ULUSLARARASI HEMATOLOJI-ONKOLOJI DERGISI ARTICLE International Journal of Hematology and Oncology

Molecular Cytogenetic Findings in Cases with Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and is usually associated with numerical and structural chromosomal changes. Although some of these changes are accepted as favorable or poor prognostic factors, the prognostic effects of others have not been well determined. In our study, we aimed to present the chromosomal changes in cases with childhood ALL and their ratios in hematologic risk groups. Thirty four patients with childhood ALL were included in the study. Subjects were diagnosed with fluorescence in situ hybridization (FISH) analysis by using standard translocation, deletion and aneuploidy probes. The chromosomal changes obtained from our analysis were classified into hematologic risk groups and their ratios were evaluated. In our study, we found that the t(12.21) translocation was the most common abnormality in minimal and standard risk groups, whereas the 9p21 deletion was the most common abnormality among high-risk patients.

Keywords: Acute lymphoblastic leukemia, Molecular cytogenetic, Chromosomal anomaly

ÖZET

Çocukluk Çaği Akut Lenfoblastik Lösemi'li Olgularda Moleküler Sitogenetik Bulgular

Akut Lenfoblastik Lösemi (ALL) çocukluk çağında görülen en yaygın malignite olup sayısal ve yapısal kromozomal değişiklikler ile birlikte görülebilmektedir.Bu değişikliklerin bazılarının iyi ya da kötü prognostik belirteç kabul edilirken bazılarının ise prognostik etkileri tam bilinmemektedir. Çalışmamızda, çocukluk çağı ALL vakalarında saptanan kromozomal değişiklikler ile hematoljik risk gruplarındaki oranlarını vermeyi amaçladık. Çalışmaya tanı almış çocukluk çağı akut lenfoblastik lösemili 34 olgu alındı. Olgulara, ALL açısından değer taşıyan standart translokasyon, delesyon ve aneuploidi probları kullanılarak floresan in situ hibridizasyon (FISH) analizleri yapıldı. Saptanan kromozomal değişiklikler vakaların hematolojik risk gruplarına göre sınıflandırılarak oranları değerlendirildi. Çalışmamızda, 9p21 delesyonu yüksek riskli hastalar arasında en sık görülen anomali iken t (12.21) translokasyonu minimal ve standart risk grubunda en sık görülen anomali olduğunu bulduk.

Anahtar Kelimeler: Akut lenfoblastik lösemi, Moleküler sitogenetik, Kromozomal anomali

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy during childhood and constitutes one third of all childhood malignancies.[1] Several chromosomal abnormalities, numerical and structural, are associated with childhood leukemia. Hyperdiploidy is characterized as a nonrandom gain of one or more chromosomes (chromosome numbers= 46-47) and is the most common cytogenetic abnormality pattern in childhood ALL.[2] Hyperdiploidy is seen in 30-40% of all cases of childhood ALL. Numeric chromosomal changes are usually encountered in chromosomes 4, 6, 8, 10, 14, 17, 18, 20 and 21.[3-6] The most common structural change is the t(12;21) (TEL-AML1) translocation, which accounts for 25% of cases of ALL.[7]

When ALL is diagnosed early, examining chromosomal changes can be useful for predicting the prognosis of the disease prior to treatment. Although it has been established that hypodiploidy (chromosome number < 46) is associated with poor prognosis, hyperdiploidy (chromosome number > 46), especially with higher chromosome numbers (chromosome numbers= 50-67), is associated with favorable prognosis. In addition, some structural changes, such as in the t(9;22) translocation, which generates the BCR/ABL fusion gene, and the MLL gene translocation, generated by the t(11q23) translocation, have poor prognosis, but the t(12;21) (TEL-AMLI) translocation has a favorable prognostic effect.[7-10] Although the deletion of the 9p21 location accounts for 15% of all childhood ALL, the prognostic value of this deletion is unknown.[11]

