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The Prevalence of Cytogenetic Abnormalities Detected by Interphase FISH Method in Chronic Lymphocytic Leukemia

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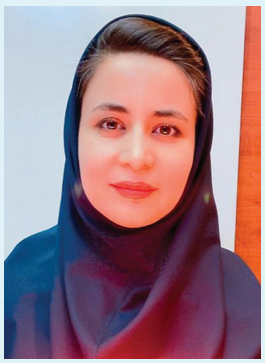
Abstract



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Background Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia. Identification of genomic aberration provides prognostic/predictive information that is helpful in the precision medicine management of these patients. The aim of this study was to determine prevalence of the most common cytogenetic abnormalities of CLL patients in the southwest region (Shiraz) of Iran and correlate with clinical prognostic parameters to clarify their prognostic value.

Materials and Methods In this cross-sectional study, 100 patients with CLL were recruited from April 2019 to October 2021. Four milliliters of anticoagulated peripheral blood was collected from each participant. The sample was used for complete blood count (CBC) test and fluorescence in situ hybridization (FISH) test. Interphase FISH (I-FISH) was performed for most common cytogenetic abnormalities, including trisomy 12, 13q14 deletion, 11q deletion, and 17p deletion on interphasic cell nuclei.

Results Among 100 patients with CLL, 33 (33%) were females and 67 (67%) were males. The mean age (mean ± standard error [SE]) of the patients was 59.00 ± 1.14 years, with a ranged of 25 to 79 years. Our analysis demonstrated that 86 (86%) patients had at least one chromosomal aberration. The most commonly detected abnormality was 13q deletion (61, 61%), followed by 17q deletion (50 cases, 50%). Trisomy 12 was detected in 14 (14%) cases and 10 cases (10%) had 11q deletion.

Conclusion The higher frequency of 13q14 and 17p anomalies in our study may be attributed to delayed medical consultations, leading to the emergence of secondary abnormalities. More studies are recommended for verifying the results.

Keywords

- FISH
- chronic lymphocytic leukemia
- cytogenetic abnormalities
- interphase
- chromosome

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Introduction

Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia with a frequency of 25 to 30% among hematological malignancies.[1,2] It is a clonally lymphoproliferative disorder characterized by progressive accumulation of mature, immunologically incompetent, and long-lived B lymphocytes.[3] Diagnosis is established based on the count, morphology, and immune phenotyping.[4] This malignancy is usually diagnosed in patients aged around 70 years and the annual incident rate rises dramatically with age.[5] The incidence of CLL is greater in men than in women, with a male-to-female ratio of 1.9:1.[6]

The clinical course of patients affected by CLL is very heterogeneous and ranges from an indolent and chronic to a rapidly progressing disease necessitating aggressive treatment.[7] This heterogeneity has a significant influence on treatment strategies, clinical approaches, and overall survival time.[7] Thus, following diagnosis, a careful risk assessment is essential to predict the course of the disease and form a basis for therapeutic decisions.

Chromosomal abnormalities can affect the clinical course and disease outcome, and thus can be used as prognostic markers.[4,8] Hence, there is a considerable interest in identifying chromosomal aberrations that could pinpoint subgroups of CLL patients who have different prognoses.[4] Cytogenetic methods including conventional cytogenetic (karyotyping) and fluorescent in situ hybridization (FISH) can be used to detect abnormalities in the genome.[9] Conventional cytogenetic analysis has been hampered by the low mitotic activity of the leukemic cells in vitro.[10]

However, interphase FISH (I-FISH) can be performed on the unstimulated cells and eliminates the need for the culture of neoplastic B cells showing low proliferation and mitotic index.[11] Accordingly, this molecular cytogenetic method in comparison with conventional karyotyping is known as a highly sensitive and robust method for detection of cytogenetic abnormalities in CLL cells. Moreover versatility, sensitivity, specificity, and resolution of the FISH technique have been largely improved. I-FISH increases the probability of detecting chromosomal disorders in CLL patients from 50 to 80% compared with conventional cytogenetic.[12] Thus, FISH is the gold standard method applied in clinical diagnostics for detection of known recurrent genomic aberrations. Cytogenetic stratification of CLL often relied on the detection by FISH of four cytogenetic markers including trisomy 12, 13q, 11q, and 17p on interphasic cells.[4]

While none of the well-defined cytogenetic abnormalities could be found in CLL, the most frequent mutations were a deletion on chromosome 13q14 (50%), a deletion on chromosome 11q22–23 (17–20%) and trisomy 12 (15%), and a deletion on chromosome 17p13. The 13q14 deletion correlates with longer survival, while trisomy 12 is associated with an intermediate prognosis. On the other hand, 11q and 17p deletions are linked to a poorer prognosis.[8,13] Accordingly, the aim of this study was to determine the frequency of the most common cytogenetic abnormalities in patients with CLL in the southwest region of Iran, utilizing the I-FISH method, specific probes were applied to investigate the centromere of chromosome 12 and the regions 13q14, 17p13, and 11q22.

