Genetic Evaluation of Childhood Acute Lymphoblastic Leukemia in Iraq Using FTA Cards

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Background. Genetic examination of childhood leukemia has not been available in Iraq. We here report the frequency of TEL-AML1, E2A-PBX1, MLL-AF4, and BCR-ABL chimeric transcripts in 264 Iraqi children newly diagnosed with acute lymphoblastic leukemia (ALL), using FTA cards impregnated with bone marrow aspirate or whole blood. ***Patients and methods.*** The diagnosis of ALL was made according to standard French-American-British morphologic criteria. Based on the results of storage temperature and duration, most of the FTA samples were preserved at 4°C for up to 6 weeks in five Iraqi hospitals and then transferred to Japan for molecular analysis. Nested reverse transcription-polymerase chain reaction was adopted for the analysis. ***Results.*** TEL-AML1 chimeric transcript product was found in 32 (12.1%) of 264 ALL patients. Eleven (4.2%) patients, 4 (1.5%) patients, and 11 (4.2%) patients had E2A-PBX1 mRNA, MLL-AF4 mRNA, and BCR-ABL mRNA, respectively. One patient had both TEL-AML1 and E2A-PBX1 fusion genes. The incidence of TEL-AML1 in Iraqi ALL children appears to be similar to or slightly higher than those of Jordan (12%) and Kuwait (7%). The prevalence and clinical findings of ALL patients with either E2A-PBX1 or BCR-ABL were comparable to the data reported elsewhere. ***Conclusion.*** International collaboration via FTA cards may be helpful to improve diagnosis and management of patients with hematological malignancies in low-income and underdeveloped countries. Pediatr Blood Cancer

Key words: Iraq; FTA cards; TEL-AML1; E2A-PBX1; MLL-AF4; BCR-ABL

INTRODUCTION

Chromosomal translocations have been identified in childhood acute lymphoblastic leukemia (ALL). The presence of these chromosomal aberrations can be used to stratify patients, decide treatment protocol, and reveal prognosis. The common risk-stratifying translocations of pediatric ALL include t(12;21)(p13;q22), t(1;19)(q23;p13), t(4;11)(q21;q23), and t(9;22)(q34;q11). These translocations encode fusion transcripts of TEL-AML1 (also termed ETV6-RUNX1), E2A-PBX1 (also termed TCF3-PBX1), MLL-AF4, and BCR-ABL (p190), respectively [1–6]. The most common translocation is t(12;21), which occurs in 20–25% of childhood ALL cases, and confers a favorable prognosis [6–9]. Another common translocation is t(1;19) with a frequency of 5–6% among childhood ALL cases [6,10]. Less common translocations are t(4;11) and t(9;22), which both infer a poorer prognosis [1,4,10].

Reverse transcription-polymerase chain reaction (RT-PCR) techniques have been developed for detection of fusion transcripts resulting from the chromosomal translocations in acute leukemia. It is generally held that the RT-PCR techniques are superior to conventional cytogenetic analysis for detection of the fusion transcripts [11,12].

In the present study, we used Flinders Technology Associates (FTA) filter papers for genetic analysis of Iraqi children with ALL [13]. The filter paper matrix of the FTA card is impregnated with chaotropic agent that denatures infectious agents, and thus samples are no longer considered a biohazard. Because of the small size of the FTA cards, they are convenient for storage in a limited space and transport of specimens [13,14]. Using FTA cards impregnated with bone marrow (BM) aspirate or whole blood, we evaluated the relative frequency of the four major transcripts described above in 264 Iraqi ALL children.

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MATERIALS AND METHODS

The study protocol was approved by the Ministry of Health in Iraq, and the institutional review board of Shinshu University School of Medicine. Five hospitals participated in this study: The Children's Welfare Teaching Hospital (CWTH) in Baghdad (a major referral center for childhood cancers in the country), Central Teaching Hospital for Children (CTH) in Baghdad, Basra Children's Specialty Hospital (BCSH) in Basra, Ibn Al Atheer

Additional supporting information may be found in the online version of this article.

