RESEARCH ARTICLE

ZAP-70 Protein Expression in B-cell Chronic Lymphoid Leukemia: a Single Center Experience from Pakistan

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Abstract

Background: Chronic lymphoid leukemia (CLL) is the most frequent type of adult leukemia. The Rai and Binet staging systems have been well recognized as standards for assessing the treatment requirements and overall survival in CLL patients. However, there is a need to seek newer prognostic markers to identify stable or progressive forms of CLL that will facilitate risk-adapted treatment strategies. Currently a molecular biomarker ZAP-70 has attracted interest as providing prognostic information in CLL patients. Objective: To determine the frequency of ZAP-70 positivity in B-CLL patients at disease presentation. Materials and Methods: From January 2011 to September 2014, 89 patients were diagnosed to have chronic lymphoid leukemia. Complete blood count was done on an automated analyzer (Cell Dyne, Abott Architect, USA), while immunophenotyping was conducted for each patient to establish the diagnosis of the disease. ZAP-70 expression was evaluated by flow cytometry. Data were compiled and analyzed by SPSS version 21. Results: Out of the total of 89 B-CLL patients, 62 (69.7%) were male and 27 (30.3%) were females with a male to female ratio of 2:1. The mean age was 57.5±12.1 years. The frequency of ZAP-70 positivity in our B-CLL patients was found to be 13.5%. ZAP-70 positivity was significantly correlated with stage III disease and high absolute lymphocytic count (P<0.05). No correlation of ZAP-70 could be established with age and gender (p>0.05). Conclusions: The frequency of ZAP-70 in our patients appears low. It is approximately half that in international data. We would recommend to screen all the newly diagnosed patients with CLL for ZAP-70 protein expression for risk stratification, family counseling and to predict overall survival.

Keywords: Chronic lymphoid leukemia - ZAP-70 - B- cell - Pakistan

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Introduction

Chronic lymphoid l eukemia (CLL) is the commonest adult leukemia in the Western world (Shanafelt, 2009; Kermani et al., 2007; Vroblova et al., 2009). CLL is characterized by proliferation, accumulation and sustained increase of morphologically mature but functionally ineffectual lymphocytes owing to defective apoptotic mechanism (Chiorazzi et al., 2005; D'Arena et al., 2007). CLL is a disease of older age, with a median age at diagnosis is 64-70 years, exhibiting strong familial predisposition (Marti et al., 2003).

Chronic lymphoid leukemia represents 22-30% of all leukemia cases with a worldwide incidence projected to be between < 1 and 5.5 per 100,000 people (Siegel et al., 2014). Australia, USA, Ireland and Italy reported to have the highest CLL incidence rates (Siegel et al., 2014). CLL is uncommon in Asian countries, such as Japan and China. It account for as few as 10 percent of all leukemia's in these regions (Siegel et al., 2014). CLL in our area is reported as least common hematological malignancy with a frequency of 0.9% (overall) and 9.7% amongst all the leukemias (Dodhy et al., 2011).

CLL has been considered as a homogeneous disease previously but current data is indicative of molecular and clinical heterogeneity (Chiorazzi et al., 2005; Hallek et al., 2008). The clinical course is very diverse, with some patient experiencing rapid disease evolution and many other living for a decade without therapeutics intervention (Sagatys and Zhang, 2012). Patients often present with localized or generalized lymphadenopathy, anemia, thrombocytopenia or constitutional symptoms (Abbott, 2004). The diagnosis of CLL does not entail the need for therapy. Different prognostic factors are important in decisions making regarding initiation of treatment, predicting the disease outcome and overall survival (Li et al., 2008).

Previously reported Rai and Binet staging system are still widely acceptable and applicable in daily practices (Vroblova et al., 2009; Mozaheb et al., 2012). But the routinely used staging system does not discriminate exactly the probable course of disease at the time of

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presentation (Vroblova et al., 2009).

New prognostic markers including ZAP-70, molecular studies and mutational status are useful predicable indicators for disease course and overall survival (Sagatys and Zhang, 2012). ZAP-70 (Zeta-chain associated protein kinase 70) is a protein normally expressed by T lymphocytes and natural killer cells. It is a part of the T cell receptor and plays a critical role in normal T-cell signaling pathway (Wang et al., 2012).

ZAP-70 protein expression on clonal B lymphocytes appears as an aberrant expression, as it is rarely expressed by normal B lymphocytes (Chiorazzi et al., 2005). It is an established prognostic tool in identifying and broadly segregate B-CLL patients into two groups (Wang et al., 2012). Beyond its widely accepted implication for determining the disease biology, its utility in counseling and management is the foremost important application (Shanafelt, 2009).

