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Detection of ETV6/RUNX1 Fusion Gene Using FISH Technique Detection in Pediatric ALL patients

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Abstract

Background One of the commonest genetic subtypes of acute lymphoblastic leukemia (ALL) is t (12;21) (ETV6/RUNX1) being associated with favorable prognosis and distinctive clinical and pathological features. There are few studies about this abnormality in Iraq.

Objective To detect the expression ETV6/RUNX1 fusion gene in B-ALL pediatric patients by using FISH technique.

Methods This cross-sectional study was conducted from April 2018 to September 2018. Forty-eight newly diagnosed children with B-ALL were enrolled in this study. Fresh peripheral heparinized blood sample (3 ml) were taken from the patient at admission before chemotherapy, and ETV6-RUNX1 probe was applied and reading done by florescent microscope.

Results The mean age of study group was (4.01±0.19) years, their median age was 4.1 years, ranging between (2-7.2) years at diagnosis, ETV6/RUNX1 chimeric transcript product was found in 19 of 48 (39.6%) pediatric B- ALL patients.

Conclusion The frequency of investigated translocation [t(12;21)/ETV6/RUNX1 in a sample of Iraqi pediatric B-ALL patients, was among the higher reported frequencies worldwide, and that ETV6/RUNX1 fusion gene is independent prognostic factor not related to other hematological and clinical parameters.

Keywords ETV6/RUNX1 fusion gene, pediatric ALL, FISH

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List of abbreviations: ALL = Acute lymphoblastic leukemia, FISH = Fluorescent in-situ hybridization, FTA cards = Flinders technology associate cards, Hb = Hemoglobin, LDH = Lactate dehydrogenase, PCR = Polymerase chain reaction, RBC = Red blood cell, WBC = White blood cell

Introduction

Genetic studies in acute lymphoblastic leukemia (ALL) had been a major contributing factor in diagnosis, prognosis therapy and shedding lights on the pathogenesis of the disease [(1)]. Therefore, classification is very important in ALL diagnosis. The six common genetic subtypes of ALL are t(1;19)(E2A-PBX1), t(12;21)(ETV6-RUNX1), t(9;22)(BCR-ABL), t(4;11) *MLL*-rearrangement and hyperdiploidy [(2)].

In 1995, two research teams discovered the t(12;21)(p13;q22) translocation, followed by other studies demonstrating that it is the most common genetic abnormality in pediatric ALL constituting about 25% of pediatric B-ALL while it was less frequent in adult ALL constituting nearly 2% [(3)].

However, by applying conventional cytogenetics, this chromosomal abnormality is barely detectable and may occur in less than 0.05% of childhood ALL. This is because the

t(12;21) is usually cryptic, and involve portions of the two chromosomes that are both small and have similar banding patterns. Therefore, it is better to be detected by fluorescence in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) [4-6].

The ETV6-RUNX1 fusion gene may arise as an early event during the prenatal period in pediatric ALL. This led to emergence of pre-leukemic clone, which after birth may give rise at low frequency to ALL after the having another necessary secondary genetic abnormality [7].

This work was done to detect the expression of ETV6/RUNX1 fusion gene in B-ALL pediatric patients by using FISH technique, and to find the correlation of ETV6/RUNX1 fusion gene to hematopathological parameters including complete blood count finding, blast count and lactate dehydrogenase (LDH).

Methods

A cross sectional study was conducted on 48 newly diagnosed B-ALL patients, who were attending Children Well Fair Teaching Hospital from April 2018 to September 2018.

The diagnosis of B-ALL depended on clinical findings, morphology and immunophenotype. The patient clinical data was obtained from patient hospital record and clinical monitoring chart.

After taking informed written consent from one or both parent, patients' samples were taken at admission. Peripheral blood collected in Na^+ heparinized tube, 1 ml of Na^+ heparinized blood sample labeled with patient name, age and date and those were stored as a fixed pellet at 4 ^oC in methanol: acetic acid (3:1) until FISH studies performed.

FISH was performed using directly labeled ETV6/RUNX1 Dual Fusion probes (Metasystem D-5115-100-OG) to show ETV6/RUNX1 fusion gene signals in cells with t (12:21) on chromosomes 21.

The orange labelled probe spans the breakpoint at 21q22(RUNX1) (646Kb) and include DNA sequence that hybridize (21q22.1), while the green labelled probe spans the breakpoint at 12p13(ETV6) (448Kb), which had DNA Sequence that hybridize (12p13).

Preparation of uncultured blood and slide preparation was done by applying standard protocol [8].

Slide reading was done by meta system fluorescents microscope using strict scoring criteria for FISH, orange RUNX1 signals are referred to as O, green ETV6 signals are referred to G, and ETV6/RUNX1 fusion signals as yellow infuse with green and orange. For each specimen, each microscopic scored 500 consecutive qualifying interphase nuclei from different area of the same slide. Samples were considered translocated positive when 4% cell showed the presence of fusion nuclei in which two probes were fused.