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Hospital for Children (IAH) in Mosul, and Nana-Kali Hospital for Hemato-Oncology (NKH) in Erbil. Written informed consent was obtained from patients or parents according to the guidelines of the Helsinki.

Diagnosis of ALL

In Iraq, the diagnosis of ALL has been made on BM aspiration smears stained with Leishman and Sudan Black B according to standard French-American-British morphologic criteria. Diagnostically difficult cases were reviewed by an Italian team guided by Dr. Anna Maria Testi (Division of Hematology, Department of Cellular Biotechnologies and Hematology, University ''La Sapienza'', Rome, Italy) through a weekly telemedicine discussion with doctors of three hospitals (CWTH, BCSH, and NKH) [15]. In some cases, final diagnosis was made by sending slides to Italy. The diagnosis of ALL was confirmed on the initial unstained BM aspiration smears of the individual patients that were transferred from Iraq and stained with May-Grünwald-Giemsa and myeloperoxidase at the Department of Pediatrics, Shinshu University School of Medicine.

Sample Collection

BM and/or peripheral blood (PB) samples were collected from 269 Iraqi patients aged $\leq$15 years (the peak was at 3 years), newly diagnosed with ALL from October 2009 to June 2011. Of a total of 269 patients, 132 cases were from CWTH, 69 cases from CTH, 31 cases from BCSH, 28 cases from IAH, and 9 cases from NKH. Since five cases were excluded because of difficulty in proving the diagnosis owing to the poor quality of their slides, 264 patients were enrolled in the study. Clinical findings at diagnosis of the children are presented in Table I. In addition, we used BM cells at onset of ALL from four Japanese patients: 3-year-old and 4-year-old females who had t(12;21) proved by FISH analysis, and 4-year-old and 13-year-old males who had t(9;22) proved by G-banding analysis.

TABLE I. Clinical Characteristics of 264 Patients With ALL

	No. of patients (%)
Sex	
Male	150 (56.8)
Female	114 (43.2)
Age (years)	
<1	9 (3.4)
1– <5	124 (47)
5– <10	89 (33.7)
≥10	42 (15.9)
WBC ($\times 10^9$/l)	
<20	135 (51.1)
20– <50	48 (18.2)
≥50	81 (30.7)
Hb (g/dl)	
<10	219 (83)
≥10	45 (17)
Platelets ($\times 10^9$/l)	
<20	112 (42.4)
20– <50	76 (28.8)
50– <100	38 (14.4)
≥ 100	38 (14.4)
FAB classification	
ALL-L 1	64 (24.2)
ALL-L 2	193 (73.1)
ALL-L 3	7 (2.7)

FTA Paper Processing

A few drops of blood from a diagnostic BM aspirate or those from PB were applied to an FTA classic card (Cat No. WB120205, GE Healthcare UK Limited, Buckinghamshire, UK) at five Iraqi hospitals. After the blood spots were dried for 1 hour at room temperature, the FTA card was kept in a special FTA envelope in a refrigerator for up to 6 weeks in two-third of the cases, and then transported by airplane to Japan. The remaining FTA cards were preserved at 4°C until 9 weeks.

Two-millimeter disks were punched out from the dried material on FTA cards using a sterile hole puncher (Harris Micro-Punch, Shunderson Communications Inc., Ottawa, Canada). Eight disks for each patient were placed in 400 μl of RNA processing buffer containing 10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA, 800 U/ml RNase out (Invitrogen, Carlsbad, CA), and 200 μg/ml glycogen, and incubated at room temperature for 15 minutes. Then, 800 μl of Isogen-LS (Nippon Gene Co., Ltd., Toyama, Japan) was added and incubated at room temperature for 15 minutes according to a modification of the manufacturer's instructions. After extraction with chloroform and isopropanol, the RNA was precipitated after 2 hours or overnight at −30°C.

