

## CORRELATION OF TRISOMY 12 WITH CLINICAL FEATURES AND OTHER LABORATORY PARAMETERS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

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### ABSTRACT

**Objective:** To determine the frequency of trisomy 12 in B-Cell chronic lymphocytic leukaemia (CLL), to correlate its association with clinico-pathologic features and to determine the role of this cytogenetic defect to the prognosis.

**Study Design:** Cross sectional study.

**Place and Duration of Study:** Haematology department, Armed Forces Institute of Pathology, Rawalpindi, from May 2017 to Aug 2018.

**Methodology:** A total of 56 newly diagnosed patients of Chronic Lymphocytic Leukaemia were included in the study. Patients were diagnosed on the basis of National Cancer Institute Working Group guidelines. A detailed history and thorough clinical examination were performed and complete blood counts, biochemical profile, bone marrow examination, immunophenotyping on bone marrow/peripheral blood samples were done for the diagnosis of Chronic lymphocytic leukaemia. Interphase FISH studies were performed on blood/bone marrow aspiration for detection of Trisomy 12 were performed.

**Results:** Out of 56 patients, trisomy was detected in 12 (10.7%) patients. Out of 7 patients with trisomy 12, five patients presented in late stages (Binet stage B and C), however this association of Trisomy 12 with Binet stage was also statistically insignificant ( $p=0.474$ ). About six with trisomy 12 were positive for CD 38, however this association was also not statistically significant ( $p=0.124$ ). Results revealed that patients having trisomy 12 underwent chemotherapy at diagnosis and during follow ups as compared to patients having other cytogenetic abnormalities. Moreover, patient with trisomy 12 develop progression in disease during course of illness, however association was statistically insignificant ( $p>0.05$ ).

**Conclusion:** Most of the chronic lymphocytic leukaemia patients with Trisomy 12 were symptomatic, presented in later Binet stages (B and C), having positive CD38 status, required chemotherapy and develop progression during course of disease.

**Keywords:** Chronic lymphocytic leukemia, Fish, Trisomy 12.

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### INTRODUCTION

In Western nations "Chronic lymphocytic leukemia (CLL)" is the most frequent kind of lymphoid malignancy, while its prevalence is relatively low in Asian countries<sup>1</sup>. According to "The American Cancer Society" 20,720 new cases of CLL were diagnosed and 3,930 deaths resulted from CLL in US in year 2019<sup>2</sup>. In Pakistan, CLL comprises 5% of all the leukaemias<sup>3</sup>.

CLL is characterized by the clonal expansion of small mature looking lymphocytes with specific (CD23 ± CD5 ± CD19) in the bone marrow, blood and lymphoid tissues<sup>4-6</sup>. Regardless of the similar transcriptional profile, morphology and immunophenotype, CLL has considerable hereditary and clinical heterogeneity where few patients show an aggressive course with poor outcome to treatment and others patients never require treatment<sup>4</sup>.

Conventional cytogenetic analysis reveal chromo-

somal aberrations in only 40-50% of patients. However, Fluorescence in situ hybridization (FISH) analysis on interphase cells can detect chromosomal changes in approximately 80% of patients with CLL, with the most well-known being Del 13q14.3, Del 11q22.3, trisomy 12, Del 17p13.1 and Del 6q23<sup>7</sup>. The most frequent cytogenetic abnormality in CLL is Del 13q14. Trisomy 12 is found in approximately 16% of CLL cases and about 20% of the CLL cases have normal karyotype<sup>8,9</sup>.

Majority of the patients with trisomy 12 have morphologically different lymphocytes with uneven nuclear contours, abundant cytoplasm and prominent nucleoli<sup>10</sup>. Researchers have proposed trisomy 12 as intermediate to unfavourable prognosis<sup>10</sup>. The survival rate of patients with trisomy 12 is worse than that of patients with del 13q14 and it is better than in patients with del 11q23 or del 17p.

The objective of this study was to determine the frequency of trisomy 12 in patients with chronic lymphocytic leukaemia and its correlation with clinical and laboratory findings. As there is scarcity of local

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data available on this topic with most of the data being from western literature, so we conducted this study for our local population.

