CYTOGENETIC FINDINGS IN ACUTE MYELOID LEUKEMIA

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Abstract- Cytogenetics has now been well established as one of the most valuable prognostic factors in acute myeloid leukemia (AML). This is the first study to describe the cytogenetic findings in Iranian AML patients. During 1998 to 2001, 104 patients with adult *de novo* AML (excluding M3) were diagnosed and treated with the standard protocols in our center. Adequate cytogenetic analysis performed on bone marrow at diagnosis was available in 39 of these patients. Clonal chromosomal abnormalities were detected in 74.4% of the patients. The chromosomal changes seen in this study in order of frequency were: t(9;22), trisomy 11 [n=4, 10.3%], trisomy 8, Abn (3q)[n=3, 7.7%], trisomy 22, monosomy 7/del (7q), monosomy X, complex karyotype [n=2, 5.1%], and t (8;21), t (6;9), trisomy 21, monosomy 5/del (5q), monosomy Y, and Abn (11q) [n=1, 2.6%]. We also categorized the patients into favorable (2.6%), intermediate (74.4%), and unfavorable (23.1%) prognostic groups based on the criteria defined by Grimwade et al in MRC-AML-10. The frequencies of different clinical and paraclinical indices were studied in these groups. Notably, complete remission (CR) rates after one cycle of chemotherapy were 60.0% and 25.0% in intermediate and unfavorable prognostic groups respectively. The overall CR rates were 83.3% and 66.6% in the mentioned groups. These findings are somewhat comparable to the results of the larger studies in other countries, suggesting the importance of cytogenetics in Iranian patients. The differences could be due to methodological variations (notably exclusion of AML-M3 in this study), and the small sample size, although ethnic and geographical differences should not be disregarded. To further clarify these results with statistical significance a larger analytical study with a greater sample size is certainly needed.

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Key Words: Cytogenetic, acute myeloid leukemia, chromosome, prognosis

INTRODUCTION

Acute myeloid leukemia (AML) with an annual incidence of 2.4 per 100,000 (1.2% of malignancies in the US), is a relatively uncommon malignant disorder (1,2). Nevertheless, cytogenetically, AML is probably the most extensively analyzed human neoplastic disease (3). Cytogenetic studies of AML have contributed substantially to our understanding of the mechanisms of leukemogenesis and will likely facilitate designing of novel therapeutic strategies (4,5). In addition, acquired cytogenetic abnormalities have been shown to represent tumor markers of diagnostic and prognostic importance (3). Many recurrent aberrations have been correlated with presenting hematologic and morphologic parameters. Selected chromosomal aberrations are now being used to categorize AML in the new World Health Organization classification of hematologic malignancies (6). Moreover, karyotypic findings at diagnosis have been repeatedly shown to be among the most valuable independent prognostic factors regarding AML (7-24). In recent years many researchers have worked on the clinical importance of cytogenetic in AML, the bulk of them have been performed in the US and European countries. This descriptive case-series study is the first to describe the cytogenetic findings in Iranian AML patients.

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MATERIALS AND METHODS

Patients and protocol

This study was based on patients registered by AML Clinical Trial of Imam Khomeini Hospital, Tehran, Iran, which was a randomized clinical trial comparing three induction of remission therapies for adult patients (age 12-60 yr) with *de novo* AML. Briefly, during 1998 to march 2001, 104 patients with adult *de novo* AML were randomized to one of three induction therapies: *A* (cytarabine100 mg/m^2 continuously infused for 7 days plus daunorubicin 45 mg/m^2/day for 3 days, i.e. 7+3), *B* (7+2 with the same formulations and dosages), and *C* (5+3 with a cytarabine of 200 mg/m^2 continuously infused for 5 days plus daunorubicin at the same dosage for 3 days). Bone marrow aspirations were performed to investigate complete remission (CR). The goal of the

base study was to compare the induction therapies using the CR rates as the final outcome.

