Journal of Sciences, Islamic Republic of Iran 17(1): 17-25 (2006) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Investigation of TEL/AML1 and BCR/ABL Fusion Genes in Patients Affected by Acute Lymphoblastic Leukaemia Using Interphase *in situ* Hybridization

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Abstract

A total of sixty patients affected with acute lymphoblastic leukaemia (ALL), including thirty children and thirty adults were studied by the conventional cytogenetics and fluorescence *in situ* hybridization (FISH) techniques. The TEL, AML1, ABL, and BCR probes were applied on interphase cells prepared from bone marrow samples. The signal distribution and the presence of the fused genes, together with the clinical features were statistically analysed. The age ranged from 16 to 42 years (mean 23±7.3) in adults and 2 to 15 years (mean 6.9±3.9) in children. In children 46% had an abnormal FISH pattern, including 23% having fused ABL/AML1, 3% and 7% with deletion and gain in TEL gene respectively; 3% and 10% having deletion and gain in AML1 gene, respectively. In adults, out of eight (27%) patients had abnormal FISH pattern, of those only 3% presented the fused TEL/AML1 gene, and the distribution of signal patterns was the same as found in children. A direct correlation were also found between the presence of fused TEL/AML1 genes and decreased WBC ($P<0.05$), however this was not significant in adults. The adults with more than 50000 WBC had a significantly lower survival period ($P<0.05$). Our results indicate that the combination of conventional karyotyping and molecular cytogenetics (FISH), and a long time follow up study could provide clinicians useful information leading more effective therapeutic management for the ALL patients.

Keywords: Acute lymphoblastic leukaemia; Fluorescence *in situ* hybridization; TEL/AML1 fusion; ABL/BCR fusion

Introduction

Acute lymphoblastic leukaemia (ALL) is a malignant clonal disease of the bone marrow (BM) in which early lymphoid precursors proliferate and replace the normal haematopoietic cells of the BM. ALL may be distinguished from other malignant lymphoid disorders by the immunophenotype of the cells, which is similar to B or T precursor cells. Immunochemistry, cyto-chemistry and cytogenetic markers are also useful for

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categorizing the malignant lymphoid clone [1].

The malignant ALL lymphoid precursor cells are arrested in an early stage of development. This arrest is caused by an abnormal expression of genes, often as a result of chromosomal translocations [2].

In acute lymphoblastic leukaemia at least 81% of all cases appear to have a clonal chromosome abnormality by cytogenetic or molecular cytogenetic analysis [2,3].

The most common genetic aberration in childhood ALL is found to be t(12;21). It is also reported that TEL/AML1 fusion occurs in the B-precursor cells of approximately 25% of ALL patients. This aberration, usually, could not be detected by conventional cytogenetic technique [3-7]. Researchers believe that this translocation may occur during the fetal development in some patients [8].

Fluorescence *in situ* hybridization (FISH) and molecular studies have shown that the TEL-AML1 fusion is not found in cases of T-lineage ALL, acute myelogenous leukaemia (AML), or non-Hodgkin lymphoma [9]. The fusion has also been detected in adult ALL with remarkably low frequencies, *i.e.*, only 0-4%, compared to those found in childhood ALL [10-12].

TEL/AML1 fusion is associated with a favorable prognosis impact of which exceeds the age and leukocyte count which are included in the present standards of risk assessment [13-15]. In childhood ALL, TEL/AML1 expression identifies a large group of low-risk patients whose relapse rates revealed to be less than 10% by modern treatment protocols [16-18].

The prognosis in patients with TEL/AML1 fusion seems to vary depending on the therapeutic protocols, recent studies have reported late relapses in this group of patients [19-21]. Cytogenetic analysis in ALL is often hampered by poor chromosome morphology and few malignant metaphases, and sometimes there are only normal metaphases found after cell culture [22,23].

Interphase fluorescent *in situ* hybridization is a sensitive method for detection of specific chromosomal aberrations that increases the detection rate of malignant cells. The development of new molecular cytogenetic methods has led to a substantial increase in knowledge concerning genetic aberrations in ALL [24-26].

About 20% of adults and 5% of children affected with ALL have a genetic abnormality called the Philadelphia chromosome arising from t(9;22).

