A Bone Marrow Study; Report of Chromosomal Variations in Hematologic Malignancies Including Acute Myeloid Leukemia, Acute Lymphoid Leukemia, and Myelodisplastic Syndrome (Northeast Iran)

Sepideh Shakeri*, MSc, Hossein Ayatollahi*, MD, PhD, Mohammad Hadi Sadeghian*, MD, PhD, Seyyede Fatemeh Shams**, MSc

*Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
**Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

Abstract

**Background:** Chromosomal aberrations which occur in different hematologic malignancies are believed to be highly applicable for identifying the prognosis and treatment protocols. We conducted the present study to investigate the chromosomal and molecular abnormalities in bone marrow of acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), and myelodysplastic syndrome patients.

**Method:** We performed this cross-sectional study in molecular pathology and cancer research center of Mashhad University of Medical Sciences (MUMS), during 2017-2019; the total number of our cases was 252. We did all the molecular and cytogenetic tests on these patients. SPSS V.16 software was utilized for the analysis of our data.

**Results:** In this research, the ALL patients were meaningfully younger than AML ones. There were significant associations between karyotype patterns and types of malignancy; normal diploid was more frequent in myelodysplastic syndrome \( (P<0.05) \). Among numerical abnormalities, trisomy 3 and monosomy 14 were the most prevalent ones.

**Conclusion:** The results of similar studies from different areas with different ethnics would help to identify new parameters for prognosis determination. Cytogenetic analysis is highly applicable for leukemia diagnosis and prognosis.

**Keywords:** Chromosome aberrations, Hematology, Cytogenetic analysis, Leukemia
Introduction
Leukemia is a clonal production of blood cells precursors in the bone marrow (BM). It is developed due to genetic mutation and chromosomal rearrangements.\(^1\) Leukemia classification is documented based on involved lineage, prognostic significance, and leukemia behavior.\(^2\)

The prognosis determination is of great importance for treatment planning. Cytogenetic and molecular assays are two powerful tools used for this purpose.\(^3\) Acute leukemia is very heterogeneous. To date, 300 chromosomal abnormalities have been identified in acute myeloid leukemia (AML).\(^4\) Cytogenetic picture of acute leukemia is much more complex in contrast to chronic ones; it is also more valuable for prognosis and diagnosis.\(^3,5\)

Cytogenetic analysis of AML and acute lymphoid leukemia (ALL) patients indicated several recurrent genetic abnormalities.\(^6\) The fourth international workshop on chromosomal abnormalities in leukemias (4IWCL) was the first large study that suggested cytogenetic tests for BM samples. These assessments are important for patients prior to starting the treatment procedure. The cytogenetic results are very important and are considered as an independent factor in AML diagnosis.\(^6,\,7\) The frequency of different chromosomal abnormalities could vary among different populations.\(^8,\,9\) This discrepancy seems to be generated from ethnicity and geographic differences. Even though conventional G banding method involves limitations for certain abnormalities, such as t (12; 21), it is very applicable in hematological malignancies.\(^10\)

In this research, we aimed to study the chromosomal abnormalities in hematologic malignancies in different ages and sex groups in northeast Iran (Khorasan Razavi province).

Method and Material
Study design and patients
This cross-sectional analysis was performed in cancer molecular pathology research center of Mashhad University of Medical Sciences (MUMS) during 2017-2019. The participants were diagnosed as AML, ALL, and myelodysplastic syndrome (MDS) patients. Peripheral and BM smears of all the patients were sent to the research center for molecular and cytogenetic analysis. Two expert hematopathologists reviewed the smears. The diagnosis was confirmed with morphology and specific staining, such as Gimsa, myeloperoxidase, molecular, and cytogenetic findings.

Ethical issues
For note, this is a report from cancer molecular pathology research center of MUMS. The patients’ information was obtained from the archives of the research center. All the ethical issues were observed to hide the patients’ name; it was approved by the ethic committee of Mashhad university of Medical Sciences. This study was one of the articles extracted from the results of project number 960039, under the ethics code of IRMUMS.fm.RC.1396.276.

