RESEARCH COMMUNICATION

Specific Chromosomal Abnormalities in Patients with Acute Nonlymphocytic Leukemia from the Islamic Republic of Iran

Movafagh Abolfazl[1]*, Isfahani Fatemeh[2], Attarian Hamid[2], Ghadiani Mojtaba[2], Mosavi Jarahi Alireza[3], Mohagheghi Mohammad Ali[4]

Abstract

Cytogenetic analysis performed at diagnosis is considered to be the most valuable prognostic factor in acute non-lymphocytic leukemia (ANLL), a very heterogeneous disease. Little data exist in Iran regarding the cytogenetic characteristics of ANLL . Therefore, cytogenetic investigations were performed for 58 patients with various subtypes of ANLL with unstimulated short term culture and high resolution cell synchronization techniques. Among the 58 evaluated patients, 45 (77.5%) showed clonal karyotypic abnormalities and the percentages of the abnormal cells were recorded within the range of 30%-100%. Some 14 were classified as M1, 20 as M2, 19 as M3 , 3 as M4, 1 as M5 and 1 as M6. The most common chromosome rearrangements were t(15;17), t(8;21) and t(9;22). Trisomy of chromosome 8 (+8) was the most frequent numerical alteration in 3 patients with M1, M2 and M6. The incidence of other chromosomal defects, including -10, DMCs , -19 , 5q- , dicentric(dic), chromatid breaks, and marker chromosomes was relatively high. Similarities and dissimilarities of our study with others may be due to the role of genetic sensitivities as well as uneven geographic distribution in the pathogenesis of ANLL. Further prospective studies are warranted to precisely elucidate ethnic differences in the pathogenesis of this disease in different populations.

Key Words: Chromosome aberration - leukemia - ANLL - Iran

Asian Pacific J Cancer Prev, **7**, 447-450

Introduction

In 1960,Nowell and Hungerford described the first chromosome abnormality in chronic myeloid leukemia. An increasing number of chromosomal aberrations is now being recognized to be non-randomly involved in hematological disorders. It has been well documented that by application of improved cytogenetic techniques ,such as high resolution banding (HRB) (Yunis,1981) using methotrexate (MTX) cell synchronization, almost 90-100 per cent of ANLL patients exhibitclonal chromosomal abnormalities.

On the basis of association of specific chromosomal changes with morphologic subtype, patients have been classified cytogenetically into various subtypes e.g. French American British (FAB) (Benett et al.,1985), FAB M1 patients 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) have comparatively long complete remissions and a long survival, and M5 patients with 11q abnormalities having a poor prognosis (Misawa et al.,1988; Fenaux et al., 1989).

Cytogenetic analysis performed at diagnosis is considered to be the most valuable prognostic factor in ANLL (Kadam et al.,1991). Since ANLL patients have not yet been cytogenetically characterized in detail in an Iranian population, the present multi-center study was conducted in Tehran. Results were compared with reports from other regions of the world to identify possible geographic heterogeneity.

Materials and Methods

Patients

During the 5 year period (2000-2005), we received bone marrow (BM) and peripheral blood (PB) samples from 58 adult patients with ANLL at initial presentation. The patients were seen in the Departments of Medical Oncology at Modares and Taleghani Hospitals and several private centers. Of these patients, 30 were males and 28 were females, ranging in age from 14 - 72 years. The diagnosis and sub classification of ANLL were based on morphologic and cytochemical studies of the PB smear, BM aspirates and biopsy specimens according to FAB co–operative group criteria (Bennett et al.,1985). Cytochemistry was done in

*1Department of Medical Genetics , 2Department of Internal Medicine , Taleghni Hospital , and .3Department of Health and Community Medicine, Shaheed Beheshti Medical University Tehran 4Cancer Research Center , Tehran University of Medical University Tehran Iran. *For Correspondence Fax: + 98(21) 22400671Email : Movafagh_a _@yahoo.com*

all cases, while immunophenotyping was considered to be necessary only in those cases that were found to be problematic.

Cytogenetic studies

In each patient,0.5-1.0 ml BM/PB was obtained and studied using ;(a)a 24–h unstimulated culture technique and (b) methotrexate cell synchronization method(Yunis,1981) with some modification. For culture ,3-5 _ 106 cells were cultured in 4 ml medium(RPMI 1640 ,Gibco-BRL Grand Island, NY,USA) supplemented with 15 per cent 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 – 5.0-h of culture . The S-phase block of synchronized cells was released after 17-h by the adding of 10^{5} M thymidin for 3.0 – 6.0-h. The processing of chromosome preparations from 24-h 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) final concentration, 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-suspend in 10 ml of 75 mM KCl (0.56%) prewarmed to 37˚C for 20 min. At this stage, 1 ml of –20˚C 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 model BH-2 light microscope. Totals of eighty well-spread metaphases were analyzed for each subject. Karyotypes were described according to SCN(1985).

