RESEARCH ARTICLE

Frequency of FLT3 (ITD, D835) Gene Mutations in Acute Myelogenous Leukemia: a Report from Northeastern Iran

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Abstract

Background: FLT3 is mutated in about 1/3 of acute myelogenous leukemia (AML) patients. The aim of the present study was to report the prevalence of FLT3 mutations and comparison with prognostic factors in AML patients in the Northeastern of Iran. Materials and Methods: This cross-sectional study concerned 100 AML cases diagnosed based on bone marrow aspiration and peripheral blood. DNA for every AML patient was extracted and underwent PCR with FLT3-ITD primers. Results: The mean age at diagnosis was 28.5 years (range, 1-66 years), 52 patients (52%) being male. Out of 100 AML patients, 21 (21%) had FLT3 mutation, (17 with FLT3-ITD, 81%, and 4 with FLT3-D825, 19%). Of the 21, 14 (66.7%) had heterozygous mutation. There was no significant difference between age, sex and organomegaly between patients with FLT3 mutation versus FLT3 wild-type. Conclusions: Our frequency of FLT3 is in line with earlier fidnings of approximately 20 to 30% and also the prevalence of FLT3-ITD is more than FLT3-D35 mutation. There was no significant difference between prognostic factors (age and sex) in the patients with FLT3 mutation versus FLT3 wild-type. The prevalence of FLT3 heterozygous mutations is more that homozygous mutations in AML patients.

Keywords: Acute myelogenous leukemia - FLT3 mutation - heterozygous mutation - Northeastern Iran

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Introduction

Acute Myelogenous Leukemia (AML) is an aggressive hematologic malignancy that is characterized by accumulation of immature myelogenous cells in the blood and bone marrow (Payandeh et al., 2015). FMS-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase with important roles in hematopoietic stem/progenitor cell survival and proliferation. It is mutated in about 1/3 of AML patients, either by internal tandem duplications (ITD) of the juxtamembrane domain or by point mutations usually involving the kinase domain (KD) (Small D, 2006). Activating mutations of FLT3 are now recognized as the most common molecular abnormality in acute myeloid leukemia, and FLT3 mutations may play a role in other hematologic malignancies as well. The poor prognosis of patients harboring these mutations renders FLT3 an obvious target of therapy (Levis and Small, 2003). The incidence of FLT3/ITD mutations varies according to age and clinical risk group, being less common in pediatric AML and in AML arising from an antecedent myelodysplastic syndrome (Scholl et al., 2008; Dicker et al., 2010). There is less of a clear pattern with FLT3/TKD mutations (Kok et al., 2013; Mead et al., 2007; Elyamany et al., 2014), which are reported to occur in approximately 7% of patients, although they seem to be more common in cytogenetically favorable risk AML (Kok et al., 2013; Mead et al., 2007). The prevalence of an ITD of the juxtamembrane domain-coding sequence and a missense mutation of D835 within the kinase domain of the FLT3 gene is 15-35% and 5-10% of adults with AML, respectively (Kiyoi and Naoe, 2006). The frequency of FLT3-D835 mutation , according to the literature, in all ages (adults and children together)is 6%-10% (Yamamoto et al., 2001).

The aim of study is to report the prevalence of FLT3 mutations and comparison of prognostic factors in AML patients with FLT3 mutations with FLT3 wild-type in the Northeastern of Iran.

Materials and Methods

Patients

In 2015 and in a cross-sectional study, 100 AML patients referred to Cancer Molecular Pathology Research Center, Ghaem Hospital, Mashhad, Iran. AML was

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diagnosed based on bone marrow aspiration and peripheral blood.

