Some Specific Chromosomal Aberrations of Hematologic Malignancies in 80 Iranian Population

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Abstract

Introduction: Most of the hematologic malignancies are heterogenous with regard to morphology, immunophenotype, and genetic rearrangements. Multiple recurrent chromosomal aberrations have been identified by conventional cytogenetic analysis, which is now widely recognized as one of the most important diagnostic and prognostic determinants in these patients.

Patients and Methods: Bone marrow samples were obtained from 80 patients with different hematologic malignancies. These consisted of 43 CML cases, 27 AML, 9 ALL and 1 MDS. In each case, cells were cultured and conventional cytogenetic analysis was performed.

Results: Among the 80 subjects, 53(66%) were abnormal and 27(34%) showed apparently normal karyotype. The various aberrations in abnormal cases were t(9;22)(q34;q11) in 43 CML (100%), Monosomy Y in 2 CML (4.6%), monosomy 7 in 1 CML (2.4%), trisomy 8 and t(15;17)(q22;q21) in 2 AML case(7.4%), t(8;21)(q22;q22) in 1 AML (3.7%) and complex karyotype in 2 CML, 1 AML , 1 ALL and 1 MDS (6%). Apart from these, some novel chromosomal abnormalities were observed in our study population.

Conclusion: The difference in the frequency of clonal chromosomal aberrations is probably the result of the applied methods for chromosome preparation and often very poor morphologic chromosome appearance, making the identification of finer structural abnormalities more difficult. Furthermore, ongoing cytogenetic studies are warranted in larger groups of hematologic malignancies to identify newly acquired chromosomal aberrations that may aid in cloning novel genes involved in the neoplastic process, ultimately helping in the development of targeted therapeutic drugs.

Keyword: Chromosomal aberration, Hematologic Malignancy, Karyotype

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Introduction

Cytogenetic analysis of malignant hematological disease is an important methodology used by clinicians and researchers, as observations of clonal chromosomal abnormalities have been shown to have both diagnostic and prognostic significance.

Conventional cytogenetic analysis of chromosome abnormalities in hematologic malignancies is hampered by the low mitotic index and poor quality of metaphases. Either bone marrow or peripheral blood cells may be used to prepare chromosome spreads for cytogenetic analysis. The identification of these nonrandom chromosomal abnormalities in association with specific hematological diseases laid the foundation for the clinical significance of cytogenetic analysis.(1, 2, 3) Recurring chromosome abnormalities are important prognostic indicators for the hematologist.(4) Cytogenetic anomalies identified in patients with hematologic malignancies are among the most important independent prognostic factors and are currently used to plan for different types of therapy. Acquired chromosomal abnormalities, structural or numerical, are detected in malignant bone marrow cells in more than 75% of patients with hematologic malignancies, with an increasing incidence due to the application of complementary detection methods provided by molecular cytogenetics.(5)

The accuracy of cytogenetic analysis has been significantly improved over the last 30 years due to

Table- 1. FAB subtypes and chromosomal abnormalities of the patients with Complex Karyotype

FAB Type	Karyotype
CML	48,XY,t(9;22)(q34;q11);+8,+Ph[18]/47XY,t(9;22)(q34;q11);+8[78]/46,XY[5]
MDS	47,XX,+8[3]/47,XX,+8,par inv(17)(p11,p13)[8]/46,XX[9]
ALL	46,XY,i(8)(q10),del(9)(p22)[14]/46,XY[1]
ALL	45,XY,t(8;14)(q24;q32),dup(1)(q22;q44),-21[21]/46,XY, ,t(8;14)(q24;q32), dup(1)(q22;q44)[1]/45,XY, t(8;14)(q24;q32),-21[6]/ 46,XY,t(8;14)(q24;q32), dup(7)(q32;q36[1]/ 46,XY,t(8;14)(q24;q32), dup(1)(q22;q44),t(11;14)(q13.2;q13), [19]/45,XY, t(8;14)(q24;q32), dup(1)(q22;q44), t(11;14)(q13.2;q13),-21[1]/46,XY, dup(1)(q22;q44), ,t(11;14)(q13.2;q13)[2]/ 45,XY, dup(1)(q22;q44), t(11;14)(q13.2;q13),-21[1]
CML	47,XY, ,t(9;22)(q34;q11),i(17)(q10),+8[19]/46,XY, t(9;22)(q34;q11),i(17)(q10)[16]/ 46,XY, ,t(9;22)(q34;q11)[15]
AML	46,XX,del(9)(q22)[45]/ 46,XX,del(6)(p)[16]/ 46,XX,del(16)(q)[7]/46,XX,[32]
CML	46,XY, t(9;22)(q34;q11)[47]/ 46,XY, t(9;22)(q34;q11), del(6)(p)[4]/47,XY, t(9;22)(q34;q11),del (6)(p);+12[1]

