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Genomic Aberrations in Egyptian Chronic Lymphocytic Leukemia Patients

HALA M. FARAWILA, M.D.*; HAMDI M. ZAWAM, M.D.**, MANAL M. MAKHLOUF, M.D.* and ZEINAB A. HASSAN, M.D.*

The Departments of Clinical & Chemical Pathology and Medical Oncology**, Faculty of Medicine, Cairo University*

ABSTRACT

Background: B-chronic lymphocytic leukemia is a heterogeneous disease with a highly variable clinical course and prognosis. Several clonal chromosomal aberrations which have different prognosis were found in CLL, the most common chromosomal abnormalities include del 13q14.3, del 11q22.3 (ATM gene), trisomy 12, del 17p13.1 (tumor suppressor gene p53) and del 13q34.

Aim of the Work: Was to determine the prevalence of the common recurring genetic defects in an Egyptian cohort of B-CLL patients and to evaluate the correlation of these genetic defects with the clinical presentation, laboratory data, response to induction therapy, disease progression, prognosis as well as overall survival.

Patients and Methods: This study included 30 B-CLL patients as well as 15 age and sex matched normal healthy subjects as a control group. All patients were subjected to Fluorescence In Situ Hybridization (FISH) technique for detection of different genomic aberrations including del 13q14.3, del 11q22.3, trisomy 12, del 17p13.1 and del 13q34.

Results: Our study revealed that the CLL FISH panel detected genomic abnormalities in 28 patients (93%). Twelve CLL patients (40%) had del 13q14.3, 7 patients (23%) had del 11q22.3, 19 patients (63%) had trisomy 12, 12 patients (44%) had del 17p13.1, while 13 patients (43%) had del 13q34. There was a statistically significant difference between CLL patients with positive and negative different genomic aberrations as regards Rai and Binet staging system and immunophenotyping including CD38 and ZAP70 ($p<0.05$) except for del 11q22.3. Also, there was a highly statistically significant difference between CLL patients with positive and negative different genomic aberrations as regards serum LDH, B2-microglobulin, number of chemotherapy cycles, response to chemotherapy and total free survival ($p<0.01$) except for the number of chemotherapy cycles in del 11q22.3 and a significant difference was found as regards overall survival ($p<0.05$). While there was no statistically significant difference as regards BM lymphocyte percent and duration of illness ($p>0.05$) except for del 13q34 which showed a significant difference ($p<0.05$).

Conclusion: Multiple genomic aberrations can play an important role in the clinical presentation, prognosis, disease progression and response to chemotherapy as well as overall survival in an Egyptian cohort of CLL patients as compared to others.

Key Words: *Del 13q14.3 – Del 11q22.3 – Trisomy 12 – Del 17p13.1 – Del 13q34 – FISH – CLL.*

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes. It is the most common form of leukemia found in adults in Western countries **[1]**. Some patients show an indolent disease and never require treatment, while in others the clinical course is aggressive requiring intensive treatment shortly after diagnosis **[2]**.

The staging systems developed by Rai et al. **[3]** and Binet et al. **[4]** have been recognized as standard methods of assessing the survival and the treatment requirements in B-CLL patients. However, these systems can not identify stable or progressive forms of the disease, especially in the early stages of B-CLL, which include most of the patients at diagnosis. The substantial heterogeneity within clinical stages has prompted for additional prognostic factors **[5]**.

Identifying chromosomal aberrations could pinpoint subgroups of patients with chronic lymphocytic leukemia who have different prognosis. These abnormalities are independent predictors of the disease progression and survival, as well as response to standard chemotherapy and monoclonal antibody therapy **[6]**.

Conventional cytogenetic analysis has been hampered by the low mitotic activity and proliferative index of leukemia cells in vitro, and

even with mitogens; metaphases are difficult to obtain. Fluorescence In Situ Hybridization (FISH) allows the detection of chromosomal aberration not only in dividing cells but also in interphase cells **[7]**. Most commonly, these abnormalities are deletions or aneuploidy, and in contrast to other low-grade lymphoid malignancies, translocations are infrequent **[8]**.

The most common chromosomal abnormalities in CLL are del 13q14.3, del 11q22.3 (ATM gene), trisomy 12, del 17p13.1 (tumor suppressor gene p53) and del 13q34. These findings have implications for the design of risk-adapted treatment strategies **[9]**.

Del 13q14.3 is the most frequent structural abnormality in CLL. Cases with this abnormality usually have mutations of the IgHV gene, classic CLL cell morphology, and good prognosis. Also, patients with del 13q34 had a similar survival to those with normal chromosomes and only one-third requires therapy **[10]**.

The ataxia telangiectasia (ATM) gene is located within the minimal region of loss at 11q22.3, suggesting that alterations in this gene may be involved in the pathogenesis of the disease. This is further supported by the finding that mutations in the ATM gene are associated with poor prognosis **[11]**.

Trisomy 12 occurs as a result of duplication of one homolog. Chromosome 12 contains the murine double minute (mdm-2) gene, and overexpression of mdm-2 could stimulate a p53 mutation, as mdm-2 binds and inactivates p53 **[12]**. While, del 17p13.1 involves the p53 locus at 17p13.1; that deletion and mutations in the p53 gene can contribute to disease progression and drug resistance **[13]**.

