

Frequency and Clinical Relevance of TEL-AML1 Fusion Gene in Childhood Acute Lymphoblastic Leukemia in Egypt

HEBA M. SHAKER, M.D.; IMAN A. SIDHOM, M.D.* and INAS A. EL-ATTAR, Ph.D.**

The Departments of Clinical Pathology, Pediatric Oncology and Biomedical Statistics & Epidemiology**, National Cancer Institute, Cairo University.*

ABSTRACT

TEL-AML1 fusion gene, resulting from 12; 21 chromosomal translocation, is believed to be the most common molecular genetic abnormality in childhood acute lymphoblastic leukemia (ALL). This study has been conducted to investigate the frequency of this fusion gene in Egyptian children suffering from ALL and to point out the different laboratory and clinical features associated with this anomaly, as well as the response of positive cases to therapy. The status of TEL-AML1 fusion gene was determined by the reverse transcriptase polymerase chain reaction (RT-PCR) in 81 children with ALL, 69 newly diagnosed and 12 in relapse. Of the newly diagnosed cases, 7 were positive for TEL-AML1 fusion gene (10.14% of all ALL cases studied, 11.67% of precursor B ALL), as well as three out of the 12 cases (25% of all ALL cases, 30% of BCP - ALL) in relapse. All positive cases belonged to the precursor B-lineage, showed an age peak between 3 and 6 years, had non-hyperdiploid DNA content and no CNS infiltration. Most of the positive cases had total leukocytic counts below $50 \times 10^9/l$, myeloid marker co-expression and good response to induction therapy. In conclusion, TEL-AML1 fusion gene identifies a subset of pediatric acute lymphoblastic leukemia associated with a number of clinical and laboratory markers of good prognosis and should thus be included in routine molecular workup of acute leukemias to confirm its impact on clinical outcome and to design suitable therapeutic regimens.

Key Words: *TEL-AML1 fusion gene - Childhood ALL - RT-PCR.*

INTRODUCTION

Cloning of the genes altered by leukemia-associated chromosomal translocations has provided critical insights into the mechanism of leukemogenesis and has led to the development of molecular approaches for the diagnosis and monitoring of patients' response to therapy [26]. In addition, it has resulted in the ability to classify leukemias into clinically relevant subgroups based on their underlying molecular ge-

netic lesions and such efforts have had their most significant impact on the medical management of pediatric patients with ALL [25]. Most common of such molecular abnormalities are the E2A-PBX1, BCR-ABL and MLL-AF4 fusion genes which are presently used as risk-stratifying features to determine appropriate therapy for pediatric ALL patients. Recently added to this list is the TEL-AML1 fusion gene resulting from t(12;21) (p13;q22) and which proved to occur more frequently than the previously mentioned molecular abnormalities [30]. By conventional cytogenetics, this abnormality could be barely detected in less than 0.05% of patients, thus other techniques such as Southern blotting, FISH and RT-PCR have been resorted to and proved their ability to easily identify it [5]. This translocation fuses the TEL gene on chromosome 12, a member of the ETS family of transcription factors [44] to AML-1 that encodes the AML-1/CBF β (core binding factor beta) transcription factor complex on chromosome 21 which is the most frequent target of myeloid associated translocations [21]. The role of the TEL-AML1 oncoprotein in leukemogenesis is still unclear, but data suggest that it directly alters the transcriptional activity of AML1, required for normal hematopoiesis [21].

TEL-AML1 Fusion was reported to occur in approximately 25% of childhood ALL [5,7,19,30,35]. The majority of positive patients range in age between 1 and 10 years at diagnosis, with a peak between 2 and 5 years. All patients display a precursor B-cell immunophenotype, in particular common ALL (CALL) and pre-B ALL, less commonly pro-B ALL. In addition, these patients are characterized by low WBC

count at diagnosis ($< 50 \times 10^9/l$), have a non-hyperdiploid DNA content (< 1.16) and most have co-expression of myeloid markers [5]. So far, this translocation has not been found in T-ALL or AML, and its frequency in adult leukemic patients is low ($< 2\%$) [18,28].

Several investigators reported an excellent prognosis in pediatric leukemia with this aberration [16,33,35,47], yet others showed up to 20% incidence in relapsed ALL cases [13,20,34]. Nevertheless, TEL-AML1 positive patients in relapse seem to remain in complete remission for longer periods of time and have a good prognosis compared to their negative counterparts [13,34].

