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Frequency of TP53 (17p13) Deletion in Patients with Chronic Lymphocytic Leukemia (CLL)

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Abstract

Objective: To determine the frequency of TP53 (17p13) gene deletion in patients diagnosed with chronic lymphocytic leukemia (CLL) and assess its correlation with age, gender, and total leukocyte count (TLC).

Methodology: This retrospective cross-sectional study was conducted at the Section of Hematology and Cytogenetics, Department of Pathology, Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore, from January 2019 to December 2021. A total of 157 CLL patients were included. Peripheral blood or bone marrow samples were analyzed using the Fluorescence In Situ Hybridization (FISH) technique to detect TP53 deletion. Statistical analysis was performed using SPSS version 21.0, and Chi-square tests were applied for significance assessment.

Results: Among the 157 CLL patients, 114 (72.61%) were males, and 43 (27.38%) were females, with a mean age of 56.44 ± 8.20 years. TP53 deletion was observed in 30 (19.11%) patients, including 23 (76%) males and 7 (23.30%) females. No significant correlation was found between TP53 deletion and age or gender ($p > 0.05$). Additionally, 18 (60%) patients with TP53 deletion had TLC $<100\times10^3$, while 12 (40%) had TLC $>100\times10^3$.

Conclusion: The frequency of TP53 gene deletion in CLL patients was 19.11%. The deletion was more prevalent in males, though statistical significance was not established. Early identification of TP53 deletion is crucial for risk stratification and treatment decisions.

Keywords: Chronic lymphocytic leukemia (CLL), TP53 gene deletion, Fluorescence In Situ Hybridization (FISH).

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Introduction

A clonal lymphoproliferative disorder called chronic lymphocytic leukemia (CLL) is characterized by peripheral B-lymphocyte counts greater than or equal to 5×10^9/L that express CD5, CD19, and CD23 while weakly expressing CD20, CD79b, and surface immunoglobulin (sIg) in the blood, bone marrow, and secondary lymphoid tissue. Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in western nations but is uncommon in Asians.[1] It is 5–10 times less in Asians.[2]

Males are more commonly affected by the condition (1.5-2:1). Asymptomatic patients make up about 80% of those who receive a diagnosis. The remaining patients frequently have neck, axillae, and inguinal generalized lymphadenopathy that is not painful. In rare cases, CLL may affect extra hematological tissues, including the skin, liver, kidney, or central nervous system.[3] The clinical course varies greatly, from very mild cases to patients with aggressive and quickly advancing disease. This heterogeneity has significant ramifications that will affect clinical approaches, treatment plans, and, ultimately, survival times following diagnosis. Trisomy 12 and deletions at 13q14, 11q22-q23, and 17p13 are frequently found in CLL cells, and these genetic changes are strongly associated with clinical outcome.[4] The definitive diagnosis of CLL is made on flow cytometry.[5] In Caucasians[3], the median age at disease diagnosis is 70 years, whereas in Asians with CLL, it is 61 years.[6]

The tumor-suppressor protein p53 is encoded by the TP53 gene, which is found on the 17p13 chromosome. This protein has many cellular functions, including controlling the cell cycle and apoptosis and promoting DNA repair in response to cellular stress signals like DNA damage. The method frequently used for TP53 (17p13) deletion detection that affects the expression of the tumor protein p53 is called Florescence In Situ Hybridization (FISH).[7] Patients who have TP53 (17p13) deletions have a poor prognosis, are resistant to chemotherapy, and negatively impact overall survival.[8] Finding out the prevalence of TP53 (17p13) deletion in chronic lymphocytic leukemia patients will aid in the generation of regional data, which is the goal of this study. Additionally, as a key component of CLL risk

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stratification, it will be useful in guiding patients' decisions regarding treatment options and prognosis counseling.

Methodology

This was a retrospective cross-sectional descriptive study covering a period from 1st January 2019 to 31st December 2021. This study was conducted at section of hematology and section of cytogenetics (Department of Pathology), Shaukat Khanum Memorial Cancer Hospital and Research Center Lahore. One fifty-seven patients aged between 35 to 75 years who have been diagnosed with chronic lymphocytic leukemia on flow cytometry at Shaukat Khanum Memorial Cancer Hospital and Research Center Lahore, before having chemotherapy were included.

