The prognostic significance of XL DLEU/LAMP (13q14 deletion) in CCL patients: a cross section study

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Abstract

Background: Many cytogenetic abnormalities were detected in CLL, one of them. Deletion of 13q14 region which is found in more than 50% of CLL patient. 13q deletion is the most common cytogenetic abnormality detected by fluorescence in situ hybridization (FISH) and has historically been associated with good prognosis. During the last years, several studies have revealed some insights in the candidate genes located at 13 q that could be responsible for CLL pathogenesis,as well as in the prognostic heterogeneity of 13q- deleted patients.

Aim of the study: evaluation of the prognostic value of 13q chromosome deletion in patients with CLL in correlation to complete blood picture, absolute lymphocyte count, prolymphocyte count and modified Rai staging.

Patients and method: This cross-sectional study was conducted on thirty adult with newly diagnosed and Denovo B-CLL patients tested for the expression of 13q deletion using Fluorescence Insitu Hybridization, from March 2018 to July 2018 and the diagnosis was document on the morphology and immunephenotyping of the peripheral blood sample using a four-color flow cytometer in the Nursing Home Hospital / flow cytometry department of the Medical City in Baghdad.

Results: XL DLEU/LAMP (13q14 deletion**)** was seen in 7 (23.3 %). 13q14 deletion was not significantly correlated to age, gender, splenomegaly, lymphadenopathy or hepatomegaly. There was highly significant difference in mean Hb between CLL patients with positive 13q14 deletion and those with no deletion (P = 0.001), being higher in patients with positive 13q14 deletion than those without deletion, 12.96 ± 1.41 g/dl versus 10.14 ±1.74 g/dl, respectively. In addition, all CLL patients with positive 13q14 deletion were free of anemia, whereas anemia (Hb < 11 g/dl) was seen in 17 (73.9 %) of patients with no deletion; the variation in 13q14 deletion according to anemia was highly significant (P = 0.001).

Conclusion: indirectly, one can suppose that chromosome 13q.14 deletion carry good prognosis in CLL patients; however, it appears better to link such deletion to survival rate in order to get better idea about its prognostic significance.

Key words: XL DLEU/LAMP (13q14 deletion), CLL. Iraq

Introduction

Chronic lymphocytic leukemia (CLL)is a malignant lymphoproliferative disorder of mature B lymphocytes, characterized by the accumulation of a monoclonal population of small mature appearing CD5+B lymphocytes in the blood, bone marrow, and secondary lymphoid organs (lymph nodes and spleen) [(1)]. CLL is the most common leukemia in adults, with a highly variable clinical course, ranging from very indolent cases to very aggressive and rapidly progressing disease [(2)]. A number of clinical and biological features have been

used to separate patients with CLL into subgroups with different prognosis and requirement of different therapeutic approaches [2]. In contrast to other B-cell malignancies, CLL is not associated with recurrent balanced chromosomal translocations. For this reason, several biological parameters have been added to the staging system to differentiate prognostic subset [3].

Many cytogenetic abnormalities were detected in CLL, one of them. Deletion of 13q14 region which is found in more than 50% of CLL patient [4]. 13q deletion is the most common cytogenetic abnormality detected by fluorescence in situ hybridization (FISH) and has historically been associated with good prognosis [5]. During the last years, several studies have revealed some insights in the candidate genes located at 13 q that could be responsible for CLL pathogenesis,as well as in the prognostic heterogeneity of 13q-deleted patients [6]. Chromosome 13q deletion is a chromosome abnormality that occurs when there is a missing (deleted) copy of genetic material on the long arm (q) of chromosome 13 [7]. The severity of the condition and the signs and symptoms depend on the size and location of the deletion and which genes are involved [6]. It is likely that the biological consequence of a unique deletion anatomy is complex, resulting in the disruption of multiple regulatory sequences [7]. Furthermore, this is likely to be the situation with 13q deletion in other tumor types, such as lymphoma, multiple myeloma, and prostate cancer, as well as deletion events in cancer in general. Here we employ genomic profiling to show that 13q deletion size is associated with disease progression [4]. In CLL the presence of 13q- conferred a favorable prognosis, with 60% of patients alive after 5 years as compared with 27% for patients with a normal FISH analysis [4, 5].