The Philadelphia (Ph) chromosome is the most frequent cytogenetic abnormality known in human leukaemias and can be detected in more than 95% of patients with chronic myeloid leukaemia (CML), in a range of 20% to 40% of adult ALL, 2% to 5% of child ALL, and in rare cases of acute myelogenous leukaemia [26-31]. The 9;22 translocation leads to a head-to-tail fusion of the abl proto-oncogene from chromosome 9 with the 5′ half of the breakpoint cluster region (bcr) sequences on chromosome 22 [32,33]. Transcription of bcr-abl results either in a 8.5-kilobase (kb) messenger RNA (mRNA) that codes for a 210-kD protein or in a 7.5-kb mRNA encoding a 190-kD protein [34,35]. For the p210 protein, exon b2 or exon b3 of the *BCR* gene (M-bcr region) is coupled to *ABL* exon 2 (b2a2 or b3a2 junction), whereas the p190-kD protein results from a break within the first intron of *BCR* (m-bcr region) splicing the first exon of the *BCR* gene to the second exon of the *ABL* gene (e1a2) [26]. Other fusion products are observed at much lower frequencies. BCR-ABL proteins demonstrate enhanced tyrosinase kinase activity compared to the normal 145-kD *ABL* gene product. Moreover, p190 exhibits a higher transforming potential than p210 in transfection assays and transgenic mouse models [35,36]. BCR-ABL expression in hemopoietic cells is known to induce resistance to apoptosis, growth factor independence, as well as alterations in cell-cell and cell-matrix interactions [37-40].

Materials and Methods

Patients

The bone marrow (BM) samples were obtained from 30 children and 30 adults with acute lymphoblastic leukaemia (ALL) consecutively diagnosed at the Hematology-Oncology and bone marrow transplantion research center, and Medical Genetics laboratory (P. Mehdipour), respectively, between June 1997 and December 2004 Tehran. ALL cases were morphologically classified according to the French-American-British (FAB) classification.

Cytogenetic Analysis

Bone marrow samples were processed directly and in short term culture according to the standard protocol [41,42]. Chromosomes were stained with trypsin-Giemsa (GTG) banding technique. Karyotypes were analysed according to the International system for Human Cytogenetic Nomencleture (ISCN 1995).The fixed cell pellets and/or slides were stored at −20°C for further FISH studies.

FISH Analysis

Previously prepared slides were pretreated with 2X standard saline citrate (SSC; 300 mmol/L sodium chloride and 30 mmol/L sodium citrate) for 2 min at 37°C and dehydrated with cold 70%, 85% and 100% ethanol for 2 min each. After air drying ten microliters

of probe mixture (probe with hybridization buffer) was added to each hybridization area, covered with 22×22 mm coverslips and sealed with rubber cement.

DNA and probe were simultaneously denatured for 5 min at 75°C. Slides were hybridized for 24 to 48 h at 37°C incubator.

After hybridization, the coverslips were removed and the slides washed in 0.4×SSC at 72°C for 2 min and in 2×SSC at room temperature for 30 s. The cells were counterstained with 10 µl of 4′-6′-diamine-2-phenylindole dihydrochloride (DAPI) and antifade in a ratio of 1:4. The image was analysed using a Lieca m-FISH. One hundred 100 nuclei were scored for TEL/AML1 and 100 for BCR/ABL probe signals Nuclei with ambiguous signals and cells with poor morphology were excluded from the scoring.

TEL and AML1 Probes

AML1/ and TEL gene probes spans the entire AML1 gene including the common breakpoints of t(12;21) and the TEL gene, respectively. This method with different colours, directly labeles TEL and AML 1 probes (LSI TEL/AML1 ES Dual colours translocation probe Vysis Inc., IL, USA). TEL probe is labeled with the spectrum Green fluorophore and the AML1 probe is labeled with the spectrum Orange fluorophore. The TEL probe begins between exons 3 to 5 and extends approximately 350 kb toward the telomere in chromosome 12. Thus the TEL probe covers the 12p13 region distal to the common breakpoints of t(12;21).

TEL/AML1 fusion in cells with t(12;21) (p12;q22) appears in the abnormal chromosome 21.

ABL and BCR Probes

The LS1 BCR/ABL Dual colour probe is a mixture of the LS1 ABL probe labeled with spectrum Orange and the LS1 BCR probe labeled with spectrum Green. The ABL probe begins between exons 4 and 5 and continues for about 300 kb toward the telomere of chromosome 9. The LS1 BCR probe begins between BCR exons 13 and 14 (major BCR exons 2 and 3) and extends toward the centromere on chromosome 22 for approximately 300 kb, extending well beyond the m-bcr region. A nucleus lacking the t(9;22) will exhibit the two orange, two green signal pattern. In a nucleus containing a simple balanced t(9;22), one orange, one green and one orange/green (yellow) fusion signal pattern will be observed.

