Prognostic Impact of TEL–AML-1 Fusion Gene on Acute Lymphoblastic Leukemia

Wijdan N. Ibrahim[1], Hassan J. Hasony[1], Jinan Gh Hassan[2]

[1]Department of Microbiology, College of Medicine, Basrah, Iraq.
[2]Department of Pediatrics, College of Medicine, Basrah, Iraq.

ABSTRACT

The TEL–AML-1 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 was conducted to investigate the frequency of this fusion gene in children suffering from ALL attending the oncology unit in the Basrah Hospital for Pediatric and Gynecology during the period May 2009 to April 2010, and point out the different laboratory features associated with this anomaly.

A total of 120 blood samples were collected (60 early-diagnosed ALL children and 60 healthy children as control group). The controls were matched with cases by age and sex. Ribonucleic acid (RNA) was successfully extracted from the fresh blood of 40 ALL cases used for the detection of TEL–AML-1 fusion gene by reverse transcriptase–polymerase chain reaction (RT-PCR).

Of the newly diagnosed ALL cases, 27.5% were positive for TEL–AML-1 fusion gene as well as 5% among the control group. All TEL–AML-1 positive cases showed an age peak between 3 and 6 years and tend to occur more frequently among females than males. The TEL–AML-1 positive cases were classified as the standard-risk group and accounted for 72.3%, while 27.3% were the high-risk group ($P < 0.05$). According to the French–American–British classification criteria, 72.3% of the high-risk and 68% of the standard-risk groups belong to the L2 stage.

The TEL–AML-1 fusion gene identifies a subset of pediatric ALL associated with a number of laboratory markers of good prognosis and should thus be considered in routine molecular work of ALL to confirm its impact on clinical outcome and to design a suitable therapy.

Key words: Acute lymphoblastic leukemia, TEL-AML-1, fusion gene

INTRODUCTION

AML-1 is normally expressed in all hematopoietic lineage and acts to regulate the expression of various genes such as granulocyte–colony stimulating factor, interleukin-3, T-cell receptors, and myeloperoxidase genes (1). Frequent translocation variants result in fusion between intron-5 of TEL and intron-2 of AML-1 (2). The t(12:21) results in the chimeric fusion gene TEL–AML-1. The basis of this selectivity is an important unresolved issue (3), but most likely reflects a selective impact of the chimeric protein on the proliferation and/or survival of B-cell precursor (4). Although the mechanism of leukemogenesis induced by TEL–AML-1 remains obscure, recent data have demonstrated the importance of both TEL and AML-1 for normal hematopoiesis, thus suggesting that the presence of TEL–AML-1 fusion protein leads to disordered hematopoietic development as a critical component (5).

Correspondence:
Wijdan N. Ibrahim
Department of Microbiology, College of Medicine, Basrah, Iraq.
e-mail: ihsanalsaimary@gmail.com

There is a persuasive evidence that TEL–AML-1 usually arises prenatally as an early or initiating mutation. The data provide convincing evidence that TEL–AML-1 translocation is the initiating event in leukemia (6,7). The latency between birth and onset of leukemia clearly demonstrates the need for important secondary and postnatal events in the promotion of ALL with TEL–AML-1 fusion (8,9) since the second nontranslocated TEL allele is often deleted in t(12:21)-positive ALL patients (up to 70%). This deletion is generally considered as the second hit in the leukemogenesis (7).

The favorable prognostic impact of TEL–AML-1 is independent of age and leukocyte count and was consistently favorable among patients treated on several different protocols. Thus, TEL–AML-1 expression identifies a large subset of B-precursors ALL patients who may be candidate for less intensive therapy (10–13). The fusion genes generated by chromosome translocation (TEL–AML-1 in ALL) primarily blocks cell differentiation. The aberrant proteins produced by these genes inhibit gene activity and differentiation by recruiting repressor molecules. These repressors include histone deacetylase enzyme (14).

Investigators have reported that almost 10%–28% of relapsed pediatric ALL patients express the TEL–AML-1 fusion, but the relapse of patients with TEL–AML-1 fusion is not always associated with poor prognosis (15).

This study aimed at determining the frequency of TEL–AML-1 fusion gene status in children with newly diagnosed ALL and to predict its association with prognostic laboratory features.

