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Evaluation of TP53 Gene Mutation in Chronic Lymphocytic Leukemia Patients

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ABSTRACT

Background: In chronic lymphocytic leukemia (CLL) disease, B cell heterogeneity and enhanced cellular proliferation play major pathogenic roles. TP53 abnormalities namely, both 17p chromosome deletion (del 17 p) and TP53mutation are considered gold standards for the clinical course of CLL and provide a better prognostic risk stratification.

Objectives: This was aimed to investigate TP53 mutations in patients with CLL and to assess their impact effect on the clinical course and hematological characteristics of these patients.

Patients and methods: Forty newly diagnosed CLL adult patients were selected and subjected to full history taking, clinical examination, peripheral blood & bone marrow examination, and immunophenotyping in addition to TP53 mutation by real-time PCR.

Results: TP53 gene mutation was positive in 20% of CLL cases, and this mutation was significantly associated with hepatosplenomegaly, lymphadenopathy, short LDT (<12 months), CD38 expression, high-grade staging, and poor response to therapy.

Conclusion: One of the most potent prognostic markers required to direct treatment decisions for CLL is the TP53 mutations, which were linked to rapid disease progression and poor outcomes.

Keywords: Chronic Lymphocytic Leukemia, 17p deletion, TP53.

INTRODUCTION

The majority of those who are affected by chronic lymphocytic leukemia (CLL) are elderly people, typically older than 70 years of age; it is also the most prevalent leukemia among adults in the Western world[1]. The median age of diagnosis may vary significantly between populations and geographical areas due to its potential to affect younger persons as well [2].

CLL is caused by the clonal proliferation of aberrant B cells that co-express T cell-specific antigens and B cell markers (CD19, CD20) (CD5) [3]. The variability in the progression and treatment requirement results from chromosomal abnormalities present in more than 75% of patients with CLL, including deletions of (13q14), del (11q23), del (17p13), and trisomy 12 [4].

TP53 aberrations can come from gene mutations that impair the function of wild-type p53 in CLL or deletion of the TP53 locus on chromosome 17 (17p13.1) [5]. P53, a tumor-suppressor protein that regulates the cell cycle and apoptosis as well as promotes DNA repair in response to cellular stress signals such as DNA damage, is encoded by the gene TP53 [5].

Chemotherapy works by causing DNA damage, which activates the TP53 pathway and causes CLL cells to apoptosis. Contrarily, chemotherapy fails to cause apoptosis in CLL cells when TP53 is damaged, which allows the disease to proceed and leads to clonal evolution as the cells continue to multiply at a steady rate [6, 7].

This was aimed to investigate TP53 mutations in patients with CLL and assess their impact effect on the clinical course and hematological characteristics of these patients.

PATIENTS AND METHODS

The observational cross-section study was conducted on 40 newly diagnosed CLL adult patients attending Ain Shams Hospitals from October 2020 to October 2021. According to WHO recommendations, the diagnosis of CLL was made using blood smears and counts, as well as immunophenotyping of circulating B-lymphocytes, which identifies a clonal B-cell population containing the CD5 antigen and characteristic B-cell markers [8].

CLL patients in the Hematology and Oncology Departments are treated according to del(17p)/TP53 mutation status. Del(17p)/TP53 mutation-positive patients are treated with (Ibrutinib 420 mg orally), while del(17p)/TP53 mutation-negative patients are treated with (Cyclophosphamide 500mg orally, Vincristine 2mg injection day one, Fludrapine 50mg orally, Rituximab500mg injection and Dexamethasone 40mg orally). The response definition after the treatment of CLL patients was based on iwCLL guidelines [9].

Ethical consent:

All participants provided written consent, and the study received Ethics Committee approval from the Faculty of Medicine at Ain Shams University. This work has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

The following was done for all patients included: a complete clinical history and thorough clinical examination. Complete blood picture using Sysmex

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XS- 1800i five-part differential hematology analyzer (Japan).

