

# A Retrospective Survey of Molecular, Cytogenetic, and Immunophenotype Data of Patients with Acute Lymphoblastic Leukemia in Northeast Iran

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## Abstract

**Background:** Excess proliferation of blood cells may lead to leukemia, which is associated with structural and numerical chromosomal aberrations. Cytogenetic findings of acute lymphoblastic leukemia can be applicable in diagnosis, prognosis, and treatment selection for patients. In the present study we have evaluated molecular, cytogenetic, and immunophenotypic findings in acute lymphoblastic leukemia patients from Mashhad, a city in Northeast Iran.

**Methods:** This cross-sectional study enrolled 124 patients with acute lymphoblastic leukemia during 2015-2017. Two expert hematopathologists confirmed the diagnosis of acute lymphoblastic leukemia in patients' peripheral blood and bone marrow smears. Molecular tests that included t(4;11), t(1;19), t(9;22)-190, and t(12;21) were done by reverse transcriptase real-time quantitative PCR. We performed karyotyping and immunophenotyping of the bone marrow samples. The data were analyzed by SPSS v.17.

**Results:** Mean age of studied cases was 20.01 years. Participants consisted of 64% males and 36% females. Cytogenetic results showed that 23.37% of participants had a normal karyotype; the other participants had the following abnormalities: hyperdiploidy (12.06%), hypodiploidy (21.55%), pseudodiploidy (24.13%), and high hyperdiploidy (18.10%). Molecular analysis of karyotype patterns indicated that 14% of the acute lymphoblastic leukemia patients had the t(12;21), 9% with t(1;19), 2.5% with t(4;11), and 2.5% had the t(9;22).

**Conclusion:** The unique findings of the present study were the presence of previously unreported novel abnormalities. These findings might be useful for oncologists and hematologists in predicting outcome, remission, survival, and treatment response in acute lymphoblastic leukemia patients.

**Keywords:** Acute lymphoblastic leukemia (ALL), Chromosomal abnormality, Cytogenetic

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## Introduction

Blood cells are generated from bone marrow precursors.<sup>1</sup> Clonal expansion of lymphoblastic precursors leads to acute lymphoblastic leukemia (ALL), which is defined by the presence of more than 20% of blasts in the bone marrow.<sup>2</sup> Radiation, chemical, and drug exposures may lead to increased blast proliferation.<sup>1</sup>

Proliferation is sometimes associated with structural and numerical chromosomal aberrations.<sup>3-5</sup> Resesarchers use various cytogenetic and molecular methods to discover chromosomal changes in ALL and other hematologic malignancies.<sup>3,4,6</sup> Chromosomal anomalies play an important role in leukemia diagnosis; they are prognostic factors used to choose the best treatment and minimal residual disease (MRD) determination.<sup>2,3,5-10</sup> These anomalies have been found in more than 70% of bone marrow involvement.<sup>4</sup> Some abnormalities have special clinical signs and morphologic changes.<sup>8,11</sup> Karyotype patterns can be associated with poor prognosis such as t(4;11), whereas others indicate good prognosis such as hyperdiploidy.<sup>12</sup> Some chromosomal changes have opposing age dependent effects such as t(9;22), which is a bad prognostic factor in adults but a good prognostic factor in children.<sup>10</sup>

Although ALL is a heterogeneous disease of adults and children,<sup>5</sup> it is more common among children under the age of 15, and especially seen in children 2-5 years of age.<sup>1</sup> Acute lymphoblastic leukemia is reported in 40% of children 1-4 years of age.<sup>13</sup> Bone marrow karyotype, immunocytochemistry (ICC), flow cytometry, reverse transcriptase real-time quantitative PCR (RT-qPCR), and FISH are suggested diagnostic techniques for ALL.<sup>1</sup>

In this study we aimed to evaluate the frequency of various chromosomal changes in ALL patients from Mashhad, Northeast Iran.