Results

Cytogenetic studies were performed in 58 patients with different subtypes of ANLL. Of them,30 were males and 28 females; The median age of these patients at the time of cytogenetic examination ,was 34 years(range 14 to 72 years). Abnormal metaphases were obtained in 45(77.5%) patients and the percentage of abnormal cells recorded within the range of 30%-100%.Out of 58 patients,14 were classified as M1,20 as M2,19 as M3,3 as M4,1 as M5 and 1as M6 (Table 1).

M1-ANLL without maturation:

The Philadelphia(Ph)translocation, t(9;22)(q34.1;q11) was the most common chromosomal rearrangement among patients with M1 subtype of ANLL. This translocation was seen as a sole clonal abnormality in 8/14 patients with M1 in 15- 25 percent of cells. Association of -10 with t(9;22) was noted in one patient. In another patient +8 was seen as a sole of anomaly with M1 subtype. Clustering of t(9;22) and Double Minute Chromosomes(DMCs) was seen in one patient. The remaining 4 patients had normal metaphases in their BM/PB specimens.

Table 1. Results and Frequency of Chromosome Patterns in 58 Patients ANLL

Chr	No.	%	FAB subtypes					
			M1	M2	M3	M4	M5	M6
t(8;21)	15	25.0		15				
t(15;17)	16	27.5			16			
t(9;22)	11	19.0	10	1				
inv/del (16)4	6.8		1		2	1		
+8	3	5.1	1	1				1
5p-	1	1.7		1				
-10	1	1.7	1					
Marker	1	1.7			1			
Dicentric	2	3.4			1		1	
Variable	1	1.7						1
Normal	13	22.4	4		6	3		1

M2- ANLL with maturation :

The specific translocation between chromosome 8 and 21,t(8;21) was seen in 15/20 patients with M2 in 14-89 percent of cells . The breakpoints were assigned as 8q22 and 21q22 in all patients . In one female patient t(8;21) was identified in combination with loss of both sex chromosome in about 50 percent of cells . Loss of Y chromosome was noted in one patient with M2 subtype without t(8;21). The 5q- abnormality was seen in combination with t(8;21) in one male patient. t(9;22) in association of two marker chromosome analyzed in one patient. An inversion of chromosome 16q with -19 and del(4) (q26-28) was seen in one male patient. Trisomy of chromosome 8 also seen in one male patient. Bone marrow of six patients resulted normal karyotype.

M3- Acute Promyelocytic Leukemia (APL):

All 16 patients with APL showed t(15;17) in 57- 100 percent of cells and the breakpoints were localized at 15q22 and 17q12. The t(15;17) was associated with other defects in 4 patients like chromatid break , dicentric and marker chromosomes . Three BM sample of this subgroup patient yielded normal karyotype.

The involvement of inv(16)(q) was observed in one patient with M4 and del(16q) in another patient in the similar subtype. Also normal pattern of karyotype was detected in M4 series of this investigations. Structural abnormality as del(11)(q23) with dic(1) was noticed in one female patient in M5 subgroup of ANLL.The cytogenetic analysis performed on BM material of one patient with M6 subtype of ANLL, revealed multiple structural and numerical abnormalities in all 19 metaphases analyzed.

Discussion

Chromosomal abnormalities in ANLL were reported for the first time by Ford et al in 1965.During the last 40 years an extensive inventory of chromosomal changes in leukemia has led to the discovery that some cytogenetic aberrations are specifically associated with different types of leukemia and have great prognostic important. In this contribution,

this study regarding chromosomal study in ANLL for the first time is reported here in the international literature.

Our results indicated 77.5% patients with ANLL show to have abnormal karyotypes. The overall frequency of chromosomal abnormalities in our study was comparable to that reported from India(Kadam et al.,1991),Argentina (Aceverdo et al.,1994) and much higher compare to the rest of the world viz., Japan (Nakamura et al.,1991)(Kuriyama et al.,2001), Saudi Arabia (Roberts et al., 1992), Singapore (Enjeti et al.,2004).

