variety annealing temperatures (58, 59, 60, 61, 62, and 63°C), and the clear distinct single band and targeted mutated band were identified.

3.3.2.3.5. Agarose Gel Electrophoresis for DNA Products

The PCR results were assessed through using electrophoresis in a (2%) of agarose gel stained with safe dye. A gel electrophoresis casting plate that met the necessary specifications was made. A particular comb was fastened to the board's sides' sealed ends in order to create wells in one side of the gel.

In order to qualify genomic DNA for the casting tray, 1% agarose gel (Norgen Biotek, Canada) was made by dissolving 1g of agarose in 10ml of 10x TBE buffer, and the volume was then finished to 100ml of distilled water. Similar to this, a 2% agarose gel was made to run the amplified DNA by microwaving it for roughly one minute and then cooling it at 50–55°C for the same amount of time. To ensure that DNA fragments fluoresce in the gel, (10 µL) of Gold View I Nuclear Staining Dye (Solarbio Science & Technology, China) was applied after that, following the manufacturer's instructions.

- By combination 100 milliliters of 10x TBE buffer with 900 milliliters of distilled water, one liter of 1x TBE was created for the electrophoretic container (tank).
- The gel was poured into a casting tray after a gel comb was inserted, and it was left to fully solidify without spilling into nearby wells.
- The first channel, left side of the gel wells, received five microliters of DNA ladder. Before being inserted into the gel wells, the DNA samples were combined with a loading dye solution in a ratio of 1:5 (2 μL of loading dye: 10 μL of DNA).

The negative electrode was placed on the side opposite the wells discovered, and the gel device lid and its surrounding electrodes were fastened together. 3-5 volts per centimeter were applied to the polymer for 45 minutes to extract genomic DNA. For products that had been amplified, the gel was first run at (45 volts) per centimeter for 15 minutes to obtain adequate resolution, until the DNA migrated over 0.5 cm in the direction of the positive electrode. The electrophoresis was then carried out for roughly an hour at (135 volts) power(Russell and Sambrook, 2001).

The DNA banding pattern was viewed using UV Transillumination (UVP, USA) at wave lengths of 240 and 366 nm(Russell and Sambrook, 2001). (The gel was illuminated from below by setting it on the transilluminator glass, and the Norgen Biotek, Canada, 100bp DNA Ladder was employed as a molecular identifier), and photographs were captured with a digital photography camera (Canon G12).

3.3.2.3.6. Molecular sequencing

Following the processes of PCR, PCR products ready to run by Sanger sequencing to analyzing the mutations and variants of TP53 gene. Forward primers of a certain kind are included in the sequencing process. Sequencing was done in the Zheen International Hospital, Genetic Department -Arbil city.

3.4. Response and outcome

Defining criteria for responses based on European Leukemia Net consensus guidelines(Alvarez-Larrán et al., 2012). For purposes of response-based analyses, Participants who attained either a complete remission (CR) or a partial remission (PR) with unfinished hematological recovery were regarded as responders. Then were calculated all parameters of this research data depending on these two groups.

Responses were evaluated based on morphologic analysis and according to established standards. An indication of less than 5% of blasts in the bone marrow was used to define CR. PR is characterized by the presence of more than 5% of blast cells in the bone marrow and a partial responding to chemotherapy in the bone marrow.

3.5 Statistical Analysis

The information from our prospective study was entered into an Excel master sheet and set up for statistical analysis by using (GraphPad Prism 9.0). The presentation of some of the data included percentage values. A Paired Student's T-test was used to comparing parameters before induction of chemotherapy among CR and PR groups, all data are expressed as mean ± standard error. Normality of data was assessed through the D'Agostino Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test. Significance was considered at a value of $p<0.05$. Unpaired The Student's t test was performed to compare the parameters before and after induction of chemotherapy using the software Microsoft Excel 2010 and the package GraphPad Prism version 9.00 for window. All of the data are expressed as the mean along with the standard error. The D'Agostino Pearson test, the Shapiro-Wilk test, and the

Kolmogorov-Smirnov test were utilized in order to examine the normality of the data. When the p-value was less than 0.05, significance was assumed to exist. Data were expressed as median and 75th percentile values, which represented the rate of occurrence of positive cells and the data. When comparing dichotomous or categorical data, Pearson's chi-squared test or Fisher's exact test were used, and Spearman's rank correlation test was used to determine the association between CD markers and other independent factors.

RESULTS

4. RESULTS

4.1. Acute Myeloid Leukemia

29 AML individuals were diagnosed over the course of a year; of these, 18 (62.07%) were males and 11 (37.93%) were females. The patients' ages varied from 5 to 80, with a mean of 38.47 years, as shown in (figure 4.1).

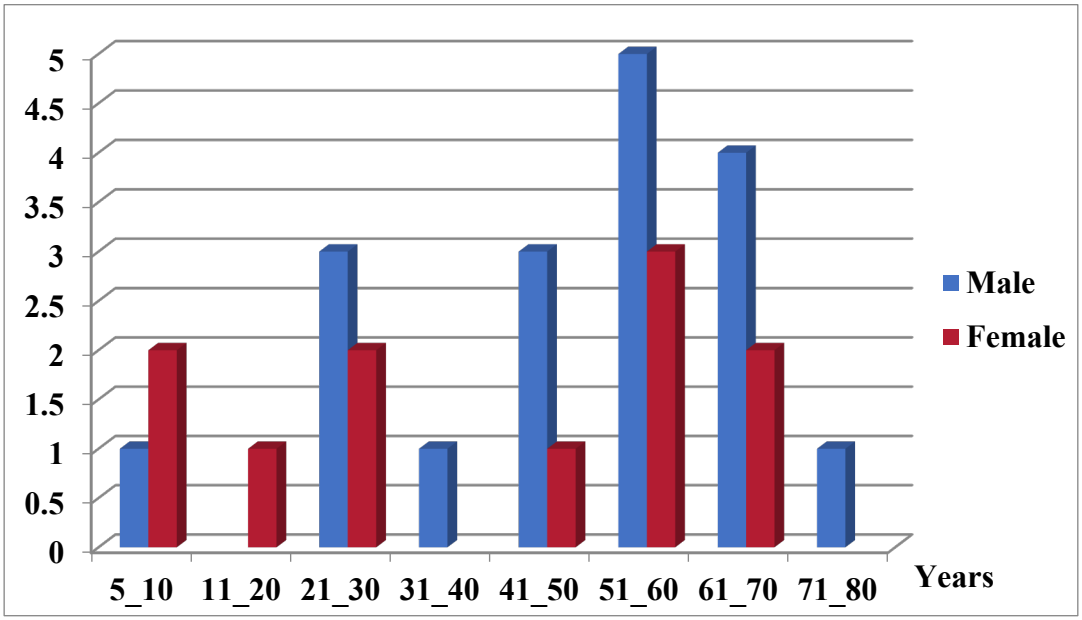


Figure 4.1: The distribution of AML cases by age and gender.

Using the FAB parameters, the morphological classification was completed. According to the FAB/WHO classification, the individuals were categorized as follows: Five instances are morphologically undifferentiated: four cases of M0, nine cases of M2, six cases each of M3, M5, and M6, and no cases of M1, M4, or M7 were found. Before beginning chemotherapy, the clinical and laboratory parameters of the study patients were addressed, and the cases were separated into 2 groups depending on the results of the post-induction bone marrow evaluation: those who

achieved CR, which included 24 (82.7%) patients, and those who achieved PR, which included 5 (17.3%) patients.

4.1.1. Complete Blood Count

Complete blood count results of median ±range for platelet counts was 38.80±279.0 in the CR group and in PR group was 268.0±288.0, and the P value of platelet count showed significant (P = 0.0207) as showed in (figure 4.2, D). While the difference among CR & PR patients for Hb, RBC and WBC were showed non-significant, as presented in (Table 4-1).

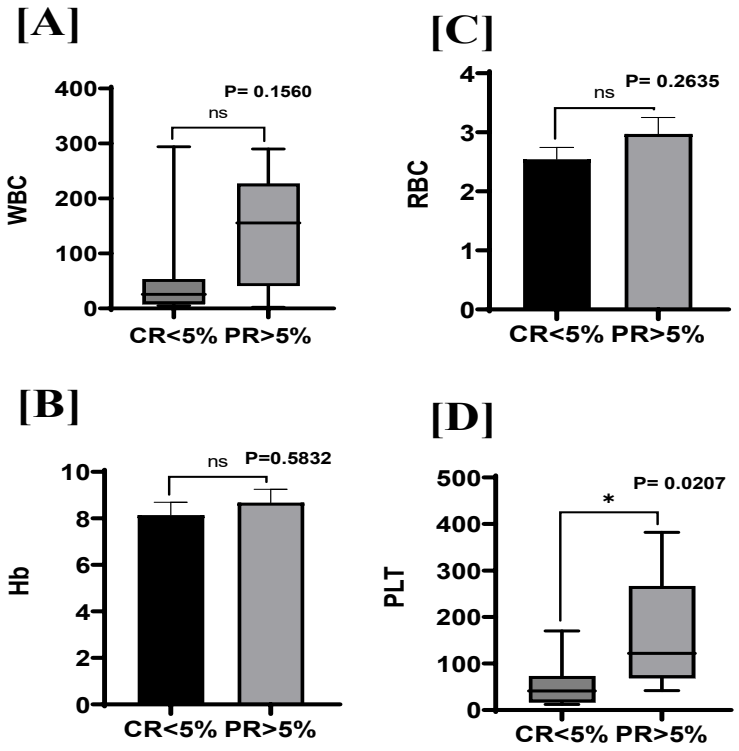


Figure 4.2: Percentage distribution of RBC, WBC, Hb(Hemoglobin) and platelets depending on CR/PR association.

Table(4.1): Hematological Parameters depends on CR/PR association in AML cases.

CBC	Mean		SE		Median		Range		P Value
	CR	PR	CR	PR	CR	PR	CR	PR	
WBC (10^9/1)					25.60	155.5	288.8	287.9	0.1560
RBC	2.542	2.972	0.202	0.280					0.2635
Hb (g/dl)	8.142	8.680	0.548	0.570					0.5832
Platelet 10^9/1					38.80	168.0	279.0	288.0	**0.0207***

WBC(White blood cells), RBC(Red blood cells), Hb(Haemoglobin), g/dl(Gram per Decilitre).

4.1.2. The Frequency of Positive Cells in AML Cases:

The most frequently positive cells in CD clusters were explained by the median immunophenotyping CD marker findings, which are shown in (figure 4.3). A total of 10 common CDs were investigated for the majority of AML patients, and they are CD13, CD33, MPO, HLADR, CD64, CD117, CD34, CD38, TdT, and CD22. The mean of these CDs is 75%, 70%, 60%, 60%, 55%, 55%, 50%, 40%, and 10%, respectively.

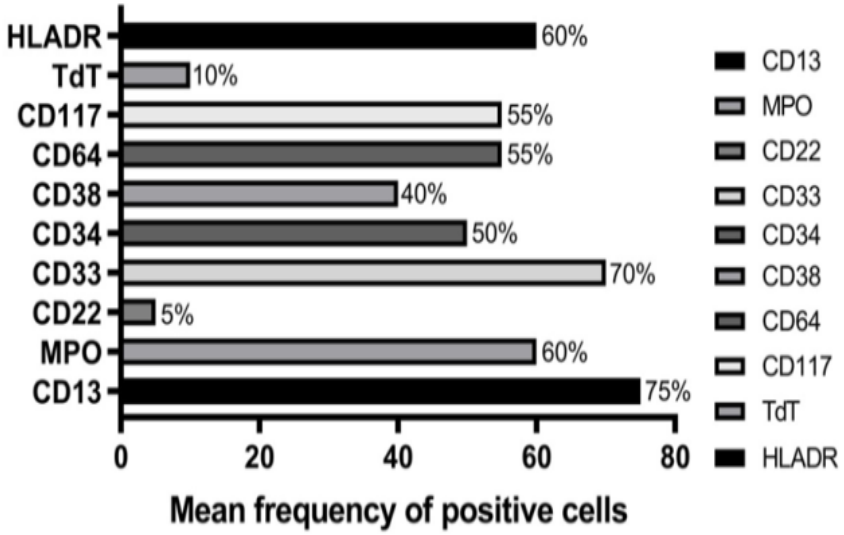


Figure 4.3: The expression of positive CD markers according to AML patients.

4.1.3. The Expression of Immunophenotyping CDs According to CR and PR Ratio:

More than 15 CDs were examined for AML diagnosis, which reported are (CD13, CD33, MPO, HLADR, CD64, CD117, CD34, CD38, TdT, and CD22), and the mean of them are (75%, 70%, 60%, 60%, 55%, 55%, 50%, 40%, 10%, and 5% respectively), but those CDs were included in CR/PR association are (CD13,CD33, HLADR, MPO, CD34, CD38, CD64, CD117, and TdT), also the percentages of blast, lymphocytes and granulocytes. The most diagnostic myeloid markers were CD13 and CD33(75% and 70%, respectively), which are the CD markers with the highest rates of expression in AML patients. The results of this research for CD13 were significant (P= 0.0012) in accordance with the relationship between CR/PR, and the results for CD33 were significant (P= 0.0012) for the same relationship(figure 4.4, A and D).

In 60% of cases, MPO and HLADR were both found to be the second most common CDs. MPO's CR/PR association p-value was significant (P=0.0103), whereas HLADR was reported non-significant (P=0.2272) based on the correlation between CR and PR ratio(figure 4.5, A & B). In (%55) of the cases, CD64 and CD117 were expressed, and the p-value for their relationship with CR/PR was highly significant (P=0.0001), as showed in (figure 4.4, C).

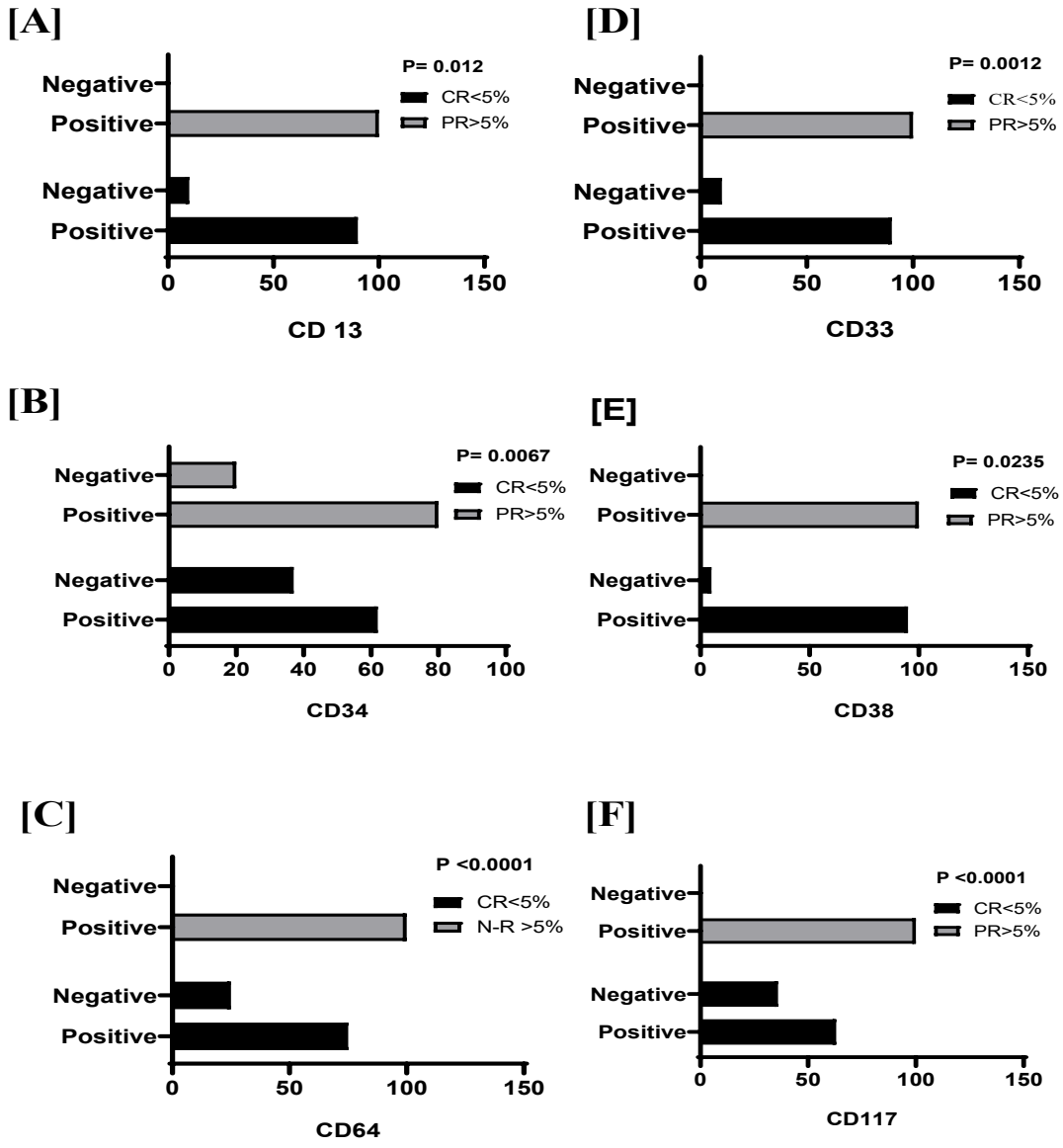


Figure 4.4: The expression of various CDmarkers(**A**: CD13, **B**:CD34, **C**: CD64, **D**: CD33, **E**: CD38 and **F**: CD117) in AML patients which categorized by CR/PR association.

