RESEARCH ARTICLE

Multiplex RT-PCR Assay for Detection of Common Fusion Transcripts in Acute Lymphoblastic Leukemia and Chronic Myeloid Leukemia Cases

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous disease which requires a risk-stratified approach for appropriate treatment. Specific chromosomal translocations within leukemic blasts are important prognostic factors that allow identification of relevant subgroups. In this study, we developed a multiplex RT-PCR assay for detection of the 4 most frequent translocations in ALL (BCR-ABL, TEL-AML1, MLL-AF4, and E2A-PBX1). Materials and Methods: A total of 214 diagnosed ALL samples from both adult and pediatric ALL and 14 cases of CML patients (154 bone marrow and 74 peripheral blood samples) were assessed for specific chromosomal translocations by cytogenetic and multiplex RT-PCR assays. Results: The results showed that 46 cases of ALL and CML (20.2%) contained the fusion transcripts. Within the positive ALL patients, the most prevalent cryptic translocation observed was mBCR-ABL (p190) at 8.41%. In addition, other genetic rearrangements detected by the multiplex PCR were 4.21% TEL-AML1 and 2.34% E2A-PBX1, whereas MLL-AF4 exhibited negative results in all tested samples. Moreover, MBCR-ABL was detected in all 14 CML samples. In 16 samples of normal karyotype ALL (n=9), ALL with no cytogentic result (n=4) and CML with no Philadelphia chromosome (n=3), fusion transcripts were detected. Conclusions: Multiplex RT-PCR provides a rapid, simple and highly sensitive method to detect fusion transcripts for prognostic and risk stratification of ALL and CML patients.

Keywords: Acute lymphoblastic leukemia - chronic myeloid leukemia - multiplex RT-PCR - risk stratification

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Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous group of clonal hematopoietic stem cell malignancies with variable clinical represents, cell morphologies, immunophenotypes, genetic alterations, prognosis, disease progressions, as well as treatment outcomes (Piwkham et al., 2015). The recent revision of the World Health Organization (WHO) 2008 classification of myeloid neoplasms and acute leukemia has been categorized chronic myeloid leukemia (CML), acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL) based on the representation of specific balanced chromosomal translocations (Vardiman et al., 2009). Conventional karyotyping could identify common chromosomal translocations in leukemia which are recognized as disease etiology, reflecting on risk-stratification and providing applicable therapeutic strategies including; AML is associated with t(8;21)(q22;q22), inv(16)(p13.1q22) or t(16;16)(p13.1;q22), t(15;17)(q22;q12), t(9;11)(p22;q23), t(6;9)(p23;q34), inv(3)(q21q26.2) or t(3;3)(q21;q26.2), t(1;22)(p13;q13); ALL is mostly involved t(9;22)(q34;q11.2), t(v;11q23), t(12;21)(p13;q22), t(5;14)(q31;q32), t(1;19)(q23;p13.3) and CML is characterized by the identification of t(9;22)(q34;q11.2) (Philadelphia chromosome) (Look, 1997; Awan et al., 2012; Xiong et al., 2013; Ye et al., 2014; Tahira et al., 2015). Although complete cytogenetic studies by karyotyping of bone marrow sample obtained from the patient has been proved to be the gold standard method (especially in CML) and proposed for an initial assessment tool to determine the baseline of patient karyotype, the test has several disadvantages such as required fresh specimen, labor intensive, time consuming, technically difficult, requires high skilled personnel, facing with contamination and culture failure, and cannot detect some minute genetic alterations (Shaikh et al., 2014). Moreover, karyotyping could not perform in treated patients who show complete

