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Geographic Heterogeneity of the *AML1-ETO* Fusion Gene in Iranian Patients with Acute Myeloid Leukemia

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Abstract

Background: The human *AML1* gene, located on chromosome 21, can be fused to the AML1-eight-twenty-one (*ETO*) oncoprotein on chromosome eight, resulting in a t(8;21)(q22;q22) translocation. Acute myeloid leukemia (AML) associated with this translocation is considered a distinct AML with a favorable prognosis. Due to the various incidences of the translocation, which is associated with geographic diversities, investigation of molecular epidemiology is important to increase the awareness of physicians and hematologists regarding the frequency this chromosomal aberration.

Methods: The patients were classified according to the French–American–British classification into eight groups: M0–M7. Determination of the prevalence of the *AML1-ETO* fusion gene was accomplished by TaqMan real-time PCR. Bone marrow samples from 113 patients with newly-diagnosed, untreated AML -M1, -M2, and -M4, and 20 healthy controls admitted to the Ghaem Hospital in Mashhad, Iran were studied.

Results: The *AML1-ETO* fusion gene was detected up 50% of the M2 subgroup and absent in the M1 and M4 subtypes and healthy controls. Comparison of the prevalence of the t(8;21) translocation with results of previous studies showed that it varies between countries. This result may be due to geographic or ethnic differences, or both.

Conclusions: The relatively high prevalence of the t(8;21) translocation in Iran was similar to that found in other Asian countries. It was closely associated with female gender, relatively young age, and FAB-M2 subtype. Its distribution varied considerably with geographic area. Therefore, further studies are needed to provide epidemiological data important for the establishment of optimal therapeutic strategies applicable to patients of each region.

Keywords: Acute myeloid leukemia, AML1-ETO, M2, Prevalence, t(8;21)

Introduction

The *AML1* gene, known as *RUNX1*, located on chromosome 21, can be fused to the eight-twenty one oncoprotein (*ETO*) or myeloid translocation gene on chromosome 8 (*MTG8*) (1). The t(8;21) translocation has often been described in acute myeloid leukemia (AML). The *AML1-ETO* gene rearrangement was first detected more than 40 years ago by Rowley (2). According to the French–American–British (FAB) classification system (3, 4), AML patients with the t(8;21) translocation generally present with AML-M2 morphology, with a minority of cases with AML-M1 or -M4 (5). The new reclassification of AML recently recommended by the WHO requires the identification of non-random chromosomal translocations as an aim for good clinical follow up (6).

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The t(8;21) translocation is detected in 7–8% of adult and 12% of childhood AML patients and 10%–22% of cases with FAB class M2. The frequency of favorable chromosomal aberrations in AML patients decreases with age. Accordingly, t(8;21) is most common in younger patients and rare in patients over 60 (7, 8). Interestingly, patients with the t(8;21) translocation have favorable prognoses and 98% attain complete remission (CR) (9).

In addition to the conventional cytogenetic techniques, the reverse transcriptase–polymerase chain reaction (RT-PCR) has been used to detect the *AML1-ETO* fusion and its transcripts (9-11). In this study, we used both methods because in some cases conventional cytogenetic techniques revealed no abnormalities while RT-PCR allowed detection of the translocation (12). According to the literature, to estimate the incidence of the t(8;21) translocation accurately, unselected consecutive groups of patients should be studied. Furthermore, the translocation prevalence can differ between countries according to their epidemiological distribution worldwide. The t(8;21) with FAB-M2 morphology is more frequent in Japan than Australia than in most other countries, with frequencies varying from 18 to 88%. The high incidence of t(8;21) in the Japan may be due to geographic differences in tumor-associated genetic abnormalities in hematologic malignancies (13).

The molecular epidemiology of AML between regions within the same country has not been thoroughly investigated. Our first objective was to describe the *AML1-ETO* fusion gene in a population of *de novo* AML patients in northeast Iran. The second was to compare our findings with those from other Asian countries to increase the awareness of physicians and hematologists regarding the frequency and nature of chromosomal aberrations that contribute to hematologic malignancies.

