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EVALUATION OF SOME CLINICAL, LABORATORY, AND GENETIC MUTATIONS OF TP53 GENE IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Abstract: The most common kind of leukemia in the world is chronic lymphocytic leukemia (CLL). Malignant clonal development and an overproduction of mature B-lymphocytes are frequently detected in older persons. TP53 mutations in CLL patients are also associated with a significantly worse survival rate and poor response to treatment. Furthermore, have been associated with poor prognostic in a range of cancers.

This cross-sectional study was conducted in Iraq and included 63 patients with newly diagnosed CLL, as well as follow-up and on-treatment cases.

Peripheral blood was tested, and the disease stage was assessed by RAI and Binet staging, as well as immunophenotyping via flow cytometry. TP53 variants were discovered by PCR amplicon sequencing using the bidirectional Sanger method.

Patients included in this study presented with lymphadenopathy, splenomegaly, and hepatomegaly.

The Rai and Binet progression stages of CLL disease are employed. The Rai staging system has five stages: 0, I, II, III, and IV, representing 17%, 40%, 10%, 22%, and 11% of patients, respectively. Similarly, Binet staging recognized sickness phases (A) 67%, (B) 11%, and (C) 22%.

TP53 mutations were 93% variant-type mutations and 7% wild-type mutations.

All possible genotypes were successfully identified, and genotype frequencies for TP53 gene variants across various SNPs in CLL patients were evaluated

The conclusion was revealed. SNP28-29insA, 38T>G SNP, and 48-49insT are all novel SNPs found in the intron sequences of the TP53 gene. All additional SNPs with no frequency are in the dbSNP database. With no significant associated risk factors for CLL.

Keywords: CLL, TP53, DNA, SNP, and PCR.

Introduction

Chronic lymphocytic leukemia (CLL) is the most widespread leukemia worldwide, accounting for 4.9 new cases every hundred thousand individuals in the United Kingdom and the United States annually. Malignant

clonal growth and an accumulation of mature B-lymphocytes are commonly found in elderly individuals, with a median diagnostic lifespan of 74[1].

CLL's clinical heterogeneity is a distinguishing feature, with a high variety of clinical courses ranging from indolent or rapidly regressing progressive pathways, in which CLL is an immunocompromised condition and a higher risk of infection-related consequences that remain fatal and life-limiting disease. [1,2,3].

CLL patients have TP53 variations, either a majority of missense or minor nonsense mutations, in the DNA-binding domain, in addition to transitions, which were discovered in methylated CpG sites [4,5].

These aberrations are also associated with a much worse survival rate and a poor reaction to chemotherapy. Also, have been linked to bad prediction in a variety of malignancies, including lymphomas [4], and more than half of all human malignancies have TP53 alterations [6].

TP53 alterations and del(17p) genes have become essential components of standard diagnostic procedures and must be carried out always before choosing a course of treatment [7].

Developing novel cancer therapy can repair the TP53 mutant protein and/or increase the function of the Tp53 wild-type protein an additional move forward in the treatment of high-risk CLL patients with TP53 mutations (8).

Aims of the study is to recognize TP53 variations in CLL patients and the relationship of TP53 variations with clinical prognosis in CLL patients

Methodology

This cross-sectional study was carried out at several centers in Iraq from October 2023 to June 2024 and comprised 63 patients with newly diagnosed cases, follow-up, and on-treatment CLL. After obtaining informed, oral agreement, complete demographic information about the enrolled patients was collected.

Each patient provided one milliliter of fresh peripheral blood and received a full blood picture count, including hemoglobin, white blood cell count, lymphocyte cell count, and platelet count. The disease stage is determined by Rai and Binet staging, as well as immunophenotyping by flow cytometry in this study.

