Interphase Fluorescence in Situ hybridization Analysis of 17p13.1 and 11q22.3 Deletions among CLL Patients

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ABSTRACT:

BACKGROUND:
Chronic lymphocytic leukemia (CLL) is a B cell neoplasm. It is the most frequent adult leukemia in European countries, comprising up to 30% of all adult leukemias with male predominance. Detection of a 17p13.1 deletion (loss of TP53) or 11q22.3 deletion (loss of ATM), by fluorescence in situ hybridization (FISH), is associated with a poorer prognosis.

OBJECTIVE:
This study aims to analyze the frequency of cytogenetics abnormalities among CLL patients.

PATIENTS, MATERIALS AND METHODS:
This study enrolled 30 adult patients presented with chronic lymphocytic leukemia visiting Baghdad teaching hospital/hematology center lab/cytogenetic unit-Baghdad /Iraq during the period extending from Mar. till Nov. 2020.The patients were diagnosed on the basis of standard clinical (Lymph node involvement and / or hepatosplenomegally), hematological and immunophenotypic criteria for diagnosis of B-CLL. Interphase FISH technique was performed on peripheral Blood samples using XL ATM/TP53 dual-color probe.

RESULTS:
We studied 30 CLL patients by I-FISH technique to detect the occurrence of cytogenetic abnormalities, eighteen (60%) had one or more cytogenetic abnormalities; Eleven (36.7%) had 17p- (including 2 with 17p- and 11q- in separate clones), Five (16.7%) had 11q-, and Two (6.7%) of patients had clonal 17p-/11q-. Twelve (40%) had neither 17p- nor 11q-. There was no statistically significant clinico pathological correlation with these cytogenetic abnormalities; p-value >0.05.

CONCLUSION:
The cytogenetic abnormalities including 17p-, 11q- and clonal 17p-/11q- among CLL patients occur within the reported figures worldwide. There was no statistically significant clinico pathological correlation with these cytogenetic abnormalities.

KEYWORDS: FISH, CLL, 17P-, 11q-, cytogenetics

INTRODUCTION:

B cell Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a diverse clinical presentation, response to treatment and survival [1]. For the cytogenetics lab, CLL is a difficult disease to obtain metaphases by conventional cytogenetics due to a very low spontaneous proliferative index of the CLL cells and poor response to mitogens; more than 50% of successful analyses were reported as normal. This reflected the normal cells, not the neoplastic cells, as FISH (molecular cytogenetics) has shown up to an 80% abnormality rate [2,3].

The most common CLL abnormalities when studied by FISH are a deletion of 13q14.3 (miR 15a and miR 16 1) [4,39], trisomy 12, deletion 11q22.3 (ATM) and deletion 17p13.1 (TP53), these abnormalities have prognostic significance with a hierarchy of best to worst prognosis include; del (13q), no FISH abnormality, trisomy 12, del (11q) and del (17p) [3,5-7,38].

The incidence of 11q- in CLL reported in various studies ranges 12%-25%, while the frequency of 17p- reported ranges 9%-15% [8,9].These lesions are always monoallelic in CLL patients [10].

The prognostic consequences of 17p- or 11q- are influenced by the integrity of the remaining allele of TP53 or ATM, respectively. Patients with progressive CLL, there is a dysfunctional mutation in the remaining allele in (80 – 90%) of patients with 17p- and in one-third of patients with 11q- ; patients with both 17p- and 11q- in the same CLL cells would have a worse prognosis than patients with either of these deletions alone [11,12,13,14].

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AIMS OF THE STUDY:

Determine the frequency of cytogenetics abnormalities among CLL patients by Interphase FISH using a metasystem XL ATM/TP53 locus-specific probe.

MATERIALS AND METHODS:

This study was conducted on a total of 30 adult patients presented with B-CLL visiting Baghdad teaching hospital/hematology center lab/cytogenetic unit-Baghdad /Iraq during the period extending from Mar. till Nov. 2020; patient characteristics were reported including age, sex, stage and treatment status. All patients were subjected to full history and clinical examination, complete blood picture (CBC), CT imaging, Immunophenotyping and FISH technique.

An interphase FISH technique were performed on peripheral blood samples and processed by the standard methods for uncultured samples, using a metasystem XL ATM/TP53 locus-specific probe to detect deletions in the long arm of chromosome 11 and in the short arm of chromosome 17. The green labeled probe hybridizes to a specific region at 11q22 covering the ATM gene. The orange labeled probe hybridizes specifically to the TP53 gene at 17p13.

