Frequency of *TP53* Mutation in B-Cell Chronic Lymphocytic Leukaemia and its Association with Lymphocyte Doubling Time

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ABSTRACT

Objective: To determine the frequency of *TP53* mutation at diagnosis of B-cell Chronic Lymphocytic Leukaemia (B-CLL) in Pakistani patients, and to investigate whether lymphocyte doubling time (LDT) of less than 1 year could be used as a surrogate marker for *TP53* mutation.

Study Design: A cross-sectional descriptive study.

Place and Duration of the Study: Department of Haematology, Liaquat National Hospital, from January 2020 to December 2022.

Methodology: Patients diagnosed with B-CLL based on the criteria set by International Workshop on Chronic Lymphocytic Leukaemia were included in the study. Clinico-haematological parameters were recorded, and *TP53* mutation analysis was performed by fluorescence *in situ* hybridisation. Patients were followed every 3-6 months after diagnosis with the recent complete blood count (CBC) reports to record CBC parameters and calculate LDT.

Results: A total of 128 B-CLL cases were evaluated, with a mean age of 62 years. Among these cases, 10 patients (7.8%) tested positive for *TP53* mutation, while 118 patients (92.2%) tested negative. During the follow-up period, 26 patients were lost to follow-up, with only one patient from the *TP53* positive group. In the *TP53* positive group, 55.6% (n=5/9) patients had an LDT of less than 1 year, indicating aggressive disease compared to 30.1% (n=28/93) patients in the negative group ($p < 0.1$).

Conclusion: *TP53* mutations may be associated with shorter LDT, indicating aggressive disease. Further research is needed to fully comprehend the relationship between *TP53* mutation and LDT in B-CLL.

Key Words: *TP53 mutation, B-Cell chronic lymphocytic leukaemia (B-CLL), Lymphocyte doubling time (LDT).*

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INTRODUCTION

B-Cell Chronic Lymphocytic Leukaemia (B-CLL) is an indolent malignancy of mature lymphoid cells. In the Western countries, it is the most prevalent leukaemia in adults, accounting for 25-35% of all leukaemia cases.[1] However, in Asia, the prevalence of CLL is relatively low, comprising of only 10% of the cases observed in the Western countries.[2,3] In Pakistan, CLL is considered a rare haematological malignancy, with an overall prevalence of 0.9% and accounting for approximately 9.7% of leukaemia cases.[4,5] This disease primarily affects older individuals, with the incidence rate rising after the age of 60, and there is a slight male predominance.[6]

CLL has a prolonged clinical course that spans over many years and displays considerable heterogeneity in clinical behaviour. While some patients experience rapidly progressive disease and require chemo-immunotherapy upon diagnosis, others undergo years of observation without any treatment or change in the clinical status. This variable clinical course underscores the significance of prognostic and predictive markers in disease management. Clinical staging such as Rai and Binet's staging, and lymphocyte doubling time (LDT) are the most commonly used and cost-effective prognostic markers. However, additional serum markers, cytogenetics, and molecular markers have gained importance over time. According to the International CLL-IPI working group, *TP53* mutation, serum Beta 2 Microglobulin >3.5 mg/L, and unmutated IGHV gene are independent prognostic factors that not only predict survival time but also provide insights into clinical behaviour, thus guiding treatment decisions.[7]

TP53 mutation is recognised as the most significant prognostic marker in CLL, supported by numerous studies and prospective clinical trials.[8,9] These findings consistently demonstrated that patients with *TP53* mutation are at a higher risk of developing rapidly progressive disease that is resistant to chemo-immunotherapy. As a result, these patients should be considered for targeted therapy when appropriate. The updated guidelines from the International Workshop on Chronic Lymphocytic Leukaemia (iwCLL) emphasised the importance of conducting *TP53* mutation analysis prior to initiating treatment.[10]

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In countries like Pakistan, performing molecular tests for every B-CLL patient undergoing treatment is a significant challenge due to the poor socioeconomic conditions. The objectives of this study were to assess the frequency of *TP53* mutation at the time of B-CLL diagnosis in Pakistani patients and investigate its relationship with lymphocyte doubling time (LDT). The rationale was to determine whether an LDT of less than 1 year could serve as a surrogate marker for *TP53* mutation.

METHODOLOGY

This was a cross-sectional, descriptive study, conducted at Liaquat National Hospital, Karachi. A total of 128 B-CLL patients who attended the haematology outpatient department (OPD) between January 2020 and December 2022 were enrolled and monitored. Inclusion criteria encompassed Pakistani patients aged between 30 to 90 years who were recently diagnosed with B-CLL, according to iwCLL criteria, which relied on immunophenotyping results.[10] Patients who had previously been diagnosed with other forms of cancer, whether haematological or non-haematological, and already had a confirmed *TP53* gene mutation were not included in the study. Additionally, patients who had undergone chemotherapy or radiotherapy as part of their treatment for any type of cancer were also excluded from the study.