MATERIALS AND METHODS

A case–control study was conducted during the period May 2009 to April 2010 on 60 consecutive children of below 15 years of age with newly diagnosed, untreated cases of acute lymphoblastic leukemia (ALL). mRNA was successfully extracted from 40 cases (20 females and 20 males). The primary diagnosis of ALL cases was based on the complete blood picture and bone marrow aspirates. An age of 1–9.99 years and white blood cell (WBC) count less than 50 x 10^9/L was considered as standard-risk criteria, with all other combinations of these features (central nervous system infiltration, hepatosplenomegaly) as high risk. The diagnosis and staging of ALL cases were based on the standard French–American–British (FAB) morphological and cytological criteria by specialist hematologist, and then referred to the leukemia treatment unit at the Basrah Hospital for Pediatric and Gynecology. Sixty healthy children matched by age and sex (31 male and 29 females) from the general population of Basrah (school children and day care centers at the city center, Abu-Alkhasib, Al-Hartha) with negative history of major illness and no history of cancers or any apparent congenital anomalies were recruited as the control group.

Blood was drawn from the veins of both patients and control; 3 mL of venous blood taken in EDTA tubes was used immediately for total RNA extraction using the QIAamp@RNA Blood Mini Kit (Qiagen GmH, Germany). All steps of the procedure were followed according to the manufacturer's instructions. Isolated total RNA was stored at −20°C in RNase-free distilled water where no degradation of RNA was detected [Qiagen (16)]. The integrity of RNA was assessed by electrophoresis through 1% agarose gel containing 1.3 iM ethidium bromide.

Polymerase chain reaction (PCR) analysis of fusion genes was based on the design of oligonucleotide primers at opposite sides of breakpoint regions, so that the PCR product contains the tumor-specific fusion sequences. However, many acute leukemia fusion genes are transcribed into fusion mRNA, which can serve as the PCR target after reverse transcription (RT) in copy-DNA (cDNA). So in this study, TEL–AML-1 fusion transcript was detected by RT-PCR techniques, which was carried out on the extracted RNA using Qiagen one-step RT-PCR Kit following the manufacturer's instructions. The PCR product from the amplification of TEL–AML-1 fusion gene was detected by two sets of primers obtained from Alpha-DNA (Canada), which were as follows:

TEL-A: (5'-TGCACCCTCTGATCCTGAAC-3') and AML-1B: (5'-AACGCCTCGCTCATCTTGC-3').

These were then electrophoresed on ethidium bromide–stained 2% agarose gel. The entire gel was subjected to equal electric current together with the internal control and ladder markers. The presence of 298 bp bands indicates breakpoint in AML-1 intron-1 whereas their absence indicates the null fusion and considered as negative result (17).

Table 1: Distribution of TEL–AML-1 fusion gene in the study population

TEL–AML-1 Fusion gene	Study group *N* (%)	Control group *N* (%)	Total *N* (%)
Positive	11/40 (27.5)	3/60 (5)	14/100 (14)
Negative	29/40 (72.5)	57 (95)	86/100 (86)
TOTAL	40 (100)	60 (100)	100 (100)

χ^2 (Chi-Square) = 10.09; df (degree of freedom) = 1; P = 0.002

Table 2: Distribution of ALL patients according to the FAB classification and risk group[a]

FAB	Standard risk *N* (%)	High risk *N* (%)	Total *N* (%)
L1	7 (28)	1 (6.7)	8 (20)
L2	17 (68)	11 (73.3)	28 (70)
L3	1 (4)	3 (20)	4 (10)
Total	25 (62.5)	15 (37.5)	40 (100)

[a]An age of 1–9.99 years and a leukocyte count less than 50 x 10/L were considered as standard-risk criteria with all other combinations of these features considered high risk.
χ^2 = 4.571; df = 2; P > 0.05

RESULTS

The distribution of TEL–AML-1 transcript in the study population is summarized in Table 1, which shows that a total of 100 children, 40 of them suffering from ALL, were successfully investigated for the presence of TEL–AML-1 fusion gene. The TEL–AML-1 fusion gene was expressed in 27.5% of ALL cases and in 5% of healthy control group. The difference was statistically significant ($P < 0.05$).

The distribution of ALL patients according to the FAB classification in relation to risk grouping is presented in Table 2. Out of the total ALL cases, 62.5% were classified as the standard-risk group and 37.5% as the high-risk group. However, according to the FAB classification, 68% of the standard-risk group belong to the L2 stage, while 73.3% of ALL cases in the high-risk group were in the L2 stage. This difference was statistically not significant ($P > 0.05$).

The distribution of TEL–AML-1 fusion gene according to sex among the study population is summarized in Table 3. No significant effect was noted with regard to sex on the distribution of TEL–AML-1 fusion genes ($P > 0.05$).

The distribution of TEL–AML-1 fusion genes according to different age groups is described in Table 4, which shows no significant effect with regard to age on the presence of fusion genes ($P > 0.05$).

