RESEARCH COMMUNICATION

Fms-Like Tyrosine Kinase 3 Mutations in Childhood Acute Leukemias and their Association with Prognosis

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Abstract

Introduction: In recent years, Fms-like tyrosin kinase (FLT) 3 has been the subject of several studies as a prognostic marker. Objective: In this study, the presence of FLT3 mutations in childhood acute leukemias patients and their association with prognosis were investigated. Materials and Methods: A total of 120 patients, 80 with acute lymphoblastic leukemia (ALL) and 40 with acute myeloblastic leukemia (AML), were included. Real time polymerase chain reaction methods on a high resolution melting analysis device were used to determine FLT3 mutations. Results: FLT3/ITD (internal tandem duplication) mutations were found in 6 (7.5%) of the patients with ALL and in 9 (22.5%) of those with AML, whereas no FLT3/TKD (trans kinase domain) mutation was evident in any case. There was no difference between the ALL patients positive and negative for FLT3/ITD with regard to overall survival (OS), event free survival (EFS) and disease free survival (DFS) (p=0.37, p=0.23, p=0.023, respectively). However, in FLT3/ITD positive and negative AML patients, there was a statistically significant difference in OS (p=0.0041), but not EFS and DFS (p=0.09, p=0.095, respectively). A significant difference was found between age and FLT3/ITD positivity (p=0.036). Conclusion: We found that FLT3/ITD positivity increased with age and that it was associated with decrease in OS in AML patients, providing further evidence that it is an independent factor negatively influencing prognosis.

Keywords: Acute leukemia - FLT3/ITD - FLT3/TKD - prognosis - childhood

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Introduction

The fms-like tyrosine kinase 3 (FLT3) gene belong to the class III receptor tyrosine kinases and is predominantly expressed on hematopoietic progenitor cells in the bone marrow, thymus, and lymph nodes (Peng et al., 2008). FLT3 receptor, which has an important role in pathogenesis of leukemia, is expressed in different levels in the cells due to the type of acute leukemias. For example, high level FLT3 expression was demonstrated in 30% of the patients with T-acute lymphoblastic leukemia (ALL) and in 70-100% of the patients with acute myeloblastic leukemia (AML) and with B-ALL (Birg et al., 1992; Carrow et al., 1996; Stacchini et al., 1996). Internal tandem duplication (ITD) and trans kinase domain (TKD) mutations from FLT3 mutations lead to ligand-independent autophosphorylation of FLT3 receptor and consequently tyrosine kinase activation. As a result of this, the pathways related to proliferation, differentiation and survey are activated and resistance to apoptosis occurs.

In recent years, FLT3 has been a subject of several studies as prognostic marker. The studies conducted with both pediatric and adult patients reported that FLT3 mutations were associated with high risk of relapse and poor prognosis in AML patients. We investigated in this study the presence of FLT3 mutations in childhood leukemias and the association of these mutations with prognosis in the patients having these mutations.

Materials and Methods

Patient Samples

This study included 120 pediatric acute leukemia patients (80 ALL, 40 AML patients) applied to the Departments of Pediatric Oncology and Pediatric Hematology of Cukurova University Medical School between December 2000 and April 2010. The samples of bone marrows of all patients were evaluated morphologically according to the French-American-British (FAB) classification. After morphological and immunological diagnosis was made, idarubicin based protocol was started in AML patients and ALL-BFM (Berlin-Frankfurt-Münster)-Turkish-2000 protocol was started in ALL patients. The ALL patients were divided into 3 groups based on the response to 2 mg/kg prednisolone therapy on the 8th day of treatment: standard risk group (SRG), medium risk group (MRG) and high risk group (HRG).

This study was approved by the local ethics committee and all parents provided written informed consent.

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Laboratory Methods

For genotypic FLT3, DNA was extracted from bone marrow or peripheral bloood samples using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany).