The current study was planned and conducted aiming at evaluation of the prognostic value of 13q chromosome deletion in patients with CLL in correlation to complete blood picture, absolute lymphocyte count, prolymphocyte count and modified Rai staging.

Patients and methods

This cross-sectional study was conducted on thirty adult with newly diagnosed and Denovo B-CLL patients tested for the expression of 13q deletion using FluorescenceInsitu Hybridization. This was conducted from March 2018 to July 2018 and the diagnosis was document on the morphology and immunephenotyping of the peripheral blood sample using a four-color flow cytometer in the Nursing Home Hospital / flow cytometry department of the Medical City in Baghdad. The lab work was done in two steps the first step was done in the FISH unit of the Nursing Home Hospital in the Medical City and the second step is done in the postgraduate pathology department in AL-Nahrain Collage of Medicine for detecting the result by using FISH technique.

From each patient a verbal consent was acquired for accepting to take the peripheral blood samples. For each patient a questionnaire form was arranged, as shown in the appendix1 including: name, age, sex, the main symptoms and physical signs especially the presence of lymphadenopathy, splenomegaly, hepatomegaly and B symptoms including (fever, weight loss and night sweating).

Clinical and laboratory information regarding age, sex, CBC, percentage of lymphocyte in peripheral blood were

obtained from patients hospital records at diagnosis.

Modified Rai staging-system was applied for staging the patients with CLL. Accordingly they were classified into 4 groups: low; Intermediate I and Intermediate II; high risk groups.

Blood samples from CLL patients were collected in a sodium heparinized tubes for evaluate of q13 deletion by FISH technique. The lab work was done in two steps , the first step was to separate lymphocytes. It was done in the FISH unit of the Nursing Home Hospital in the Medical City. The second step was slide preparation and determination of the deletion by FISH technique. It was done in the post graduate lab of the Pathology Department in AL-Nahrain Medical Collage.

Clinical and laboratory information regarding age, sex, CBC, percentage of lymphocyte in peripheral blood were obtained from patients hospital records at diagnosis, and also physical examination regarding hepatosplenomegally, lymphadenopathy and the presence of B symptoms (fever, weight loss, night sweating) were done at time of the taking the blood samples.

Results

The statistical analysis in the current study was based on enrollment of 30 patients with CLL. The mean age of patients was 63.87 ±8.01 years and the age has ranged from 46 to 75 years. According to gender the study included 20 males and 10 females, accounted for 66.7 % and 33.3 %, respectively; the male: female ratio was 2:1, table 1. Patients with CLL were categorized according to physical findings into those with Lymphadenopathy, those with splenomegaly and those with hepatomegaly, 16 (53.3 %), 9 (30 %) and 5 (16.7 %), respectively. Hematological findings are summarized in table 2.

Stage I disease has been identified in 10 (33.3 %), stage II disease has been seen in 3 (10.0 %), stage III disease has been reported in 12 (40.0 %) and stage IV disease has been observed in 5 (16.7 %), table 3. Therefore, intermediate risk (stages I and II) has been seen in 13 (43.3 %), while high risk (stages III and IV) has been identified in 17 (56.7 %), table 3.

According to XL DLEU/LAMP (13q14 deletion**)**, patients were classified in to those with positive marker expression and those with negative marker expression, 7 (23.3 %) and 23 (76.6 %), respectively. 13q14 deletion was not significantly correlated to age, gender, splenomegaly, lymphadenopathy or hepatomegaly, tables 4 and 5.

There was highly significant difference in mean Hb between CLL patients with positive 13q14 deletion and those with no deletion (P = 0.001), being higher in patients with positive 13q14 deletion than those without deletion, 12.96 ± 1.41 g/dl versus 10.14 ±1.74 g/dl, respectively. In addition, all CLL patients with positive 13q14 deletion were free of anemia, whereas anemia (Hb < 11 g/dl) was seen in 17 (73.9 %) of patients with no deletion; the variation in 13q14 deletion according to anemia was highly significant (P = 0.001), as shown in table 6.