## METHODOLOGY

It was a cross sectional study conducted at the department of haematology Armed Forces Institute of Pathology from May 2017 to September 2018. The ethics review committee approval was sought before the conduct of study. A total 56 patients newly diagnosed as B-CLL of all ages and either gender were enrolled in the study. Patient were diagnosed as CLL as per National Cancer Institute Working Guidelines for CLL (Lymphocytosis  $>5 \times 10^9/L$  and characteristic immunophenotype (CD19<sup>±</sup>, CD5<sup>±</sup>, CD23<sup>±</sup>, CD20 weak positive and weak expression of either kappa or lambda). The non-probability consecutive sampling technique was employed. Patients who were already on treatment and patients who lost to follow up were excluded from the study.

A detailed history and thorough clinical examination were performed and complete blood counts, biochemical profile, bone marrow examination, immunophenotyping on bone marrow/peripheral blood samples were done for the diagnosis of Chronic lymphocytic leukaemia. Interphase FISH studies were performed on blood/bone marrow aspiration for detection of Trisomy 12 and other cytogenetic abnormalities. Clinico-pathological features of patients with Trisomy 12 were compared with those of other cytogenetic abnormalities. SPSS-23 was used to analyze data. The Laboratory parameters, WBC count, Hb levels, Platelet count, CD-38 and ZAP-70 status were examined alongwith personal and BINET stage data. Patients were followed up (mean follow up-12 months) for disease progression or dependence of chemotherapy. The progression of disease here was defined as stage progression, increase in B2 microglobulin and Lymphocyte Doubling Time  $<12$  months.

Flourescent in Situ Hybridization analysis: Blood and bone marrow specimens were analyzed through interphase FISH studies. Specimen was collected and cultured at 37°C in two intervals for 24 hours then after adding 200ul colchicine for 1 hr and centrifuged at 1500 rpm for 8 min. Afterwards, slide was prepared for FISH study. The slide underwent further procedures such as Meta system XL DLEU/LAMP/12CEN probe, denaturation at 74°C for 5 min, hybridization at 37°C for 18 hrs and co-denaturation at 74°C. The slide was washed and air dried. 10 to 20 ul of DAPI-2 counter stain was added and it was frozen at -20°C for

1 day. A total of 500 nuclei were analyzed per probe set using a fluorescent microscope with an orange green spectrum filter. Positive trisomy 12 was denoted by three green signals and two orange signals (fig-1).

Data was entered and analyzed by SPSS-23. Descriptive statistics such as mean & SD was computed for numeric variables whereas frequencies and percentages were computed for qualitative variables. The chi-square test was applied between the variables.  $p$ -value  $<0.05$  was taken as statistically significant.

## RESULTS

A total of 56 patients were included in the study with age ranged between 38-87 years. The median age of the patients with CLL was 60 years. Many patients 45 (80.4%) were males and 11 (19.6%) were females with a male to female ratio of 4:1. Majority of them were diagnosed incidentally on routine blood counts. Presenting signs and symptoms, blood counts & staging are shown in table-I. About 28 patients had Binet stage A, 23 had Binet stage B & 5 had stage C. CD38 expression was positive in 33 cases. ZAP 70 was negative in all cases.

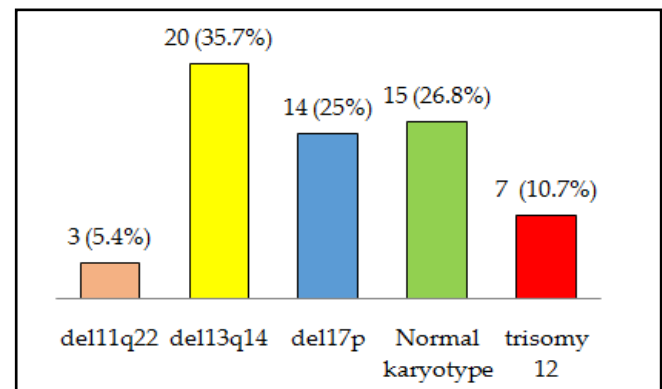


Figure: Frequency distribution of cytogenetic abnormalities.