DNA was extracted from blood samples; The molecular weight and integrity of the isolated DNA were estimated using 2% agarose gel electrophoresis. Then Primer was Prepared, and after that optimization of the PT53 PCR product was labored by different annealing temperature gradients of 55-65 C adopted, furthermore a single PCR fragment was chosen for amplification, which was expected to extend 722 bp of the TP53 genes protein. Lastly, to optimize the PCR condition of The PT53 gene product, only the primer annealing temperature at 62°C was employed.

DNA sequencing of PCR Amplicons detected by bidirectional Sanger technique. following the sequencing company's advice (Macrogen Inc. Geumchen, Seoul, South Korea). And chromatographs generated from ABI sequence files (Applied Biosystems Sequence) were additionally estimated, to ensure that the notes and alterations were not caused by PCR or sequencing manufactured objects. By comparing the viewed DNA sequences of the investigated samples with the recovered neighboring DNA sequences of the NCBI Blastn engine {(NCBI) The National Center for Biotechnology Information, (BLAST) The Basic Local Alignment Search Tool}, essential situations and other details of the recovered PCR fragments were recognized.

Results

Table (1) shows the sociodemographic distribution of the 63 Chronic Lymphocytic Leukemia (CLL) patients included in this study, with 54% men and 46% females. Patients' ages range from 52% under 65 to 48% over 65 years.

Diagnosed CLL patients observed (47) 75% newly diagnosed patients, (12) 19%old patients on treatment, and (4) 6%old patients on follow-up consecutively.

Table 1: Sociodemographic characteristics of the CLL Patients (n=63)

Socio-demographic variables		CLL (No.= 63)	
		Fr.	%
Gender	Male	34	54
	Female	29	46
Age (Years)	< 65, *n* (%)	33	52
	≥ 65, *n* (%)	30	48
CLL patients	Newly diagnosed	47	75
	Old patients on follow-up	4	6
	Old patients in therapy	12	19

The clinical presentation demonstrated in Table (2**)** of patients enriched in this study was 84% lymphadenopathy,25%splenomegaly, and,8% hepatomegaly.

Table 2: Overview of Clinical Presentations and Complications in CLL Patients of Study Group.

Clinical & Complications		CLL (No.= 63)	
		Fr.	%
Lymphadenopathy	Yes	53	84
	No	10	16
Splenomegaly	Yes	16	25
	No	47	75
Hepatomegaly	Yes	5	8
	No	58	92

The hematological characteristics of the patients shown in Table (3) showed that the mean WBC count was 58.5 × 10^9/L, with a range of 6.62 to 466 × 10^9/L. The mean values of the absolute lymphocyte count were 50.4x10^9/L, hemoglobin was 11.9/dl, and the mean platelet count was 198.6x10^9/L.

Table 3: Descriptive statistic of the hematological parameter among 63 patients with CLL.

Hematological parameter	Minimum	Minimum	Mean	±SD	Median	IQR
WBC (x 10^9/L)	6.62	466	58.5	76.1	37.9	29.5
ALC (x 10^9/L)	3.3	465	50.4	86.7	20.3	34.5
Haemoglobin (g/dl)	6.8	16	11.9	2.05	12.2	2.8
Platelet count (x 10^9/L)	39	518	198.6	85	197	78

ALC: Absolute lymphocyte count; WBC: white blood count; SD: Standard Deviation; IQR: interquartile range.

The Rai and Binet progression stage of the CLL illness is shown in Table (4). Five stages make up the Rai staging system: 0, I, II, III, and IV, which correspond to 17%, 40%, 10%, 22%, and 11% of the patients, respectively. In a similar vein, the Binet staging identified illness stages (A) 67%, (B) 11%, and (C) 22%, respectively.

Table 4: Staging of CLL Progression According to Rai Staging System and Binet Staging System.

Staging System		CLL (No.= 63)	
		Fr.	%
Rai Staging	0	11	17
	I	25	40
	II	6	10
	III	14	22
	IV	7	11
Binet Staging	A	42	67
	B	7	11
	C	14	22

The types of transformations found in our CLL patients are identified and displayed in Table (5), where 93% (59 patients) have variant-type mutations and 7% (4 patients) have wild-type mutations.

