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Molecular diagnosis of hematological malignancies by RT-PCR

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ABSTRACT

Detection of genes, which are coding chimerical proteins, by using reverse transcriptase-polymerase chain reaction (RT-PCR) has a crucial role in diagnosis, treatment and prognosis of leukemias. In this study, RT-PCR and nested PCR techniques were performed, in 43 hematological malignancy cases, using primers specific for t(1;19)(q23;p13), t(4;11)(q21;q23), t(8;21)(q22;q22), t(9;22)(q34;q11)p210, t(9;22)(q34;q11)p190, t(12;21)(p13;q22), t(15;17)(q22;q21) translocations and for inv(16)(p13;q22). Four adult chronic myelogenous leukemia (CML) cases and two pediatric acute lymphoblastic leukemia (ALL) cases were shown to have t(9;22)p210 transcripts. Two adult CML cases, and two adult acute myeloid leukemia (AML) cases and two pediatric ALL cases were found positive for t(9;22)p190 transcripts. Additionally, one adult CML patient had p210 and p190 transcripts together. Translocation t(12;21) in 3 pediatric ALL cases, t(8;21) in an adult AML case, t(15;17) in an adult AML and inv(16) in an adult AML case were detected. Chromosomal aberrations of t(1;19) and t(4;11) were not observed in any case of our patient group. Our findings, evaluated with clinical outcome, are consistent with previous reports. As a sensitive technique, RT-PCR analysis is very useful with the case for whom cytogenetical analysis is not possible due to insufficient metaphase.[Turk J Cancer 2005;35(3):113-118].

KEY WORDS:

RT-PCR, chromosomal aberrations, and hematological malignancy

INTRODUCTION

Numerical and structural abnormalities of chromosomes are quite frequent and are an important pathogenetic phenomenon in hematological malignancies (1,2). Molecular studies, performed on these chromosomal rearrangements, revealed crucial data about tumorigenesis mechanisms (3). Functions and activities of many proto-oncogenes and tumor suppressor genes localized on or around the breakpoints of chromosomal abnormalities are altered by formation of fusion genes coding for chimerical proteins (4).

After the first discovery of translocation t(9;22)(q34;q11) which is between breakpoint cluster region (BCR) gene on chromosome 22 and ABL gene on chromosome 9, many chromosomal aberrations causing fusion genes in cancer have been shown by cytogenetic techniques (5-8). Discovery of molecular techniques have led to more precise determination of percentage of cases with certain chromosomal aberrations. Translocation t(9;22) is observed in 95% of CML (chronic myelogenous leukemia) patients, in 2-10% of pediatric AML (acute myeloid leukemia) cases, and in 20-50% of adult ALL (acute lymphoblastic leukemia) cases. In addition, this translocation might be seen in less than 2% of AML cases and rarely seen in lymphoma and myeloma cases. Different transcription products, namely p190, p210, and p230, might arise from different breakpoints on BCR gene (9-11).

Inv(16)(p13;q22) is found in almost all AML-M4Eo subgroup patients. This translocation is formed due to fusion of core binding factor beta (CBFB) gene and myosin heavy chain 11 (MYH11) gene (9). Translocation t(15;17)(q22;q21) is detected in acute promyeloid leukemia (APL) M3 patients which constitute 10% of all AML cases. In t(15;17) translocation, a chimerical protein is formed by fusion of promyelotic leukemia (PML) gene on 15q22 and retinoic acid receptor alpha (RARA) gene on 17q21. This fusion protein is observed in 98% of APL cases (12). Translocation t(8;21)(q22;q22) is the product of fusion of eight twenty-one (ETO) gene on chromosome 8 and acute myeloid leukemia 1 (AML1) gene on chromosome 21. This translocation is found in 18% of AML-M2 cases (13). Translocation t(12;21)(p13;q22) is formed by fusion of TEL/ETV6 gene on chromosome 12 and AML1/CBFA2 on chromosome 21 and is observed in 22% of pediatric ALL cases (14). Fusion of AF4 gene on chromosome 4 with MLL gene on chromosome 11 causes formation of t(4;11)(q21;q23) translocation, which is seen in 50-70% of infant ALL cases and in approximately 5% of adult ALL cases (9,15). Translocation t(1;19)(q23;p13) is formed as a result of fusion of PBX1 gene on chromosome 1 and E2A gene on chromosome 19 and is observed in 3% of adult ALL cases (16).