Peripheral blood smear examination and bone marrow aspiration (when available) were done. Immunophenotyping for CD5, CD19, CD23, surface immunoglobulin, CD22a, CD79b, FMC7, and CD38 using Navios Flowcytometry from Beckman Colter (USA) was done on either BM or peripheral blood samples. TP53 mutation was assessed by real-time PCR using Rotor-Gene Q MDx, Germany.

DNA extraction and genotyping:

ThermoFisher Scientific's GeneJET™ Whole Blood Genomic DNA Purification Mini Kit was used to extract total DNA from an EDTA-treated blood sample (USA).

DNA analysis was done using TP53 _72 Genetic Polymorphism Pro72Arg (rs1042522) kit supplied by Syntol, Moscow, Russia. The volume of each kit component per 1 sample was 10µ of 2.5XReaction mix, 10µ of 2.5XDiluent, 0.5µ of Taq DNA-polymerase, 5 U/µL. 5 µL of analyzed and control samples were added. PCR cycling conditions: An initial denaturation step at 95°C for 3 min, followed by 10 cycles at 95°C for 15 seconds and 10 cycles at 63°C for 40 seconds. The final extension of 30 cycles at 95°C for 15 seconds and 30 cycles at 63°C for 40 seconds was carried out. The allelic discrimination plate read was evaluated, the fluorescence channel FAM, and HEX for each allele were inputted, and the allele types were confirmed.

Statistical analysis

Using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA), the current study's statistical analysis was carried out. The mean, SD, and range of quantitative values were displayed. Frequency and percentage were used to express qualitative data. To evaluate the association between qualitative variables, the chi-square test was performed. The Mann-Whitney test was employed when the quantitative data was not normally distributed, while the Student t-test was used to compare the mean and SD of 2 sets of normally distributed data. When the P-value is less than 0.05, it is considered statistically significant.

RESULTS

This study was carried out on 22 males and 18 females. Their age ranged from 33 to 85 years old with a Mean ± SD of 60.30 ± 11.78. 35.0% of the patients presented with lymphadenopathy, and 18-19% of them presented with hepatomegaly and or splenomegaly. Most of the patients have high-grade staging (62.5%) and LDT >12(67.5%). Cases achieved CR was 17 (42.5%) **(Table 1).**

Table (1): Demographic and clinical data of the studied patients

		No.= 40
Age (years)	Mean±SD	60.30 ± 11.78
	Range	33 – 85
Sex	Female	18 (45.0%)
	Male	22 (55.0%)
Lymph nodes	No	26 (65.0%)
	Yes	14 (35.0%)
Splenomegaly	No	21 (52.5%)
	Yes	19 (47.5%)
Hepatomegaly	No	22 (55.0%)
	Yes	18 (45.0%)
Staging	Low grade	15 (37.5%)
	High grade	25 (62.5%)
Lymphocyte doubling time (LDT)	LDT >12	27 (67.5%)
	LDT <12	13 (32.5%)
Response 2 months after completion of therapy.	CR	17 (42.5%)
	PD	12 (30.0%)
	PR	11 (27.5%)

CR: complete remission PD: progressive disease PR: partial remission.

Leukocytosis in the studied patients ranged from (11.2 – 267.22) x 10^3 cell/µL (the median was 32.5 x 10^3 cell/µL). Hb ranged from 5.1 – 18.5 g/dL with a mean of 10.92 g/dl. Platelet count ranged from (48 – 382) x 10^3 cell/µL with a mean of 192.53 x 10^3 cell/µL. PB Lymphocytes ranged from 60 – 97 %with 5 – 36 % atypical lymphocytes and 1 – 9% prolymphocytes. 62.5% of patients have High smear cells >30%. Besides, the BM Lymphocytes % range was (16 – 98) **(Table 2).**