The purpose of the present study is to assess the TEL-AML1 fusion gene status in Egyptian pediatric ALL cases as regards frequency and association with known prognostic laboratory and clinical features, as well as the response of positive patients to induction therapy.

PATIENTS AND METHODS

Bone marrow and/or peripheral blood mononuclear cells were available for analysis from 81 children (≤ 16 years), 69 newly diagnosed and 12 in relapse, who were received at the outpatient clinic and pediatric ward of the National Cancer Institute, Cairo University, during the period from October 1999 to October 2000.

Diagnosis of ALL was based on standard French-American-British (FAB) morphological and cytochemical criteria [3,4]. Immunophenotyping was carried out using the EPICS XL flow cytometer from Coulter. Antibodies were purchased from Becton & Dickinson, Coulter Clone, Dako and Serotec. They included antibodies specific to lymphoid-associated antigens (CD2, CD3, CD4, CD5, CD7, CD8, CD19), myeloid/monocytic antigens (CD13, CD14, CD15, CD33), in addition to HLA-DR and CD10. Results were considered positive when 20% or more of the malignant cells expressed a particular antigen (10% or more in case of CD10 expression).

TEL-AML1 fusion transcript was detected by the reverse transcriptase-polymerase chain reaction (RT-PCR) technique. Total RNA was isolated from the patients' blood or bone marrow samples using the QIAamp® RNA Blood

Mini Kit (Qiagen GmbH, Germany). Reverse transcription of the isolated RNA and amplification of the chimeric TEL-AML1 gene fragment were performed in a 20- μ l single-step reaction using the Qiagen One-Step RT-PCR Kit (Qiagen GmbH, Germany) according to "the standardized RT-PCR protocol for analysis of fusion gene transcripts from chromosome aberrations in acute leukemia" [43]. RNA was reverse transcribed at 42°C for 45 minutes and the resulting cDNA was amplified using the previously described set of primers [43]: TEL-A (5'-TGCACCCTCTGATCCTGAAC-3') and AML-1-B (5'-AACGCCTCGCTCATCTTGC-3') (Fig. 1). After an initial melting step at 95°C for 30 seconds, 35 amplification cycles of 60 seconds at 94°C, 60 seconds at 65°C and 60 seconds at 72°C were performed. No final extension was needed and the PCR reaction was stopped at 16°C or room temperature. PCR products were analyzed on a 2% agarose gel and visualized by ethidium bromide staining.

Patients with newly diagnosed ALL were treated according to the NCI treatment protocol modified from the study XIII BH of St. Jude Children's Research Hospital. It included 6 weeks of induction, 2 weeks of consolidation and 120 weeks of continuation therapy. Induction therapy consisted of prednisone, vincristine (VCR), daunomycin, asparaginase, etoposide (VP-16), aracytin (Ara-C), in addition to triple intrathecal (IT) therapy for CNS prophylaxis. Consolidation therapy included 2 courses of high dose methotrexate (HDMTX), 6-mercaptopurine (6-MP) and triple IT therapy. Continuation therapy consisted of extended triple IT therapy and 15 cycles of an 8-week course of VP-16+cyclophosphamide (CTX), 6-MP+MTX, MTX+Ara-C, dexamethazone (Dex)+VCR, VP-16+Ara-C, 6-MP+HDMTX, VP-16+Ara-C, Dex-VCR. During continuation therapy, reinduction was given in the form of VCR, daunomycin, Dex, HDMTX, 6-MP and triple IT therapy. HDMTX was replaced by MTX while VP-16 was substituted by 6-MP after week 54 to minimize late drug effects. For relapsing ALL, re-induction therapy consisted of VCR, daunomycin, Dex, asparaginase and triple IT therapy for 4 weeks, after which patients were evaluated for response. Those who achieved second complete remission continued treatment on the St Jude protocol for relapsing ALL (ALL R16). Bone marrow was evaluated

at day 43 post-induction for newly diagnosed cases and 4 weeks following reinduction for relapsing cases to assess response to therapy. Good response to therapy was reported when the bone marrow blast percentage was <5%. Patients evaluable for assessment of response to therapy were those who attained complete remission (CR), partial remission (PR), or were resistant to therapy (R) by day 43 post-induction. Those who died early during induction therapy or were lost to follow-up were not evaluable for assessment.