FAB: French-American-British, CML: Chronic Myelogenous Leukemia, MDS: Myelodysplastic Syndrome, ALL: Acute Lymphoblastic Leukemia, AML: Acute Myelogenous Leukemia.

Table- 2. Chromosomal abnormalities in the 80 patients successfully karyotyped

Parameter	No. of patients	CML	AML	ALL	MDS
Karyotypes	80	43	27	9	1
Normal	27		20	7	
Abnormal	53	43	7	2	1
Single	42	37	5		
Double	6	4	1	1	
Complex	5	2	1	1	1
Monosomy Y	4	2	2		
Monosomy 7	1	1			
t(15;17)(q22;q21)	2		2		
trisomy 8	4	2	1		1
t(9;22)(q34;q11)	43	42		1	

technical advances regarding culture methodology and banding techniques.(6, 7)

High resolution chromosome analysis, introduced in 1976 by Yunis, involves synchronization of dividing cells in prophase or prometaphase, resulting in longer chromosomes with multiple bands.(8) At this level of resolution (over 600 bands per chromosome), structural abnormalities of 3-5Mb of DNA can be detected, while alterations smaller than 3Mb and translocations involving telomeric regions are extremely difficult to identify.(9) Furthermore, high resolution chromosome analysis is labor-intensive and has the limitation of the inconsistency of band resolution. The possible presence of multiple abnormal clones, the poor quality of metaphases and the low mitotic index associated with the disease have been widely recognized as the major problems associated with applying conventional cytogenetic analysis to hematologic malignancies.

Patients and Methods

A total of 4 ml of bone marrow (BM) aspirates were obtained from 80 patients with different hematological malignancies who visited the Department of Hematology- Oncology and Stem Cell Transplantation at Shariati Hospital, from 2006 to 2008. All the cell cultures and cytogenetic analysis were performed in our center with the support of grants from the Tehran University of Medical Sciences.

Bone marrow cells were cultured for 24 hours in RPMI 1640 supplemented with 20% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and streptomycin. After a 24-hour incubation, colcemid was added at a final concentration of 0.1 μg/ml for 20 minutes. Then, the cells were treated with hypotonic KCl (0.075 M) for 12–15 minutes and fixed with methanol/acetic acid (3:1). Metaphase chromosomes were banded using the conventional GTG banding technique and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN).(10) Each time, the 10 to 20 chromosomal spread was analyzed even if the quality was not optimal; from these, twenty metaphases were analyzed with Leica software whenever possible to demonstrate the clonal nature of the aberrations. A karyotype was considered complex if there was an involvement of three or more chromosomes.

Results

We report herein cytogenetic studies on 80 Iranian patients with hematological malignancy; 43 CML, 27 AML, 9 ALL, and one MDS case. Of these, 51 (60.7%) were males and 29 (39.5%) were females (Figure- 1). The age distribution was in the range of 3-70 years and the mean age was 40 years. Patients were divided into different ranges of disease and age as shown in Figure- 2. Of the 80 patients, which were successfully karyotyped, 27 (34%) had normal karyotype and 53 patients (66%) had a