Recurrent translocation
ALL recurrent mutations, such as t (1; 19), t (4; 11), t (12; 21), t (9; 22) p190, and AML, including t (8; 21), t (15; 17), t (16; 16), inv (16), t (6; 9), and t (9; 11), were performed for the cited patients. Moreover, we did qRT-PCR. The molecular steps were applied according to Ayatollahi’s study about quantitative evaluation of WT1 in AML patients.\(^11\) No molecular studies were carried out for the MDS cases. The primary diagnosis of MDS was done according to the morphology of BM smears and the patient clinical manifestations. All the chromosomal abnormalities of the MDS individuals were observed and confirmed in the BM karyotype of patients.
Cytogenetic and analysis
BM samples were cultured for conventional cytogenetic assessment and the applied method has been described in Ayatollahi’s study, in detail. The cultured samples were harvested (incubation, adding hypotonic solution, washing by fixative). The prepared slides were stained employing G banding method and evaluated by an expert cytotechnologist; at least 10 metaphases were checked for the possible abnormalities. The results were reported according to ISCN 2017.

Definition of karyotype patterns
The results of BM karyotype were classified as normal diploid, hypo and hyper diploid, complex karyotype, and pseudo-diploid. Normal karyotype or diploid karyotype is defined by the presence of each pair of chromosome 1-23. Hypo and hyper diploid are known by the presence of less and more than 46 chromosomes, respectively. Complex karyotype is identified by 3 or more than 3 clonal or structural aberrations. Pseudo diploid is the presence of 46 chromosomes with numerical or structural abnormalities.

Age, sex, clinical diagnosis, and type of cytogenetic abnormality were the involved variables for the statistical analysis.

Statistical analysis
The entire statistical assessments were done employing SPSS V16. Chi square test was applied for finding the differences between the parameters. P<0.05 was considered to be statistically significant.

Results
A total number of 252 patients with hematologic malignancies, including 76 AML (30.15%), 147 ALL (58.33%), and 28 MDS (11.11%), were studied in this research. The mean age of the studied individuals was 22.38. The analysis of conventional karyotype indicated 50.79% (128) normal diploid, hypo-diploid of 4.36 %, hyper-diploid of 9.52 %( 24), pseudo-diploid of 20.63% (52), and complex karyotype of 14.88 %( 37). Table 1 represents the age, sex distribution, and the frequency of different karyotype.

Cytogenetic data and age
Patients age classification has been defined as the following in other researches in two groups of pediatrics (<15) and adults (≥15). According to the low frequency of adults in ALL and low frequency of pediatrics in AML groups, we were not successful to categorize the population based on age definition. A significant difference was observed between the acute leukemia and mean age (P value: 0.00); in other words, the mean age of the ALL individuals was meaningfully lower than that of the AML ones (P value<0.0). The sex ratio (male/female) in the ALL group was slightly higher than that in the AML group (1.7 VS 1.3); sex distribution was not significantly different between the two types of acute malignancies (P>0.05). Moreover, MDS was more frequent among the men in our study.

Cytogenetic data and malignancy type
The initial analysis showed a meaningful relation between the malignancies and karyotype pattern. Normal diploid karyotype was meaningfully higher in the MDS cases in comparison with the AML cases (P< 0.00); 79.3% of the MDS showed normal diploid template. Table 2 depicts the abnormal karyotype results of the MDS patients. Chromosome 17 abnormalities were observed in 83.33% of the MDS patients with abnormal karyotype. Hyper diploid karyotype was more prevalent in the ALL individuals (P< 0.07). The pseudodiploid pattern was meaningfully more frequent in the AML patients compared to that in the MDS and ALL patients (P< 0.01 and 0.00). Figure 1 demonstrates the amount of chromosomes loss and gain, based on
cytogenetic results. According to the evaluation of the numerical abnormalities in the acute leukemia cases, monosomy of chromosome 14 and trisomy of chromosome 3 were the most prevalent numerical abnormalities. Regarding the cytogenetic findings of the present study, figure 2 shows various types of structural chromosomal abnormalities in the ALL and AML groups. Double minutes are acentric chromosome fragments which rise during cell division; it was found in two ALL and one MDS case.

**Molecular data**
As it was mentioned, recurrent translocations of ALL and AML were run for each patient. No t (6; 9) was found in the studied individuals. The most frequent recurrent translocations were t (12; 21) and t (15; 17), which were detected in 10.20% and 14.47% of the ALL and AML individuals, respectively.