Frequency, clinical and laboratory features in cases with and without TEL-AML1 fusion gene were compared using the two-tailed Fisher's exact test and the Mann-Whitney test. Variables tested included gender, age, total leukocytic count (TLC), immunophenotype, myeloid marker co-expression, DNA index, organomegaly, CNS infiltration and response to therapy during the first 43 days following induction. Differences were considered significant when the *p*-value was ≤ 0.05 .

RESULTS

The TEL/AML1 fusion transcript was detected by a single-round RT-PCR reaction. On a 2% agarose gel, the product of such reaction appeared as a 298 bp or a 259 bp band (Fig. 2). The smaller band size is due to the location of the AML1 breakpoint in intron 2 instead of intron 1 (Fig. 1) [43].

A total of 81 children suffering from ALL referred to the NCI, Cairo University, over a period of 12 months (October 1999-October 2000) were successfully investigated for the presence of TEL-AML1 fusion gene. They included 69 newly diagnosed (fresh) cases and 12 cases presenting in relapse. TEL-AML1 fusion gene was expressed in 10 of the 81 cases examined (12.35%), 7 newly diagnosed (10.14%) and 3 (25%) in relapse. The frequency of TEL-AML1 transcript in the studied ALL cases is summarized in table (1).

TEL-AML1 positive patients were 3 girls and 7 boys, with ages ranging from 3 to 15 years with a peak between 3 and 6 years. All positive cases were strictly limited to B-lineage ALL (1 pro-B, 6 CALL and 3 Pre-B). Their total leukocytic counts (TLC) ranged from 3 to 90 $\times 10^9/l$ with a median of 16.6 $\times 10^9/l$. Non-

hyperdiploid DNA content (< 1.16) was detected in all positive cases. The myeloid antigens, CD13 and CD33, were expressed in 5/10 (50%) and 4/10 (40%) TEL-AML1 positive cases, respectively. None of the cases showed CNS infiltration. None of the newly diagnosed cases showed massive organomegaly (> 5 cm bcm), while 33% of relapsed cases showed massive organomegaly. As regards response to therapy, all 5 evaluable newly diagnosed cases responded well during the first 43 days following induction therapy while none showed slow response to therapy, as opposed to 9% of the TEL-AML1 negative patients who responded slowly to therapy. Of the two evaluable relapsing cases, 1 showed good response after 4 weeks of reinduction therapy and the other showed slow response. Detailed clinical and laboratory data of the 10 TEL-AML1 positive cases are shown in table (2).

Differences between TEL-AML1 positive and negative cases, as regards clinical and laboratory features as well as response to therapy, are shown in tables (3&4).

Table (1): Frequency of TEL-AML1 transcripts in studied cases.

| | No. of patients examined | TEL-AML1 positive cases (%) |
|--------------------------------------|--------------------------|-----------------------------|
| <i>Newly diagnosed cases (n=69):</i> | | |
| Pro-B (CD 19+) | 10 | 1 (10.0%) |
| CALL (CD19+, CD10+) | 31 | 4 (12.9%) |
| Pre-B (CD19+, CD10+, clg+) | 19 | 2 (10.5%) |
| T-ALL | 9 | 0 |
| | 69 | 7 (10.14%) |
| <i>Relapsed cases (n=12):</i> | | |
| Pro-B | 1 | 0 |
| CALL | 5 | 2 (40%) |
| Pre-B | 4 | 1 (25%) |
| T-ALL | 2 | 0 |
| | 12 | 3 (25%) |
| Total | 81 | 10 (12.35%) |

clg: Cytoplasmic immunoglobulin.

Table (2): Detailed demographic, clinical and laboratory data of TEL-AML1 positive cases.