RT-PCR

RT-PCR was performed according to the procedure described previously [12,16]. cDNA was synthesized from total RNA with 0.5 U of AMV Reverse Transcriptase (TaKaRa RNA PCR Kit (AMV) Ver.3.0, Takara Bio Inc., Ohtsu, Japan) in RT-buffer containing random hexamers and deoxynucleotide triphosphates (dNTPs-100 μM each) at 42°C for 20 minutes. The cDNA was then heated to 95°C for 5 minutes to inactivate the reverse transcriptase, cooled to 4°C for 5 minutes, and stored at −30°C. The integrity of RNA extracted from the FTA samples and the correct synthesis of the cDNA were evaluated by amplification of the GAPDH mRNA. Every sample that showed amplification of the control GAPDH gene was considered to be valid for analyses.

All Iraqi BM and/or PB samples on FTA filter papers were analyzed and reevaluated at least once, together with REH cell line for t(12;21)/TEL-AML1, RCH-ACV cell line for t(1;19)/E2A-PBX1, MV4-11 cell line for t(4;11)/MLL-AF4, or TOM-1 cell line for t(9;22)/BCR-ABL as positive control cells. These cell lines were purchased from DSMZ (Braunschweig, Germany). Normal healthy PB cells were used as negative controls.

To detect the four types of transcriptional products, we used nested RT-PCR [11]. The first round of nested PCR was performed in a total volume of 25 μl of reaction mixture containing 2.5 μl of 10× PCR Gold Buffer, 2 mM $MgCl_2$, 200 μM dNTP, external primers (FASMAC Co., Ltd., Atsugi, Japan) at 500 nM each, 5 U/μl of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA), and 1 μl of cDNA template.

The PCR conditions were 95°C for 10 minutes followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C. One microliter of the dilution was subjected to second PCR amplification in a 25 μl reaction buffer containing 500 nM

each internal (nested) primer (FASMAC Co., Ltd.). The nested PCR was performed under the same conditions, except for 55°C as the annealing temperature for E2A-PBX1. Preliminary experiments using the patient samples and the leukemic cell lines revealed that a combination of 40 cycles of first-round PCR and 30 or more cycles of second-round PCR was optimal for detection of the TEL-AML1 and BCR-ABL mRNA. The primers used are listed in Supplemental Table I. Ten microliter of the second PCR product was electrophoresed on 3% ethidium bromide-containing agarose gels, and photographed under ultraviolet light.

Statistical Analysis

The chi-square test was used to determine the frequency of ALL cases with TEL-AML1, E2A-PBX1, MLL-AF4, or BCR-ABL rearrangement between Iraq and other countries. We used the unpaired *t*-test to determine the significance of differences in clinical findings of ALL children with a chimeric transcript between Iraq and other countries.

RESULTS

Since it has been demonstrated that FTA cards provide an easy way to prepare total RNA from a variety of samples stored in the short term at room temperature and in the longer term at −20°C or −80°C, we conceived of using the cards for genetic analysis of Iraqi children with ALL [13]. First, we examined storage temperature and duration by which extraction of RNA was possible from REH cells carrying TEL-AML1 fusion mRNA that were infiltrated into the FTA matrix. A total volume of 125 μl of culture medium containing 500–10,000 REH cells per μl was applied to the FTA cards, and kept at −30°C, 4°C, or 37°C for 2–6 weeks. As presented in Figure 1, GAPDH mRNA was detectable when at least 500 cells per μl were infiltrated into the FTA matrix and kept at either −30°C or 4°C for up to 6 weeks. On the other hand, 37°C was not suitable for RNA storage.

To evaluate the sensitivity of nested PCR for TEL-AML1 mRNA, various ratios of mixture of REH cells with normal PB cells were applied to the FTA matrix, and kept at either −30°C or 4°C for 6 weeks. When the mixture of 2.5% or more REH cells with normal PB cells was applied to the FTA cards, second PCR with internal TEL-AML1 primers yielded clear bands at both temperatures, as presented in Figure 2A. The TEL-AML1 transcript, as well as GAPDH mRNA, was detectable in the FTA samples that were kept at 4°C for 40 weeks. We examined the relative expression levels, using BM cells of two Japanese children who had t(12;21) detected by FISH analysis, and those of two Japanese children who had t(9;22) detected by G-banding analysis. As shown in Figure 2B–D, similar results were obtained in the mixture of 2.5% or more BM cells of each patient sample with normal PB cells.