CLL cells often exhibit multiple cytogenetic abnormalities that may be involved in the pathogenesis of the disease, prognosis, disease progression and alter the sensitivity of CLL cells to chemotherapy agents. These findings have implications for the design of risk-adapted treatment strategies **[14]**.

Aim of the work:

The aim of this work was to study the prevalence of the common recurring genetic defects in an Egyptian cohort of B-CLL patients including del 13q14.3, del 11q22.3 (ATM gene), trisomy 12, del 17p13.1 (tumor suppressor gene p53) and del 13q34. We also aimed to evaluate the correlation of these genetic defects with the clinical presentation, laboratory data, response to induction therapy, disease progression, prognosis as well as overall survival of these patients.

PATIENTS AND METHODS

Patients:

The present study was conducted on 30 patients with B-chronic lymphocytic leukemia, their ages ranged between 42 and 80 years with a mean of 57.96±10.55 and a median of 56 years. They were 23 male (77%) and 7 female (23%). Patients were diagnosed and selected among cases referred to the Haematology Clinic and the Haematology-Oncology Unit at El-Kasr El-Aini Hospital, Cairo University. Fifteen age and sex matched normal healthy individuals were also included as a control group.

The diagnosis of leukemia was based on complete history taking, clinical examination for organomegaly and lymphadenopathy and laboratory investigations for diagnosis and prognosis of B-CLL including complete blood count, bone marrow aspirate, immunophenotyping of lymphoid cells, serum LDH, B2-microglobulin and special laboratory investigations (for patients and controls) for detection of different genomic aberrations including del 13q-14.3, del 11q22.3 (ATM gene), trisomy 12, del 17p13.1 (tumor suppressor gene p53) and del 13q34 using Fluorescence In Situ Hybridization (FISH) technique according to the method described by Nascimento et al. **[15]**.

Patients were studied prior to chemotherapy and followed up after induction chemotherapy. Patients were treated by one of the following lines of chemotherapy depending on age, performance status and stage of disease; chlorambucil (CIb) and prednisone: CIb was given orally at dose of 0.2mg/kg/day and predinisone 20mg/m^2/day. Cyclophosphmide, vincristine and prednisone (CVP): Cyclophosphmide 400 mg/m^2 IV on days 1-3, vincristine 1.4mg/m^2 IV on days 1 and oral prednisone 400mg/m^2 on days 1-5. Fludarabine, rituximab and cyclophosphamide: Fludarabine 25mg/m^2 IV, rituximab 375mg/m^2 IV and cyclophosphmide 250mg/m^2 IV on days 1-3. Response to induction chemotherapy was assessed according to the criteria proposed by the National Cancer Institute (NCI)-

sponsored working group prior to study SWOG-9108 **[16]**.

Evaluation of response to chemotherapy had been made according to the following criteria; Complete remission (CR): Asymptomatic patients with no organomegaly or lymphadenopathy. Lymphocyte count <4x10^3/µl, neutrophils >1.5x10^3/µl, hemoglobin >11gm/dl, platelets count >100x10^6/µl and bone marrow lymphocytes <30%. Partial remission (PR): More than 50% decrease in organomegaly or lymphadenopathy plus one of the following: Neutroplils >1.5x10^3/µl, hemoglobin >11gm/dl and platelets count >100x10^6/µl. Progressive disease (PD): New lesion or >50% increase in organomegaly or lymphadenopathy, circulating lymphocytes revealing >50% increase.

Methods:

1- Sample collection:

Seven milliliters of venous blood were collected from each patient and each individual of the control group by sterile venipuncture under aseptic precautions and divided as follows: 2ml on ethylene diamine tetra-acetic acid (EDTA) sterile vacutainer for performing a complete blood picture and immunophenotyping, the remaining 5ml on sodium-heparin sterile vacutainer for the study of different genomic aberrations by FISH technique.

2- Detection of different genomic aberrations using FISH technique:

I- Cell culturing and fixation:

Under the laminar flow, peripheral blood lymphocytes were cultered. The culture media (Gibcobrl) comprised of 5ml RPMI culture medium, 50µl pencillin-streptomycin (1%), 1ml fetal calf serum (20%), 1ml patient's cell-rich plasma. After 2 hours, 100µl colcemide (Boehringer) was added followed by 5ml hypotonic solution then 4 times wash with methanol-acetic acid.

II- *FISH technique for the study of different genomic aberrations:*

Cytogenetic abnormalities using FISH technique was detected using five DNA probes in two sets (Abott Vysis, Downers Grove, IL, USA):