| No. | Sex/ age (yrs) | FAB | TLC (X 10 ⁹ /l) | DNA index | Pheno- type | Surface marker expression | | | | | Hepato- megaly (cm bcm) | Spleno- megaly (cm bcm) | CNS infiltration | Response in 43 days |
|-----|----------------------|-----|-------------------------------|--------------|----------------|---------------------------|----------|-----|----------|----------|-------------------------------|-------------------------------|---------------------|------------------------|
| | | | | | | CD 19 | CD 10 | clg | CD 13 | CD 33 | | | | |
| 1 | F/15 | L2 | 8.0 | 1.00 | Pre-B | 94 | 86 | 91 | 23 | 63 | 0 | 0 | Neg | CR |
| 2 | M/3 | L2 | 23.0 | 0.98 | CALL | 71 | 49 | 6 | 2 | 1 | 5 | 3 | Neg | LFU |
| 3 | M/3 | L2 | 90.0 | 0.98 | Pro-B | 95 | 7 | 3 | 2 | 6 | 5 | 5 | Neg | CR |
| 4 | M/10 | L1 | 23.0 | 1.00 | CALL | 95 | 94 | 5 | 71 | 71 | 3 | 0 | Neg | CR |
| 5 | F/3 | L2 | 10.0 | 1.00 | CALL | 49 | 44 | 12 | 6 | 1.0 | 5 | 1 | Neg | CR |
| 6 | M/5.5 | L2 | 3.0 | 0.96 | Pre-B | 72 | 53 | 53 | 39 | 18 | 0 | 0 | Neg | CR |
| 7 | F/4.5 | L2 | 68.0 | ND | CALL | 86 | 83 | 3 | 14 | 6 | 4 | 0 | Neg | D |
| 8 | M/6 | Rel | 8.0 | 0.98 | Pre-B | 90 | 50 | 82 | 4 | 3 | 2 | 5 | Neg | R |
| 9 | M/6 | Rel | 4.5 | 1.10 | CALL | 95 | 77 | 5 | 84 | 83 | 0 | 0 | Neg | CR |
| 10 | M/6 | Rel | 34.0 | 0.96 | CALL | 95 | 50 | 2 | 83 | 83 | 8 | 8 | ND | D |

clg: Cytoplasmic immunoglobulin; bcm: below costal margin; CR; Complete remission; R: Resistant to therapy; D: Died during induction; LFU: Lost follow-up; ND: Not done.

Table (3): Clinical and laboratory features of the newly diagnosed pediatric ALL cases (n=69) in relation to TEL-AML1 fusion gene status.

| Feature | TEL-AML1 positive (n=7) | | TEL-AML1 negative (n=62) | | p-value | |
|----------------------------|-------------------------|------|--------------------------|-------|---------|---------|
| | Frequency | % | Frequency | % | | |
| Gender | Females | 3 | 57 | 34 | 55 | 0.696 |
| | Males | 4 | 43 | 28 | 45 | |
| Age (years) | ≤ 10 | 6 | 86 | 48 | 77 | 1.000 |
| | > 10 | 1 | 14 | 14 | 23 | |
| TLC (X 10 ⁹ /l) | < 50 | 5 | 71 | 36 | 58 | 0.693 |
| | ≥ 50 | 2 | 29 | 26 | 42 | |
| DNA index | < 1.16 | 6/6* | 100 | 45/49 | 92 | 1.000 |
| | ≥ 1.16 | 0/6 | 0 | 4/49 | 8 | |
| Immunophenotype | BCP-ALL | 7 | 100 | 53 | 85 | 0.582 |
| | T-ALL | 0 | 0 | 9 | 15 | |
| CD13 | Negative | 4 | 57 | 52/52 | 100 | 0.001** |
| | Positive | 3 | 43 | 0/52 | 0 | |
| CD33 | Negative | 5 | 71 | 46/51 | 90 | 0.196 |
| | Positive | 2 | 29 | 5/51 | 10 | |
| Hepatomegaly (cm bcm) | ≤ 5 | 7 | 100 | 54 | 87 | 0.312 |
| | > 5 | 0 | 0 | 8 | 13 | |
| Spleno- megaly (cm bcm) | ≤ 5 | 7 | 100 | 47 | 76 | 0.141 |
| | > 5 | 0 | 0 | 15 | 24 | |
| CNS infiltration | Negative | 7 | 100 | 53/57 | 93 | 1.000 |
| | Positive | 0 | 0 | 4/57 | 7 | |
| Response to therapy | Good (< 43 days) | 55 | 100 | 43/47 | 91 | 1.000 |
| | Slow (> 43 days) | 0/5 | 0 | 4/47 | 9 | |

* : Frequency/number of cases tested.

** : Significant result at $p < 0.05$.

BCP-ALL : B-cell precursor ALL.

bcm : Below costal margin.

