CLINICO-PATHOLOGICAL FEATURES IN PATIENTS OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH DEL 11q22

Saqib Hussain Korejo, Chaudhary Altaf, Hamid Saeed Malik, Rafia Mahmood, Ayesha Khurshid, Ali Ahmed

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To determine the frequency of DEL 11q22 in patients of Chronic Lymphocytic Leukemia and clinico-pathological features in Chronic Lymphocytic Leukemia with DEL 11q22.

Study Design: Cross sectional analytical study.

Place and Duration of Study: Department of Hematology, Armed Forces Institute of Pathology Rawalpindi, from Apr 2017 to Jul 2018.

Methodology: A total of 61 newly diagnosed cases of Chronic Lymphocytic Leukemia were included. Diagnosis was made according to National Cancer Institute Working Group guidelines for diagnosis of Chronic Lymphocytic Leukemia. After relevant history and clinical examination; complete blood counts, biochemical profile, bone marrow examination, immunophenotyping on bone marrow or peripheral blood samples and Interphase FISH studies on blood or bone marrow specimens for detection of DEL 11q22 were carried out.

Results: Deletion 11q22 was detected in 7 (11.4%) of Chronic Lymphocytic Leukemia patients. Median white cell count was 152 x 10^9/L (74-156) and absolute lymphocyte count was 105.5 x 10^9/L (74-139) which was higher than patients of Chronic Lymphocytic Leukemia without DEL 11q22. According to Rai staging, 3 (42.9%) patients presented in advanced stage Rai III and 2 (28.6%) patients were in Rai I & Rai II. All patients of Chronic Lymphocytic Leukemia with DEL 11q22 presented with B-symptoms and lymphadenopathy. Bulky disease was found in 2(28.5%) of patients. CD38 expression was in 3 (42.8%) cases.

Conclusion: Frequency of DEL 11q22 in Chronic Lymphocytic Leukemia in our population was found to be 11.4%. All patients of Chronic Lymphocytic Leukemia with DEL 11q22 presented with B-Symptoms and lymphadenopathy.

Keywords: Chronic lymphocytic leukemia, DEL 11q22, Fluorescence in situ hybridization (FISH).

INTRODUCTION

Chronic Lymphocytic Leukemia (CLL) is the most common lymphoproliferative disorder and constitutes around 30% of adult leukemia's. In Western world, CLL is the most common form of leukemia[1,2]. It is characterized by proliferation and bone marrow infiltration by mature clonal small lymphocytes. Lymphocytic infiltration is also seen in lymphoid tissue[3]. It is a disease of elderly with a median age at diagnosis of 72 years[4]. Men are more commonly affected than women. Many of the patients are asymptomatic and diagnosed incidentally on routine blood counts. Most of them are observed without treatment until they develop some symptoms or there is any evidence of disease progression. Around 80% of the patients present with lymphadenopathy while splenomegaly occurs in 50% at the time of diagnosis[5]. Patients may present with B symptoms such as fever, fatigue, night sweats and weight loss or symptoms of anemia and infection[6]. According to National Cancer Institute Working Guidelines, diagnosis of CLL requires absolute clonal lymphocytosis of >5 x 10^9/L with a characteristic immunopheno-type CD19+, CD5+ and CD23+. There is generally weak expression of CD20 and clonal lymphocytes are Kappa or Lambda light chain restricted. This is the characteristic immunophenotype which is necessary for diagnosis of CLL[7].

Commonly two clinical staging systems are used for prognosis, Rai and Binet staging; both

Correspondence: Dr Saqib Hussain Korejo, Department of Haematology, AFIP Rawalpindi Pakistan
Email: saqibkorejo@hotmail.com
Received: 17 Sep 2018; revised received: 16 Jan 2019; accepted: 21 Jan 2019

are classified on the basis of absolute lymphocytosis, lymphadenopathy, organomegaly (splenomegaly and hepatomegaly) and the presence of anemia or thrombocytopenia[8]. Cytogenetic chromosomal abnormalities are considered as important prognostic markers with specific prognostic significance[9].

Conventional cytogenetics is utilized to detect 40-50% of cytogenetic abnormalities in CLL. However, use of fluorescent in situ hybridization (FISH) can increase the yield upto 80%. The common cytogenetic abnormalities include deletion 13q, 11q, 17p and trisomy. These all have specific prognostic significance[9].

