RESEARCH ARTICLE

Low Frequency of ETV6-RUNX1 (t 12; 21) in Saudi Arabian Pediatric Acute Lymphoblastic Leukemia Patients: Association with Clinical Parameters and Early Remission

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Abstract

Background: Pediatric acute lymphoblastic leukemia (pALL) patients at King Abdulaziz Medical City represent a pure Saudi Arabian population. ETV6-RUNX1 positive pALL patients have good prognosis as compared to ETV6-RUNX1 negative counterparts. Therefore, frequencies of these two patient groups have a huge consideration in treatment strategies of pALL in a given population. Different geographical locations have been reported to have different frequencies of ETV6-RUNX1 ranging from 10% in Southeast Asia to 30% in Australia. Aim: Therefore, the objective of this study was to establish the ETV6-RUNX1 status of Saudi Arabian pALL patients and its association with clinical parameters and early remission. Materials and Methods: Clinical parameters and ETV6-RUNX1 status (using FISH technique) of pALL patients attending the Pediatric Oncology Clinic, King Abdulaziz Medical City, Riyadh from 2006 to 2011 were studied. Comparisons between ETV6-RUNX1 positive and negative groups were accomplished using chi-square test or Fisher's exact test. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC). Results: Out of 54 patients, 33 were male and 21 were females (ratio 1.57:1). B- and T-cell lineages were found in 47 (87%) and 7 (13%) patients respectively. Only 5 (9.3%) patients were ETV6-RUNX1 positive while 49(80.7%) were ETV6-RUNX1 negative. All ETV6-RUNX1 patients (100%) were of B-cell lineage and 80% (4/5) were in the 3-7 year age group. None of the ETV6-RUNX11 patients had ≥5% blasts (no remission) at day 14 as compared with 9% in the ETV6-RUNX1 negative group (Figure 1).Conclusions: Frequency of ETV6-RUNX1 positive patients (less than 10%) in our pALL patients is much lower than reported for most European countries, North America, Australia and Japan while it is in accordance with ETV6-RUNX1 frequencies from Egypt (11.6%), Pakistan (10%), Spain (2%) and India (5-7%). This shows ethnic differences in genetics of pALL as well as higher frequencies of ETV6-RUNX1 positive pALL mostly in more industrialized countries, probably due to some industrial pollutants or westernized lifestyle.

Keywords: Acute lymphoblastic leukemia - early remission - ETV6-RUNX1 - translocation - Saudi Arabia

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Introduction

Acute Lymphoblastic Leukemia (ALL) is the cancer of lymphoid lineage of blood forming pluripotent progenitor cells in the bone marrow (Bhojwani et al., 2015).ALL is characterized by an abnormal huge number of immature lymphocytes or blasts in blood and bone marrow (Ceppi et al., 2015). It is the most common cancer in children worldwide accounting for 25% of all cancers (Pui et al., 2015). National Cancer Institute reported a sharp peak in ALL incidence among children aged 2 to 3 years (>80 cases per million per year), with rates decreasing to 20 cases per million for ages 8 to 10 years. The incidence of ALL among children aged 2 to 3 years is approximately fourfold greater than that for infants and is nearly tenfold greater than that for adolescents aged 16 to 21 years (Ribera & Oriol, 2009). In Saudi Arabia, leukemia has been reported to be third most common cancer in males (Al-Ahmadi & Al-Zahrani, 2013) with ALL being the most common type of leukemia (Alghamdi et al., 2014; Al-Mutlaq et al., 2015). Unlike most of the western populations, Saudi Arabia has 41.7% of the population under 15 years of age, which put a large number of the population at risk of childhood cancer specifically ALL

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(Al-Mutlaq et al., 2015).

Many genetic abnormalities are found in Childhood ALL, which have implications in differential diagnosis/ classification, prognosis, drug selection at different phases of anti-leukemic treatment and in accessing the efficacy of the treatment (Cooper & Brown, 2015). ETV6-RUNX1 ((t (12; 21) (p13; q22)) is one of the most common chromosomal abnormalities in pediatric ALL patients and is associated with favorable prognosis and prolonged survival in majority of the patients (Moorman et al., 2014). In most of the European countries, US, Japan and Australia, ETV6-RUNX1 frequency has been reported to be 20-33% among pediatric ALL (Amor et al., 1998; Iqbal &Tanveer 2007; Awan et al., 2012; Inaba et al., 2013; Iqbal, 2014). Nevertheless, lower frequencies of ETV6-RUNX1 have been reported in some geographical regions (Kwong & Wong., 1997; Eguchi-Ishimae et al., 1998; Garcia-Sanz et al., 1999; Tsang et al., 2001; Rahman et al., 2006; Chung et al., 2010; Faiz & Qazi., 2010; Mazloumi et al., 2012; Iqbal et al. 2014). This shows ethnic differences in occurrence of this prognostically important genetic lesion.