Statistical analysis

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 25. p value of >0.05 was considering to be non-significant, <0.05 was consider to be significant.

Results

The mean age of the study group was (4.01±0.19) years (mean±SE), their median age was 4.1 years, ranging between (2-7.2) years at diagnosis, majority of patient were 3 and 4 years old. Among 48 patients, 18 were females, representing (37.5%) and 30 were males representing (62.5%).

ETV6/RUNX1 fusion gene expression was positive in 19 patients representing (39.6%). While it was negative in 29 patients representing (60.4%) (Figures 1 and 2).

Regarding gender distribution (Table 1), 16 (53.3%) male patients were negative and 14 (46.7%) male patients were positive for the fusion gene, regarding female patients; 13 (72.2%) were negative and 5 (27.8%) were positive (P = 0.2) for the fusion gene. There was no significant difference in relation to gender between ETV6/RUNX1 positive cases and ETV6/RUNX1 negative cases (P = 0.20).

WBC count was significantly higher in ETV6/RUNX1 positive cases than in negative

cases, while hemoglobin level, platelet count, blast percent and LDH level showed no significant difference between positive and negative cases (table 2).

There was no significant difference in regard to clinical feature between ETV6/RUNX1 positive and negative groups (table 3).

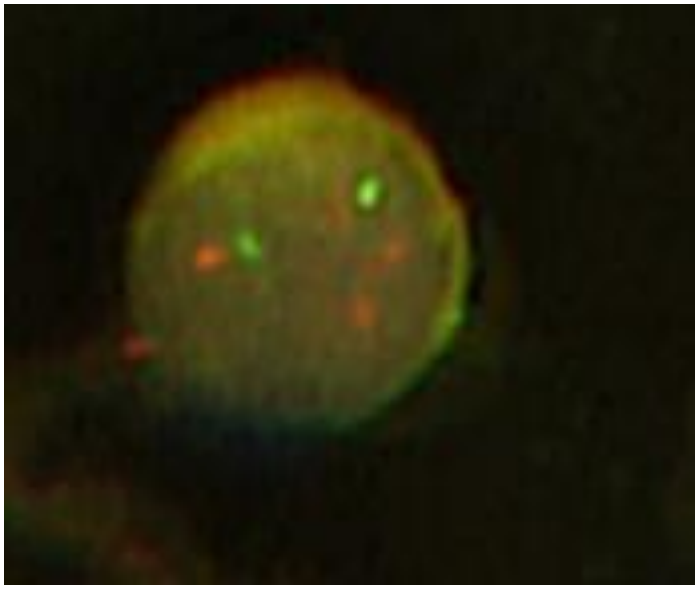

Figure 1. Fluorescent microscope image showing normal cells without fusion gene expression, 2 green signals for ETV6 gene, 2 orange signal for RUNX1 gene

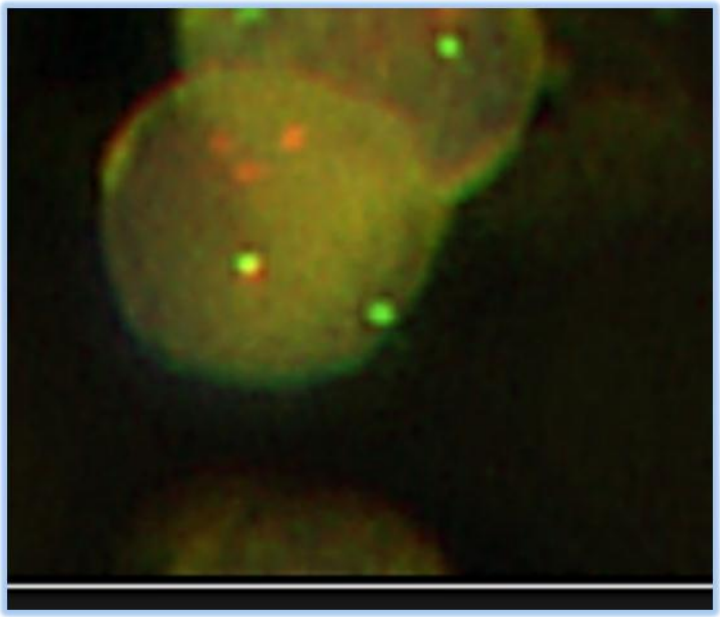

Figure 2. Fluorescent microscope image showing ETV6/RUNX1 fusion gene (green, orange and yellow)

Table 1. ETV6/RUNX1 fusion gene expression in relation to gender

Parameter	Variable	Male (n=30)	Female (n=18)	P
ETV6/Runx1	-ve N (%)	16 (53.3%)	13 (72.2%)	0.20 *NS
	+ve N (%)	14 (46.7%)	5 (27.8%)	