There was no significant difference in mean total WBC count between CLL patients with positive 13q14 deletion and those with no deletion, 24000 (6000) versus 21000 (10000) (P = 0.597). In addition, there was no significant difference in mean absolute lymphocyte count between CLL patients with positive 13q14 deletion and those with no deletion, 40.5 (64.9) versus 63.8 (42.6) (P = 0.898). Moreover, when CLL patients were categorized into those with absolute lymphocyte count ≤50 and

those with absolute lymphocyte count > 50, there was no significant difference in proportion of patients with positive 13q14 deletion and those with no deletion (*P* = 0.392), as shown in table 7. There was no significant difference in mean platelet count between CLL patients with positive 13q14 deletion and those with no deletion, 150.0 (146.0) versus 147.0 (100.0) (*P* = 0.734). Moreover, when CLL patients were categorized into those with thrombocytopenia (platelet < 100) and those without thrombocytopenia (platelet count ≥ 100), there was no significant difference in proportion of patients with positive 13q14 deletion and those with no deletion (*P* = 1.000), as shown in table 8.

The positive 13q14 deletion was significantly higher in patients with intermediate stage than patients with high stage, 46.2 % versus 5.9 %, respectively (*P* = 0.032), table 9. On the other hand, positive 13q14 deletion according to stage of disease was as following: stage I (40.0 %), stage II (66.7 %), stage III 0 (0.0 %) and stage IV (20.0 %), that is, the lower the stage, the more likely is 13q14 deletion, table 4.10; however, Chi-square was not valid because more than 20 % of cells had expected count less than 5; therefore, Spearman correlation test was carried out instead and the results were shown in figure 1; in which the correlation was negative (*r* = 0.332) and the level of significance was border line (*P* = 0.073) which is very close to 0.05.

Table 1: Demographic characteristics

Characteristic		**Value**
Age (years)	Mean ±SD	63.87 ±8.01
	Range	46-75
	46-50	4 (13.3%)
	51-55	0 (0.0%)
	56-60	3 (10.0%)
	61-65	9 (30.0%)
	66-70	9 (30.0%)
	> 70	5 (16.7%)
Gender	Male, *n* (%)	20 (66.7%)
	Female, *n* (%)	10 (33.3%)

Table 2: Hematological characteristics of patients with CLL

Characteristic	**Statistic**	**Value**
HB	Mean ± SD	10.80 ±2.04
	Range	6 - 15

	Anemia (Hb <11), *n* (%)	17 (56.7 %)
WBC $X10^9$/L	Median (IQR)	22500 (9250)
	Range	8000 -30,000
PLT $X10^9$/L	Median (IQR)	148.5 (103.0)
	Range	40 -330
	Thrombocytopenia (platelet < 100), *n* (%)	5 (16.7 %)
Absolute lymphocyte count $X10^9$/L	Median (IQR)	62.30 (47.35)
	Range	8 -185
	ALC > 50, *n* (%)	18 (60.0 %)

HB: Hemoglobin; WBC: white blood cells; PLT: platelet; SD: standard deviation; IQR: inter-quartile range

Table 3: Rai staging of CLL patients

Stage	Risk	*n*	%
I	Intermediate	10	33.3
II	Intermediate	3	10.0
III	High	12	40.0
IV	High	5	16.7

Table 4: Association between age of patients with CLL and 13q14 deletion

Characteristic		**13q14 deletion**		***P***
		Positive *n* = 7	**Negative *n* = 23**	
Age (years)	Mean ±SD	61.57 ±6.37	64.57 ±8.45	0.396 † NS
	≤70 years, *n* (%)	0 (0.0 %)	5 (21.7 %)	0.304 ¥ NS
	> 70 years, *n* (%)	7 (100.0 %)	18 (78.3 %)	
Gender	Male, *n* (%)	5 (71.4 %)	15 (65.2 %)	1.000 ¥ NS
	Female, *n* (%)	2 (28.6 %)	8 (34.8 %)	

n: number of cases; SD: standard deviation; †: Mann Whitney U test; ¥: Fischer exact test; NS: not significant at $P \leq 0.05$