A total of 200 nuclei were analyzed per probe set, patients were classified into four groups based on the hierarchical classification of FISH defects [3]: 1) 17p- and 11q- in the same clonal CLL cells (double hit) (1O1G), 2) 17p-(1O2G), 3) 11q-(1G2O), and 4) neither 17p- nor 11q- (2O2G).

Statistical analyses were performed using SPSS statistical package for Social Science (version 20.0 for windows, SPSS, Chicago, IL, USA).

Unpaired Student's t-test and Chi-square test were used to study the relation of the presence of cytogenetic abnormalities and age, gender, RAI, LAP groups.

P value of < 0.05 was considered statistically significant.

RESULTS:

A total of 30 CLL cases were enrolled; their ages ranged between (30-67) years with a (mean ±SD) of (56.17±8.45) and a median of 57 years. They included 21(70%) males and 9(30%) females; M:F ratio was 2.3:1; Table (1).

Out of the 30 CLL patients studied, Eleven (36.7%) had 17p- (10-75% of interphase nuclei). Five (16.7%) had 11q- (15-80% of interphase nuclei). Two (6.7%) of patients had both 17p- and 11q- in the same cells ("double hit") including one patient with isolated 17p- as well as 17p- and 11q- ("double hit") in a sub-clone, and another patient with isolated 11q- as well as 17p- and 11q- ("double hit") in a sub-clone. Twelve (40%) had neither 17p- nor 11q- ; Table (2), Table (3).

Majority of patients with 17p- were male 8(72.7%); with a Mean ±SD (median) 56.3±5.4 (56.0) years and 7 (63.6%) presented with LAP, 4 (36.4%) were in low and high risk Rai stage.

Three (60%) of patients with 11q- were male with a Mean±SD (median) of 48.2±16 (54) years and 4(80%) presented with LAP; 4 (80%) were in intermediate risk Rai stage.

All patients with both 17p- and 11q- were males 2(100%) and diagnosed with more advanced stage disease 1(50%) were in intermediate and 1 (50%) were in high risk Rai stage, 2(100%) presented with LAP with a Mean±SD (median) of 58.5±4.9 (58.5) years.

There was no significant relation between the presence of different cytogenetic abnormalities and gender, RAI and LAP groups (p value > 0.05).Age did not differ between different cytogenetic abnormalities groups (p value > 0.05); Table (2).

Table 1:The distribution of age and gender of 30 enrolled cases.

NO. of Cases	30
Age(years): Range = Mean ± SD = Median =	 30-67 56.17±8.45 57
Gender: Male = Female = M:F	 21(70%) 9(30%) 2.3:1

Table 2:Patient characteristics.

	17p- only 11(36.7%)	11q- only 5(16.7%)	17p-&11q- 2(6.7%)	Neither 12(40%)
Gender Male Female P=0.75	8(72.7%) 3(27.3%)	3(60%) 2(40%)	2(100%) 0(0.0%)	8(66.7%) 4(33.3%)
Rai.stage Low Inter. High P=0.10	4(36.4%) 3(27.3%) 4(36.4%)	1(20%) 4(80%) 0(0.0%)	0(0.0%) 1(50%) 1(50%)	6(50%) 6(50%) 0(0.0%)
LAP +ve -ve P=0.44	7(63.6%) 4(36.4%)	4(80%) 1(20%)	2(100%) 0(0.0%)	6(50%) 6(50%)
Age Mean±SD (median)years P=0.108	56.3±5.4(56.0)	48.2±16(54.0)	58.5±4.9(58.5)	59.0±5.3(60.5)

Table 3:Patient demographics and FISH test (%) result.

Case No.	Age at DX.	Gender	Rai stage	LAP	11q-(ATM)	17P-(TP53)	17P- & 11q-
1.	51	M	3	+	24%	15%	-
2.	56	F	3	+	-	26%	-
3.	55	M	4	+	55%	-	13%
4.	60	M	4	+	10%	20%	-
5.	56	M	2	+	-	10%	-
6.	45	F	3	+	-	50%	-
7.	54	M	0	-	-	-	-
8.	52	M	1	+	-	-	-
9.	61	M	0	-	-	-	-
10.	57	M	1	+	35%	-	-
11.	62	M	0	-	-	12 %	-
12.	55	F	0	-	-	-	-
13.	54	M	1	+	15%	-	-
14.	60	M	1	+	-	-	-
15.	54	F	0	-	-	25%	-
16.	62	M	2	+	-	50%	10%
17.	55	M	0	-	-	11%	-
18.	50	F	0	-	-	-	-
19.	57	M	1	+	-	20%	-
20.	58	M	0	-	-	14%	-
21.	62	F	1	+	-	-	-
22.	65	M	1	+	-	-	-
23.	30	F	1	+	20%	-	-