Materials and Methods

Patients

In this cross-sectional study, a total of 100 patients with CLL were recruited from Dr. Daneshbod Pathobiology Laboratory (Shiraz, Iran) between April 2019 and October 2021. All the patients had a definite diagnosis of CLL based on their clinical records, cell morphological assessment, and immunophenotypic assays verified by two expert hemato-oncologists and hematopathologists. Peripheral blood samples were collected from patients. Written informed consent was obtained from all the patients before sampling. The study was approved by the local ethics committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1399.471).

Blood Sampling

From each patient, 4 mL of peripheral blood was collected into ethylenediaminetetraacetic acid (EDTA) treated tubes. This anticoagulated sample was used for the complete blood count (CBC) test and I-FISH slide preparation.

CBC Index Analyzing

CBC indexes of all the participants were measured using a Sysmex KX-21N automated hematology analyzer (Sysmex Corporation, Kobe, Japan).

Interphase Fluorescent In Situ Hybridization

For FISH analysis, slides were prepared from peripheral bloods as follows: 1 mL of peripheral blood was mixed with 9 mL of ammonium chloride hemolysis buffer and kept on ice for 10 minutes. The tubes were centrifuged for 10 minutes at 570 relative centrifugal force (RCF). The supernatant was aspirated and the cells were washed in 1x phosphate buffer saline (PBS) and centrifuged for 10 minutes at 570 RCF. The cells were re-suspended in the hypotonic KCl buffer (0.075 M) for 10 minutes at 37°C, followed by fixation using Carnoy's solution three times. Finally, the suspension was mixed carefully and spread in the same way as used for karyotype studies.[14] The slides were let to be dried at room temperature (RT). After slide preparation, the cells were aged by incubation overnight at 37°C and dehydrated in a decreasing alcohol series. To indicate del 17p13, del13q14, del 11q22, and trisomy12, dual-color locus-specific probes were purchased from MetaSystem Company (Altlussheim, Germany). FISH was performed according to the manufacturer's instructions. Evaluation of the FISH signals was performed using an Olympus BX43 fluorescence microscope (Olympus, Tokyo, Japan). The results were reported according to ISCN 2020 (imaging software). Peripheral samples from healthy age- and sex-matched individuals were used to calculate the cutoff values. Results were considered clonal when the percentage of cells with any given chromosome abnormality exceeded the established cutoff value. Normal cutoff values were established at 5% for $11q^-$, $17p^-$, $13q^-$, and

2% for +12. For each case, at least two technologists scored analyzable interphases nuclei. A minimum of 200 interphase nuclei were analyzed for each probe.

Statistical Analysis

The data were analyzed using the IBM SPSS package version 23 (IBM Corp., United States). The normal distribution of continuous variables including age, white blood cell (WBC), platelet, and hemoglobin was tested using the Shapiro–Wilk test. The significance of various cytogenetic subgroups with clinical variables was evaluated by Student's *t*-test, chi-squared test, and Mann–Whitney *U* test depending on the type of variants. A *p*-value less than 0.05 was considered statistically significant.

Results

Demographical Characteristics of Patients

Among 100 patients with CLL, 67 cases (67%) were males and 33 (33%) were females. The mean age (mean ± standard error [SE]) of the patients was 59 ± 1.14 years, with a range of 25 to 79 years. The mean age of the female patients was 56.45 ± 2.35 years (range: 25–77 years). The mean age of the males was 60.25 ± 1.23 years (range: 36–79 years). Approximately 11% of CLL patients were younger than 45 years.

Complete Blood Count Index

The analysis of CBC indexes indicated that WBCs count ranged from 3.8 to 493.19 × 109/L (mean ± SE = 47.8 ± 6.12 × 109/L). The platelet counts of the patients ranged from 25 to 518 × 109/L with a mean ± SE value of 173.75 ± 8.03 × 109/L. The hemoglobin concentration of the patients ranged from 5.58 to 18.8 g/dL, with a mean value of 13.6 ± 0.25 g/dL (►Table 1).