Table 5: Association between clinical signs and 13q14 deletion in patients with CLL

Sign	**Total *n* = 30**	**13q14 deletion**		***P*** ¥
		Positive *n* = 7	**Negative *n* = 23**	

		n	%	*n*	%	
LAP	16	4	25.0	12	75.0	Reference
Splenomegaly	9	2	22.2	7	77.8	1.000 NS
Hepatomegaly	5	1	20.0	4	80.0	1.000 NS

n: number of cases; LAP: Lymphadenopathy; ¥: Yates correction; NS: not significant at $P \leq 0.05$

Table 6: Association between anemia and 13q14 deletion in CLL patients

Hb	**Positive** ***n*** **= 7**	**Negative** ***n*** **= 23**	***P***
Mean ±SD	12.96 ± 1.41	10.14 ±1.74	0.001 † HS
Anemia (Hb < 11), *n* (%)	0 (0.0%)	17 (73.9 %)	0.001 ¥ HS
No anemia, *n* (%)	7 (100.0 %)	6 (26.1 %)	

n: number of cases; SD: standard deviation; †: Mann Whitney U test; ¥: Fischer exact test; HS: highly significant at $P \leq 0.01$

Table 7: Association between 13q14 deletion and total WBC and absolute lymphocyte counts in CLL patients

Characteristic		**13q14 deletion**		***P***
		Positive ***n*** **= 7**	**Negative** ***n*** **= 23**	
WBC	Median (IQR)	24000 (6000)	21000 (10000)	0.597 † NS
Absolute lymphocyte counts	Median (IQR)	40.5 (64.9)	63.8 (42.6)	0.898 † NS
	≤50, *n* (%)	4 (57.1 %)	8 (34.8 %)	0.392 ¥ NS
	> 50, *n* (%)	3 (42.9 %)	15 (65.2 %)	

n: number of cases; IQR: inter quetile range; †: Mann Whitney U test; ¥: Fischer exact test; NS: not significant at $P \leq 0.05$

Table 8: Association between 13q14 deletion and platelet counts in CLL patients

Platelet count X1000/cc	**13q14 deletion**		***P***
	Positive ***n*** **= 7**	**Negative** ***n*** **= 23**	
Mean ±SD	150.0 (146.0)	147.0 (100.0)	0.734 † NS
Thrombocytopenia < 100	1 (14.3 %)	4 (17.4 %)	1.000 ¥

≥ 100	6 (85.7 %)	19 (82.6 %)	NS

n: number of cases; IQR: inter quartile range; †: Mann Whitney U test; ¥: Yates correction; NS: not significant at *P* ≤ 0.05

Table 9: Association between 13q14 deletion and Rai staging in CLL patients

Characteristic		**Total *n* = 30**	**13q14 deletion**		***P***
			Positive *n* = 7	**Negative *n* = 23**	
Risk	Intermediate	13	6 (46.2 %)	7 (30.4 %)	0.032 ¥ NS
	High	17	1 (5.9 %)	16 (69.6 %)	
Stage	I	10	4 (40.0 %)	6 (26.1 %)	0.039 £ NV
	II	3	2 (66.7 %)	1 (4.3 %)	
	III	12	0 (0.0 %)	12 (52.2 %)	
	IV	5	1 (20.0 %)	4 (17.4 %)	

n: number of cases; †: Mann Whitney U test; ¥:Yates correction; £: Chi-square test; NS: not significant at *P* ≤ 0.05; NV: Not valid since more than 20 % of cells have expected count < 5

