DOI: https://doi.org/10.53350/pjmhs2023173746

ORIGINAL ARTICLE

Patients with Chronic Lymphocytic Leukemia are more Likely to Have TP53 Gene Mutations

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ABSTRACT

Objective: The purpose was to quantify the prevalence of Tp53 gene mutation in chronic lymphocytic leukemia patients..
Study Design: Cross-sectional/ Descriptive study
Place and Duration: This study was conducted in multiple centres including Mayo Hospital Lahore and Liaquat University of Medical and Health Sciences in the duration from August, 2022 to January, 2023.
Methods: Total 112 cases of chronic lymphocytic leukaemia were included in this study. All the patients were received at laboratory for TP53 mutation analysis. To identify the TP53 mutation using the FISH method, five ml of whole blood or one ml of bone marrow aspirate sample were taken. Data input and analysis were done with the help of the statistical package for social sciences 22.
Results: The mean age of the patients was 52.8±6.52 years and had mean BMI 23.7±14.52 kg/m^2. Seventy-three (65.2%) patients were males and 39 (34.8%) cases were females. There were 52 (46.4%) patients educated and 60 (53.6%) cases were non educated. We found frequency of Tp53 gene mutation in 17 (15.2%) cases in which 11 cases were males and 6 cases were females. Among 17 cases of Tp53 gene mutation, 8 cases had age >50 years, 6 cases were aged between 30-50 years and 3 cases were between 18-30 years.
Conclusion: We concluded in this study that frequency of Tp53 gene mutation in patients of chronic lymphocytic leukaemia was 15.2% in which majority were males and had age >50 years.
Keywords: CLL, Tumour suppressor gene, Chronic lymphocytic leukaemia, FISH, TP53, Fluorescence in Situ hybridization

INTRODUCTION

Chronic lymphoid leukemia (CLL) is defined by a highly variable disease course, with some patients surviving for over a decade without the need for treatment and others experiencing rapid disease progression and adverse effects despite receiving operational chemoimmunotherapy.[1] A portion of this variation can be attributed to the many genetic variations that are present in CLL individuals.[2] Removals in chromosome 17p [del(17p)] that cause the loss of the TP53 gene, which produces the neoplasms-suppressor protein p53, are particularly associated with a bad prognosis. Del(17p) or no TP53 mutations are likewise associated with a poor prognosis.[3] We shall collectively call to all of these changes as TP53 aberrations.

The greatest prognostic and predictive indicators used to determine a patient's course of treatment for CLL are TP53 abnormalities, which are also linked to significantly reduced survival and poor chemoimmunotherapy response.[4] Alemtuzumab and allogeneic hematopoietic stem cell transplantation were the only successful therapies available up until recently for individuals with CLL with TP53 abnormalities. There are currently several new small-molecule inhibitors on the market, such as the BCL2 inhibitor venetoclax, the phosphatidylinositol 3-kinase (PI3K) inhibitor idelalisib, and the Bruton tyrosine kinase (BTK) inhibitor ibrutinib, which are effective in treating individuals with TP53 abnormalities.[5,6] Consequently, identifying TP53 abnormalities is crucial to selecting the best course of action for CLL patients.[7,8]

The use of highly sensitive NGS sequencing technologies has aided in the detection of TP53 mutations, with the potential to find variants with allelic parts (VAFs) below the standard cutoff of 10%, which was published by the European Studies Action on Chronic Lymphocytic Leukemia, also (ERIC) in 2018 [9] and above which mutations in TP53 should be reported clinically. However, there is disagreement on the clinical and biological significance of these small clones.