Data was obtained from HIS after IRB approval which included patients fulfilling the required criteria. Originally, five ml of peripheral blood or bone marrow in EDTA tube was collected for detection of TP53 gene deletion by using FISH technique. Hypotonic solution was added to the sample and then sample was fixed. Centrifugation was performed and nucleated cells were separated from the rest of the sample. The FISH panel included probes for the detection of TP53 (17p13) deletion. The nucleated cells were heated up to 73 Celsius to convert double stranded DNA to single stranded, hybridization was done using commercially prepared TP53 gene probes and detection of TP53 (17p13) gene was conducted by microscopic examination of interphase nuclei. In a normal cell, the expected pattern for a nucleus hybridized with the p53 (17p13) probe is a two orange (2O) two green (2G) signal pattern. In an abnormal cell containing the deletion, two green (2G) and one orange (1O) signal pattern was observed.

Data was entered and statistical analysis was done in SPSS version 21.0. Proportion ratio of males and females in study population was determined. Mean and Standard deviation for age were calculated. Frequency and percentage of Tp53 deletion in CLL was computed in total study population and also with gender distribution and age groups. Effect modifiers i.e., age, gender and WBC count were compared with respect to TP53 deletion status to see correlation. Chi-square test was applied to see the significant difference among these, with P <0.05 taken as significant value at 95% confidence interval.

Results

Out of 157 chronic lymphocytic patients 43 (27.38%) were females and 114 (72.61%) were males. Their age range was years in female and years in males. The mean age of all patients was 55.4±8.20 years; in males mean age was 55.03±8.15 years, while in female the mean age was 56.55±8.35 years. Age distribution and frequency is mentioned in Table I. Tp53 gene deletion in patients with chronic lymphocytic leukemia was found in 30 (19.11%) patients. Among these patients 23 (76%) were males and 7 (23.30%) were females Table II. Rest of males and females were negative for TP53 gene deletion. Table III showed 18(60%) patients with Tp53 deletion had TLC <100×10^3 and 12(40%) patients with Tp53 deletion had TLC>100×10^3. To check the effect modifier through stratification with Tp53 gene deletion with different demographic parameter of the study age & gender finally, both age and gender were not statistically significant with TP53 gene deletion P-Value found ≥ 0.05 at 95% confidence interval.

Table I: Age Distribution and TP53 Gene Deletion Status in Chronic Lymphocytic Leukemia Patients.

Age groups	TP53 gene deletion	
	Positive	Negative
35-45 Years	06 (20%)	14 (11%)
46-55 Years	07 (23.3%)	40 (31.49%)
56-65 Years	12 (40%)	64 (50.39%)
66-75 Years	05 (16.6%)	09 (7.08%)
Total	30	127

Chi-square=5.114 P=value=0.164

Table II: Gender-Wise Frequency of TP53 Gene Deletion in Chronic Lymphocytic Leukemia Patients.

Gender	TP53 deletion		
	Positive	Negative	Total
Male	23 (76%)	91 (71.65%)	114 (72.61%)
Female	07(23.3)	36 (28.34%)	43 (27.38%)
Total	30	127	157
Gender	**TP53** deletion		
	Positive	**Negative**	**Total**
Male	23 (76%)	91 (71.65%)	114 (72.61%)
Female	07(23.3)	36 (28.34%)	43 (27.38%)
Total	30	127	157

Chi-square= 0.3066 P value= 0.579

Table III: Correlation of Total Leukocyte Count (TLC) With TP53 Gene Deletion in Chronic Lymphocytic Leukemia Patients

TLC	TP53 gene deletion	
	Positive	Negative
TLC<100×10^3	18 (60%)	72 (56.60%)
TLC>100×10^3	12 (40%)	55 (43.30%)
Total	30	127

Chi-square= 0.1085 P value= 0.7419

Discussion

In this study, the prevalence of TP53 gene deletion in patients with chronic lymphocytic leukemia was investigated. The prevalence of TP53 gene deletions and their results are largely unknown in developing Asian nations, including Pakistan. Treatment for CLL patients with TP53 gene deletion (TP53 mutation) is difficult because these patients do not respond well to standard therapies and have suboptimal progression-free and OS outcomes. As a result, these patients need alternative therapies, with novel agents and targeted therapy possibly leading to better results.[3,9,10] Therefore, early detection of TP53 gene deletion can assist in identifying high-risk cases that should receive appropriate treatment to increase their survival.