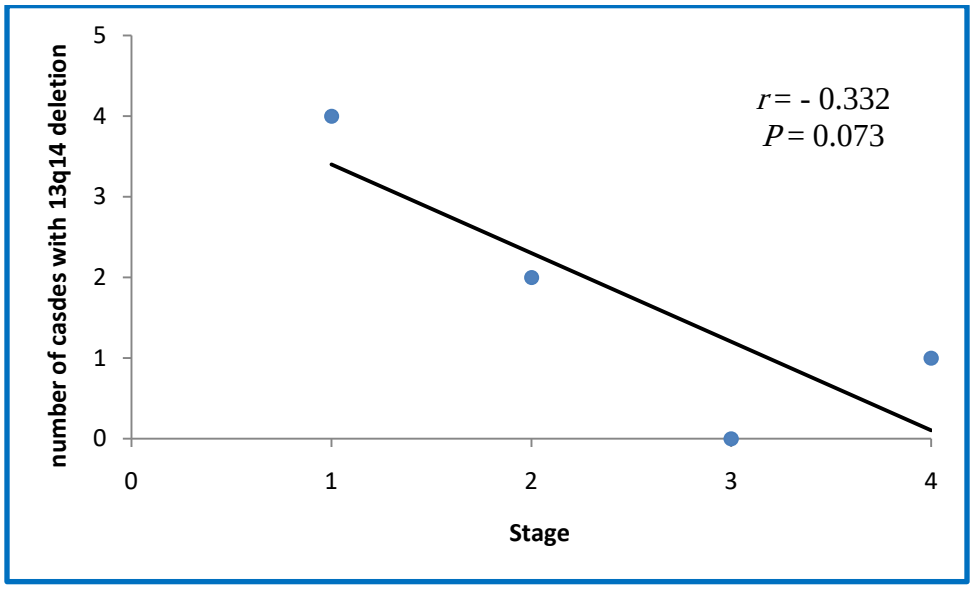

Figure 1: Correlation between Rai stage and marker expression

Discussion

In the current study, the level of hemoglobin in CLL patients has been in the range of 6 to 15 g/dl and the mean was 10.80 ± 2.04 g/dl. In one study, the mean baseline hemoglobin level in patients with CLL was 9.8 g/dl and the range was form 7.2 to 11 g/dl [8]. In another study, the mean hemoglobin level of CLL patients was 9.5 g/dl and the range was from 3.9 to 15.2 g/dl [9]. In a further study, mean hemoglobin level at baseline was 10.4 g/dl [10]. Therefore, the level of hemoglobin in patients with CLL, enrolled in the current study, is comparable to that reported by previous studies. In addition, anemia, defined as Hb < 10 g/dl, has been indentified in 56.7 % of CLL cases participating in this study. In one study, anemia was seen in 26 % of cases [11]; a figure that is lower than that reported in the current study. In general, anemia may be observed in 15 to 30% of patients with CLL and it often results from bone marrow infiltration, even though it can also be attributed to an autoimmune phenomenon [12].

The identification of anemia is important clinically since prognosis in CLL patients is partly determine by the existence and severity of anemia, therefore, anemia is one of the clinical parameters that have been incorporated within the two widely used clinical staging system for CLL, namely Rai and Binet [12]. In the current study, the mean platelet count has been 166.93 ±82.26 $X10^3$/cc and the range has been from 40 - 330 $X10^3$/cc; patients with thrombocytopenia have accounted for 5 out of 30 patients (16.7 %). Those results were comparable to many studies [12-14]). In one study, thrombocytopenia was identified in 18 % of patients with CLL (11), a figure that is approximately similar to that of the present study; however, it is lower than that described by Hasan *et al.* who stated that thrombocytopenia was identified in 60 % of cases [15]. Thrombocytopenia is usually attributed to bone marrow failure however autoimmunity was not seen in our patients. In this study, the leukocyte count has been in the range of 3000 -30000 and the mean was 19783.00 ±7583.65. According to several authors, high leukocyte count is associated with poor prognosis [12, 16]. The absolute lymphocyte count has been in the range of 8 -185 X 10^3/cc and the mean has been 69.34 ±44.11; patients with absolute lymphocyte count > 50 have accounted for 18 (60.0 %). In one study, the mean lymphocyte count in CLL patients was 186.68 X 10^3/cc and it ranged from 5.03 to 869 X 10^3/cc [9]. Absolute lymphocyte count has been used as a prognostic factor and high lymphocyte count of more than 50,000/cc has been linked to poor prognosis (17, 18). Therefore, identification of patients with high lymphocyte count may aid in better disease staging and hence strict prognostic directed treatment options.