Table 5: Distribution of Transformation Types Among CLL Patients.

Patients	Transformation type	
	Wild Type	Variant type
No. of Patients (63)	4 (7 %)	59 (93 %)

The molecular study of mutations protein TP53 was the first stage in bidirectional Sanger sequencing findings for TP53 PCR products. This key method enabled us to identify genetic variants among CLL cases. The sequencing findings were then edited, aligned, and evaluated using BioEdit Sequence Alignment Editor Software Version 7.1, along with sequences from the reference database. To validate the reliability and relevance of our findings, we compared them to the National Center for Biotechnology Information (NCBI) database.

In this study, we effectively identified all probable genotypes and examined genotype frequencies for the TP53 gene across several SNPs, as revealed in Table (6**)** of CLL patients, observing variant SNP 28-29insT 22 at 35%, SNP 38T>G 25 at 40%, SNP 48-49 ins T(1) at 2%, SNP 133G>A rs2151018195 (GA) 5 at 8%, SNP rs2151018158 (25) at 40%, SNP rs2151017599 (30) at 48%, SNP rs2151013504 (2) at 3%, SNP rs2151012813 (9) at 14%, SNP rs1597359927 (47) at 75%, and SNP rs1057522354 (48) at 76% of patients.

These results offer a thorough summary of the genetic variants in the TP53 gene that are distributed across our patients with CLL.

Table 6: Examining Genotype Frequencies in CLL Patients

Genotype TP53			CLL (No.= 63)	
			Fr.	%
28-29insT	T	Wild type	41	65
	TT	Mutant allele	22	35
38T>G	TT	Wild type	38	60
	TG	Mutant allele	25	40
48-49insT	T	Wild type	62	98
	TT	Mutant allele	1	2
133 G>A rs2151018195	GG	Wild type	58	92
	GA	Mutant allele	5	8
137 T>C rs2151018158	TT	Wild type	38	60

	TC	Mutant allele	25	40
178 T>G rs2151017599	TT	Wild type	33	52
	TG	Mutant allele	30	48
354 A>C rs2151013504	AA	Wild type	61	97
	AC	Mutant allele	2	3
403 T>C rs2151012813	TT	Wild type	54	86
	TC	Mutant allele	9	14
410 A>G rs1597359927	AA	Wild type	16	25
	AG	Mutant allele	47	75
420 G>T rs1057522354	GG	Wild type	15	24
	GT	Mutant allele	48	76

The sequencing chromatogram of the detected variant, as well as its extensive comments, were noted. The chromatogram of this sequence was presented relative to its location in the PCR amplicon. The detected SNPs were 28-29insA, 38T>G, and 48-49insT shown in (figure 1).