Conventional cytogenetic techniques can provide information about chromosomal changes, confirm clinical diagnosis, and are used to classify chromosomal abnormalities for determining prognosis and promoting treatment strategies. With leukemias, poor or decreased quality of metaphase preparations obtained from cultures of bone marrow cells and blood cells makes cytogenetic analysis of chromosomes difficult. Additionally, the detection of some chromosomal anomalies, such as the t(12;21) translocation, can be challenging. In early diagnoses of leukemia, molecular cytogenetic studies can not only provide cytogenetic information but are also useful in detecting translocations and hyperdiploidy when cultures are negative or when cultures have poor quality of metaphases.[12-14]

In this study, we utilize molecular cytogenetic analysis (FISH) to present the rates of numerical and structural chromosomal changes in patients with childhood ALL.

MATERIALS AND METHODS

Thirty-four patients recently diagnosed with childhood ALL at Uludag University Medical Faculty, Department of Pediatric Hematology, were included in the study. To evaluate numerical and structural chromosomal changes, bone marrow samples were sent to Uludag University Medical Faculty, Department of Medical Genetics, for conventional and molecular cytogenetic analysis (FISH). In the FISH analysis, 200 well-hybridized cell signals were evaluated with each available probe. The bone marrow samples were processed with fixative after KCl administration. Nuclear smears were laid on plates and suspended in 2x SCC with 0.01 N HCl and pepsin. DNA was denatured in 70% formamide. Prepared samples were hybridized with centromeric X, Y, 4, 6, 7, 8, 10, 18 probes and locus-specific 5, 9, 11, 12, 14, 15, 16, 17, 21 and 22 probes. Samples were stained by DAPI II (125 ng/ml in antifade) and analyzed by Quips Imaging Systems (Applied, UK) with single, (aqua, gold, blue, red, green and Dapi), double (red/green) and triple (Dapi/red/green) filters on a Nikon E 600 fluorescence microscope in the FISH Laboratory of Uludag University Medical Faculty, Department of Medical Genetics. The molecular cytogenetic findings are shown in Figure 1.

RESULTS

Thirty-four cases of childhood ALL were included in this study. The ages of the patients were between 2 years and 16 years (median=5); 21(62%) of the patients were male, and 13(38%) of the patients were female. At the time of diagnosis, the white blood cell counts of the patients ranged from 840x103/mm^3 to 257x103/mm^3 (median=103x103/mm^3). Eighteen of the patients had chromosomal changes, including hyperdiploidy (chromosome numbers= 47-67) in 9(26%) cases and a t(12:21) translocation in 11(32%) cases. Two of the patients with a t(12;21) translocation also had concomitant hyperdiploidy. Although the t(11q23) translocation has prognostic significance, it was seen in only one patient. None of the patients ex-