Table (4): Clinical and laboratory features of the relapsing ALL cases (n=12) in relation to TEL-AML1 fusion gene status.

| Feature | | TEL-AML1 positive (n=3) | | TEL-AML1 negative (n=9) | | p-value |
|-----------------------------------|----------|-------------------------|-----|-------------------------|-----|---------|
| | | Frequency | % | Frequency | % | |
| Gender | Females | 0 | 0 | 2 | 22 | 1.000 |
| | Males | 3 | 100 | 7 | 78 | |
| Age at initial diagnosis (years) | ≤ 10 | 3 | 100 | 4 | 44 | 1.000 |
| | > 10 | 0 | 0 | 5 | 56 | |
| TLC (X 10 ⁹ /l) | < 50 | 3 | 100 | 6/9 | 67 | 0.509 |
| | ≥ 50 | 0 | 0 | 3/9 | 33 | |
| DNA index | < 1.16 | 3 | 100 | 5/6* | 83 | 1.000 |
| | ≥ 1.16 | 0 | 0 | 1/6 | 17 | |
| Immunophenotype | BCP-ALL | 3 | 100 | 7 | 78 | 1.000 |
| | T-ALL | 0 | 0 | 2 | 22 | |
| CD13 | Negative | 1 | 33 | 6/6 | 100 | 0.083 |
| | Positive | 2 | 67 | 0/6 | 0 | |
| CD33 | Negative | 1 | 33 | 6/7 | 86 | 0.183 |
| | Positive | 2 | 67 | 1/7 | 14 | |
| Hepatomegaly (cm bcm) | ≤ 5 | 2 | 67 | 7 | 78 | 1.000 |
| | > 5 | 1 | 33 | 2 | 22 | |
| Splenomegaly (cm bcm) | ≤ 5 | 2 | 67 | 6 | 67 | 1.000 |
| | > 5 | 1 | 33 | 3 | 33 | |
| CNS infiltration | Negative | 2/2 | 100 | 7 | 78 | 1.000 |
| | Positive | 0/2 | 0 | 2 | 22 | |
| Duration of 1st CR (months) | ≤ 36 | 1 | 33 | 4 | 44 | 1.000 |
| | > 36 | 2 | 67 | 5 | 56 | |
| Site of relapse | BM | 3 | 100 | 6 | 67 | 0.509 |
| | Combined | 0 | 0 | 3 | 33 | |
| Response to reinduction (4 weeks) | Good | 1/2 | 50 | 2/6 | 33 | 1.000 |
| | Slow | 1/2 | 50 | 4/6 | 67 | |

* : Frequency/number of cases tested. CR : Complete remission.
 BCP-ALL : B-cell precursor ALL. BM : Bone marrow (hematological) relapse.
 bcm : Below costal margin. Combined : Bone marrow + CNS and/or testicular relapse.

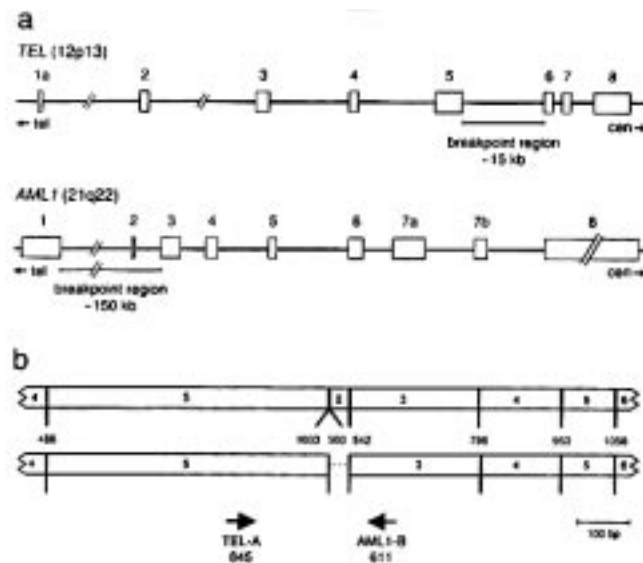


Fig. (1): (a) Schematic diagram of the structure of the TEL and AML1 genes involved in t(12;21) (p13;q22). The centromere (cen) and telomere (left) orientation, exon numbering and relevant breakpoint regions are indicated. (b) Schematic diagram of the TEL-AML1 fusion transcripts. Most t(12;21)-positive patients have the larger transcript because of a breakpoint in AML1 intron 1, but alternative splicing can cause skipping of AML1 exon 2, leading to two PCR products in some patients. In a minority of patients, the AML1 breakpoint is located in intron 2, resulting in a shorter transcript without AML1 exon 2. The arrows indicate the relative position of the two primers used; the numbers below the primers refer to the 5' nucleotide position of each primer [43].