**Novel karyotypes**
Novel karyotypes were detected in 25 patients (9.92%). 23 cases out of 25 (92.00%) de novo karyotypes belonged to the acute leukemias group (AML&ALL).

**Discussion**
This study described the series of children and adolescents from Iran with hematologic disorders, including AML, MDS, and ALL. Our subjects were characterized with various cytogenetic aberrations. Most of the leukemia patients were male. Normal diploid pattern and t (15;17) were the most frequent karyotype and molecular findings among the studied population.

Cytogenetic information (cytogenetic profile) is highly applicable for leukemia diagnosis. The crucial role of conventional cytogenetic, particularly BM karyotype, is elucidated for hematologists and oncologists in order to determine the prognosis and indicate the treatment protocols. Despite all its limitations, bone marrow karyotype is widely used in the cancer cytogenetic labs for leukemia diagnosis. In accordance with previous studies, the sex ratio (male/female) revealed a slight male predominance which was observed in all the three hematologic neoplasms; evaluating 140 patients, Koliipoli reported male/female ratio of 1.33. It seems that further frequency of malignancies in male gender is attributed to weak cellular immune function compared to females.

Cytogenetic abnormalities were detected in 54.7% of all the AML cases; this statistic is comparable to other studies from various geographic regions (range of 52–80% abnormalities). t (15;17) was the most prevalent chromosomal abnormality among the Iranian de novo AML patients with a percentage of 14.7%, which is comparable with that observed in China (14.5%) 22, Korea (8.6%) 4, and other studies in Asian populations. Consistent with the present findings, t (15; 17) was the most usual chromosomal change (15.32%) in Manola’s report from Greece; t (8;21) was in the second place too (13.7%).

In de novo AML patients, normal karyotype pattern was detected to be 45.3%, which is similar to other studies. Complex karyotype was found in 8% of the studied patients, which is noticeable in the previous studies in China (8%) by Cheng, and in Korea (12.5%) by Byun and colleagues. 22 Stephen, in the assessment of 1592 cases of AML, reported normal and complex karyotype in 42% and 21% of the cases, respectively. Sierra et al. from Spain believes that karyotype patterns of AML are various in different areas; this difference is attributed to the genetic variations and races.

In spite of the few studies on cytogenetic profile of MDS, cytogenetic disorders are found in half of the MDS cases; specifically, complex karyotypes are frequent in MDS individuals. Despite previous reports about karyotype pattern of MDS, the present study...
found a significant relationship between normal karyotype and MDS syndrome ($P=0.0$). According to table 2, 5 out of 6 (83.33%) MDS individuals with abnormal karyotype demonstrated various types of chromosome 17 abnormalities, including trisomy, deletion, and iso-chromosome. The remarkable involvement of this chromosome reveals its important role. It seems that the located genes on chromosome 17 have tremendous effects on the disease emergence and the prognosis; it could be a striking issue to follow-up. The study on MDS patients by Wang from China suggested 1q trisomy as the most abnormal occurrence, which is associated with poor prognosis. Due to the lack of information, we cannot follow the patients to identify the prognosis.

In the category of ALL, cytogenetic results represented 52.3% abnormal patterns. Reviewing the results of the present and previous papers displayed a higher frequency of abnormal karyotype in the ALL against AML cases. Meanwhile, the statistical analysis did not confirm any meaningful relations concerning this matter ($P=0.7$). The patients with structurally complex karyotype had a significantly higher relapse-free survival than the individuals with other abnormal karyotypes, such as t (4; 11), low hypodiploidy, and t (9; 22); they are known as higher risk cytogenetic features of ALL. The higher frequency of abnormal karyotype seems to be the cause of worse prognosis and outcome of ALL.

Strickland et al., in the prospective treauputic trial about AML individuals, reported that chromosome 1, 2 followed 8 and 15 monosomies are the least common ones. Chromosome 17 was more frequently lost (48%). However, Luquet in the retrospective survey of 38 hyper diploid karyotype reported trisomy of chromosome 8 as the most frequent (68%) gain among AML patients. It was reported in 10.5% of AML, including adults and pediatrics in the Kopolini’s report. Nevertheless, in the present research, the gain and the loss of this chromosome was not significantly high or low. This difference might be due to the frequency of two types of acute leukemias in the two studies. The previously published data described a hypothesis about the essential role of trisomy 8 in generating AML, which is in contrast to the fact that hyper diploid karyotypes are associated with proper prognosis compared to hypo ones; it emphasizes the effect of gene dosage. Consequently, the gain and the loss impress the prognosis compatible to the affected genes. The analysis of gene dosage was performed for certain disorders, AML for instance. Meanwhile, according to the frequent occurrence of numerical abnormalities in leukemias, it is suggested to be analyzed completely.