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cytogenetic response (CyR) after induction therapies (Goldman, 2005). In the past decade, fluorescent in situ hybridization (FISH) technique using fluorescent-labeled probes specific to unique chromosomal translocation has been shown superior advantages over conventional karyotyping including high sensitivity and specificity, can be performed on both dividing and non-dividing cells (metaphase/interphase cells), fast, can analyze many cells in the same time, and could be a monitoring test for the detection of minimal residual disease (MRD) in leukemia (Krauter et al., 1998; Elmaagacli, 2007; Park et al., 2008; Testoni et al., 2009; Mazloumi et al., 2012). Nevertheless, several limitations of FISH have been observed such as not a screening test, requires specific probes, low resolution in particular probe set, and less sensitivity than the PCR-based technology. At present, the reverse transcriptase-PCR assay (RT-PCR) has been shown to be a powerful sensitive, specific, and rapid test for detection of genetic alterations including recurrent chromosomal translocations in leukemia (Scurto et al., 1998; van Dongen et al., 1999; Harrison, 2000; Liang et al., 2002; Elia et al., 2003; Yang et al., 2010). Moreover, several studies demonstrated that RT-PCR has greater sensitivity than conventional karyotyping and equivalent to FISH for detecting and monitoring of MRD in several types of leukemia (Colleoni et al., 2000; Sarriera et al., 2001; Raanani et al., 2004). Furthermore, in almost last two decades, several multiplex RT-PCR systems have been developed for routinely used as a screening test for revealing recurrent genetic alterations in leukemia based on its ability to simultaneously detect multiple genetic aberrant/chromosomal translocations (van Dongen et al., 1999; Salto-Tellez et al., 2003; Dunlap et al., 2012; Xiong et al., 2013). In this report, we described our routine multiplex RT-PCR assay for detecting and screening recurrent translocations in CML and ALL patients..

Materials and Methods

Patient samples

A total of 214 diagnosed ALL samples from both adult and pediatric ALL and 14 cases of CML patients (154 bone marrow and 74 peripheral blood samples) who registered for leukemia diagnosis at Department of Pathology, Ramathibodi hospital, Mahidol University, Bangkok, Thailand during January 2010 to April 2015 were collected and subjected to this study. Diagnostic criteria was based on the representation of hematologic findings, histology, the presence of leukemic blast (lymphoid lineage) and immunophenotypes. Complete cytogenetic study according to the recently revised WHO 2008 classification of myeloid neoplasms and acute leukemia (Vardiman et al., 2009) was done.

Cytogenetic study

Complete cytogenetic study or karyotyping was performed at Human Genetic Laboratory, Department of Pathology, Ramathibodi hospital using G-banding technique after short term culture without mitogen activation. On-screen karyotyping was performed on 20-30 metaphases using Ikaros software, MetaSystems, Germany. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2013) (Shaffer et al., 2013).

RNA extraction, RNA quantification, and cDNA synthesis

Total RNA was extracted from peripheral blood and bone marrow samples using QIAamp RNA Blood Mini Kit (Qiagen) according to the manufacturer's recommendations. RNA concentration and qualification were determined by NanoDrop 2000 (Thermo Scientific, MA, USA) spectrophotometer. One nanogram of RNA was reverse transcribed to complementary DNA (cDNA) using SuperScript® VILO™ cDNA synthesis kit (Life Technologies, USA) according to the manufacturer's recommendations.

Multiplex PCR

Single-round multiplex PCR reaction specific for amplification of four common chromosomal translocations in ALL (TEL-AML1, MLL-AF4, E2A-PBX1, and BCR-ABL; p190), the additionally BCR-ABL; p210 in CML, and internal control HPRT (hypoxanthine ribosyltransferase) genes was performed to amplify cDNA generated from RNA isolated from ALL patients. Primers details are described in Table 1. Optimal multiplex PCR condition was following: 95° C for 10 minutes, 35 cycles of 95° C for 30 seconds, 65° C for 30 seconds, 72° C for 30 seconds, and final extension at 72° C for 10 minutes. PCR was held at 4° C until continue to further steps. 10 μl of PCR products were detected by automated electrophoresis, QIAxcel Advanced system (QIAGEN, Germany).

Quality Control

Multiplex RT-PCR assay was performed using leukemia cell line as positive controls for specific chromosomal translocations (K562 cell line with MBCR-ABL p210 and SUP-B15 cell line with mBCR-ABL p190). For the other three translocations, cDNA from patients positive for cryptic translocations by conventional cytogenetics including, t(12;21)(p13;q22) and t(1;19)(q23;p13.3) was used as positive controls for TEL-AML1 and E2A-PBX1, respectively. For t(4;11)(q21;q23) positive control, plasmid DNA carrying MLL-AF4 was used.