Out of 56 patients, 20 patients had deletion 13q14 (35.7%) in CLL patients, followed by normal karyotype in 15 patients (26.8%), del17q in 14 patients (25%), trisomy 12 in 7 cases (10.7%) & del11q22 in 3 patients (5.4%) (figure).

The mean age showed statistically significant difference when compared with trisomy 12 and without trisomy 12 ( $p=0.048$ ). Out of 7 patients with trisomy 12, five patients presented in late stages (Binet stage B and C), however this association of Trisomy 12 with Binet stage was also statistically insignificant ( $p=0.474$ ). About six with trisomy 12 were positive for CD38, however this association was also not statistically significant ( $p=0.124$ ) (table-II).

These patients were followed up for progression of disease and chemotherapy dependence for 14 months. Results revealed that patients having trisomy 12 underwent chemotherapy at diagnosis and during follow up more frequently as compared to patients having other cytogenetic abnormalities. Moreover, results showed statistically insignificant ( $p$ -value>0.05) relationship of trisomy 12 with disease progression and chemotherapy dependence during course of disease (table-III).

**Table-I: Descriptive Statistics.**

Variables	Statistics
<b>Quantitative</b>	<b>Mean ± SD</b>
Age in years	60.01 ± 9.68
WBC (x 10 <sup>9</sup> /L)	106.42 ± 81.52
ALC (x 10 <sup>9</sup> /L)	92.86 ± 78.50
Hb (g/dl)	11.02 ± 2.33
Platelets (x 10 <sup>9</sup> /L)	164.26 ± 92.29
<b>Qualitative</b>	<b>n (%)</b>
<b>Gender</b>	
Male	45 (80.4%)
Female	11 (19.6%)
<b>Clinical &amp; Physical Presentation</b>	
Asymptomatic (Incidental finding)	22 (5.3%)
Cellulitis (Rt Forearm)	2 (3.5%)
Chest Infection	2 (3.5%)
Chest Pain	1 (1.7%)
Fever	22 (39.2%)
Weakness	5 (8.9%)
Incidental findings	19 (33.9%)
Progression pallor	1 (1.7%)
Weight loss	7 (12.5%)
Respiratory tract infection	1 (1.7%)
Headache	2 (3.5%)
Lymphadenopathy	35 (62.5%)
Splenomegaly	6 (10.7%)
Hepatomegaly	1 (1.8%)
<b>Binet Stage</b>	
A	28 (50%)
B	5 (8.9%)
C	23 (41.1)
CD38	33 (58.9%)
ZAP70	-

**DISCUSSION**

In the current study we have assessed 56 patients with CLL. The average age of the patients was 60.01 ± 9.68 ranging from 38-87 years. Gunawardana *et al.* Evaluated the clinical and biological characteristics in Asian & White patients with CLL and reported the median age of patients as 70 (25-92) years in Whites while 61 (29-85) years in Asians<sup>11,12</sup>. In a local study conducted by Rashid *et al.*, the reported mean age of

the patients was 65.8 ± 1.5 years and majority of the patients belonged to 71-80 years age group<sup>3</sup>. In another study, Ehsan *et al.*, found the average age as 62.84 years in the patients with CLL<sup>13</sup>. This shows that more or less age distribution in CLL patients is almost same among different populaces and is certainly a disease of