Ultimately, this study evaluated the hematologic malignancies structurally and numerically. We used conventional cytogenetic and routine molecular study for the cited aim. It is suggested to design and perform similar studies with a larger population and utilizing more up to date techniques in order to gain further accurate results.

**Conclusion**

This paper was a report from chromosomal variations in different hematologic malignancies. It would help hematologists for searching new genes in affected chromosomes or even effects of chromosomal changes on oncogenic genes. Performing survival studies and statistical analysis are suggested in various racial and geographical populations. The results could help to focus on some new topics for the determination of the pivotal parameters.
about the generation and prognosis of the disease.

Acknowledgment
We sincerely thank the staff of cancer molecular pathology research center of MUMS for their professional cooperation.

Conflict of Interest
None declared.

References


Table 1. Age, sex, mutation, and karyotype distribution of the patients with hematologic malignancy

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>AML</th>
<th>ALL</th>
<th>MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age</td>
<td>28.71±19.76</td>
<td>10.74±13.55</td>
<td>49.00±17.28</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>(43)</td>
<td>(94)</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>57.3%</td>
<td>42.7%</td>
<td>63.9%</td>
</tr>
<tr>
<td>karyotype</td>
<td>Normal diploid</td>
<td>45.3%</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>Hyper diploid</td>
<td>5.3%</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>Hypo diploid</td>
<td>2.7%</td>
<td>(2)</td>
</tr>
<tr>
<td></td>
<td>Complex karyotype</td>
<td>10.7%</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td>Pseudo diploid</td>
<td>36.0%</td>
<td>(27)</td>
</tr>
<tr>
<td></td>
<td>Recurrent translocations</td>
<td>t(1;19)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t(4;11)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t(9;22)p190</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t(12;21)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t(8;21)</td>
<td>5.26%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t(15;17)</td>
<td>14.47%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inv(16)</td>
<td>3.94%</td>
</tr>
</tbody>
</table>

AML: Acute myeloid leukemia
ALL: Acute lymphoblastic leukemia
MDS: Myelodysplastic syndrome

Table 2. Abnormal karyotype of the MDS patients

<table>
<thead>
<tr>
<th>num</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47,xy,+6[5],46,xy[15]</td>
</tr>
<tr>
<td>2</td>
<td>46,xy,t(17q)[8]/46,xy[7]</td>
</tr>
<tr>
<td>3</td>
<td>46,xy,+y[3]/del(16)(q22),del(17)(p12,p13)[16],46,xy[24]</td>
</tr>
<tr>
<td>4</td>
<td>46,xx,del(17)(p12)[10]</td>
</tr>
<tr>
<td>5</td>
<td>66-70,xy,t(2,4)(q11,q13),t(14,21)(q22,q22),+1,+1,+2,+2,+3,+6,+6,+9,+9,</td>
</tr>
<tr>
<td></td>
<td>+11,+13,+14,+14,+17,+19,+20,+20,+21,+21,+21,+21,+22,2-6 dmin [cp25]/48,</td>
</tr>
<tr>
<td></td>
<td>xyt(14,21)(q22,q22)+r(7)(p22q36)+8,2-4dmin[cp5]</td>
</tr>
<tr>
<td>6</td>
<td>45-46,xy,t(2,12)(q31,p13),dic(3,6)(q11,q11),-6,del(17)[5]/46,xy[10]</td>
</tr>
</tbody>
</table>

*dmin: Double minute
Figure 1. This figure shows chromosomal gain and loss in acute leukemias (ALL & AML).
AML: acute myeloid leukemia
ALL: acute lymphoblastic leukemia
Figure 2. This figure shows the frequency of structural and numerical chromosomal variations in acute leukemias (AML&ALL).

AML: acute myeloid leukemia
ALL: acute lymphoblastic leukemia