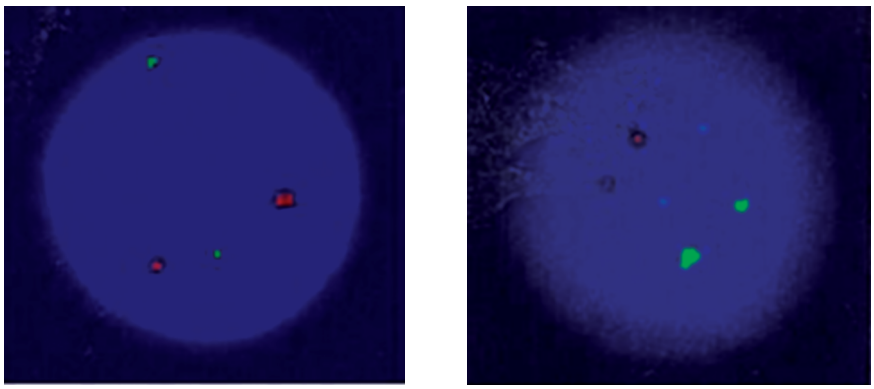
- Probe set 1: Allows assessment of the following chromosomal regions; LSI p53 (17p13.1) and LSI ATM (11q22.3) in two orange and two green signals respectively.
- Probe set 2: Allows assessment of the following regions; LSI D13S319 (13q14.3), LSI 13q34 (13q34), CEP12 (12p11.1-q11) in one mix as two orange, two aqua and two green signals respectively.
- The two probes were set up separately on 2 different slides prepared from fixed cell pellets for each patient. Hybridization was done using Vysis hybrit TM according to the manufacturer's protocols and rapid wash procedure was performed, then 10µl 4-6-diaidino-2-phenylindole dihydrochloride (DAPH II) counterstain were added to target areas and covers applied to slides.

III- *Detection of hybridization signals:*

Detection of hybridization signals were performed using epi-thrombin fluorescence Olympus microscope equipped with filters capable of simultaneously passing DAPI/FITC/Texas red and Quips spectra Vysis hardware and software. The hybridized probes fluoresce with bright intensity both in interphase nuclei and on metaphase chromosomes. Two hundred interphase cells were counted and the results taken according to the signals detected in a score system in which the number of bright distinct fluorescent spots in each nucleus was recorded as 0, 1, 2, 3, 4 signals.

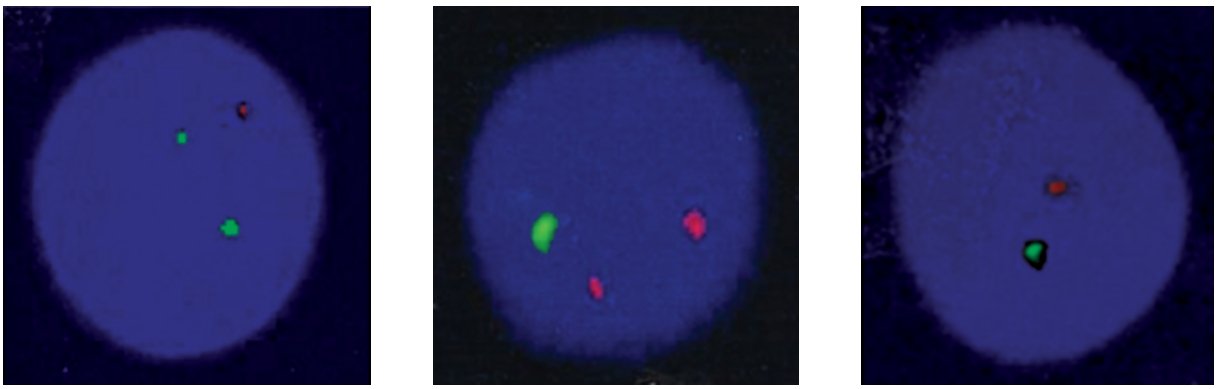
IV- *Interpretation of results:*

- Normal: In interphase nuclei of normal cells, the probe set 1 generally appears as two distinct orange signals and two green signals for p53 (17p13.1) and ATM (11q22.3) respectively as shown in Fig. (1a), Probe set 2 appears as two distinct orange signals, two aqua and two green signals for D13S319 (13q14.3), 13q34 (13q34), CEP12 (12p11.1-q11) respectively as shown in Fig. (1b). The signals may appear split or diffused. Occasionally, the probe may show extra-signals (diffuse or split) depending upon the condensation of the DNA and the relative distance between chromatids.
- Deletion: p53 (17p13.1), ATM (11q22.3), D13S319 (13q14.3) and 13q34 (13q34) areas appear as single orange, green, orange and aqua signal respectively as shown in Figs. (2,3) using the same technique.
- Trisomy: CEP12 (12p11.1-q11) area appears as 3 green signals as shown in Fig. (3b,c).



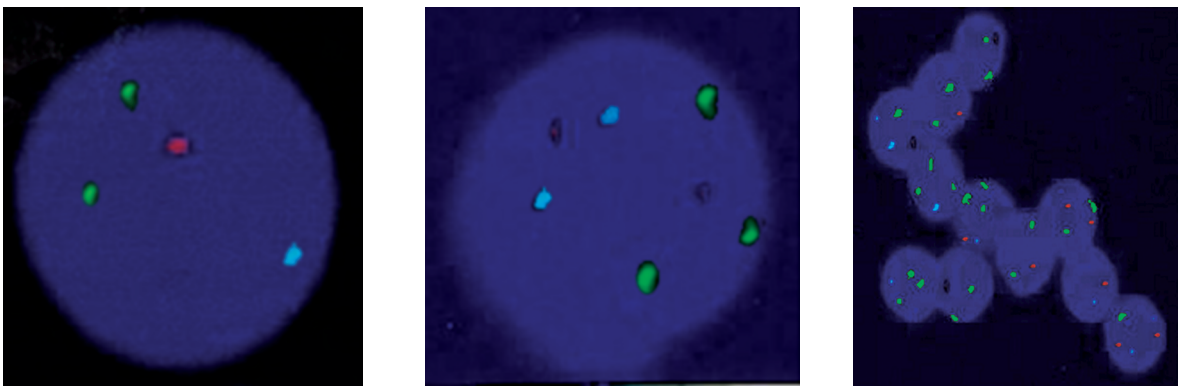
(A) **(B)**

Fig. (1): FISH technique using: (a) Probe set 1 (LSI P53 and LSI ATM) showing normal chromosome 17p13.1 and chromosome 11q22.3 in two orange and two green signals respectively. (b) Probe set 2 (LSI D13S319, LSI 13q34 and CEP12) showing normal chromosome 13q14.3, chromosome 13q34 and chromosome 12 in two orange, two aqua and two green signals respectively.