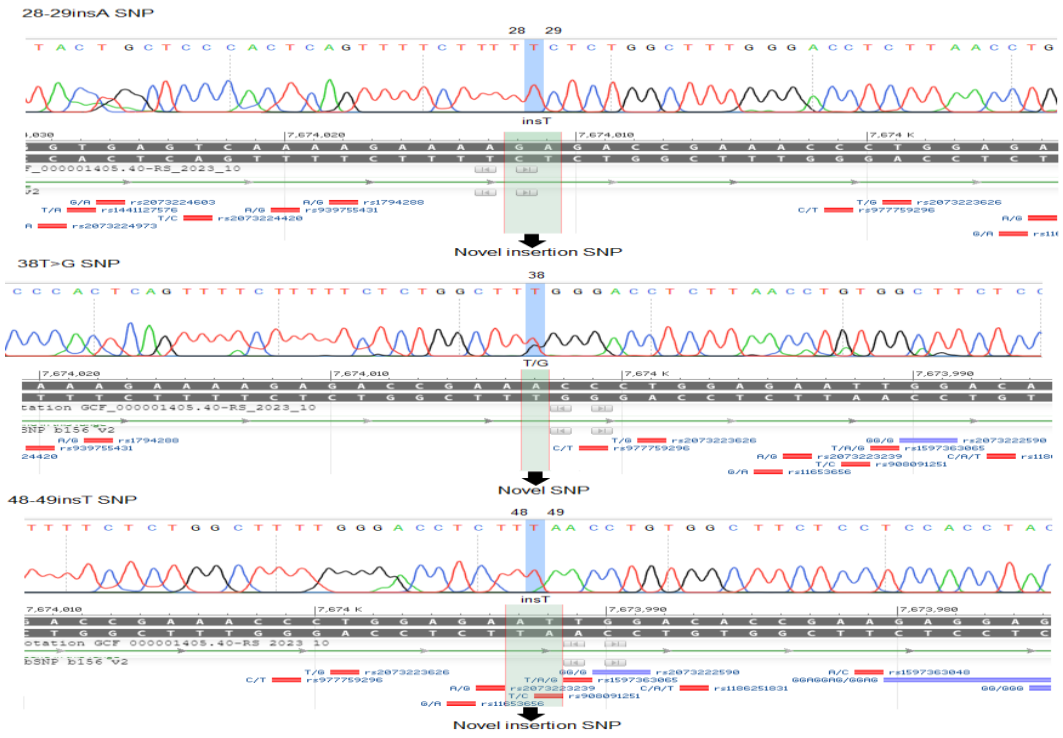

Figure 1. The SNP's novelty checking of TP53 genetic single nucleotide polymorphisms of 28-29insA, 38T>G, 48-49insT SNPs using the dbSNP server. The identified known and novel SNPs are marked accordingly. GenBank acc. no. NC_000017.11 was used in the positioning of the highlighted substitution SNPs. The position of the targeted sequences was found in the negative strand.

The observed 133G>A SNP was originally deposited as rs2151018195. This SNP is also found in the intron sequences of the TP53 gene (https://www.ncbi.nlm.nih.gov/snp/rs2151018195). However, no occurrences of the deposited rs2151018195 SNP in the dbSNP database were detected in the dbSNP server. The discovered 137T>C SNP was originally deposited as rs2151018158. This SNP is also present in the TP53 gene's intron sequences (https://www.ncbi.nlm.nih.gov/snp/rs2151018158). However, no frequency of the deposited rs2151018158 SNP in the dbSNP database was identified in the dbSNP server. They are shown in figure (2).