* Chi square test, NS: Non-Significant (P>0.05)

Table 2. White blood cell, hemoglobin, Platelet count and Lactate dehydrogenase in relation to ETV6/RUNX1 fusion gene expression

Variable		-ve N=29	+ve N=19	P
WBCs (10^9/L)	Mean±SE	39.92±4.83	60.72±8.11	0.04 *
	Median (Range)	35 (2.8-96)	69 (2.5-133)	S
Hb (mg/dl)	Mean±SE	9.54±0.27	9.19±0.34	0.42 *
	Median (Range)	9.4 (6.8-12.3)	9.2 (7.1-12.2)	NS
Platelets (10^9/L)	Mean±SE	129.14±10.62	120.95±10.45	0.84 *
	Median (Range)	111 (65-280)	110 (51-200)	NS
Blast cell (%)	Mean±SE	65.0±4.79	67.05±5.99	P=70 *
	Median (Range)	72 (14-95)	79 (11-95)	NS
LDH (IU)	Mean±SE	948.34±78.27	1033.2±92.55	0.49 *
	Median (Range)	989 (110-1882)	1110 (156-1781)	NS

*Mann Whitney test *significant (p<0.05), NS: Non-Significant (P>0.05)

Table 3. Clinical features in relation to ETV6/RUNX1 fusion gene expression

Symptoms and signs	-ve N=29 N (%)	+ve N=19 N (%)	P
Fever	19 (65.5%)	12 (63.2%)	0.87 * NS
Hepatosplenomegaly	14 (48.3%)	10 (52.6%)	0.77 * NS
Pallor	12 (41.4%)	8 (42.1%)	0.96 * NS
Vomiting	13 (44.8%)	5 (26.3%)	0.20 * NS
Weight loss	5 (17.2%)	4 (21.1%)	0.74 * NS
Jaundice	3 (10.3%)	4 (21.1%)	0.31 * NS
Lymphadenopathy	2 (6.9%)	2 (10.5%)	0.66 * NS
Nausea	2 (6.9%)	2 (10.5%)	0.66 * NS
Anorexia	2 (6.9%)	2 (10.5%)	0.66 * NS
Lethargy	3 (10.3%)	0 (0.0%)	0.15 * NS
CNS involvement	2 (6.9%)	0 (0.0%)	0.24 * NS
Bone pain	0 (0.0%)	1 (5.3%)	0.21 * NS

* Chi square test, NS: Non-Significant (P>0.05)

Discussion

In this study, t(12;21)/ETV/RUNX1 was detected by using FISH technique in 19/48 patient representing (39.6%). In Iraq, focus on these translocation done by two studies dealing with ETV6/RUNX1 fusion gene expression, Salih study that was done at 2015 using RT-PCR on 47 children to evaluate different types of translocation in ALL, revealed the presence of molecular abnormalities in (51.06%) patients; (27.65%) had ETV6/RUNX1, Other study done by Al-Kzayer et al. during 2012 using flinders technology associate (FTA) card on Iraqi children and it was conducted in Japan where ETV6/RUNX1fusion gene was detected in (12.1%) [9,10].

The result of current study goes with other study that showed the ETV/RUNX1 is the most frequent translocation of ALL [9]. While in Jordan the frequency of this fusion gene was 12.4% and in Kuwait it was 7% [11,12]. This difference may be related to the technique used in the studies where the latter two studies depend on cytogenetic analysis.

In relation to gender, the frequency of fusion gene was much higher in male than female, table (1), this result agreed with other studies, which found higher male/female ratio, and disagreed with other [10,13].

In present study, there was no significant correlation of the fusion gene with clinical features (table 3), however other published studies had showed that, fever, hepatomegaly, splenomegaly and LAP were more common features but CNS and testicular involvement less frequent [14].

Regarding hematological parameters only WBC count show significant difference between ETV6/RUNX1 positive cases and negative cases being higher in positive fusion gene. Other parameters including Hb, platelet bone marrow blast percent, and LDH level had no significant correlation with the presence of fusion gene. These results disagree with other studies showing that positive fusion gene cases do not have high WBC [14].

Therefore, we may conclude that ETV6/RUNX1 fusion gene is independent prognostic factor not related to other hematological and clinical parameters.

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Author contribution

Dr. Mahdi collected the cases data, performed the blood preparations, FISH procedure, statistical analyses reviewing the manuscript, Dr. Hameed and Dr. Al-Mamoori have role in study design and concept, work supervision, editing and reviewing the manuscript. Mahmood and Dr. Qassim provide technical support for FISH procedure, digital imaging using fluorescent microscope, involved in study design and reviewing the manuscript.

Conflict of interest

No conflict of interest.

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