ZAP-70 expression is the predictor for the need of treatment initiation. Positive patients have poor prognosis with shorter progression free survival, decreased overall survival and aggressive disease course (D'Arena et al., 2007). Whereas patients with ZAP-70 negativity have shown good prognosis, prolonged treatment free survival and most important overall survival is lengthened (Shanafelt, 2009). ZAP-70 negative patients demonstrate slowly progressing disease pace, can be reassured and may not need any treatment in their lifetimes (Shanafelt, 2009).

The rational of our study is to evaluate the prevalence of ZAP-70 positivity in Pakistani B-cell Chronic lymphoid leukemia patients and to determine its correlation with age, gender, stage of disease and hematological parameters.

Materials and Methods

This prospective cross sectional study, extended from January 2011 to September 2014. 89 patients with established chronic lymphoid leukemia were enrolled in the study. An informed consent was obtained from all participating patients.

Patients with other lymphoid neoplasm (both B and T-cell lineages) were excluded. Patients with another associated malignancy or having relapsed/ refractory CLL were also excluded.

Demographic data including age, gender and medical history were recorded. Hematological parameter included hemoglobin, WBC counts, absolute lymphocytic count and platelets were determined by Cell Dyne Ruby (Abott Architect, USA). Immunophenotyping was done by flow cytometry or immunohistochemistry to diagnose chronic lymphoid leukemia (CD19, CD20, CD5 and CD23 positivity and FMC7, cyclin D1 and BCL2 to be negative for diagnosis of B-CLL). ZAP-70 analysis was done by flow cytometry technique, result reported as positive or negative. Ethical approval was given by ethical and research committee of Liaquat National Hospital, obtained prior to the study.

Data analysis

Data was compiled and analyzed using SPSS version 21. The results were expressed as mean±SD for quantitative variables and qualitative variables are presented as frequency and percentages. Student't' test was applied for the comparison of mean. Data were considered statistically significant at P value < 0.05. Chi-square test was applied to assess the correlation.

Results

A total of 89 confirmed chronic lymphoid leukemia patients using the non probability consecutive sampling were included in this study.

Out of total 89 patients, 62(69.7%) were male and 27(30.3%) were females with male to female ratio of 2:1. The mean age was 57.5±12.1 (range 35-80) years. The mean hemoglobin, white cell count and platelets were 10.3±2.55g/dl (range 2.7-15.20); 98.2±73.6 $x10^9$/L (range 16-389) and 200.1 ± 91.1 $x10^9$/L (range 71-560) respectively. The mean absolute lymphocytic count was 91.88±69.43 $x10^9$/L (range 14-380).

According to Rai staging, 7 patients (7.9%) were in stage 0, 16 patients (18%) had stage 1, 22 patients (24.7%) were in stage II and 30 patients (33.7%) in stage III, while 14 patients (15.7%) were in stage 1V of disease.

ZAP-70 was found to be positive in 12 (13.5%) patients, while 77 (86.5%) were negative. The comparative analyses of ZAP-70 positive and negative patients are shown in table-1.

Patients were stratified on the basis of gender, age and absolute lymphocytic count to see the effect and association of these modifiers with ZAP-70 positivity.

Based on gender, 8(12.9%) male patients were found to be positive for ZAP-70 while remaining 54(87.1%) were negative. In females, 4(14.8%) patients were positive for ZAP-70 while 23(85.2%) patients were found to be negative. There was no significant association between ZAP-70 and gender (p>0.05).

Patients were stratified into two groups according to age. Group 1 with age <60 years and group 2 with age ≥60 years. In group 1, 7(15.9%) were positive while remaining patients 37(84.1%) were negative for ZAP-70. In group 2, 5(11%) patients were positive for ZAP-70 while 40(89%)

Table 1. Comparative Analysis of ZAP-70 Positive and Negative Patients

Parameters	ZAP-70 positive n=12	ZAP-70 negative n=77	P-value
Age	51.67±10.34	58.36±12.18	0.05
Hemoglobin	10.19±1.99	10.30±2.63	0.7
Total leukocytic count	86.16±59.42	100.04±75.68	0.7
Platelets	207.66±102.53	197.62±90.02	1
Absolute lymphocytic count	96.92±73.24	59.58±13.64	0.01*

patients were found to be negative. No correlation could be established between ZAP-70 positivity and age (p>0.05).

However comparative analysis revealed statistically significantly high absolute lymphocytic count of 96.92±73.24 in ZAP-70 positive patients group as compared with negative group that was 59.58±13.64 (p=0.01). Another important pertinent finding was positive correlation of ZAP-70 protein expression with advanced clinical disease. Strong positive correlation was detected with Rai stage III of disease and ZAP-70 protein expression (p=0.004).