Written informed consent was obtained from each patient for their participation in the study, including data collection and laboratory investigations. A detailed medical history, physical examination, ECOG performance status, and Binet's staging were documented for each patient. Diagnostic and prognostic investigations included complete blood count (CBC), peripheral blood smear, immunophenotyping *via* flowcytometry on peripheral blood, abdominal ultrasound/computed tomography (CT) scan of the whole body, serum creatinine, serum lactate dehydrogenase levels, serum uric acid, serum alanine transaminase levels, serum calcium and direct antiglobulin test. Additionally, *TP53* mutation analysis was performed using the Fluorescence *in situ* Hybridisation (FISH) technique right after diagnosis.

Lymphocyte doubling time (LDT) refers to the duration required for lymphocytes to double in number from the count observed at diagnosis. An LDT of 12 months or less indicated rapidly progressive disease with a poor prognosis, while an LDT of more than 12 months suggested slowly progressive disease and a favourable prognosis.[10,11] Patients were followed up every 3-6 months after diagnosis, with recent CBC reports. Absolute Lymphocyte Count (ALC) was recorded at the initial visit (baseline) and during each subsequent follow-up visit to calculate the LDT and evaluate disease progression. The median time from diagnosis to the follow-up visits was approximately 10 months (range: 7-13 months), which was used to calculate the LDT.

The sample size (n=82) for the study was determined using the open epi sample size calculator, considering a 95% confidence level. The prevalence of the outcome variable was derived from a previous study. Non-probability consecutive sampling was employed as the sampling technique.

Data compilation and analysis were performed using IBM SPSS version 22. Qualitative variables were presented in frequencies and percentages, while quantitative variables were expressed as mean ± SD. The relationship between categorical variables was assessed using the Pearson Chi-square ($\chi 2$) test or Fisher's exact test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 128 patients who had recently been diagnosed with B-CLL participated in the study. Among these patients, 89 (69.5%) were males, and 39 (30.5%) were females. The mean age of the patients was 62 years, ranging from 35 to 88 years. *TP53* mutation analysis was conducted on all patients using the FISH technique with peripheral blood samples. The results showed that 10 patients (7.8%) tested positive for the mutation, while 118 patients (92.2%) tested negative.

Table I presents the clinical and laboratory parameters of patients diagnosed with B-CLL, with and without *TP53* mutation. A comparison between these groups revealed significant associations between *TP53* mutation and age over 62 years (p=0.03), advanced clinical stage (p=0.03), baseline ALC (p=0.007), and follow-up ALC between 12-18 months after diagnosis (p=0.001). However, *TP53* mutation did not show a significant association with gender (p=1.0), LDT (p=0.1), LDH (p=0.7), direct antiglobulin test (p=1.0), serum creatinine level (p=0.3), serum ALT levels (p=0.7), and serum calcium (p=0.3).

During the follow-up period, 26 patients were lost to follow-up, with one patient from the *TP53* positive group and 25 patients from the negative group. In the *TP53* positive group, 55.6% (n=5/9) of patients had an LDT of less than 1 year, indicating aggressive disease, compared to 30.1% (n=28/93) of patients in the negative group (55.6% *vs.* 30.1%; p <0.1). Similarly, in the *TP53* mutation with B-CLL group, 44.4% (n=4/9) of patients had an LDT of more than 1 year, while 69.9% (n=65/93) of patients in the *TP53* negative group had an LDT of more than 1 year (44.4% *vs.* 69.9%; p <0.1). Figure 1 shows the study variable (*TP53* mutation status) and their associations with LDT.

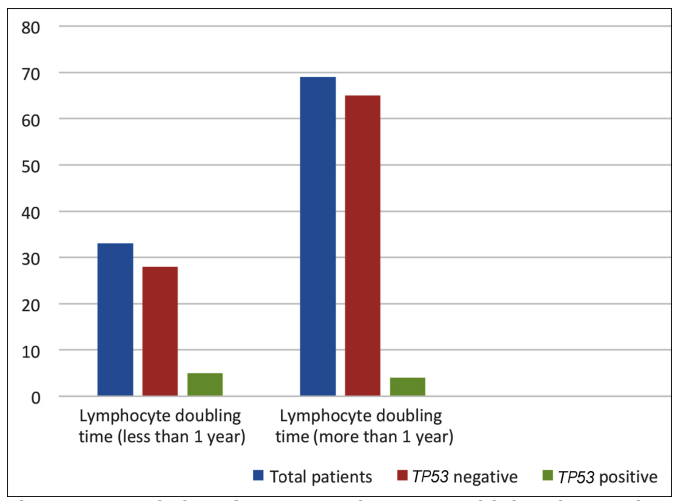

Figure 1: Association of *TP53* mutation status with lymphocyte doubling time.

Table I: Characteristics of patients according to *TP53* mutation status.

