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Evaluating The Frequency Of Flt3-Tkd Among Patients Suffering Acute Myeloid Leukemia In Baghdad Province, Iraq

Israa Jasim Alswaili[1], Hadideh Mabudi[2,*], Elham Konar[2]

1 Department of Genetics, Science and Research Branch, Islamic Azad University, Tehran, Iran.

2 Department of Genetics, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.

Abstract

Background: Molecular basis of acute myeloid leukemia (AML) is a mutation in genes that regulate cell proliferation and differentiation. Mutation in the FMS-like tyrosine kinase 3 (FLT3) receptor gene is one of the most common mutations in AML, which causes abnormal proliferation and survival of leukemic cells.This study aimed to diagnose and determine the frequency of FLT3- tyrosine kinase domain (TKD) mutation in patients with AML.

Methods: Patients with AML were evaluated for FLT3-TKD mutation with Sanger sequencing.

Results: 50 patients including 27 (54%) male and 23 (56%) female were included. The mean age was 28.7 ±9.01 years. Among all patients, just 3 (6%) subjects have FLT3-TKD mutation. There are no significant differences for gender and age between patients with mutation and without FLT3-TKD mutation (P-Value =0.53) and (P-Value =0.32), respectively.

Conclusion: Current survey indicated that the FLT3-TKD has a low incidence among AML patients in Baghdad. Further analysis with larger sample size, disease subtype evaluation, and treatment response is recommended.

Keywords: Acute myeloid leukemia; AML; FLT3-TKD mutation; FMS-like tyrosine kinase 3

Introduction

Acute myeloid leukemia (AML) is one of the most prevalent poor prognosis leukemia in adults, characterized by the proliferation of malignant myeloid progenitor cells. With increasing age, its incidence has increased with a median age of 68 years (1). Exposure to the DNA damaging agents, previous therapies causes AML development; however, most patients suffer from an idiopathicform of the disease.Several factors influence disease progression, including age, symptoms in disease onset, genetic abnormalities, and comorbidities. This is one of the main explanations forAML heterogonous.

Among them, the mutations, chromosomal translocations, and cytogenetic abnormalities have the most impacts on prognosis.However,intensive chemotherapy and stem cell transplantation have shown favorable outcomes in AML but not in all patients. These therapeutics influence underlying factors, that the most important of which is the existence of genetic defects.

AML patients with normal karyotype have recurrent genetic aberrations ofFMS-like tyrosine kinase 3 (FLT3), CEBPA, NPM1, RUNX1, TET2, IDH1/IDH2, DNMT3A, ASXL1, MLL, and WT1 mutations are more prevalent(2). Of these, FLT3 is more prevalent.The FLT3 mutation is seen in two forms: successive internal duplication (FLT3 / ITD) near the membrane below the receptor and the other in the tyrosine kinase domain (FLT3-TKD). Mutation in FLT3 cause continuous tyrosine phosphorylation resulting in activation of the tyrosine kinase receptor. FLT3-ITD mutation is the most common form of FLT3 defect that has been shown to cause poor prognosis in AML patients, while there is little and old data about the effect of FLT3-TKD on disease progression(3).There is evidence that patients with FLT3-TKD mutation respond better to chemotherapy at the time of AML diagnosis than patients with FLT3-ITD(4). Nevertheless, due to low prevalence, it is difficult to explain the effect of FLT3-TKD on the AML process (5). In this regard, due to the high prognostic value of FLT3 gene mutation in patients with AML, there is a strong focus on developing drugs that have the best outcome in AML with FLT3 mutations(6,7).

Material and Methods

Patients and study design

In the current survey, the AML patients were included. The selection of participants was as follow diagnostic of AML without considering the type of AML based on the FAB classificationand normal karyotype at the time of diagnosis. There are no restrictions for gender and age. Patients with normal karyotype but with other mutations were excluded. The study was carried out accordingto the Ethics Committee of Iran's Ministry of Health andMedicalEducation guidelines.All participants were assigned the consent form.

MutationAnalysis

DNA was extracted from peripheral blood cells using Magcore automated nucleic acid extraction (Switzerland). The purity and concentration of the extracted DNA were measured using the Nanodrop spectrophotometer (Thermo).The first nucleotide G of codon 835 was exclusively substituted with T (Asp835Tyr) in FLT3-TKD mutationwas detected by following primers: forward primer: AGTGAGGATTGCACTCAAAGG, and reverse primer: GTTTGTTGCACATCATCATGGC.

PCR Reaction

FlexCycler Thermocycler carried out the PCR reaction as follows: 12.5 µL Master Mix 2X (Taq Mix Red, PCR bio, UK), 1µL DNA (100ng), 0.5 µL forward primer. 0.5 µL reverse primer, 0.25 µL forward control primer, 0.25 µL reverse control primer, and H2O up to a final volume of 25 µL. The PCR reaction was performed as follows: initial denaturation at 95 ^{o}C for 5 min, followed by 30 cycles of 95^{o} C for 60 sec, 62^{o}C for 45 sec, 72^{o}C for 60 sec, and 72^{o}C for the final extension.