Conventional metaphase cytogenetic analyses underestimate chromosomal aberrations in CLL patients, as the detection of abnormalities is limited by the low mitotic activity of CLL cells, whereas FISH analysis using interphase cells can identify up to 80% of chromosomal abnormalities. Thus, FISH is considered much more sensitive for CLL, with better ability to detect genomic aberrations, which makes it the preferred method for reliably identifying chromosomal aberrations in CLL.[7]

To the best of our knowledge, there is very little available FISH-based data regarding the frequency of TP53 gene deletion among patients with CLL in Pakistan. In this context, Shaukat Khanum Memorial Cancer Hospital and Research Center Lahore is a large institution in Pakistan, which treats a large number of patients from throughout the country who have various ethnic backgrounds. Therefore, we evaluated the frequency of TP53 gene deletion (TP53 mutation) among patients who were treated at our center.

The present study included 157 newly diagnosed cases of CLL, with 30 patients (19.11%) being positive for TP53 gene deletion. These results agree with the findings of a relatively local studies conducted by Mehmood R[11] *et al and* Wu SJ[12] *et al* who reported a frequency of 18.5% and 20.5% in population of Pakistan and Taiwan respectively. However, relatively low frequency of TP53 gene deletion was reported by Stilgenbauer S[13] *et al* in CLL8 trial (11.5%), Takahashi K[14] *et al* in Finland (14%), Xia Y[15] *et al* and Chan TS[16] *et al* in China (15% and 8.2%), Dicker F[17] *et al* in Germany (13.5%) and Qadir H[18] *et al* in Pakistan (14%).

According to World health organization 2016 review Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in the Western world, but is significantly less frequent in Asia. The median age of diagnosis in the USA, Europe and Australia is 70 years of age. The present study revealed a patient age range of 35–75 years, with a mean age of 55 years. The study conducted at Agha Khan University Karachi Pakistan by Qadir H [18] et al has reported median age of 56 years similar to our findings. Numerous studies conducted around the world regarding chronic lymphocytic leukemia (CLL). According to studies conducted internationally including India[19], Finland[13,14], China[15,16], Taiwan[12] and Germany[17] median age of diagnosis of chronic lymphocytic patients was 61 years, 65years, 64 years, 62 years and 63 years respectively. Studies conducted locally at Armed Forces Institute of Pathology[11] showed median age at the time of diagnosis of chronic lymphocytic leukemia 64 years respectively.

The present study revealed a male: female ratio of chronic lymphocytic leukemia (CLL) approximately 2.6:1 (72.61% vs 27.38%). Other studies revealed male to female ratio as follows: Agrawal N[19] *et al* (3:1), Takahashi K[14] *et al* (2.2:1), Chan JS[16] et al (2.6:1) and Mehmood R[11] et al (3:1). These studies reported male to female ratio close to our study, showing male predominance.

While there are some differences when our findings are compared to those from other global regions, our findings generally concur with those of other local studies. Geographical, racial, and/or ethnic differences in disease biology as well as genetic factors may be responsible for these variations. These variations may be caused by Pakistan's status as a developing nation, which could restrict patients' access to tertiary care centers and make it more likely that their illnesses will not be discovered until they are quite far along.

Conclusion

In conclusion, the frequency of TP53 gene deletion was relatively high among patients with CLL who visited our center in Pakistan. TP53 gene deletion in chronic lymphocytic leukemia is the worst prognostic marker and patients with TP53 gene deletion do not respond to the standard chemotherapy of CLL. The data was collected before initiation of treatment for CLL as it could alter the

treatment regime of the patients. Most of the patients had high total leukocyte count (TLC) along with high absolute lymphocyte count (ALC). The incidence of TP53 gene deletion (TP53 mutation) is higher in Pakistan as compared to other Asian countries.

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