## Materials and Methods

This cross-sectional study included 124 ALL patients who referred to the Cancer Molecular

Pathology Research Center during 2015-2017. This clinical study was approved by the Ethics Committee of Mashhad University of Medical Sciences. Prepared bone marrow and peripheral blood smears of all cases were stained by Giemsa and myeloperoxidase, and evaluated by 2 expert hematopathologists for confirmation of malignancy. Molecular tests such as RT-qPCR for ALL recurrent translocations were also performed. Table 1 lists the patients' clinical and laboratory assessments.

### Cytogenetic analysis

Bone marrow aspirates, as the preferred sample for conventional cytogenetic analysis, were collected in heparinized containers and transported on ice to the Cytogenetics Laboratory after collection. If the bone marrow aspirate was not available, we cultured BM sample or the patient's peripheral blood when there were >20% blasts in the sample. Cell counts were performed for optimization of the cell culture. Patients' sterile bone marrow samples that had been placed in heparin were cultured in RPMI 1640 supplemented with 1% L-glutamine, 20% fetal bovine serum (FBS), and 1% pen strep for 24

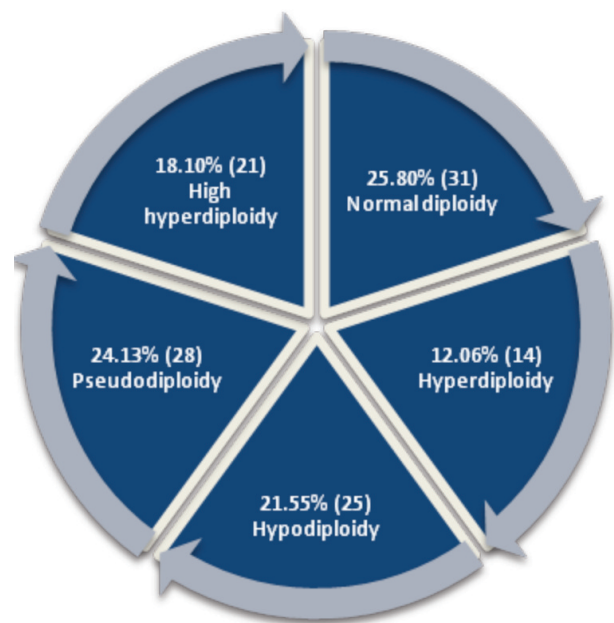


Figure 1. Numerical abnormalities (n=120).

and 48 h. Two separate tubes were cultured for each patient. Bone marrow culture, harvest procedure, and slide preparation were performed according to Shakeri et al.<sup>4</sup> Karyotype was reported according to the International System of Human Cytogenetic Nomenclature (ISCN 2016) at the time of analysis. A minimum of 20 metaphases must be analyzed if there were no clonal abnormalities. Chromosomal aberrations must be clonal when present in a minimum of 2 cells with the same extra chromosomes or structural aberrations, or at least 3 cells with the same chromosome loss. We classified the numerical abnormalities as hyperdiploid (47–50 chromosomes), high hyperdiploid (>50 chromosomes), pseudodiploid (46 chromosomes with structural or numeric abnormalities), diploid (normal 46 chromosomes), or hypodiploid (<46 chromosomes). Sometimes the processed samples yield no or only a few analyzable metaphase cells, or the karyotypes obtained are of poor quality and unable to be interpreted. These are limitations of conventional cytogenetic analysis.