Prevalence of DEL 11q22 in various studies has been estimated between 10% to 20% and it is with mutation in ATM gene. ATM protein has specific role in regulation of DNA damage response pathway; for that reason, it is noteworthy that DEL 11q22 has been related with resistance to DNA destructive chemotherapy[10]. Patients with DEL 11q22 tends to be younger, have significant lymphadenopathy with rapid disease progression, and demonstrate somewhat shorter survival compared with those without 11q22 deletion. Patients with 11q22 have short time to treatment, short remission duration and poor survival.

As most of the data is from western literature, we planned this study with an objective to determine the frequency of 11q22 deletion in our population and to study the clinico-pathological features in our CLL patients with 11q22 deletion.

METHODOLOGY

This cross-sectional analytical study was conducted at Armed Forces Institute of Pathology from 20th April 2017 to 30th July 2018. All newly diagnosed cases of CLL were included in the study. Sample size was calculated by using WHO calculator. Patients were selected by purposive non-probability sampling. Diagnosis of CLL was done as per National Cancer Institute Working Guidelines for diagnosis (Lymphocytosis >5 x 10^9/L,CD19+, CD5+, CD23+, CD20 weak positive[11,12] and expression of either kappa or lambda). All previously treated patients were excluded from the study. Study was conducted after the approval of Institutional ethical review committee. Relevant history was recoreded and clinical examination was done, Complete blood counts, Bone marrow examination, Immunophenotyping from bone marrow or peripheral blood, FISH from blood or bone marrow samples and other relevant investigations were also carried out. Bulky disease at initial presentation was assessed by physical examination (PE) (defined as any node/nodal mass ≥5 cm and/or spleen ≥6 cm), CT scan and MRI criteria were not used because of unavailability to all patients.

Interphase FISH studies were performed on blood or bone marrow specimens processed by standard methods for cultured samples. Three ml sample was collected in sodium heparin tube.

It was cultured at 37°C without Phytohaemagglutin in for 24 hours and then after adding 200ul colchicine for 1 hr. It was then centrifuged at 1500 rpm for 8 min. Supernant was discarded and 10% KCL was added followed by centrifugation. Three washes were given with glacial acetic acid and methanol in 3:1. The clear pellet was placed on the slide. Slide was prepared for FISH by treatment with ascending concentration of alcohol followed by 10% saline sodium citrate buffer (SSC solution). The slide was air dried in a dark place. 10ul of Meta system XL probe specific for 11q22 was applied to the sample on the slide and Cover slip was applied and sealed with rubber cement. Denaturation at 74°C for 5 min, hybridization at 37°C for 18 hrs and co-denaturation at 74°C for 5 minutes were done. The slide was washed and air dried.

10 to 20ul of DAPI-2 counter stain was added and it was frozen at -20°C for 1 day. A total of 500 nuclei were analyzed per probe set using a fluorescent microscope with an orange green spectrum filter. Positive DEL 11q22 was denoted by one green signal and two orange signals. FISH analysis was shown in figure.

Collected data was entered and analyzed by SPSS version 20. Test of normality (kolmogrove and shipro test) to check whether data was parametric or non parametric was applied. Descriptive statistics (median IQR) was computed for quantitative non parametric variables. Frequency and percentages were computed for categorical variable (gender, cytogenetics etc) and association for categorical variables of CD38 and cytogenetics computed by chi square test. Test of significance applied to validate (Mann Whitney U-test) hypothesis. A *p*-value of ≤0.05 was considered as a significant value.

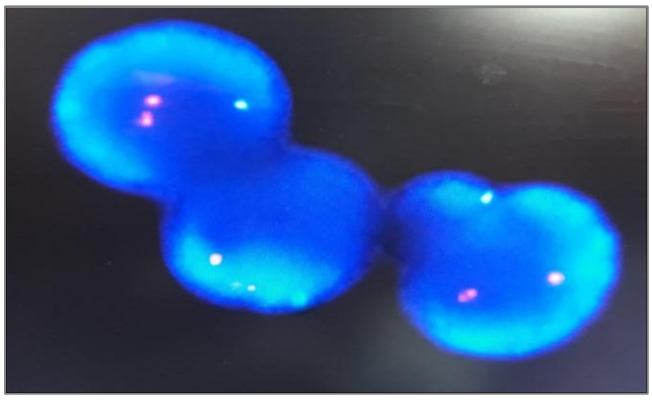

Figure: Positive Del 11q22 was denoted by one green signal and two orange signals.