(A) **(B)** **(C)**

Fig. (2): FISH technique using probe set 1 (LSI P53 and LSI ATM) showing: (a) Del 17p13.1 and normal chromosome 11q22.3 in one orange and two green signals respectively. (b) Normal chromosome 17p13.1 and del 11q22.3 in two orange and one green signals respectively. (c) Del 17p13.1 and del 11q22.3 in one orange and one green signals respectively.



(A) **(B)** **(C)**

Fig. (3): FISH technique using probe set 2 (LSI D13S319, LSI 13q34 and CEP12) showing: (a) Del 13q14.3, del 13q34 and normal chromosome 12 in one orange, one aqua and two green signals respectively. (b) Normal chromosome 13q14.3, chromosome 13q34 and trisomy 12 in two orange, two aqua and three green signals respectively. (c) Del 13q14.3, del 13q34 and trisomy 12 in one orange, one aqua and three green signals respectively.

For each probe, two hundred interphase cells of the control subjects were counted. Means and standard deviations (SD) of the percentage of the nuclei with hybridization signals were calculated. Results were considered abnormal, if the percent of nuclei with the abnormal hybridization signal >2SD from the mean.

Our cut off values were as follows:

10% for del 13q14.3 (D13S319), 7% for del 11q22.3 (ATM gene), 5% for trisomy 12 (12p11.1-q11), 10% for del 17p13.1 (p53) and 9.5% for del 13q34.

Statistical analysis:

Data was analyzed using SPSS win statistical package version 15. Numerical data were expressed as a mean, standard deviation (SD) and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher's Exact test was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using the Mann-Whitney test (non-parametric *t*-test). Relation between numerical variables was tested using Pearson product-moment correlation coefficient. A *p*-value less than 0.05 was considered significant and a *p*-value less than 0.01 was considered highly significant.

RESULTS

The patients' characteristics are displayed in Tables (1,2).

Different genomic aberrations detected in B-CLL patients are shown in Table (3).

Table (4) demonstrates the correlation of different genomic aberrations with clinical, laboratory, staging systems and prognostic parameters in B-CLL patients.

Table (1): Clinical and laboratory data of 30 B-CLL patients.

Item	B-CLL Patients (No. 30)
Age (years)	57.96±10.55 (42-80)*
Gender	
Male	23 (77%)**
Female	7 (23%)
Clinical data	
Hepatomegaly	18 (60%)
Splenomegaly	21 (70%)
Lymphadenopathy	7 (90%)
Laboratory data	
Hb gm/dl	9.42±2.49 (5.50-14.20)
TLC x 10^9/L	66.39±48.78 (9.80-212.30)
Platelets x 10^9/L	156.33±84.24 (30.0-373.0)
PB lymphocytes %	78.33±16.66 (40-98)
PB absolute lymphocytic count x10^9/L	62.84±51.90 (5.60-191.07)
Immunophenotyping	
CD38	12 (40%)
ZAP-70	13 (43%)

* Mean ± SD (range).
** No. (%)

Table (2): Different staging systems, prognostic parameters and treatment outcome in 30 B-CLL patients.

Item	B-CLL Patients (No. 30)
Different staging systems	
Rai staging system:	
Stage Modified stage	
0 Low risk	1 (3%)*
I Intermediate risk	8 (27%)
II Intermediate risk	3 (10%)
III High risk	8 (27%)
IV High risk	10 (33%)
Binet staging system:	
A	7 (23%)
B	10 (33%)
C	13 (44%)
Different prognostic factors	
Serum LDH U/L	612.45±215.27 (258.00-952.00)**
B2-microglobulin mg/dl	3.13±0.98 (1.80-4.80)
BM lymphocyte %	48.16±16.75 (35.00-75.00)
Duration of illness in years	5.28±2.16 (2.00-12.00)
Number of chemotherapy cycles	3.92±2.80 (0.00-9.00)
Treatment outcome	
Initial response to chemotherapy	
Complete remission (CR)	14 (47%)
Partial remission (PR)	10 (33%)
Progressive disease (PD)	6 (20%)
Total free survival (TFS) (months)	41.85±27.20 (6.00-120.00)
Overall survival (months)	45.73±29.38 (7.00-120.00)
Alive	26 (87%)
Death	4 (13%)

* No. (%).
** Mean ± SD (range).

Table (3): Different genomic aberrations detected in 30 B-CLL patients by FISH technique.

Item	B-CLL Patients (No. 30)
Cytogenetic abnormalities	28 (93%)*
Del 13q14.3	12 (40%)
Del 11q22.3	7 (23%)
Trisomy 12	19 (63%)
Del 17p13.1	12 (40%)
Del 13q34	13 (43%)

* No. (%).