This simple probe design detects the 5′ BCR /3′ ABL gene fusion useful for detecting samples with a high percentage of cells possessing this translocation.

Results

The distribution of patients, sex, age and haematologic characteristics is presented in Table 1. The results of the conventional cytogenetics and FISH are given in Table 2. In the present study, the ALL patients included 30 adults and 30 children. As far as sex concerns, adult patients included 70% (21/30) male and 30% (9/30) female and children.

Karyotypic Analysis

As far as the conventional karyotyping concerns, the mode of clones and the chromosome abnormalities is presented in Tables 3 and 4.

FISH Analysis

Distribution of the observed signals is presented in Tables 5 and 6.

BCR/ABL Probe Analysis

Thirteen per cent (4/30) of adults and 7% (2/30) of children revealed to present fusion of BCR/ABL genes, among those two patients were diagnosed as the mosaics (Fig. 1).

In addition 7% (2/30) of adults patients showed the deleted ABL gene and 3.5% (1/30) presented the lack of BCR gene. Children affected with ALL, had an extra ABL gene and another patient had an extra copy of BCR gene without BCR/ABL fusion (Fig. 1).

TEL/AML1 Probe Analysis

The occurrence of TEL/AML1 fusion was detected in 3.5% (1/30) of adult and 23% (7/30) in children affected with ALL (Fig. 1).

The abnormal signal pattern in adult patients included 1 lacking TEL gene, 2 with an extra copy of TEL gene, one with lack of AML1 and 3 patients with an extra copy of AML1 gene.

Patients included 57% (17/30) male and 43% (13/30) female.

The age ranged from 16 to 42 years (mean 23±7.3) in adults and 2 to 15 years (mean 6.9±3.9) in children. The ratio of blast morphology in our patients was compatible with FAB classification (L1/L2) in adults and children were found to be 13/17 and 14/16, respectively.

The WBC count ranged between 200/µl-96000/µl in adults with 9 out of 30 patients having over 40000/µl and 1000/µl-42000/µl in children with 2 out of 30 patients having over 40000/µl.

Table 1. The clinical description of the children patients

Patient No.	Sex	Age at diagnosis (years)	Initial WBC count	FBA Classification	Survival (months)
1	F	14	7100	L_1	35
2	M	11	9000	L_2	18
3	F	5	7000	L_2	70
4	M	4	6700	L_1	MD*
5	F	15	3600	L_1	24
6	F	12	19000	L_2	31
7	F	5	12000	L_1	28
8	M	3	4500	L_2	MD
9	F	4	4200	L_1	64
10	M	5	5000	L_2	58
11	M	5	4300	L_2	61
12	F	12	42000	L_1	15
13	M	5	7600	L_1	38
14	M	2	18700	L_1	44
15	M	5	6400	L_2	57
16	F	11	12200	L_2	42
17	M	4	6300	L_1	8
18	M	4	24380	L_2	48
19	M	4	6800	L_2	35
20	F	14	17000	L_1	25
21	F	5	6600	L_2	118
22	M	5	32000	L_2	42
23	F	7	4000	L_1	62
24	M	8	8400	L_2	25
25	M	12	11000	L_2	34
26	F	5	6000	L_2	5
27	F	4	10200	L_2	43
28	M	11	8500	L_1	35
29	M	3	11700	L_1	44
30	M	4	4200	L_1	MD

* Missing data

The children patients included 3.3% (1/30) with lack of TEL gene, 7% (2/30) with gain of TEL, 1 with a deleted AML1 and 3 with gain of AML1 gene.

Discussion

The conventional cytogenetics and FISH analysis were performed on a group of 60 ALL patients in order to study the incidence of t(9;22) (q34;q11.2) and t(12;21) (p13;q22) as well as their correlation with the clinical findings.

Fluorescence *in situ* hybridization has become a powerful tool in cytogenetic analysis. Recent reports have shown that in ALL patients, especially in children, translocations for instance for chromosomes 12 and 21 occur in high frequency and is easily missed by conventional cytogenetic methods [43,44].