Discussion

Chronic lymphoid leukemia is a heterogeneous hematopoietic malignancy with highly variable disease course (Dohner et al., 1995; Hallek et al., 1999; Seiffert et al., 2010; Baliakas et al., 2013). Many patients do not need treatment, whereas some requires intensive treatment early after diagnosis or with disease progression. Thus, it is important to develop sensitive stratification markers to identify patients with inert versus progressive disease (Hamblin et al., 1999). Historically prognosis of patients with CLL has been based solely on clinical features.

In the last two decades a number of biological prognostic parameters have been identified. Amongst them, the expressions of ZAP-70 in neoplastic B lymphocytes are increasingly being recognized as of paramount importance to identify attenuated versus progressive CLL, with the potential to facilitate for risk adapted treatment. ZAP-70 protein expressions have been identified as a robust predictor of disease progression and poor overall survival in B-CLL (Rassenti et al., 2004; Crespo et al., 2003).

The present study has shown ZAP-70 positivity in 13.5% of Pakistani B-CLL patients. To the best of our knowledge, this is the first report from our part of the world. Previously published studies from Pakistan on CLL addressed the epidemiological, clinical and hematological markers but did not look into ZAP-70 protein expression and its possible correlations (Junaid et al., 2011; Khan et al., 2014; Rafiq et al., 2014).

ZAP-70 positivity in CLL has been observed from various racial backgrounds ranging from 25% to 57% (Gogia et al., 2013). When compared with earlier reports, our results are in consensus with regional study from India, revealing 25% positivity for ZAP-70 protein expression (Gogia et al., 2013). In parallel to our findings, their study also demonstrated no correlation of ZAP-70 with maternal characteristics including age and gender (Gogia et al., 2013).

However the prevalence was quite high in Egyptian study that disclosed 47.4% ZAP-70 positivity rate in CLL patients (el-Sharnouby et al., 2006). The study disclosed a strong association of ZAP-70 positivity with high lymphocytic count and advanced disease stage which is in concurrence to our results (el-Sharnouby et al., 2006).

Furthermore study reported from Spain also revealed ZAP-70 positivity in 37% of patient (Moreno and Montserrat, 2008). The author emphasized that ZAP-70 predicts treatment free survival, progression-free survival and most importantly overall survival (Moreno and Montserrat, 2008). These considerations suggest that ZAP-70 expression in CLL may reflect an activation state of the malignant clone that is associated with progressive disease. Thus, a laboratory test for ZAP-70 expression could be an important adjunct in the patients overall management. The author also correlated ZAP-70 expression with mutation status and concluded that patients who coexpress unmutated status and ZAP-70 would have the poorest prognosis, in comparison with those having both markers negativity (Moreno and Montserrat, 2008).

Gribben JG from Rome had reported no statistical correlation between gender distribution and ZAP-70 positivity similar to our findings, however slightly high prevalence (36%) was determined in their study (Gribben, 2008).

Other studies from Italy and China by D'Arena, et al and Qi RJ et al have reported the frequency of ZAP-70 positivity in 36% and 40.4% patients respectively (D'Arena et al., 2007; Qi et al., 2009). In accordance to our findings, they also reported significant relation between advanced clinical stages with ZAP-70 positive expression (D'Arena et al., 2007).

The dissimilarity in frequency of ZAP-70 between us and west could be attributed to difference in genetics makeup, ethnic origin and geographical distribution between different populations. Consequently the frequency of ZAP-70 is around half as compared to other studies. Therefore, we may conclude that majority of our patient not necessitate therapy, with probably prolonged overall survival. Furthermore, the expression of ZAP-70 appears to be constant over time, it should be used at the time of diagnosis to identify patients who are at increased risk for early disease progression (Rassenti et al., 2004).

We would like to mention limitations of our study as well. Firstly the sample size in our study is small. A large sample would be a better indicator of ZAP-70 prevalence in our population. Secondly we did not perform the test in our institution. We sent our samples in different laboratories. It is possible that they lacked interlaboratory standardization and uniformity of reporting leading to unusually low ZAP-70 positivity in our study. Despite the limitations mentioned above, strength of this study is the fact that this is first local study reported from our part of world. Thus it provides essential local informative data for prognostic stratification in our setup.

In conclusion, ZAP-70 is an established poor prognostic marker in B-CLL patients. Providentially ZAP-70 prevalence in our patients is much less as compared with regional and international studies. We would recommended to screen all the newly diagnosed patients with CLL for ZAP-70 status for risk stratification, family counseling and to predict the overall survival. Future studies should incorporate immunoglobulin status along with ZAP-70 for better and more meaningful stratification in these patients.

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