Molecular analysis

For mutation detecting, DNA was extracted from 100 bone marrow biopsy with preparation with QIAamp DNA tissue reagent according to the QIAGEN protocol (Figure 1). The concentration and quality of the DNA were analyzed with NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). A ratio value of 1.8 was considered to indicate DNA purity. As FLT3 mutations encoded by GATATC nucleotides sequence, which exhibits an EcoRV restriction, are detected by Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR), followed by electrophoresis on 2.5% agarose gel and Ethidiom Bromide staining. Primers had been designed by Blast software (NCBI.GOVE site) and had been synthesized by METABION Company (Germany). The following primers were amplified ITD mutation region; 14 Forward 5' GCAATTTAG-GTATGAAAGCCAGC-3' and 15 Reverse 5' CTTTCAGCATTTTGACGGCAACC-3' were used over 35 cycles that include 1 minute 94 C°, 30 seconds 60C° and 90 seconds 72C°. The following primers were performed for TKD mutation; 17 Forward 5' -CCGCCAGGAACGTGC-TG-3' and 17 Reverse 5'-GCAGCCTCACATTGCCCC-3' over 35 cycles that include1 minute at 94 C°, 1 minute at 66 C° and 90 seconds at 72 C°, too. The master mix was composed of 1μl DNA (1μg/ml), 10 Pmol of each primer, 10mmol dNTP, 2.5 Unit EX- Taq DNA polymerase (Takara-Japan) in the buffer (10 mmol/l Tris–HCl (pH 8.3), 50 mmol/l KCl and 1.5 mmol/l MgCl2). PCR procedure was performed in ABI verity thermo cycle machinery, Amplified products were incubated with EcoRV restriction enzyme overnight in 37C° and electrophoresis was done on agarose gel as described before. ITD mutations were detected by agarose gel electrophoresis of the PCR products. This protocol usually demonstrates a wild-type band (328bp) and a larger-size band that was the ITD mutation. TKD mutations were detected by electrophoresis of the amplified products following digestion with Eco RV(Thermo scientific, (EU) Lithuania). The amplified products of wild-type alleles were digested into two bands (68 bp and 46 bp) by Eco RV. When amplified products contain TKD mutations, undigested bands (114 bp) were visualized on agarose gel electrophoresis. Inclusion of a negative control is essential to ensure complete digestion by Eco RV, therewith eliminating the possibility of false-positive results in patient samples (Kooshyar et al., 2015).

Statistical analysis

The data were analyzed with SPSS version 19 software that T-test was used for comparison of the means and Chi-square other values. P<0.05 was considered statistically significant.

Results

The mean age at diagnosis was 28.5 years (range, 1-66 years) that 52 patients (52%) were male (Table 1). Out of 100 patients with AML, nineteen patients (19%) had hepatomegaly and 21 patients (21%) had splenomegaly at diagnosis. Twenty-one patients (21%) had FLT3 mutation.

The prevalence of FLT3 mutations based on site has been shown in Table 2. There was two type of mutation for FLT3, FLT3ITD and FLT3-D835. Out of 21 patients with FLT3 mutation, 17 patients (81%) had FLT3-ITD mutation and 4 patients (19%) had FLT3-D825 mutation. Out of 17 patients with FLT3-ITD mutation, site of mutation in 7 patients (41.2%) was homozygous and in 10 patients (58.8%) was heterozygous and also in 4 patients with

Table 1. Baseline Variables of Patients (n=100)

Variables	n(%)	Mean±SD	Range
Age, years		28.5±18.6	1-66
Sex			
Male	52(52)		
Female	48(48)		
Hepatomegaly			
Yes	19(19)		
No	81(81)		
Splenomegaly			
Yes	21(21)		
No	79(79)		
FLT3			
Mutation	21(21)		
Wild-type	79(79)		

Table 2. Prevalence of FLT3 Mutations Based on Site

Mutation	Homozygous	Heterozygous	Total
FLT3-ITD	7(41.2)	10(58.8)	17(81)
FLT3-D835	0	4(100)	4(19)
Total	7(33.3)	14(66.7)	21(100)

Table 3. Correlation between Variables in Patients with/without FLT3 Mutations

Variables	FLT3 mutation N=21	FLT3 wild-type N=79	P-value
Age, years	29.6±16.3	28.2±19.2	0.76
Sex, n(%)			0.43
Male	10(47.6)	41(42.6)	
Female	11(52.4)	37(47.4)	
Organomegaly			0.42
Yes	5(25)	16(20.3)	
No	15(75)	63(79.7)	

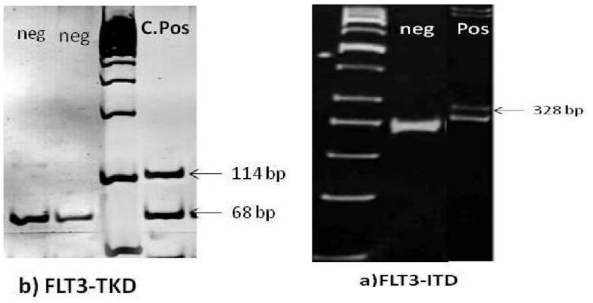

Figure 1. Electrophoresis of PCR Products Related to FLT3-ITD/TKD. (a) PCR product of the FLT3/ITD mutation in the juxtamembrane domain. Positive control (C.Pos) is included. (b) EcoRV restricted of positive control related to FLT3-TKD

FLT3-D835 mutation, site of mutation in all patients was heterozygous. Therefore, out of 21 patients with FLT3 mutation, 14 patients (66.7%) had heterozygous mutation.

