Frequency of Zap-70 and CD38 in Newly Diagnosed Cases of B-Cell Chronic Lymphocytic Leukemia

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Abstract: Introduction: Amongst all chronic lymphoproliferative disorders B-CLL is the most common. Clinical behavior of CLL is very variable and in order to identify the clinical spectrum there is a need for risk adaptive prognostic markers which will further facilitate in management strategy. Currently available molecular biomarkers ZAP-70 & CD-38 have gain much interest in providing useful prognostic information in patients diagnosed as B-CLL.

Objective: To assess ZAP-70 and CD 38 positivity in patients diagnosed with B-CLL.

Material & Methods: This was a cross sectional study conducted between Jan 2014 to July 2018 at NIBD, Karachi. Patients with B-CLL meeting the inclusion criteria were enrolled in the study. All patients gave informed consent. Immunophenotyping data analysis was done on FASC Calibur flowcytometry and Cell Quest software. We defined ZAP-70 and CD38 positivity as the presence of this marker on at least 20% and 30% of the B-CLL cells respectively. All required information was collected on pre designed Performa.

Results: Total of 101 patients diagnosed as B-Cell CLL were included in the study. Median age of enrolled patients was 64 years (range 43-85 years). Majority of the patients i.e. 69 out of 101 (68.32%) were males and the rest 32 (31.68%) were female. ZAP-70 was positive in 2.97% of patients (03/101), while CD 38 positivity was detected in 33.66% (34/101).

Conclusion: ZAP-70 & CD-38 positivity was present in 2.97% & 33.66% of newly diagnosed B-CLL patients respectively. Screening for these biological markers in all newly diagnosed CLL patients will result in more dependable identification of disease clinical behavior.

Keywords: ZAP-70, CD-38, Chronic lymphocytic leukemia, Flowcytometry, Immunophenotyping, Prognosis.

INTRODUCTION

B Cell-Chronic Lymphocytic Leukemia (B-CLL), accounts for the most common chronic leukemia of adult in the western [1, 2]. However, in Asian countries it is uncommon and accounts for less than 10% cases [3]. Similarly, in Pakistan it has frequency of 9.7% amongst all the leukemia [4]. It is usually predominates in elderly with median age of onset of 65 years [5], however 10% of CLL patients are usually less than 55 years of age. The phenotypic presentation of this disease is extremely heterogeneous ranging from indolent disease to rapidly progressive requiring immediate treatment. Majority of patients have stage 0 or 1 at presentation (Rai/Binet staging system). New biological prognostic markers such as ZAP-70 & CD-38 are independent predicable indicators of disease behavior [6]. ZAP-70 protein (defined as positive on at least 20% of B- cells), is actually a Zeta chain associated protein kinase. It has normal expression in T lymphocytes and natural killer cells, whereas on clonal B lymphocytes it appears as an aberrant marker [7, 8]. Patients with ZAP-70 positivity at diagnosis have poor prognosis as it is associated with aggressive nature of disease requiring early management with chemotherapy, short progression free survival and decreased overall survival [9, 10].

CD-38 is a type II trans-membrane glycoprotein that functions as a receptor that induces proliferation & increases survival of CLL cells [11]. CD-38 positivity (defined as its presence on at least 30% of CLL cells) is an independent negative prognostic marker for clinical behavior in CLL [12, 13]. These patients have more aggressive disease and also show diminished response to chemotherapy [11]. In a study conducted by Gogia A, *et al*. ZAP-70 & CD-38 positivity was detected in 25 & 36% of Indian B cell CLL patients respectively [14]. On the other hand study conducted by Rozina *et al*. showed 13.5% ZAP-70 positive cases in Pakistani B-CLL patients [9].

Our study was designed to see the frequency of both CD-38 & ZAP-70 positivity in our population, which would be helpful for risk stratification, patient counselling and compare it with local and international data.

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MATERIAL & METHODS

Patients with B-Cell CLL meeting the inclusion criteria (All cases of B-Cell Chronic Lymphocytic Leukemia diagnosed on Flow cytometry at NIBD & BMT, Both inpatient & outpatient cases, Age > 40 years & < 90 years, both gender) were enrolled in the study. An informed written consent was taken from all patients enrolled in study after explaining the purpose and procedure of the study. 3cc of peripheral blood was collected in EDTA tube. Whole blood was incubated at room temperature for 15 minutes with flourochrome conjugated antibodies including CD 5, CD 19, CD 20, CD 23, CD 79a, CD 11c, CD 25, FMC 7, CD 10, CD 3, CD 4, CD 8, CD 38, anti kappa light chain, anti lambda light chain & ZAP 70. Using RBC lysing solution, RBCs were lysed. Leukocytes were washed with PBS solution twice. Tubes were then analyzed using BD FACS Caliber. Cell Quest Pro Software was used to acquire 10,000 events from each tube. Signals from each flour chrome were digitalized & plotted on histograms of forward & side scatter. Gate was applied at lymphocyte population & analyzed for the presence of antibodies. All information was collected on predesigned Performa. Data was analyzed on statistically software SPSS version 17. Frequency & percentage were computed for categorical data like CD-38 & ZAP-70.

RESULTS

A total of 101 patients with B-Cell CLL were enrolled in the study. The median age of study population was 64 years (range 43-85 years). Majority of patients i.e., 69 out of 101 (68.32%) were males (Fig. **1**).
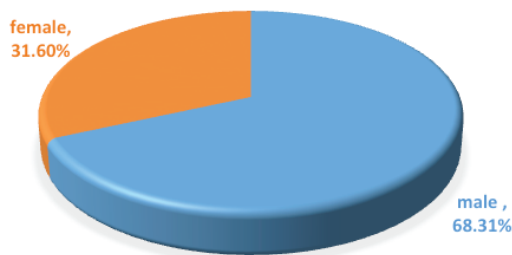

Fig. (1). Gender Distribution.

