Geographic Heterogeneity of Cytogenetic Characteristics of Acute Myeloid Leukemia in the early detection: A Comparative Study of Iranian and Indian adult Patients

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Abstract

Background: Acute Meyloid Leukemia (AML) in adults is known to be a heterogeneous disease with diverse chromosome abnormalities. Some of these chromosome abnormalities are found with a high incidence in populations from specific geographical areas and ethnic societies. Therefore, we studied the cytogenetic features of AML cases in contrasting societies of Iran and India.

Methods: Cytogenetic investigation was performed in various subtypes of AML with unstimulated short-term culture and High Resolution Cell Synchronization with some modification.

Results: Cytogenetically, Iranian M3 displayed a higher frequency of t(15;17) than Indian M3 (33.8% vs 19.3%) followed by M2 [t(8;21) (27.7% vs 16.2%)] and M1 [t(9;22) (16.0% vs 11.3%)]; whereas, inv(16)11q23 and numerical chromosomal aberrations in chromosome 5,7,8 occurred more frequently in Indian than Iranian.

Conclusion: These findings represented different cytogenetic characteristics of t(15;17) between the two populations. This is the first systematic cytogenetic study of an ethnic Iranian population. Extensive biological studies of AML in Iran and India and various countries to be needed to clarify the role of genetic as well as geographic heterogeneity in the pathogenesis of AML.

Keywords: acute myeloid leukemia, t(15;17), geographic, ethnic, detection

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Introduction

Acute Myeloid Leukemia is a heterogeneous group of diseases which includes several discrete syndromes with characteristic clinical, morphological, phenotypic and cytogenesis features [1-2]. On the basis of association of specific chromosomal changes with morphologic subtype, patients have been cytogenetically classified into various subtypes e.g French American British (FAB) classification [3]: FAB M1 patient with t(9;22), FAB M2 with t(8;21), FAB M3 Promyelocytic with t(15;17), FAB M4 Eo (M4 with eosinophilia) with inv(16) who have long and complete remissions and a long survival, M5 patients with 11q abnormalities who have a poor prognosis [4]. But in the course of time and due to availability of more data, it became clear that some of these chromosomal abnormalities are found in individuals from specific geographical areas and ethnic groups. Apart from the geographic heterogeneity, application of processing techniques such as MTX synchronization and culturing of BM cells for 24 hours has indicated the significance of cytogenetics analysis in the culture cells.

AML patients have not yet been cytogenetic characterized in detail in an Iranian population. Our study was the largest on cytogenetics of AML ever performed in Iranian population [5]. In the present investigation, the karyotype pattern of Iranian and Indian AML patients who were detected at early stages, was studied in order to identify possible geographic heterogeneity.

Methods

We studied 65 adult patients with AML as initial diagnosis in the department of medical oncology at two major referral hospitals (Modares and Taleghani hospitals) and several private centers at Tehran during the year 2000 and 2006. Also, 33 Indian adult patients with newly diagnosed, untreated AML were self evaluated (by own) in the department of Hematology, Postgraduate Institute for Medical Education and Research (PGIMER) in the Northern part of India (1989-1992). We also included 29

***Table 1**: Patients' characteristics*

	Iran	India	Statistical Comparison *(P- Value)*
No. of patients	65	62	
Age (Years) Range	15 – 72	15 – 75	
Mean	47.3	45.1	0.2059 NS
SD	9.5 *	10.0 *	
Sex (M/F)	39:26	38:24	0.8817 NS

*Approximately 1/6 of range

***Table 2**: Incidence of chromosomal abnormalities in both populations*

	t (15;17) No(%)	t (8;21) N0(%)	t (9;22) N0(%)	inv(16) N0(%)	$11q^{23}$ N0(%)	5/7/8 N0(%)	Others N0(%)	Normal N0(%)
Iran	22(33.8%)	18(27.7%)	11(16.9%)	2(3%)	2(3%)	4(6%)	5(7.6%)	13(20%)
Indian	12(19.3%)	10(16.2%)	7(11.3%)	2(3.2%)	4(6.4%)	5(8%)	6(9.6%)	5(8%)
Statistical Comparison *(P- Value)*	0.03362 df=1 S	0.1747 df=1 NS	0.5123 df=1 NS	0.9617 df=1 NS	0.4328 df=1 NS	0.7400 df=1 NS	0.7397 df=1 NS	0.0943 df=1 NS

NS: Non-Significant, S: Significant, NO: Number, t:translocation

cases of AML who were previously reported by Kadam et al (1991)[6] in the central coast part of India for firm conclusions. The patients in this study were seen in the department of medical oncology at Tata Memorial Hospital between 1988 and 1989. In the present study, Cytomorphology, Cytochemistary and Cytogenetics was done in all cases, while immunophenotyping was considered only in those cases that were found to be problematic.

Cytogenetics

In each patient, 0.5-1.0 ml of BM/PB was obtained and studied using (a) a 24–hrs unstimulated culture technique and (b) methotrexate cell synchronization method [7] with some modification. For culture, 3-5 × 106 cells were cultured in a 4 ml medium (RPMI 1640 ,Gibco-BRL Grand Island, NY,USA) supplemented with 15% heat inactivated fetal bovine serum (Gibco-BRL Grand Island, NY,USA) at 370C in an atmosphere containing 5% CO2. For MTX synchronization, BM/PB cells were synchronized with 10-7 M MTX after 1.0 to 5.0 hrs of culture. The S-phase block of synchronized cells was released after 17 hrs by adding 10-5 M thymidin for 3.0 to 6.0 –hrs. The processing of chromosome preparations from 24-hrs cultures as well as from MTX synchronized cultures was performed according to standard methods. Briefly, the cultured cells were then treated with colcemide (Gibco-BRL Grand Island, NY, USA at the final concentration of 10 µg/ml and incubated at 37oC for an additional 3 min. The contents of the tube were then centrifuged for 10 min at 1000 rpm and re-suspended in 10 ml of 75 mM KCl (0.56%) prewarmed to 37oC for 20 min. At this stage, 1 ml of Carnoys Fixative (3:1 methanol : acetic acid) was added to the tube, and this fixation step was repeated four times. Ten slides were prepared for each culture and stained for 3 min with Giemsa. Slides were examined with an Olympus BH-2 light microscope. Eighty well-spread metaphases were analyzed for each subject and karyotypes were described according to ISCN [8].

