ORIGINAL ARTICLE

FREQUENCY OF TP53 GENE MUTATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Chronic lymphocytic leukaemia (CLL), an indolent but malignant lymphoproliferative disorder, is characterized by unregulated and uninhibited growth of mature monoclonal lymphocytes, with deletion of 17p containing TP53 gene being the most important prognostic factor. TP53 mutations, reported in 10% of CLL cases, seem to have a direct correlation to a more advanced stage and aggressive transformation of CLL. **Methods:** This was a retrospective cross-sectional descriptive study limited to a period from 1st June 2013 to 30th June 2016, conducted at Section of haematology, Department of Pathology and Laboratory Medicine, Aga Khan University Hospital, Karachi. One thirty-nine cases of CLL received for TP53 mutation analysis at the Aga Khan University hospital clinical Laboratory were included in the study. Five ml of whole blood or one ml of bone marrow aspirate sample in EDTA tube was collected for the detection of TP53 mutation by the FISH technique. Statistical package for social sciences 21 was used for data entry and analysis. **Results:** Of the 139 chronic lymphocytic leukaemia patients, 43 (31%) were females and 96 (69%) were males. The mean age of all patients was 56.3±10.84 years. Tp53 gene mutation in patients with chronic lymphocytic leukaemia was found only in 19(13.7%) patients. Among these patients 15 (10.9%) were male and 04(2.9%) were females. Age and gender were not statistically significant with Tp53 mutation with a *p*-value $\geq$ 0.05 at a 95% confidence interval. **Conclusion:** In a cohort of Pakistani patients with Chronic lymphocytic leukaemia, TP53 gene mutation was found in 19 (13.7%).

Keywords: Chronic lymphocytic leukaemia; CLL; Tumour suppressor gene; TP53; Fluorescence in Situ hybridization; FISH

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INTRODUCTION

Chronic lymphocytic leukaemia (CLL), an indolent but malignant lymphoproliferative disorder, is characterized by uninhibited growth of mature lymphocytes which are classically monoclonal in origin. Primarily a disease of the elderly, with an average age of 70 years at diagnosis[1–3], CLL has a lower incidence in Asian countries such as China and Japan when compared to the West.[4–6]

Chronic lymphocytic Leukaemia has a range of clinical manifestations, with some patients exhibiting no symptoms or change in clinical status even when untreated while others have rapid disease onset and fatal progression.[7] Outcomes have a direct correlation to various prognostic factors including staging, bone marrow histologic pattern, and lymphocyte doubling time. Molecular prognostic factors have also been important in determining outcomes, the most extensively investigated and validated being the cytogenetic, immunoglobulin heavy chain variable (IGHV) gene mutational status, and CD38 and ZAP-70 expression.[8,9] Of these molecular markers, the deletion of 17p containing TP53 gene has consistently proven to be an indicator of rapid disease progression, refractory to treatment.[10,11]

The p53 tumour suppressor gene resides on chromosome 17 band p13.1. Mutations that inactivate p53 promote prolonged cancer cell survival and proliferation, chemotherapy resistance, and disease progression.[12] This mutation, although a feature of other haematological malignancies, is characteristic for aggressive Non -Hodgkin's lymphoma[13], progressive Chronic lymphocytic Leukaemia[14], and B-cell chronic prolymphocytic leukaemia[15,16]

TP53 mutation, occurring in approximately 10 percent of patients with newly diagnosed CLL, is associated with more advanced stages of the disease and subsequent transformation of CLL to its aggressive phase which is often refractory to conventional chemotherapy.[17,18] Current international guidelines recommend screening all CLL patients for deletion of 17p containing theTP53gene so they can be evaluated for alternative treatment therapies, as they have consistently shown poor response to conventional chemotherapy.[19,20] However, there is

no local study on TP53 mutations in chronic lymphocytic leukaemia to date.

The purpose of this study is to determine the frequency of TP53 mutation in chronic lymphocytic leukaemia patients in the Pakistani population. This will generate local data and help in risk stratification. This will further help in counselling the patients and their families about the prognosis.

MATERIAL AND METHOD

This was a retrospective cross-sectional descriptive study covering a period from 1st June 2013 to 30th June 2016. The study was conducted at Section of haematology, The Aga Khan University Hospital Karachi. One thirty-nine newly diagnosed cases of CLL received for TP53 mutation analysis at Aga Khan University hospital clinical Laboratory were included in the study.