The importance of clonal chromosome aberrations, either, by the conventional or molecular cytogenetic analysis has been previously reported in ALL patients [45]. Among these, hyperdiploidy and presence of Philadelphia chromosome were considered as

Table 2. The clinical description of the adult patients

Patient No.	Sex	Age at diagnosis (years)	Initial WBC count	FBA Classification	Survival (months)
1	M	24	50000	L_2	24
2	F	28	86000	L_2	15
3	M	19	52000	L_1	29
4	M	21	200	L_2	MD*
5	M	42	34000	L_1	31
6	M	17	14000	L_1	35
7	M	18	11800	L_2	38
8	M	18	11000	L_2	24
9	M	28	4200	L_2	28
10	M	19	5800	L_2	30
11	F	21	8800	L_2	29
12	M	17	13000	L_1	21
13	F	24	5900	L_1	36
14	M	21	10300	L_2	22
15	F	22	43000	L_1	17
16	M	37	50000	L_1	21
17	M	40	67000	L_2	25
18	M	21	36000	L_2	36
19	M	23	47000	L_2	33
20	F	16	32000	L_1	41
21	M	33	96000	L_1	17
22	F	18	13300	L_2	32
23	F	23	42000	L_2	37
24	M	16	19000	L_1	43
25	F	23	20800	L_3	31
26	M	20	22200	L_1	36
27	M	16	8600	L_1	29
28	M	16	3800	L_1	34
29	F	16	84000	L_2	21
30	M	33	45000	L_2	27

* Missing data

Table 3. Frequency of different karyotypic clones among 30 adults and 30 children affected with ALL

Patients	Karyotypic mode				
	Diploidy (%)	*Hyperdiplpidy (%)*	*Hypodiploidy (%)*	*Polyploidy*	*Total*
Adults	7(23)	14(47)	9(30)	–	30(100)
Children	3(10)	18(60)	6(20)	3(10)	30(100)

Table 4. Frequency of type of chromosome aberrations

Patients	Type of aberration			
	Numerical		Structural	
	Gain (%)	*Loss (%) total*	*P arm*	*Q arm total*
Adults	19(63)*	11(37)**	6(21)***	24(79)****
Children	21(70)	9(30)	7(23)	23(77)

* Chromosomes involved: 2, 12, 16, 19 and 21; ** Chromosomes involved: 16, 17 and 22; *** Chromosomes involved: 2, 3 and X; **** Chromosomes involved: 3, 6, 9 and 22

Table 5. Summary of the signal patterns of the TEL/AML1 probe by FISH analysis

TEL/AML1 probe	Adults (n=30) Frequency (%)	Children (n=30) Frequency (%)
Normal	22(73)*	16(54)
Abnormal	8(27)	14(46)
TEL/AML1 fusion	1(3)	7(23)
TEL deletion	1(3)	1(3)
TEL gain	2(7)	2(7)
AML1 deletion	1(3)	1(3)
AML1 gains	3(10)	3(10)

* Statistical analysis normal/abnormal FISH ($p<0.02$)

Table 6. Summary of the signal patterns of the BCR/ABL probe by FISH analysis

BCR/ABL probe	Adults (n=30) Frequency(%)	Children (n=30) Frequency(%)
Normal FISH	21(70)*	26(87)
Abnormal FISH	9(30)	4(13)
BCR/ABL fusion	4(13)	1(3)
ABL deletion	2(7)	1(3)
ABL gain	1(3)	1(3)
BCR deletion	1(3)	0(0)
BCR gains	1(3)	1(3)

* Statistical analysis normal/abnormal FISH ($p<0.02$)

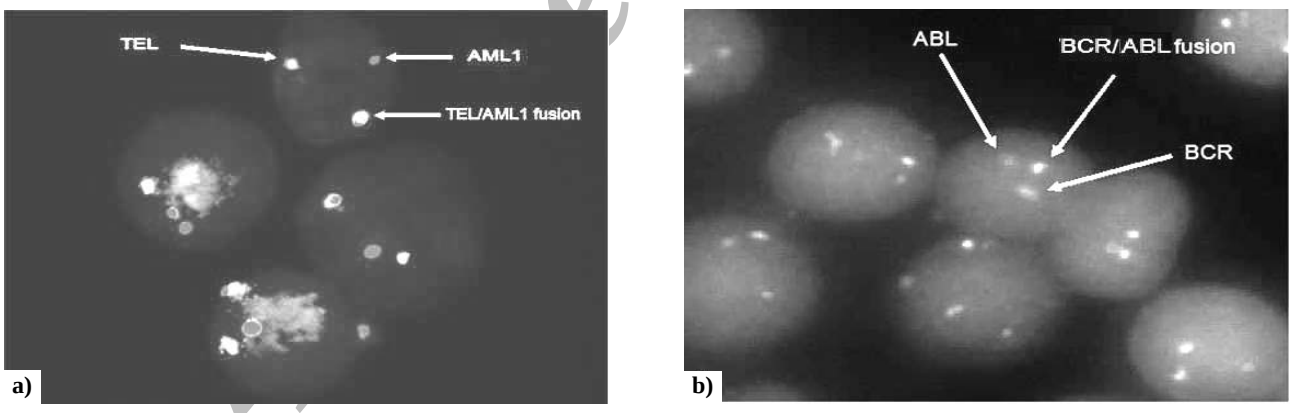

Figure 1. a) TEL/AML1 fusion in bone marrow cells in a patient affected with ALL; b) BCR/ABL fusion in bone marrow cells in a patient affected with ALL.

prognostic markers, especially, in childhood ALL [35].