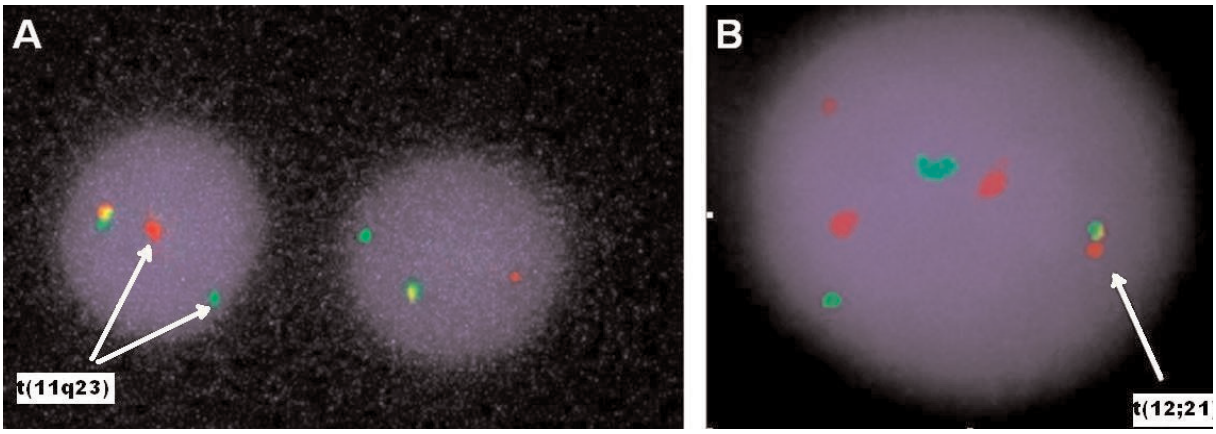

Figure 1. Interphase appearance of FISH in different ALL patients. **(A)** The separate presentation of red and green signals on chromosomes with a t(11q23) translocation (arrows) in case 1. **(B)** The synchronous presentation of hyperdiploidy and a t(12;21) translocation(arrow) in case 18.

hibited hypodiploidy or a t(9;22) translocation. The 9p21 deletion was evaluated in 22 patients, and 8(36%) of them exhibited homozygous (n=2) or hemizygous (n=6) deletions (Table 1). When risk groups were evaluated, the t(12;21) (TEL-AML 1) translocation was the most commonly encountered chromosomal change in standard-risk and minimal-risk groups, whereas high-risk patients commonly had 9p21 deletions (Tables 2 and 3).

DISCUSSION

Randomized chromosomal changes are encountered in 80% of ALL patients, and some of these chromosomal changes, either numerical or structural, have prognostic importance.[15] In this study, some of the patients illustrated both types of chromosomal changes. It has been reported that the coincidence of the t(12;21) translocation and hyperdiploidy decreases the risk of treatment failure.[16] Additionally, the MLL gene rearrangement, the t(9;22) translocation and hypodiploidy indicate poor prognosis in ALL patients. Detection of chromosomal changes is useful for confirming diagnoses, aiding with prognosis suggestions, and guiding treatment protocols.

We detected chromosomal changes in 74% of our patients (25/34). The most common chromosomal abnormality was the t(12;21) translocation, which was detected in 11 of our patients. It has been suggested that the t(12;21) translocation is a favorable prognostic factor in the short term; however, the utility of the t(12;21) translocation as a favorable prognostic factor in the long term and its ability to predict relapse are unknown.[17,18] We also detected different types of translocations; a double t(12;21) translocation was detected in one of our patients, and a 12p13 deletion was seen in two patients. The prognostic importance of simultaneously occurring t(12;21) translocations, 12p13 deletions, hyperdiploidy and double fusions is currently unknown. Thus, we suggest that more information should be obtained from patients with different variants of t(12,21) translocations. The role of the t(12,21) translocation in prognosis, incidence of relapse and follow-up should also be evaluated. Consistent with the literature, in our study, hyperdiploidy was detected in 26% of ALL patients, with the most common copy gains seen in chromosomes 4, 6, 10, 21 and X (7, 9). Previous studies have suggested that gaining a copy of chromosomes 4, 10 or 17 is associated with favorable prognosis; however, trisomy of chromosome 5 confers poorer outcome among high-hyperdiploid patients.[19,20]

In our study, we did not detect poor prognostic changes, such as the t(9;22) translocation or hypodiploidy. However, one of our patients had a t(11q23) translocation. The t(11q23) translocation is a poor prognostic factor, accounting for 2-4% of childhood ALL, and it is expressed in 80% of all infants with ALL.[21] The 9p21 deletion was evaluated in 22 patients, and eight of them expressed either a homozygous deletion or a hemizygous deletion. Among these patients with a 9p21 deletion, six patients exhibited a hemizygous deletion and two patients exhibited a homozygous deletion.

Table 1. Demographic features and molecular cytogenetic findings in patients with ALL.