Real Time Polymerase Chain Reaction (RT-PCR)

DNA samples were amplified for FLT3/ITD using the previously described primers 11F, 5'-GCAATTTAGGTATGAAAGCCAGC-3', and 12R, 5'-CTTCAGCATTTTGACGGCAACC-3'. The primers of 20F, 5'-CCGCCAGGAACGTGCTTG-3', and 20R, 5'-GCAGCCTCACATTGCCCC-3' were used for FLT3/TKD. One hundered nanograms of DNA was amplified in a 50 µl reaction containing 0.3 µmol/L each of each primer and 1X high fidelity master mix (Roche Diagnostic, Germany) (containing Tag and Tgo DNA polymerases, 0.2 mmol/L each dNTP, and 1.5 mmol/L MgCL2). The amplification performed on the LightCycler 480 device (Roche diagnostic, Germany) and entailed an initial denaturation of 94 °C for 2 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 64 °C for 30 seconds, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 5 minutes.

High-Resolution Melting Analysis (HRMA)

All FLT3 amplificons were subjected to HRMA. High resolution melting dye master mix was added to each amplificon at a final concentration of 10 µl of each sample was heated in a glass capillary on the LightCycler 480 device (Roche Diagnostic, Germany) at 95 °C for 1 minute and then cooled to 40 °C for 2 minutes with a temperature transition of 20 °C/second. Samples were then heated at 0.2 °C/second from 60 to 90 °C. This glass capillary-based single sample high resolution melting instrument collects fluorescence data at a rate of approximately 25 data points per 1°C. Results were analyzed as fluorescence versus temperature graphs with LightCycler 480 Software.

Statistical analysis

All statistical analysis were done using SPSS ver. 10.01 (statistical package for social sciences) software. Pearson Chi-Square test was used to assess the data of the patients and Kaplan-Meier test was used to evaluate the survival time of the patients. In addition, one-way ANOVA test and Mann-Whitney U test were used. The level of significance was set to a p value <0.05.

Results

The mean age of ALL patients was 84.3 ± 48.4 months (range, 2-210 months); and the mean age of AML patients was 86.0 ± 60.6 months (range, 9-192 months). Of ALL patients, 47 patients (58,8%) were boys and 33 patients (41,3%) were girls; of AML patients, 19 (47,5%) were boys and 21 patients (52,5%) were girls. The distribution of the patients according to FAB morphologic classification and properties of their final status were shown in Table 1.

Of 80 patients diagnosed with ALL, 12 patients (15%) relapsed during follow up. Of the patients relapsed, 6 patients (50%) died and 6 patients (50%) are still alive.

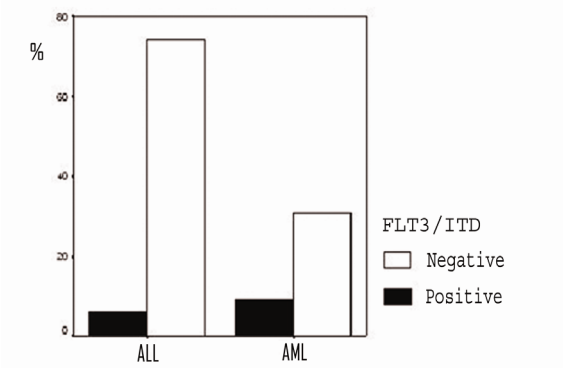

Figure 1. Rates of FLT3/ITD Positivity in ALL and AML Patients

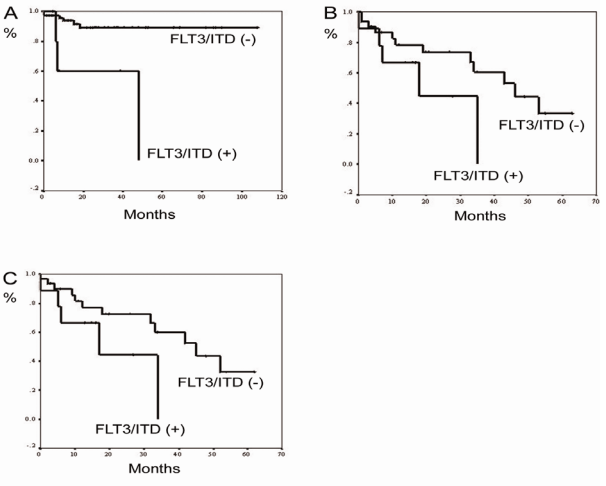

Figure 2. Kaplan-Meier analyses of the AML patients according to FLT3/ITD positivity. A: Overall survival, B: Event free survival, C: Disease free survival

Of 40 patients with AML, 12 (30%) relapsed. Two of the patients relapsed (16,6%) died. Among these relapsed patents, 3 patients (25%) completed the therapy, 6 patients (50%) continue chemotherapy and one patient (8,3%) receives maintenance therapy.

