

## ORIGINAL ARTICLE

# CHROMOSOMAL ABNORMALITIES IN LEUKEMIA IN IRAN: A PILOT STUDY

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### Abstract

**Objective-**To carry out cytogenetics investigations on bone marrow and peripheral blood samples obtained from patients with leukemia.

**Methods-**A total of 35 bone marrow or blood samples from all types of leukemia patients was referred to the Cytogenetic Laboratory of Tehran University of Medical Sciences. The referral centers were the Hematology and Oncology Centers in Shariati Hospital and the Children's Medical Center. Cell culturing including high resolution (HR) and Giemsa banding (G-bands by trypsin using Giemsa stain; GTG) were carried out according to standard protocols. Chromosome analysis was performed following international system for human cytogenetics nomenclature (ISCN) guidelines (1995).

**Results-**Among the 28 cases, chromosomal abnormality rate was 50% in acute myelogenous leukemia (AML), 80% in acute lymphocytic leukemia (ALL), 83% in chronic myelocytic leukemia (CML) and 100% in the Lymphoproliferative disease (LPD) groups. Two patients in the myeloproliferative disease (MPD)/CML group had normal karyotype and were therefore treated as MPD. The types of observed chromosomal abnormality were translocation 15/17 in AML-M3 patients, double trisomy 8 and 13 in an AML patient, hyperdiploidy of 50-55 of chromosomes in a child with ALL, a double abnormality of Philadelphia and t 7/8 in a CML patient at blast stage and a complex variant Philadelphia translocation of a t4/9/22 in a CML patient.

**Conclusion-**Despite being a pilot study with a small number of samples, the majority of patients demonstrated chromosomal abnormalities comparable to previously reported cases in other countries. The type of chromosomal abnormality was relevant to diagnosis and stage of the disease.

**Keywords** • Leukemia • acute • chronic • chromosomal abnormality • lymphocytic • myelogenous

### Introduction

Cytogenetic investigation on malignant tumors is a well-established procedure. In 1960, with the discovery of the Philadelphia chromosome in chronic myelogenous leukemia (CML) patients, Hungerford and Nowell showed that certain neoplasias could have specific chromosome rearrangements.

They also proved Boveri's theory on clonal

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origin in cancer. The cytogenetic investigation has a crucial role in establishing the diagnosis, staging and prognosis in neoplasia.<sup>1</sup>

Progress in cytogenetic techniques such as banding<sup>2</sup>, high resolution<sup>3</sup> and molecular cytogenetics including fluorescent *in situ* hybridization (FISH)<sup>4,5</sup> have revolutionized the interpretation of chromosome rearrangements in neoplasia.

According to the Catalog of Chromosome Aberrations in Cancer<sup>5</sup>, chromosome rearrangements have been reported in more than 22,000

## Chromosomal Abnormalities in Leukemia in Iran: A Pilot Study

**Table 1.** Summary of result in leukemic patients.

Final diagnosis (No. of patients)	No. with abnormal karyotype (%)	Subtype of leukemia (No. of patients)	Type of chromosomal abnormality
AML* (12)	6 (50)	AML-M3 (5)	15/17 translocation
		AML-M2 (1)	47, XY, +8/47, XY, +13/48, XY, +8, +13/ 46, XY
ALL† (5)	4 (80)	-----	-----
LPD‡ (3)	3 (100)	Referred as large cell lymphoma (1)	Deletion of long arm of chromosome 7 at q36 region
		Referred as LPD	Fifty-two percent of the cells contained double chromosome abnormalities including loss of chromosome Y and an isochromosome 7q
		Referred as lymphoma	All cells had deleted 6q
CML§ (6)	5 (83)	-----	Philadelphia chromosome translocation in all 5 cases
MPD   (2)	0	-----	Philadelphia negative

\*Acute Myelogenous Leukemia, † Acute Lymphocytic Leukemia, ‡ Lymphoproliferative Disorders, § Chronic Myelogenous Leukemia, || Myeloproliferative Disease

types of neoplasia.<sup>6</sup> Some forms of leukemia have specific chromosome aberrations. For example, translocation 15/17 is specific for AML-M3, while inversion 16 is associated with AML-M4E. However, with progression of the disease, new clonal changes can occur and some of the cytogenetic findings are significant for the prognosis and outcome of the treatment. For example, in AML patients, inversion 16 and translocation 15/17 can have a long remission period and good prognosis but monosomy 5 and 7 are usually associated with poor prognosis in AML.<sup>7-9</sup>

In this study, chromosomal investigation was carried out in 35 bone marrow and peripheral blood samples in leukemic patients and chromosomal findings were assessed in relation to diagnosis, stage and prognosis of the disease.

### Materials and Methods

A total of 35 specimens (31 bone marrow and 4 peripheral blood) from patients with leukemia were received from the Genetic Department of Tehran University of Medical Sciences. The patients were referred from the Hematology and Oncology Sections of Shariati Hospital and the Children's Medical Center, Tehran University of Medical Sciences, and were of five different types of leukemia (acute lymphocytic leukemia, AML; ALL, myeloproliferative disease, CML; MPD and Lymphoproliferative disorders; LPD). Bone marrow aspirate (1mL) was collected in 5mL

sterile transport medium containing heparin and antibiotics. In the event of a dry tap and fibrosis, peripheral blood was collected.