Materials and Methods

Patients

We analyzed bone marrow (BM) samples from 179 newly-diagnosed patients with untreated AML and 20 healthy controls admitted to Ghaem Hospital in Mashhad, Iran. All study subjects gave informed consent approved by the Ethics Committee of the Mashhad University of Medical Sciences (MUMS). The control group contained unrelated healthy volunteers without leukemia. Giemsa, myeloperoxidase, and non-specific esterase -stained BM samples were examined. Morphologies were determined were according to the revised FAB classification (3, 4) and patients were classified into eight AML subtypes as follows: M0, M1, M2, M3, M4, M5, M6, and M7.

Cytogenetic studies

Chromosomes of BM cells from cultures from 113 patients with AML-M1, -M2, and -M4 were generally analyzed within 24 h of diagnosis (6). Metaphase chromosomes were banded by the conventional Giemsa banding technique. Karyotypes were analyzed according to the International System for Human Cytogenetics Nomenclature (ISCN) (14).

Detection of AML1-ETO fusion transcript

Mononuclear cells (MNC) from BM samples of 113 AML M1, M2, and M4 patients, and 20 control subjects were isolated using Ficoll solution (Cedarlane, Ontario, Canada) and stored at -70 ºC. Total RNA was extracted using the RNX-Plus™ kit according to the manufacturer's protocol (Cinnagen, Tehran, Iran). RNA was used as the template for cDNA synthesis (15). The *AML1-ETO* and the control gene *ABL* amplified singly from the same cDNA. The real-time PCR was performed with the fluorescent TaqMan technology. Universal primers and probes for *AML1-ETO* and *ABL* control gene were used according to Fujimaki *et al*. 2000 (9). The real-time PCR was performed in a final volume of 25 µl including TaqMan Universal PCR Master Mix (Takara. Bio, Shiga, Japan), 300 nM of each primer, 200 nM of ABL or 100 nM of *AML1-ETO* probe (Applied Biosystems, Weiterstadt, Germany), and 1 µl of cDNA. The fluorescence intensity of the reporter label was normalized using the rhodamine derivative ROX as a passive reference label present in the buffer solution. The reaction parameters were 2 minutes at 50 ºC for AmpErases UNG treatment (to prevent the reamplification of carryover PCR products), 30 seconds at 95 ºC (to inactivate UNG and activate AmpliTaq Gold Polymerase), followed by 40 cycles of 4 seconds at 95º C (denaturation) and 32 seconds at 60 ºC (annealing and extension). All reactions were performed on the StepOne real-time PCR system (Applied Biosystems, Foster City, USA).

Statistical analysis

The statistical significance of relationships between sex and age, and AML-M2 and t(8;21) were analyzed with the student's t-test, chi-square test, and Fisher exact test. Statistical significances were based on *p* values. Data was analyzed using SPSS (ver. 11.5).

Results

The frequencies of the FAB morphological subtypes in AML patients were as follows: M0 = 1.67%, M1 = 20.67%, M2 = 17.88%, M3 = 16.76%, M4 = 24.58%, M5 = 16.76%, M6 = 1.12%, and M7 = 0.56%. As expected, the *AML1-ETO* fusion was found predominantly in the M2 subtype and was absent from the M1, M4, and control groups. The *AML1-ETO* fusion was detected in 16 of 32 AML-M2 patients. The characteristics of the 32 patients with M2 morphology involved in the study are summarized in Table 1.

Table 1. Clinical and genetic characteristics of AML-M2 patients
F=female; M=male; WBC=white blood cells; += The t(8;21) detected by conventional cytogenetic methods and/or molecular analysis; Ab= t(8;21) translocation was not detected by conventional cytogenetic methods and/or molecular analysis; NA=cytogenetic data was not available.