Results

Multiplex PCR assay potentially used as a screening and monitoring test for ALL and CML

A total of 228 samples (154 bone marrow aspirate and 74 peripheral blood samples) collected from ALL (n = 214) and CML (n = 14) patients were analyzed using conventional karyotyping and subsequently examined by the established multiplex PCR. Multiplex PCR following with gel electrophoresis was performed as described in material and method section. The interpretation of the finding was shown in figure 1. All samples were positive for a housekeeping gene (HPRT) by the multiplex PCR (except lane 4 which is a plasmid control DNA of MLL-AF4). The established multiplex PCR was able to detect

BCR-ABL fusion in all 14 samples collected from CML patients. Interestingly, this method was able to detect BCR-ABL fusion transcripts in 2 treated CML patients who showed negative result for Philadelphia chromosome by conventional genetic study (No.3 and 23 in Table 2). These results indicated that our proposed multiplex PCR could be potentially used as a routine screening and monitoring test for CML. In diagnosed ALL samples, 32 samples were positive for the generated multiplex PCR analysis (15.00 %) whereas 182 samples (85.00 %) showed negative results. Within the positive group, the most prevalent cryptic translocation observed in our tested samples was the mBCR-ABL (p190) with 8.41 % (18/214). Other genetic rearrangements detected by the established multiplex PCR in ALL were following, TEL-AML1 with 4.21% (9/214), E2A-PBX1 with 2.34% (5/214), whereas MLL-AF4 exhibited negative result in all tested samples (Figure 2). Furthermore, the generated multiplex PCR assay was able to identify those fusion transcripts (except MLL-AF4) in 14 samples which could not be detected by the routinely conventional cytogenetic analysis (Table 2). To gather, our established multiplex PCR assay could be potentially used as a combined test with conventional cytogenetic study for screening and detecting common cryptic chromosomal translocations as well as a monitoring test for ALL and CML.

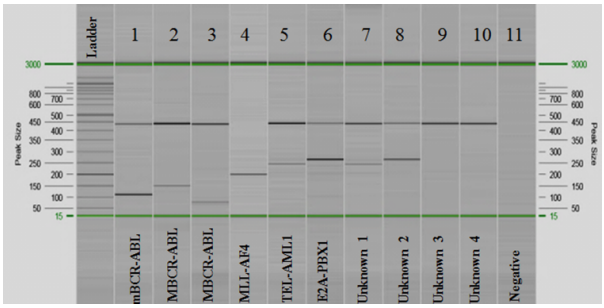

Figure 1. Gel Electrophoresis of Multiplex PCR Products. The upper band (440 bp) represents the HPRT internal control gene. Lane 1-6 are positive controls for each cryptic translocation including mBCR-ABL (108 bp), MBCR-ABL b3a2 (149 bp), MBCR-ABL b2a2 (74 bp), MLL-AF4 (200 bp), TEL-AML1 (241 bp), and E2A-PBX1 (262 bp), respectively from cell lines or the patients (except lane 4 is plasmid control of MLL-AF4). Lane 7-8 are positive patient samples with TEL-AML1 and E2A-PBX1 fusion transcript, respectively. Lane 9-10 show negative result from leukemia patients and lane 11 is negative control from distilled water

PCR sequencing revealed genetic breakpoint of TEL-AML1 and E2A-PBX1 sharing between individual ALL patients

To further confirm the positive results by our proposed multiplex PCR and to investigate the structure of recurrent chromosomal translocations in each patient, we performed PCR sequencing assay specific to each fusion gene including TEL-AML1 and E2A-PBX1. We were able to sequence 3 of 9 cDNA samples from TEL-AML1 positive patients (33.33 %) and 3 of 5 cDNA samples from patients with E2A-PBX1 (60.0 %), respectively. We observed that