Fluorescent In Situ Hybridization Abnormalities

As summarized in ►Table 2, our analysis demonstrated that 86 (86%) patients had at least one chromosomal aberration. The most common abnormality was del13q, solely (23%) or accompanied by other abnormalities (61%). Deletion 11q had the lowest frequency and was only observed along with other abnormalities (10%; ►Fig. 1). Patients were classified based on good, intermediate, and poor prognoses as shown in ►Table 3.

Discussion

The use of FISH on interphase cells with specific deoxyribonucleic acid (DNA) probes improved the identification of clonal aberrations by up to 80% in patients with CLL.[12] Some of these aberrations can affect the clinical course and disease outcome. Thus, it is believed that chromosomal abnormalities can be available as prognostic markers.[4] Cytogenetic stratification of CLL often relies on the detection of four most common cytogenetic markers (trisomy 12 and deletions of 13q, 11q, and 17p) in interphasic cell nuclei by the FISH method.[4] The increased detection rate of I-FISH was mainly attributed to revealing deletions of 13q14.3 and 17p13, which are often cytogenetically cryptic.[15] The capability of FISH to identify submicroscopic aberrations clarifies why del (13)(q14.3) was more common in FISH results compared with $^{+}$12 in karyotype analysis.[15] Similarly, del(17)(p13.1) and del(11)(q22.3) were more frequently detected by FISH than by karyotyping.[15]

This research investigates the frequency of prevalent cytogenetic abnormalities in patients with CLL and their association with clinical prognostic parameters, aiming to clarify their prognostic importance. Our results revealed that 86 (86%) patients had at least one chromosomal aberration. Hemoglobin was insignificantly higher in male patients than in female patients. Hemoglobin showed a gender-dependent parameter, with higher concentration in males.

The most frequently observed abnormality was del13q14, with a prevalence of 23% for a single abnormality and 61% for those coexisting with other abnormalities. Similar to our results, Rahimi et al reported that 45.5% of Iranian CLL cases display chromosomal abnormalities; specifically, 40.90% of them had the deletion of the 13q14 chromosome.[16] Other studies also reported this deletion as the most frequent and early genomic alteration in CLL with a prevalence of 40 to 65%.[8,17–20] Within this deleted region, genes related to miR-15a and miR-16–1 microRNAs are located, which have been shown to impact the tumor suppressor activity of CLL. Additionally, other genes such as DLEU7 are placed in the 13q region.[16,21] It has been shown that patients with sole deletion of 13q14 have good prognosis; however, some forms lead to bad outcomes.[18] Accordingly, in the present study, 61% patients (41% males and 20% females) had del13q14, while the prevalence of del13q14 was found to be higher in women compared with men (60 vs. 31.56%) in other studies.[16] In our study, we had no significant difference between hemoglobin <11 and del13q14. ►Table 4 Furthermore, a study reported that individuals with del13q were more likely to experience anemia.[16] In this regard, however, we found no significant difference between WBC mean and del13q14. The results of Rahimi et al indicated that patients with del13q14 exhibited a higher WBC count.[16]

Table 1 Clinical and biological variables in male versus female CLL patients

Clinical and biological variables	Male (mean ± SE)	Female (mean ± SE)	*p*-value
Age	60.25 ± 1.23	56.45 ± 2.35	0.005
White blood cell count (×10^9/L)	47. 59 ± 8.3	48.28 ± 8.05	0.94
Hemoglobin (g/dL)	14.34 ± 0.32	12.11 ± 0.26	0.002
Platelets count (×10^9/L)	157.96 ± 7.31	205.33 ± 18.12	0.002

Abbreviations: CLL, chronic lymphocytic leukemia; SE, standard error.