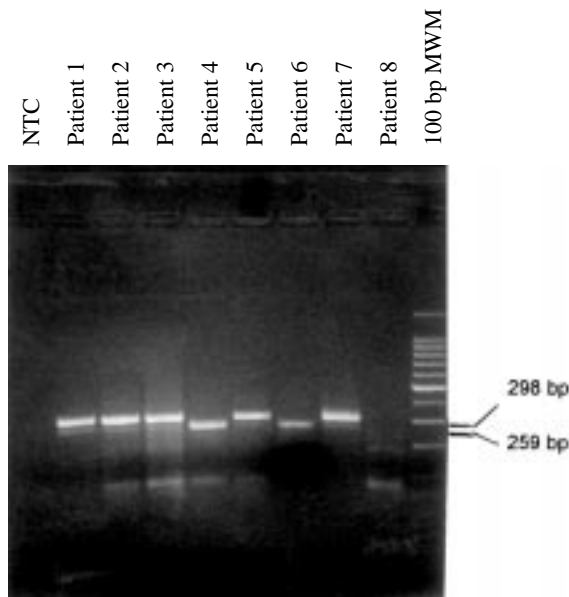


Fig. (2): Ethidium bromide-stained 2% agarose gel showing TEL-AML1 transcripts in patients 1-7 using the TEL-A and AML1-B primers. Patients 1,2,3,5 & 7 show one PCR band of 298 bp indicating a breakpoint in AML1 intron 1. Patients 4 & 6 show one PCR band of 259 bp resulting from an AML1 intron 2 breakpoint [43]. Patient 8 is negative for TEL-AML1 fusion transcript. NTC is the no-template control with water added instead of RNA to monitor contamination during the PCR reaction. A 100-bp molecular weight marker (MWM) is added in the last lane for band size estimation.

DISCUSSION

In our series, TEL-AML1 fusion gene occurred in 10 of the 81 pediatric ALL cases examined (12.35%), 7/69 (10.14%) newly diagnosed and 3/12 (25%) in relapse. Other studies on newly diagnosed ALL cases reported frequencies varying from 2 to 33% [5,6,9,10,14,15,18,19,30,32,39,41,47]. The highest frequency (33%) was reported by a French group of researchers [6] who also reported loss of heterozygosity (LOH) of ETV6 in most cases positive for TEL-AML1 fusion gene, a finding reported earlier by Raynaud et al. [28] who suggested that such association provided a further proliferative advantage to leukemic cells. The lowest frequency (2%) came from the Spanish group of Garcia-Sanz et al. [10] who initially reported the absence of TEL-AML1 fusion gene in their 38 pediatric cases studied by RT-PCR and FISH techniques. While their report was in press, they analyzed three more samples from children with ALL and found only one case to be positive for such aberration, thus raising the frequency in

their series from 0% to 2%, which remains the lowest frequency reported for TEL-AML1 fusion gene in childhood ALL. Between these two extremes, our frequency in newly diagnosed cases (10.14%) seems to lie on the "low-side" of the average reported frequencies, comparable to results from India (9%) [14], Hiroshima, Japan (10%) [9], United Kingdom (11%) [39] and Kyoto, Japan (13%) [20]. Higher percentages were recorded in Brazil (18%) [18], Italy and Germany (19%) [5], the Czech Republic (22%) [47] and USA (22% & 27%) [19,32].

If only B-lineage newly diagnosed ALL cases are considered (60 cases), our frequency rises to 11.67%, obviously because the TEL-AML1 fusion gene has been negative in all our T-ALL cases. Thus, excluding T cases raises the percentage of TEL-AML1 positivity in our study, as well as others [10,18,20], all of which failed to detect such fusion gene in their T-ALL cases.