RESULTS

A total of 61 patients were included in the study. Age of patients ranged between 39-87 years. Out of these, 48 (78.68%) were males and 13 (21.3%) were females with a male to female ratio of 3.7:1. DEL 11q22 was detected in 7 (11.4%) of CLL patients in our study. Clinical characteristics, blood counts, staging and prognostic markers were summarized in table-I.

All patients of CLL with DEL 11q22 presented with B-Symptoms and lymphadenopathy. No case was diagnosed incidentally on routine blood counts. Bulky disease was seen in 2 (28.5%) of patients. CD38 expression was positive in 3 (42.8%) cases. ZAP 70 was negative in all cases. When laboratory parameters between patients of CLL with and without 11q22 DEL were compared. There was significant difference among median values of hemoglobin concen-tration, WBC, ALC and Platelet count but these were not statistically significant. Clinico-pathological features with and without 11q22 DEL are CLL was summarized in table-II.

Table-I: Clinico-hematological characteristics of chronic lymphocytic leukemia patients (n=61).

Characteristics	n (%)
Clinical Features	
Symptoms	
Fever	24 (39.3)
Weight loss	5 (8.19)
Physical Findings	
Asymptomatic	21 (34.42)
Lymphadenopathy	46 (75.4)
Splenomegaly	23 (37.7)
Hepatomegaly	7 (11.4)
Median Blood Counts on Presentation	
WBCs count (x 10^9/L)	103 (179)
ALC (x 10^9/L)	93.04 (234)
Haemoglobin conc g/dl	11.01 (15.1)
Platelets (x 10^9/L)	120 (352)
Binet Stage	
A	32 (52.4)
B	3 (4.91)
C	26 (42.6)
Rai Staging	
Rai 0	14 (23)
Rai I	14 (23)
Rai II	8 (13.1)
Rai III	12 (19.7)
Rai IV	13 (21.3)
CD38 expression	32 (52.4)
ZAP70	-

DISCUSSION

Fluorescence in situ hybridization (FISH) is the main and broadly used method for cytogenetic risk stratification in newly diagnosed patients of CLL. Importance of FISH is well recognized in the prognostication of patients with chronic lymphocytic leukemia. DEL 11q22 is regarded as a high risk prognostic marker in patients of CLL. Although DEL 11q22 in CLL has very good response to chemo- immunotherapy, but progression free survival is shorter than that seen without DEL 11q22[11-16].

Our study included 61 patients of CLL. The study has revealed many findings regarding

CLL with de11q22. In our study frequency of DEL 11q22 was 11.4% of the patients while Thomas and Colleagues have reported frequency of DEL 11q22 in CLL Chinese patients as 11.4 % and our study is in agreement with this study[17]. However frequency of DEL 11q22 reported by Angel *et al*[18], in Spain was reported 9.7% and by Rahimi *et al* in Iran was 9.09%[19]. These percentages are lower in comparison with our study. The difference can be due to ethnic, geographical and demographic reasons.

In our study, age of the patients of CLL with DEL 11q22 ranged between 52-66 years with a median age of 63 years. Rahimi *et al* have demonstrated median age of patients to be 63 years which matches our result[19]. In another study, Jain *et al* found median age of 60 years which is close to our findings[16]. Dohner *et al* reported median age of 58 years in patient of CLL with DEL 11q22 which is on the lower side then our study[20].

In our study, males were more common as compared to female with a M:F ratio is 3.69:1. Our observation matches with the study conducted by Preetesh *et al*[16] who has reported M:F ratio of 3.66:1. Dohner *et al*[20] has also reported male predominance.

We found median WBC count of 150 x 10^9/ L. Jain *et al* 2012 reported median white blood cell count of 33.8 x 10^9/L[21]. However, Jain *et al*

Table-II: Clinico-pathological features of CLL patients with 11q22 deletion and without 11q22 deletion and comparison of median values of lab parameters.