**Table-II: Clinico-pathological features with trisomy 12 and without trisomy 12.**

Variables	Trisomy 12		p-value
	With (n=7)	Without (n=49)	
<b>Age Groups</b>			
≤50 years	3 (30%)	7 (70%)	0.605
>50 years	4 (8.7%)	42 (91.3%)	
Mean (SD)	53.28 (9.51)	60.97 (9.41)	0.048
<b>Gender</b>			
Male	6 (13.3%)	39 (86.7%)	0.703
Female	1 (9.1%)	10 (90.9%)	
<b>Hb Levels</b>			
≤ 12 g/dl	6 (17.1%)	29 (82.9%)	0.175
>12 g/dl	1 (4.8%)	20 (95.2%)	
Mean(SD)	1.14 (0.37)	1.40 (0.49)	0.181
<b>WBC Level</b>			
≤150 (x10 <sup>9</sup> /L)	7 (15.9%)	37 (84.1%)	0.14
>150 (x10 <sup>9</sup> /L)	-	12 (100%)	
Mean (SD)	1.00 (0.001)	1.24 (0.43)	0.145
<b>ALC Level</b>			
≤150 (x 10 <sup>9</sup> /L)	7 (15.2%)	39 (84.8%)	0.187
>150 (x 10 <sup>9</sup> /L)	-	10 (100%)	
Mean(SD)	1.00 (0.001)	1.20 (0.40)	0.194
<b>Platelet Count</b>			
≤150 (x10 <sup>9</sup> /L)	4 (12.1%)	29 (87.9%)	0.918
>150 (x10 <sup>9</sup> /L)	3 (13%)	20 (87%)	
Mean(SD)	1.42 (0.53)	1.40 (0.49)	0.92
<b>CD38</b>			
Positive	6 (18.2%)	27 (81.8%)	0.124
Negative	1 (4.3%)	22 (95.7%)	
<b>ZAP70</b>			
Positive	-	-	N/A
Negative	7 (12.5%)	49 (87.5%)	
<b>Binet Stage</b>			
A	2 (7.1%)	26 (92.9%)	0.474
B	1 (2%)	4 (80%)	
C	4 (17.4%)	19 (82.6%)	

**Table-III: Relationship of trisomy 12 with disease progression and chemotherapy dependence.**

	Trisomy 12		Total	p-value
	Yes	No		
<b>Progression Disease</b>				
Yes	5	24	29	0.666
No	2	25	27	
<b>Chemotherapy Dependence</b>				
Yes	5	24	29	0.666
No	2	25	27	

older age. In this study, majority of patients were males (80.4%) whereas 19.6% were females. In the study by Chan *et al*, showed similar results with respect to gender i.e. males were (73%) and females (27%)<sup>14</sup>. Similar results were found in the study by Ehsan *et al*, with a male to female ratio of 4.6:1<sup>13</sup>. Another study showed that males constitute 42% in Whites as compared to 53% in Asians<sup>12</sup>.

Hence male gender, is an established potential risk factor for the development of CLL. One of the serious conditions related with increased morbidity and worse prognosis is anemia among CLL patients. In our study the mean Hb level was  $11.02 \pm 2.33$  g/dl, the WBC ( $\times 10^9/L$ ) was  $106.42 \pm 81.52$ , ALC ( $\times 10^9/L$ ) was  $92.86 \pm 78.50$  & Platelets count ( $\times 10^9/L$ ) was  $164.26 \pm 92.29$ . Almost similar findings were observed in the study conducted at Karachi, Pakistan which showed mean hemoglobin level as  $10.8 \pm 2.4$  g/dl with platelets  $197.8 \pm 103.2 \times 10^9/L$  & TLC as  $91.5 \pm 87.8 \times 10^9/L$ <sup>15</sup>. Another study showed mean Hb level as  $9.8$  g/dl  $\pm 2.62$ . TLC was divided into groups which showed that majority of the CLL patients in the range of  $50000-100000 \times 10^9/L$  and in their study about 71.3% of the patients had Platelet count of  $>00 \times 10^9/L$ <sup>3</sup>.

In the present study, the most common presentation of CLL patients is lymphadenopathy (62.5%), however splenomegaly and hepatomegaly were observed in 10.7% and 1.8% of the cases. About 39.9% of the patients presented with fever and 33.9% of the patients had incidental findings.

In a study conducted by Dabaghao *et al*. Fever was reported as the most frequent presentation (48%) and 73% of the patients had hepatomegaly on examination<sup>16</sup>. In another study, fever was present in 26% of the cases, jaundice in 23%, hepatomegaly in 19% and lymphadenopathy in 8%<sup>17</sup>. Rashid *et al*, found splenomegaly in 87.3% & lymphadenopathy in 62.7% of the CLL patients<sup>3</sup>.