Cytogenetic analyses

Cytogenetic studies were performed on bone marrow samples of 39 patients obtained at presentation prior to induction therapy using standard G-banding technique in two qualified cytogenetic laboratories in Tehran. Bone marrow samples were cultured using standard methods; a minimum of 20 cells were fully analyzed to look for clonal abnormalities, using International System for Human Cytogenetic Nomenclature criteria (25). Karyotypes were considered normal if no clonal abnormalities were detected in any of the cells. Complex karyotype was defined by the presence of a clone with three or more abnormalities (22). Patients were divided into three different prognostic groups of favorable, intermediate, and unfavorable, according to published criteria by MRC AML 10 trial (23) (Table 1). The frequencies of different clinical and paraclinical indices were studied in these prognostic groups (Table 3).

Criteria for treatment outcome

Complete remission was defined according to the standard criteria (26) with peripheral blood neutrophil count ≥ 1500/µL, platelet count ≥ 100,000/µL, no blast cell in peripheral blood smear, marrow cellularity more than 20% with trilineage maturation, and marrow blast less than 5%, with no Auer rod visible.

Statistical methods

This study is a descriptive case-series study, with its results depicted as frequency tables, and distribution histograms.

RESULTS

Adequate cytogenetic analysis was available in 39 of the patients (Age: 12-60 yr; F/M: 20/19). Clonal chromosomal abnormalities were detected in 29 (74.4%) of these patients, and 10 (25.6%) had normal karyotypes. The chromosomal changes in order of frequency were: t (9;22), trisomy 11 [n=4, 10.3%], trisomy 8, Abn(3q)[n=3, 7.7%], trisomy 22, monosomy 7/del (7q), monosomy X, complex karyotype[n=2, 5.1%], and t (8;21), t (6;9), trisomy 21, monosomy 5/del (5q), monosomy Y, and Abn (11q) [n=1, 2.6%].

Table 1. Medical research council risk categorization criteria, Grimwade et al, MRC AMl 10, 1998

Chromosomal risk group	Abnormalities	Comment
Favorable	Inv (16)/t (16;16)/del (16q), t (15;17), t (8;21)	Either alone or with other abnormalities
Intermediate	Normal, 11q23 abn, +8, del (9q), del (7q), +21, +22, other structural/numerical abnormality	Without any favorable/unfavorable abnormality
Unfavorable	Del (5q)/-5, -7, abn (3q), complex karyotypes (≥ 5 chromosomal abnormalities)	Either alone or with other intermediate/ unfavorable abnormality

Table 2. Clinical and laboratory characteristics in different prognostic groups

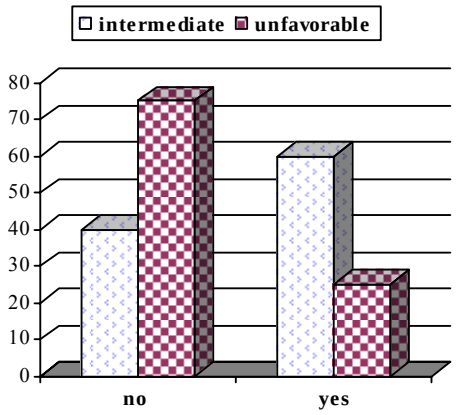
	Favorable*	Intermediate	Unfavorable
Age (year)			
Median	19	41.5	38.0
Range		16-60	12-60
Sex (% of female)	Male	48.3	66.7
Median WBC (10^9/L)	48.9	16.8	7.6
Median Hct (%)	13	25	27
Median Plt (10^9/L)	47	57	40.5
Hepatomegaly** (%)	100	40.0	33.3
Splenomagaly (%)	100	73.1	57.1
Lymphadenopathy (%)	100	44	12.5
Temp > 38ºC on admission	100	20.7	25
FAB Morphology			
M_2 (%)		57.1	77.8
M_4 (%)	100	25	22.2
M_5 (%)		10.7	
M_6 (%)		7.1	
Immunophenotype			
CD34+ (%)	100	65	80
CD7+ (%)		45.5	66.7

* This group includes only one patient
** Liver span > 12 cm

Table 3. Relative and absolute frequencies of different cytogenetic aberrations compared with those in MRC-AML 10 study, Grimwade et al, 1998