		Deletion with 11q22 Deletion (n=7) (Median IQR)	Deletion without 11q22 Deletion (n=54) (Median IQR)	*p*-value
Median age (year)		63 (52-66 years)	59 (38-75)	0.964
Rai staging	Rai 0	-	14 (25.9%)	
	Rai I	2 (28.6)	12 (22.2%)	
	Rai II	2 (28.6)	6 (11.1%)	
	Rai III	3 (42.9)	9 (16.7%)	
	Rai IV		13 (24.1%)	
Absolute lymphocyte count x 10^9/L (median)		105.5 (74-139)	77.54 (8.4-234)	0.330
WBCs count x 10^9/L (median)		152 (74-156)	90.9 (9.3-234)	0.085
Hemoglobin (g/dl) (median)		10.3 (8.1-11.2)	11.1 (4.9-15.2)	0.135
Platelets x 10^9/L (median)		111.9 (106-118)	160 (50-352)	0.154
B-Symptoms				
Yes		7 (100%)	27 (50%)	
No		-	27 (50%)	
Physical Examination				
Lymphadenopathy				
Cervical		7 (100%)	38 (70.3%)	
Submandibular		1 (14.2%)	8 (14.1%)	
Axillary		5 (71.4)	12 (22.2%)	
Inguinal		4 (57.14%)	15 (27.7%)	
Unremarkable		-	14 (25.9%)	
Organomegaly				
Splenomegaly		2 (28.5%)	22 (40.7%)	0.44
Hepatomegaly		2 (28.5%)	4 (7.4%)	
Bulky disease on PE		2 (28.5%)	4 (74%)	
CD38		3 (42.8%)	29 (53.7%)	
ZAP70		-	-	

demonstrated WBC count of 30 x 10^9/L[16]. These studies showed low median WBC count in comparison with our findings. Reasons for high WBCs count in our population may be that our patients presented late to the tertiary care hospitals as the facilities are available at few centres in Pakistan. We have reported median absolute lymphocyte count of 105.5 x 10^9/L while Jain *et al* in 2015[16] demonstrated median ALC 30 x 10^9/L and in 2012 Jain *et al*[21] has reported median ALC of 27x 10^9/L. Both the studies showed very low median ALC count when compared with our study.

Majority patients in series with DEL 11q22 CLL presented with advanced clinical stage Rai III (42%) and Rai II (28.6%) and I (28.6%). Dohner *et al*[20] reported 10.5% in Rai stage III and 15.2% in Rai stage IV. However, Jain *et al* 2015[16] has demonstrated that 8% patients in Rai III and 9% in Rai IV. Results of both these studies results were not comparable with our findings; this may be due ethnic and demographic changes.

Bulky disease was seen in 2 (28.6%) of our patients. Jain *et al* 2015[16] has demonstrated bulky disease in 9% which is low in comparison with our study. However, Jain *et al* in 2012[21] has again reported 6.4% bulky disease by physical examination which is also not in accordance with our study.

CD38 was positive in 3 (42%) of patients in our study. Angel *et al*[18] has reported 55% positive cases which is higher than our study. Jain *et al*[16] has reported CD38 positivity in 54% of patients with DEL 11q22. Both studies have showed slightly higher expression of CD38 but is comparable with our study.

CONCLUSION

The frequency of DEL 11q22 in CLL in our population was 11.4%. The WBC count and ALC count was also high when compared with the patients of CLL without 11q22 deletion.

CONFLICT OF INTEREST

The study has no conflict of interest to be declared by any author.