The results for CD34 and CD38 were (50%) and (40%, respectively), and the P values for the CR and PR associations for both were significant (P=0.0067 and (0.0235) (figure 4.4, B & E), respectively). Less frequently occurring CDs in our cases were CD22 and TdT (10% and 5%, respectively). TdT marker showed significant (P=0.0209) CR/PR ratio, but in PR group the number of negative CDs higher than positive CDs, as shown in (figure 4.4 and 4.5).

According to the CR/PR association, other factors such as the lymphocyte percentage were slightly significant (P=0.0391), while the blast and granulocyte percentages were not (P=0.0818, and P=0.5077, respectively) (figure 4.5, D). In this study, additional CDs for AML cases were reported as being negative, including (CD2, cytoplasmic CD3, CD7, CD10, CD19 and cytoplasmic CD79 A).

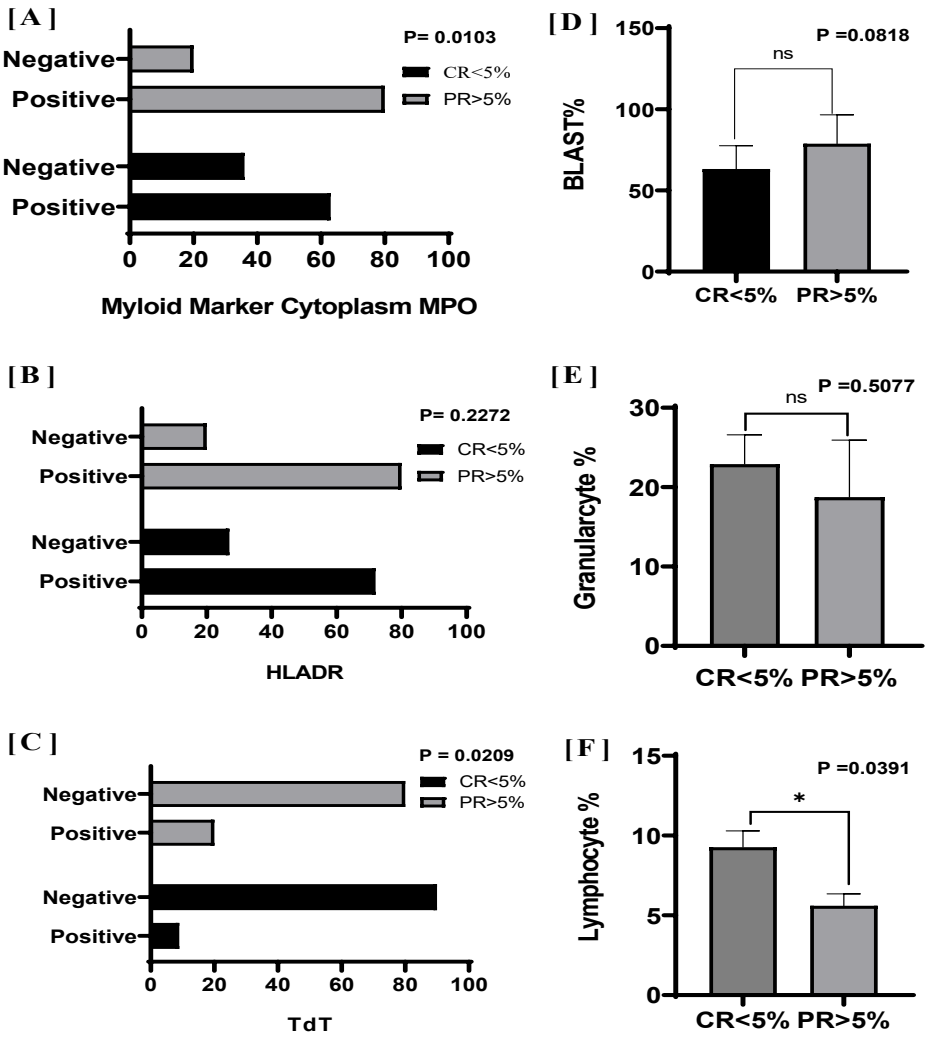


Figure 4.5: The expression of various antibody markers(**A**: MPO, **B**: HLADR and **C**: TdT), **D**: Blast%, **E**: Lymphocyte% and **F**: Granulocyte% from Flow Cytometry reports in AML patients categorized by CR/PR association.

4.1.4. Bone Marrow Aspiration Result According to CR and PR Ratio:

The hypercelularity of CR group is 90% but for PR group was %100, also the percentage of fragments in CR group was 10% but no fragments were seen in the PR group, p value of the CR/PR ratio indicated significant (0.0068)(figure 4.6, A). The

blast percentage were diagnosed in the bone marrow, was showed slightly significant(P=0.0365) according to the CR/PR association, as reported in (Table 4.2) and (figure 4.6, B & C).

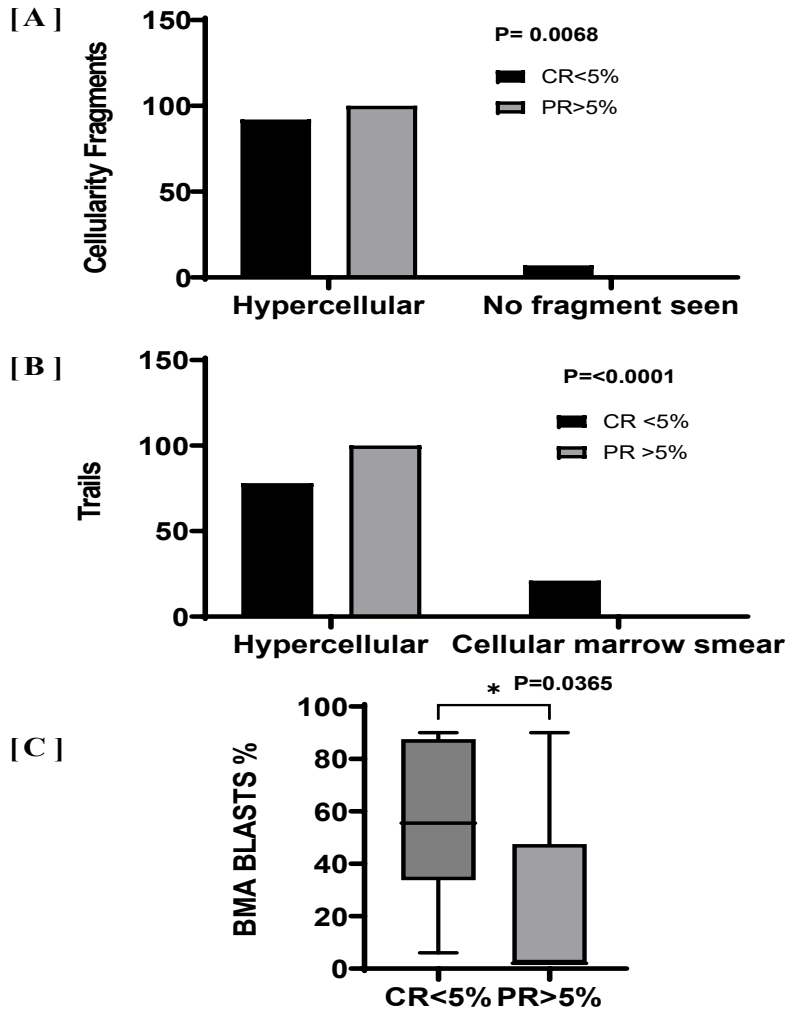


Figure 4.6: Considerable difference of Blast percentage, Trials and Cellularity fragments composition from bone marrow reports, depending on CR/PR association**.**

Table(4.2): Bone marrow cellularity and Bblast% results depend on CR/PR association in AML cases.

	Mean	SE	Median	Range	

Flow Cytometry	CR	PR	CR	PR	CR	PR	CR	PR	P Value
Cellularity Fragments									**0.0068***
Trails									**<0.0001****
BLASTS					55.50	2.000	84.00	88.00	**0.0365***

4.1.5. Analysis of P53 Mutations

In twenty samples screened and run for the detection of P53 mutations in exon 5, and found P53 mutations in all 20 cases (100%) as demonstrated in (figure 4.7).

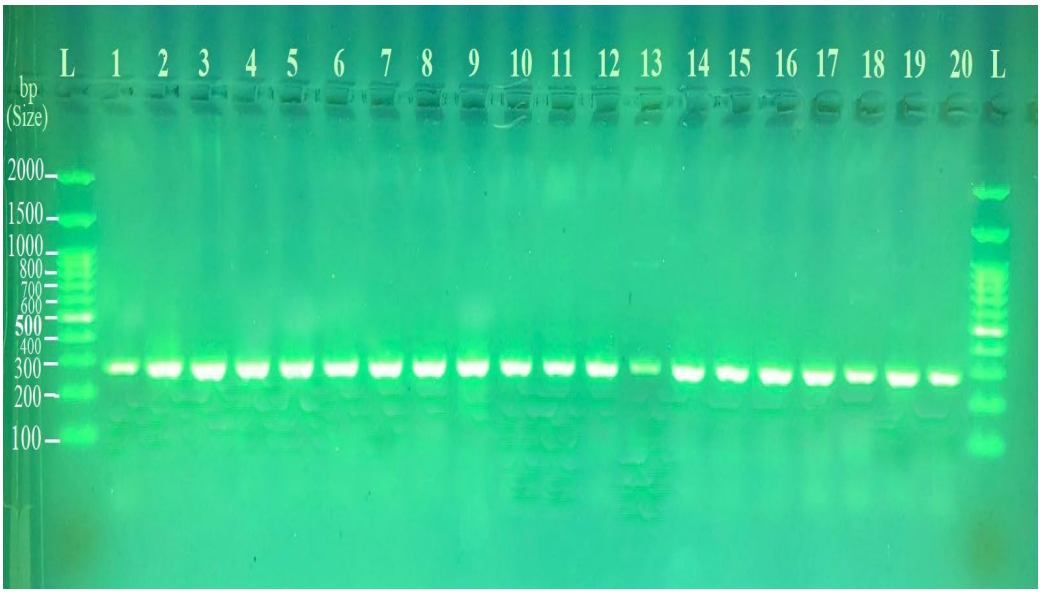


Figure 4.7: Agarose gel electrophoresis (2%) demonstrated corresponding to amplification in exon 5 of P53 mutation in 20 AML patients. Lane L: 100 bp DNA ladder. Lanes (Patient 1 to Patient 20) exhibit positive PCR DNA amplicons sizes (282 bp) at temperature 55**°C**, which indicate that al of 20 individuals with P53 mutation.

P53 mutations were found in all 20 of the samples that were screened and evaluated for P53 mutation detection in exon 6; the mean positive range for these mutations is 100%, as demonstrated in (figure 4.8).

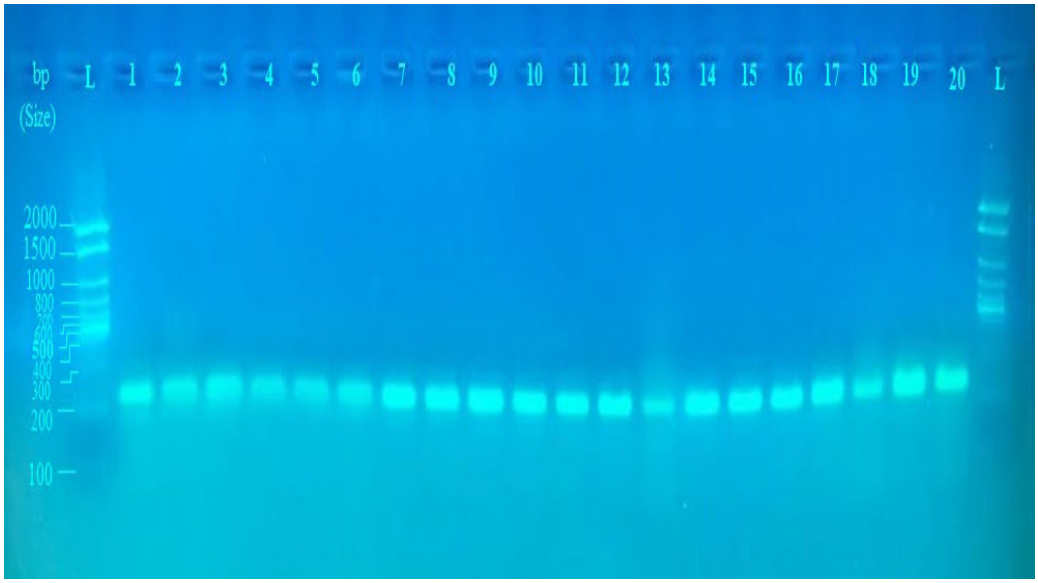


Figure 4.8: Agarose gel electrophoresis (2%) demonstrated corresponding to amplification in exon 6 of P53 mutation in 20 AML patients. Lane L: 100 bp DNA ladder. Lanes (Patient 1 to Patient 20) exhibit positive PCR DNA amplicons sizes (221 bp) at optimized temperature 62**°C**, which indicate that al of 20 individuals with P53 mutation.

After analyzing 20 samples out of 29 for the detection of P53 mutations in exon 7, we found P53 mutations in all 20 cases, and all mutated bands were very clear. This means that the positive range is 100%, as seen in (figure 4.9).