We then examined whether 264 Iraqi children newly diagnosed with ALL had any of the four types of translocations (TEL-AML1, E2A-PBX1, MLL-AF4, and BCR-ABL mRNA), using BM or PB samples applied to the FTA matrix. Since refrigerators, but not −30°C freezers, were available in all five Iraqi hospitals, most of the FTA samples were preserved at 4°C for up to 6 weeks, and then transferred to Japan for molecular analysis. GAPDH mRNA was successfully amplified from all 264 samples. As presented in Figure 3, TEL-AML1 chimeric transcript product (181 bp) was found in 32 (12.1%) patients. On May-Grünwald-Giemsa staining combined with FISH, BM cells of two Iraqi patients with TEL-AML1 fusion transcript displayed a fusion signal for t(12;21), whereas those of two patients with no TEL-AML1 mRNA were negative. Eleven (4.2%) patients, 4 (1.5%) patients, and 11 (4.2%) patients had E2A-PBX1 mRNA (289 bp), MLL-AF4 mRNA (approximately 270–290 bp), and BCR-ABL mRNA (190 bp), respectively. The sequence of MLL-AF4 fusion transcript in three patients was identical to that of MV4-11 cells. As presented in Table II, the age of the patients with TEL-AML1 ranged from 1.7 years to 12 years. More than 90% of cases with

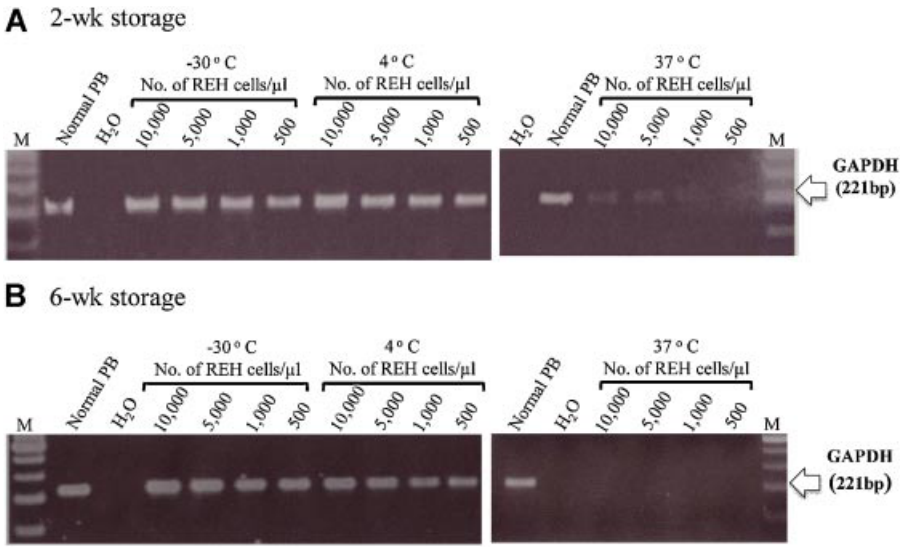

Fig. 1. Storage temperature and duration by which extraction of GAPDH mRNA from REH cells infiltrated into the FTA matrix was possible. A total volume of 125 μl of culture medium containing 500–10,000 REH cells per μl was applied to the FTA cards, and kept at −30°C, 4°C, or 37°C for 2 weeks (**A**), or 6 weeks (**B**). RNA was extracted from eight punched disks per dried material. Normal healthy PB cells treated with no FTA cards were used as a control. GAPDH mRNA is shown as a 221 bp band. M, 100 bp size ladder.