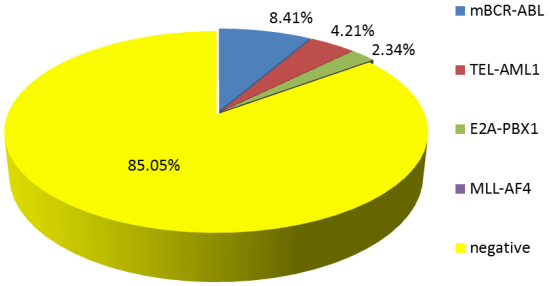

Figure 2. Distribution of the Fusion Gene Transcripts in Diagnosed ALL Performed by Multiplex PCR. The mBCR-ABL (e1a2; p190) is the most common chromosomal translocation identified in the study (8.41%). For other transcript types, TEL-AML1 and E2A-PBX1 are observed in 4.21% and 2.34%, respectively. However, MLL-AF4 is not able to be detected in this study

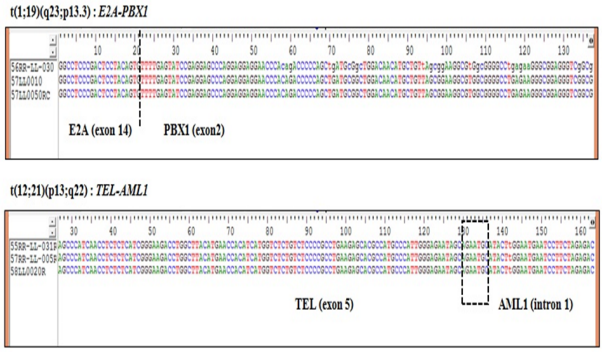

Figure 3. Direct Sequencing Results from Patient Samples with E2A-PBX1 and TEL-AML1 Positive. Common variants of E2A-PBX1 (E2A exon 14 fused to the exon 2 of PBX1) and TEL-AML1 (TEL exon 5 fused to intron 1 of AML1) were observed in this report

Table 1. PCR Primers for Multiplex PCR Amplification of Common Translocations in ALL and CML

Chromosomal translocation	Fusion gene	Primer sequence (5'-3')	Reference sequence
t(12;21)(p13;q22)	TEL-AML1	CGAGGACGGGCTGCATAG AACGCCTCGCTCATCTT GNM_001754.4	NM_001987.4
t(1;19)(q23;p13.3)	E2A-PBX1	GGCCTCCCGACTCCTACAGT TCGTATTTCTCCAGCTCCGTATGNM_001204961.1	NM_003200.3
t(4;11)(q21;q23)	MLL-AF4	CCGCCCAAGTATCCCTGTAA CACAATGGACTTCATTGGAGTAGGTNM_001166693.1	NM_001197104.1
t(9;22)(q34;q11.2)	mBCR-ABL	CGCAAGACCGGGCAGAT ACTCAGACCCTGAGGCTCAAAGNM_007313.2	NM_021574.2
t(9;22)(q34;q11.2)	MBCR-ABL	AAGAAGCTGTCGGAGCAGGA ACTCAGACCCTGAGGCTCAAAGNM_007313.2	NM_004327.3
House-keeping (control) gene	HPRT	TCAGGCAGTATAATCCAAAGATGGT TTAAACAACAATCCGCCCAAANM_000194.2	NM_000194.2