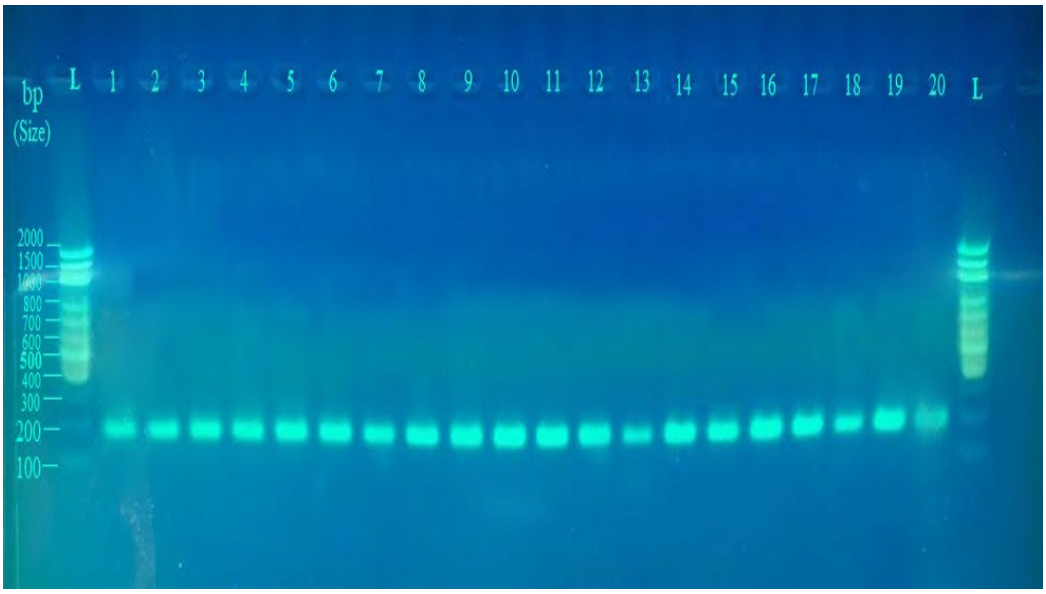


Figure 4.9: Agarose gel electrophoresis (2%) demonstrated corresponding to amplification in Exon 7 of P53 mutation in 20 AML patients. Lane L: 100 bp DNA ladder. Lanes (Patient 1 to Patient 20) exhibit positive PCR DNA amplicons sizes (182 bp) at optimized temperature 62**°C**, which indicate that al of 20 individuals with P53 mutation.

All 20 of the patients with P53 mutations that we screened and went through for exon 8 P53 mutation detection had the mean positive range of 100%, as demonstrated in (figure 4.10).

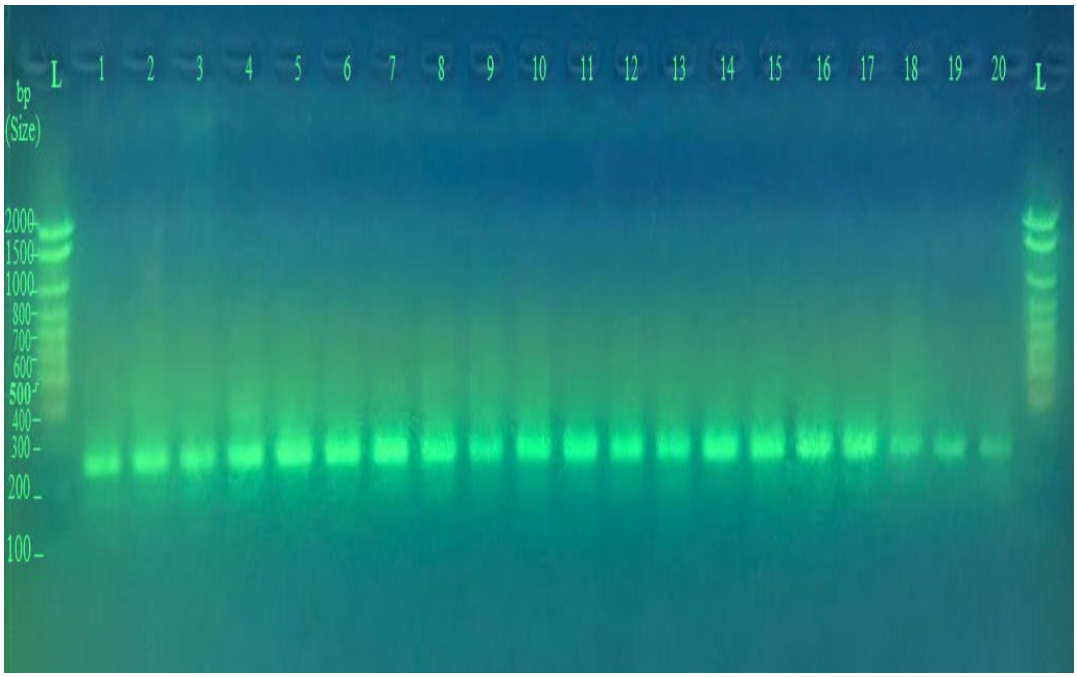


Figure 4.10: Agarose gel electrophoresis (2%) demonstrated corresponding to amplification in exon 8 of P53 mutation in 20 AML patients. Lane L: 100 bp DNA ladder. Lanes (Patient 1 to Patient 20) exhibit positive PCR DNA amplicons sizes (236 bp) at optimized temperature 59**°C**, which indicate that al of 20 individuals with P53 mutation**.**

4.1.6. Molecular Exon Sequencing

In the present study, PCR products of 5 AML patients was analyzed to detect the TP53 gene mutations in exons 5, 6, 7 and 8 by

using Sanger sequencing(Table 4.3). PCR products analyzed by Sanger sequencing only with forward primers.

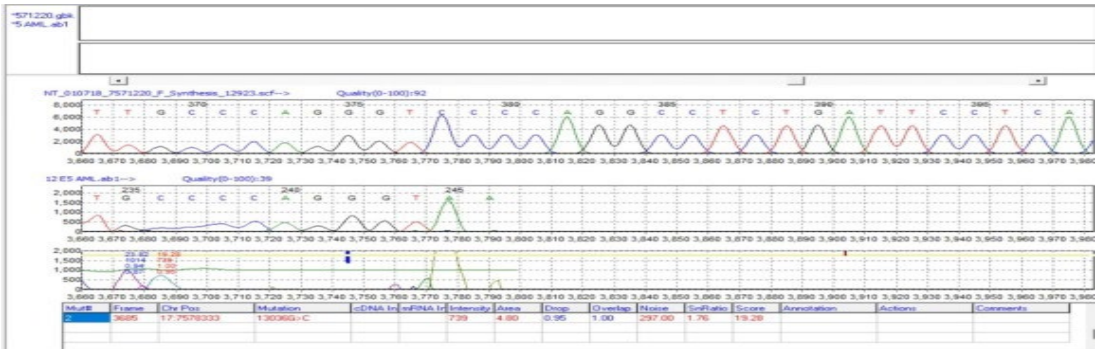
Table(4.3): Sanger sequencing analysis of TP53 gene mutations in exons 5, 6, 7 and 8 of AML patients.

NO.	Sample File No.	Gene	Exons	Mutation1	Mutation2	Mutation3
1	13	TP53	5			
2	14	TP53	5			
3	17	TP53	5	**13107G>GC, 176R>R/P$8**	**13186G>GT, 202P>P/P$7**	
4	6	TP53	5			
5	9	TP53	5	**13234G>GA$8**	**13244G>GC$62**	
6	13	TP53	6	**13158G>GC, 193R>R/P$7**	**13234G>GA$10**	
7	16	TP53	6			
8	17	TP53	6	**13159A>AG, 193R>R/R$52**		
9	2	TP53	6	**13107G>GC, 176R>R/P$11**	**13235T>TA$9**	
10	5	TP53	6	**13107G>GC, 176R>R/P$10**	**13235T>TA$9**	
11	18	TP53	7			
12	19	TP53	7			
13	2	TP53	7			
14	4	TP53	7			
15	7	TP53	7	**13899T>TA$15**		
16	1	TP53	8	**14216G>GC, 242G>G/A$8**		
17	13	TP53	8	**14289A>AG, 266E>E/E$7**		
18	19	TP53	8			
19	3	TP53	8	**14216G>GC, 242G>G/A$9**	**14239T>TG, 250F>F/V$6**	**14289A>AG, 266E>E/E$7**
20	9	TP53	8			

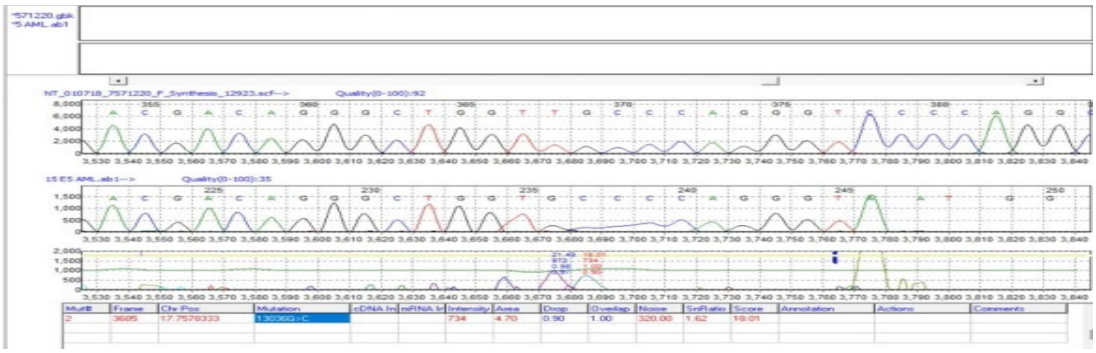
For exon 5 was randomly from total 20 PCR products selected 5 samples(13, 14, 17, 6 and 9). Sequencing reults showed 4 mutations in 2 samples, sample (17) have 2 mutations, 1 shift from G to GC (13107G>GC), & shift from G to GT (13186G>GT). 2 mutations in sample (9), shifts from G

to GA (13234G>GA$8) & G to GC (13244G>GC$62), that shifts cause changes in A.A molecules.

[A]



[B]



[C]

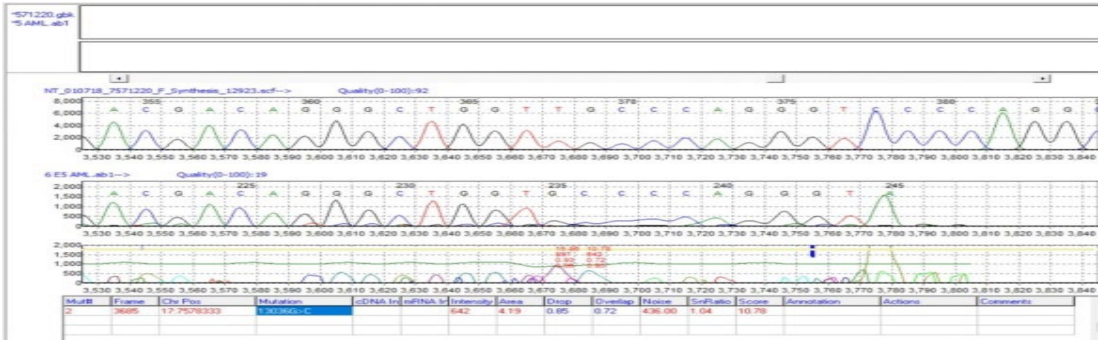


Figure 4.11. Show 4 mutations on the TP53 gene in exon 5 of AML patients.

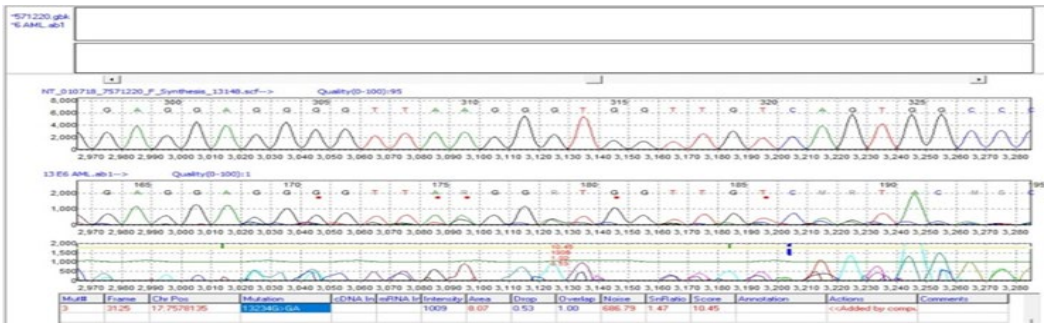
A) Shifts from G to GC (13107G>GC,176R>R/P$8) in sample (3 & 5).

B) Shifts from G to GT (13186G>GT, 202P>P/P$7) in sample number (3).

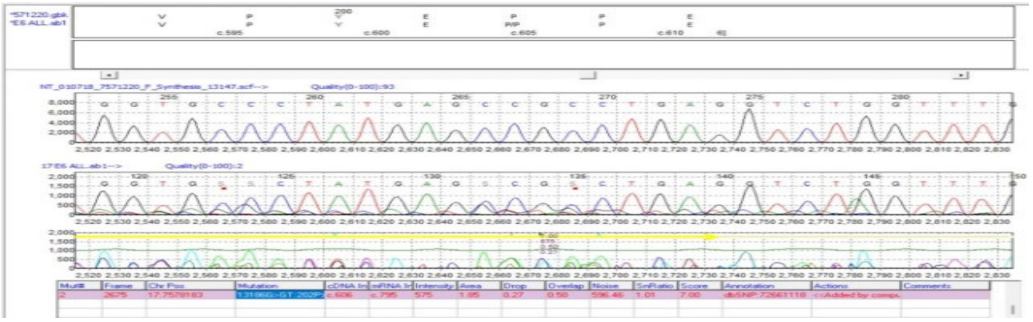
C) Shifts from G to GA (13234G>GA$8) in sample number (5).

For exon 6 was casually from total 20 PCR products selected 5 samples(13, 16, 17, 2 & 5). Sequencing reults showed 7 mutations in 4 samples, sample(13) have 2 mutations, first shifts from G to GC (13158G>GC), & 2nd shift from G to GA (13234G>GA$10). Sample (17), shifts from A to AG (13159A>AG). Sample (2 & 5) have same mutations, one shift from G to GC (13107G>GC), and 2nd shift from T to TA (13235T>TA$9), which causes changes in amino acid molecules.

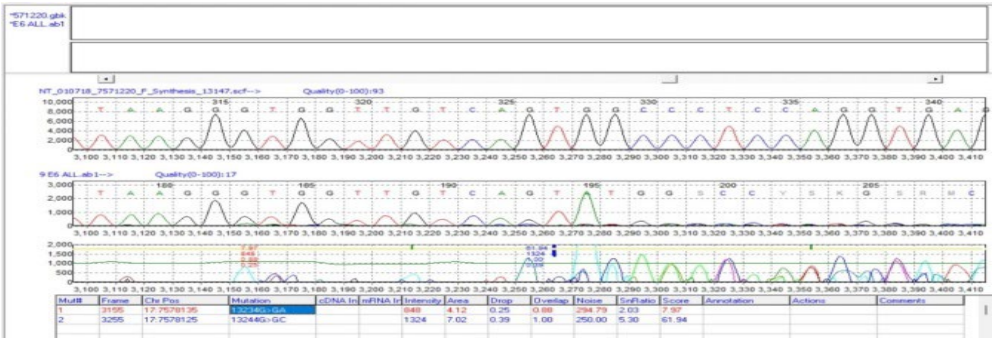
[A]



[B]



[C]



[D]

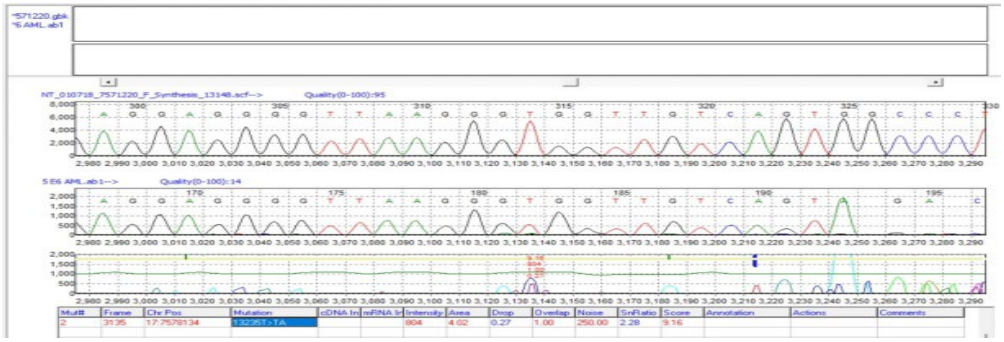


Figure 4.12. Show 5 mutations on TP53 gene in exon 6 of AML patients.
A) Shift from G to GC (13158G>GC,193R>R/P$7), in sample (13, 2 & 5).
B) Shift from G to GA (13234G>GA$10), in sample numer(13).
C) Shift from A to AG(13159A>AG, 193R>R/R$52) in sample number (17).
D) Shifts from T to TA (13235T>TA$9) in sample number (2 & 5).