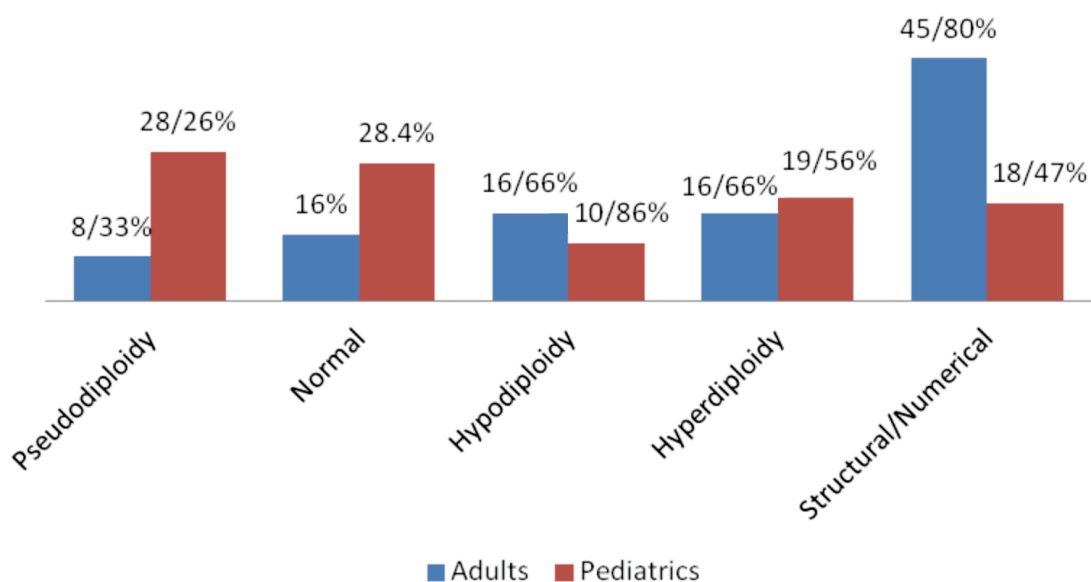
### Molecular tests

We used RT-qPCR to identify the most frequent fusion gene in ALL. RNA was extracted from bone marrow or peripheral blood with TRIzol (Invitrogen, Cergy, France). We determined the RNA concentration by measuring the density at 260 nm with a Spectrophotometer. RNA was stored at -80°C until use. The cDNA synthesis protocol for RT-qPCR was optimized according to the BIOMED-1 protocol.<sup>14</sup> All RT-qPCR reactions were performed on a StepOne Applied Biosystem (Applied Biosystems, Foster City, CA, USA) according to the Slovak et al. protocol.<sup>15</sup>

We performed molecular analysis for ALL translocations as follows: t(1;19) with E2A-PBX1; t(4;11) with MLL-AF4; t(12;21) with TEL-AML1; and t(9;22) with BCR-ABL p190.

### Immunophenotype

We used the following monoclonal antibodies (MoAbs) CD10, CD19, CD3, cIgM, and TdT for ICC assessments. Reactivity with MoAbs was assessed by indirect immunofluorescence. According to surface antigen expression, we classified the B-cell precursor ALL as pre-B ALL (CD19+, CD10-, CD20-, cyIgM-, TdT+);



**Figure 2.** Distribution of cytogenetic abnormalities in pediatric and adult patients.

common (CD19+, CD10+, cyIgM-, TdT+); and pre-B (CD19+, CD10+/-, cyIgM+, TdT+). CD3 positive cases were classified as T-ALL. We divided the patients into two groups, B-ALL and T-ALL, based on the immunophenotype findings.

### Statistical analysis

We reported descriptive statistics that included the frequency of different chromosomal abnormalities, age, and sex. We used SPSS version 17 for statistical analysis. The student's t-test was also used. *P*-values less than 0.5 were considered statistically significant.

### Results

The sample consisted of 124 ALL patients between 7 months and 46 years of age. The mean age was 20.01 years. There were 64% males and 36% females in this study with a male to female ratio of 1.82. In terms of age, 100 out of 120 cases were  $\leq 15$  years of age (pediatric group) and 24 out of 120 were  $> 15$  years of age (adult group). According to the patients' medical records, the most common findings at the time of the physical examination were hepatomegaly (14%), splenomegaly (20%), lymphadenopathy (14%), purpura (12%), and fever (38%). Laboratory results were as follows: mean hemoglobin (6.72 g/dl), mean white cell count ( $60776 \times 10^9/L$ ), mean platelet count ( $81100 \times 10^9/L$ ), and red blood cells ( $2.85 \times 10^9/L$ ).