Five ml of whole blood or one ml of bone marrow aspirate sample in EDTA tube was collected for the detection of TP53 mutation by FISH technique. Using standard cytogenetic protocols samples consisting of interphase nuclei or metaphase spreads were fixed to glass slides. The resulting specimen DNA, denatured to its single-stranded form, could now hybridize with the p53 (17p13.1) single colour probe. Following hybridization and counterstaining, detection of TP53 (17p13.1) gene was conducted by microscopic examination of interphase nuclei. An abnormal cell containing the characteristic deletion, one orange signal pattern is observed whereas, in a normal cell, the nucleus was hybridized with the p53 (17p13.1) probe resulting in a two orange (2O) signal pattern. All the data was recorded in pre-designed *pro forma.*

Data was entered and then analysed by using SPSS version 21. Mean and Standard deviation for age was calculated. The proportion ratio of males and females in the study population was also calculated. The frequency and percentage of Tp53 mutation in CLL was computed in the entire study population along with gender distribution and age groups. Effect modifier was compared to TP53 like age and gender to see the impact on TP53. A Chi-square test was applied to see a significant difference. p-value <0.05 taken as significant value at 95% confidence interval.

RESULTS

Out of 139 chronic lymphocytic patients, 43 (31%) were females and 96 (69%) were males. Their age range was 37–82 years in females and 30-86 years in males. The mean age of all patients was 56.3±10.84 years; in males 55.6±11.11 years, while in the female the mean age was 58.11±10.12 years. The frequency of different age groups was found to be 10 (7.2%) in age 30–40 years, 34 (24.5%) were in 41–50 years of age, most of the patients 48 (34.5%) were found in 51–60 years of age, 35 (25.1%) were in 61–70 years & finally, 12 (8.7%) were found in age >70 years recruited in the study. Tp53 gene mutation in patients with chronic lymphocytic leukaemia was found only in 19 (13.7%) patients. Among these patients 15 (10.9%) were male and 04 (2.9%) were females. To check the effect modifier through stratification with Tp53 mutation with a different demographic parameter of the study age & gender finally, both age (p-value=0.208) and gender (p=0.316) were not statistically significant with TP53 mutation.

DISCUSSION

This study was designed to assess the prevalence of TP53 gene mutation in patients diagnosed with Chronic Lymphocytic Leukaemia. Currently, there is a scarcity of information about the incidence of TP53 mutations and their outcomes, in developing countries, particularly Pakistan.

According to the World health organization 2014 review, although Chronic lymphocytic leukaemia (CLL) continues to be the most prevalent leukaemia in the west, its incidence is significantly lower in Asia. The median age of diagnosis in Europe and Australia and the United States is approximately the same at 70 years of age. Of these patients, roughly a quarter are younger than 65 and approximately 6% younger than 50 years, with a male to female ratio of approximately 1.3:1 to 1.7:1.[21]

According to the Indian study median age of diagnosis of chronic lymphocytic patients is 61 years, out of total 95 patients, 75 were males and 20 were females, among them thirty patients were 55 years or less and 65 were more than 55 years of age.[22]

In this study on the Pakistani cohort, in Aga Khan University Hospital the median age was 56 years of age. Out of 139 Pakistani Chronic lymphocytic patients, from 30–40 years were 10 subjects (7.2%); from 41–50 years were 34 (24.5%); from 51–60 years were 48 (34.5%); from 61–70 years were 35 (25.1%) and >70 years were 12 (8.7%) subjects. No patient was seen below 30 years of age. The majority of the patients were between 51–60 years. In our study, the male to female ratio was (69% male, 31% female) respectively. The incidence of CLL differs by geographical location. The incidence of CLL in Asian countries, China and Japan, in particular, is significantly lower, occurring at a frequency that is roughly 10 percent of that seen in the West[23–26]; an observation attributed mostly to Environmental factors.[27,28]

TP53 mutations can be detected by a variety of different methods, with sensitive tests such as FISH detecting chromosomal abnormalities in the majority of patients diagnosed with CLL.[29] In this study, mutations were identified by using the FISH technique. Whole-genome sequencing of CLL cases suggests that an average CLL genome contains numerous genetic alterations, many of which are not apparent on standard cytogenetics.[29]

In our study on a Pakistani cohort, 13.7% of TP53 mutations were seen. This is in contrast to a mutation rate of 10% seen in US and Chinese cohorts, 14.5% for Italian, and 13.5% for German Cohorts of patients with CLL.[16,25,26]

CONCLUSION

Tp53 gene mutation was detected in 19/139 (13.7%) patients. The overall incidence of Tp53 mutations, although higher in Pakistani patients, was still within the globally reported range.

AUTHORS' CONTRIBUTION

HQ: Conceptualization of study design and write-up. NN: Data collection, editing. NQ: Data analysis and interpretation. SNA: Write-up. HT: Proof reading, editing. AQ: Literature search, editing.

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