For exon 7 was casually from total 20 PCR products selected 5 patients(18, 19, 2, 4 and 7). Sequencing reults showed only one mutations in in sample number (7), this mutation was shift from T to TA (13899T>TA$15) which causes changes in amino acid molecules.

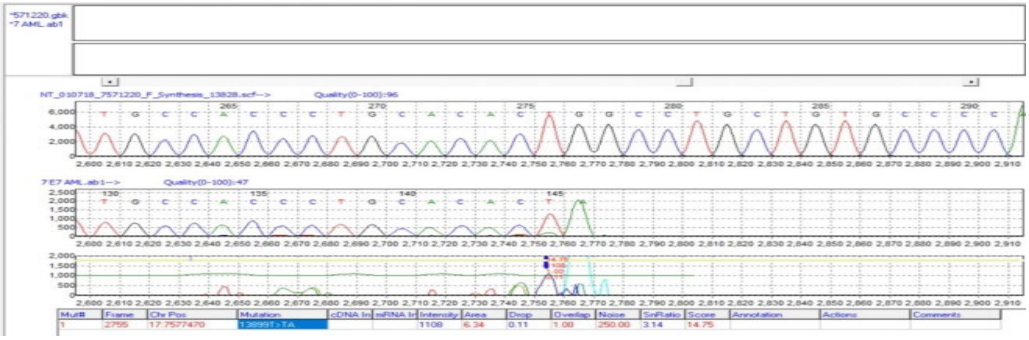
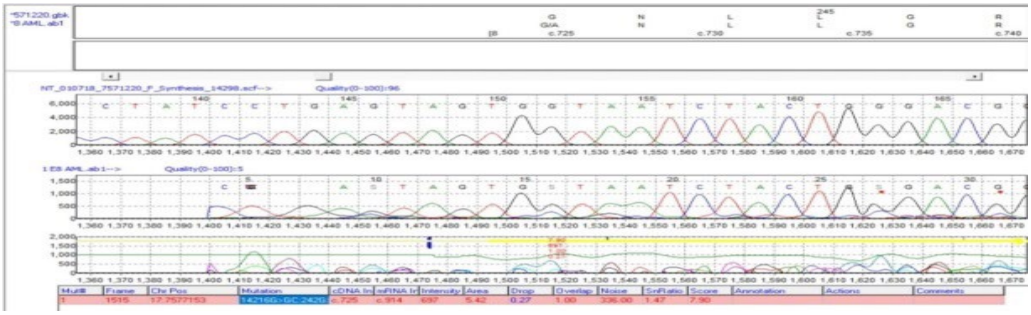


Figure 4.13: Show a mutation on the TP53 gene in exon 7 of AML sample number(7), which shift from T to TA (13899T>TA$15).

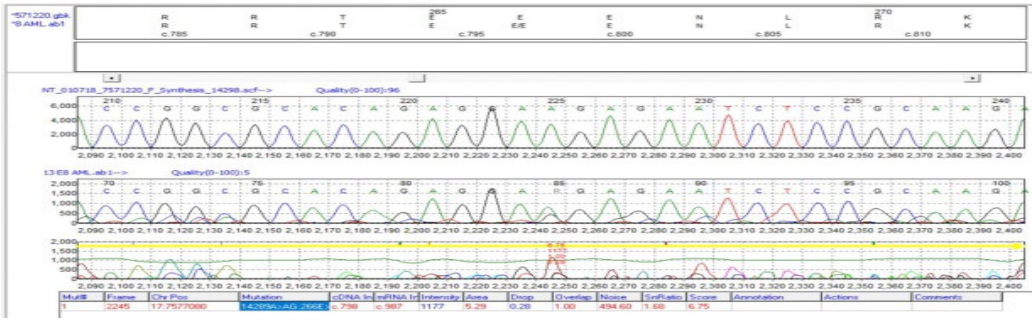
For exon 8 was casually from total 20 PCR products selected 5 samples(1, 13, 19, 3 and 5). Sequencing reults showed mutations in samples (1, 13 and 3), in those have 5 mutations, which sample (1) have only 1 mutation,

shifts from G to GC (14216G>GC), and patient 13 have 1 mutation which shift from A to AG (14289A>AG). For the patient number three have 3 mutations which one of them shifts from G to GC (14216G>GC), the second shifts from T to TG (14239T>TG,250F>F/V$6), and 3rd shift from A to AG(14289A>AG), those mutations was cause changes in A.A molecules.

[A]



[B]



[C]

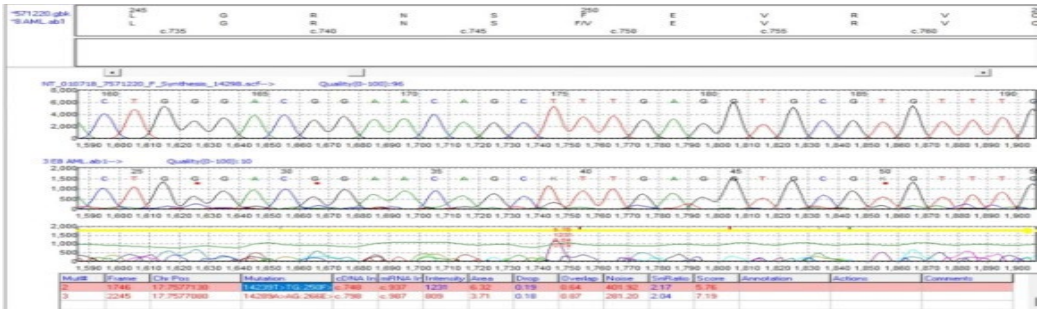


Figure 4.14: Show 5 different mutations on the TP53 gene in exon 8 of AML A) shift from G to GC (14216G>GC, 242G>G/A$8) of sample number(1).

B) shift from A to AG (14289A>AG,266E>E/E$7) in sample (13 & 1).

C) shift from G to GC (14216G>GC) & T to TG (14239T>TG) in sample (3).

4.2. Acute Lymphoblastic Leukemia

Over the course of a year, we gathered 32 diagnosed ALL cases, of which 14 (43.75%) were males and 18 (56.25%) were females. The patients' ages varied from 1 to 25 years old, with a mean patient age of 7.34 years, as shown in (figure 4.26).

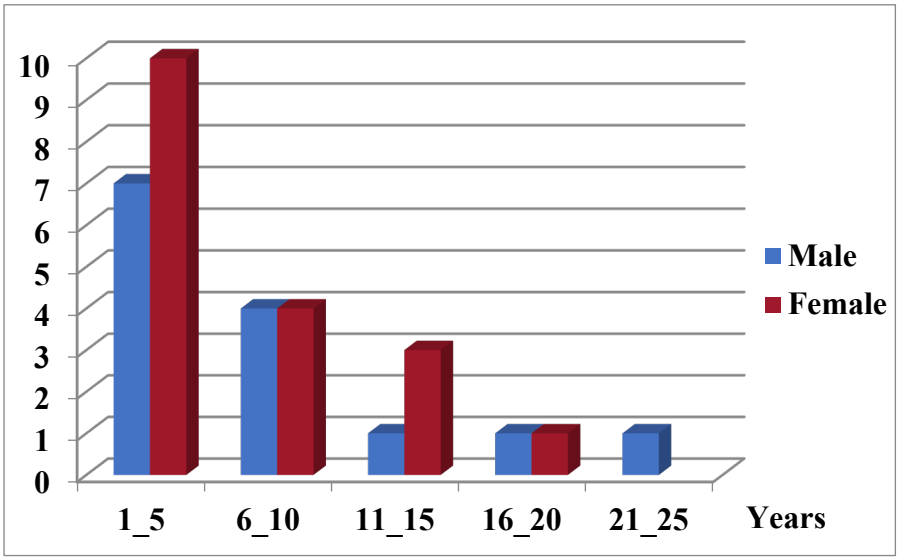


Figure 4.15: Age and Gender distribution of ALL patients.

The morphological classification of patients included in this study was not identified in the hospital reports, but ALL sub-types were identified, 27(84.37) of them are B-ALL subtype and 5(15.63) of cases are T-ALL subtype, overall 32 ALL patients. Since about 80 percent of ALL arise from the B-cell lineage and from 15 to 20 percent of ALL cases arise from the T-cell lineage 5. The clinical and laboratory parameters of ALL studied patients were addressed before

starting of chemotherapy & cases are classified depending on post induction B.M evaluation to 2 groups; patients who achieved CR which includes (78.1%%) patients & those with PR (21.9%) patients**.**

4.2.1. Complete Blood Count(CBC) results according to CR/PR associatioan in ALL cases:

The results of complete blood count parameters are statistically showed non-significant depending on the CR/PR association, and the P-values of WBC, RBC, Hb and platelets count are (0.5709, 0.5295, 0.6633, and 0.1062 respectively) as presented in (Figure 4.16- A, B, C, & D) and (Table 4.4).

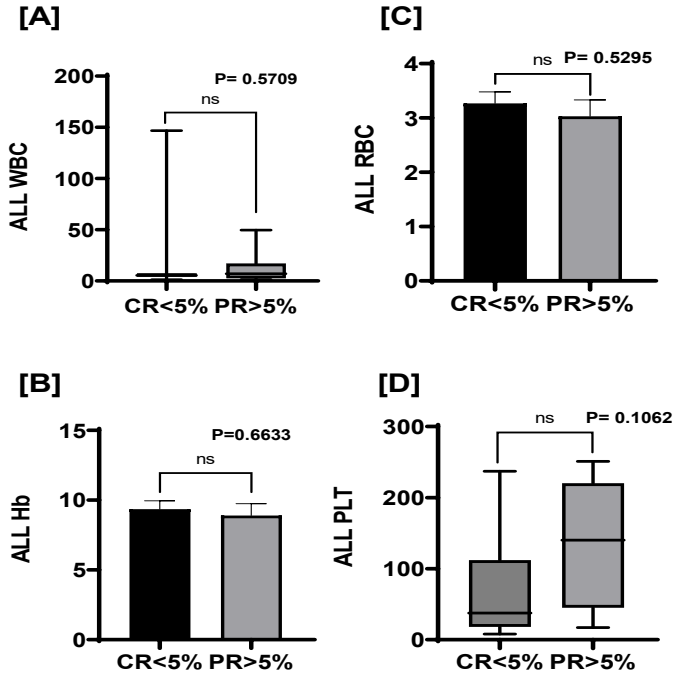


Figure 4.16: Percentage distribution of RBC, WBC, Hb(Hemoglobin) and platelets depending on CR/PR association.

Table (4.4): Hematological Parameters depends on CR/PR association in ALL patients.

CBC	Mean		SE		Median		Range		P Value
	CR	PR	CR	PR	CR	PR	CR	PR	
WBC (10^9/1)					5.500	7.050	145.5	48.20	0.5709
RBC	3.267	3.031	0.212	0.301					0.5295
Hb (g/dl)	9.357	8.900	0.595	0.847					0.6633
Platelet 10^9/1					37.50	140.0	229.0	234.0	0.1062

WBC(white blood cells), RBC(red blood cells), Hb(haemoglobin).

4.2.2. The Frequency of Positive Cells

The examined CDs for most of ALL patients are 14 CD markers, were the mean frequency of positive cells of immunophenotyping results for cluster CDs, showed that the most frequent to less frequent CDs (CD19, CD79a, TdT, HLADR, CD10, CD22, CD34, CD2, CD7, and CD13), the frequency of them are (95.24%, 95.24%, 95%, 90%, 85.71%, 80%, 50%, 20%, 10%, and 9.09% respectively), other CDs (T Lymphoid Specific Marker Cytoplasmic CD3, CD14, CD15, CD33 and MPO) reprted as negative in all ALL patients, which are showed in (Figure 4.17)

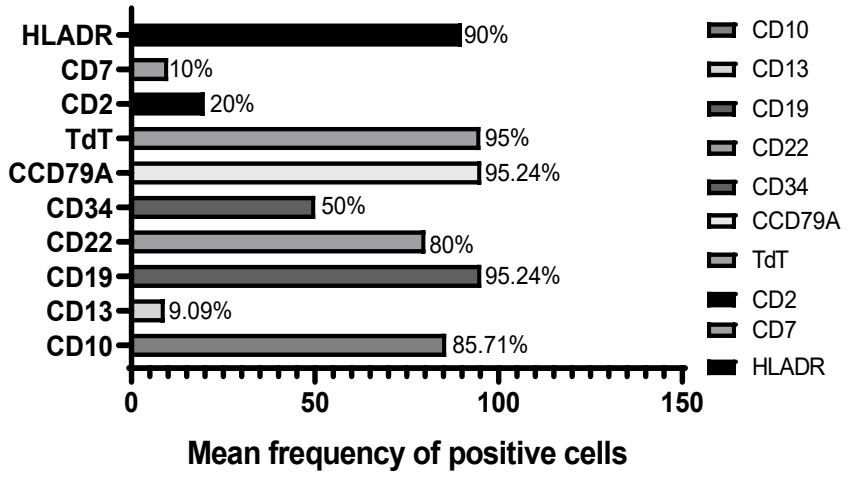


Figure 4.17: Expression of positive CD markers according to ALL patients.

4.2.3. The Expression of Immunophenotyping CDs

Cluster CDs are a group of membrane proteins that are mainly found on the surfaces of white blood cells (WBCs) and are critical for identifying and segregating different lymphocyte and leukocyte types. The Nanakaly hospital found that more than 14 CD markers, including CD2, CD3, CD10, CD13, CD14, CD15, CD19, CD22, CD33, CD34, CD79a, MPO, HLADR, and TdT, as well as the percentages of blast, lymphocyte, and granulocyte, were necessary for the detection of ALL disease. The most prevalently expressed CD markers in ALL patients were CD19, CD79A, and TdT, which were also the most definitive lymphoid markers (95.24%, 95.24%, and 95%, respectively) (figure 4.18, B & F) and (figure 4.19, B).

The results of CD19 confirmed a significant relationship between the CR/PR ratio ($P=0.0003$), CD79A confirmed a strongly significant relationship for the same association ($P<0.0001$), and TdT is highly significant ($P<0.0001$), as shown in (Figure 4.19, B). In 90% of cases, HLADR are found to be the second most

common CDs, and based on the correlation between CR and PR ratio in ALL cases, the p-value for HLADR was being highly significant (P<0.0001) (figure 4.19, A). CD10 was expressed in 85.71 percent of patients, and the correlation between CR and PR p values for both was highly significant (P<0.0001). Given that the positivity rate for the CR group is 100% and the result of the PR group shows that 80% of CDs are positive and 20% of CDs are negative, this result is unwelcome. CD22 was expressed in (80%) of cases, and depending on the association of CR/PR, p-values were strongly significant (P<0.0001)(Figure 4.18, E).