Table (4): Correlation of different genomic aberrations with clinical, laboratory, staging systems and prognostic parameters in 30 B-CLL patients.

Item	Del 13q14.3	Del 11q22.3	Trisomy 12	Del 17q13.1	Del 13q34
Age	0.412 (0.04)*	0.036 (NS)	–0.469 (0.02)	–0.147 (NS)	0.395 (0.03)
Gender	0.216 (NS)	0.051 (NS)	0.187 (NS)	0.182 (NS)	0.171 (NS)
Clinical data					
Hepatomegaly	–0.182 (NS)	0.125 (NS)	0.503 (0.03)	–0.158 (NS)	–0.018 (NS)
Splenomegaly	–0.471 (0.01)	0.396 (0.03)	0.014 (NS)	0.091 (NS)	–0.192 (NS)
Lymphadenopathy	–0.506 (0.009)	0.597 (0.008)	0.562 (0.03)	0.124 (NS)	–0.494 (0.04)
Laboratory data					
Hb gm/dl	0.115 (NS)	–0.055 (NS)	–0.374 (0.01)	–0.016 (NS)	0.392 (0.02)
TLC x 10^9/L	–0.032 (NS)	0.181 (NS)	0.432 (0.04)	0.157 (NS)	–0.466 (0.009)
Platelets x 10^9/L	0.205 (NS)	–0.018 (NS)	–0.355 (0.03)	–0.369 (0.02)	0.137 (NS)
PB lymphocytes %	–0.417 (0.03)	0.182 (NS)	0.186 (NS)	0.040 (NS)	–0.042 (NS)
Absolute lymphocytic count	–0.482 (0.01)	0.229 (NS)	0.092 (NS)	0.129 (NS)	–0.002 (NS)
Immunophenotyping					
CD38	–0.650 (0.003)	0.031 (NS)	0.794 (0.001)	0.438 (0.02)	–0.634 (0.004)
ZAP-70	–0.789 (0.001)	0.135 (NS)	0.635 (0.004)	0.399 (0.01)	–0.739 (0.001)
Different staging systems					
Rai staging system	–0.482 (0.004)	0.339 (0.03)	0.625 (0.001)	0.475 (0.04)	–0.617 (0.001)
Binet staging system	–0.597 (0.001)	0.350 (0.01)	0.537 (0.002)	0.428 (0.02)	–0.540 (0.002)
Different prognostic factors					
Serum LDH U/L	–0.483 (0.002)	0.425 (0.001)	0.689 (0.002)	0.746 (0.003)	–0.726 (0.002)
B2-microglobulin mg/dl	–0.495 (0.007)	0.384 (0.002)	0.562 (0.003)	0.524 (0.005)	–0.503 (0.001)
BM lymphocyte %	–0.162 (NS)	0.124 (NS)	0.219 (NS)	0.203 (NS)	–0.619 (0.02)
Duration of illness in years	–0.369 (0.04)	0.281 (NS)	0.324 (0.03)	0.189 (NS)	–0.918 (0.008)
No. of chemotherapy cycles	–0.571 (0.005)	0.237 (NS)	0.867 (0.001)	0.817 (0.003)	–0.723 (0.004)
Total free survival	0.625 (0.002)	–0.576 (0.01)	–0.426 (0.02)	–0.682 (0.002)	0.553 (0.009)
Overall survival	0.446 (0.01)	–0.407 (0.02)	–0.365 (0.04)	–0.435 (0.03)	0.487 (0.02)

* *r*-value (*p*-value). NS: Non-significant.

Statistical comparison between CLL patients with positive and negative different genomic aberrations as regards age, gender and clinical data was studied. Comparison revealed no significant difference as regards age, gender and clinical data ($p>0.05$) except for age which showed significant difference in del 13q14.3, trisomy 12 and del 13q34 positive and negative patients ($p=0.04$, 0.03 and 0.04 respectively), while hepatomegaly showed significant difference in trisomy 12 only ($p=0.04$), and splenomegaly showed significant difference in del 13q14.3 and del 11q22.3 ($p=0.02$ and 0.03 respectively), and lastly, lymphadenopathy showed significant difference in del 13q14.3, del 11q-22.3, trisomy 12 and del 13q34 ($p=0.03$, 0.02, 0.04 and 0.04 respectively).

Regarding laboratory data, comparison between CLL patients with positive and negative different genomic aberrations revealed no significant difference between different groups ($p>0.05$) except for P.B. absolute lymphocytic count in del 13q14.3 which showed significant difference ($p=0.03$), haemoglobin level in trisomy 12 which showed highly significant difference ($p=0.008$), platelets count in del 17q13.1 which showed significant difference ($p=0.04$)

and lastly, haemoglobin level and total leucocyte count in del 13q34 which showed significant difference (p=0.03 and 0.02 respectively). While regarding immunophenotyping, comparison revealed a significant difference between CLL patients with positive and negative different genomic aberrations as regards CD38 and ZAP-70 (p<0.05) except for del 11q22.3, in which CD38 and ZAP-70 expression was almost absent in del 13q14.3 and del 13q34 positive cases but increased in trisomy 12 and del 17q13.1 positive cases. No significant difference was found as regards other immunophenotyping markers (p>0.05).