RT-PCR is a very fast technique and unlike classical cytogenetic techniques, results can be obtained when the available patient material is in low quantities and qualities. In this study, detection of well-defined and frequently seen chromosomal rearrangements were performed by using RT-PCR and nested PCR system with translocation specific primers in 43 hematological malignancy patients.

MATERIALS AND METHODS

In this study, RT-PCR analysis technique, previously standardized by van Dongen et al. (9), for detection of most frequent 8 chromosomal rearrangements of leukemias were performed. Studied rearrangements are t(1;19)(q23;p13), t(4;11)(q21;q23), t(8;21)(q22;q22), t(9;22)(q34;q11)p210, t(9;22)(q34;q11)p190, t(12;21)(p13;q22), t(15;17)(q22;q21), and inv(16)(p13;q22).

RNA isolations were performed for 7 CML, 19 ALL, 11 AML, 1 juvenile-myelomonocytic leukemia (J-MML), 2 lymphoma, 2 myelodysplastic syndrome (MDS) and 1 CLL (Chronic Lymphocytic Leukemia) patients' peripheral blood samples by using QIAmp RNA isolation kit according to manufacturer's recommendations. RNA samples of different patients, that had been previously analyzed in another center by molecular techniques and were known to have these 8 chromosomal rearrangements, were used as positive controls in this study. Using ABL gene specific primer RNA, samples were checked on 3% agarose gel. RT-PCR coupled nested-PCR analysis was performed using fusion gene specific primers (9).

RESULTS

Clinical diagnosis, age and results of RT-PCR analysis of 43 patients included in this study are given in tables 1 and 2, and a gel image of RT-PCR analysis is given in figure 1.

From the Pediatric Hematology department, a total of 19 ALL, 5 AML, 2 lymphoma and 1 J-MML cases were referred. Transcripts for t(9;22)p210 translocation were detected in 2 (11%) ALL cases, diagnosed at the ages of 7 and 16 years. In patient 3 and patient 16, translocation t(9;22)p190 (11%) was observed. TEL/AML1 fusion gene was shown in 3 of 19 (16%) pediatric ALL cases. Translocations of other fusion genes were not observed in any of the pediatric cases.

Sixteen adult hematological malignancy cases were screened for the presence of the fusion transcripts. Seven of the cases were diagnosed as CML. Transcripts for t(9;22)p190 and t(9;22)p210 translocations were found in 1 (14%) and 4 (57%) CML cases, respectively. In patient 31, who was diagnosed as CML at the age of 48, both p190 and p210 transcripts of t(9;22) (14%) translocation was detected simultaneously. Translocation t(9;22)p190 was observed in 2 of 6 (33%) AML cases. Translocations t(15;17), t(8;21), and inv(16) were found positive in 3 AML patients, for each translocation the frequency was 17%. None of the screened translocations were detected in 2 MDS and 1 CLL patients who were also included in the study.

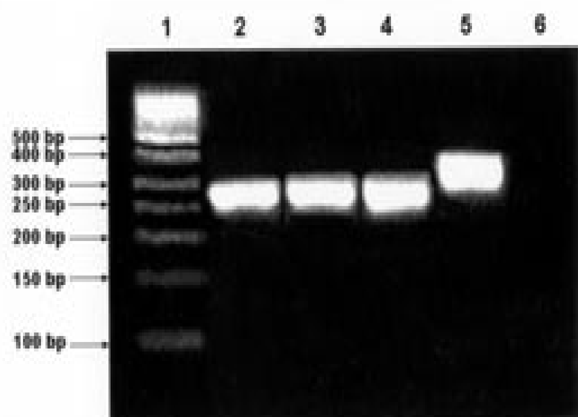

Fig 1. RT-PCR gel result. Lane 1: 50 base pair ladder; Lane 2-5: t(9;22)p210 positive cases; Lane 6: Negative control

DISCUSSION

Diagnoses of leukemias have been improved by classical karyotyping, FISH and PCR analysis (3). As numerical chromosomal aberrations and unknown balanced alterations are detectable solely by cytogenetic analysis; this type of conventional methods form an important platform for molecular characterization of genetic aberrations.