24.	65	F	0	-	-	-	-
25.	65	M	2	+	-	-	-
26.	67	F	2	+	80%	-	-
27.	65	M	1	+		75%	
28.	33	M	0	-	70%	-	-
29.	57	M	0	-	-	-	-
30.	62	M	1	+	-	-	-

DISCUSSION:

Cytogenetic analysis at the time of CLL diagnosis is important for optimal patient prognostic stratification, predicting survival and treatment decision. The use of FISH is important in determining the incidence and the clinical relevance of karyotypic abnormalities in CLL patients, especially when there are no enough mitosis for conventional cytogenetic analysis. Analysis of aberrant chromosomal region with specific DNA probes using I-FISH detect 80% of clonal aberration among CLL patients [15].

In this study we used I-FISH methods to detect deletions in 11q22.3 and 17p13.1 and whether there is correlation between the presence of these abnormalities and clinicopathological characteristics.

Genomic aberrations such as 11q22 (ATM) and 17p (TP53) deletions, trisomy chromosome 12 and loss of 13q14 region provide prognostic impact which is important in assignment of therapeutic protocol among CLL patients [16,17].

This study revealed that the cytogenetics abnormalities of all 30 cases were 18/30(60%) (Table-2); other studies found the cytogenetics abnormalities in 100% of cases [18, 19].Stilgenbauer et al. [20] and Haferlach et al. [21] found that the genomic aberrations were detected in 92% and 85.2% of cases respectively; whereas cytogenetic aberrations were reported in 62% and 55% by Quijano et al [22] and Lu et al. [23] respectively; this discrepancy in reported results could be attributed to variation in number of studied cases. The TP53 abnormalities have been detected in10-15% of patients using single strand conformational polymorphism and polymerase chain reaction sequencing and in 26% using FISH technique [24-26].Deletion of chromosome 17p13 involving TP53,atumor suppressor gene involved in cell cycling and cell death is the strongest single predictor of bad prognosis and resistance to treatment[27].

In the current study, positive CLL patients with only 17p13 deletion were 11/30(36.7%) of cases (Table-2).In the literature, studies using FISH technique, showed wide variability of results. Doneda et al [18] and Eid et al. [19] reported TP53 deletion in 100% and 20% respectively of CLL patients. Sindelarova et al. [9] and Xu et al. [28] documented TP53 deletion in 16% and 16.8% respectively. Abdel Salam et al. [29]; Dickinson et al. [30] and Gozzetti et al. [31] reported TP53 deletion in 10% of CLL cases. Whereas other studies reported lower levels of detection: 8.7% [32], 8% (22), 7% [33] and 3.4% [34].

Deletion of chromosome 11 occur in high proportion of newly diagnosed and untreated CLL (24.6%) thus representing the second most frequent aberration after 13q14 involvement [35] .In our study 5/30(16.7%) of cases showed 11q22 (ATM) deletion (Table-2), in acceptance with Greipp et al [33] who reported ATM deletion in 11%. Zhu et al. [36] and Doneda et al. [18] reported ATM deletion in 30% and 58% of cases respectively. In the current study 2/30 (6.7%) showed clonal 17p-/11q- (Table-2) whereas Greipp et al [33] documented clonal 17p-/11q- in 1% of cases.

In the current study there was no statistically significant clinicopathlogical correlation with these cytogenetic abnormalities; p-value >0.05.On the contrary Xu et al. [37] had shown patients with advanced- stage disease had del 17p13 more frequently than patients at earlier stages; Doneda et al. [18]showed a statistical correlation between 11q22 (ATM)deletion and the presence of lymphadenopathy.

The discrepancy between the results of various studies is due to the variation in sample size, ethnic group studied, age of patients, stage of disease and the different techniques used to detect these abnormalities.

CONCLUSION:

The frequency of 17p-, 11q- and clonal 17p-/11q- among CLL patients laid within the reported figures worldwide. There was no statistically significant clinicopathological correlation with these cytogenetic abnormalities.

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