TP53 mutations in CLL may have an effect on prognosis, according to a number of investigations.[10,11] The relevance of the TP53 mutation in connection to the 17p deletion has been difficult to define, though, due to a lack of comprehensive genetic investigation, a limited sample size, and cohort variability. The majority of investigations have discovered TP53 mutations in 5% of individuals who do not have a 17p deletion (the only known TP53 mutation). Large prospective studies are required to validate the prognostic impact of mutant TP53 in light of the other known prognostic variables.[12] The US Eastern Cooperative Oncology Group 2997 study was the first prospective trial fully disclosed that examined the prognostic impact of TP53 mutations and 17p deletion to date. This study was unable to demonstrate the prognostic importance of TP53 mutations. Interpretation is challenging since actual mutations were combined with suspected mutations and germline polymorphisms.[13]

The Next Generation Reading (NGS) technology provides depth (LoD 1%) or super duper-deep (LoD 1%) DNA sequencing, allowing the discovery of mutations much below Sanger's LoD. A part of the introns as well as all of the splice sites may be sequenced using NGS techniques, allowing for the simultaneous investigation of the exonic regions. NGS techniques could be useful for completely genotyping somatic mutations in CLL.[14,15]

MATERIAL AND METHODS

This Cross-sectional/ Descriptive study was conducted at This study was conducted in multiple centres including Mayo Hospital Lahore and Liaquat University of Medical and Health Sciences in the duration from August, 2022 to January, 2023 and comprised of 112 newly diagnosed cases of CLL received for TP53 mutation. After receiving informed, written consent, comprehensive demographic information on the enrolled cases was collected. Patients <18 years of age, pregnant females and those did not provide any written consent were excluded.

For the TP53 mutation FISH method, five milliliters of whole blood or one milliliter of bone marrow aspirate sample were obtained. Standard cytogenetic procedures were used to fix samples with interphase nuclei or metaphase spreads on glass slides. The DNA from the final specimen may now hybridize with the single-color p53 (17p13.1) probe after being denatured to become single-stranded. After hybridization and counterstaining, interphase nuclei were examined under a microscope to identify the TP53 (17p13.1) gene. One orange signal pattern is shown in

an aberrant cell that has the typical deletion, but two orange (2O) signal patterns are seen in a normal cell when the nucleus was hybridized with the p53 (17p13.1) probe. Each piece of information was entered into a pre-made proforma.

Using SPSS version 22, data was input before being analyzed. Age was determined using the mean and standard deviation. It was also computed what percentage of the study population was male and female. In addition to calculating gender distribution and age categories, the frequency and percentage of Tp53 mutation in CLL were calculated throughout the full research population. Age and gender were used as effect modifier comparisons to see how they affected TP53. To find a significant difference, a Chi-square test was used. p-values below 0.05 are considered significant at a 95% confidence level.

RESULTS

The mean age of the patients was 52.8±6.52 years and had mean BMI 23.7±14.52 kg/m^2. Seventy-three (65.2%) patients were males and 39 (34.8%) cases were females. There were 52 (46.4%) patients educated and 60 (53.6%) cases were non educated.(table 1)

Table-1: Baseline details of enrolled cases

Variables	Frequency	Percentage
Mean Age (years)	52.8±6.52	
Mean BMI (kg/m^2)	23.7±14.52	
Gender		
Male	73	65.2
Female	39	34.8
Education Status		
Educated	52	46.4
Non-educated	60	53.6

We found frequency of Tp53 gene mutation in 17 (15.2%) cases in which 11 cases were males and 6 cases were females.(table 2)

Table-2: Frequency of gene mutation among all cases

Variables	Frequency (n=112)	Percentage
Tp53 Gene mutation		
Yes	17	15.2
No	95	84.8
Gender		
Male	11	9.8
Female	6	5.4

Among 17 cases of Tp53 gene mutation, 8 cases had age >50 years, 6 cases were aged between 30-50 years and 3 cases were between 18-30 years.(figure1)