Table 2. Data Summary of Samples Positive for the Established Multiplex PCR

No	Sample	Age (Year)	Sex	Karyotyping	Diagnosis	Multiplex PCR
1	PB	25	M	46,XY,t(9;22)(q34;q11.2)[6]/46,XY[25]	CML	mBCR-ABL (p190)
2	PB	32	F	46,XX,-2,del(2)(p21p23),der(9)t(9;22)(q34;q11.2) t(2;22)(q21;q13),add(19)(q13.4),der(22)t(9;22),+mar[17]/46,idem,der(6)t(1;6)(q21;q21),der(7)t(6;7)(q21;p22)[6]/46,XX[5]	ALL	mBCR-ABL (p190)
3	BM	26	M	46,XY[30]	CML	mBCR-ABL (p190)
4	BM	42	F	46,XX,der(1)t(1;9)(p13;p13),add(7)(p15),der(9)t(1;9)(p22;p13), t(9;22)(q34;q11.2)[11] /46,XX[18]	ALL	mBCR-ABL (p190)
5	BM	14	M	50,XY,+X,+5,-9,+21,+2mar[2]/46,XY[33]	ALL	mBCR-ABL (p190)
6	BM	9	F	46,XX,-3,der(9)add(9)(p22)t(9;22;18)(q34;q11.2;q21.1),der(9)t(3;9)(p13;p22),del(16)(q12-13q22),der(18)t(9;22;18),der(22)t(9;22;18),+r[8]/46,XX[23]	ALL	mBCR-ABL (p190)
7	PB	ND	M	46,XY,t(9;22)(q34;q11.2)[26]/46,XY,der(9)t(9;11)(p13;q23)t(9;22)(q34;q11.2),der(11)t(9;11),der(22)t(9;22)[1]/46,XY[3]	ALL	mBCR-ABL (p190)
8	BM	41	M	46,XY[20]	ALL	mBCR-ABL (p190)
9	PB	80	F	46,XX,t(9;22)(q34;q11.2)[19]/46,sl,t(2;14)(p11.2;q11.2)[1]/45,sdl1,der(15;16)(q10;p10)[7]/46,XX[3]	CML	mBCR-ABL (p190)
10	PB	53	M	45,XY,dic(9;20)(p13;q11.2),t(9;22)(q34;q11.2)[16]/45,sl,dic(9;20), +inv(20)(p13q13.1)[5]/46,sdl1,+r[9]	ALL	mBCR-ABL (p190)
11	PB	43	F	46,XX,t(9;22)(q34;q11.2)[3]/44~46,X,inv(3)(q21q26.2), ?t(4;14)(q31;q32),der(9)del(9)(p13)t(9;22)(q34;q11.2), t(9;22), del(15)(q22),add(18)(q21),+add(19)(p13),inc[cp5]/45,XX, inv(3), ?t(4;14),der(9)del(9)t(9;22),add(18),der(22)t(9;22), inc[1]/46,XX[7]	ALL	mBCR-ABL (p190)
12	PB	23	M	46,XY,t(9;22)(q34;q11.2)[4]/47~48,XY,+1,dic(1;6)(p13;q27),add(2) (q21),t(2;5)(p13;q31),der(5)t(2;5)(q21;q31),add(8)(p11.2), del(8)(p21p23),+del(8)x2,-9,del(14)(q24q32), der(22)ins(22;?)(q11.2;?)[cp24]/ 46,XY[2]	ALL	mBCR-ABL (p190)
13	BM	43	F	46,XX,t(9;22)(q34;q11.2)[9]/46,XX[22]	ALL	mBCR-ABL (p190)
14	BM	43	F	46,XX[30]	ALL	mBCR-ABL (p190)
15	BM	43	F	46,XX,t(9;22)(q34;q11.2)[8]/46,XX[35]	ALL	mBCR-ABL (p190)
16	BM	42	M	46,XY[30]	ALL	mBCR-ABL (p190)
17	PB	43	M	ND	ALL	mBCR-ABL (p190)
18	BM	54	M	46,XY[34]	ALL	mBCR-ABL (p190)
19	BM	54	M	"46,XY,-9,t(9;22)(q34;q11.2),inv(20)(p13q13.1),+r[1]/46,idem, t(5;15)(p13;q21)[1]/ 46,idem,der(6)inv(6)(p11.2q13)del(6)(q13q15), + der(22)t(9;22),-r[5]/46,idem,der(6)inv(6)del(6)[2]/ 47,idem, der(6)inv(6)del(6),+der(22)t(9;22)[13]/47,idem,der(6)inv(6)del(6), +der(22)t(9;22),-r,+mar[1]/47,idem,+der(22)t(9;22)[1]/46,XY[3]	ALL	mBCR-ABL (p190)
20	BM	54	M	"47,XY,der(6)inv(6)(p11.2q13)del(6)(q13q15),-9,t(9;22)(q34;q11.2), inv(20)(p13q13.1),+der(22)t(9;22),+r[28]/48,idem,+17[1]/ 49,idem,+18,+mar[1]	ALL	mBCR-ABL (p190)
21	BM	43	F	41~44,X,inv(3)(q21q26.2),?t(4;14)(q31;q32),der(9)del(9)(p13) t(9;22)(q34;q11.2),+del(11)(q21q23),add(13)(p11.2),+add(14)(q24), del(15)(q22),add(16)(q12-13),i(17)(q10),add(18)(q21), der(22)t(9;22),inc[cp13]//46,XY[14]	ALL	mBCR-ABL (p190)
22	BM	52	M	46,XY,t(9;22;10)(q34;q11.2;q22)[33]	CML	MBCR-ABL (p210)
23	BM	49	M	46,X,add(Y)(q11.23)[32]	CML	MBCR-ABL (p210)
24	BM	11	F	46,XX,t(9;22)(q34;q11.2)[17]/46,XX[13]	CML	MBCR-ABL (p210)
25	BM	12	F	46,XX,t(9;14)(q34;q11.2)[31]	CML	MBCR-ABL (p210)
26	PB	42	F	46,XX,t(9;22)(q34;q11.2)[1]/45,idem,-16,-20,+mar[3]/49,idem, +add(8)(p23),+10,+17[1]/46,XX,t(9;22)(q34;q11.2),inc[1]/46,XX, t(9;22)(q34;q11.2),add(18)(q23),inc[1]/49,XX,t(9;22)(q34;q11.2), +17,inc[1]	CML	MBCR-ABL (p210)