One of the clinical staging systems is Binet staging system which is used to evaluate the prognosis of CLL patients. In the present study, majority of the patients had Binet stage A (50%), followed by stage C (41.1%) and stage B (8.9%). Almost similar findings were observed in study conducted among Chinese population, which showed that most of the patients had Binet stage A (56.1%) followed by stage C (22.2%) and stage B (21.7%)<sup>14</sup>. However in another study, majority of the patients with CLL had Binet stage C (20 cases), 7 patients had stage A & 4 patients had stage B<sup>14</sup>.

In the last few years it has been observed that CLL patients with unmutated immunoglobulin heavy chain gene (IgHV) usually have shorter survival rate and more aggressive disease<sup>18</sup>. CD38 is a reliable and useful marker which is associated with CLL because usually it remains stable over time, even after chemotherapy, and it can be estimated easily in flow cytometry laboratories<sup>11</sup>. CD38 was found to be correlated with IgHV mutational status<sup>19</sup>. Recently, ZAP70, a member of syk family protein tyrosine kinases (PTKs); which shows an important part in T-cell development and T-cell antigen receptor (TCR), has shown association with B-cell receptor in CLL patients<sup>20,21</sup>. Hence, CD38 and ZAP70 are the significant prognostic markers in patients with CLL<sup>22</sup>. In the present study, CD38 expression was positive in 33% of cases, however ZAP 70 was negative in all the patients. However, in another research 42.5% of the patients with CLL showed high levels of CD38 and 47.5% of the patients were ZAP-70 positive<sup>23</sup>. In a study conducted at India, CD38 & ZAP-70 positivity were reported in 36% and 25% of the patients respectively<sup>24</sup>.

FISH is one of the most powerful and widely used prognostic tools in patients with CLL and the third most common cytogenetic abnormality in CLL is trisomy 12<sup>25</sup>. Döhner *et al*. proposed hierarchical prognostic model in which trisomy 12 was labelled as an intermediate risk marker. In the present study, trisomy 12 was detected in 10.7% of the patients with CLL. In a study conducted by El-Kinawy *et al*, trisomy 12 was detected in 19.3% of the patients<sup>23</sup>. Bagir *et al*, detected trisomy 12 in 36 cases. However, the patients with trisomy 12 had better overall survival rate as compared to patients with del (11q23) or del (17p) while it is poorer as compared to patients with del (13q14)<sup>11</sup>.

In this study, we have stratified trisomy 12 with respect to age, gender, blood laboratory findings, ZAP 70 and CD38. However, no significant statistical association was found between stratified groups ( $p>0.05$ ). In a study by Matutes *et al*, about 18% of the patients had trisomy 12, majority of those patients were males<sup>28</sup>, and most of them had Binet stage A (64%). In this study majority of patients with trisomy 12 were males (6 cases), 100% of the patients had WBC  $\leq 150$  ( $\times 10^9/L$ ), 85.7% of the patients had positive CD38, none of the patient had ZAP70 and 57.1% of them had Binet stage C. In another study conducted by Abruzzo *et al*, majority of the CLL patients with trisomy 12 were males (80%), had WBC  $\leq 150$  L/G (75%), ZAP positive in 62%

and CD38 <30% in 67% of the cases. Riches *et al.* found CLL patients with trisomy 12 had statistically significant expression of CD38 as compared to other major cytogenetic abnormalities ( $p < 0.01$ ).

In this study, 5 CLL patients with trisomy 12 were chemotherapy dependent and showed progression of disease. Among them 1 patient died during the follow up period of 1 year. Sagatys *et al.*, observed in their study that survival rate of CLL patients with trisomy 12 is poorer as compared to patients with del (13q14) and it is better in patients with del (11q23) or del (17p).

### CONCLUSION

Frequency of trisomy 12 was found to be 7 (10.7%). Most of the CLL patients with Trisomy 12 were symptomatic, presented in later Binet stages (B and C), having positive CD38 status, required chemotherapy and develop progression during course of disease. Therefore, this cytogenetic abnormality may be associated with unfavourable prognosis. The insignificant association when comparing with patients without trisomy 12 is due to presence of other unfavourable markers (Del 17p and 11q22) in that group. So Investigation of Trisomy 12 by FISH should therefore be included in the panel of prognostic markers in patients with chronic lymphocytic leukaemia.

### CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any authors.

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