Table 2 Frequency of chromosomal abnormality in male and female patients with CLL

Chromosomal abnormalities	Mean age ± SD	Frequency (male/female)	*p*-value
Sole chromosomal abnormality			
del13q	61.17 ± 11.64	13/10	0.31
del 11q	–	0/0	–
del17p	55.4 ± 15	7/8	0.08
trisomy 12	55.34 ± 7.23	3/0	0.54
Two chromosomal abnormalities			
del13q + del 11q	60.67 ± 6.11	1/2	0.25
del13q + del17p	57.37 ± 9.44	17/7	0.8
del13q + trisomy 12	70.25 ± 7.23	3/1	0.99
del17p + del 11q	60.33 ± 13.87	2/1	0.99
del17p + trisomy 12	67.33 ± 9.81	2/1	0.25
del 11q + trisomy 12	–	0/0	–
Three chromosomal abnormalities			
del13q + del 11q + del17p	54.67 ± 9.9	3/0	0.54
del13q + del 11q + trisomy 12	75	1/0	0.99
del13q + del17p + trisomy 12	58	2/0	0.99
del17p + del 11q + trisomy 12	–	0/0	–
del13q + del17p + del 11q + trisomy 12	–	0/0	–
No chromosomal abnormality			
del13q/del 17p/del 11q/trisomy 12	59.55 ± 11.25	9/2	0.33

Abbreviations: CLL, chronic lymphocytic leukemia; SD, standard deviation.

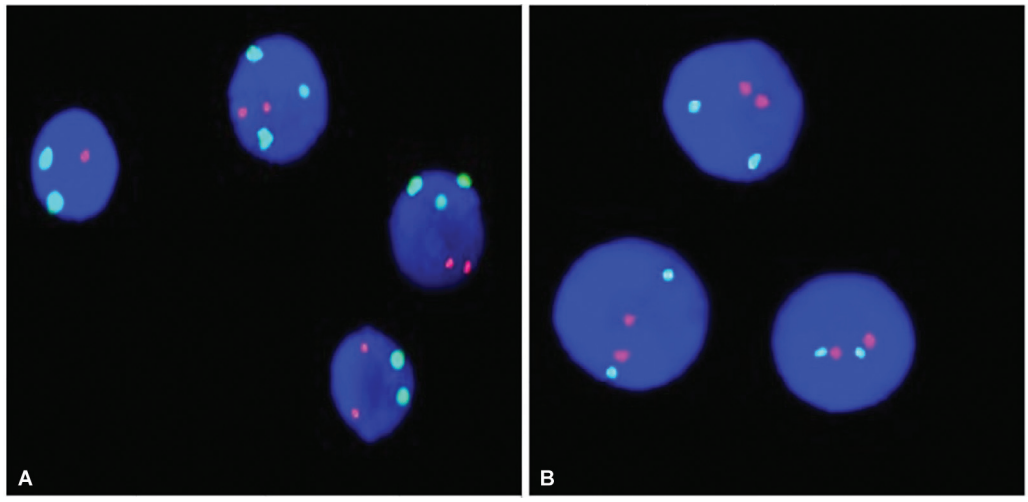

Fig. 1 Interphase fluorescent in situ hybridization. (**A**) Trisomy 12(three green signal) and deletion 13q14(single red signal). (**B**) normal 17p13 (two red signals) and 11q22(two green signals).

Deletion of the short arm of chromosome 17 is a commonly acquired abnormality in CLL often observed after treatment.[4,19] The incidence of del17p ranges from 5 to 9% in individuals who have recently been diagnosed with CLL, but it can reach up to 50% in those with relapsed/refractory (R/R) cases.[8,22] In our study, 15 and 35% of cases had del17p solely and with other abnormalities, respectively. High frequency of this abnormality in our study in comparison with other reports might be related to a delay in initial medical attention, thereby manifesting a secondary abnormality. Concordantly, Rahimi et al reported that 13.63% of CLL cases displayed the deletion of 17p.[16] Gogia et al reported del17p in 11.4% of treatment-naive Indian patients with CLL utilizing the FISH technique.[23] The 17p deletion often involves the region that encodes the TP53 gene.[24] Deletion of chromosome 17p might represent the most aggressive CLL subset

Table 3 CLL patients with good, intermediate, and poor prognoses

Prognosis classification	Frequency	Hemoglobin (mean ± SD)	Platelet count (mean ± SD)
Good prognosis			
del13q	23 (13 males and 10 females)	13.8 ± 1.9	157 ± 71.8
Intermediate			
Trisomy 12	3 (3 males and 0 females)	15.45 ± 3.4	163 ± 23.8
del13q + trisomy 12	4 (3 males and 1 female)	14.8 ± 0.52	190 ± 51.4
Poor prognosis			
del 11q Sole	0	–	–
del17p	15 (7 males and 8 females)	12.7 ± 2.5	160.46 ± 72.9
del13q + del 11q	3 (1 male and 2 females)	12.26 ± 2.3	329.6 ± 93.3
del17p + del 11q	3 (2 males and 1 female)	11.8 ± 1	119 ± 98.5
del13q + del 11q + del17p	3 (3 males and 0 females)	14.8 ± 1.5	161.3 ± 99.7
del13q + del 11q + trisomy 12	1 (1 male and 0 females)	15.4	54
del13q + del17p + trisomy 12	2 (2 males and 0 females)	15.9	130
del17p + del 11q + trisomy 12	0	–	–
del13q + del17p + del 11q + trisomy 12	0	–	–