Table (2): Hematological parameters of CLL studied patients

No.= 40		
TLC (x 10^3 cell/µL)	Mean±SD	32.5 ± 7.1
Hb (g/dl)	Mean±SD	10.92 ± 2.49
Platelet (x 10^3 cell/µL)	Mean±SD	192.53 ± 4.52
PB Lymph. %	Mean±SD	85.75 ± 7.85
Atypical lymph. %	Mean±SD	9.5 ± 1.81
Prolymph%	Mean±SD	3.60 ± 0.84
BM Lymph. %	Mean±SD	66.18 ± 15.04
Smear cell	Low smear cell < 30%	15 (37.5%)
	High smear cell >30%	25 (62.5%)

TLC: Total leukocytic count PB: peripheral blood BM: bone marrow Hb: hemoglobin IQR: IQR: Interquartile range (difference between 1st and 3rd quartiles)

Most of the patients (85.0%) presented with atypical Immunopheno- typing. CD38 was negative in about 72.5% of patients. 40.0 % of patients have cytogenetic abnormalities. The P53 gene mutation was positive in 20 % of patients (8 cases), 6 were homozygous cases, and 2 were heterozygous **(Table 3).**

Table (3): Immunophenotyping and genetic parameters of CLL studied patients.

		No.= 40
Immunopheno typing score	Typical	6 (15.0%)
	Atypical	34 (85.0%)
CD38	Negative	29 (72.5%)
	Positive	11 (72.5%)
Karyotyping abnormality	Negative	21 (52.5%)
	Positive	16 (40.0%)
	Failed	3 (7.5%)
P53 mutation	Negative	32 (80.0%)
	Positive	8 (20.0%)

TP53 gene mutation was significantly associated with clinical prognostic data namely lymphadenopathy, splenomegaly, and hepatomegaly with a p-value of (0.008, 0.001, 0.007) respectively. Also, a short LDT (<12 months) was significantly detected in 100% of patients with mutant TP53 (p-value 0.000), and a high-grade staging was significantly increased in mutated TP53 CLL cases (100.0% of cases; p-value 0.014). Regarding the response to therapy, no cases of mutated TP53 patients achieved CR (0.0%). No statistically significant association was elicited between TP53 gene mutation and age or sex (P-value > 0.05) **(Table 4).**

Table (4): TP53 mutation status in relation to demographic and clinical data

		TP53 mutation		**Test value**	**P- value**	**Sig.**
		Negative	**Positive**			
		No.= 32	**No.= 8**			
Age (years)	Mean±SD	60.13 ± 12.69	61.00 ± 7.65	-0.186•	0.854	NS
	Range	33 – 85	50 – 71			
Sex	Female	13 (40.6%)	5 (62.5%)	1.237*	0.266	NS
	Male	19 (59.4%)	3 (37.5%)			
LN	No	24 (75.0%)	2 (25.0%)	7.033*	0.008	HS
	Yes	8 (25.0%)	6 (75.0%)			
Spleen	No	21 (65.6%)	0 (0.0%)	11.053*	0.001	HS
	Yes	11 (34.4%)	8 (100.0%)			
Liver	No	21 (65.6%)	1 (12.5%)	7.298*	0.007	HS
	Yes	11 (34.4%)	7 (87.5%)			
Staging	Low grade	15 (46.9%)	0 (0.0%)	6.000*	0.014	S
	High grade	17 (53.1%)	8 (100.0%)			
LDT	LDT >12	27 (84.4%)	0 (0.0%)	20.76*	0.000	HS
	LDT <12	5 (15.6%)	8 (100.0%)			
Response 2 months after completion of therapy.	CR PD PR	17 (53.1%) 5 (15.6%) 10 (31.2%)	0 (0.0%) 7 (87.5%) 1 (12.5%)	16.08*	0.000	HS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
*: Chi-square test; •: Independent t-test

Regarding hematological parameters, CLL patients with mutant TP53 presented a significant leukocytosis (P-value 0.007), low Smear cell count (P-value 0.000), and a significant increase in atypical lymphocyte percentage (P-value 0.032) which contributes to the poor prognosis. No statistically significant association was detected between p53 mutation and Hb concentration, platelet count, and lymphocyte percent whether in PB or BM in this study (P-value > 0.05 for each) **(Table 5).**

Table (5): TP53 mutation status in relation to hematological parameters of the studied patients.