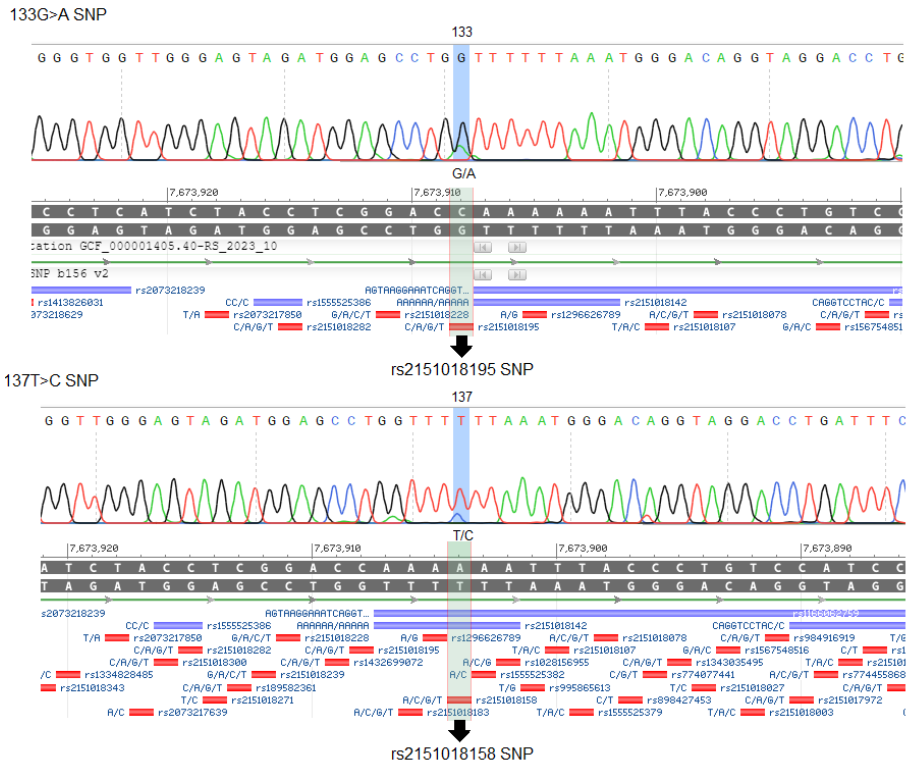

Figure 2. The SNP's novelty checking of TP53 genetic single nucleotide polymorphisms of 133G>A and 137T>C SNPs using the dbSNP server. The identified known and novel SNPs are marked accordingly. GenBank acc. no. NC_000017.11 was used in the positioning of the highlighted substitution SNPs. The position of the targeted sequences was found in the negative strand.

The observed 178T>G SNP was originally deposited as rs2151017599. This SNP may also be detected in the intron sequences of the TP53 gene (https://www.ncbi.nlm.nih.gov/snp/rs2151017599). However, no frequency of the deposited rs2151017599 SNP in the dbSNP database was detected in the dbSNP server, as shown in Figure (3).

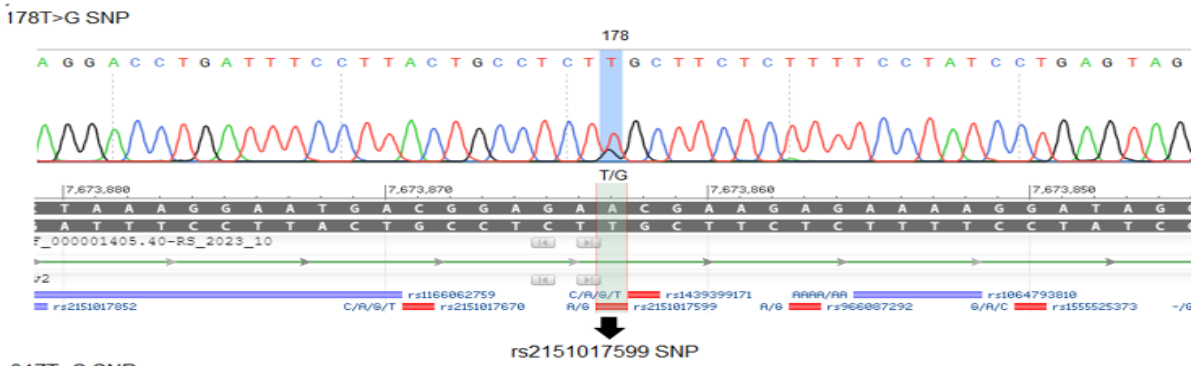

Figure 3. The SNP's novelty checking of TP53 genetic single nucleotide polymorphisms of 178T>G, using the dbSNP server. GenBank acc. no. NC_000017.11 was used in the positioning of the highlighted substitution SNPs. The position of the targeted sequences was found in the negative strand.

The detected SNPs were rs2151013504 (located in the intron sequences of the TP53 gene (https://www.ncbi.nlm.nih.gov/snp/rs2151013504)), SNP rs2151012813 (also located in the intron sequences) (https://www.ncbi.nlm.nih.gov/snp/rs2151012813)., and SNP rs1597359927(also located in the intron sequences), as shown in figure (4).

