

**Original article****TEL-AML-1 fusion gene in children with acute lymphoblastic in basra pediatric oncology center****J.G. Hassan***Department of paediatrics, college of medicine, Basrah, Iraq.*

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ABSTRACT

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 has been conducted to investigate the frequency of this fusion gene in children suffering from ALL attending the oncology unit in Basra hospital for pediatric and gynecology during the period from May 2009 to April 2010, and point out the different laboratory features associated with this anomalies. 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) has been successfully extracted from 40 ALL cases fresh blood used for the detection of TEL-AML-1 fusion gene by reverse transcriptase- polymerase chain reaction (RT-PCR). Of 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 3-6 years and tend to occur more frequent among female than males. TEL-AML-1 positive cases that classified as standard risk group were accounted for 72.3% while 27.3% were high risk group ($P<0.05$) and according to the French- -American-British (FAB) classification criteria, 72.3% of high risk and 68% of standard risk groups belong to L2 stage. 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 suitable therapy.

1. 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(IL-3), T-cell receptors and myeloperoxidase(MPO) genes. Frequent translocation variants result in fusion between intron-5 of TEL and intron-2 of AML-12. The t(12,21) result in chimeric fusion gene TEL-AML1 (Roumier et al., 2003). The basis of this selectivity is an important unresolved issue (Kazunori et al., 2006), but most likely reflects a selective impact of the chimeric protein on the proliferation and/or survival of B-cell precursors (Arthur et al., 2004). Although the mechanism of leukemogenesis induced by TEL-AML1 remains obscure, recent data have demonstrated the importance of both TEL and AML-1 for normal hematopoiesis, thus suggesting that the presence of TEL-AML1 fusion protein leads to disordered hematopoietic development as a critical component (Rubnitz et al., 1999).

There is a persuasive evidence that TEL-AML1 usually arise prenatally as an early or initiating mutation. The data provide convincing evidence that TEL-AML1 translocation is the initiating event in leukemia. 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-AML1 fusion (Charles et al., 2007; Jaro-ova et al., 2002). , since the second non-translocated 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.

The favorable prognostic impact of TEL-AML1 is independent of age and leukocyte count and was consistently favorable among patients treated on several different protocols. Thus TEL-AML1 expression identifies a large subset of B-precursor ALL patients who may be candidate for less intensive therapy (Andersen et al., 2002; Andersen and Look, 2005; Fabio and Maurizio, 2008; Artigas et al., 2006). The fusion genes generated by chromosome translocation (TEL-AML1 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 (Jukka, 2001) .

Investigators have reported that almost 10-28% of relapsed paediatric ALL patients express the TEL-AML1 fusion, but the relapse of patients with TEL-AML1 fusion is not always associated with poor prognosis (Anthony et al., 2001).

This study aimed at determining the frequency of TEL-AML1 fusion gene status in children with newly diagnosed ALL and to predict its association with prognostic laboratory features.

2. Materials and methods

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

Blood was drawn from the veins of both patients and control, 3ml of venous blood taken in EDTA tubes used immediately for total RNA extraction using QIAamp® RNA blood mini kit (Qiagen GmbH Germany). All procedure steps were followed according to the manufacturer's instructions. Isolated total RNA stored at -20°C in RNAase free distilled water where no degradation of RNA been detected (Qiagen), (Marks et al., 1996). The integrity of RNA were assessed by electrophoresis through 1% agarose gel containing, 1.3 μM ethidium bromide.

Polymerase chain reaction (PCR) analysis of fusion genes is based on the design of oligonucleotide primers at opposite side of breakpoint regions, so that the PCR product contains the tumor specific fusion sequences. However, many acute leukemia, fusion genes is 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 reverse transcriptase-PCR(RT-PCR) techniques which was carried out on the extracted RNA using Qiagen one step RT-PCR kit following the manufacturer's instructions (Qiagen). PCR product from amplification of TEL-AML-1 fusion gene was detected by two sets of primers obtained from Alpha-DNA (Canada) which was, TEL-A, (5'-TGCACCTCTGATCCTGAAC-3') and AML-1B, (5'-AACGCCTCGCTCATCTTGC-3') 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 indicating breakpoint in AML-1 intron-1 whereas their absence indicating the null fusion and considered as negative result (Shaker et al., 2001).