		TP53 mutation		Test value	P- value	Sig.
		Negative No.= 32	Positive No.= 8			
TLC (10^3 cell/µL)	Mean±SD	26.75 ±5.81	126.50± 28.1	-2.689≠	0.007	HS
Hb(g/dl)	Mean±SD	11.15 ± 2.61	10.00 ± 1.80	1.176•	0.247	NS
Plat (10^3 cell/µL)	Mean±SD	197.53 ± 42.4	172.50 ± 39.32	0.711•	0.482	NS
PB Lymph. %	Mean±SD	86.44 ± 7.50	83.00 ± 9.15	1.111•	0.274	NS
Atypical lymph %	Mean±SD	9 ±1.81	17.5 ± 3.5	-2.149≠	0.032	S
Prolymph%	Mean±SD	3.63 ± 0.87	3.50 ± 0.83	0.162•	0.872	NS
BM Lymph. %	Mean±SD	63.59 ± 13.21	76.50 ± 6.27	-1.316•	0.196	NS
Smear cell	Low smear cell < 30%	7 (21.9%)	8 (100.0%)	16.66*	0.000	HS
	High smear cell >30%	25 (78.1%)	0 (0.0%)			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
•: Independent t-test; ≠: Mann-Whitney test, *: Chi-square test

All patients with positive TP53 mutation had cytogenetic abnormality (P-value 0.001) and atypical Immunophenotyping. Moreover, CD38 was significantly detected in cases with TP53 gene mutation (P-value 0.000) **(Table 6).**

Table (6): TP53 mutation status in relation to Immunophenotyping and genetic parameters of the studied patients.

		P53 mutation		Test value*	P- value	Sig
		Negative No.= 32	Positive No.= 8			
Immunopheno typing score	Typical	6 (18.8%)	0 (0.0%)	1.765	0.184	NS
	Atypical	26 (81.2%)	8 (100.0%)			
CD38	Negative	28 (87.5%)	1 (12.5%)	18.056	0.000	HS
	Positive	4 (12.5%)	7 (87.5%)			
Karyotyping abnormality	Negative	21 (65.6%)	0 (0.0%)	15.000	0.001	HS
	Positive	8 (25.0%)	8 (100.0%)			
	Failed	3 (9.4%)	0 (0.0%)			
	Failed	3 (9.4%)	0 (0.0%)			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant, *: Chi-square test

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DISCUSSION

As a result of the very variable nature of the disease course associated with CLL, some patients can go more than 10 years without requiring treatment, while others still experience poor outcomes after receiving effective chemoimmunotherapy [10]. This genetic heterogeneity, in particular the del 17p mutation that results in the deletion of the TP53 gene, which codes for the tumor-suppressor protein p53, is partially accounted for by the genetic abnormalities found in CLL patients [11].

In the TP53 gene's coding region, several single nucleotide polymorphisms (SNPs) have been found and thoroughly characterized. Rs1042522 (p.Pro72Arg), one of the missense SNPs, is prevalent in all global populations [12]. This study's main objectives were to evaluate TP53 gene mutations in CLL patients and investigate how they affected the patients' prognostic, clinical, and hematological characteristics.