The frequency distribution of ZAP-70 and CD-38 positivity is presented in Table **1**.

Table 1. Frequency Distribution of Zap-70 and CD-38 Positivity.

ZAP-70 POSITIVTY	FREQUENCY (n)	PERCENTAGE (%)
ABSENT	98	97.03%
PRESENT	03	2.97%
CD 38 POSITIVTY	**FREQUENCY (n)**	**PERCENTAGE (%)**
ABSENT	67	66.34%
PRESENT	34	33.66%
TOTAL	101	100%

Only 03 (2.97%) out of 101 patients were ZAP-70 positive. While CD-38 positivity was seen in 34 patients (33.66%). Majority of the CD 38 positivity was seen in stage 3 and stage 4 disease i.e. 61.7% and 29.4% respectively (Table **2**).

Table 2. Frequency of CD-38 According to the RAI Staging System of Disease.

Stage	CD-38 Positive	CD-38 Negative
Stage II	03	34
Stage III	21	18
Stage IV	10	15
Total	34	67

Flow cytometric analysis showing positive expression of CD-38 and ZAP-70 on B-cell CLL markers CD5, CD19 (Fig. **2**).
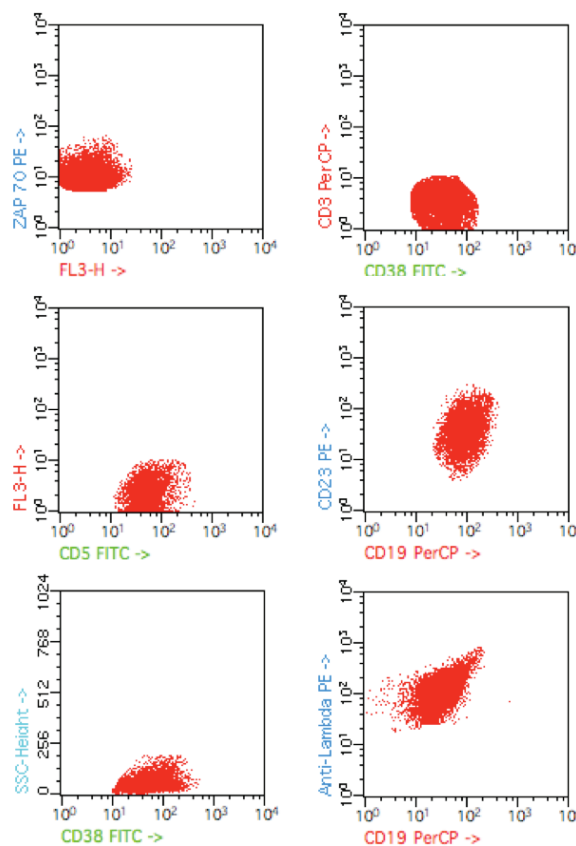

Fig. (2). Flow Cytometric Analysis Showing Positive Expression of CD-38 and ZAP-70 on B-Cell CLL Markers CD5, CD19.

DISCUSSION

A number of significantly new biological markers have been discern in B-Cell Chronic Lymphocytic Leukemia in the last two decades. Among them CD-38 and ZAP-70 expression in neoplastic B lymphocyte have independent predictable

prognostic significance due their association with aggressive nature of disease, poor response to conventional chemotherapy, short progression free survival and decreased overall survival. ZAP-70 positivity in CLL has been reported from 36 to 57% in various studies. A Study conducted by Zeeshan *et al.* has showed Pakistani population to have 13.5% ZAP-70 positive CLL cases, whereas our study ZAP-70 positivity was found in only 2.97% of B-CLL cases. Study conducted by Gogia A, *et al.* from India revealed ZAP-70 positivity in 25% patients [14-17]. Another study from China by Qi RJ, *et al.* had reported the frequency of ZAP-70 to be 40.4% in all B-CLL cases. Screening for ZAP-70 at the time of diagnosis can be beneficial at the early identification of that subgroup of CLL patients who will be the candidate for early intensive management because of the aggressive nature of their disease.

In our study, CD-38 positivity was seen in 34 patients (33.66%) as compared to various studies that have reported CD-38 positivity in CLL ranging from 29% to 60%. Gogia A, et al. reported it to be 29% [14-17]. While Nihal *et al.* from Egypt reported it in 42.5% patients [18-20]. Majority of our patients with CD38 positivity had Rai stage 3 and 4 at the time of diagnosis.

Various studies have been published from our part of the world about the epidemiology, clinical and hematological markers and staging in CLL patients but even after the extensive search of literature we found that no study has been conducted so far in Pakistan that addresses the frequency of both CD-38 & ZAP-70 simultaneously. Limitation of our study include small sample size. A larger group of cohort would be a better representative of ZAP-70 & CD-38 prevalence in our population.

CONCLUSION

In our study, we concluded that ZAP-70 & CD-38 positivity was present in 2.97% & 33.66% of newly diagnosed B-CLL patients respectively. Moreover, CD-38 positivity was seen in more frequently in stage 3 and stage 4 of the disease at the time of diagnosis. So early screening for both CD38 and ZAP-70 in all newly diagnosed B-Cell CLL patients are surrogate markers of disease clinical spectrum which can guide about risk stratification, treatment outcome and overall survival.

CONFLICT OF INTEREST

Declared none.

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