REFERENCES

1. Frenzel LP, Reinhardt HC, Pallasch CP. Concepts of chronic lymphocytic leukemia pathogenesis: DNA damage response and tumor microenvironment. Oncol Res Treat 2016; 39(1-2): 9-16.
2. Rodrigues CA, Goncalves MV, Ikoma MR, Lorand-Metze I, Pereira AD, Farias DL, et al. Diagnosis and treatment of chronic lymphocytic leukemia: recommendations from the Brazilian Group of Chronic Lymphocytic Leukemia. Rev Bras Hematol Hemoter 2016; 38(4): 346-57.
3. Zeeshan R, Sultan S, Irfan SM, Kakar J, Hameed MA. Clinico-hematological profile of patients with B-chronic lymphoid leukemia in Pakistan. Asian Pac J Cancer Prev 2015; 16(2): 793-6.
4. Burger JA, Tedeschi A, Barr PM, Robak T, Owen C, Ghia P, et al. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. NEJM 2015; 373(25): 2425-37.
5. Ghahfarokhi HJ, Ashoori S, Akbari MT, Lotfizadeh M, Karimi A, Teimori H. ZAP70 Expression Within Del6q21, Del11q13 and Del17p13 Cytogenetic Subgroups of Iranian Patients with Chronic Lymphocytic Leukemia. Iran Red Crescent Med J 2014; 16(11): 45-50.
6. Oscier D, Dearden C, Eren E, Fegan C, Follows G, Hillmen P, et al. GuiDelines on the diagnosis, investigation and management of chronic lymphocytic leukemia. Br J Haematol 2012; 159(5): 541-64.
7. Nabhan C, Rosen ST. Chronic lymphocytic leukemia: A clinical review. JAMA 2014; 312(21): 2265-76.
8. Teimori H, Ashoori S, Akbari MT, Naeini MM, Chaleshtori MH. FISH Analysis for Del 6q21 and Del 17p13 in B-cell Chronic Lymphocytic Leukemia in Iranians. Iran Red Crescent Med J 2013; 15(2): 107-12.
9. Wu SJ, Lin CT, Agathangelidis A, Lin LI, Kuo YY, Tien HF, et al. Distinct molecular genetics of chronic lymphocytic leukemia in Taiwan: Clinical and pathogenetic implications. Haematologica 2017; 102(6): 1085-90.
10. Stilgenbauer S, Bullinger L, Lichter P, Döhner H. Genetics of chronic lymphocytic leukemia: genomic aberrations and VVH gene mutation status in pathogenesis and clinical course. Leukemia 2002; 16(6): 993.
11. Jiang Y, Chen HC, Su X, Thompson PA, Liu X, Do KA, et al. ATM function and its relationship with ATM gene mutations in chronic lymphocytic leukemia with the recurrent Deletion (11q22.3-23.2). Blood Cancer J 2016; 6(9): e465.
12. Eid OM, Eid MM, Kayed HF, Mahmoud WM, Mousafa SS, Ismail MM, et al. Detection of cytogenetics abnormalities in chronic lymphocytic leukemia using FISH technique and their prognostic impact. Gulf J Oncol 2014; 1(15): 68-75.
13. Chastain EC, Duncavage EJ. Clinical prognostic biomarkers in chronic lymphocytic leukemia and diffuse large B-cell lymphoma. Arch Pathol Lab Med 2015; 139(5): 602-7.
14. Puiggros A, Blanco G, Espinet B. Genetic abnormalities in chronic lymphocytic leukemia: where we are and where we go. Biomed Res Int 2014; 2014: 435983.
15. Nabhan C, Raca G, Wang YL. Predicting Prognosis in Chronic Lymphocytic Leukemia in the Contemporary Era. J Am Med Assoc Oncol 2015; 1(7): 965-74.
16. Jain P, Keating M, Thompson PA, Trinh L, Wang X, Wierda W, et al. High fluorescence in situ hybridization percentage of Deletion 11q in patients with chronic lymphocytic leukemia is an independent predictor of adverse outcome. Am J Hematol 2015; 90(6): 471-7.
17. Chan TS, Lee YS, Del Giudice I, Marinelli M, Ilari C, Cafforio L, et al. Clinicopathological features and outcome of chronic

lymphocytic leukemia in Chinese patients. Oncotarget 2017; 8(15): 25455-68.
18. Hernández JÁ, Hernández-Sánchez M, Rodríguez-Vicente AE, Grossmann V, Collado R, Heras C, et al. A low frequency of losses in 11q chromosome is associated with better outcome and lower rate of genomic mutations in patients with chronic lymphocytic leukemia. Public Library Sci One 2015; 10(11): e0143073.
19. Rahimi H, Sadeghian MH, Keramati MR, Jafarian AH, Shakeri S, Shams SF, et al. Cytogenetic abnormalities with interphase fish method and clinical manifestation in chronic lymphocytic leukemia patients in north-east of Iran. Int J Hematol Oncol Stem Cell Res 2017; 11(3): 217-24.
20. Dohner H, Stilgenbauer S, James MR, Benner A, Weilguni T, Bentz M, et al. 11q Deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood 1997; 89(7): 2516-22.
21. Jain P, Trinh LX, Benjamini O, Lerner S, Wang X, Ferrajoli A, et al. Deletion 11q Abnormality in Patients with Chronic Lymphocytic Leukemia (CLL) May Not Have Poor Clinical Outcomes and Bulky Disease (clinical and radiological) At Presentation–Clinical Characteristics of (n=172) Previously Untreated Patients with CLL and Del 11q Cytogenetic Abnormality. Am Soc Hematol. 2012; 120(21): 2890-90.