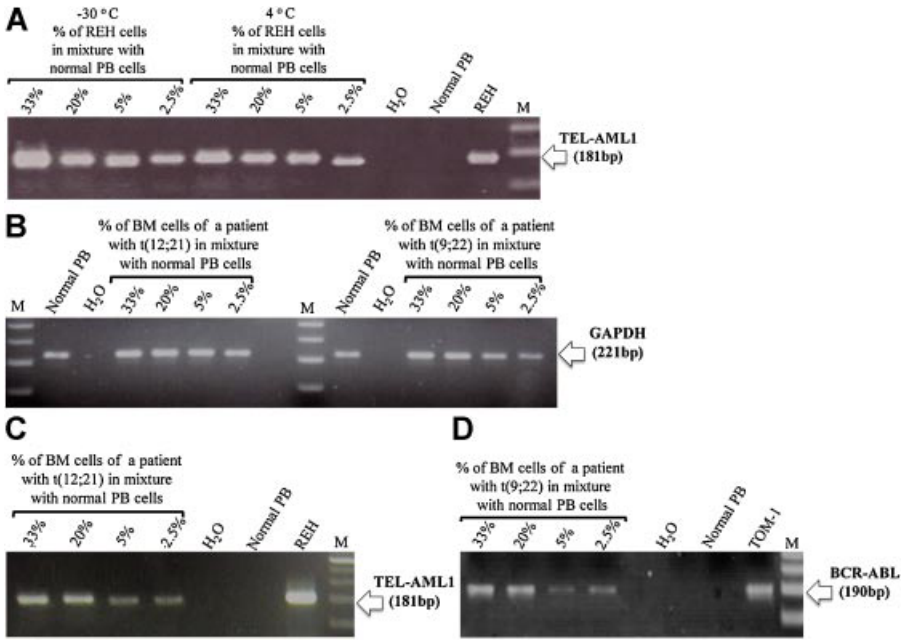

Fig. 2. Nested RT-PCR for TEL-AML1 mRNA or BCR-ABL mRNA from the various ratios of mixture of leukemic cells with normal PB cells that were applied to the FTA cards. **A**: A total volume of 125 μl of culture medium containing 10,000 cells (mixture of 2.5–33% REH cells with normal PB cells) per μl was applied to the FTA cards, and kept at −30°C or 4°C for 6 weeks. **B**–**D**: A mixture of either BM cells of a Japanese patient possessing t(12;21) or BM cells of a Japanese patient possessing t(9;22) with normal PB cells was applied to the FTA cards, and kept at 4°C for 2 weeks. RNA was extracted from eight punched disks per dried material. RNA extracted from leukemic cell lines treated with no FTA cards was used as a positive control. Normal healthy PB cells treated with no FTA cards were used as a negative control. TEL-AML and BCR-ABL fusion transcripts are shown as a 181 bp and 190 bp band, respectively. M, 100 bp size ladder.

TEL-AML1 rearrangement in Iraq belonged to a favorable age group, with 71.9% of these cases diagnosed in patients aged between 2 and 6 years. The ratio of males to females was 1.3. The WBC counts of 31 patients were 1×10^9/L to less than 100×10^9/L, whereas the value of the remaining patient was higher than 300×10^9/L. Patient no. 205 had both TEL-AML1 and E2A-PBX1 fusion genes. In 11 patients with E2A-PBX1 fusion transcript, the age ranged from 1.8 years to 9.6 years. The ratio of males to females was 1.8. The WBC counts were 2.9×10^9/L to 107×10^9/L with a median of 18×10^9/L. In 11 patients with BCR-ABL mRNA, the age ranged from 3 years to 14 years. The ratio of males to females was 2.7. The WBC counts were 4.9×10^9/L to 530×10^9/L with a median of 40×10^9/L.