Most of the fore-mentioned researchers used the RT-PCR technique for basic analysis of TEL-AML1 fusion gene, while some complemented their analysis by Southern blotting or fluorescent in situ hybridization (FISH). However, the varying frequencies in any method used indicates that the different methodologies employed have more or less close sensitivity and specificity levels in detecting the fusion gene in question, so they cannot be held responsible for such variations. International variation in incidence rates of TEL-AML1 fusion gene could be attributed to genetic and/or environmental factors that may contribute to pathogenesis of the disease [10,18].

In our series, the relatively low frequency of TEL-AML1 positivity in newly diagnosed precursor B-ALL cases (11.67%) may be an additional explanation to the inferior prognosis of our cases compared to western reports. As opposed to a 5-year event-free survival (EFS) of 79% for CALL and 71% for pre-B ALL reported by Pui et al. [23] and an estimated 6-year EFS of 77% for CALL and 67% for pre-B ALL in a BFM study [29], an Egyptian study reported a 5-year EFS of 64% for CALL and 52% for pre-B ALL [36]. Thus, in addition to the suggested causes of the inferior outcome of BCP-ALL in developing countries, i.e. modest living conditions and altered patterns of infection during infancy [11], it seems that underlying molec-

ular mechanisms may play an additional role in the pathogenesis and in determining the clinical outcome of this leukemia subset.

As regards the frequency of TEL-AML1 fusion gene in relapsed cases, our results showed a 25% frequency in the 12 cases of relapsed ALL received during the period of our study (30% if only Bcs. ALL is considered). Shurtleff et al. [35] were among the first researchers to identify TEL-AML1 fusion gene in pediatric ALL and to use it to define a "subgroup of patients with excellent prognosis". This quote was supported by a number of studies that reported low frequencies of this aberration among relapsed cases, varying from 3 to 10% [16,31,47]. However, the prognostic significance of this aberration has been disputed by other studies based on the relatively high incidence found in relapsed cases in their cohorts, which reached 19-28% [13,20,34], a finding which is comparable to our result in relapsed ALL. In contrast to the short period of our study (a maximum of 12 months), other groups followed their cases long enough and could demonstrate a significantly longer duration of first complete remission (CR) with a median of 46 months versus 26 months in TEL-AML1 negative cases [13,16,31,34,47], a finding which still puts TEL-AML1 positive cases ahead of negative ones regarding prognosis. The enormous variation between TEL-AML1 frequencies in relapsed cases reported by different studies may be attributed to the small sample sizes in each study, which is subject to strong inaccuracies and deviations. Moreover, when studies are carried out retrospectively, selection biases that may lead to misinterpretation may sometimes be unavoidable [5].

Clinical and laboratory features with recognized prognostic value in childhood ALL include age, sex, initial total leukocyte count, degree of organomegaly and early response to therapy [22,24,40]. Patient age and leukocyte count at diagnosis provide the most important prognostic information and are used by all major oncology centers to determine the risk status of patients. To facilitate comparisons of clinical trials performed by different investigative groups, the National Cancer Institute (USA) sponsored a workshop to define uniform risk criteria for children with B-lineage ALL [37]. By consensus, the participants recommended an age of 1.00 to 9.99 years and a leukocyte count

of less than $50 \times 10^9/l$ as standard risk criteria, with all other combinations of these features considered high risk. Our results conform with this consensus as well as with other reports on clinical features of TEL-AML1 positive cases [13,33,47], thus assigning our TEL-AML1 positive patients to the standard risk group. All our patients were within this age, except for one 15-year old adolescent. TEL-AML1 was negative in all infants below one year of age, where such abnormality was reported to be very rare [32].

The total leukocytic count (TLC) was below $50 \times 10^9/l$ in 5 out of our 7 TEL-AML1 positive patients (71%) and in all 3 relapsed cases. Only 2 newly diagnosed cases (29%) had a TLC over $50 \times 10^9/l$, while none exceeded $100 \times 10^9/l$. This is in accordance with previous studies [13,15,20] that demonstrated that most of, if not all, their cases were below $50 \times 10^9/l$. Again, this criterion places our TEL-AML1 positive cases in the standard risk group regarding response to therapy and incidence of relapse [37].

No significant difference could be detected regarding the gender of patients positive or negative for TEL-AML1 fusion gene, either the newly diagnosed or the relapsed cases. Seven patients of the 10 TEL-AML1 positive children were males [4 newly diagnosed (57%) and 3 (100%) in relapse]. Boys have been reported to have higher incidence of relapse, which could be explained only in part by relapse in the testis, as the rate of bone marrow relapse was also found to be higher in males, as is the case in our series [17].