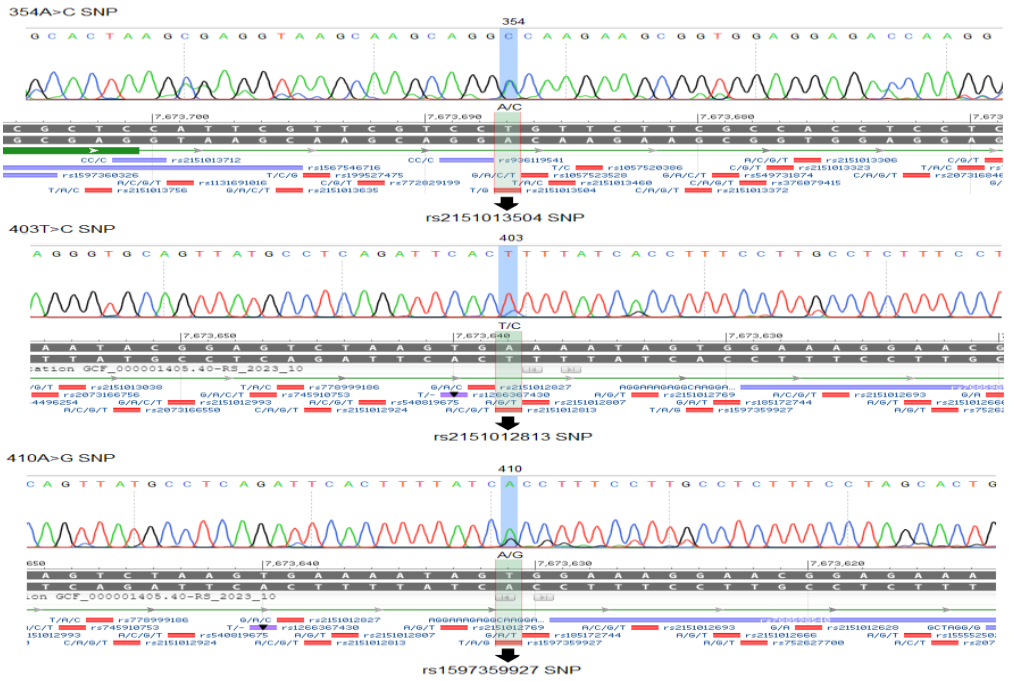

Figure 4. The SNP's novelty checking of TP53 genetic single nucleotide polymorphisms of 354A>C, 403T>C, and 410A>G using the dbSNP server. The identified known and novel SNPs are marked accordingly. GenBank acc. no. NC_000017.11 was used in the positioning of the highlighted substitution SNPs. The position of the targeted sequences was found in the negative strand.

The 420G>T SNP discovered was originally deposited as rs1057522354 in the TP53 gene's intron sequence (https://www.ncbi.nlm.nih.gov/snp/rs1057522354), however, has an extremely low frequency (T=0.000004) in the dbSNP database and dbSNP server, as shown in Figure 5.