DISCUSSION

During the last three decades, the war-related conditions in Iraq have resulted in a marked deterioration in medical services including diagnosis and management of cancer patients. Iraqi oncologists still depend on primitive morphological stains (Leishman and Sudan Black B) to make a diagnosis of ALL. Flow cytometric analysis and conventional/molecular genetic studies are not yet available. Lack of optimal diagnostic procedures, shortage of chemotherapeutic agents, inadequate supportive care, a high degree of treatment abandonment, and higher rates of relapse exert a strong negative impact on the survival rates of pediatric ALL in Iraq [15,17,18]. Such circumstances have created helplessness in dealing with relatively curable diseases like ALL among Iraqi health care providers as well as patients and their families. In 2004, two Japanese NGO groups, Japan Iraq Medical Network (JIM-NET) and Japan Chernobyl Foundation (JCF), started to support the five main Iraqi oncology centers by providing chemotherapeutic agents and medical equipment. To improve management of Iraqi children with cancers, Italian physicians of University ''La Sapienza'' in Rome together with an Italian organization (INTERSOS) began consultation for diagnostically difficult cases at three Iraqi hematology centers via online telemedicine in 2006 [15]. In 2008, induction treatment consisting of a 7-day prephase with daily oral prednisolone was initiated according to a collaborative project between Pediatric Oncology Unit of Central Welfare Teaching Hospital in Baghdad and the Italian oncologists described above. At the same time, the Japanese doctors proposed genetic classification of ALL because of the importance of chromosomal translocations within the leukemia cell on response to chemotherapy and prognosis. Because of the lack of G-banding and fluorescence in situ hybridization (FISH) techniques in Iraq, we conceived of using FTA cards easily transportable for molecular analysis in Japan. The present study demonstrated that RNA could be extracted and used for molecular analysis, if the dried blood spots on the FTA cards were kept in refrigerators for up to 40 weeks. Accordingly, the use of FTA chemically treated paper may be an attractive alternative to the collection of blood in tubes for obtaining genetic information of hematologic malignancies in low-income and underdeveloped countries.

In the current study, 32 (12.1%) of 264 Iraqi children newly diagnosed with ALL were positive for the TEL-AML1 transcript according to nested RT-PCR. This frequency is significantly lower