Although there are few reports about the biology and clinical outcome of childhood ALL from Saudi Arabia, reports regarding the clinical characteristics and clinical outcome of the prognostically important genetic abnormalities are lacking. Therefore, this study was carried out to find out the association of ETV6-RUNX1 positive and ETV6-RUNX1 negative pediatric ALL patients with clinical features and treatment outcome in pediatric ALL patients from King Abdulaziz Medical City, National Guard Health Affairs, Riyadh, Saudi Arabia, which are a true representation of Saudi ethnicity (Wikipedia for Saudi Arabian National Guard, 2015) and therefore best sample to show if there are ethnic differences in genetic epidemiology (e.g., ETV6-RUNX1 frequency) of Saudi pediatric ALL from other ethnic groups in the world.

Materials and Methods

Patients

This study was conducted at Division of Pediatric Hematology/Oncology Department of Oncology / King Saud Bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City, National Guard Health Affairs, Riyadh, Saudi Arabia. Inclusion criteria were all

Table 1. Association of Patient Characteristics with day 14 Response in Pediatric Acute Lymphoblastic Leukemia Patients

			Response Day 14		
Covariate	Statistics	Level	Poor Response N=4	Good Response N=45	P-value*
gender	N (Row %)	female	1 (5.26)	18 (94.74)	0.555
	N (Row %)	male	3 (10)	27 (90)	
Age	N (Row %)	<= 2 Years	0 (0)	9 (100)	0.471
	N (Row %)	3 - 7 Years	2 (7.69)	24 (92.31)	
	N (Row %)	8 - 15 Years	2 (14.29)	12 (85.71)	
T-cell	N (Row %)	Negative	2 (4.65)	41 (95.35)	0.016
	N (Row %)	Positive	2 (33.33)	4 (66.67)	
B-cell	N (Row %)	Negative	2 (33.33)	4 (66.67)	0.016
	N (Row %)	Positive	2 (4.65)	41 (95.35)	
WBC	N (Row %)	<=30000	1 (2.7)	36 (97.3)	0.014
	N (Row %)	> 30000	3 (25)	9 (75)	
TEL-AML1	N (Row %)	Negative	4 (9.09)	40 (90.91)	0.482
	N (Row %)	Positive	0 (0)	5 (100)	

* The p-value is calculated by chi-square test

Table 2. Association of Patient Characteristics with day 29 Response in Pediatric Acute Lymphoblastic Leukemia Patients

			Response Day 14		
Covariate	Statistics	Level	Poor Response N=1	Good Response N=50	P-value*
gender	N (Row %)	female	0 (0)	21 (100)	0.398
	N (Row %)	male	1 (3.33)	29 (96.67)	
Age	N (Row %)	<= 2 Years	0 (0)	9 (100)	0.328
	N (Row %)	3 - 7 Years	0 (0)	26 (100)	
	N (Row %)	8 - 15 Years	1 (6.25)	15 (93.75)	
T-cell	N (Row %)	Negative	0 (0)	45 (100)	0.006
	N (Row %)	Positive	1 (16.67)	5 (83.33)	
B-cell	N (Row %)	Negative	1 (16.67)	5 (83.33)	0.006
	N (Row %)	Positive	0 (0)	45 (100)	
WBC	N (Row %)	<=30000	0 (0)	40 (100)	0.054
	N (Row %)	> 30000	1 (9.09)	10 (90.91)	
TEL-AML1	N (Row %)	Negative	1 (2.17)	45 (97.83)	0.739
	N (Row %)	Positive	0 (0)	5 (100)	

* The p-value is calculated by chi-square test

pediatric Acute Lymphoblastic Leukemia (ALL) patients between the ages of 2 to 15 years. Study was approved by King Abdullah International Medical Research Centre (KAIMRC), National Guard Health Affairs, Riyadh, Saudi Arabia.