Case	Age (Years)/ Sex	WBC ($\times 10^9$/l)	FAB	t(8;21)	AML1-ETO fusion transcript
1	16/M	15.7	M2	+	+
2	9/F	14.6	M2	+	+
3	36/M	12.3	M2	+	+
4	24/M	63.2	M2	+	+
5	3/F	256.7	M2	Ab	Ab
6	9/M	39.7	M2	Ab	Ab
7	10/M	10.4	M2	Ab	Ab
8	9/F	198	M2	Ab	Ab
9	10/F	3.3	M2	NA	+
10	12/F	50.8	M2	NA	Ab
11	47/F	18.9	M2	NA	+
12	33/F	1	M2	Ab	Ab
13	24/F	15.2	M2	NA	+
14	6/M	10.2	M2	NA	+
15	48/F	7.4	M2	Ab	Ab
16	54/M	16.3	M2	+	+
17	13/F	111	M2	+	+
18	2/M	11.1	M2	Ab	Ab
19	18/M	29.3	M2	Ab	Ab
20	8/F	37.7	M2	NA	Ab
21	58/M	18.2	M2	Ab	Ab
22	17/M	5.4	M2	NA	Ab
23	52/F	8.8	M2	Ab	Ab
24	34/M	14.2	M2	Ab	Ab
25	12/M	48.9	M2	Ab	Ab
26	30/F	14.6	M2	+	+
27	30/F	6.2	M2	NA	+
28	10/F	166	M2	+	+
29	5/F	16.8	M2	+	+
30	35/F	1.5	M2	Ab	Ab
31	44/M	16.9	M2	+	+
32	19/M	6.9	M2	+	+

Of total AML-M2 cases, 17 were females and 15 were males. The AML-M2 age groups were defined as follows: children; 1-18 years, adult; 19- 60 years. The age range was 2–54 and the median age was 17.5 with a standard deviation (SD) of 1.6. The white blood count (WBC) ranged from 1-198 with a median of 15.4 (10^9/l) (SD = 6.02).

The M1, M2, and M4 subgroups were diagnosed by conventional cytogenetics and/or real-time PCR. Cytogenetic results were available for 24 of 32 patients with the AML-M2 morphology. Of those 24 patients, 11 (45.8%) had a t(8;21) translocation, two (8.33%) had other cytogenetic abnormalities, and the 11 remaining patients were cytogenetically normal. Of 16 patients with the t(8;21) translocation, the age ranged from 6-54 years and the median age was 23.6. The fusion was seen in seven children and nine adults. In addition, the female to male ratio in fusion-positive patients was 1.2:1. Our data revealed no statistically significant correlations between age or sex and the *AML1-ETO* fusion ($p > 0.05$) (Table 2).

Table 2. Sex ratio and age groups in different AML categories
NS= not significant. *$p < 0.05$ was considered significant

Category	Male (%)	Female (%)	Child group (%)	Adult group (%)
AML	96/179 (53.6)	83/179 (46.3)	51/179 (28.5)	128/179 (71.5)
AML-M2	15/32 (46.9)	17/32 (53.1)	17/32 (53.1)	15/32 (46.9)
AML-M2+t(8;21)	7/16 (43.7)	9/16 (56.2)	7/16 (43.7)	9/16 (56.2)
p-Value	NS*	NS	NS	NS

Discussion

AML includes numerous distinct syndromes with typical clinical, morphological, phenotypic, and cytogenetic characteristics. The chromosomal aberrations, best illustrated by the t(8;21) translocation in FAB M2 disease (16), result in exclusive molecular rearrangements that provide insights into the pathogenesis of each of these diseases. Thesecould potentially identify etiological factors involved in leukemogenesis. Further description of the AML subtypes in different human populations may allow a better understanding of the genetic and environmental factors incorporated into their genesis (13). For this reason, we studied the laboratory features of AML cases in northeast Iran.

The t(8;21) translocation is one of the most common genetic abnormalities in AML and accounts

for 5-10% of AML cases and 10-22% of cases with M2 morphology (8). The main cause of increasing interest in the recognition of *AML1-ETO* is its involvement in relatively good prognoses with remission rates of 80% and disease-free survival of 60% (5, 17). Diagnostic cytogenetic and molecular methods are generally recognized as the most important prognostic predictors in AML (18). Some previous reports revealed that several t(8;21) translocation variants were unrelated and conferred a poor prognoses. In contrast, a relatively low percentage of patients were detected to be positive by molecular studies but negative by conventional cytogenetics (19, 20). Therefore, our study utilized both conventional cytogenetics and real-time PCR, although several cases in our study lacked cytogenetic data due to improper storage or transport conditions, or because of the absence of metaphases.