### Cytogenetics studies

We performed the G-banding techniques on 124 samples from the ALL patients. We were unable to analyze 4 (3.3%) samples due to the absence of cell growth (mitotic index (MI) zero). Karyotype analysis indicated that 74.2% of patients presented with chromosomal alterations in their bone marrow and 25.8% had a normal karyotype. Immunophenotype analysis showed that 91.6% of patients had B-ALL and 6.6% T-ALL. We classified the B-ALL cases as pro-B (33.3%), early pre-B (16%), and pre-B (45%). A total of 74.1% of patients had the following chromosomal abnormalities: numerical (30%),

**Table 1.** Recurrent chromosomal translocations.

Chromosomal abnormality	Patients (n)	Percent
t(12;21) (p13;q22)	17	14
t(1;19) (q23;p13)	11	9
t(4;11) (q21;q23)	3	2.5
t(9;22) (q34;q11)	3	2.5

structural and numerical (23.3%), and structural (20.8%). Analysis of ploidy indicated that 31 (25.8%) out of 120 patients had a normal diploid karyotype, 25 (20.8%) were pseudodiploid, 25 (20.8%) were hypodiploid, 14 (11.6%) had hyperdiploid, and 21 (18.3%) patients had high hyperdiploid (Figure 1). In the current study, there were 14 out of 120 (11.6%) patients identified by the traditional definition of complex karyotype with more than or equal to 3 clonal aberrations and a structurally complex karyotype.<sup>16</sup>

There were unusual or novel cytogenetic abnormalities in 20.8% of patients; these cases had hyperdiploidy or hypodiploidy with additional modifications. We also included cell types of the patients and observed that all patients had B-ALL, with the exception of one patient who was T-ALL. Table 2 lists the novel abnormalities and type of lineage involvement.

There were no statistically significant relationships observed between sex and age of patients when their disease onset. ( $P > 0.5$ ). There was no distinction between males and females in either the pediatric or adult group. We did not observe any significant differences between the B and T lineages and the patient groups ( $P > 0.5$ ). Thus, lineage involvement was not meaningfully relevant in adults and pediatrics.

Table 1 summarizes the recurrent chromosomal translocations found in the study patients. According to SPSS analysis, there were no statistically significant relationships between age and recurrent translocations ( $P > 0.5$ ). The normal karyotype was the most common karyotype among the 2 groups.

### Discussion



Acute lymphoblastic leukemia is a heterogeneous disease at the clinical and genetic levels. Although ALL can occur at any age, it is more prevalent in childhood and accounts for 33% of all malignancies in children under 14 years of age.<sup>16</sup> Major chromosomal abnormalities are the prognostic factors for patients seen in follow-up.

In the present study we analyzed and compared the cytogenetic and molecular test results of 124 ALL adult and pediatric patients. We successfully propagated 96.66% of the bone marrow samples. This rate significantly differed from previous studies. Safaei et al., in a study from Fars Province, Iran reported a success rate of 84.5% for their cultures.<sup>2</sup> Another survey in Brazil has reported success in 91% of 100 cultures.<sup>17</sup> The impact of poor metaphase cytogenetics in ALL, particularly among those with the L1 morphology, is critical. The RT-qPCR studies provide valid data for metaphase analysis.

We divided the patients into 2 groups according to age, as previously reported. The mean age of the studied individuals was 20.01 years; it was 5.57 years in the pediatric group and 34.46 years in the adult group. Ceppi et al. reported mean ages of ALL children of 6.5 years and 7.3 years.<sup>18, 19</sup> In the current study there were 69% male ALL cases and 35% female ALL cases, which agreed with previous researches.<sup>2,5,6,19,20</sup> Although this finding indicated that ALL is more common in males, Ceppi et al. have reported ALL in 56% of females in their study. Generally, the larger number of males is evident.<sup>18</sup>