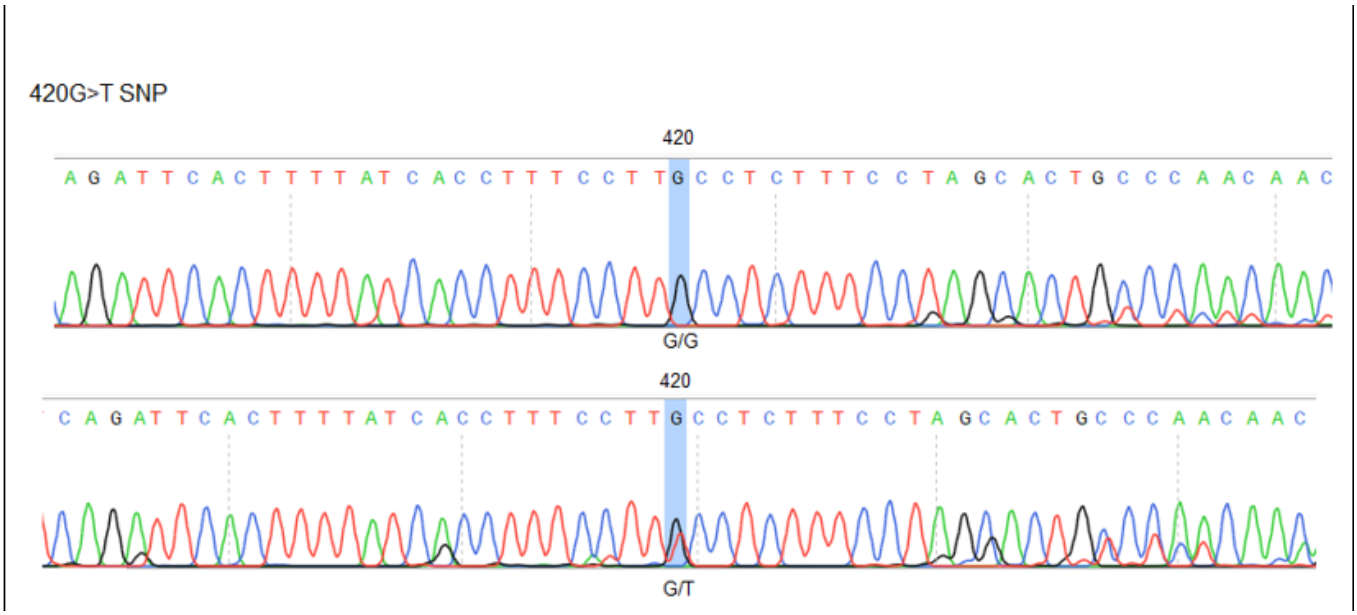

Figure 5. the pattern of the detected 420G>T, SNPs within the DNA chromatogram of the targeted 722 bp amplicons of the TP53 gene. The identified SNPs are highlighted according to their positions in the PCR amplicons.

Discussion

The TP53 gene produces the protein p53, which is essential for cell cycle arrest and subsequent apoptosis after DNA damage (9).

Currently, more than 200 TP53 single nucleotide polymorphisms (SNPs) are being documented.

(http://www-p53.iarc.fr/). A number of them have been proposed to contribute to the vulnerability to various forms of malignancy and they may have cumulative impacts on clinical outcomes (10,11).

TP53 abnormalities, such as TP53 variations and chromosome 17p deletion, have factually been the best indicators of adverse results for individuals with chronic lymphocytic leukemia (CLL) following initial-line chemoimmunotherapy (12).

Males (34) account for 54% of CLL patients in our research, whereas females (29) account for 46%. Another study revealed that Men are twice as likely to get CLL as women (13).

The patients in this research were about equal in age, with 52% under 65 and 48% over 65. The median age was 61 years. While the median age was about 61 years in another research (14). Moreover**,** at diagnosis, the average age is 72 years old. Elderly people are the primary target of CLL patients. At 80 years of age, the annual prevalence rate increases to 30 per 100000 people (13).

In the current study clinical presentations showed lymphadenopathy in 53 (84%), while another study demonstrated that About half of the symptomatic individuals have lymphadenopathy-related symptoms (3). As well as, lymphadenopathy (60%) in another study (15).

Splenomegaly (25%) was impacted in this study. the primary clinical manifestations were splenomegaly (64.8%) (15)**.** Korubo et al detected that above 90% of cases had splenomegaly, which is higher than the results of 49.1% of patients discovered by Basabaeen et al in Sudan (17,18).

Hepatomegaly reveals (8%). also, Agrawal N et al discovered hepatomegaly (63%) at the time of diagnosis (19). It may be seen in around 20% to 21.9% of individuals at the time of diagnosis (20,21).