In this study 60 patients with ALL (30 adults and 30 children) were investigated for BCR/ABL and TEL/AML1 fusion genes using Interphase FISH. The correlation between these findings and patients' clinical features were studied.

Alterations of Signal Patterns and Fusion of TEL/AML1 and BCR/ABL

Translocation of chromosomes 12 and 21 using FISH was found in 3.3% and 23% in adult and child patients, respectively while the conventional cytogenetic

technique revealed a normal karyotype. However, our result is in concordance with 1-4% as previously reported [44-46].

The presence of the both fused genes, either as a sole anomaly, or accompanied by aneusomies of signals, including the gain or loss of the genes are important to be considered.

No significant correlation was found between the presence of Ph+ and the survival period, either in adults, or in children. However a child (#13) presenting aneusomies of signals as the sole anomaly, *i.e.*, without any fusion and with the loss of AML1 and ABL as well as additional copies of TEL, AML1, and ABL, presented with a normal WBC count and L1 FAB classification, revealed to have a survival period of 235 months from the time of diagnosis.

The presence of a Ph chromosome in adults was accompanied by high WBC, L2 FAB-classification and old age.

Clinical Implications

Previous studies have frequently stated that the t(12; 21) is a marker related to a good prognosis [24-28].

In our series, most patients with the translocation were disease free during the follow-up studies. This is due to the presence of t(12;21). In this study all patients with the t(12; 21) (p13; q22) had pre-B ALL and all of them remained at the first remission and no relapse has occurred. The number of TEL/AML1 fused genes signals for one adult patient (#9) was 75 (out of 100 cells), whereas the number of signals for TEL/AML1 in 7 child patients with ALL was 33 to 48 (out of 100 cells). In this study, seven children patients with t(12;21) were characterized with age ranging between 2 and 8 years (only one patient was 15 years old), having less than 50 chromosomes, and WBC ranging from 1000/µl to 13500/µl. Five had similar immuno-phenotypes, positive for DR, CD10, CD13, CD19, while the other two patients had a CD10, CD19 immuno-phenotype, negative for DR and CD13. These results are in concordance with the previous reports [15-19].

The abnormalities of ABL and BCR genes (aneusomies and translocation) were found in 18% (11/60) of ALL patients including adult and children. The ph chromosome was detected in 13% (4/30) of adults and 3.3% (1/30) of children by FISH techniques. However, 3.3% (1/30) of children and 10% (3/30) of adults had the ph chromosome by conventional cytogenetic analysis. The application of FISH has confirmed the results observed by the conventional cytogenetic technique except in one patient.

One adult and 1 child patient had extra copy of BCR gene. Another child patient was lacking the BCR gene.

The incidence of BCR/ABL fusion, and the signal distribution of ABL and BCR in the present Iranian ALL patients, regarding chromosomal gains and losses, are in concordance with previous reports. However, there is lower incidence of hyperdiploidy with more than 50 chromosomes in ph-positive ALL in this study, compared with other reports [45-48]. In adults and children, the incidence of the fused BCR/ABL gene in our patients were in concordence with the previous studies, but, the incidence is lower than that reported from Hong Kong and CALGB studies [25].

There was a significant inverse correlation between the increased age of ALL children and a higher survival period ($P<0.05$, Table 1); and also a direct correlation between the presence of fused TEL/AML1 genes and decreased WBC ($P<0.05$), but this was not significant in adults. The adults with more than 50000 WBC had, significantly, a lower survival period ($P<0.05$), of whom, the patient #9 had the fused TEL/AML1, accompanied by additional materials, including TEL, ABL, and BCR genes with 43000 WBC and survival time of 55 months.

The combination of conventional karyotyping and molecular cytogenetics (FISH), and a long time follow up study could provide the Haematologists/oncologists a valuable package of information which could lead to appropriate therapeutic management for the benefit and a better style of life for ALL patients.

Acknowledgement

We would like to thank the patients and their family for help in providing the bone marrow samples. This work was supported by the department of medical genetics of the Tehran University of Medical Sciences.

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