	Age	Gender	Leukocyte count	Risk group	t(9;22)	t(11q23)	t(12;21)	9p21 deletion	Numerical changes
1	13	M	125000	HRG	N	P	N	N	N
2	16	M	140000	MRG	N	N	N	ND	N
3	3	F	7600	MRG	N	N	N	ND	Hyperdiploidy
4	2	F	4500	MRG	N	N	N	ND	Hyperdiploidy
5	4	F	4200	SRG	N	N	P	ND	N
6	5	F	15300	SRG	N	N	N	ND	Hyperdiploidy
7	4	F	12000	MRG	N	N	N	ND	Hyperdiploidy
8	16	F	10300	MRG	N	N	N	ND	N
9	3	M	6320	MRG	N	N	P	N	N
10	3	M	2700	MRG	N	N	N	ND	N
11	9	M	7200	MRG	N	N	N	ND	Hyperdiploidy
12	4	F	4900	MRG	N	N	P	ND	Hyperdiploidy
13	16	M	2800	HRG	N	N	N	ND	N
14	6	M	19300	MRG	N	N	P	N	N
15	14	M	2980	MRG	N	N	N	Hemizygote	N
16	13	M	52000	MRG	N	N	N	Hemizygote	N
17	4	F	2400	MRG	N	N	N	ND	N
18	9	M	28700	MRG	N	N	P	N	Hyperdiploidy
19	3	M	4000	MRG	N	N	P	N	N
20	4	M	840	MRG	N	N	P	N	N
21	13	M	80000	HRG	N	N	N	Homozygote	N
22	3	F	68000	HRG	N	N	N	N	N
23	7	F	14000	HRG	N	N	N	Hemizygote	Hyperdiploidy
24	5	M	2700	MRG	N	N	P	N	N
25	4	M	44700	MRG	N	N	N	N	Hyperdiploidy
26	10	M	10300	MRG	N	N	P	N	N
27	3	F	257000	MRG	N	N	N	N	N
28	9	M	14700	MRG	N	N	N	N	N
29	4	F	4200	HRG	N	N	N	Hemizygote	N
30	7	M	30000	HRG	N	N	N	Homozygote	N
31	13	M	43300	MRG	N	N	N	Hemizygote	N
32	9	M	4500	MRG	N	N	N	N	N
33	4	M	7950	SRG	N	N	P	N	N
34	3	F	140000	MRG	N	N	P	Hemizygote	N

M= Male, F= Female, MRG= Minimal risk group, SRG= Standard risk group, HRG= High risk group, N= Negative, P; Positive, ND= No data

Prognostic significance is limited to homozygous deletions of 9p21, which can be explained by loss of heterozygosity. Unlike homozygous deletions, hemizygous deletions might not be sufficient to turn off the function of the CDKN2A and the CDKN2B genes. Kim et al. suggested that homozygous deletion of the CDKN2A and CDKN2B genes is a poor prognostic factor in adult but not in childhood B-lineage ALL.[11,22]

In conclusion, high incidences of chromosomal changes are detected at the time of diagnosis in children with ALL. When conventional cytogenetic preparations lack metaphases or the metaphases are of poor quality, additional molecular cytogenetic analyses can be performed to support the diagnosis of ALL, as well as to provide useful information to aid in the choice of treatment protocols and follow-up protocols.

Table 2. The distributions of molecular cytogenetic [t(9,22), t(11q23), t(12;21) and numerical changes] findings according to risk groups in ALL patients(n=34).

	MRG (n=24)(%)	SRG (n=3)(%)	HRG (n=7)(%)
t(9;22) translocation	0(0)	0(0)	0(0)
t(11q23) translocation	0(0)	0(0)	1(14)
t(12;21) translocation	9(37)	2(66)	0(0)
Numerical changes	7(29)	1(33)	1(14)

MRG; Minimal risk group, SRG; Standard risk group, HRG; High risk group

Table 3. The distribution of 9p21 deletions in risk groups in ALL patients(n=22).

MRG (n=15)(%)		SRG (n=1)(%)	HRG (n=6)(%)
9p21 deletion	4(27)	0(0)	4(66)

MRG; Minimal risk group, SRG; Standard risk group, HRG; High risk group

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