Table (5) displays statistical comparison between CLL patients with positive and negative different genomic aberrations as regards different staging systems. A significant difference was found as regards Rai and Binet staging system (p<0.05), in which del 13q14.3 and del 13q34 were common with early Rai and Binet stages while del 11q22.3, trisomy 12 and del 17q13.1 were common in late stages.

Table (5): Comparison between 30 B-CLL patients with positive and negative different genomic aberrations as regards different staging systems.

Item	Del 13q14.3		Del 11q22.3		Trisomy 12		Del 17q13.1		Del 13q34	
	+ve (No.12)	–ve (No.18)	+ve (No.7)	–ve (No.23)	+ve (No.19)	–ve (No.11)	+ve (No.12)	–ve (No.18)	+ve (No.13)	–ve (No.17)
Rai staging system:										
0	1 (100%)*	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)
I	7 (87.5%)	1 (12.5%)	0 (0%)	8 (100%)	1 (12.5%)	7 (87.5%)	1 (12.5%)	7 (87.5%)	8 (100%)	0 (0%)
II	1 (33%)	2 (67%)	1 (33%)	2 (67%)	3 (100%)	0 (0%)	1 (33%)	2 (67%)	2 (67%)	1 (33%)
III	2 (25%)	6 (75%)	3 (37.5%)	5 (62.5%)	5 (62.5%)	3 (37.5%)	4 (50%)	4 (50%)	1 (12.5%)	7 (87.5%)
IV	1 (10%)	9 (90%)	3 (30%)	7 (70%)	10 (100%)	0 (0%)	6 (60%)	4 (40%)	1 (10%)	9 (90%)
p-value	0.03		0.02		0.001		0.03		0.001	
Binet staging system:										
A	6 (86%)	1 (14%)	1 (14%)	6 (86%)	2 (28.5%)	5 (71.5%)	0 (0%)	7 (100%)	7 (100%)	0 (0%)
B	4 (40%)	6 (60%)	1 (10%)	9 (90%)	7 (70%)	3 (30%)	3 (30%)	7 (70%)	5 (50%)	5 (50%)
C	2 (15%)	11 (85%)	5 (38.5%)	8 (61.5%)	10 (77%)	3 (23%)	9 (69%)	4 (31%)	1 (7%)	12 (92%)
p-value	0.002		0.009		0.001		0.02		0.001	

* No. (%).

Statistical comparison between CLL patients with positive and negative different genomic aberrations as regards prognostic factors is shown in Table (6). Comparison revealed a highly significant difference as regards serum LDH, B2-microglobulin and number of chemotherapy cycles (p<0.01) except for number of chemotherapy cycles in del 11q22.3 which showed insignificant difference (p>0.05). Del 13q14.3 and del 13q34 positive cases showed lower serum LDH, B2-microglobulin levels and decreased number of chemotherapy cycles, while del 11q22.3, trisomy 12 and del 17q13.1 positive cases showed higher serum LDH, B2-microglobulin levels and increased number of chemotherapy cycles. While no significant difference was found between CLL patients with positive and negative different genomic aberrations as regards BM lymphocyte percent and duration of illness (p>0.05) except for del 13q34 which showed significant difference (p=0.04 and 0.02 respectively), as decreased BM lymphocyte percent and duration of illness was found in positive del 13q34 cases.

Statistical comparison between CLL patients with positive and negative different genomic aberrations as regards treatment outcome (initial response to chemotherapy), total free survival and overall survival was studied. There was a highly significant difference as regards treatment outcome and total free survival (p<0.01), as del 13q14.3 and del 13q34 positive cases had a higher incidence of favorable outcome (complete remission) and higher total free survival, while del 11q22.3, trisomy 12 and 17q13.1 positive cases had a higher incidence of unfavorable outcome (partial remission and progressive disease) and lower total free survival. Lastly, a significant difference was found as regards overall survival (p<0.05), in which del 13q14.3 and del 13q34 positive cases showed longer survival and lower death rate than del 11q22.3, trisomy 12 and del 17q13.1 positive cases.

Table (6): Comparison between 30 B-CLL patients with positive and negative different genomic aberrations as regards prognostic factors.

Item	Serum LDH U/L	B2-microglobulin mg/dl	BM lymphocyte %	Duration of illness in years	Number of chemotherapy cycles
Del 13q14.3					
+ve (No.12)	331.21±54.39*	2.02±0.89	39.42±3.84	5.21±3.11	3.21±2.77
–ve (No.18)	680.22±116.19	4.13±0.35	57.00±18.81	6.35±1.68	6.50±1.84
p-value	0.003	0.004	0.34	0.08	0.002
Del 11q22.3					
+ve (No.7)	744.78±174.02	3.84±0.46	53.00±19.13	6.73±1.67	5.10±2.60
–ve (No.23)	362.09±61.79	1.98±0.18	38.50±4.94	4.13±3.19	2.09±1.46
p-value	0.001	0.001	0.09	0.23	0.14
Trisomy 12					
+ve (No.19)	784.62±174.03	4.10±0.75	57.66±20.52	5.62±1.66	5.87±2.09
–ve (No.11)	506.50±164.87	2.18±0.95	38.66±3.05	4.69±2.35	2.73±1.52
p-value	0.001	0.001	0.25	0.06	0.001
Del 17q13.1					
+ve (No.12)	763.00±180.97	3.81±0.70	51.21±18.23	5.15±1.18	6.38±1.94
–ve (No.18)	557.07±208.70	1.84±0.95	35.98±3.01	4.34±2.50	3.42±2.24
p-value	0.007	0.003	0.38	0.35	0.002
Del 13q34					
+ve (No.13)	352.44±58.06	1.98±0.62	38.50±4.94	4.72±1.25	2.55±1.50
–ve (No.17)	600.00±172.02	3.53±0.35	58.01±19.97	6.10±3.57	6.50±1.84
p-value	0.001	0.001	0.04	0.02	0.005

* Mean ± SD.