27	PB	43	F	45,XX,-7,t(9;22)(q34;q11.2)[29]/46,XX[2]	CML	MBCR-ABL (p210)
28	BM	42	F	48~49,XX,del(6)(q13q21),+8,+add(8)(p23),t(9;22)(q34;q11.2),+10, add(11)(q25),add(15)(p13),+17,add(18)(q23),del(20)(q11.2)[cp27]/ 46,XX[2]	CML	MBCR-ABL (p210)
29	BM	42	F	49,XX,+add(8)(p23),t(9;22)(q34;q11.2),+10,+17[13]/48,idem,-add(8),add(15)(p13),der(18)t(8;18)(q13;q23),del(20)(q11.2)[4]/ 49,idem,-add(8),+10,add(15),der(18)t(8;18),del(20)[1]/ 49,idem, del(6)(q13q21)[2]/49,idem,-add(8),+8,add(11)(q25)[1]	CML	MBCR-ABL (p210)
30	BM	18	M	45,XY,der(7;9)(q10;q10)t(9;22)(q34;q11.2),del(11)(q21q23), der(22)t(9;22)[20]/ 45,idem,add(19)(p13.1)[1]/46,idem,+der(1;2)(q10;p10),add(8)(p11.2)[7]/46,XY[2]	CML	MBCR-ABL (p210)
31	PB	53	M	46,XY,t(9;22)(q34;q11.2)[17]/46,idem,del(10)(q22q26), t(14;16)(q32;p11.2)[10]/ 46,XY[4]	CML	MBCR-ABL (p210)
32	PB	38	M	46,XY,t(9;22)(q34;q11.2)[5]/46,XY,del(9)(p13p22)[20]/46,XY[6]	CML	MBCR-ABL (p210)
33	BM	2	M	46,XY,del(6)(q15q25)[6]/46,XY[25]	ALL	TEL-AML1
34	BM	7	F	"45,X,-X,der(1)del(1)(p34.1)?t(1;1)(q32p34.1), del(4)(q12), del(5) (q31q35),del(6)(q15q23),add(8)(q24.3),del(9)(p13p22), inv(13) (q12q32),add(17)(p13),?del(20)(q11.2q13.1), add(21)(q22)[16]/ 46,idem,+mar1[1]/46,idem,add(1)(p32), +mar2[1]/46,idem,+X,-14, +mar3[1]/46,XX,del(1)(p13p22 or p22p32) ,add(2)(p23),add(4)(q21), del(4)(q12),add(5)(q31), add(8) (q24.3), add(9)(p13), t(14;21)(q24;q22),?t(17;17)(q23;q25)[4]/46,XX[7]	ALL	TEL-AML1
35	BM	11	M	45,XY,?dic(14;18)(p11.2;p11.2)[24]/46,XY[6]	ALL	TEL-AML1
36	BM	2	M	47,XY,t(2;12)(p13;p13),+mar[1]/46,XY[13]	ALL	TEL-AML1
37	BM	2	M	ND	ALL	TEL-AML1
38	BM	2	F	46,XX,+21,der(21;21)(q10;q10)[40]/46,idem,del(12)(p11.2)[1]	ALL	TEL-AML1
39	BM	3	F	ND	ALL	TEL-AML1
40	BM	3	F	46,XX[33]	ALL	TEL-AML1
41	BM	3	F	45,X,-X,del(4)(q13q25),add(11)(p15)[29]/46,XX[2]	ALL	TEL-AML1
42	PB	6	M	46,XY[8]	ALL	E2A-PBX1
43	BM	9	M	46,XY [31]	ALL	E2A-PBX1
44	PB	2	M	ND	ALL	E2A-PBX1
45	BM	11	F	46,XX[2]	ALL	E2A-PBX1
46	PB	3	F	48,XY,+1,dic(1;9)(p13;p22),t(1;9)(q21;p13.3),+add(19)(q13.3), +mar[22]/47,idem,-2,-5,add(7)(q11.2),add(17)(p11.2),+mar2[1]	ALL	E2A-PBX1