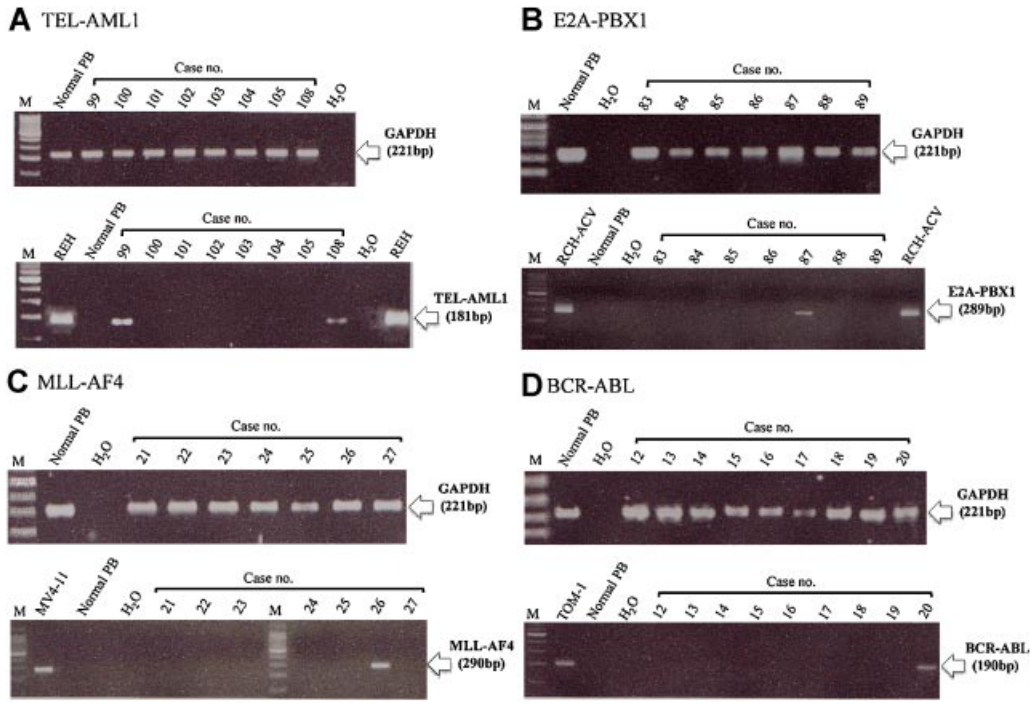

Fig. 3. Detection of TEL-AML1, E2A-PBX1, MLL-AF4, or BCR-ABL fusion transcript in 264 Iraqi children newly diagnosed with ALL, using BM or PB samples applied to the FTA matrix. Most of the FTA samples infiltrated with BM and/or PB cells were preserved at 4°C for up to 6 weeks at Iraqi hospitals, and then transferred to Japan for molecular analysis. GAPDH mRNA (221 bp) was amplified from all 264 samples. We used REH, RCH-ACV, MV4-11, and TOM-1 cells treated with no FTA cards as positive controls for detection of TELAML1, E2A-PBX1, MLL-AF4, and BCR-ABL transcripts, respectively. Normal healthy PB cells treated with no FTA cards were used as negative controls. Cases no. 99 and 108 were positive for TEL-AML (181 bp). Case no. 87 was positive for E2A-PBX1 (289 bp). Case no. 26 was positive for MLL-AF4 (approximately 290 bp). Case no. 20 was positive for BCR-ABL (190 bp). M, 100 bp size ladder.

than the values in Western countries (16–27% in USA and Europe) [7,8,19,20]. One possibility is an underestimation of the frequency of pediatric Iraqi ALL patients with TEL-AML1 by the use of FTA card-archived samples, since there was no difference in the percentage of ALL children at the ages of 1–9 years between Iraq (81.1%) and European countries (80.8%) [20]. In this regard, it is demonstrated that mRNA integrity from the dried material on FTA cards is sufficient to allow amplification by RT-PCR [21]. Actually, GAPDH mRNA was successfully amplified from all 264 Iraqi FTA samples. Nested RT-PCR that we used in the study is a well-recognized technique for increasing both the sensitivity and the specificity for target detection as well as minimizing unwanted non-specific amplification products [22]. The incidence of TEL-AML1 in Iraqi ALL children appears to be similar to or slightly higher than the data reported by investigators in Jordan (12.4%) [23] and in Kuwait (6.7%) [24], both of which are Arab countries adjacent to Iraq. In addition, there is a significant difference in the frequency of t(12;21)(p13;q22) in pediatric B-precursor ALL between Western countries and Asian countries [7,8,19,25]. Different from the result of ALL with TEL-AML1, the prevalence of Iraqi ALL patients with E2A-PBX1 or BCR-ABL was comparable to the data obtained from Western patients [26,27]. Taking these findings together, a significant difference in the frequency of pediatric ALL patients possessing TEL-AML1 between Iraq and Western countries may be related to geographic or ethnic variation in the genotype of ALL. More than 90% of cases with TEL-AML1 rearrangement in Iraq belonged to a favorable age group with 71.9% of these cases diagnosed in patients aged between 2 and 6 years. Additionally, 31 (96.9%) cases had no hyperleukocytosis at presentation. These clinical findings of Iraqi ALL patients with TEL-AML1 are consistent with those reported elsewhere [20,28]. In developed countries, it has been demonstrated that patients with this translocation have a superior clinical outcome, with relapse-free survival rates approaching 90% upon testing a variety of drug regimens [6,9]. Thus, disclosure of Iraqi ALL children with TEL-AML1 may provide important information to persuade the patients not to abandon treatment and to undergo chemotherapy with sufficiently assorted effective medicines.

A low percentage (1.5%) of patients with ALL possessed MLL-AF4 transcript. This seems to be due largely to the limited number of infants in our study. It is likely that poverty and illiteracy of the parents as well as lack of adequate medical services result in death from progressive disease before referral to the oncology centers in Iraq.