FLT3/TKD point mutation checked among acute leukemia patients was not found in any of 120 patients. In contrast, FLT3/ITD mutation was found in 15 (12,5%) of 120 patients. The rates of FLT3/ITD among ALL and AML patients were shown in Figure 1.

FLT3/ITD was found in 6 (7.5%) of 80 ALL patients. Of the patients, who were positive for FLT3/ITD, four were boys and two were girls. Three of them had ALL-L1 subtype, two patients had ALL-L2, and one patient had ALL-L3 subtype. Of these patients, three were in MRG, two patients were in SRG and one patient was in HRG group. Two patients relapsed and among them, three patients, including these two relapsed cases, died. One patient had central nervous system involvement. Among the patients who survived, one patient has already continued maintenance therapy and two patients have already received chemotherapy. Comparison of ALL patients in whom FLT3/ITD was determined and those in whom that was not determined with regard to OS, EFS and DFS did not reveal any statistically significant differences (p=0.37 for OS, p=0.23 for EFS, p=0.23 for DFS).

FLT3/ITD was found in 9 (22,5%) of 40 AML patients.

Table 1. Characteristics and Last Clinical Situations of ALL and AML Patients

	ALL n:80							AML n:40				
Male	47 (58.8%)							19 (47.5%)				
Female	33 (41.3%)							21 (52.5%)				
Age (mean ± SD, month)	84.5 ± 48.1							86.0 ± 60.6				
SRG	25 (31.3%)											
MRG	38 (47.5%)											
HRG	17 (21.2%)											
Subgrups (FAB)	L1	L2	L3	MLL	M0	M1	M2	M3	M4	M5	M6	M7
n	44	27	7	2	10	8	6	1	6	6	2	1
%	55	33,8	8,8	2,5	25	20	15	2,5	15	15	5	2.5

	ALL		AML	
	n	%	n	%
Follow-up without chemotherapy	12	15	12	30
With induction/ consolidation treatment	30	37.5	11	27.5
With maintenance treatment	28	35	9	22.5
Dead	9	11.3	8	20
Out off follow-up	1	1.25	-	-

ALL; acute lymphoblastic leukemia, AML; acute myeloblastic leukemia, SD; standart deviation, SRG; standard risk group, MRG); medium risk group, HRG; high risk group, FAB; French-American-British, MLL; mixed lineage leukemia.

Table 2. The Clinical and Laboratory Characteristics of ALL Patients According to FLT3/ITD Positivity

	FLT3/ITD (+)	FLT3/ITD (-)	P value
Age (mean ± SD,month)	132 ± 46.2	80.7 ± 46.4	0.015
Male (n - %)	4 (66.7)	43 (58.1)	> 0.05
Female (n - %)	2 (33.3)	31 (41.9)	
Hemoglobin (g/dl)	8.06 ± 1.99	8.02 ± 2.57	> 0.05
Leucocyte (/mm^3)	54,633 ± 76,955	54,996 ± 74,379	> 0.05
Platelets (/mm^3)	89,000 ± 77,079	110,889 ± 126,892	> 0.05
LDH (U/L)	949 ± 743	2,147 ± 3,225	> 0.05
Uric acid (mg/dl)	5.33 ± 1.13	6.11 ± 5.38	> 0.05
AST (U/L)	31.7 ± 12.7	49.5 ± 46.7	> 0.05
ALT (U/L)	28.2 ± 16.4	34.5 ± 50,30	> 0.05
BUN (mg/dl)	13.7 ± 2.3	14,29 ± 12.2	> 0.05
Creatinine (mg/dl)	1.7 ± 3.1	0.5 ± 0.42	> 0.05

ALL; acute lymphoblastic leukemia, SD; standart deviation, LDH; lactate dhydrogenase, AST; aspartat aminotransferase, ALT; alanine aminotransferase, BUN; blood urea nitrogen.