The WBC count ranged from 6.62 to 466 × 10^9/L, with a mean of 58.5 × 10^9/L in this study. Korubo et al found that above than 50% of the patients had WBC counts, above 100 × 10^9/L, indicating a substantial tumor burden, and prognostic markers (17).

Absolute lymphocyte count (ALC)from 3.3 to 465 x10^9/ L, With a mean of 50.5 x10^9/L in the current study.

Diagnosis of CLL involves peripheral blood lymphocytosis, absolute lymphocyte count (ALC) of > 5 × 10^9/L, and clonality verified by immunophenotyping (IMPT). And, if the ALC is over 100 × 10^9 /L, a poor prognostic indicator (17).

There are 14 patients at 22% having anemia with low Haemoglobin (Hb) of less than 10 g/dl in this presentation, Hb which is a very important prognostic indicator. Patients classified as high-risk under the Rai (stage III) and Binet (stage C) categories had hemoglobin values less than 10–11 g/dL (21)**.**

Rafiq et al declared a lower level of Hb (7.4±1.6 g/dL) in individuals with CLL, is linked to worse survival rates, lower quality of life, higher risk of chemotherapy-related damage, and more frequent blood transfusions (22).

Patients had 11% thrombocytopenia in our study, while Agrawal et al revealed that 18% of the patients had thrombocytopenia (19).

Nearly most of our asymptomatic patients are detected at an early stage according to the Rai staging, at stage one 40%, and Binet staging at A level 67%. while, In the Danish National CLL registry, 80% were Binet Stage A (23). Also, in presenting study reveals that in high-risk Rai staging (3-4) there are (21) at

33%, and in Binet staging group C (14) at 22% of patients, other studies indicated that 54 (49.1%) and 42 (38.18%) patients presented at Rai high-risk and Binet C stages, respectively (18).

The observed SNP 28-29insA was not before published in the dbSNP database, detected in the intron sequences of the targeted TP53 gene (NC_000017.11; g.7674011-7674012insA) however. there are 22 mutant alleles at 35%, while the wild type is 65%.

In addition to the recognized, 38T>G SNP has not before been recorded in the dbSNP database, identified in the intron sequences of the targeted TP53 gene (NC_000017.11; g.7674003) with mutant allele 40%, and 48-49insT SNP was not earlier registered in the dbSNP database, discovered in the intron sequences of the targeted TP53 gene (NC_000017.11; g.7673993-7673994insT) with only one mutant SNP at 2%.

These SNPs are novel SNPs situated in the intron sequences of the targeted TP53 gene. Concerning the recognized 133G>A SNP (substitution), it was found that this detected SNP was previously deposited as rs2151018195. This SNP is also located in the intron sequences of the TP53 gene in only 5 of the patients at 8%. (https://www.ncbi.nlm.nih.gov/snp/rs2151018195). However, no frequency of the placed rs2151018195 SNP in the dbSNP database was seen in the dbSNP server. As well, this SNP was not reported in any publication.

However, all other SNPs rs such as rs2151018158, rs2151017599, rs2151017599, rs2151013504, rs2151012813, rs1597359927, and rs1057522354 no frequency of the deposited in the dbSNP database was seen in the dbSNP server. Also, there are no published publications.

However, A large number of polymorphic variations were found in CLL samples, several of which had substantial relationships with CLL formation and progression (24, 25).

Furthermore, several single nucleotide polymorphisms (SNPs) in TP53 variations have been linked to an increased risk of developing different types of cancer and to a cumulative impact on medical results. (26). However, the majority of TP53 mutations deactivate (27)**.**

Conclusions:

1. Our patients have 93% mutated genes.
2. SNP28-29insA, 38T>G SNP, and 48-49insT are novel SNPs situated in the intron sequences of the targeted TP53 gene.
3. All other detected SNPs in the TP53 gene sequencing had no significant risk association with CLL.

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