DISCUSSION

B-cell chronic lymphocytic leukemia (B-CLL) is a B-cell neoplasm characterized by an indolent course with progressive splenic and lymph node enlargement associated with chronic lymphocytosis **[17]**. Patients with B-CLL follow heterogeneous clinical courses. Some survive for a long time without therapy, while others die rapidly despite aggressive treatment **[18]**.

Chromosomal abnormalities play a major role in the pathogenesis of CLL, the study of leukemia specific cytogenetic abnormalities has contributed greatly to the clinical diagnosis and identification of specific chromosomal abnormalities are important for predication of the disease progression and survival, as well as response to chemo and monoclonal antibody therapy and for stratification of patients into the appropriate treatment protocols **[17]**.

The most important numerical and structural abnormalities found in CLL include trisomy 12 and deletions in several chromosome regions, such as 13q14.3, 13q34, 11q22.3 and 17p13.1 as well as other less frequently occurring aberrations. They have been reported to be of significant prognostic value in B-CLL **[19]**. A correlation between high-risk cytogenetic aberrations and unmutated IgVH genes has been found **[10]**.

This study included 30 B-CLL patients, their ages ranged between 42 and 80 years. They were 23 male (77%) and 7 female (23%). Fifteen age and sex matched normal healthy subjects were included as a control group. They were all subjected to FISH technique for detection of different genomic aberrations including del 13q14.3, del 11q22.3, trisomy 12, del 17p13.1 and del 13q34.

Our study detected genomic abnormalities in 28/30 patients (93%). This result was nearly similar to the results of other reasearchers who have reported the frequency of abnormalities as 81%, 82% and 80% respectively **[7,20,21]**. While other investigators detected the frequency as 52%, 68% and 75% respectively **[15,22,23]**. Their results were varied and lower than our results, this may be due to the difference of their cut off values or the difference of the number of patients they have studied.

In the present study, the most frequent abnormality was trisomy 12 (63%) in 19/30 patients which is higher than what was reported

by other investigators who detected chromosome 12 abnormalities in 19%, 23%, 23.3% and 25.5 % of CLL patients respectively **[21,23-25]**. The difference may be due to the higher number of their patients.

The second most frequent abnormality was involving chromosome 13 either of the loci 13q14.3 or 13q34. It was detected in 12/30 patients (40%) having del 13q14.3 and 13/30 patients (43%) having del 13q34. In concordance with our study, other researchers have detected this abnormality in 41%, 42% 43% and 45% of patients respectively **[6,21,24,25]**. Also, abnormalities of chromosome 17p13.1 (p53) was detected in almost equal percent to del 13q14.3 and del 13q34, it was detected in 12/30 patients (40%). Lower percentage of this abnormality 12%, 20%, 10% and 16% respectively were previously detected by other investigators **[6,15, 24,25]**. While others almost did not find p53 abnormality except in 3% and 2% of patients respectively **[19,23]**.

Finally, the least frequent abnormality was involving chromosome 11q22.3 (ATM locus). We found deletion in only 7/30 CLL patients (23%). This result is in accordance with previous studies which recorded this abnormality in 25%, 18%, 13% and 10.5% of their cases respectively **[19,21,24,25]**. Also, recent studies found ATM deletion but at a lower percent of 5% and 7% respectively **[9,15]**.

In the present study, our results showed a significantly higher age with del 13q14.3 and del 13q34 and lower age with trisomy 12 and insignificant correlation regarding age with other cytogenetic aberrations. This comes in agreement with recent studies which could not elicit a significant correlation regarding age **[15,24]**, while other researchers reported that patients with positive genomic aberrations showed significant difference when compared to negative patients regarding their age and found that favorable alterations (del 13q14.3, del 13q34) were seen more frequently among older patients, while unfavorable alterations (del 11q22.3, trisomy 12, del 17p13.1) were more frequently observed among younger patients and this matched our results in del 13q14.3, trisomy 12 and del 13q34 **[20,22]**.

Earlier studies found a significant correlation with gender and stated that there was a prevalence of female in the worse prognostic group (del 11q22.3, trisomy 12, del 17p13.1) and prevalence of male in the group with a better prognosis (del 13q14.3, del 13q34) **[15]**, but this is the contrary of our results as we could not find a significant correlation with gender, while our results are in agreement with what was previously reported in other studies **[24,25]**.

As regards clinical data, Patients with trisomy 12 were significantly presented almost all with hepatomegaly, and patients with del 13q14.3 were significantly less prone to present with splenomegaly while patients with del 11q22.3 were more prone to present with huge splenomegaly. and lastly, patients with del 13q14.3 and del 13q34 were less likely to have multiple lymphadenopathy while patients with del 11q22.3 and trisomy 12 were more likely to have multiple generalized lymphadenopathy. Other studies found a significant difference between the CLL patients with positive and negative different genomic aberrations regarding splenomegaly and lymphadenopathy as we detected and showed that del 11q22.3 and trisomy 12 were more likely to have splenomegaly and multiple bulky lymphadenopathy **[20,26]**.