Although nested RT-PCR using the FTA cards appears to be a useful method for detecting representative chimeric translocations in childhood ALL patients in Iraq, the present study would have more significance if other technical approaches, such as FISH, were used. The shortcoming of using the FTA cards is an inability to identify genetic abnormalities other than the specified translocations. Thus, flow cytometric, conventional cytogenetic, and FISH analyses are necessary for the complete diagnosis of ALL. International collaboration via FTA cards may be helpful to

TABLE II. Clinical Characteristics of ALL Patients With TEL-AML1, E2A-PBX1, MLL-AF4, or BCR-ABL Fusion Gene

Case no.	Age (years)	Sex	WBC ($\times 10^9$/l)	% of blasts in PB	Hb (g/dl)	Plts ($\times 10^9$/l)	FAB
TEL-AML1 (n = 32)							
25	3	F	1	0	3.9	10	L2
36	2.5	M	7.7	59	10.5	2	L1
38	7.5	M	78.2	85	4.5	8	L2
51	2.5	M	28	0	6.5	58	L1
72	4.5	M	3.2	7	8.9	2	L2
90	5	F	3.1	6	8.2	47	L2
99	2.5	M	7.7	44	8	110	L2
108	5.5	F	7.3	71	5	5	L2
111	4	M	30	85	3.3	6	L2
113	1.8	M	14.5	36	8.7	80	L2
118	5	F	6.6	25	4	65	L1
133	4	F	72.7	75	6.8	10	L1
134	3.5	F	10.7	40	7.7	27	L1
140	9	F	7.8	52	6.7	124	L1
143	3.7	F	15.2	86	8.3	16	L2
146	3	M	17.5	80	3	11	L2
149	1.8	M	14.5	32	8.7	35	L2
156	5	F	2.9	15	5.8	24	L2
160	2	F	336	84	7	26	L2
162	12	M	7.4	60	10.4	57	L2
167	6	M	50	85	6.4	10	L2
169	3.4	F	14.2	45	2.8	41	L2
205[a]	2.8	M	18	40	9.4	54	L2
211	11	M	4.1	44	10.9	106	L1
228	5	M	25	94	7	160	L1
235	3.5	M	9	8	5.6	10	L1
239	4.5	M	40.6	80	5.3	11	L1
241	5.2	F	38	85	5.7	117	L2
244	7	F	29	91	7.2	257	L2
247	7	M	2.9	94	9.2	0.2	L2
262	1.7	F	22.7	70	7	25	L2
263	4	M	4.2	54	9.9	2	L1
E2A-PBX1 (n = 11)							
23	4	F	17	48	6.8	49	L1
39	2.7	F	7.5	23	6.2	50	L2
87	9.6	M	50	70	9.3	9	L2
159	3	M	53	92	10.5	28	L2
168	3	F	78	75	10.1	30	L2
177	1.8	M	107	86	4.6	11	L2
178	3	M	90	51	5.1	17	L2
187	6.5	F	16	32	8	30	L2
205[a]	2.8	M	18	40	9.4	54	L2
208	3	M	2.9	0	3.7	42	L1
209	3.8	M	3.3	19	4.5	23	L2
MLL-AF4 (n = 4)							
26	0.3	F	5	10	7	10	L2
190	2.8	M	220	90	4.3	16	L2
212	2.4	F	145	86	5.9	11	L2
267	0.9	M	28	78	6.8	10	L1
BCR-ABL (n = 11)							
2	10	M	90	88	9.8	140	L2
20	7	F	10.9	56	8	108	L2
31	3.5	F	12	75	10	79	L1
75	3.3	F	17	52	8.1	15	L2
132	3	M	4.9	80	7.6	145	L2
138	7	M	200.9	82	11	77	L2
157	4.5	M	40	92	6	18	L2
181	8	M	36.4	95	8	12	L2
193	13.5	M	530	90	10.3	23	L2
216	14	M	460.8	98	9	10	L2
248	14	M	480	98	8	30	L2

[a]Case no. 205 had both TEL-AML1 and E2A-PBX1 fusion genes.

improve diagnosis and management of patients with hematologic malignancies in underdeveloped countries.

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