FAB-associated abnormalities viz., t(8;21), t(15;17), t(9;22), t/del(11), inv(16) were seen in our ANLL patients. Among these, t(8;21) and (15;17) appeared to be specifically associated with subtypes M2 and M3, respectively. In this study,M2 was the predominant FAB subtype(34.5%) followed by M3 (32.7%), M1(24%), M4(5%), M5(1.72), M6(1.72), which is in accordance with studies reported from Mumbai (Kadam et al.,1991), Germany (Klause et al.,2004) and Thailand (Auewarakul et al.,2005).

Specific chromosomal rearrangements associated with FAB – M1 subtype such as t(9;22) (Ph-chromosome) were seen in 8/14 with M1 and 1/14 with M2 in the present investigation. Presence of Philadelphia (Ph) chromosome in a case of ANLL for the first time was reported by Sonta et al (1976).Other series in the literature indicated that t(9;22) is not very specific for patients with the M1 phenotype but can be associated with M2 or M4 patients , occasionally with M6 but never with M3 and M5 patients (Woods et al ., 1985; Mitelman ,1985;Kadam et al ., 1991; Mauritzson et al ., 2002).Other structural and numerical abnormalities such as +8,-10 and DMCs was detected with M1 series in the present study(Suzuki et al.,2000; Arnand.,2005). The presence of these anomalies along with FAB specific changes in the cells, resulting in formation of predominant clones in the BM, may indicate additional adverse leukemogenic changes .

Translocation between chromosome 8 and 21 either as a sole chromosomal anomaly or often with loss of sex chromosome is described in this study and in the literature. The break points involved in t(8;21) and the frequency observed in the present study are essentially similar to those found in ANLL – M2 elsewhere (FIWCL,1984; Kadam et al ., 1991; Lai et al ., 2005). Significantly, Japanese patients with M2 displayed a higher frequency of t(8;21) than Australians (Nakase et al ., 2000). The occurrence of sex chromosomes, loss of Y chromosome, 5q- , -8 , -19,inv(16q) in the present findings as a additional chromosomal abnormality is an adverse factor for prognosis of t(8;21) in ANLL (FIWCL, 1984 ; Woods et al ., 1985 ; Kadam et al ., 1991; Lai et al ., 2005) . The first case of Co- expression of t(8;21) and t(15;17) with other structural aberration was reported by own finding (Movafagh et al., 1996).

Acute promyelocytic leukemia (APL) is a distinct clinicomorphological class of ANLL. During the present study , cytogenetic analysis was performed on 19 patients with APL-M3 subgroup of ANLL and the presence of diagnostic chromosomal rearrangement designated as t(15;17), either independently or associated with other clonal defects was predominantly encountered in 16(27.5%) out of 19 patients . It is reasonable to state that the incidence of specific chromosomal anomaly i.e., t(15;17) in ANLL –M3 is similar to data available in the literature (Fitzgerald et al., 1983; FIWCL,1984; Roberts et al.,1992; Acevedo et al.,1994; Nakause et al.,2000; Kuriyama et al., 2001; Mauritzson et al.,2002; Harani et al.,2005).

The occurrence of the inv(16q)/del(16) abnormality in M4 and M5 subtype of present findings in patients with monocyte proliferation indicated the strong correlationship between morphology and associated chromosome changes(FIWCL,1984; Yunis et al.,1984; Kadam et al.,1991; Roberts et al.,1992; Klaus et al.,2004; Enjeti et al., 2004; Harani et al.,2005).

The random chromosome abnormalities presented here and reported by several researchers could not supported any specific clonal abnormalities for M6 subgroup of ANLL patients (Klaus et al., 2004). Trisomy of chromosome 8 was most frequent numerical alteration in 3 patients with M1,M2 and M6 in the present study.+8 was the most common numerical abnormality in all FAB subgroup and its distribution among the FAB nomenclature is uneven (Fenaux et al.,1989;Kadamet al.,1991;Roberts et al.,1992 ;Mauritzsom et al., 2004; Klaus et al., 2004; Enjeti et al., 2004; Qui et al.,2005).

The relevant literature from different Asian countries along with our study reported here, confirmed that the incidence of chromosomal abnormalities varies considerably (Roberts et al., 1992; Dashinamurthy et al., 2000; Suzuki et al ., 2000; Enjeti et al., 2004; Harani et al., 2005; Qui et al., 2005).

It can be concluded that the number of these aberrations in the present study are not large enough to identify them as a nonrandom chromosomal abnormalities in any subgroup of ANLL patients. Similarities and dissimilarities of present findings with other researchers may be due to uneven geographic distribution, populations with different ethnic origin and, possibly, different chromosomal sensitivities to breakage.

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