In the current study, stage I disease has been identified in 10 (33.3 %), stage II disease has been seen in 3 (10.0 %), stage III disease has been reported in 12 (40.0 %) and stage IV disease has been observed in 5 (16.7 %). Therefore, intermediate risk (stages I and II) has been seen in 13 (43.3 %), while high risk (stages III and IV) has been identified in 17 (56.7 %). This fact reveals that more than have of patients enrolled in the current study have advanced stage disease.

The XL DLEU/LAMP probe detects deletions on chromosome 13q. The orange labeled probe hybridizes to the DLEU locus region at 13q14.2, including D13S319 and the green probe hybridizes to the LAMP locus at 13q34 [19]. In the present study, chromosome 13.q deletion was identified in Positive 7 (23.3 %), whereas, 23 (76.6 %) CLL cases have no deletions. It has been found that deletions in the 13q 14 region are deleted in more than halve of CLL patients,

being the most common chromosomal abnormality in CLL [20, 21]. The prognosis and clinical course of CLL are heterogeneous. Conventional banding techniques in CLL are hampered by the low mitotic index of the neoplastic cells. The introduction of interphase cytogenetics using fluorescent *in situ* hybridization (FISH) has greatly increased the sensitivity of cytogenetic analyses. The most frequently deleted region in B-CLL is located in 13q14.3 distal to RB1.

In the current study, there was no significant difference in mean age between CLL patients with chromosome 13.q14 deletion and those without such deletion (P = 0.396). Some authors have linked survival to age at diagnosis and have found that prognosis is better in younger patients [22, 23]; however, we failed to find an association between age and chromosome 13.q14 deletion following thorough search in available published articles dealing with CLL [24-26]. In the current study there was no significant association between chromosome 13.q14 deletion and hepatomegaly or splenomegaly. The presence of splenomegaly and hepatomegaly indicates progression of the disease toward more advancing stage and is correlated with less favorable prognosis. Most of studies describing the prognostic value of chromosome 13.q14 deletion in CLL have linked this type of deletion to survival rate rather than to other prognostic factors such as splenomegaly or hepatomegaly [24 -26]. In the current study, there was highly significant difference in mean Hb concentration between CLL patients with chromosome 13.q14 deletion and those without deletion (P = 0.001), being lower in patients without deletions. In addition, all CLL patients with chromosome 13.q14 deletion were free of anemia, whereas anemia (Hb < 11 g/dl) was seen in 17 (73.9 %) of patients without with chromosome 13.q14 deletion. Anemia is a poor prognostic factor and therefore, indirectly, one can conclude that chromosome 13.q14 deletion in CLL is associated with favorable prognosis. Several studies have pointed to similar results [24-26].

In the current study, there was no significant difference in mean total WBC count and mean absolute lymphocyte count in correlation to chromosome 13.q14 deletion. High leukocyte count and high absolute lymphocyte counts have been linked to less favorable prognosis by some authors [12, 16-18]; however, for the best of our knowledge none of the authors dealing with chromosome 13.q14 deletion in CLL have linked such deletion to leukocyte or lymphocyte counts [24-26]. In the present study, there was no significant difference in mean platelet count between CLL patients with 13q14 chromosomal deletion and those without, further more there was no significant association between thrombocytopenia and 13q14 chromosomal deletion. The lack of association between 13q14 chromosomal deletion and thrombocytopenia may indicate that such deletion is an independent prognostic factor since a number of authors have shown favorable survival rate in association with 13q14 chromosomal deletion [27-30]. The current study revealed that CLL patients with chromosome 13q.14 deletion have better Rai staging than those without. Therefore, indirectly, one can suppose that chromosome 13q.14 deletion carry good prognosis in CLL patients; however, it appears better to link such deletion to survival rate in order to get better idea about its prognostic significance.

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