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Frequency of Deletion 13q14.3 and Its Impact on Outcome in Patients of Chronic Lymphocytic Leukemia; A Single-Centered Institutional Study from Pakistan

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Abstract

Objectives: To evaluate the frequency and prognostic significance of this mutation in the Pakistani population.
Methodology: A retrospective study was conducted at Aga Khan University Hospital, Karachi, Pakistan, from January 2015 to December 2022. A total of 150 patients of all ages, diagnosed with chronic lymphocytic leukemia (CLL) according to the National Cancer Institute Working Guidelines for CLL (lymphocytosis >5 x 10^9/L, CD19+, CD5+, CD23+, CD20 weakly positive, and expression of either kappa or lambda light chains), who received treatment or follow-up at Aga Khan University Hospital, were included in the study. Hematological parameters and FISH study data were obtained from the hospital's electronic records. Progression-free survival (PFS) and overall survival (OS) were determined using Kaplan-Meier analysis, and the effects of mutations detected via FISH on prognosis and outcomes in CLL patients were evaluated.
Results: The most common mutations in the patient sample were deletion 13q14.3 (27%, n=28 [out of 150]), deletion 11q22 (13%, n=10), trisomy 12 (7%, n=6), and TP53 mutations (6%, n=4). In total, 47% (n=99) of patients had no detectable mutations on FISH. Patients with a deletion 13q14.3 mutation had a higher mean progression-free survival (128.9 months, 95% CI: 114.4–143.5) compared to the overall patient sample (68.1 months, 95% CI: 31.5–68.4), as well as a longer mean overall survival (127.0 months, 95% CI: 112–141) compared to the overall patient sample (67.0 months, 95% CI: 44.9–73.6).
Conclusion: Our study demonstrates that deletion 13q14.3 is the most common mutation in Pakistani CLL patients and is associated with a better prognosis in this population.
Keywords: Chronic lymphocytic leukemia, deletion 13q14.3, prognosis.

Introduction

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder characterized by the progressive accumulation of dysfunctional monoclonal B-lymphocytes. It is the most common adult leukemia in the Western world.[1–3] The accumulation of CD5-positive monoclonal lymphoid cells in bone marrow, spleen, and lymph nodes is a characteristic feature of chronic lymphocytic leukemia.[4] Males are more frequently affected than females, and individuals between sixty-five to seventy-two years of age are more affected.[5] The presentation of chronic lymphocytic leukemia varies greatly ranging from indolent to aggressive disease.[1] Most individuals have no symptoms, while others may have painless widespread lymphadenopathy or, less frequently, present with various infections. In certain cases, CLL may transform into Hodgkin's lymphoma or Richter's syndrome, which are more aggressive hematological malignancies.[1–3]

CLL is initially diagnosed using a complete blood count with differential, a peripheral blood smear, and immunophenotyping.[6] When both of the following criteria are present, CLL can be diagnosed using the 2018 International Workshop on CLL (iwCLL) update of the National Cancer Institute guidelines, which includes. [4,7]; 1) absolute B lymphocyte count in the peripheral blood 5 x 10^9/L, sustained for at least three months, with a predominant population of morphologically mature-appearing small lymphocytes. 2) flow cytometry of the peripheral blood demonstrating immunoglobulin light chain restriction (kappa or lambda) and, 3) the following pattern of markers: expression of B cell-associated

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antigens (CD19, CD20, and CD23); and expression of CD5.

Rai and Binet are two staging systems that categorize patients of chronic lymphocytic leukemia based on physical examination and peripheral blood counts into three risk groups (low, intermediate, and high). These traditional staging systems identify the overall survival and people in the early stages of the disease who would require therapy. Yet, modern biological approaches are already replacing them and aiding in the establishment of a better prognosis and therapy. Prognostic serum indicators such as the soluble cluster of differentiation (CD) 23, cell surface proteins such as CD38, cytoplasmic proteins such as zeta chain associated protein kinase 70, and other cytogenetic investigations are among the novel biological approaches.[8,9]