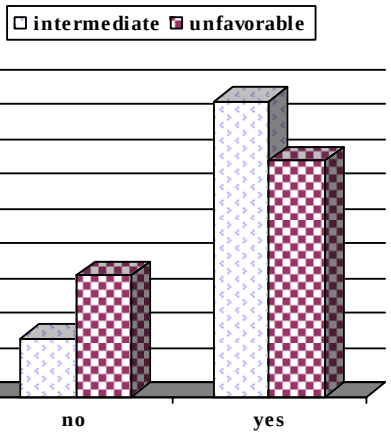
Cytogenetic aberration	MRC-AML 10		This study	
	percent	Total number	percent	Total number
All patients		1,612		39
No abnormality	42	680	25.6	10
T (15;17)	12	198	---	---
+8	9	148	7.7	3
T (8;21)	8	122	2.6	1
Complex	6	95	5.1	2
-7/del (7q)	6	93	5.1	2
11q23	4	60	12.8	5
Inv (16)	4	57	---	---
+21	3	45	2.6	1
Abn (3q)	3	40	7.7	3
-5/del (5q)	4	54	2.6	1
Del (9q)	4	52	---	---
+22	2	22	5.1	2
Other numerical	14	219	28.2	11
Other structural	23	366	28.2	11

We also categorized the patients into favorable (2.6%), intermediate (74.4%), and unfavorable (23.1%) prognostic groups based on the criteria defined by Grimwade, et al in MRC-AML-10. The frequencies of different clinical and paraclinical indices were studied in these groups (Table 3). Notably, complete remission (CR) rates after one cycle of chemotherapy were 60.0% and 25.0% in intermediate and unfavorable prognostic groups respectively (Fig. 1). The overall CR rates were 83.3% and 66.6% in the mentioned groups (Fig. 2).



	No	Yes	Total
Prog-favorable	1 (100)	---	1 (100)
Nostic intermediate	8 (40.0)	12 (60.0)	20 (100)
Group unfavaorable	6 (75.0)	2 (25.0)	8 (100)
Total	15 (51.7)	14 (48.3)	29 (100)

Fig. 1. CR at the end of first course



	No	Yes	Total
Prog-favorable	---	1 (100)	1 (100)
Nostic intermediate	3 (16.7)	15 (83.3)	18 (100)
Group unfavaorable	2 (33.3)	4 (66.7)	6 (100)
Total	5 (20.0)	20 (80.0)	25 (100)

Fig. 2. Compelete remission rates at the end of second course of induction chemotherapy. The numbers in parentheses are in percent