Hyperdiploidy, defined as DNA content more than 1.16 times that of a normal cell, has consistently exerted a favorable influence on treatment outcome in patients with B-lineage ALL [42]. All our 9 tested TEL-AML1 positive cases had a DNA index below 1.16, a finding supported by other studies [30,32,33]. To better explain the discrepancy between hyperdiploidy as a favorable factor and its absence in TEL-AML1 positive cases claimed to predict good prognosis, Rubnitz et al. [32] compared the outcome among patients with either rearranged TEL, germline TEL with hyperdiploidy and germline TEL without hyperdiploidy by the Southern blot technique. The rearranged TEL group and the germline TEL group with hyperdiploidy fared significantly better than the

germline TEL group without hyperdiploidy. Thus, hyperdiploidy and TEL rearrangement together identify a majority of B-lineage ALL patients with favorable prognosis. Moreover, TEL rearrangement appears to predict a favorable outcome independent of other risk-stratifying features.

As has been mentioned before, all our newly diagnosed TEL-AML1 cases were strictly B-lineage ALL, mostly CALL and Pre-B ALL and less commonly Pro-B ALL (Table 1). This is in accordance with other reports [20,32,33,35, 45], so TEL-AML1 fusion appears to be unique to B-cell progenitor ALL. The basis of such selectivity is an important issue that remains unresolved [1], but most likely reflects a selective impact of the chimeric protein on the proliferation and/or survival of B-cell precursors [12].

Although TEL-AML1 fusion gene occurs only in B-precursor ALL, 3/7 of our newly diagnosed cases (42%) and 2/3 relapsed cases (67%) expressed the myeloid surface markers CD13, CD33, or both. Similarly, myeloid surface marker expression was reported in 50% of cases in the study of Rubnitz et al. [33], in 23% of the series of Baruchel et al. [2] and in 25% of the cases studied by Borkhardt et al. [5]. By contrast, McLean et al. [19] detected no myeloid surface marker expression in the cases they analyzed. Myeloid antigen co-expression may be explained on the basis of the involvement of the AML1 gene in myeloid leukemias [21], hence the expression of myeloid-associated markers. Although in several studies, mainly in adults, expression of myeloid antigens in ALL has been found to be associated with poor outcome [38,46], this feature was not prognostically important in other studies [5,8,33] as proved by the good clinical outcome of the TEL-AML1 positive patients showing such co-expression. This further identifies TEL-AML1 fusion gene positive cases as a separate entity with unique clinical and laboratory features that deserve further study to assess their impact on prognosis.

Neither our newly diagnosed nor our relapsed TEL-AML1 positive cases showed CNS infiltration. Other studies also reported a low incidence of CNS infiltration in TEL-AML1 positive cases, ranging from 0-8% [18,32]. This can be considered an additional factor in favor of TEL-AML1 fusion gene as a marker for good prognosis.

Response to therapy, defined in our series as the achievement of complete remission by day 43 following induction, was noted in all 5 evaluable TEL-AML1 positive newly diagnosed cases and 1 of the 2 evaluable cases in relapse. In spite of the lack of statistical significance of these results when compared with TEL-AML1 negative cases, newly diagnosed cases showed a predilection to good response to therapy to be confirmed by surveying a larger cohort.

The clinical outcome and prognostic significance of TEL-AML1 fusion gene cannot be inferred from our study due to the short period of follow-up for our patients (a maximum of 12 months). A longer follow-up period is definitely needed to assess the impact of this fusion gene on the clinical outcome of patients positive for this fusion.

In conclusion, we report a frequency of 10.14% of TEL-AML1 fusion gene in our newly diagnosed pediatric ALL cases (11.67% of precursor B-ALL) and a 25% frequency in relapsing cases. Positive cases bear features for good prognosis, namely the 1-10 year age group, total leukocytic count less than $50 \times 10^9/l$, precursor B phenotype, negative CNS infiltration and good response to therapy. TEL-AML1 positive patients can thus be considered a distinct clinical entity which deserves thorough molecular screening and long-term prospective clinical trials to further clarify the prognostic impact of this fusion and accordingly design treatment protocols with the least deleterious effects on the growth and development of children suffering from ALL.

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