Immunophenotyping can provide valuable prognostic information in ALL patients. We have observed that B-ALL was 91.6% and T-ALL was 6.6% in the current study. Definite lineage involvement was undetermined in 2 cases, which had the B/T (mixed) phenotype. A literature search revealed similar findings. Gil reported 93% B-ALL and 7% T-ALL immunophenotype in 2013. They found that the majority (83%) of B-ALL were related to the pre-B stage.<sup>16</sup> Another study in Nicaragua found B-cell involvement in 81% of cases and T-cell in 3% of the cases.<sup>18</sup> The findings

were the same as the current study where the majority (45%) of B-cell were in the pre-B stage. Previously, patients with T-ALL (10%-15% of childhood ALL) had a worse prognosis compared to those with B-ALL. However, with current treatment, the prognosis of T-ALL has improved.<sup>21</sup>

We observed that 27 out of 124 (22.5%) patients had a normal karyotype, from which 16.6% were adults and 26% were children. An Indian study evaluated the cytogenetic profile of de novo B-ALL and reported that 37.2% had the normal karyotype.<sup>22</sup> A comparison of our results with other studies showed a different frequency for the normal karyotype. Nizzamani et al. reported patients with a normal karyotype of 47% and Safaei et al. reported that 46.15% had normal karyotype.<sup>2,5</sup> These differences appeared to be the result of population differences such as age or possibly genetics.

Assessment of chromosomal rearrangement distribution showed that numerical abnormalities were present in 30% and structural abnormalities in 20.8% of the ALL study patients. Analysis of ploidy showed that 22.5% had normal diploidy, and the rest were pseudodiploidy (20.8%), hypodiploidy (20.8%), hyperdiploidy (11.6%), and high hyperdiploidy (17.5%). Another research in adult and pediatric cases reported numerical abnormalities of 61.7% and structural abnormalities of 53.8%.<sup>2</sup> There were more numerical abnormalities than structural abnormalities, which was similar to the current study.

We observed that 11.6% of the study patients had the complex karyotype. Bhandari et al. reported that 6 out of 215 (2.8%) B lineage ALL patients had the complex karyotype.<sup>22</sup> Motlló et al. reported that children with the structurally complex karyotype had a highly unfavorable prognosis with 2-year overall survival. Patients with structurally complex karyotype had significantly higher relapse-free survival compared to children with the t(4;11), hypodiploidy and t(9;22) karyotypes, which are known as higher risk cytogenetic features.<sup>23</sup> Another study has reported 20.51% with the complex karyotype.<sup>24</sup> The lower