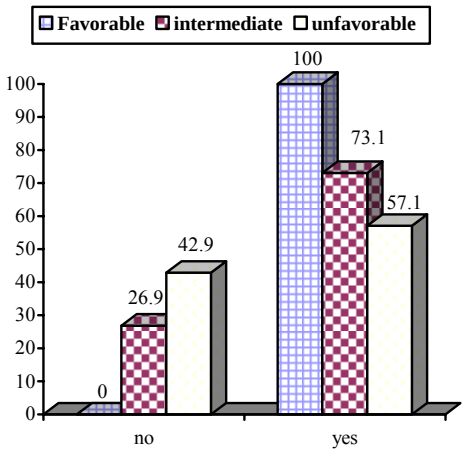


Fig. 3. Relative frequencies of splenomegaly in different prognostic groups

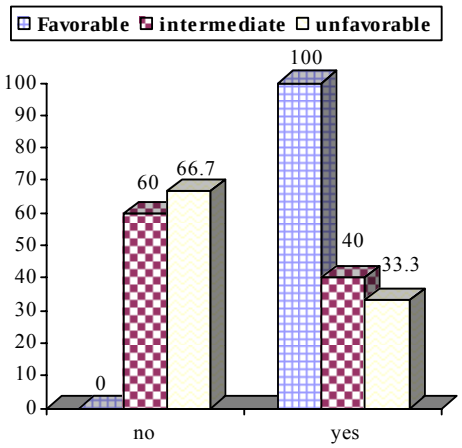


Fig. 4. Relative frequencies of splenomegaly in different prognostic groups

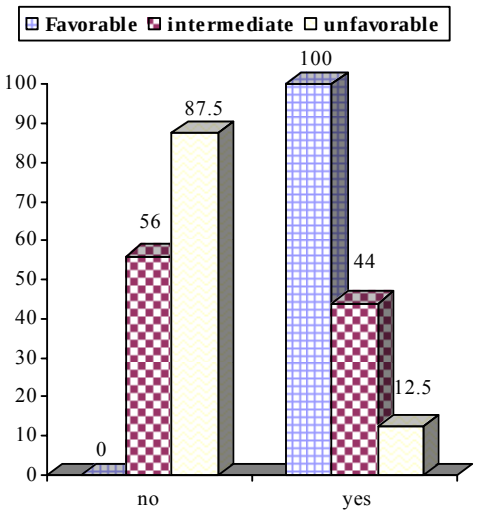


Fig. 5. Relative frequencies of lymphadenopathy in different prognostic groups

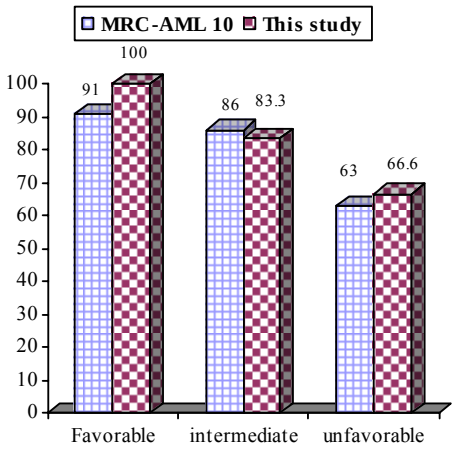


Fig. 6. Overall complete remission rates: a comparison with MRC-AML 10 study

Also interesting was the distribution of these groups in relation to organomegaly and lymphadenopathy: leukocytosis, hepatomegaly, splenomegaly, and lymphadenopathy are all seen in a greater proportion of patients with intermediate than unfavorable chromosomal changes (Fig. 3,4,5).

DISCUSSION

As there was only one patient with favorable prognostic group we did not include it in our discussion due to its low reliability. The results described above suggest some points the most important of which is the lower CR rate in unfavorable prognostic group than in intermediate prognostic group (25.0% vs. 60.0% after one cycle of chemotherapy and 66.7% vs. 83.3% overall). These results are compared with that of Grimwade study in Figure 6. Clonal chromosomal abnormalities were detected in 74.4% of our patients, the frequencies of which are compared with those in Grimwade study in Table 3. As shown the results are somewhat concordant, however some differences are seen: 1. There was no t (15;17) in our patients, which was clearly predictable due to exclusion of AML M3 in our study, 2. Abnormalities of chromosome 16, either as inv (16) or t (16;16) were not detected in our patients (vs. 4% in Grimwade study), 3. There was only one patient with t (8;21) in our study (vs. 8% in Grimwade study), 4. Changes of chromosome 11 including trisomy 11 were found in 12.8% of our patients which is greater than that of Grimwade study, and 5. Philadelphia chromosome, i.e. t (9;22), was detected in 10.3% of our patients, again greater than that of Grimwade study. The differences could be due to methodological variations (notably exclusion of AML M3), and the small sample size, although ethnic, and geographical differences should not be disregarded.

Clinical and laboratory characteristics in different prognostic groups are summarized in Table 3. As stated above leukocytosis, organomegaly, and lymphadenopathy are all seen in a greater proportion of patients with intermediate than unfavorable chromosomal changes. It may be due to chance, or alternatively may suggest a hypothesis that proliferative activity of leukemic blasts is more in intermediate group, resulting in their more sensitivity to the used chemotherapeutic agents. Very recently it was shown that proliferative activity of leukemic blasts was significantly more in favorable than in unfavorable prognostic groups (27). There were some limitations in this study. The major limitation was the small sample size: AML is a relatively rare disease and thus a longer period of time is needed. Another limition was that the patients were randomly treated with one of three somewhat different therapeutic protocols. Performance of cytogenetic study had no relation with therapeutic protocol, however, due to relatively small sample size, an error might have occurred from inequality of proportions of the mentioned protocols in each chromosomal prognostic group. To overcome this possibility, one should analyze the data of individual therapeutic groups separately, not practically applicable when the sample size is small. In conclusion cytogenetic seems to be the most important prognostic factor also in adult Iranian patients with *de novo* AML. To further clarify these results with statistical significance a larger analytical study with a greater sample size is certainly needed.

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