The correlation between variables in patients with/without FLT3 mutations has been shown in Table 3. There was no significant difference between age, sex and organomegaly between patients with FLT3 mutation versus FLT3 wild-type (P>0.05).

Discussion

One study with bone marrow samples from 116 children with newly-diagnosed AML (Ruan et al., 2011), reported that FLT3/ITD and FLT3/TKD mutations were detected in 9 cases (7.8%) and 13 cases (11.2%). Another study (McCormick et al., 2010), showed that 38 of 114 patients with (diagnosis/relapse) specimens of AML (33%) were positive for FLT3 mutations (33 (86.8%) FLT3/ITD, 5(13.2%) FLT3/TKD). Quentmeier et al.(2003) screened 69 AML patients for FLT3 mutations that four cases (5.8%) showed FLT3-ITD mutation, none carried a D835 point mutation. The presence of ITD was analyzed in diagnostic samples of 176 AML patients and the D835 mutation in 135 of these patients. In all, 41 (23%) patients harbored FLT3 mutations, with 34 (19.3%) of them positive for FLT3-ITD, and seven (5%) positive for FLT3-D835 mutation (Chillón et al., 2004). Elyamany et al.(2014) selected 97 patients with AML at the diagnosis that the mean age 37.8 years (range, 1-82 years). Of the 97 AML patients, 47 were males (48.5%); 18 cases (18.55%) were positive for FLT3 mutations. In these 18 FLT3-positive cases, 14 cases (77.8%) had FLT3-ITD and 4 cases (22.2%) were found to contain the FLT3-D835 mutations. Also, one study was conducted in Saudi Arabia for FLT3 mutations in AML patients that showed frequency of 20.15% (26/129) (Gari et al., 2008). Two other studies (Gilliland and Griffin, 2002; Stirewalt and Radich, 2003), showed that mutations of FLT3 have been detected in about 30% and approximately 25% of patients with AML. Alvarado et al. (2014) examined 69 FLT3-mutated patients with AML that 87% of patients had an ITD mutation, 7% had a D835/I836 mutation, and 6% had combined ITD and D835/I836 mutations. In 67 AML patients with FLT3 mutation from Southern India, FLT3-ITD mutations were found in 55 (82.1%) cases, the TKD (Asp 835) mutation in 11 (16.4%) cases, and in one (1.5%) case we observed a combined mutation (Sarojam et al., 2014). Out of 100 patients in this study(mean age 28.5 years, range(1-66 years), 52% male), 21 patients had FLT3 mutation that 17 patients (81%) had ITD mutation, 19% D835 mutation and none carried combination of both mutations. The prevalence of FLT3 mutation is approximately different based on ethnicity and area of geography in the studies. Lee et al. (2007) reported that the comparison between FLT3-ITD positive versus FLT3 wild-type, displayed trends for increased of presence of organomegaly. Dehbi et al.(2013) presented 33 normal karyotype AML patients (13 males and 20 females) with a median age of 30 years (range, 3-58 years).The mean age of the FLT3-ITD group was 37 years against 30 years for the non-mutated group. Also, it didn't find any significant association between the FLT3-ITD mutation with age, sex, hemoglobin level, white blood cell count, platelets, and blasts percentage. Although, the percentage of organomegaly was more in this study for FLT3 mutation group versus FLT3 wild-type group, but this difference was no significant. Also, there was no significant difference between two groups with age and sex.

Dehbi et al. (2013) showed that patients who presented with the FLT3-ITD mutation were older than the group with wild type (37 years vs. 30 years); this observation was similar to result of Auewaraku's study (Auewarakul et al., 2005). This study showed that there was no significant difference between FLT3 mutation group versus FLT3 wild-type group for age (29.6 versus 28.2 years). FLT3 gene mutations were somewhat equally distributed in both males (49%) and females (51%) (Sarojam et al., 2014) that our study confirmed it (47.6% male and 52.7% female). In most cases for AML, FLT3 mutation is heterozygous (Gari et al., 2008). Sarojam et al. (2014) reported that all mutants (ITD and TKD) in their study were heterozygous for FLT3 mutation that our study confirmed these results (66.7% heterozygous).

In conclusions, The frequency of FLT3 in a lot of studies is approximately between 20 to 30% and also the prevalence of FLT3-ITD is more than FLT3-D35 mutation. There was no significant difference between prognostic factors (age and sex) in the patients with FLT3 mutation versus FLT3 wild-type. The prevalence of FLT3 heterozygous mutations is more that homozygous mutations in AML patients.

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