Tissue culturing was carried out using standard protocols.<sup>1</sup> Four cultures including overnight clocemid, 24 h, 48 h and synchronized 24 h using FUdR/Uridine block were set up. The cell density used was 10<sup>6</sup>/mL. All cases were routinely evaluated by Giemsa banding (GTG). About 10-30 metaphase spreads were examined. An abnormal clone was defined as a minimum of two cells for structural or a gain in chromosome number and minimum of three cells for a chromosome loss. ISCN guidelines<sup>10</sup> were followed for chromosome nomenclature.

### Results

The karyotype was obtained in 28 patients (80%). The number of abnormal karyotypes, subtypes of leukemia, and types of chromosomal abnormalities are shown in Table 1. As it is evident half of the patients with AML had karyotype abnormalities. All of the patients with LPD and more than three-fourth of patients with CML and ALL displayed some kind of karyotype abnormality.

Among the AML patients 5 cases were of AML-M3 of whom 3 had 15/17 translocations. One patient with AML-M2 had an abnormal karyotype containing four different cell lines: 47,XY, +8/47, XY, +13/48, XY, +8, +13/ 46, XY. This 68-year-old patient was diagnosed as MDS at

presentation but consequently progressed to AML-M2 and died shortly after.

Concerning the ALL patients, in a 13-year-old boy who had been diagnosed, as ALL, most cells were hyperdiploid with 50-55 chromosomes. In another case, a 5-year-old child, 30% of the examined metaphase spreads contained a dicentric translocation between chromosomes 9 and 12.

All patients in the LPD group had abnormal karyotypes. One case was referred as large cell lymphoma, in whom 30% of the metaphase spreads had deletion of the long arm of chromosome 7 at the q36 region. In another case, a 25 year old man, 52% of the cells contained double chromosome abnormalities including loss of chromosome Y and an isochromosome 7q. The last case with LPD was a 16-year-old boy referred as lymphoma. All his cells displayed 6q depletion.

In the CML group, 5 out of 6 patients had the Philadelphia translocation. A 15-year-old boy with clinical features of CML in this group had normal karyotype. One of the specimens was a bone marrow sample obtained from a 34-year-old woman with CML at blast stage. All the metaphase spreads in addition to standard Philadelphia translocation (t 9/22) contained a translocation between chromosomes 7 and 8. On the other hand, this patient contained only the Philadelphia translocation at presentation. The chromosomal changes confirmed the progression of the disease from chronic to blast stage. Another case was a 27-year-old man at CML blast stage. All the metaphase spreads contained the variant Philadelphia translocation involving chromosomes 4, 9 and 22. Two other patients had the standard q/22 Philadelphia translocation.

Finally, one patient was suspected for CML. However this patient was Philadelphia negative. As a result was classified, as MPD and bone marrow transplantation was not carried out.

## Discussion

This study, despite being a pilot inquiry, and in fact due to the small number of cases should be regarded as a case series, signifies the status of chromosomal investigation in the determination of diagnosis, stage and prognosis of disease in patients with leukemia. The cytogenetic findings can have valuable contribution in the management of patients with leukemia.

Regarding the AML cases, Overall, 70% of AML-M3 patients are reported to have 15/17

translocation.<sup>11</sup> Both trisomies 8 and 13 have been reported in AML and MDS. Patients with more than one chromosome aberration have poor prognosis.<sup>12</sup>

In the ALL cases, the figures are comparable to similar studies.<sup>5</sup> Patients with hyperdiploid cells with 50-55 chromosomes are usually pre-B cell ALL and have favorable prognosis.<sup>13</sup> Patients with dicentric translocation between chromosomes 9 and 12 are usually B-cell type ALL and have also a good prognosis.<sup>14</sup>

In LPD group, abnormalities of chromosomes 7 at q36-q37 are associated with T-cell lymphoma. Ten percent of patients with non-Hodgkin's lymphoma lack the Y chromosomes.<sup>7</sup> Isochromosome 7q has been reported in secondary ALL.<sup>15</sup> Deletion of 6q has been reported in T-cell leukemia and lymphoma.<sup>16</sup>

Five to ten percent of patients with chronic myeloproliferative disorders with clinical findings similar to CML, lack the Philadelphia chromosome but contain the hybrid between C-ab1 and C-bcr at the molecular level.<sup>17</sup> Considering the patient with Philadelphia translocation at the presentation that progressed to blast stage, it is indicated that 75% to 80% of CML patients show new chromosomal changes with evolution of the disease. Such karyotypic changes can occur even six months prior to clinical manifestation of the disease and thus can be valuable in the management of the patients.<sup>18</sup> Five to 10% of CML cases contain a variant Philadelphia translocation.<sup>19</sup>

Finally, patients like the one being Philadelphia negative and classified as MPD usually have better prognosis.<sup>9</sup>

It is recommended that extended studies with more number of patients be carried out in order to obtain a more clear view of the chromosomal abnormalities in leukemia in Iran.

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## Chromosomal Abnormalities in Leukemia in Iran: A Pilot Study

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