Genomic abnormalities as detected by fluorescence in situ hybridization (FISH) are present in most CLL cases at diagnosis, and additional abnormalities are acquired with disease evolution. In the pre-treatment evaluation of patients with CLL, we routinely performed FISH of the peripheral blood for the four common chromosomal abnormalities that can be detected in approximately 80 percent of CLL tumors.[10] This includes deletion (13q14.3), trisomy 12, deletion Tp53, and deletion (11q22). The most prevalent chromosomal aberration associated with CLL, by FISH, is the deletion of 13q14.3, which accounts for nearly fifty to sixty percent of all cases. It has a generally favorable prognosis; however certain types have resulted in adverse consequences. The magnitude of the deletions, whether large or small, monoallelic or biallelic, and deletion of 13q14.3 in the telomeric or centromeric region all have prognostic significance. This distinction is critical in deciding clinical outcomes for each patient.[5,11] However, there is a lack of literature when it comes to the outcomes of CLL in the Pakistani population, and in particular, the impact that the aforementioned mutations may have on the prognosis in this population, given the unique local mix of factors. Moreover, the prevalent mutations in CLL patients in the Pakistani population have not been assessed before. Therefore, this study aimed to evaluate the frequency and prognostic significance of deletion 13q14.3 in terms of survival in patients with chronic lymphocytic leukemia.

Methodology

A retrospective cohort study was conducted at Aga Khan University Hospital, Karachi, Pakistan, involving patients from January 2015 to December 2022. A total of 150 patients of all ages, diagnosed with chronic lymphocytic leukemia (CLL) according to the National Cancer Institute Working Guidelines for CLL (lymphocytosis >5 x 10^9/L, CD19+, CD5+, CD23+, CD20 weakly positive, and expression of either kappa or lambda light chains), who received treatment or follow-up at Aga Khan University Hospital, were included in the study. Patients with discordant histology or transformed lymphoma were excluded.

Complete medical history and clinical data were obtained from electronic medical records. The study received approval from the Ethical Review Committee (ERC) of Aga Khan University (ERC #:2023-8710-24917) and the College of Physicians and Surgeons of Pakistan (CPSP). Patient confidentiality was maintained throughout the data collection process. Information on demographics, CLL staging, hematological parameters, mutation analysis by FISH, and clinical outcomes related to deletion 13q14.3 were recorded using a standardized proforma.

The key variables evaluated included hemoglobin, total leucocyte count (TLC), absolute lymphocyte count (ALC), platelet count, direct Coombs test, reticulocyte count, uric acid, lactate dehydrogenase (LDH), beta-2 microglobulin levels, FISH mutation analysis for CLL (including deletion 13q14.3, deletion 11q22, deletion TP53, and trisomy 12), Eastern Cooperative Oncology Group (ECOG) performance status, comorbid conditions, and staging according to the RAI and BINET systems.

For the evaluation of deletion 13q14.3 by FISH, the LSI D13S25 (13q14.3) FISH probe (Abbott, USA) was used to hybridize the patient's interphase nuclei. This probe has 100% specificity and 100% sensitivity, with a calculated cutoff value for the 2G1R signal pattern of 3%.

Progression-free survival (PFS) was defined as the duration of time during which the patient remained alive without disease progression or relapse after remission. Overall survival (OS) was defined as the time (in months) from diagnosis to the date of death or last follow-up.

Data were analyzed using SPSS version 20. Continuous variables were assessed using independent sample t-

tests, while categorical data were analyzed using Fisher's exact test. Kaplan-Meier survival curves were generated to illustrate patients' PFS and OS. The log-rank test was used to compare median survival times. Survival rates with corresponding 95% confidence intervals (CIs) were reported. The Cox regression model was applied to adjust P-values and hazard ratios (HRs) for significant factors, including demographic variables, baseline hematological and clinical parameters, and specific FISH-detected mutations. A significance level of $P \leq 0.05$ was used for all analyses.

Results

Our study included 150 patients diagnosed with chronic lymphocytic leukemia (CLL), with an average age of 66 years (SD±12 years). Among these patients, 48 (32%) were female and 102 (68%) were male. All patients had comorbidities, with the most common being diabetes (37%, n=56), ischemic heart disease (IHD) (15%, n=22), and hypertension (49%, n=73).