Sequencing

Sanger sequencing was carried out toconformational PCR reactions. All positive samples were sequenced by ABI-3130 XL (USA). The result of the sequences data was visualized by UGENE software.

Statistical analysis

The Chi-square test was used to determine the relationship between qualitative variables. Odds ratio (OR) with 95% confidence interval (95%CI) were calculated. A p-value considered less than 0.05 (P<0.05). An unconditional logistic regression analysis will be used to control possible confound in factors. Data management and analysis were performed using SPSS software (V24).

Results

Fifty patients were recruited for this study. Of these, 27 (54%) were male, and 23 (56%) were female. The mean age was 28.7 ±9.01 years. All patients were diagnosed according to the FAB classification. The most frequent AML type was M3 (32%) and followed by M2 (26%), M4 (20%), M5 (13%), M0 (3%), M7 (3%),M6 (2%), and M1 (1%), respectively(Figure 1). 15 patients(30%) underwent bone marrow transplantation after recovering completely. 14 patients (28%) died due to related-treatment adverse events, and 3 patients (6%) expired during treatment (Table 1).Among all patients, just 3 (6%) subjects have FLT3-TKD mutation (Figure2). There are no significant differences for gender and age between patients with mutation and without FLT3-TKD mutation (P-Value =0.53) and (P-Value =0.32), respectively.

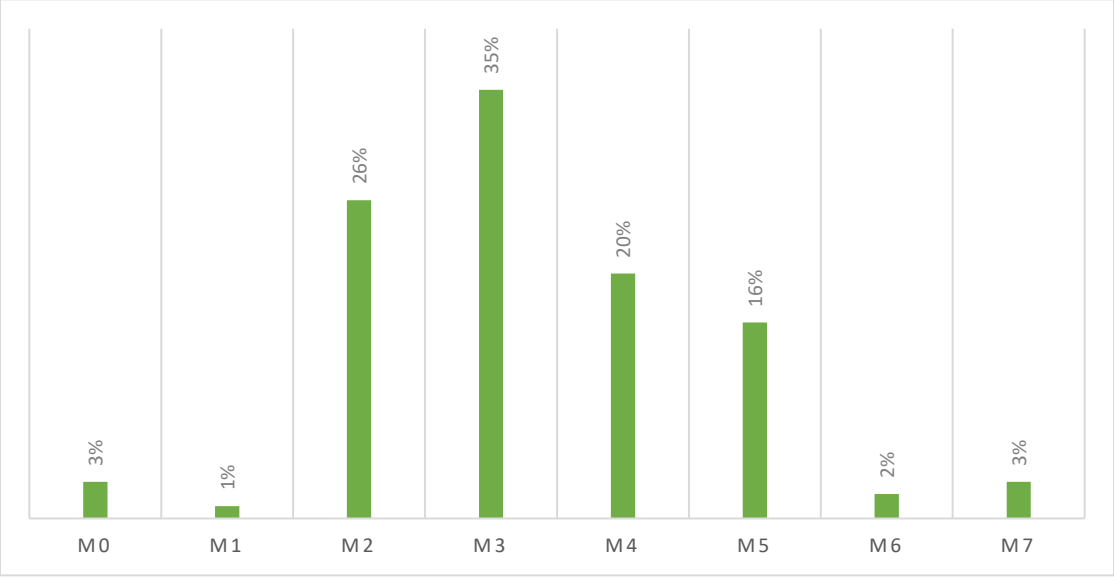

Figure 1. AML subtype frequency.

Table 1. Clinical information of patients.

Variables	Reports
Gender (%)	
Male	27 (54)
Female	23 (46)

Mean age ±SD (year)	28.7 ±9.01
Subtype AML frequency (%)	
M0	3%
M1	1%
M2	28%
M3	35%
M4	20%
M5	16%
M6	2%
M7	3%
Number of death patients (%)	
Related-treatment death	14 (28)
During treatment	3 (6)
Recovered patients (%)	15 (30)