Table 3. The Clinical and Laboratory Characteristics of AML Patients According to FLT3/ITD Positivity

	FLT3/ITD (+)	FLT3/ITD (-)	p value
Age (mean ± SD,month)	97.6 ± 53.5	82.6 ± 62.9	>0.05
Male (n - %)	4 (44.4)	15 (48.4)	>0.05
Female (n - %)	5 (55.6)	16 (51.6)	
Hemoglobin (g/dl)	7.52 ± 2.22	7.33 ± 1.63	>0.05
Leucocyte (/mm^3)	45,598 ± 68,434	29,863 ± 33,155	>0.05
Platelets (/mm^3)	134,000 ± 160,918	81,000 ± 94,568	>0.05
LDH (U/L)	1,639 ± 2,723	1,919 ± 4,005	>0.05
Uric acid (mg/dl)	3.40 ± 1.46	4.78 ± 2.84	>0.05
AST (U/L)	14.5 ± 17.0	36.9 ± 25.6	>0.05
ALT (U/L)	19.1 ± 12.6	26.2 ± 37.2	>0.05
BUN (mg/dl)	12.7 ± 3.6	10.9 ± 5.93	>0.05
Creatinine (mg/dl)	0.42 ± 0.17	0.44 ± 0.22	>0.05

AML; acute myeloblastic leukemia, SD; standart deviation, LDH; lactate dhydrogenase, AST; aspartat aminotransferase, ALT; alanine aminotransferase, BUN; blood urea nitrogen.

Of the patients who were positive for FLT3/ITD, four patients were boys and five were girls. Two of them had AML-M1 subtype, one patient had AML-M2, one patient had AML-M7, one patient had AML-M6, and two patients had ALM-M0 subtype. Two of these patients relapsed and five patients, including these relapsed cases, died. Among the patients who survived, three patients have already continued maintenance therapy and one patient is under follow up after completion of the therapy. OS of AML patients positive for FLT3/ITD was found to be 67,5% at 12th month, 67,5% at 24th month and 33% at 36th and at 48th month. OS of AML patients negative for FLT3/ITD was higher, 90% at 12th, at 24th and at 36th months. With regard to OS, the difference between AML patients in whom FLT3/ITD was determined and those in whom that was not determined was statistically significant (p=0.0041). With regard to EFS and DFS, there was no difference between AML patients in whom FLT3/ITD was

determined and those in whom that was not determined (p=0.09 for EFS; p=0.095 for DFS) (Figure 2).

In whole study group, the mean age of the patients in whom FLT3/ITD was determined was 111,3 ± 52,0 months (range, 18-180 months) whereas the mean age of the patients in whom FLT3/ITD was not found was 81 ± 51,8 months (range, 2-210 months). The difference between them was statistically significant (p<0.05). The mean age of the patients positive for FLT3/ITD was 132 ± 46,16 months (range, 60-180 months) among ALL patients and 97,56 ± 53,48 months (range, 18-180 months) among AML patients, however, the difference was not found to be statistically significant (p= 0.22).

In our study, no significant relationship was found between laboratory results and FLT3/ITD positivity in both ALL and AML patients (p>0.05) (Table 2,3).

Discussion

FLT3 activation mutations were first recognized in AML patients and are the most common somatic mutations in that disease. In the studies conducted with adult AML patients, the incidence of FLT3/ITD varies from 15% to 35% (Kottaridis et al., 2001; Yamamoto et al., 2001; Frohling et al., 2002; Schnittger et al., 2002; Thiede et al., 2002), and it was reported to be 5-9% for FLT3/TKD (Abu-Duhier et al., 2000; Fenski et al., 2000; Abu-Duhier et al., 2001; Yamamoto et al., 2001; Thiede et al., 2002; Gari et al., 2008). In pediatric studies, the incidence of FTL3/ITD among AML patients is lower than that of adults, it was found to be approximately 5-16% (Iwai et al., 1999; Kondo et al., 1999; Xu et al., 1999; Meshinchi et al., 2001). The incidence of FLT3/TKD was found to be 6,7% in one of two pediatric studies conducted by Meshinchi et al., (2003; 2006), and to be 7% in the other study. In a study performed by Peng et al., (2008) in 2008 with 120 adults newly diagnosed with leukemia, the incidence of FLT3/ITD mutation was found to be 25% in AML and 7% in ALL, similar to results of our study, and they suggested that FLT3/ITD positivity was associated with poor prognosis.