**Table 2.** Novel abnormalities in patients.

Sex	Age (years)	Cell-type	Novel abnormalities
F	6	B-cell	48,xx,+8,+21,del(22)(q13)/46,xx
M	1	B-cell	44,xy,+y,t(1;3)(p36,q21),-14,-18,-5,-9,-17
M	1	B-cell	47-48,xy,t(6;11)(p11,q13),del(6)(p12),del(11)(p12)del(11)(q14),+6,+11
M	5	B-cell	46,xy,del(6)(q21),+6,+11,t(12;21)(p13,q22),-14,-16/46,xy
F	33	B-cell	43-45(2n),XX,t(1,3)(q21,q29),t(1,20)(p13,p13),t(9,22)(q34,q11),del(9)(q12),-19(cp15)
M	5	T-cell	46-49,xy,-x,+xx,t(7;9)(p16,q24),del(5)(q13),dic(6,3)(q11;q11),x2,add(7)(p22),+17,+20[cp15]
M	5	B-cell	39-46(2n),xy,der(10),t(10,12)(p15,q12),t(12,21)(p13,q22)[cp5]
M	5	B-cell	46,XY,t(2;9),del(5q),dic(3;6)
M	19	B-cell	42-44(2n)xy,-x,t(1,14)(q23;q13),t(1,17)(q32,p13),del(6)(q21),-10,-13,add(14)(q32),-8,-20[cp20]
M	3	B-cell	47,xy,del(4)(p12),+6,+8,del(8)(q12),-11,-17,del(22)(q12),+mar/79-86(4n)
M	18	B-cell	mos,45,xy,t(2,6)(q31;q26),-17,46,xy
M	16	B-cell	46,xy,der5 t(5,11)(q12;p11), del(9p),t(16,18)
M	17	B-cell	46,xy,t(7,14)(q32,q32),46,xy
M	23	B-cell	46,xy, dic(1,15)(p11,p11),dup(3)(q21;q29),+1\46,xy,dup(3)(q21;q29),der(18),t(1,18)(q10;q11)
F	28	B-cell	46,xy,del(17)(p12),del(1)(q21),+3,-5
M	15	B-cell	45-46,xy,t(2;12)(q31;p13),dic(3;6)(q11;q11),-6, del(17)(p12)/46,xy
F	6	B-cell	47,xx,t(4,11)(q21,q23),del(4)(q22),+4
F	2	B-cell	48,XX,del(3)(q12),+8+21
M	4	B-cell	46-50,XY,t(1;7)(q32;p12),t(3;6)(q11;p12),del(3)(p13),+3,del(4)(q22),del(4)(p12),+4,+5,+6,add(17)(q25)
F	14	B-cell	42-43,+1,dup(1)(p31p32)t(1;3)(q21;p12),+3,del(3)(q21q28),-4,-5,del(6)(q21),-7,del(7)(q32),-9,-11,-21
F	22	B-cell	45-46,del(x)(q24),dup(1)(q31q43),del(6)(q21q23),add(9)(p?),dic(13;21)(p13;q22),-14,del(17)(p11),+21
F	11	B-cell	t(1;19),del(6q),t(3;6)
M	12	B-cell	48,xy,dup(1),+1,+5,+21
F	3	B-cell	54,xx,+x,dup(1q21q31)
M	2	B-cell	46,XY,t(2;21)(p11;q11)

frequency of patients with the complex karyotype in the current study in comparison with the 2 mentioned studies might be due to the low number of cases. We observed novel karyotypes in 20.8% (25 out of 124) of patients compared to another study that reported 11.4% (10 out of 88) of patients had a novel karyotype.<sup>2</sup>

The t(12;21)(p13;q22) chromosomal rearrangement is one of the most common in B-ALL that occurs in 15%-25% of children and confers a favorable prognosis.<sup>25</sup> In the present study, we have detected the t(12;21) chromosomal rearrangement in 14% of patients. Cytogenetic results from an Iranian study revealed that t(12;21) was the most common abnormality (13%).<sup>2</sup> In the current study, t(12, 21) was also the most common

abnormality, which was seen in 17% of cases. t(12;21) was more prevalent than other recurrent translocations in our study. t(1;19)(q23;p13) are commonly seen in 5% of childhood B-ALL cases and is associated with poor outcomes.<sup>26</sup> We have observed that 9% of our patients had t(1;19). Of interest, one adult patient had t(1;19). t(9;22)(q34;q11) is detected in 3%-5% of pediatric ALL and 25% of adult ALL cases. The Philadelphia (ph) chromosome was present in 2.5% of B-ALL patients in the current study. Nizzamani reported the ph chromosome in 6% of ALL cases,<sup>5</sup> whereas it was present in 3%-5% of cases in a study by Schultz et al.<sup>27</sup> Historically, ALL patients with the (ph) chromosome have an extremely poor outcome. t(4;11)(q21;q23) is

present in 1%-2% of childhood B-ALL and has a poor prognosis.<sup>28</sup> We noted that 7.6% of our pediatric patients had t(4;11), which seemed more prevalent than usual.

## Conclusion

Advances in cytogenetic and molecular technologies have enriched our current understanding of childhood T-ALL and B-ALL, although conventional karyotyping plays a significant role in detection of numerous recurrent abnormalities. The unique finding of the present study was the presence of novel abnormalities in 2 cases. These abnormalities have not been previously reported by researchers of ALL cases in Northeast Iran. The findings of the present study may be useful for oncologists and other physicians to predict outcome, remission, survival, and treatment response in ALL patients.

## Conflict of Interest

None declared.

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