Along with other laboratory parameters for diagnosis and risk stratification, patients were tested for ETV6-RUNX1 using interphase fluorescent in situ hybridization (FISH) as a part of routine laboratory findings.

Interphase fluorescent in situ hybridization (FISH)

Selection Of Material: Vysis ETV6/RUNX1 DF FISH Probe Kit (Abbot Laboratories, Illinois, USA) was used to detect ETV6/RUNX1 fusion oncogene resulting from t(12;21)(p13;q22). FISH procedures were carried out according to manufacturer's instructions.

Pre-hybridization, Hybridization and Post-hybridization: WBCs were washed with 1X PBS. Cells were fixed in methanol/acetic acid, dropped on slides, and air dried. The slides were pretreated with "0.01% pepsin + 0.02 M HCl" at 37°C for 10 min. The cells and probes were denatured on a heating plate together at 78°C for 10 min. Hybridization was performed overnight at 37°C. Posthybridization washing was done in 23 SSC containing 50% formamide for 7 min at 42°C followed by two washes in 23 SSC (42°C for 7 min). The slides were covered by Vectashield (Vector Laboratories, Burlingame, CA) containing 0.5 g/ml DAPI.

FISH Analysis: Slides prepared by FISH were analyzed using CytoVision 7.0 system (Applied Imaging, Biosciences Centre, Newcastle, UK).

Treatment protocol & Clinically Follow-up

CCG1991 protocol was used for standard risk patients while CCG1961 protocol was used for high risk patients. Number of blasts at day 14 and day 29 of the treatment were also calculated as a part of routine clinical follow-up.

Response criteria

Complete remission (CR or M1) was defined as: Normal bone marrow (with <5% blasts and >25% cellularity), neutrophil counts >1.5×109/l, platelet count > 100×109/l, and all extramedullary disease resolved. Anything less than CR was considered as incomplete remission (M2 or M3).

Statistical analysis

The association demographic data, clinical and laboratory parameters and ETV6/RUNX1 status was statistically studied using was performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC). Comparison between ETV6-RUNX1 positive and negative groups done using chi-square test or Fisher's exact test.

Results

Out of 54 patients, 33 were male and 21 were females (ration1.57:1). B- and T-cell lineage was found in 47 (87%) and 7 (13%) patients respectively. Overall, B-cell lineage and WBC count less than 30,000 were significantly associated with complete remission (M1) at day 14 and day 29 (Tables 1 & 2).

Only 5 (9.3%) patients with ETV6-RUNX1 positive while 49(80.7%) were ETV6-RUNX1 negative. Mean age of ETV6-RUNX1 positive and ETV6-RUNX1 negative patients was 5.90±0.495 and 3±0.316, respectively (<0.001). Moreover, 80% (4/5) of ETV6-RUNX1 positive patients were in 3-7 year age group while 46.94% (23/49) ETV6-RUNX1 negative patients in this age group. Furthermore, though 36.73% of ETV6-RUNX1 negative patients were in 8-15 years age group, no ETV6-RUNX1 positive patients were in this age group (Table 3, Figure 1).

Table 3. Demographics and Clinical Characteristics of ETV6-RUNX1 Positive and ETV6-RUNX1 Negative Pediatric Acute Lymphoblastic Leukemia Patients

Parameters	ETV6-RUNX1		P-value*
	Negative (n=49)	Positive (n=5)	
Gender			
Female	19 (38.78)	2 (40)	0.957
Male	30 (61.22)	3 (60)	
Age			
Mean±SEM	5.90±0.495	3±0.316	<0.001
Median	5	3	0.088
Range	1-13	2-4	
<= 2 Years	8 (16.33)	1 (20)	0.239
3 - 7 Years	23 (46.94)	4 (80)	
8 - 15 Years	18 (36.73)	0 (0)	
T-cell			
Negative	42 (85.71)	5 (100)	0.365
Positive	7 (14.29)	0 (0)	
B-cell			
Negative	7 (14.29)	0 (0)	0.365
Positive	42 (85.71)	5 (100)	
WBC (X103)			
Mean±SEM	28.61±8.64	10.68±5.12	0.083
Median	9.40	6.40	0.63
Range	0-400	3-30	
<=30	38 (77.55)	4 (80)	0.900
> 30	11 (22.45)	1 (20)	
Response @ 14			
Poor Response	4 (9.09)	0 (0)	0.482
Good Response	40 (90.91)	5 (100)	
Response@ 28			
Poor Response	1 (2.17)	0 (0)	0.739
Good Response	45 (97.83)	5 (100)	