TP53 gene mutation was positive in 20 % of patients (8 cases), which is higher than in the western literature [13]. **Rossi *et al.*** [13] reported TP53 mutation in only 10% of patients at diagnosis, this may reflect a difference in disease biology. **Leroy *et al.*** [14] found that the most frequent aberration affecting the TP53 gene in CLL, del 17p coupled with TP53 mutations, accounts for almost two-thirds of cases. Even though TP53 aberrations are less common at diagnosis, they are present in 40–50% of instances of advanced or therapy-refractory CLL, underscoring the necessity to reevaluate TP53 status before beginning each line of therapy because the clones may grow during relapse and/or disease progression [15]. Given this situation, the use of NGS avoids patients

from being incorrectly labeled as having normal TP53 because of its increased sensitivity when examining TP53 aberrations [16].

TP53 gene mutation was significantly associated with clinical prognostic data namely lymphadenopathy, splenomegaly, and hepatomegaly. Also, a short LDT (<12 months) and a high-grade staging of CLL cases were significantly detected in all cases of TP53 mutation in this study. Similarly, **Xia *et al.*** [17] reported an advanced Binet stage associated with TP53 mutation.

Moreover, regarding response to therapy, no cases of mutated P53 patients achieved CR, similar results were observed by **Zenz *et al.*** [18]. The function of p53 in maintaining genomic stability likely is what causes cohorts of patients with TP53 abnormalities to respond poorly to therapy and have worse survival outcomes [19]. The greatest unfavorable prognostic variables were discovered to be TP53 abnormalities [20]. Additionally, it has been discovered that among newly diagnosed patients, TP53 anomalies are strong and substantial indicators of a quicker time to initial therapy commencement [20]. Additionally, substantial differences in progression-free survival or overall survival were linked to the amount of TP53 changes [21]. According to **Cherng *et al.*** [21], in the era of targeted medicine, low-burden TP53 mutations should not be disregarded when determining the genetic risk of CLL. The variable allele frequency threshold may also have significant effects on how clinically CLL is managed [22].

The karyotypes and immunophenotyping of all patients with positive TP53 mutations were aberrant. **Rigolin *et al.*** [23] observed a strong correlation between aberrant karyotyping and advanced CLL stages, the need for treatment, as well as a worse prognosis and shorter overall survival [23, 24]. Notably, its predictive significance is modulated by the types of identified abnormalities [24]. Curiously, a longitudinal study revealed that the TP53 mutations were acquired before karyotype evolution, highlighting the genetic instability associated with the TP53 mutation and suggesting that it likely contributed to the emergence of complex karyotypes [25].

Furthermore, CD38 was positive in (87.5%) of cases harboring TP53 mutations in this study. Normal B cells express CD38, a transmembrane glycoprotein that is thought to play a key role in apoptosis. Additionally, CD38 promotes B cell survival and proliferation, which indicates a poor prognosis because this threshold has predicted lower response rates and a shorter time between diagnosis and treatment [26]. In this setting, prognostic markers like CD38 and zeta-associated protein (ZAP-70) are thought to provide information on the expected development of the disease in an untreated individual [27]. Additionally, all of the patients that were investigated exhibited abnormal immunophenotypes (where some patients have a brighter expression of CD20 and surface immunoglobulin). Other poor prognostic factors, such as high expressions of CD38, unmutated immunoglobulin heavy chain gene (IGHV), and ZAP-70 expression, have been seen more frequently in patients with 17p deletions [28], according to **Rassenti *et al.*** [28]. They also noted that atypical immunophenotyping has occasionally been seen in these patients.

The majority of patients with 17p deletion still require treatment and have poor outcomes, despite reports of a tiny subfraction of individuals with 17p deletion who have a reasonably indolent course (usually with mutant IGHV and no therapeutic indication) [28].

CONCLUSION

Understanding the pathophysiology of CLL requires a thorough understanding of TP53 aberrations. It is now more widely understood that TP53 mutations and deletion 17p have a role in high-risk disease and poor prognosis. As a result, it's crucial to take TP53 anomalies into account when managing and stratifying these patients' therapies. Our findings needed to be confirmed by larger-scale research on other variants.

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