TEL-AML1 gene rearrangements were conserved between individual patients. This common breakpoint was sparing through exon 5 of TEL and fused to the end of exon 2 on AML1 (Figure3). Likewise, all patients with E2A-PBX1 positive showed similar breakpoint structures which fused exon 14 of E2A to exon 2 of PBX1 gene. The results indicated that our established multiplex PCR is specific to detect the recurrent chromosomal translocations and able to identify common structurally genetic breakpoints of TEL-AML1 and E2A-PBX1 in ALL patients.

Discussion

Conventional cytogenetic, FISH, and molecular genetic testing play an important role for the diagnosis, risk stratification, planning of the effective therapeutic strategies, and disease monitoring in hematological malignancies. The detection of Philadelphia (Ph) chromosome by karyotyping is recognized as a genetic hallmark of CML (> 90 % of CML) and the test becomes a gold standard method for the first line diagnosis of CML (Tahira et al., 2015). In addition, approximately 5 % of ALL patients (childhood) harbor Ph chromosome which is recognized as a poor prognosis marker in ALL (Look, 1997). At the present, Imatinib which inhibits the tyrosine kinase activity of BCR-ABL fusion protein becomes an effective standard therapy for CML. The drug is able to induce complete hematologic response as well as complete cytogenetic response (CCyR) in a majority of patients and approximately 80 % of patients show complete remission. Thus, molecular genetic testing such as PCR-based technology is necessary for the monitoring of minimal residual disease in CML who displays the disappearance of Ph chromosome (CCyR). In this report, we were able to develop a highly sensitive multiplex PCR to detect BCR-ABL fusion transcripts including two variants of

MBCR-ABL (p210) and mBCR-ABL (p190) specific for CML and ALL, respectively. We demonstrated the concordance results of the application of the established multiplex PCR and conventional cytogenetic study to the identification of BCR-ABL fusion transcripts in CML and ALL patients (Table 2). Consequently, this method was able to be used as a screening/preliminary test for the detection of BCR-ABL fusions in patients who suspects to be CML or ALL. Furthermore, our proposed method was able to detect BCR-ABL fusion transcripts in 3 ALL and 1 CML patients who accounts for CCyR after treatments. This results indicated that our generated multiplex PCR is a potential method used as a monitoring test for Ph positive CML and ALL patients after receiving standard therapies. Recently, quantitative RT-PCR (qRT-PCR) specific for the measurement of BCR-ABL (both p210 and p190) fusion transcripts has been developed and wildly used for monitoring of MRD in Ph positive CML and ALL (Gabert et al., 2003; Erba, 2015). The technique becomes a powerful tool for observing the response of those patients at the molecular level and guiding the clinician to detect the earliest molecular relapse. Although Imatinib provides highly effective therapeutic outcome to treat Ph positive CML patients, about 30 % of patients become Imatinib resistant due to acquire mutations of ABL tyrosine kinase domain (TKD) leading to disease relapse. Therefore, mutation analysis of ABL TKD is critical for the mutation screening before the treatment as well as during Imatinib treatment (Branford et al., 2003; Hughes et al., 2009; Kim et al., 2009; Wongboonma et al., 2012; Elias et al., 2014). In summary, the combination of comprehensive techniques including complete cytogenetic study, FISH, PCR-based technology is essential for the achievement of the best therapeutic outcome in treatments of Ph positive CML and ALL.