Statistical analysis

The difference between two groups was analyzed with student's t-test,chi-square and Fisher Exact test and calculation was based on P-value. Data analysis was performed by SPSS (version 11.5,Inc.USA).

Results

Cytogenetics study was performed in 65 Iranian and 62 de novo patients with different subtypes of AML. In the Iranian group, 39 were males and 26 were females with a mean age of 47.3 years of age (ranging from 15 to 72) at the time of cytogenetic examination, and the percentage of abnormal cytogenetic cells was recorded to be between 30 to 100%. Of 62 cases in the Indian group, at the time of diagnosis, 38 were males and 24 were females with a mean age of 45.1 (ranging from 15 to 75). In this group of patients, percentage of abnormal karyotype cells was between 92 to 100%. The cytogenetic results in both groups were correlated with various subgroups of FAB classification.

There was no significant difference in age and sex distribution between Iranian patients and Indian patients (Table 1). In this study, the frequency of

chromosomal abnormalities according to FAB morphological classification was compared between the two countries which is shown in Table 2. Cytogenetically , Iranian M3 subtype displayed a higher frequency of t(15;17) than Indian M3 (33.8% vs 19.3%, P value =0.0362). A striking difference in the incidence of t(8;21), t(9;22) and normal karyotype i.e 46XX,46XY was found in our series versus Indian population. Co-expression of two FAB-specific chromosome changes, t(15;17) and t(8;21), was detected in a case of AML in the series of Indian patients. There was no significant difference between the two groups in the incidence of inv(16)11q23 and additional non-specific chromosomal abnormalities ,such as trisomy of chromosomes 5,7,8. In each group, repeated bone marrow aspiration and peripheral blood samples of 17 cases were not available for karyotyping, due to lack of mitoses or inappropriate preparations.

Discussion

Among myeloid malignancies, acute myeloid leukemia in adults is known to be a heterogeneous disease with diverse morphologic, immunophenotypic, clinical and cytogenetic characteristics [1-2]. More precise delineation of the subtypes of AML occurring in different human populations may allow a better understanding of the genetic and environmental factors involved in their genesis. For this reason, we studied, laboratory features of AML cases occurring in contrasting societies of Iran and India.These cytogenetic findings can have valuable contribution in the management of patients with leukemia in both populations.

The Iranian cases had occurred in an ethnically homogenous Asian (Middle East) population residing in a predominantly urban and industrialized area whereas the Indian cases were collected from two large provinces in the Northern and central coast part of India, and therefore represented a different population as compared to Iranians. The major finding of this research work was an increased frequency of cases of M3-FAB-AML with t(15;17) chromosomal abnormality in Iranians as compared to Indians. The malignant promyelocytes in APL are associated with a specific reciprocal translocation between chromosomes 15 and 17 . The t(15;17) abnormality is a distinct sub-type of FAB-M3 in AML. Geographic heterogeneity and occurrence of chromosomal abnormality with t(15;17) is confirmed by the reviewed data in the Fourth International Workshop [9] and relevant data is available in International literature [3-10-11-12-13-14-15].

With respect to the geographic heterogeneity in the incidence of t(15;17) in APL patients reported in the literature, our findings in this study and the previous case study in Iran [10] are in close agreement with reports from the Hispanic population of the United States and Spain [11], Latin America and Brazil [12-13] and from Asian populations of Japan, Taiwan, Singapore and Turkey [14-15-16-17-18]. The different incidence of t(15;17) AML in Iranian and Indian societies suggests a genetic susceptibility to this chromosomal changes in Iranian people or an environmental factors, or both.

The t(8;21) chromosome abnormality is a distinct and specific subtype of FAB-(M2-AML) classification. The frequency of M2 subtype of FAB with t(8;21) structural chromosome abnormality was relatively more common among Iranian patients compared to Indian patients. The different incidence of t(8;21) in Iranian (28%) and Indian (16%) societies is essentially similar to a comparative study of AML patients in Japanese and Australian populations [19]. The rang of reported incidence of t(8;21) in FAB-M2 subtype was 58-88% in Asian cases [19], 19-54% in Europe, and 12-27% in the USA [20-21]. Chromosomal abnormalities have also been observed in different geographic regions and among different ethnic groups, with a high incidence of t(8;21) in Morocco [22], Tunisia [23-24], Oman [25], China [26], Russia [27] and South Korea [28-29-30]. Interestingly, the first case of co-expression of t(8;21) and t(15;17) associated with other structural and numerical aberrations was reported through self-finding in a series of Indian population [31]. The additional chromosome abnormalities were frequently reported in t(8;21). The first case of t(8;21) acute myeloid leukemia was recently reported in India[32]. Other chromosomal abnormalities were found with similar frequencies in the two populations.

Conclusion: Similarities and dissimilarities of the present study between Iranian and Indian populations and other reports could be due to methodological variations, although geographic differences, different chromosomal sensitivity to breakage, and different ethnic origins must not be disregarded.Extensive biological studies are needed in Iran and India and other countries to help provide epidemiological clues which may play a role in the pathogenesis of AML in different geographic regions of the world.

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