Age is critical risk factor in AML. Although the differences in incidences of the t(8;21) translocation between Iranian children and adults with AML-M2 were not statistically significant, it was designated as an ‘‘age specific cytogenetic abnormality’’ in other studies (18, 21-23). Although the mean age of the AML subjects in our study was 41, it is interesting to note that most patients with t(8;21) were under 30, as was noted in another report (24).

In our study the incidence of t(8;21) was higher in females than in males; however, in some other studies t(8:21) was more prevalent in children and males. (23, 25, 26).

Clinical characteristics of t(8;21) AML varied with ethnicity. This result implies that direct adoption of treatment plans based on clinical analyses conducted in western countries may not be advisable for Asian populations (27). In addition, data from Asia is lacking, even though Asia contains over 60% of the world’s population (26, 28). Data from similar varied populations might be critical in diagnosing the effect of ethnic, environmental, and geographical factors in AML-associated chromosome aberrations. To establish optimal therapeutic strategies appropriate for Iranian patients, understanding of clinical features and outcomes in Iranian patients with the t(8;21) translocation is critical. Previous studies noted higher incidences of the t(8:21) FAB-M2 subtype in Asia than in other countries with rates of 58-88% in Asia, 19-54% in Europe, and 12-27% in the USA (29-32).

Table 3. Comparison of frequencies of t(8;21) between the present study and non-Iranian series of AML patients

Country	AML no.	t(8;21)/AML (%)	FABM2/AML (%)	t(8;21)/M2 (%)	Med age	Female/ Male Ratio	Method of detection	Reference
Iran	179	16/179 (8.9)	32/179 (17.9)	16/32 (50)	31	1.1:1	PCR/ CC	Recent Study
Iran	58	15/58 (25.9)	20/58 (34.4)	15/20 (75)	25	1.1:1	CC	Movafagh *et al.*[33]
Oman	70	7/70 (10)	22/70 (31.4)	7/22 (31.8)	25	1.9:1	CC	Udayakumar *et al.*[27]
China	629	54/629 (8.6)	132/629 (21)	35/132(26.5)	43	1.2:1	CC	So *et al.*
South Korea	29	7/29 (24.1)	14/29 (48.3)	6/14 (42.9)	36	1:1.1	CC	Koo *et al.*[35]
Japan	494	53/494 (10.7)	160/494 (32.4)	53/160(33.1)	50.7	1.3:1	CC	Nakase *et al.*[13]
Hong Kong	30	4/30 (13.3)	15/30 (50)	2/15 (13.3)	-	-	CC	Chan *et al.*[34]
Malaysia	24	4/24 (16.6)	8/24 (33.3)	4/8 (50)	24	1:1	CC	Rosline *et al.*[36]
Taiwan	32	7/32 (21.9)	16/32 (50)	7/16 (43.7)	36	1:1.3	CC	Tien *et al.*[32]

Med=Median; CC=Conventional cytogenetic.

We also compared our results including the prevalence of the *AML-TEO* fusion in AML and AML-M2 subtype with other recent reports from Asia. Our findings revealed similarities and differences between Iranian and other reported AML patients (Table 3).

After molecular analysis, we noted the relatively high frequency of the *AML1-ETO* fusion in AML-M2 cases (50%) but this was lower than was reported for patients in Tehran with *de novo* AML (75%) (33). This difference may be due to the small sample size of the previous study. In contrast, the incidence of t(8;21) in AML-M2 in our study was higher than those from Hong Kong, Japan, Oman, and China studies (13, 26, 28, 34), and similar to those found in South Korea, Malaysia, and Taiwan studies (32, 35, 36). Patient sample sizes varied in most studies. Differences in inclusion and exclusion criteria and variations in diagnostic techniques can also affect data accuracy. On this subject, large single-center studies with reasonably uniform populations make available useful information to accompany multicenter studies. Taken together, in accordance with the variety of the clinical features among t(8;21) AML patients from Asia and western countries, clinicians should be aware of potential clinical differences between ethnicities. Further studies on differences in clinical features among different ethnicities, including Iranians, are needed. The different incidences of the t(8;21) translocation in Asia strongly suggest a genetic susceptibility to this chromosomal aberration in Asian people, or an environmental factor, or both. More investigation is required to recognize the genetic and/or environmental elements responsible for this increased prevalence of the t(8;21) translocation in Asian AML patients.

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