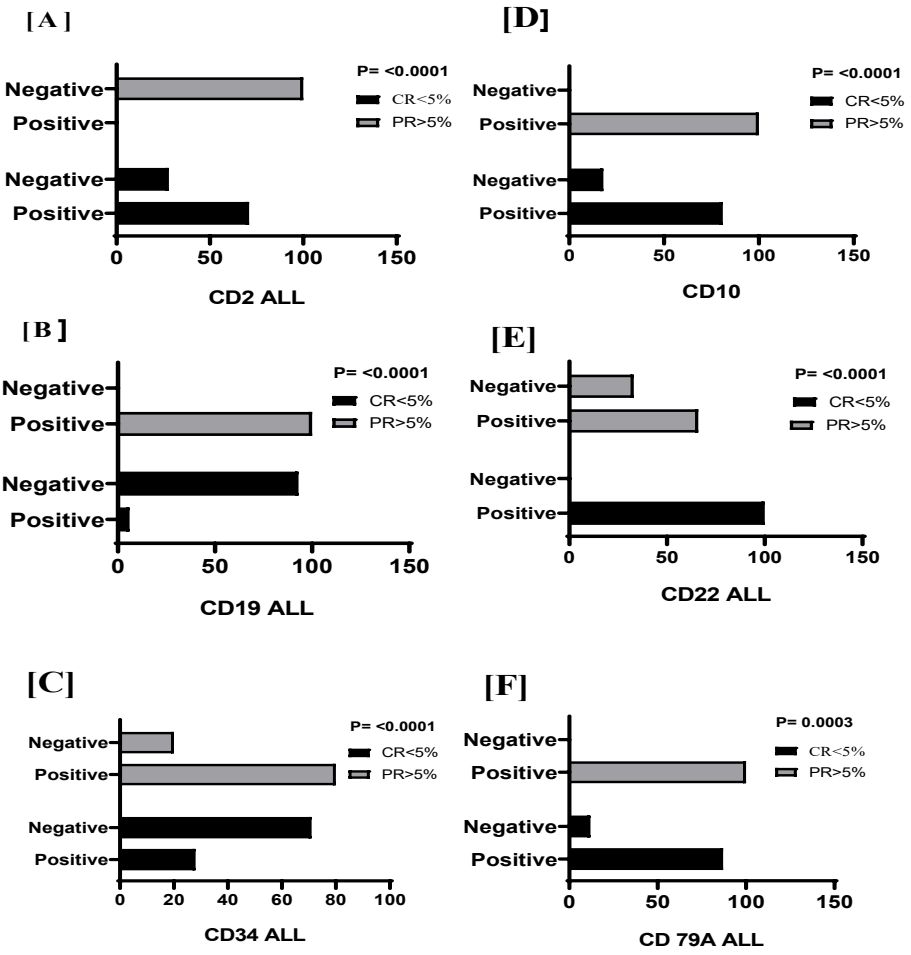


Figure 4.18: The expression of various CDmarkers(CD2, CD10, CD19, CD22, CD34 and CD79A) in ALL patients which categorized by CR/PR association.

In fifty percentage of the patients, CD34 was only moderately expressed, and the p-value for the relationship between CR/PR was highly significant (P<0.0001). In ALL instances, CD2 were the least frequently expressed CDs; their mean frequency was 20%, and their results were also highly significant (P 0.0001) when compared to the correlation between CR/PR ratio(figure 4.19, C).

Other variables such as blasts, lymphocytes, and granulocytes demonstrated non-significant (P=0.1442, P=0.6076 and P=0.1790, respectively) dependence on the CR/PR relationship, as shown in (figure 4.19- C, D and E). In this study, many additional CDs were recorded as negative for ALL patients, including (T Lymphoid Specific Marker Cytoplasmic CD3, CD14, CD15, CD33 and MPO).

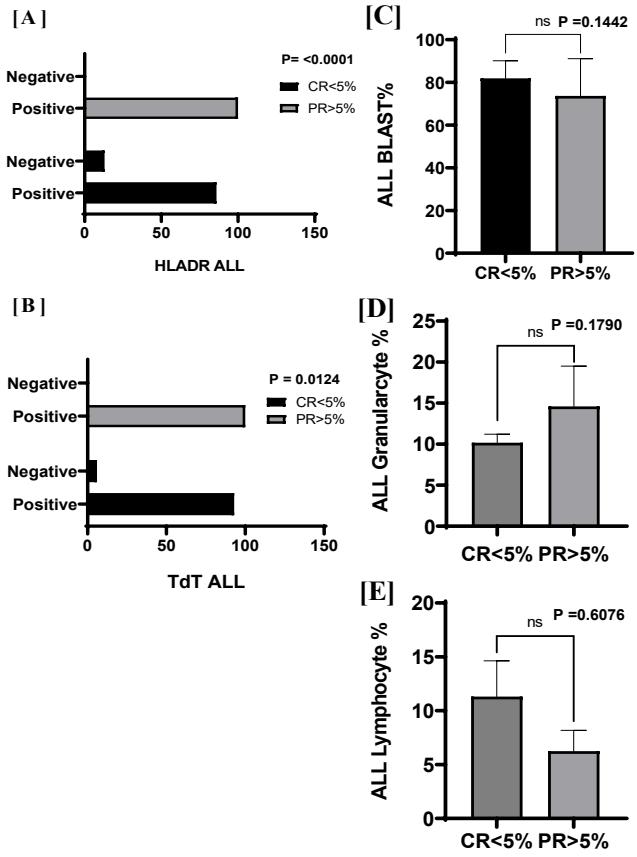


Figure 4.19: The expression of various antibody markers(A; HLADR and B; TdT), C; Blast%, D; Granulocyte% and E; Lymphocyte% in ALL patients categorized by CR/PR association.

4.2.4. Bone Marrow Aspiration Result According to CR and PR Ratio:

The reports of bone marrow aspiration was included in our study. One of parameters is hypercelularity of CR group is 40% but for PR group was 85%, while normocelluarity were seen only in CR group, the p value of this ratio showed strongly significant (P<0.0001) (figure 4.20, A & B). The blast percentage in the bone marrow according to the CR/PR association was reported as non-significant(P=0.1450), as presented in (Figure 4.20, C) and (Table4.5).

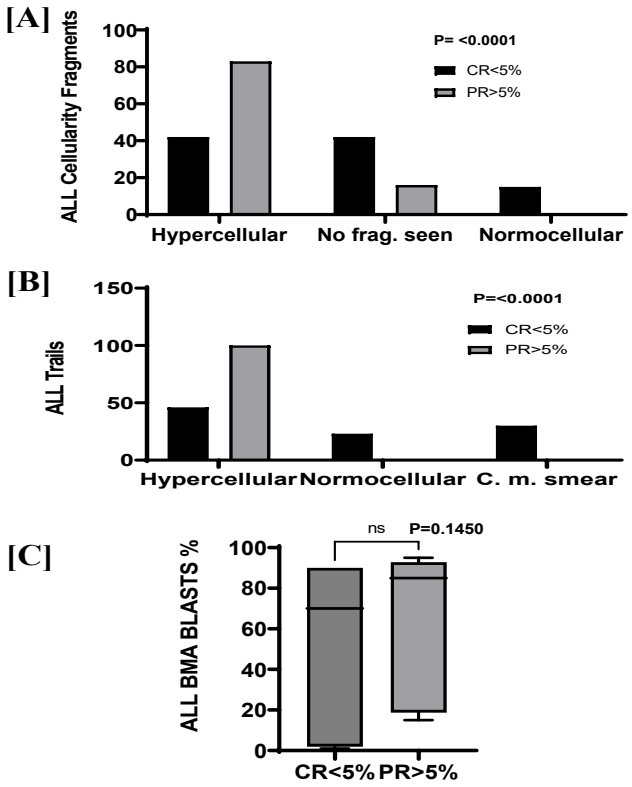


Figure 4.20: Considerable difference of Blast percentage and Cellularity fragments composition, depending on CR/PR association**.**

Table (4.5): Bonw marrow reports depending on CR/PR association in ALL patients.

No.	Bone Marrow Aspiration	P value
1	Cellularity Fragments	**<0.0001 ******
2	Trials	**<0.0001 ******
3	BLASTS	0.1450

4.2.5. Analysis of P53 Mutations

Twenty of the total 32 ALL cases were investigated in order to identify P53 mutations in exons 5 to 8. Following DNA extraction and PCR, 20 DNA samples that had already been verified by bone marrow aspiration and FCM were performed in order to see if P53 mutation bands were present.

Twenty samples were screened and analyzed for the presence of P53 mutations in exon 5. All 20 patients (100%) had these mutations, as demonstrated in (figure 4.21).

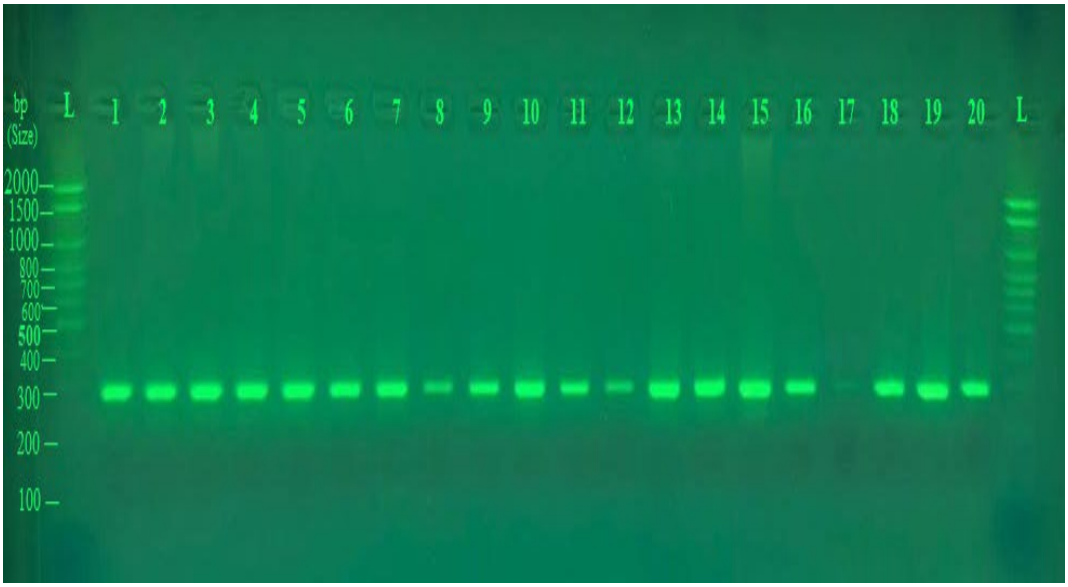


Figure 4.21: Agarose gel electrophoresis (2%) demonstrated corresponding to amplification in exon 5 of P53 mutation in 20 ALL patients. Lane L: 100 bp DNA ladder. Lanes(Patient 1 to Patient 20) exhibit positive PCR DNA amplicons sizes (282 bp) at temperature 55**°C**, which indicate that all of 20 individuals with P53 mutation.

Twenty specimens were screened and run for the presence of P53 mutations in exon 6. All 20 individuals had these mutations, meaning that 100% of the samples tested positive(figure 4.22). In all cases showed clear mutated bands, while in cases number 12 and 17 showed light bands.

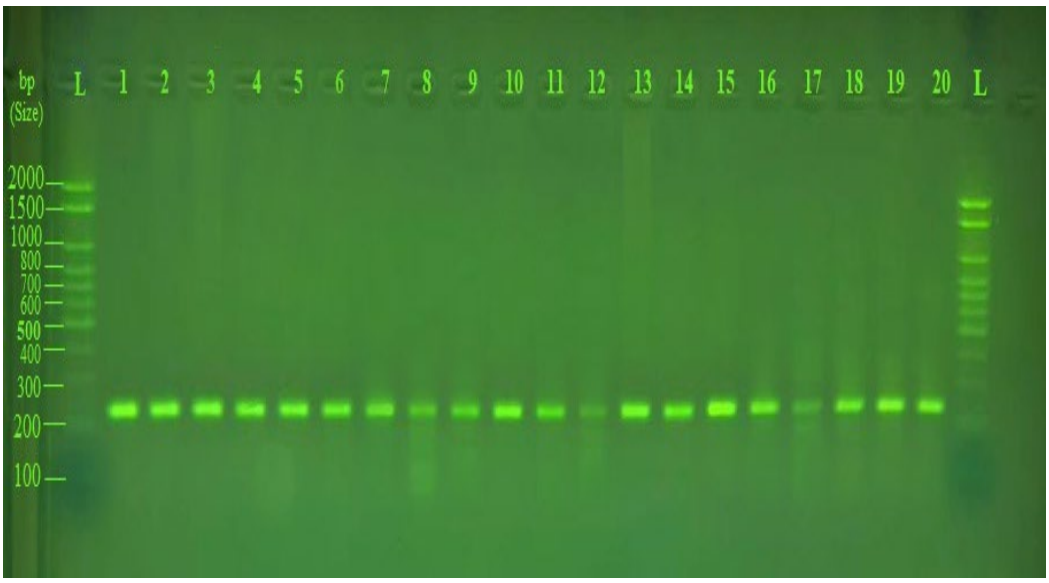


Figure 4.22: Agarose gel electrophoresis (2%) demonstrated corresponding to amplification in exon 6 of P53 mutation in 20 ALL patients. Lane L: 100 bp DNA ladder. Lanes Patient 1 to patient 20) exhibit positive PCR DNA amplicons sizes (221 bp) at optimized temperature 62°C, which indicate that all of 20 individuals with P53 mutation.

After examining 20 samples out of 29 for the presence of P53 mutations in exon 7, we discovered that all 20 cases had these mutations. All of the mutated bands were also very clear, with the exception of sample number 12's band, which revealed a light band due to the sample's lower than normal average purity (purity = 1.65)

of DNA. As can be seen in, this indicates that the favorable range is 100%. (figure 4.23).

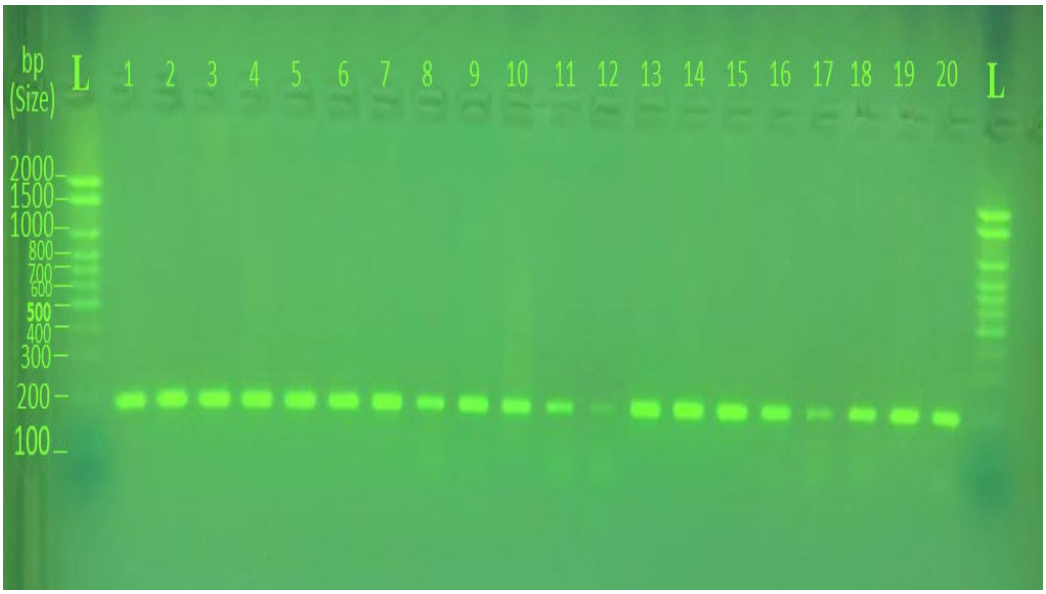


Figure 4.23: Agarose gel electrophoresis (2%) demonstrated corresponding to amplification in Exon 7 of P53 mutation in 20 ALL patients. Lane L: 100 bp DNA ladder. Lanes (Patient 1 to patient 20) exhibit positive PCR DNA amplicons sizes (182 bp) at optimized temperature 62**°C**, which indicate that all of 20 individuals with P53 mutation.

The mean positive range for exon 8 P53 mutant detection was 100% in all 20 of the patients with P53 mutations that we screened and examined. This is shown in (figure 4.24). Because no samples from the CR and PR group were flagged as negative.