In spite of recent advances in classical karyotype analysis, difficulties in obtaining enough number of good quality metaphases have not been completely overcome. Molecular cytogenetic techniques, such as FISH analysis, when compared with cytogenetic techniques, although have some superiority in terms of time consumed, and easiness, these

Table1
RT-PCR analysis results of pediatric cases

Patient No	Clinical diagnosis	Age (years)	Translocations that were found positive
1	ALL	16	-
2	ALL	7	t(9;22)p210
3	ALL	16	t(9;22)p190
4	ALL	3	t(12;21)
5	ALL	8	-
6	ALL	3	t(12;21)
7	ALL	4.5	-
8	ALL	10	t(12;21)
9	ALL	3 months	-
10	ALL	3.5	-
11	ALL	16	t(9;22)p210
12	ALL	4	-
13	ALL	3	-
14	ALL	3	-
15	ALL	3	-
16	ALL	1	t(9;22)p190
17	ALL	6	-
18	ALL	6	-
19	ALL	3	-
20	AML	3	-
21	AML	2	-
22	AML	7	-
23	AML	5	-
24	AML	2	-
25	Lymphoma	16	-
26	Lymphoma	8	-
27	J-MML	5	-

techniques have disadvantages of having false positivity depending on specific probes, in addition to above mentioned difficulty (4). These limitations gave rise to increased usage of PCR techniques in leukemia diagnosis and prognosis (17-19). There are two main advantages of this method. First, this technique might be applied in less than two days, and one can perform PCR reactions of different aberrations and different patients at the same time. Second, in detection of known submicroscopic aberrations, usage of this method provides more certain results and ensures detection of one malignant cell among one million normal cells. Moreover, molecular techniques can be used when the material is insufficient for classical karyotyping.

In this study, RT-PCR and nested-PCR methods were applied for detection of fusion genes at mRNA level. In our study group, t(1;19), t(4;11) translocations have not been seen in any patient. Translocation t(1;19) has been shown in 3-6% of B-ALL cases, previously. Additionally, this translocation has been seen in ALL, T-ALL and AML cases, rarely (less than 1%). For this reason, observing no individual with this translocation might be considered as expected. Likewise, our patient group contains only one infancy ALL case, in which, translocation t(4;11) has been mainly observed (20). Inv(16) chromosomal alteration is related to AML-M4Eo subgroup and this chromosomal aberration is observed in one of 11 AML cases in this study (9).

Translocation t(12;21), which is detected especially in pediatric ALL cases, was shown in 3 of 19 cases. In a previous study, frequency of t(12;21) translocation was reported as 39% among pediatric ALL cases (14). This translocation is observed, more frequently, at 3-5 years of age (9). In our study, 2 of t(12;21) positive cases were 3 years old and one case was 10 years old. These findings confirm that t(12;21) translocation should be routinely screened in all pediatric ALL cases.

P210 product of t(9;22) coding BCL-ABL fusion gene with increased tyrosine-kinase activity, was detected in 5 of 7 adult CML cases and 2 of 19 pediatric ALL cases. Positivity of p210 product was reported in almost all adult CML cases and 20-30% of all Philadelphia positive pediatric ALL cases. Translocation t(9;22)p190 product, occurring due to different break points, was observed in adult ALL and in adult AML cases. Our results are consistent with the previous reports' findings that p190 product was present in 22% of adult ALL cases and in 1% of adult AML cases (10). Although p190 transcript is specific to ALL this

Table2
RT-PCR analysis results of adult cases

Patient No	Clinical diagnosis	Age (years)	Translocations that were found positive
28	CML	23	-
29	CML	24	t(9;22)p210
30	CML	33	t(9;22)p210
31	CML	48	t(9;22)p210, t(9;22)p190
32	CML	60	t(9;22)p210
33	CML	26	t(9;22)p190
34	CML	40	t(9;22)p210
35	AML	29	t(9;22)p190
36	AML	40	t(15;17)
37	AML	32	t(8;21)
38	AML	35	t(9;22)p190
39	AML	52	-
40	AML	55	Inv(16)
41	MDS	46	-
42	MDS	63	-
43	CLL	76	-

transcript has been rarely detected in CML patients. In our patient group presence of p210 was shown in 2 (out of 7) CML patients one of which has also p190 transcript. In fact, it has been reported that, all CML patients at the time of diagnosis have some p190 products by alternative splicing mechanism in addition to p210 transcript (21,22). As a consequence, it might be stated that both p190 and p210 transcript status of all pediatric and adult ALL, AML and CML cases should be checked routinely for diagnostic, prognostic and therapeutic purposes.

Each PCR reaction has 10^{-2} sensitivity, and nested PCR analysis increases this sensitivity to 10^{-4} (9,19). Therefore, for detection of leukemic genetic alterations, PCR methods are fast, sufficient and feasible, and PCR methods should be used as supplementary to cytogenetic analysis, for efficiency. PCR method is also suitable for detection of other newly identified translocations by designation of new specific primers (9). Molecular detection and use of these translocations in treatment has an additional importance especially in cytogenetically problematic cases.

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