Patients' characteristics	All n (%)	*TP53* mutation positive n (%)	*TP53* mutation negative n (%)	p-value
Patients	128	10(7.8)	118(92.2)	
Age (mean)	62.37	69	61.8	0.03
Gender				
Male	89(69.5)	7(70)	82(69.5)	1.0
Female	39(30.5)	3(30)	36(30.5)	
Binet's stage				
A	81(63.3)	3(30)	78(66.1)	0.03
B	18(14.1)	3(30)	15(12.7)	
C	29(22.7)	4(40)	25(21.2)	
ALC at presentation (mean)	70.4x10^9/L	110 x10^9/L	67.3 x10^9/L	0.002
ALC (b/w 12-18 months)	65.5 x10^9/L	130.5 x10^9/L	59.5 x10^9/L	0.005
LDT				
≤12 months	33(32)	5(55.6)	28(30.1)	0.1
>12 months	69(67.6)	4(44.4)	65(69.9)	
Direct antiglobulin test				
Positive	4(3.4)	0(0)	4(3.6)	1.0
Negative	115(96)	9(100)	106(96.4)	
LDH (Mean)	298U/L	279 U/L	300 U/L	0.7
Uric acid (mean)	5.8mg/dl	6.2mg/dl	5.4mg/dl	0.1
Serum creatinine (mean)	0.9 mg/dl	0.9 mg/dl	0.9 mg/dl	0.6
Serum calcium (mean)	9.98 mg/dl	16.67 mg/dl	9.42 mg/dl	0.3
ALT (mean)	25.26 U/L	21.56 U/L	25.57 U/L	0.7

DISCUSSION

The present study aimed to investigate the frequency of *TP53* mutation in patients with B-CLL at the time of diagnosis and assess its association with LDT. In this study, 10 patients (7.8%) tested positive for *TP53* mutation. This frequency aligned with the western studies reporting a range of 3.7 to 8.5% of patients detected positive for *TP53* in CLL.[12,13] These findings contrast with a local study showing a relatively high number of *TP53* -positive patients, i.e. 13.7%.[14] Another local study also demonstrated similar findings, with 18.5% of B-CLL patients having 17p deletion.[15] Similarly from the neighbouring country, India, Kadam Amare *et al*. reported 22% frequency of *TP53* in CLL.[16]

The relatively low frequency observed in this study may be attributed to the fact that mutation status was examined in newly diagnosed patients regardless of their clinical stage or treatment indication. These findings support a previously published Swedish study where Zainuddin *et al*. reported a 3.7% frequency (n=10/268) of CLL patients detected positive for *TP53* mutation, concluding that *TP53* mutation is rare at disease onset.[13] These results endorsed the notion that *TP53* mutation develops during disease progression. However, in resource-limited countries, the use of *TP53* mutation analysis is limited due to its high cost which has made its utility debatable.

The results of this study showed that patients with *TP53* mutation had a higher proportion of patients (55.6%) with LDT less than 1 year compared to those without *TP53* mutation (30.1%). Conversely, patients without *TP53* mutations had a higher proportion (69.9%) of patients with LDT more than 1 year compared to 44.4% of patients with *TP53* mutation. Although these results suggested that *TP53* mutation may be associated with a shorter LDT in CLL but statistical significance was not observed. Therefore, LDT may complement *TP53* aberrations in CLL prognostification but it cannot replace them.

Furthermore, *TP53* positive patients were older and had higher baseline ALC. Additionally, significant association between positive *TP53* mutation and the doubling of ALC count between 12 to 18 months indicated a rapidly progressive nature of the disease in mutation-positive patients. These findings emphasised the crucial role of early determination of mutation status, particularly in patients with a high baseline ALC count, age over 60 years, and high-risk disease (such as Binet Stage C). Patients who test positive for *TP53* mutation should be closely monitored under strict surveillance. These findings were consistent with some of the previously reported studies.[17,18] This study contributed to the growing body of literature supporting the association between *TP53* mutations and advanced-stage disease, which may have implications for the clinical management and treatment decisions for patients with CLL.[19,20]

However, it is important to acknowledge the limitations of this study, including the small sample size and the loss of 26 patients to follow-up. The exclusion of these patients may have potentially impacted the result. Moreover, the impact of *TP53* mutation on LDT may be influenced by other factors, such as age, gender, and stage of the disease. Further studies with larger sample size and longer follow-up periods are needed to confirm findings and explore the potential mechanism underlying the association between *TP53* mutation and LDT in CLL. The clinical implications of this finding warrant additional investigation, as it could potentially impact treatment decision-making and patient prognosis.

CONCLUSION

There was a relatively low frequency of *TP53* mutation at the time of B-CLL diagnosis, specifically at 7.8%. Patients with a positive *TP53* mutation tended to experience an earlier increase in lymphocyte count, with lymphocyte count doubling between 12 to 18 months, compared to patients without the *TP53* mutation. *TP53* mutations may be linked to a shorter LDT in patients with CLL.

ETHICAL APPROVAL:

An approval was obtained from the Ethical Review Committee (ERC) of Liaquat National Hospital and Medical College with approval number 0581-2020 LNH – ERC. Data confidentiality was guaranteed that under no circumstances, patient's name, identity and any other associated information will be disclosed to any other person.

PATIENTS' CONSENT:

All participants provided written informed consents prior to participation.

COMPETING INTEREST:

The authors declared no competing interest.

AUTHORS' CONTRIBUTION:

SMA: Conception of the work, design, data collection, interpretation, and analysis, drafting of the initial version.
SMI: Conception, data analysis, review, and editing.
NR: Review and editing.
All authors approved the final version of the manuscript to be published.

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