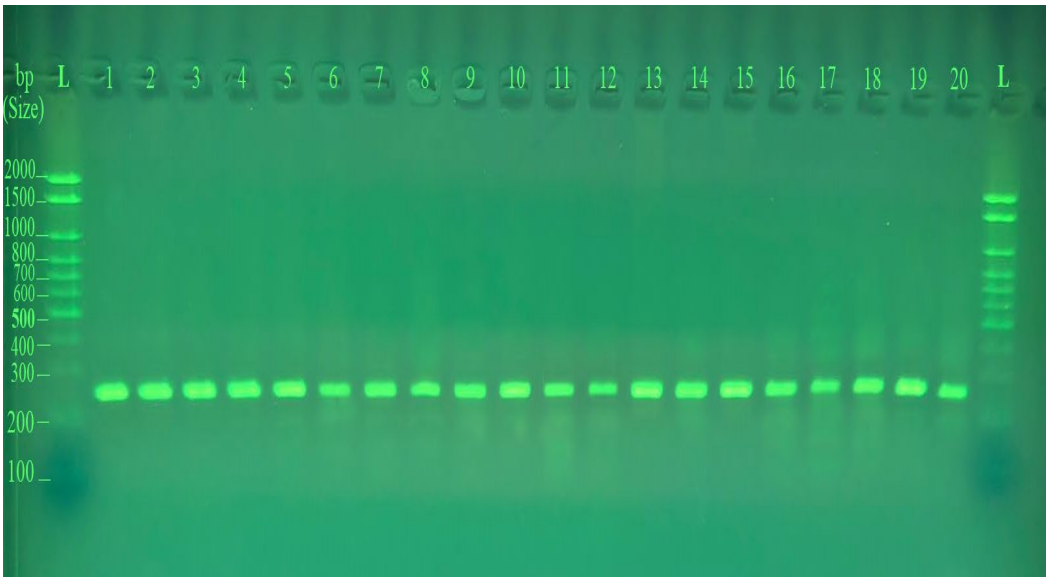


Figure 4.24: Agarose gel electrophoresis (2%) demonstrated corresponding to amplification in exon 8 of P53 mutation in 20 ALL patients. Lane L: 100 bp DNA ladder. Lanes (Patient 1 to patient 20) exhibit positive PCR DNA amplicons sizes (236 bp) at optimized temperature 59**°C**, which indicate that al of 20 individuals with P53 mutation.

4.2.6. Molecular Exon Sequencing

In the present study, PCR products of 5 Acute lymphoblastic leukemia patients was analyzed to detect the TP53 gene mutations in exons 5 to 8 by using Sanger sequencing, as Snger sequencing results presented in the (Table 4.6). PCR products analyzed by Sanger sequencing only with forward primers.

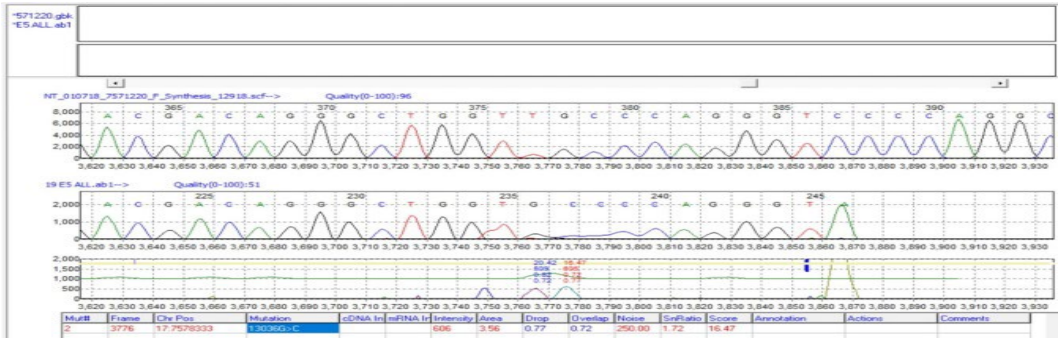
Table(4.6): Sanger sequencing analysis of TP53 gene mutations in exons 5, 6, 7 and 8 of ALL patients.

No.	Sample File No	Gene	Exons	Mutation 1	Mutation 2
1	14	TP53	5		
2	15	TP53	5		
3	16	TP53	5	**12834A>AT,112K>K/M$9**	**13045C>A$151**
4	19	TP53	5	**13035T>TG$20**	**13036G>C$16**
5	9	TP53	5		
6	13	TP53	6		
7	14	TP53	6		
8	17	TP53	6	**13107G>GC,176R>R/P$8**	**13186G>GT,202P>P/P$7**
9	6	TP53	6		
10	9	TP53	6	**13234G>GA$8**	**13244G>GC$62**
11	1	TP53	7	**13899T>TA$7**	
12	11	TP53	7		
13	12	TP53	7		

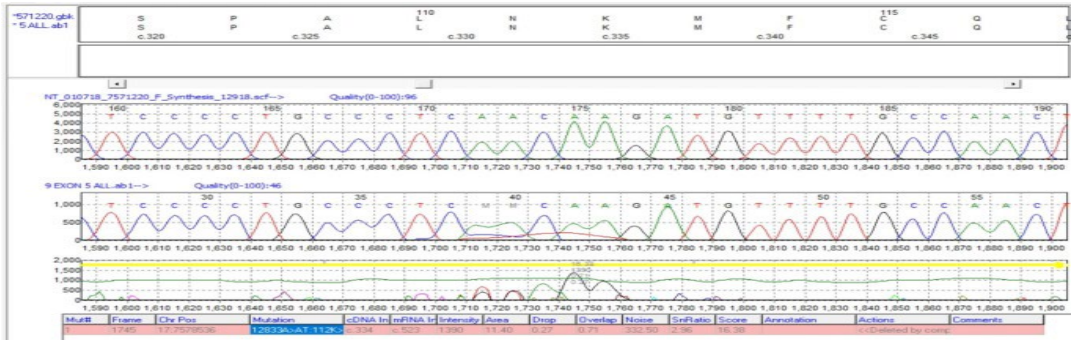
14	3	TP53	7	**13899T>TA$10**	
15	6	TP53	7	**13899T>TA$5**	
16	14	TP53	8		
17	16	TP53	8		
18	2	TP53	8		
19	20	TP53	8		
20	9	TP53	8		

For exon 5 was randomly from total 20 PCR products selected 5 patients(14, 15, 16, 19 and 9). Sequencing reults showed 4 mutations in in 2 patients, which sample(16) have 2 mutations, one shift from A to AT (12834A>AT), and other shift from C to A (13045C>A$151). 2 different mutations in patient(19), Which shift from T to TG (13035T>TG$20), another shift from G to C (13036G>C$16), that shifts cause changes in amino acid molecules.

[A]



[B]



[C]

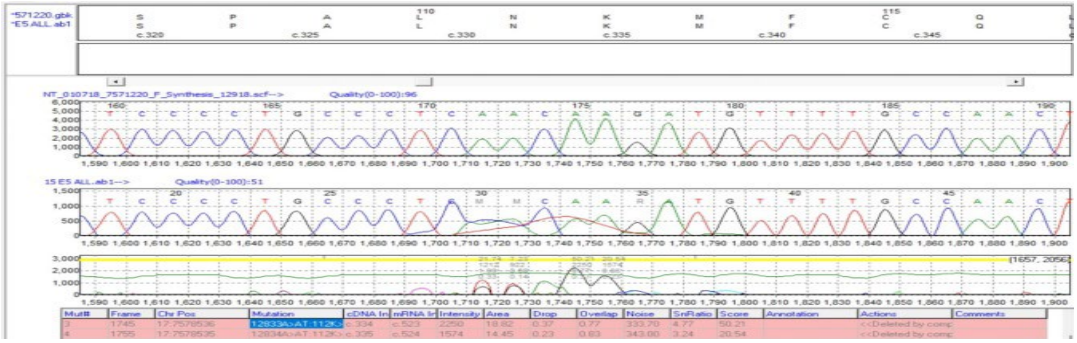


Figure 4.25: Show 4 mutations on TP53 gene in exon 5 of ALL patients. A) Shift from A to AT (12834A>AT,112K>K/M$9) Sample number (16).

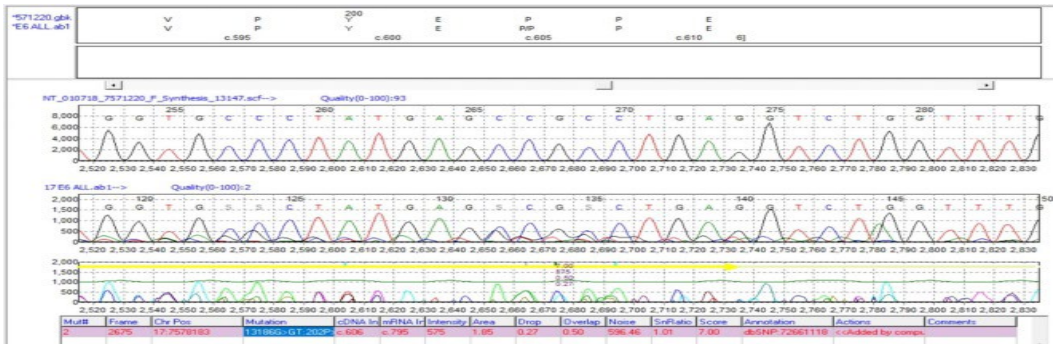
B) shifts from C to A (13045C>A$151), and Patient number (19),

C) shifts from T to TG (13035T>TG$20) in patient number (19)

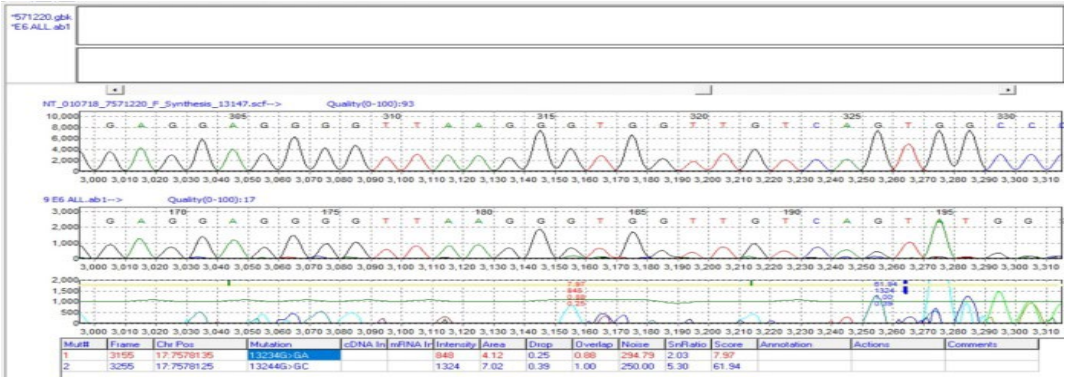
For exon 6, from total 20 PCR products selected 5 patients(13, 14, 17, 6 and 9). Sequencing reults showed 4 mutations in 2 samples, sample 17 have 2 mutations, 1st shift from G to GC (13107G>GC), & 2nd from G to GT

(13186G>GT). Patient (9) have same repeated mutations, first shifts from G to GA (13234G>GA$8),& 2nd shifts from G to GC (13244G>GC$62).

[A]



[B]



[C]

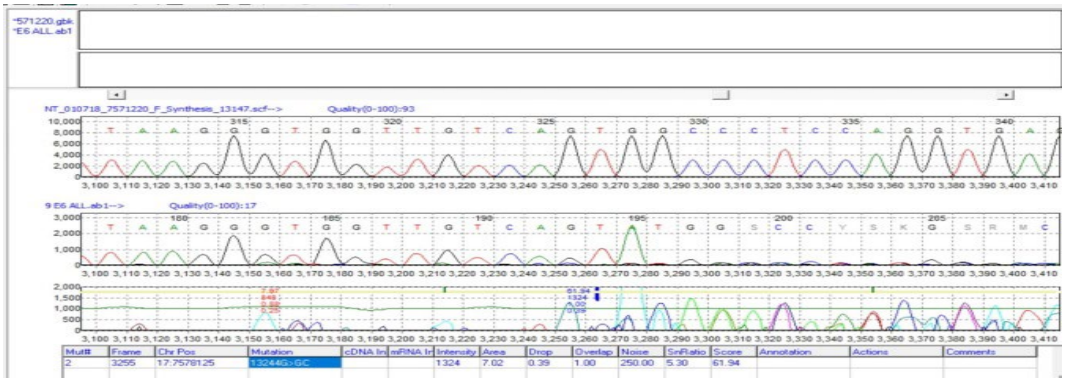


Figure 4.26: Show 4 mutations on the TP53 gene in exon 6 of ALL patients. A) Shifts from G to GC (13107G>GC), in sample number (17 & 9)

B) Shifts from G to GT (13186G>GT) in sample number (17).

C) Shifts from G to GA (13234G>GA$8) in sample number (9).

For exon 7 sequencing was randomly from total 20 PCR products selected 5 patients(1, 11, 12, 3 and 6). Sequencing results showed same mutations in all 3 patients(1, 11 and 6), those mutations was shifts from T to TA (13899T>TA$7, 13899T>TA$10 and 13899T>TA$5), as presented in (Figure 4.27), which causes changes in amino acid molecules.

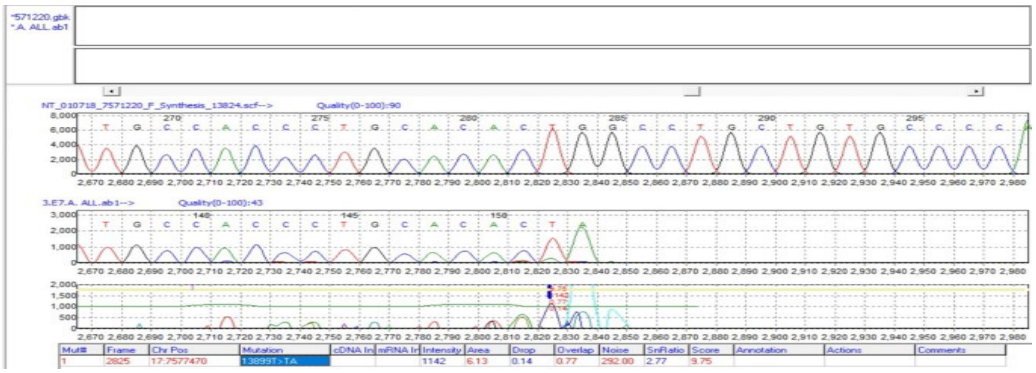


Figure 4.27: Show the same mutations on the TP53 gene in exon 7 of ALL patients. Patients number (1, 11 and 6), all have the same type of mutation, shifts from T to TA (13899T>TA$7, 13899T>TA$10, 13899T>TA$5), which causes changes in amino acid molecules.

For exon 8 sequencing was randomly from total 20 PCR products selected 5 patients(14, 16, 2, 20 and 9). The sequencing reults showed that no mutations were present in all 5 samples.

DISCUSSION

5-DISCUSSION

5.1 Acute Myeloid Leukemia

Acute Myeloid Leukemia (AML) is a diverse collection of diseases that can manifest with various morphologic, immunophenotypic, and cytogenetic traits. The identification of these patterns may be crucial for a more accurate prognosis evaluation and an effective treatment plan (Aggarwal and Weinberg, 2021).

The average age of the AML patients was 38.47 years old, with 18 men and 11 women participating. Of the 29 AML patients, 24 cases (82.7%) experienced a complete remission(CR), while 5 cases (17.3%) experienced a partial remission(PR). According to (Pouls RK et al, 2012), who carried out a study in Erbil city, 94 adult patients were diagnosed as AML, 58 of them were males and 36 were females; studied patients ranged between 16 and 75 years with a mean age (±SD) of 33.8±21.3 years.