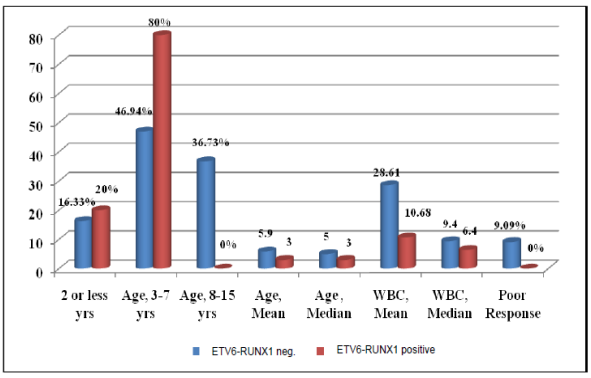

Figure 1. Comparison of Clinical Parameters of ETV6-RUNX1 positive and ETV6-RUNX1 Negative Pediatric Acute Lymphoblastic Leukemia Patients from Saudi Arabia

All ETV6-RUNX1 patients (100%) were of B-cell lineage as compared to 85.71% (42/49) of ETV6-RUNX1 negative patients (p=?). Similarly, none of the ETV6-RUNX1 positive patients was T-cell lineage as compared to 14.29% T-cell lineage ETV6-RUNX1 negative patients. Similarly, none of ETV6-RUNX1 positive patients had ≥5% blasts (no remission) at Day 14 as compared to 9% patients from ETV6-RUNX1 negative group (Table 3, Figure 1).

Discussion

Our study shows a low representation of ETV6-RUNX1 fusion oncogenes among pediatric ALL patients from King Abdulaziz Medical City, National Guard Health Affairs, Riyadh, Saudi Arabia. Most of ETV6-RUNX1 positive (80%) patients belonged to 3-7 years of age group and allETV6-RUNX1 positive showed early remission (day 14) as compared to 9% of ETV6-RUNX1 negative patients not showing day 14 remission.

Frequency of ETV6-RUNX1 in this study is in accordance with previous study from middle-east region reporting 14.7 % ETV6-RUNX1 frequency in pediatric ALL patients (Al-Mulla et al., 2014) although it is much lower than the 20-33% frequency in Europe, US, Japan and Australia (Shurtleff et al., 1995; Harbott et al., 1997; Kobayashi et al., 1997; Amor et al., 1998).

TEL-AML1 frequencies vary greatly in different countries of South East Asia and Southern Asia. A recent study from Pakistani reported the frequency of ETV6-RUNX1 in pediatric ALL patients to be 10.2% (Iqbal, 2014/10). ETV6-RUNX1 frequency has been found to be 17.1% in Korean pediatric ALL population (Chung et al., 2010), Taiwan 17% (Liang et al., 1996) and Malaysia 19% (Gill et al., 2005). In Egypt, ETV6-RUNX1 positivity among pediatric ALL has been reported to be 11.6% (Shaker et al., 2001). Although most of the studies reported higher ETV6-RUNX1 frequencies in Japan, one study from Hiroshima Japan reported it to be 10% (Eguchi-Ishimae et al., 1998). Contrary to other European countries, 2% ETV6-RUNX1 frequency has been reported in Spanish pediatric ALL patients (Garcia-Sanz et al., 1999).All of these studies indicate great ethnic and geographical variations in frequency of this prognostically important genetic abnormality specifically and genetics of pediatric ALL generally, which can have a significant bearing on global pediatric ALL management strategies (Wesołowska-Andersen et al., 2015).

In conclusion, like most of the other developing countries of the region, Saudi Arabia pediatric ALL patients have lower frequencies of ETV6-RUNX1 fusion oncogene than the developed countries like US, Europe, Japan and Canada. Further multicenter studies are required to find out frequencies of other prognostically significant genetic abnormalities in Saudi pediatric ALL patients in order to better plan and manage this highly curable disease in the kingdom.

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