Our findings demonstrated no significant difference between CLL patients with positive and negative different genomic aberrations regarding laboratory data except for a significant lower P.B. absolute lymphocytic count in patients with del 13q14.3, lower haemoglobin level in patients with trisomy 12, lower platelets count in patients with del 17q13.1 and lastly, higher haemoglobin level and lower total leucocyte count in patients with del 13q34. These results are in concordance with that recorded by previous studies which reported lower haemoglobin level, platelets count and higher total leucocyte count in del 11q22.3, trisomy 12, del 17p13.1 and higher haemoglobin level, platelets count and lower total leucocyte count in del 13q14.3, del 13q34 **[20]**. Also, recent studies reported no significant correlation between cytogenetic aberrations and P.B. absolute lymphocyte count and this comes in agreement with our results except for del 13q14.3 **[24]**.

In the current study, a significant difference was found between CLL patients with positive and negative different genomic aberrations regarding CD38 and ZAP-70 except for del 11q22.3. CD38 and ZAP-70 expression is almost

absent in cases with del 13q14.3 and del 13q34 but increased in cases with trisomy 12 and del 17q13.1. In aggreement with these results, recently reported researches could elicit as well a significant correlation regarding CD38 and reported that trisomy 12, del 17p13.1 and del 11q22.3 tended to present with high CD38 expression in comparison to the other aberrations. But, on the contrary, they could not elicit a significant correlation regarding ZAP-70 as we detected **[15,24,27]**, while earlier studies reported a significant correlation **[27]**, this may be attributed due to technical factors as different monoclonal antibodies and different gating strategies used in flowcytometry detection of ZAP-70.

Our findings showed a significant difference between CLL patients with positive and negative different genomic aberrations regarding Rai and Binet staging system, as patients with del 13q14.3 and del 13q34 tended to be A Binet stage and 0, I Rai stages, while in patients with del 11q22.3, trisomy 12, del 17p13.1 B, C Binet and II, III, IV Rai stages prevailed. This is in agreement with a number of previous studies **[13,15,19,20]**. In contrast to our results, other investigators showed insignificant difference **[6,24,25]**. This may be due to their large sample size compared with smaller sample size in our study.

As regards prognostic factors, our results demonstrated that cases with del 13q14.3 and del 13q34 showed a highly significant lower serum LDH, B2-microglobulin levels and decreased number of chemotherapy cycles, while cases with del 11q22.3, trisomy 12 and del 17q13.1 showed a highly significant higher serum LDH, B2-microglobulin levels and increased number of chemotherapy cycles. These results are in accordance with that of other researchers **[13,20]**. On the other hand, recently reported researches found insignificant difference **[24]**. This may be due to the difference of their normal values or the number of patients they have studied. Also, recent studies stated no significant differences between patients with positive and negative different cytogenetic aberrations regarding B.M. lymphocyte percent and duration of illness. This comes in consistency with our results, except for del 13q34 which showed a significant lower BM lymphocyte percent and duration of illness **[24]**.

Earlier studies reported that the percentage of cells with del 11q22.3, trisomy 12 and del 17q13.1 increased during follow-up period in CLL patients with signs of progressive disease, drug resistance and require more chemotherapy cycles and had shorter treatment-free intervals, while del 13q14.3 and del 13q34 do not carry an adverse prognostic significance or disease progression and require less therapy and had longer treatment-free intervals **[19,27-29]**. This comes in agreement with our results, as we found that cases with del 13q14.3 and del 13q34 showed a significant decreased number of chemotherapy cycles, good prognosis, favorable treatment outcome and better response to chemotherapy as well as longer total free survival, while cases with del 11q22.3, trisomy 12 and del 17q13.1 showed increased number of chemotherapy cycles, poor prognosis, unfavorable outcome and poor response to chemotherapy as well as shorter total free survival.

Lastly, a significant difference was found between CLL patients with positive and negative different genomic aberrations regarding overall survival ($p<0.05$), as cases with del 13q14.3 and del 13q34 showed longer survival and lower death rate than cases with del 11q22.3, trisomy 12q11.1 and del 17q13.1. In concordance with these results, previous studies have reported that the survival time was significantly shorter in patients with trisomy 12, p53 or ATM gene deletion, while patients with deletion at chromosome 13 had longer survival time **[19,20,25,29]**.

In conclusion, our study categorized chromosomal aberrations into two groups; first with favorable prognosis alterations with no adverse disease progression, better therapy response and longer survival which are 13q deletion whether 13q14.3 or 13q34, and second the unfavorable alterations with signs of disease progression, drug resistance and shorter survival were more frequently with del 11q22.3 (ATM), trisomy 12 and del 17q13.1 (p53). Additionally, chromosomal aberrations with poor prognosis tended to express CD38 and ZAP-70 proteins. Finally, multiple genomic aberrations can play an important role in the clinical presentation, prognosis, disease progression and response to chemotherapy as well as overall survival in an Egyptian cohort of CLL patients as compared to others.

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