Baseline hematological parameters are summarized in Table I. B symptoms were present in 54(35%) of patients. The majority of patients had an Eastern Cooperative Oncology Group (ECOG) performance status of either 3 (45%, n=67) or 2 (43%, n=65), with fewer patients having a status of 1 (2.6%, n=4) or 4 (9.3%, n=14). According to the Binet staging system, patients were nearly evenly distributed between stage A (36%, n=54) and stage B (37%, n=57), with a smaller proportion in stage C (26%, n=41). In terms of RAI staging, most patients were classified as stage 2 (38%, n=58) or stage 1 (23%, n=36).

Deletion 13q14.3 was the most frequently detected mutation by fluorescence in situ hybridization (FISH), occurring in 27% (n=28) of patients. This was followed by deletion 11q22, found in 13% (n=10) of patients. Trisomy 12 and TP53 mutations were the least common, detected in 7% (n=6) and 6% (n=4) of patients, respectively. (Table II)

Using a Kaplan-Meier survival analysis, we were able to estimate the mean progression free survival for our patient sample to be 68.1 months (95% CI: 31.5-68.4), while the median overall survival was estimated to be 67.0 months (95% CI: 44.9-73.6). (figure 1 a & b)

Table I: Patient demographics.

Variables	Mean ± SD
Age	65.5 ± 12.3
Gender	**N (%)**
Male	102(68%)
Female	48(32%)
B symptoms	54 (35%)
ECOG	
1	4(2.6%)
2	65(43%)
3	67(45%)
4	14(9.3%)
Binet staging	
A	54(36%)
B	57(37%)
C	41(26%)
RAI Staging	
0	10(0.06%)
1	36(23%)
2	58(38%)
3	28(18%)
4	10(0.06%)
deletion 11q22	10 (13%)
deletion 13q 14.3	28 (27%)
Tp53 mutation	4 (6%)
Trisomy 12	6 (7%)
No mutation	99(47%)
Haemoglobin (mg/dL)	11.1 ± 2.0
Absolute lymphocyte count (/µl)	778 ± 297
Reticulocyte count (%)	2.3 ± 3.1
LDH (IU/L)	486 ± 339
Beta-2 macroglobulin levels (mg/dl)	405 ± 249
Uric Acid level (mg/dl)	6.9 ± 3.8

Table II: Mutations in our sample. (total N=150)

Mutation	**N (%) [out of sample of 150]**
deletion 11q22	10 (13%)
deletion 13q 14.3	28 (27%)
Tp53 mutation	4 (6%)
Trisomy 12	6 (7%)
No mutation	99(47%)

For those with a deletion 13q14.3 mutation, the median PFS was 128.9 months (95% CI: 114.4-143.5), while the median OS was 127.0 months (95% CI: 112-141), both of which were significantly higher than the corresponding values for the overall patient sample. (figure 2 a & b).

Cox-regression revealed none of the moderating variables to have a significant effect on PFS, except for IHD, which conferred an increased hazard for progression (Hazard Ratio: 3.18, 95% CI: 1.05-9.61). As for the overall survival, higher uric acid levels at presentation were associated with an increased hazard of death (HR: 1.13, 95% CI: 1.02-1.25), while the presence of B symptoms at diagnosis was

associated with better OS (HR: 0.216, 95% CI: 0.087-0.535).

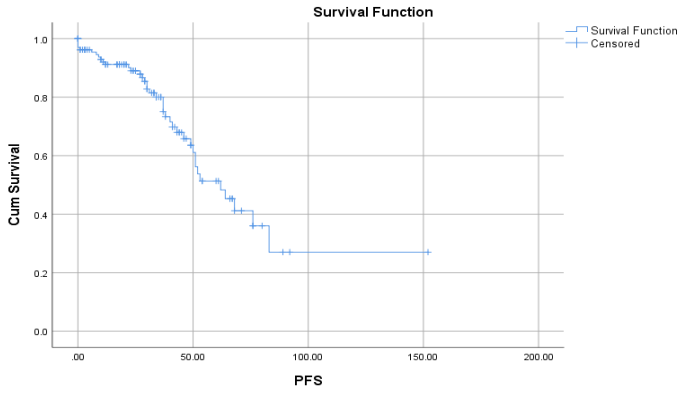

Figure 1 (a). Progression free survival in patients with CLL.