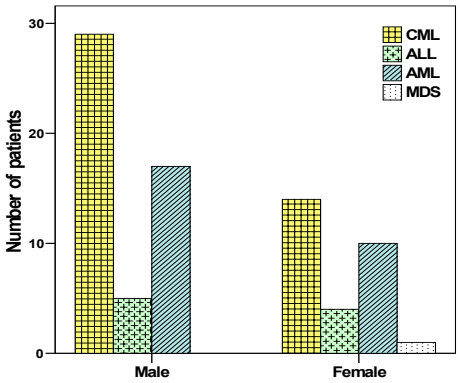

Figure-1. Sex distribution of different disease groups in 80 Iranian patients

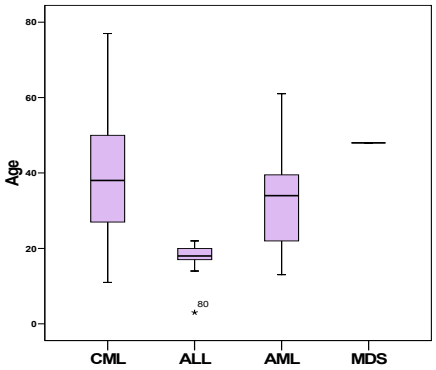

Figure- 2. Age distribution of different disease groups in 80 Iranian patients

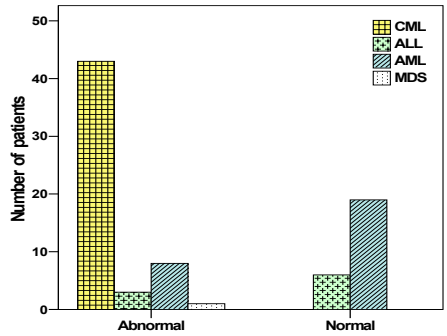

Figure- 3. Normal and abnormal karyotype between different patients groups

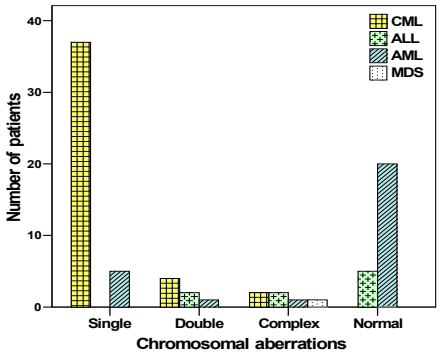

Figure- 4. Single, double and complex karyotype between different groups of patients

chromosomal abnormality (Figure- 3). Some of the patients has single, double or complex karyotypes with one, two, three or more than chromosomal aberrations (Figure- 4). The most frequent chromosomal abnormalities were t(9;22)(q34;q11) in 43 CML (100%). Monosomy Y was found in 2 CML (4.6%) and in 2 AML (7.4%), monosomy 7 in 1 CML (2%), trisomy 8 in 2 CML (4%), 1 AML (3.5%) and 1 MDS. Chromosomal translocation of t(15;17)(q22;q21) was observed in 2 AML (7%) cases, t(8;21)(q22;q22) in 1 AML (3.7%) and complex karyotypes in 2 CML(4%), 1 AML(7%), 1 ALL (11%) and 1MDS. Some chromosomal abnormalities of the patients with complex karyotyoe are shown in Table-1. In addition to these non-random chromosomal abnormalities, some rare abnormalities were also encountered (Table-2). Karyotyping results of some chromosomal aberration in our patients are shown in Figures-5 A, B, C, D.

Discussion

For the past three decades, cytogenetic studies of hematological disorders indicate that each and every case is equally and critically important. There are an increasing numbers of balanced rearrangements associated with distinct cases and clinical features, suggesting that chromosomal abnormalities reflect basic differences in leukemia biology. Furthermore, clonal cytogenetic abnormalities are one of the most important factors in predicting clinical outcomes in leukemia and are used to guide risk-adapted treatment strategies.(11) In this study, 52.5% of the patients had at least one chromosome abnormality. The frequency of chromosomal aberrations reported in the literature is extremely variable, ranging from 51 to 90%.(12, 13, 14, 15) This difference in the frequency of clonal chromosomal aberrations is probably the result of the applied methods for chromosome preparation and often very poor morphologic chromosome appearance, making the identification of finer structural abnormalities more difficult. According to the double and complex chromosomal aberration, we found 7.5% of our patients with double and 6.5% with more than two chromosomal aberrations. We also found some recurring chromosomal aberrations like trisomy 8 in two CML, one AML and one MDS of our patients. From 27 AML patients that were successfully karyotyped, seven patients had abnormal karyotypes. The t(15;17) abnormality was identified in 7.4% of our AML patients. In some other reports, however, t(15;17) is identified in 11–15% of AML.(12,13,16)

From 43 CML patients that had been karyotyped, we found 43 abnormal karyotypes. From 43 CML