Xu et al., (1999), in a study with 194 pediatric patients (87 AML patients, 60 ALL patients, 32 MDS patients and 15 patients with juvenile chronic myeloid leukemia) in which RT-PCR method was used, found FTL3/ITD mutations in the rates of 13,8% in AML and 3,3% in ALL, lower than the rates found in our study. It was reported that those patients in whom FLT3/ITD was found died within 44-72 months.

In our study, we found FLT3/ITD mutation in 15 (12,5%) of 120 pediatric patients with acute leukemia. We found that mutation positive in 9 (22,5%) of AML patients and in 6 (7,5%) of ALL patients. In our study, FLT3/IDT mutation rate was found to be higher than that reported in some studies mentioned above in the pediatric literature. Although the number of the studies with ALL patients is limited, our results are similar to the rates in adult patients.

In our study, OS was found to be reduced in FLT3/ITD positive AML patients. In several studies in which PCR was used, as we did, it was demonstrated that FLT3/ITD positivity was associated with reduced OS (Kottaridis et al., 2001; Thiede et al., 2002). In two studies evaluating one thousand patients, similar to our study, it was shown that FLT3/ITD expression was related to reduced OS 4 years after cessation of comparable therapies (Kottaridis et al., 2001; Yamamoto et al., 2001). In another study with 224 AML patients who had normal cytogenetics and were followed up for an average duration of 34 months, it was found that the patients expressing FLT3/ITD mutation had shorter OS (Frohling et al., 2002).

In our study, we did not find any statistically significant difference between AML patients in whom FLT3/ITD was determined and those in whom that was not determined with regard to EFS and DFS. This situation may be related to few numbers of positive patients. In the studies with adult AML in the literature, a significant reduction in OS, EFS and DFS (Kottaridis et al., 2001; Levis and Small, 2003). In a study evaluating 461 pediatric AML patients, survival rate of AML patients with FLT3/ITD was 19%, whereas that rate was as higher as 58% in the patients with wild-type FLT3 receptor. In this study, the rate of remission induction was 40% and EFS was only 7% in the patients with FLT3/ITD mutation; whereas in the patients who did not have mutation, remission induction rate and EFS were found to be 74% and 44%, respectively (Nakao et al., 1996).

In a recent meta-analysis with AML patients, it was asserted that FLT3/ITD mutations markedly shortened OS and that they were effective as well as ITD to shorten DFS (Yanada et al., 2005). A study conducted by Yamamoto et al., (2001) with 201 pediatric AML patients showed that FLT3/TKD positivity shortened DFS. Since FLT3/TKD mutation was not found in our study, its effect on prognosis couldn't have been assessed. In the literature, there are few number of the studies reporting statistically significant results on the association of TKD with prognosis, since FLT3/TKD positivity is less common than ITD. Therefore, further studies are needed to determine whether or not it does have an effect on prognosis (Thiede et al., 2002).

It was determined an association between age and incidence of FLT3/ITD mutation in acute leukemic patients. As in our study, the frequency of mutations increase as age advances. In pediatric studies, it has been denoted that FLT3/ITD incidence increases over 10 years of age and becomes closer to adult frequency. However, in the studies with adults, it was found that frequency did not change with age (Parcell et al., 2006).

In a study conducted by Thiede et al., (2002) including 979 AML patients, it was shown that both mutations of FLT3/ITD and FLT3/TKD was associated with high leukocyte count. In a study conducted by Peng et al. (2008), ITD positivity was found to be related to high leukocyte count and elevated LDH level; and they showed that FLT3/ITD was an independent prognostic factor in AML. In a study performed by Kottaridis et al., (2001), it was highlighted high leukocyte count and the increase in the risk of relapse with the presence of FLT3/ITD in the patients. Additionally, they indicated that this situation was inversely related to OS, EFS and DFS, so it was a poor prognostic factor. In a study conducted by Yanada et al. (2005), it was suggested that FLT3/ITD was a strong independent marker of poor prognosis in pediatric AML.

In our study, no significant association was found between FLT3/ITD positivity and laboratory results, particularly leukocyte count and LDH levels, because of insufficient number of the patients.

In conclusion, we found in our study that FLT3/ITD positivity increased as age advanced in childhood acute leukemias and that FLT3/ITD positivity was associated with a decrease in OS. In accordance with the data in literature, we concluded that FLT3/ITD positivity was an independent risk factor influencing prognosis negatively.

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