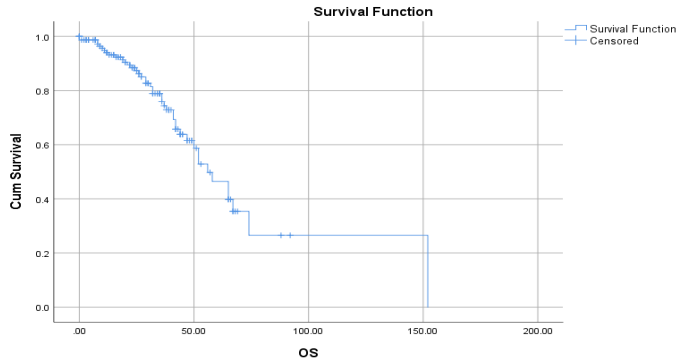

Figure 1 (b). Overall survival in patients with CLL.

Discussion

Despite previous studies having established favorable outcomes for CLL compared to other malignant hematological disorders[12], research concerning molecular genetics lags in lower-middle income countries[13], the impact of the 13q14 deletion on patient outcomes is therefore unassessed. Our study is the first from Pakistan to determine the frequency of the 13q14 deletion and measure its associated outcomes.

Our study comprising of 150 CLL patients from a large tertiary care center finds nearly half of patients to have no mutation, over a quarter with the 13q14.3 deletion and 13% with the 11q22 deletion. A study Pakistan on idiopathic acquired aplastic anemia patients found similar frequencies [11], suggesting a genetic existence within our population. The percentage however is lower compared to results outside Pakistan where more than 50% of patients of CLL have had the deletion.[14] The high prevalence of deletion 13q14.3 in comparison to others is present. The two least common mutations in the Pakistani population were deletion Tp53 and trisomy 12, are in line with other studies.[7] This corroborates the limited existing literature relevant to the Pakistani population, and helps clinicians be more cognizant of the factors and mutations influencing outcomes in Pakistan.

All participants had a diagnosed comorbid condition, most commonly hypertension, this is further confirmatory of studies from Pakistan showing high prevalence of NCDs, an important observation that could potentially affect outcomes in our population. [15] In addition, in the sample, a considerably high prevalence of B symptoms was also found, which are typically found in lymphoma patients [16]. This was comparable to a Pakistani study which has found a 51% occurrence in Non-Hodgkin Lymphoma patients.[17] Therefore, these results seem to suggest that clinicians should be aware of such symptoms as a common presenting complaint in the Pakistani population. However, further studies need to be conducted to ascertain the prognostic relevance of B symptoms in CLL patients. Additionally, with a mean of 0.30, ECOG. Our study found 14 individuals to be completely disabled and 67 to be capable of only limited self-care. Our results are concurrent with evidence [18] that CLL has a profound impact on the patient's life and further on the quality of life (QOL), regardless of disease stage. QOL studies have been conducted for CLL patients in high income countries [18,19] but such data is lacking in LMICs. Our study also finds a similar gap in literature. Therefore, in view of our results, further studies are needed to evaluate the impact on QOL, as unique socioeconomic factors in the Pakistani setting may serve to create QOL outcomes that differ significantly from those in other settings.

In our cohort, the estimated mean progression-free survival was 68.1 months. The deletion 13q14.3 generally has a good prognosis but some forms have led to unfavorable outcomes, but this is dependent on size, allelicity and region of deletion.[20] We have observed a significant variation in the overall and progression-free survival means of CLL patients who carry the deletion 13q14.3 mutation. Patients with CLL who have a mutation in deletion 13q14.3 have an overall survival rate of 127.0 months. According Nabhan et al, the median survival in CLL patients with deletion 13q14.3 was 133 months.[21] Although not significant in our results, there were other factors as well which can determine the overall

survival and these include, high serum lactate dehydrogenase and beta-2 microglobulin levels, extent of infiltration of bone marrow, and splenomegaly.[22]

Conclusion

Our study finds compelling evidence of the presence of deletion 13q14.3 in the Pakistani population with an overall survival of 127 months. We recommended that future studies assess the impact of mutation of quality of life and address more detailed genetic-level information to gain more clarity over the concept matter in the context of the Pakistani population, for whom there is a paucity of genetic research for CLL.

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