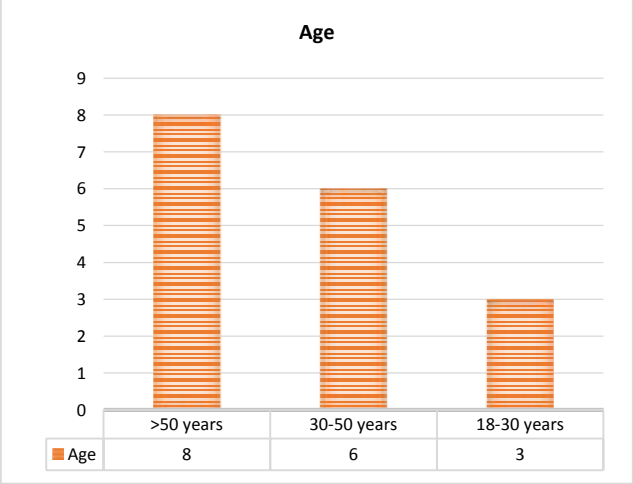

Figure-1: Age distribution of Tp53 gene mutation cases

DISCUSSION

The most current TP53 recommendations were published by the European Research Initiative in Chronic Lymphocytic Leukaemia in 2018[16]. These recommendations state that only TP53 mutations over the consensus Sanger sequencing-like criterion of 10% VAF ought to be clinically reported. When reporting alterations from five to ten percent VAF, it should always be noted that their clinical significance is uncertain. This advice is still relevant in 2021, and the absence of clinical data and technological challenges in finding low-burden mutations[17] are the key justifications for keeping this cautious criterion for reporting. It is necessary to revisit the 10% VAF criterion in order to increase the population of patients qualified for this form of treatment because TP53 gene mutations continue to be a common indication for treatment with targeted medicines in many nations. The patient cohorts who need to be treated in accordance with CLL recommendations for TP53 testing must provide enough evidence for this review.[18]

The purpose of this study was to evaluate the frequency of TP53 gene mutation in individuals with Chronic Lymphocytic Leukaemia. There is currently a dearth of knowledge on the prevalence of TP53 mutations and their effects in developing nations, notably Pakistan. In current study 112 patients with chronic lymphocytic leukaemia were included. The mean age of the patients was 52.8±6.52 years and had mean BMI 23.7±14.52 kg/m^2. Seventy-three (65.2%) patients were males and 39 (34.8%) cases were females. The findings of our study was comparable to the prior studies.[19,20] According to an Indian research, the average age at which a patient with chronic lymphocytic leukemia is diagnosed is 61 years old. Of the 95 patients who were studied, 75 were men and 20 were women. Of these, 30 patients were under the age of 55 and 65 were over 55.[21]

In a recent research, the overall incidence of spontaneous TP53 mutations [5% (24/469 individuals)] and del(17p) [2.3% (11/469 individuals)] in the O-CLL1 cohort and their relative distribution are comparable to those of earlier findings.[22,23] The bulk of TP53 alterations were point mutations, which changed just one amino acid in the P53 protein's DNA binding region. Both the patient groups with Mut/Del and Mut/noDel shared this reality. Three amino acid alterations (p.Arg175His, p.Pro278Arg, and p.Ile195Thr) shared by the Mut/noDel and Mut/Del patient groups were discovered among the nearly 2,000 amino acid substitutions brought on by a point mutation in the TP53 gene.[22]

In current study, frequency of Tp53 gene mutation was found in 17 (15.2%) cases in which 11 cases were males and 6 cases were females. Geographical differences in CLL prevalence exist. China and Japan in particular have a far lower incidence of CLL than Western nations do, with incidence rates that are around 10% lower overall; this finding is mostly ascribed to environmental causes.[23-25] Numerous techniques may be used to identify TP53 mutations, although the majority of CLL patients are identified with chromosomal abnormalities by sensitive testing like FISH. In this work, the FISH method was used to locate mutations. According to whole-genome sequencing of CLL patients, the average CLL genome has a large number of genetic changes, many of which are not visible with conventional cytogenetics.[26]

CONCLUSION

We concluded in this study that frequency of Tp53 gene mutation in patients of chronic lymphocytic leukaemia was 15.2% in which majority were males and had age >50 years.

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