3. 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 was successfully investigated for the presence of TEL-AML-1 fusion gene. 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).

Table 1
Distribution of TEL-AML-1 fusion gene in 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)

X² =10.09 df =1 P =0.002

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

Table 2
Distribution of ALL patients according to FAB classification and risk.

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)

An age Of 1-9.99 years and a leukocyte count less than 50x10/L considered as standard risk criteria with all other combinations of these features considered high risk.

X² = 4.571 df=2 P>0.05

The distribution of TEL-AML-1 fusion gene according to sex among the study population is summarized in Table-3. There was no significant effects to sex on the distribution of TEL-AML-1 fusion genes (P>0.05).

4. Discussion

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood. Cure of many of these children is difficult to predict and is considered an individual response of the patients to chemotherapy (Fatih et al., 2001). 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 (Fatih et al., 2001; Mosad et al., 2008; Einav, 2003). Chromosomal abnormalities in childhood ALL had important significance related to diagnosis, management and prognosis. Understanding of leukemogenesis is enhanced by identification of specific chromosomal alterations which pinpoint sites for molecular studies to identify genes involved in the transformation and proliferation of leukemic cells (Einav, 2003; Nina, 2006).

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)

X² = 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)	3 (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 2-33% (23-30). The highest frequency (33%) was reported by a French group (22) and the lowest came from Spanish group by Garcia-Sanz et al (23). Our figure (27.5%) in newly diagnosed ALL cases seems to lie on the high side of the reported frequencies. Comparable to our results that reported from Brazil (18%) (24), Italy and Germany (19%) (29), the Czech Republic (22%) (27) and USA (22% and 27%) (McLean et al., 1996; Rubnitz et al., 1997). Lower percentages were reported from India (9%) (28) and UK (11%) (30). Most of the fore-mentioned researchers used the RT-PCR technique for basic analysis of TEL-AML-1 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, but can be attributed to the usage of different sets of primers. Furthermore by using RT-PCR strategies, we found that a significant proportion of healthy individuals (5%) harbor the TEL-AML-1 gene rearrangement. These cases have same residency which may give an idea that the area was exposed to same environmental factors like radiation or due to exposure to an outbreak of viral infection. However, another study reported a frequency of 8.8% (Gary, 2001). It seems that underlying molecular mechanisms may play an additional role in the pathogenesis and in determining the clinical outcome of this subset (Greaves, 1997).

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 do we have to report or follow-up on RT-PCR positivity for a known oncogene in healthy individuals?, At a minimum investigators engaged in such analyses should consider 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 (Gary, 2001).

Clinical and laboratory features with recognized prognostic value in the childhood ALL includes age, sex, initial total WBC count, degree of organomegaly and early response to therapy (Greaves, 1997; Pui, 1995). 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 arbitrary³⁵. We found that 62.5% of TEL-AML-1 positive cases were classified as 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 the unnecessary combination of drugs with their side effect and acute toxicity and late-occurring adverse events in addition to the cost of these drugs on the patient³⁶. Our results is consistent with other reports on clinical features and significance of TEL-AML-1 positive cases, (Zuna et al., 1999; Harbott, et al., 1997). In addition the favorable impact of TEL-AML-1 is independent of age and leukocyte count¹¹. However, all the TEL-AML-1 positive cases in the study and control groups were below 6 years old, thus assigning our TEL-AML-1 positive patients to the standard risk group. This can be considered as factors in favour of TEL-AML-1 fusion gene as a marker for good prognosis.

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