DISCUSSION

ALL is the most common malignancy of childhood. Cure of many of ALL children is difficult to predict and is considered an individual response of a patient to chemotherapy (18). It is likely that this clinical heterogeneity reflects a diverse pathogenesis of leukemia. The molecular basis of childhood ALL is largely unknown. Furthermore, it is likely that significant advance in the treatment of childhood ALL will depend on a better understanding of the molecular events that causes the disease (18–20). Chromosomal abnormalities in childhood ALL had important significance related to diagnosis, management, and prognosis. Understanding of leukemogenesis is enhanced by the identification of specific chromosomal alterations that pinpoint sites for molecular studies to identify genes involved in the transformation and proliferation of leukemic cells (20,21).

Table 3: Distribution of TEL–AML-1 fusion gene in relation to sex

TEL–AML-1 fusion gene	Study (ALL) group		Control group	
	Male *N* (%)	Female *N* (%)	Male *N* (%)	Female *N* (%)
Positive	5 (25)	6 (30)	1 (3.2)	2 (6.9)
Negative	15 (75)	14 (70)	30 (96.8)	27 (93.1)
Total	20 (100)	20 (100)	31 (100)	29 (100)

χ^2 = 0.125, df = 1, NS; EFT = 0.425, df = 1, NS

Table 4: Distribution of TEL–AML-1 fusion genes according to age of ALL patients

Age groups (years)	TEL–AML-1 fusion genes			
	Study group		Control group	
	Positive *N* (%)	Negative *N* (%)	Positive *N* (%)	Negative *N* (%)
< 5	6 (54.5)	18 (62.1)	1 (33.3)	36 (63.2)
5–10	5 (45.5)	7 (24.1)	2 (66.7)	18 (31.6)
>10	0 (0)	4 (13.8)	0 (0)	3 (5.3)
Total	11 (27.5)	29 (72.5)	3 (5)	57(95)

EFT = 2.8, df = 2, P > 0.05

In the present study, the frequency of TEL–AML-1 gene fusion was 27.5% in patients with ALL and 5% in the control group. Other studies on newly diagnosed ALL cases reported frequencies varying from 2% to 33% (23–30). The highest frequency (33%) was reported by a French group (22) and the lowest came from a Spanish group by Garcia-Sanz et al (23). The present-study figure (27.5%) in newly diagnosed ALL cases seems to lie on the higher side of the reported frequencies. Comparable to the present-study results were those reported from Brazil (18%) (24), Italy and Germany (19%),29 the Czech Republic (22%) (27), and the United States (22% and 27%) (25,26). Lower percentages were reported from India (9%) (28) and the United Kingdom (11%) (30). Most of the aforementioned researchers used the RT-PCR technique for the basic analysis of TEL–AML-1 fusion gene, while some complemented their analysis by southern blotting or fluorescent in situ hybridization. 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, but can be attributed to the usage of different sets of primers. Furthermore, by using RT-PCR strategies, it was found that a significant proportion of healthy individuals (5%) harbor the TEL–AML-1 gene rearrangement. These cases have same residency that may give an idea that the area was exposed to same environmental factors such as radiation or due to exposure to an outbreak of viral infection. However, another study reported a frequency of 8.8% (31). It seems that the underlying molecular mechanisms may play an additional role in the pathogenesis and in determining the clinical outcome of this subset (32).

This report and others like it raise a number of interesting questions. Does the presence of an oncogenic fusion gene detected by RT-PCR confer an increased risk to develop leukemia? Or, what obligation does one have to report or follow-up on RT-PCR positivity for a known oncogene in a healthy individual? At a minimum, investigators engaged in such analyses should consider the design and implementation of studies that would allow for assessment of relative risk of leukemia based on the presence of RT-PCR detectable fusion genes (31).

Clinical and laboratory features with recognized prognostic values in the childhood ALL include age, sex, initial total WBC count, degree of organomegaly, and early response to therapy (32,33). These variables have consistently emerged as independent predictors of outcome in almost all therapeutic studies. Age and WBC count are continuous variables, and discrete thresholds used for risk stratification are somewhat arbitrary (34). It was found that 62.5% of TEL–AML-1 positive cases were classified as the standard-risk group, while 37.5% of them had been classified as high-risk patients in spite of harboring TEL–AML-1 rearrangement. These high-risk patients will be subjected to unnecessary combination of drugs with their side effects and acute toxicity and late-occurring adverse events in addition to the cost of these drugs on the patient (35). The present-study results are consistent with other reports on clinical features and significance of TEL–AML-1 positive cases (27,36). In addition, the favorable impact of TEL–AML-1 is independent of age and leukocyte count.11 However, all the TEL–AML-1 positive cases in the study and control groups were below 6 years old, thus assigning the TEL–AML-1 positive patients to the standard-risk group. These can be considered as factors in favor of TEL–AML-1 fusion gene as a marker for good prognosis.

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