The M2 subtype of AML was the most prevalent subtype in our research data, accounting for 9 cases (31% of subtypes), which was a bit more frequent than the frequency of 27-29% reported in other studies(Pouls et al., 2012, Ghosh et al., 2003). The M3 was the second common subtype is AML was 20%, which are similar to results were reported by(Pouls et al., 2012) in Erbil and by (SALIM and JALAL, 2018) in Baghdad. The M5 proportion of AML was 4(13.7%), it is higher than the results which reported by (SALIM and JALAL, 2018). Four cases of subtype M0(13.7%) and only one case of M6 subtype were reported in our study, similar to our results reported for M6 subtype in (Pouls et al., 2012) and smaller results was reported by (Al Allawi, 1990) for M0 subtype. Patients receiving intensive chemotherapy had higher CR and CR/CRi(Complete remission with incomplete count recovery) rates

than those receiving HMA alone, but these rates were still low when compared to the 85% CR rate for TP53 wild-type AML earlier reported(Daver et al., 2023), this result is slightly higher than our reported. As far as shared CR/CRi and CR rates go, IC recorded the highest rates (46% and 43%, respectively)(Daver et al., 2023). Estey et al, 2013 reported lower than our results, the AML response to chemotherapy is extremely variable with CR rates ranging from 50% to 80%(Estey, 2013).

5.1.1 Complete Blood Count Results

The platelet count was 38.80±279.0 in the CR group and was 268.0±288.0 in the PR group, and the P values for platelet count were significant (P = 0.0207). Sadek et al, in 2020 reported the level of platelets which measured the level was 59.31±8.07 and the P-values were strongly significant (P=0.000) (Sadek et al., 2020). The findings of additional parameters, such as Hb, RBC, and WBC, were non-significant.

5.1.2 Immunophenotypin CD Results

As the most diagnostic myeloid markers, CD13 and CD33 had mean expression levels of CDs that were the highest in this study (75% and 70%, respectively). SALIM and JALAL et al, in 2018 reported greater CD expression values in Duhok city, with CD13 and CD33 being the most commonly expressed markers (92.6% and 85.2%, respectively) (SALIM and JALAL, 2018). In our study, the CD13 and CD33 results revealed a significant association between the CR/PR ratio (P=0.0012 for CD13 and P=0.0012 for CD33, respectively). According to the correlation between the CR and PR ratio in this study, both MPO and HLADR were found to be the second most common CDs in 60% of cases, and the CR/PR association p value for MPO was significant (P=0.0103), whereas HLADR was reported non-significant

(P=0.2272), because the percentage of positive and negative CDs are approximately similar in CR and PR group.

The prevalence of the commonly expressed myeloid-related antigens in AML patients was comparable to that found in the literature for CD13 (92.6 vs 60-90%), CD33 (85.2% vs 70-90%) and MPO (73.2% vs 0-75%), but slightly higher for CD117 (92.6% vs 60-70%)(Osman et al., 2015). Bain et al, 2010 in UK, they reported greater than our results for MPO, the MPO was expressed in 73.2% of AML patients, the sensitivity of flow-cytometry in the identification of MPO can be improved when using the 3% cut-off instead of the 10% cut-off(Bain and Haferlach, 2010).

In 55% of cases, CD64 and CD117 were expressed, and the correlation between their CR/PR p-values was highly significant (P 0.0001). Sadek et al, reported smaller results for CD64 which expressed in only 4 cases (11.8%)(Sadek et al., 2020). Compared to CD13 or CD33, CD117 is more specific for myeloid origin and CD13 is more specific than CD33(Sadek et al., 2020). Bian et al, indicated that the myeloid marker CD117 were the most frequent expressed antigens in the study reported in UK (Bain and Haferlach, 2010). Sadek et al, reported that CD34 in almost all cases are positive(Sadek et al., 2020). Bradstok et al, reported the hemopoietic progenitor cell markers CD34 were detected in 42% cases(Bradstock et al., 1994). According to (Geller et al, 1990), patients with leukemia cells expressing CD34 had a complete remission (CR) incidence of 59%, and the p-value of CD34 expression (P = 0.008) was significant in predicting treatment response(Geller et al., 1990). Keyhani et al, investigated CD38 expression in 304 AML and its strongly significant (P<0.001) (Keyhani et al., 2000).

In our study, CD22 and TdT were the least common CDs to be expressed (10% and 5%, respectively).SALIM and JALAL et al, found that the expression of the TdT marker was 5% lower in Duhok(SALIM and JALAL, 2018). In this study, other CDs were reported as being non-significant, including (CD2, Cytoplasmic CD3, CD7, CD10, CD19 and Cytoplasmic CD79 A).

5.1.3 Bone Marrow Aspiration Reports

The spread between their median blast percentages, which ranged from 84.00 to 88.00. The CR group's hypercelularity was 90%. The CR group's hypercellularity percentage was 80% and the marrow showed 20% of cellular marrow smears, whereas the PR group's hypercellularity was 100%. *Lindsely et al*, studied 15 cases of AML and documented changes in the bone marrow's cellularity following induction therapy(Lindsley et al., 2015). Of these cases, 5 (33.3%) had hypocellularity, 2 (13.3%) had normal cells, and 3 (20%) had hypercellularity**.**

5.1.4. *P53* mutation and mutation frequency

The *TP53* gene, which has been well studied in other hematological malignancies like acute myeloid leukemia(AML), is the one that is most commonly mutated in cancer. Advanced disease phases are typically linked to TP53 deletions or mutations(Stengel et al., 2014).

In the present study, the *P53* distribution in acute myeloid leukemia for exon 5 is represented so high 100%(20/20), its mean the *P53* mutated in all cases, and the achievement results of CR and PR showed non-significant. *Welch et al*, reported the *TP53* mutation reported in 9/21 (47%) of AML patients (Welch et al., 2016), it is lower than our results. The prevalence of *TP53* mutations in CK-AML, where they are the most frequent genetic lesion, is

approximately 70%. The higher *TP53* mutation rate in these entities (21% to 33%) corresponds with the increased CK frequency in elderly de novo AML patients, AML, postmyeloproliferative disorders, and t-AML, indicating mut*P53*-induced genomic destabilization(Prokocimer et al., 2017).

In this study, the *P53* distribution in AML disease for exon 6 of *P53* mutation is resulted high 100%, it means the *P53* were mutated in all cases. Bally et al, reported in 62 individuals with high risk MDS or AML, they examined TP53 mutations, 23 cases (37.1%) had a TP53 mutation, and 18 of those patients (78.3%) had a complex karyotype (Bally et al., 2014). Schottelius et al, worked on potential mechanisms causing a disruption of the controlled expression of wild-type P53 in myeloid AML phenotype in the German city of Freiburg, they reported in their study. In primary leukemic cells from 50 individuals, P53 transcript accumulation, nucleotide sequence, and gene structure were examined. Reverse transcriptase (RT)-PCR they used to find P53-specific transcripts in 16 of 23 AML patients(Schottelius et al., 1994).

In our study, the P53 mutaions were detected in exon 7 was showed high, 100%(20 of 20), and the achievement results of CR and PR showed non-significant, because no one of cases were reportd as negative from CR and PR group. Previously *Seliger et al*, founded a high frequency of P53 expression (58%) in patients with AML(Seliger et al., 2000). Sarma et al, reported in Guwahati India , P53 mutations with aberrant phenotypes were discovered in 21 (58.3%) cases of AML. 141 of 234 (60%) individuals had TP53 variants. DNA sequence analysis revealed TP53 mutations in 60% of cases, which is consistent with earlier findings(Sarma et al., 2015). TP53 was deleted and/or mutated in 70% of the 234 CK-AMLs in our dataset, making it the gene in this AML subgroup that has been altered the most frequently (Rücker et al., 2012).

In the current study, the P53 mutaions were detected in exon 8 was showed very high, is 100%, and the achievement results of CR and PR showed non-significant, because no one of cases were reportd as negative from CR and PR group. The prevalence of TP53 mutations in CK-AML, where they are the most frequent genetic lesion, is approximately about 70%.26 (Prokocimer et al., 2017). In 70.2% of TP53-mutated AML patients, which have more harmful TP53 CN loss was found in 70.2% of TP53-mutated AML patients, which have more harmful TP53 mutations. There were 442 patients in the TP53-mutant AML cohort (422 in the retrospective group and 20 in the prospective group), 336 of whom (76%) had one TP53 mutation, and 106 of whom (24%) had more than one TP53 mutation(Tashakori et al., 2022)**.** A TP53 mutation was found in the remaining allele in fifty of the 57 instances demonstrating the loss of one TP53 allele. A TP53 mutation was also discovered in 33 of 50 instances with two copies of TP53. Consequently, 78% of AML with a complicated aberrant karyotype had TP53 mutations (Haferlach et al., 2008). The most recent research, published in January–March 2023, the study conducted on myeloid leukemia and found that 36/82 (46%) of the subjects had the TP53 mutation (Rogers et al., 2023).

The present study reslts of P53 mutation in exons (5, 6, 7 and 8) according to CR and PR groups indicates that, patients who completely responsed to chemotherapy induction, are still genetically have a P53 muations in all of 20 AML patients.

5.1.5. Molecular Exon Sequencing

To determine the sequences of small mutated fragments of human DNA, we used a Sanger sequencing technique. From total 20 PCR products selected

5 samples for each exons(5, 6, 7 and 8). at least exons from the fourth to the ninth, including splicing sites, should be covered in the analysis, even if the optimal range goes from the second to the eleventh(Minervini et al., 2016).

In the present study, sequencing results showed mutation in 10/20 samples in AML patients, its mean the percentage of mutated samples is %50. In all 20 samples reported 17 mutations in 10 samples, as in exon 5 includes 4 mutations in 2 samples, and in exon 6, was reported 7 mutations in 4 samples, only one mutation was present in exon 7 and in exon 8 stated with 5 mutations in 3 DNA samples.

The polymorphism variant G>GC, which was observed seven times in six samples, is the most prevalent in AML patients. A>AG and T>TA, which were each found three times in three mutant samples, are the second-most frequent polymorphisms. Two distinct samples both revealed G>GA. While T>TG and G>GT alterations were also discovered in the current investigation.

5.2. Acute Lymphoblastic Leukemia

The most typical form of pediatric leukemia is ALL. One-third of all cancers found in adolescents under 15 years old are caused by it (Clarke et al., 2016). Epidemiological research has demonstrated that a number of genetic variables are very important in the leukemogenesis process. The use of modern risk-directed treatment results in high survival rates. According to earlier research, ALL patients had 5-year event-free survival rates >80% and total survival rates >90% (Bhatia et al., 2002). The present study was incorporated 32 cases of ALL, 14 (43.75%) were males and 18 (56.25%) were females, their ages was ranged from 1 to 25 years old, and overall mean age of the patients was 7.34 years old.

According to *Kawamura et al*, whom discovered 22 ALL cases with the t(l; 19) at various Japanese universities, as reported in May 1995 (Kawamura et al., 1995). These varied in age from 2 to 14 years, and there were 7 males and 15 females. According to (Leong et al., 1999), They conducted a research in London, UK, where 14 adult patients with B-lineage ALL (median age 29 years; range 3-54 years) received their diagnosis. (Lymphoblasts were obtained and characterized from 80 children with B cell precursor ALL, 21 with T cell ALL, and three newborns, aged 0-21 years at diagnosis (Felix et al., 1992).

Overall of 32 ALL patients in the current study, (78.1%) of patients achieved a CR and 7(21.9%) were achieved a PR. (93%) obtained as a CR, (4%) as a PR, and (2%) passed away during induction, according to results that were higher than ours in 2002 (Hoelzer et al., 2002).

The morphological subclassification of ALL patients included was not identified in the hospital reports, but the ALL sub-types were identified, 27(84.37) of them are B-ALL subtype and 5(15.63) of cases are T-ALL subtype, this results ensure that the frequency of B-ALL and T-ALL in its frequent range (Mühlbacher et al., 2014). They were used immunophenotyping to examine a total of 19 cases, of which 13 (68.4%) cases displayed the typical B-ALL phenotype and six of them were identified as pro-B-ALL. (Mukhopadhyay et al., 2013) in India, they reported that, out of a total of 500 patients, T-ALL accounted for 50.4% of cases and pro-B, B-cell precursor ALL for 47.6%. According to *Chiaretti et al* reports, between 15 and 20 percent of ALL cases come from the T-cell bloodline, while approximately 80 percent of ALL cases originate from the B-cell lineage(Chiaretti et al., 2014b).

5.2.1. Complete Blood Count Results

The P-value result of hematologic remission in all complete blood count parameters, such as WBC, RBC, Hb and platelets of ALL patients were included in our study were showed non-significant, depending on the CR/PR association. According to Salmoiraghi et al, This study did not reveal correlation with clinical features, such as gender, hemoglobin, Leukocyte count, platelets, percentage of blasts at diagnosis, and clinical risk class, as had been previously described(Salmoiraghi et al., 2016). According to Moueden et al., J Blood Lymph 2018 from January, the P-value findings for Hb, WBC, and platelets were not significant (P=0.26, P=0.33, and P=0.55, respectively) (Moueden et al., 2018).

5.2.2. Immunophenotyping CD Results

The mean positive frequency results of CD19, CD79A and TdT were the most expressed CD markers in this study for ALL patients (95.2%. 95.2% and 95% respectively). Depending on the correlation of CR/PR ratio, the results of CD19, CD79A and TdT were strongly significant($P<0.0001$, $P=0.0003$ & $P=0.0124$ respectively). In B-lineage ALL the most important markers for diagnosis, differential diagnosis and subclassification are CD19, CD20, CD22, CD24, and CD79a. Early B-lineage indicators include CD19, CD22 (cytoplasm and membrane), and CD79a (Coustan-Smith et al., 1998).

According to *Khurram et al*, the Medical Journal of the Islamic World Academy of Sciences, CD19, CD79A, and TdT had higher accuracy in diagnosing B-ALL patients than we reported (98.18%, 98.18%, and 90.90% of patients were positive, respectively)(Khurram et al., 2010). *Rezaei et al,* reported the prevalence of TdT in B-ALL was reported to be 86.2% in the Iranian Journal of Pathology in 2020, slightly lower than our finding. Along with surface(s) CD19, cCD79a, and CD10, cytoplasmic CD22 has been

suggested by the WHO as a powerful B lymphoid lineage-associated marker. 90% of the time, HLADR was found to be the second most common CD(Rezaei et al., 2020). According to *Khurram et al*, they are reported greater percentage than our results for HLADR(98.18%) biomarker(Khurram et al., 2010).

CD10 was the third expressed CD in ALL patients, were frequently expressed in 85.71% of cases, and the association of CR/PR p-value showed strongly significant (P<0.0001). MM Khurram, et al, 2010, they reported greater percentage than our results for CD10 is (100%) in B-ALL cases (Khurram et al., 2010). CD19 + CD79a, cytoplasmic CD22, and CD10 are used to diagnose B-lineage cells. (one or two of the latter according to staining intensity of CD19) (Matutes et al., 2011). *Rezaei et al*, for the frequency of CD10 in B-ALL was reported to be 79.4% in the Iranian Journal of Pathology in 2020, which is marginally lower than our finding(Rezaei et al., 2020). The result of CD22 in the present study was 80%. The positive frequency of CD34 were showed 50% and the P-value of CR/PR eatio were strongly significant (P<0.0001). *Khurram et al*, in cases of B-ALL, they reported a slightly higher percentage than our findings for CD34 (52.7%), and a slightly lower percentage than our results (44.44%) for patients with T-ALL(Khurram et al., 2010). *Rezaei et al*, reported that the frequency of CD34 in B-ALL is 60.9%, slightly greater than our reported(Rezaei et al., 2020).

CD2 is another marker were its frequency is (20%). Less frequent CDs was reported in our study for ALL was CD7 and CD13 (10% and 9.09% respectively. Khurram et al, reported the same percentage for CD13(9.09%) in B-ALL cases, and slightly greater than our result(11.11%) in T-ALL patients(Khurram et al., 2010). Other CDs were reported as negative in this study for ALL patients, was including (Cytoplasmic CD3, , CD10, CD19, CD33, CD38, CD64 and MPO).