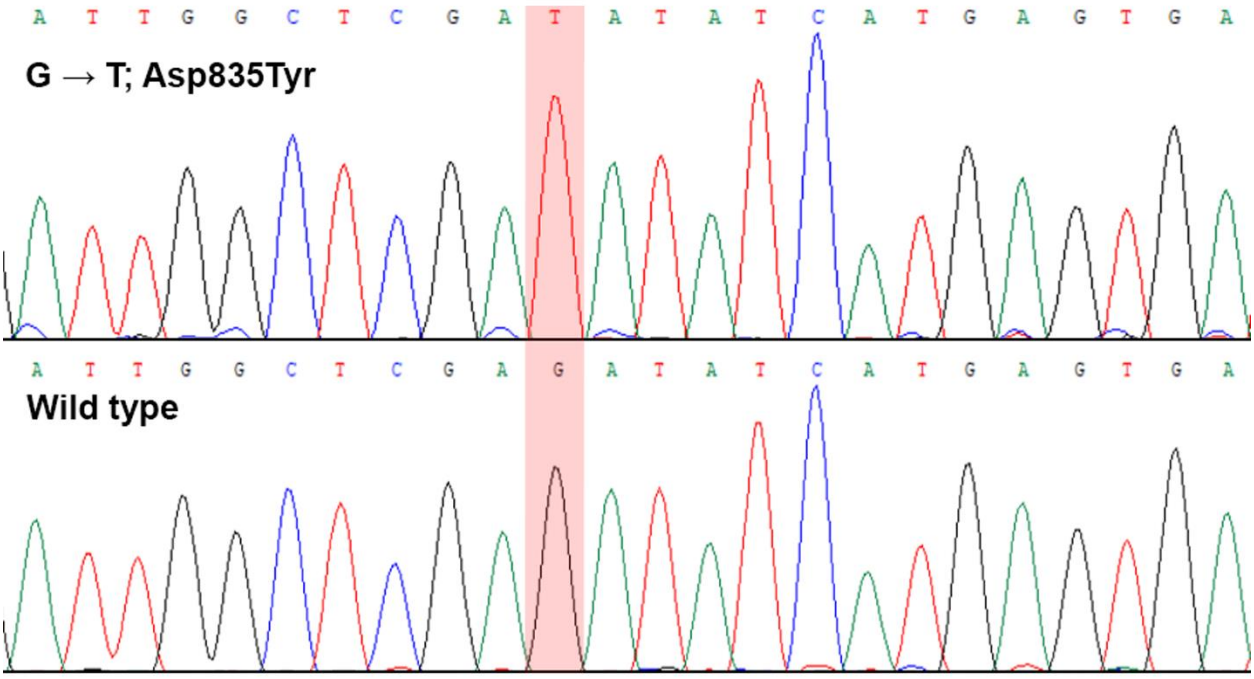

Figure 2. The sequencing result of FLT3-TKD mutation.

Discussion

Despite numerous studies about FLT3 mutations in AML, there is a lack of reports about the FLT3-TKD in Baghdad. In the current survey, 50 patients with AML were evaluated for the FLT3-TKD mutation. Our finding indicated that the frequency of FLT3-TKD in Baghdad is very low. This is in accordance withElyamany et al., who investigated 90 AML patients and reported that just 4.12% of patients suffer from FLT3-TKD mutation(8). In the same study with a larger sample size in China,just 4% of patients have

FLT3-TKD mutation (9). In contrast,Qiu et al. have shown that 17.7% of their study population have FLT3-TKD and also, they demonstrated that this gene defect has an association with response to treatment(4).

FLT3 is a family member of tyrosine kinase receptor class III (RTK III) that includesC-Kit, C-fms, and PDGFR.FLT3 have a considerable expression in hematopoietic stem cells which seems to play an essential role in hematopoiesis. In a normal situation, there is a need for growth factors (interleukin-3) for stimulation, whereas mutation in FLT3, there in independent cell growth following expression of interleukin-3 receptor (CD123) on hematopoietic stem cells; which is no expression in normal (10). FLT3-TKD mutations are small mutations that occur in the FLT3 activation loop, usually as a result of point mutations in the D835 codon orI836 codon deletion.These substitutions result in continuous phosphorylation of tyrosine, resulting in activation of the tyrosine kinase receptor.

Due to the low rate of mutation and studied patients, we cannot accurately define the relationship between this mutation and FAB subgroups. In a study in Japan that set out to determine the prevalence of FLT3-TKD in AML and its impact on disease progression, their results showed, in agreement with our result, the frequency was low without association with prognosis (11). It was revealed that the FLT3-TKD is influenced by the geographical region, so that the prevalence of FLT3-TKD is lower than in Europe, which is in line with our finding (11). Despite knowing this fact, there is an increasing interest in investigating the role of FLT3-TKD in disease progression. A recent investigation showed that secondary TKD mutations arise after using FLT3 inhibitors in patients with single FLT3-ITD mutated AML(12); hence, evaluating FLT3-TKD is recommended during treatment.

It was demonstrated that there is an association between FLT3-TKD, gender, and age; in females and older age, the incidence of FLT3-TKD increases(13).In the event that,in the present survey, AML patients without any restriction for age were evaluated, which can be an effect on the results. In patients with FLT3-TKD, a percentage of patients are resistant to the first and second generation of FLT3-TKD inhibitors, especially patients with D835Y(14,15). One explanation for this is the failure to identify these specific substitutions. On the other hand, it was revealed that patients with low FLT3-TKD mutation showed false-negative sequencing results. It causes a proliferation of investigations to introduce favorable sensitivity and specificity methods to detect FLT3-TKD low level (16).

The main limitation of the present survey is that due to the low frequency of TKD mutation, we could not find any association between FLT3-TKD and AML subtype. Lack of data about the response to treatment is the other main limitation.

Conclusion

The current survey indicated that theFLT3-TKD has a low incidence among AML patients in Baghdad. Considering this issue that this mutation has a crucial role in response to treatment, in this regard,further analysis with a larger sample size, evaluating with disease subtype, and response to treatment is recommended.

Ethics approval and consent to participate

All procedure performs in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or compare ethical strand.

Consent for publication

All patients are assigned a consent form.

Conflict of interest

The authors declare no conflict of interest.

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