Abbreviations: CLL, chronic lymphocytic leukemia; SD, standard deviation.

displaying treatment failure.[25] In fact, del17p are thought to represent approximately 40% of the cases that are resistant to treatment. Thus, patients with 17p deletion are categorized as the highest-risk group with the shortest survival rate.[8,26] In addition, according to Nelson et al, the majority of cases in the poor prognostic FISH groups, specifically those with sole del17p and del11q, were found to have advanced disease with Rai stage III or IV.[27]

Another chromosomal abnormality found in CLL is trisomy 12. Our results showed this trisomy was detected (solely in 3 patients and coexisting with other abnormalities in 10 patients). This aberration was detected in 10 to 20% of patients in studies.[28] Trisomy 12 is frequently associated with atypical lymphocyte morphology, advanced disease, and an aggressive clinical course.[29]

An additional genetic alteration in CLL is the deletion of the long arm of chromosome 11, which is observed in 5 to 20% of patients.[8,30,31] Approximately 20% of CLL patients have del 11q, and an increased risk of relapse after undergoing chemotherapy.[32] In our study, deletion 11q had the lowest frequency (sole abnormality = 0, coexisting with other abnormalities = 10). Rahimi et al also reported that 9.09% of cases demonstrated the deletion of 11q.[16] This region, which includes the ataxia-telangiectasia mutated (ATM) gene, has been extensively studied in CLL.[33] The ATM tumor suppressor gene codes for a protein that acts upstream of p53 in the DNA damage response pathway. However, it has been found in 8 to 30% of patients with 11q deletions, indicating that other genes play more significant role in these deletions.[33] BIRC3, a gene located near the ATM gene, is one of these genes that play a role in 11q deletion.[34] However, a study by Rose-Zerilli et al suggested that ATM mutations have a greater impact on the progression-free survival and overall survival of 11q-deleted patients undergoing first-line therapy, compared with BIRC3 deletion.[35]

In conclusion, our results indicated that most patients with CLL had chromosomal abnormalities and patients with normal I-FISH test were rare. Deletion of 13q14 and 17p regions had the highest frequency in CLL patients irrespective of gender. The elevated occurrence of this irregularity in our study may be associated with the delay in initial medical attention, leading to the manifestation of a secondary abnormality. More studies with more participants are recommended for verifying the results. Additionally, parallel application of karyotype, I-FISH, and metaphase fluorescence in situ hybridization (mFISH) is suggested.

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Conflict of Interest
None declared.

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Table 4 Hemoglobin, platelet, and white blood cell (WBC) index in chronic lymphocytic leukemia (CLL) patients with chromosomal abnormalities

Chromosomal abnormalities	Hemoglobin (>11 or <11), *p*-value	Platelet (>10,000 or <100,000), *p*-value	White blood cell (mean)
Sole chromosomal abnormality			
del13q	0.44	0.9	0.9
del 11q	–	–	–
del17p	0.64	0.25	0.9
Trisomy 12	0.9	0.9	0.58
Two chromosomal abnormalities			
del13q + del 11q	0.27	0.9	0.9
del13q + del17p	0.7	0.1	0.1
del13q + trisomy 12	0.9	0.9	0.78
del17p + del 11q	0.9	0.06	0.37
del17p + trisomy 12	0.27	0.41	0.53
del 11q + trisomy 12	–	–	–
Three chromosomal abnormalities			
del13q + del 11q + del17p	0.9	0.41	0.9
del13q + del 11q + trisomy 12	0.9	0.16	–
del13q + del17p + trisomy 12	0.9	0.9	0.53
del17p + del 11q + trisomy 12	–	–	–
del13q + del17p + del 11q + trisomy 12	–	–	–
No chromosomal abnormality			
del13q/del 17p/del 11q/trisomy 12	0.32	0.9	–

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