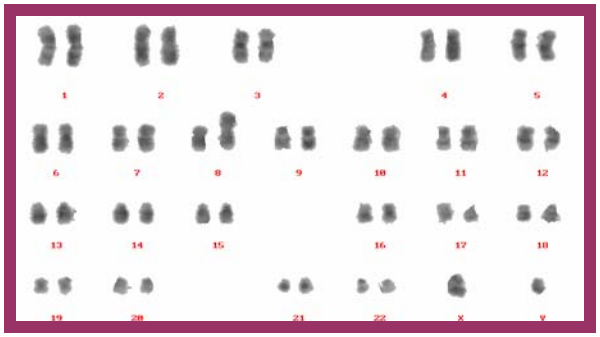

Figure- 5A. Karyotype of a CML patient with complex chromosomal aberration 46,XY,i(8)(q10),del(9)(p22)[14]/46,XY[1].

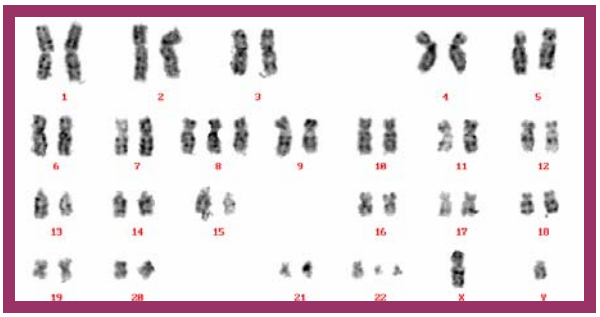

Figure 5B. Karyotype of a CML patient with complex chromosomal aberration 48,XY,t(9;22)(q34;q11);+Ph;+8[18]/47,XY,t(9;22)(q34;q11);+8[78]/46,XY[5].

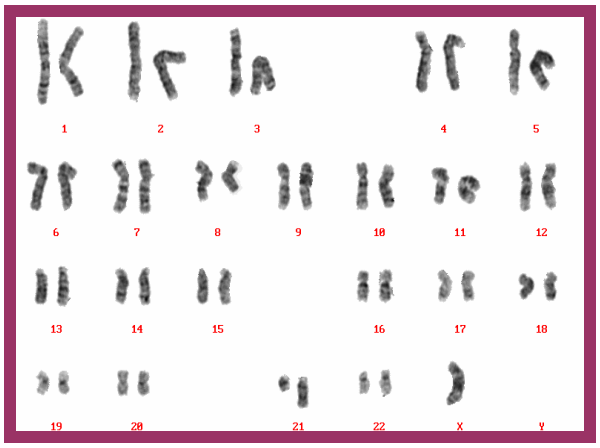

Figure 5C. Karyotype of a AML patient with chromosomal translocation 45,X,t(8;21)(q22;q22).

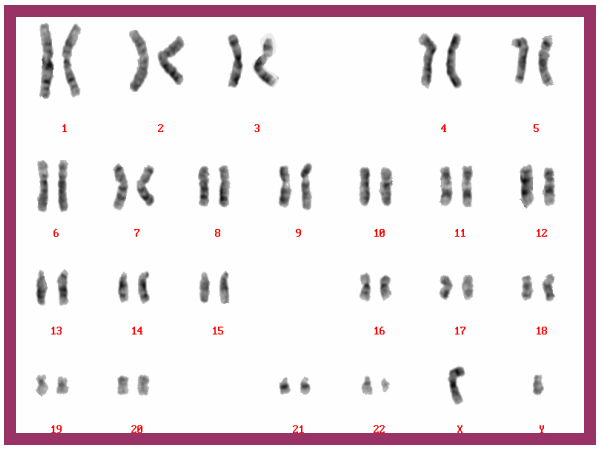

Figure 5D. Karyotype of a CML patient with chromosomal translocation 46,XY,t(9;22)(q34;q11).

patients with abnormal karyotypes all had Philadelphia chromosome but only 4.6% of them had complex karyotypes with trisomy 8 and monosomy Y. One ALL patient also had Philadelphia chromosome. We also showed one MDS patient with +8 and inv(17). This following cytogenetic aberration was reported in 15-20% of MDS and 5-10% treatment-related MDS.(10)

In conclusion, we are reporting some of the incidence of the chromosomal abnormalities which vary considerably among our patients with different hematological malignancies, from the time we have set up our own cytogenetic analysis.

Future large, prospective, and randomized trials are warranted to provide stronger evidence regarding the clinical relevance of chromosomal aberrations and its molecular pathogenesis.

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