5.2.3 Bone Marrow Aspiration Results

The blast percentages p-value were showed non-significant (0.1450). The hypercelularity of CR group is 40% but for PR group was %80, the percentage of normocellular in CR group was 17% but no normocellulars were seen in the PR group. Changes were happened in the composition of trials in the bone marrow results makes the data to be strongly significant (P<0.0001), the CR group hypercellularity percentage was 48%, normocellularity percentage is 25% and the marrow showed 30% of cellular marrow, but PR group hypercellarity was 100%. *Belurkar et al*, studied 50 cases of ALL and documented changes in the bone marrow's cellularity after induction therapy in a study that was published in the International Journal of Scientific and Research Publications. Of these, 23 cases (46%) had hypocellularity, 15 cases (30%) had normal cells, and 5 cases (10%) had hypercellularity(Belurkar et al., 2015).

5.2.4. P53 mutation and mutation frequency

In the present study, we aimed that to asses the frequency and prognostic value of P53 mutation in ALL individuals. For that, we systematically analyzed 20 patients with ALL disease, to detect the P53 mutation based on achievement of CR and PR chemotherapy induction.

In the present study we are analyzed exons 5 to 8 of P53 gene, because most of P53 mutations in human tumors occur within 4 exons located in these regions (exons 5-8). We analyzed twenty DNA smaples of ALL cases in exon

5 of P53 gene, the results showed that the distribution of P53 mutation were detected in 100% (20/20) of individuals, and the result of achievement CR/PR association indicated non-significant, because all cases from CR and PR were resulted positive.

Patients with relapsed B-cell ALL (28 of 98) and T-cell ALL (6 of 13) both found the sequence changes of TP53 (Yu et al., 2020). If analyses are not limited to TP53 mutations and deletions but also take into account promoter hypermethylation, other studies indicated a much higher proportion of TP53 alterations in ALL (30 to 40%)(Stengel et al., 2014). Our results for detection the distribution of P53 mutations in exon 6 of ALL is 100% (20/20), its represented as a high frequency of mutation in ALL cancer, and the result of achievement CR/PR association indicated non-significant, because all cases from CR and PR were resulted positive. In 54% of ALL cases, the TP53/RB tumor suppressor pathway was active (Zhang et al., 2011).

In the present study, the P53 distribution in ALL for exon 7 is 100%, its mean the P53 mutated in all cases, and the achievement results of CR and PR showed non-significant. the spread of TP53 mutations found by direct sequencing in T-cell ALL and B-cell precursor ALL in relapsed disease. The preponderance of TP53 sequence alterations were found in exon 8 (62%), with at least two of the changes affecting codons 281, 282, and 297, and in exon 7 (29%), with four of the changes affecting codon 248 (Hof et al., 2011)**.** *Sarma et al*, in Guwahati of India, worked on 36 (59.2%) cases of B-ALL and 6 (66.7%) cases of T-ALL, respectively, were documented to have aberrant phenotypes(Sarma et al., 2015). Patients with complex karyotypes and those who have lost chromosomes 17/17p, 5/5q, or 7/7q have an even higher incidence of TP53 abnormalities, ranging from 70% to 80% (Kulasekararaj et al., 2013).

In this study, the P53 mutaions were distributed in exon 8 was showed high, is 100%, and the achievement results of CR and PR showed non-significant, because no one of cases were reportd as negative from CR and PR group. Additionally, TP53 variants were found in 8 of 106 (7.5%) non-low hypodiploid ALL cases but only in 10 of 11 adult low hypodiploid cases (90.9%)(Holmfeldt et al., 2013). In 10 of 11 (91%) adult cases with TP53 mutations, all of which were found in the DNA-binding domain, the nearly universal alteration of TP53 in low-hypodiploid ALL was also verified. The TP53 mutations were somatic in all adult hypodiploid ALL cases, even though half of these mutations had previously been connected to LFS (Comeaux and Mullighan, 2017). *Muhlbacher et al*, in 2014 reported, in 93% (27/29) of the patients, they found TP53 mutations to be highly prevalent. Due to chromosome 17 monosomy, the second TP53 allele was lost in 26/27 individuals with TP53 mutation. The low median overall mortality (18.5 months) could be attributed to the frequent TP53 mutations. As a result, ALL with low hypodiploidy exhibits a typical pattern of chromosome losses and a strikingly high incidence of TP53 mutations(Mühlbacher et al., 2014). *Prokocimer and Rotter*, Published in the German city of Freiburg, they investigated the potential causes of the acute leukemia of lymphoid (ALL) phenotype and the disruption of the controlled production of wild-type P53. In primary leukemic cells from 50 individuals, P53 transcript accumulation, nucleotide sequence, and gene structure were examined. Reverse transcriptase (RT)-PCR identified P53-specific transcripts in 26/26 (100%) cases of ALL(Prokocimer and Rotter, 1994). According to the most recent research, adult LH-ALL is characterized by somatic TP53 biallelic inactivation, which is present in almost all cases (98%), and they discovered TP53 mutations in 34% of patients' post-treatment remission data (Kim et al., 2023).

The present prospective study reslts of P53 mutation in exons 5 to 8, according to CR and PR groups indicates that, patients who have complete response tp medication are genetically mutated for P53 gene in all 20 ALL individuals .

5.1.5. Molecular Exon Sequencing

We employed a Sanger sequencing method to identify the sequences of tiny altered chunks of human DNA. Five samples were chosen from all of the 20 PCR products' exons (5, 6, 7, and 8). Even though the ideal range spans from the second to the eleventh exon, the study should at least encompass exons from the fourth to the ninth, including splicing sites(Minervini et al., 2016).

In the current study, sequencing data revealed mutation in 7/20 samples from ALL patients, with a mean mutation rate of 35%. In total 20 samples, there were reported 11 mutations in 7 samples, with exon 5 having 4 mutations in 2 samples and exon 6 having 4 mutations in 2 samples. Exon 7 had three mutations, and exon 8 had no found variants.

The two polymorphism variants T>TA and G>GC, both of which were found twice in two distinct samples, are the most prevalent among ALL patients. In contrast, the current investigation also discovered mutations like A>AT, T>TG, G>GT, G>GA, G>C, and C>A.

CONCLUSIONS

In the present study concluded the follwings:

1- The overall CR rate exceeding (72.4% for AML patients and 78.1% for ALL patients). Defining the prognostic factors described in this study may have particular relevance, especially in light of the fact that of all the current large studies, this prospective trial includes more intensive postremission therapies than others.
2- The biggest findings were reported in this study, is the P53 mutation frequency in AML (20/20)100% and ALL (20/20)100% patients.
3- The most immunophenotyping CDs were frequently reported as positive in AML are (CD13, CD33, HLADR and MPO) and in ALL are (CD10, CD19, CD22, CD79A, TdT and HLADR).

RECOMMENDATIONS AND FUTURE WORKS

In the present study recommended the followings:

1. Investigation of TP53 protein expression by the Western blotting and ELISA techniques in Leukemia patients.
2. Molecular quantification of P53 protein gene expression by using RT-PCR for diagnosis AML and ALL Patients.
3. Sequencing is another molecular techniques for P53gene mutation detection in AML and ALL using next generation sequencing (NGS) or Sanger Sequencing methods.

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پوختە

نەخۆشییە شێرپەنجەییەکانی خوێن لەنێو ئەو نەخۆشییە زۆرانەدان کە گۆڕانی پرۆتینی ٥٣ پیشان دەدەن. لە شێرپەنجەی خوێنی تونددا، گۆڕانکارییەکانی جینی پرۆتینی ٥٣ ئاماژەن بۆ پێشبینییەکی خراپ. ئێمە هەڵسەنگاندنێکی گشتگیرمان ئەنجامدا بە بەکارهێنانی سکرینکردنی گۆڕانی پرۆتینی 53 لە ڕێگەی گۆڕانکارییەکانی خوێن، ڕاپۆرتەکانی مۆخی ئێسک، کاردانەوەی زنجیرەی پۆلیمیراز، و ئەلکترۆفۆڕێزی جێڵ. لە توێژینەوەکانی ئێستادا بۆ هەڵسەنگاندنی چەندێتیی بازدانی پرۆتینی 53 لە نەخۆشانی شێرپەنجەی خوێنی توندی مایلۆید و شێرپەنجەی خوێنی توندی لیمفۆید. جینی سەرکوتکەری وەرەم (پرۆتینی وەرەمی 53) ڕۆڵێکی گرنگ دەگێڕێت لە پاراستنی هاوسەنگی جینۆمی لە ڕێگەی کۆنترۆڵکردنی پیربوونی خانەیی، مردنی بەرنامەدارێژراوی خانە، پرۆسەی گۆڕانکاری خۆراک و چاککردنەوەی ناوکە ترشی(دی ئێن ئەی). لەم توێژینەوەیە هەڵسەنگاندنمان بۆ ٦١ نەخۆش کردووە لەنەخۆشانی نەخۆشخانەی نانەکەڵی لە شاری هەولێر، لەڕێکەوتی ١ی تەممووزی ٢٠٢١، تا ١١ی ئازاری ٢٠٢٢. ئێمە بەراوردمان کرد لەسەر بنەمای پارامێتەرەکانی خوێن، فینۆتایپینگ سی دیەکان و ڕاپۆرتەکانی مۆخی ئێسک بۆ کۆی گشتی ٦١ حاڵەتی شێرپەنجەی خوێنی(٢٩نەخۆشی شێرپەنجەی خوێنی مایلۆید و ٣٢ حاڵەتی شێرپەنجەی خوێنی توندی لیمفۆید) لە قۆناغی دوای هێنانەکایەوە بەپێی پەیوەندی وەڵامدانەوەی تەواو/وەڵامدانەوەی بەشەکی، بۆ توێژینەوەکە ناویان تۆمارکرا، لەم 40 حاڵەتە (20 لە شێرپەنجەی خوێنی توندی مایلۆید و 20 لە شێرپەنجەی خوێنی توندی لیمفۆید) بوون، بۆ بەدواداچوون بۆ دەستنیشانکردنی بازدان و گۆڕانی خانەیی. لە کۆی 61 نەخۆش (29 نەخۆشی شێرپەنجەی خوێنی توندی مایلۆید 24(82.7%)

وەڵامدانەوەی تەواویان هەبوو بۆ دەرمانی کیمیایی و 5(17.3%) وەڵامدانەوەی بەشەکیان هەبووبۆ دەرمان. لە کۆی ٣٢ نەخۆشی شێرپەنجەی خوێنی لیمفۆبڵاستیکی توند 25(79.3%) وەڵامدانەوەی تەواویان هەبوو وە 7(20.7%) وەڵامدانەوەی بەشەکیان هەبوو. لە دیارترین ئەنجامەکانی توێژینەوەکەمان (100%) (20/20) لە نەخۆشانی شێرپەنجەی خوێنی توندی مایلۆیدی و (100%) (20/20) لە نەخۆشانی شێرپەنجەی خوێنی توندی لیمفۆبڵاستیک، ڕووداوی بازدان تیایاندا ڕوویداوە، کە ئەمەش ئەنجامێکی بەرز و بەرچاوە. ئەوەی جێگای باسە تەنانەت ئەو نەخۆشانەی کە وەڵامدانەوەی تەواویان هەبووە بۆ دەرمانی کیمیایی، ڕێژەی بازدان (100%) بووە تیایاندا. زۆرترین سی دی پۆزەتیف لە نەخۆشانی شێرپەنجەی خوێنی توندی مایلۆید، بریتین لە

(CD34، CD117، CD64، HLADR، MPO، CD33، CD13)وە ڕێژەی سەدیان بریتین لە

(75%، 70%، 60%، 60%، 55%، 55% و 50%)

بەپێی پەیوەندییەکانی وەڵامدانەوەی تەواو/وەڵامدانەوەی بەشەکی ئەو سیدییانە لە ڕووی ئاماڕییەوە وەCD64)، CD117، CD13، CD33، CD34، MPO، TdTوCD38 ،(گرنگیان نیشاندا (<0.0001)، 0.0001 >، 0.0012، 0.0012، 0.0067، 0.0103، 0.0235 0.0209، pvalue (زۆرترین سی دی لە نەخۆشانی شێرپەنجەی خوێنی لیمفۆبڵاستی توند، بریتین لە

P-valueوە CD19) ، CD79a، TdT، HLADR، CD10، CD22وCD34)

ئەوان بریتین لە (95.24%، 95.24%، 95%، 90%، 85.71%، 80% و 50%) و بەپێی پەیوەندییەکانی وەڵامدانەوەی تەواو/وەڵامدانەوەی بەشەکی ، ئەو نیشاندەرانەی سی دیەکان لە ڕووی وەCD2)، CD10، CD19، CD22، CD34، CD79Aو(TdT ئاماڕییەوە گرنگیان نیشان دا (<0.0001.)، 0.0001>، 0.0001 >، 0.0001>، 0.0001>، 0.01240.0003 بەP-value (

لە قۆناغی دوای هاندانی گۆڕانکارییەکانی مۆخی ئێسک لە حاڵەتەکانی شێرپەنجەی خوێنی توندی مایلۆید بەزۆری زیاد خانەیی بوو (100%) لە گروپی وەڵامدانەوەی تەواو، وە زیاد خانەیی (85%) لە گروپی وەڵامدانەوەی بەشەکی، و بەپێی ڕێژەی وەڵامدانەوەی تەواو/وەڵامدانەوەی بەشەکی بە شێوەیەکی سووک دەرکەوت گرنگە بۆ پارچە خانەییەکان (0.0068)، P-value ، هەروەها ڕێژەی بلاست گرنگ بوون0.0365.

لە حاڵەتەکانی شێرپەنجەی خوێنی توندی لیمفۆبڵاستیکدا، ڕاپۆرتەکانی مۆخی ئێسک بە شێوەیەکی سەرەکی زیادە خانەیی بوون (100%) لە گروپی وەڵامدانەوەی تەواو وە زیاد خانەیی (45%)، لە گروپی ڕزگاربوونی بەشەکی، وە بە پێی پەیوەندی وەڵامدانەوەی تەواو/وەڵامدانەوەی بەشەکی ، بەهای بەهێزو بەرچاوی نیشان دا (<0.0001). P-value

ئەنجامی بازدان لە پڕۆتینی 53 گۆڕانکارییەکی زۆری هەبووە لە حاڵەتەکانی شێرپەنجەی خوێنی توندی مایلۆید و شێرپەنجەی خوێنی توندی لیمفۆید، وە ئەنجامی سی دیەکان بە شێوەیەکی بەرچاو دەربڕدراون لە شێرپەنجەی خوێنی توندا، هەروەها ئەنجامەکان لە مۆخی ئێسک زیادە خانەییان نیشان دا.

.

زانکۆی پۆلیتەکنیکی هەولێر
ERBIL POLYTECHNIC UNIVERSITY

هەڵسەنگاندنی گۆڕانی جینی *P53* و شێوازەکانی فینۆتایپی بەرگری لە نەخۆشانی شێرپەنجەی خوێنی توند لە نەخۆشخانەی نانەکەلی- هەولێر

نامەیەکە

پێشکەش بە ئەنجومەنی کۆلێژی تەکنیکی تەندروستی هەولێر کراوە لە زانکۆی پۆلیتەکنیکی - هەولێر

وەک بەشێک لە پێداویستیەکانی بەدەستهێنانی پلەی ماستەر لە بەشی شیکاری نەخۆشیەکان

لە لایەن

اشقي محمد كريم

بەکالۆریۆس لە شیکاری نەخۆشیەکان

بە سەرپەرشتیاری

د. نوشیروان صادق محمد

هەولێر- کوردستان-عێراق

نەورۆز ٢٧٢٣ ک ڕەمەزان ١٤٤٤ ک مارس ٢٠٢٣ ز