Prospective outcome of the identification of genetic alterations involving in the development of ALL was that we were able to use the generated multiplex PCR to detect recurrent chromosomal translocations including t(9;22)(q34;q11.2) (BCR-ABL; p190), t(12;21)(p13;q22) (TEL-AML1), and t(1;19)(q23;p13.3) (E2A-PBX1). In this study, the most prevalent translocations identified in diagnosed ALL were t(9;22)(q34;q11.2) (BCR-ABL; p190) which was accounted for 8.41 % (18/241) cases of all tested samples. The majority of Ph positive ALL cases in this study was observed in adult (16/18) whereas one childhood ALL was positive for BCR-ABL. These finding supported that BCR-ABL positive ALL progressively increases with the age (approximately 5 % in childhood and 20-50 % of adult ALL) (van Dongen et al., 1999; Elia et al., 2003). For other fusion transcripts, we could identify 4.21 % of TEL-AML1 and 2.34 % of E2A-PBX1 in tested samples which all of them were observed in childhood ALL (age 2-11; median age = 4 for TEL-AML1 and 6 for E2A-PBX1). The translocation t(12;21)(p13;q22) resulting in the formation of TEL-AML1 was recognized as the most cryptic translocation frequently identified in ALL (approximately 25 % in childhood ALL) and could not be detected by conventional cytogenetic assay (van Dongen et al., 1999; Zelent et al., 2004; Zafar, 2014). However, in Iraqi pediatric ALL patients, TEL-AML1 and E2A-PBX1 transcripts were detected for 20.8 and 16.7 %, respectively by real-time PCR (Kadhom et al., 2015). Although lower frequency of TEL-AML1 was observed in our report which may be affected by the distribution and the selected of patient samples, we could demonstrate the similar result of the stage of development/age onset (age between 2-11 at diagnosis) of TEL-AML1 compared to other publications. These supported evidences that TEL-AML1 is occurred early in prenatal or during gestation period and recognized as the initiating event for the development of TEL-AML1 positive pediatric ALL (Greaves et al., 2003; Greaves and Wiemels, 2003). Moreover, we performed direct sequencing method to confirm and investigate the transcriptional variants of ALL samples positive for TEL-AML1. Similarly, common variant of TEL-AML1 fusion transcripts was observed in this report when compared to other publications which exon 5 of TEL fuses to the end of exon 2 on AML1 (van Dongen et al., 1999; Wiemels and Greaves, 1999; von Goessel et al., 2009). Likewise, fewer frequency of cryptic translocation t(1;19)(q23;p13.3) (E2A-PBX1) was previously reported (Crist et al., 1990; van Dongen et al., 1999; Sudhakar et al., 2011; Bhatia et al., 2012). In addition, common breakpoint region formation of exon 14 of E2A with exon 2 of PBX1 gene was the same as other reports (Mellentin et al., 1990; Wiemels et al., 2002). This indicated the consistence of E2A-PBX1 fusion transcript formation in ALL which may contribute to similar disease phenotypes as well as clinical outcomes (Borowitz et al., 1993; Yang et al., 2010). Nevertheless, in this work we were not be able to detect cryptic translocation of t(4;11) (q21;q23) (MLL-AF4) in the tested samples. MLL-AF4 fusion transcripts and other MLL rearrangements are recognized as poor prognosis markers in acute leukemia (Krivtsov and Armstrong, 2007; Muntean and Hess, 2012). In contrast to TEL-AML1 and E2A-PBX1, there are at least 10 fusion variants of MLL-AF4 that are able to be identified in ALL due to different breakpoint structures (van Dongen et al., 1999). This could explain the negative result for detection of MLL-AF4 using our established multiplex PCR in different selected samples . To improve the range of MLL-AF4 fusion transcript detection, new molecular assay is optimized for amplification of common variants of MLL-AF4 and other MLL rearrangements frequently observed in acute leukemia.

In conclusion, we proposed that our generated multiplex PCR for routine use is a specific, highly sensitive, and fast method for screening and monitoring of recurrent chromosomal translocations in CML and ALL patients. This assay will provide an efficient tool for the clinicians to evaluate genetic status of the patients prior to clinical management decision.

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