

Molecular Study of FLT3-ITD Mutation in Iraqi Adult Acute Myeloid Leukemia Patients; Its Correlation with Clinicopathological Parameters

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Abstract: Acute myeloid leukemia (AML) is a hematological malignancy of myeloid progenitor cells characterized by abnormal proliferation, inhibition of differentiation and expansion of leukemic cells prevented at the early step of hematopoiesis. Detection of molecular markers has become a smart tool to further division of patients in AML subgroups. The Fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutations are found in about 20-25% of AML patients and it is associated with increased transcript level of FLT3 and with unfortunate prognosis in adult AML patients. This study aims to find FLT3-ITD mutation in Iraqi adult newly diagnosed AML patients using real time-PCR technique and gel electrophoresis post PCR procedure as well as to evaluate the relationship of FLT3-ITD mutation with clinicopathological parameters including age, gender, total WBC count and FAB subtypes of the disease. FLT3-ITD mutation was found in 1.88% of AML patients. FLT3-ITD mutation was not related to any clinical variables including age, gender, total WBC count and FAB subtypes of the disease with statistical significance. These findings suggest low rate of FLT3-ITD is found in Iraqi adult AML patients and no correlations are established between FLT3-ITD mutation and any clinical variables of AML including age, gender, total WBC count and FAB subtypes of the disease.

Keywords: Acute Myeloid Leukemia, FLT3-ITD, Real Time- PCR

1. Introduction

Acute myeloid leukemia (AML) is a heterogenous hematological malignancy involving the clonal expansion of myeloid blasts in the bone marrow and peripheral blood with possible spread to liver and spleen. [1] AML is generally a disease of elder people and is infrequent before the age of 45. The mean age of a patient with AML is about 67 years. AML is somewhat more common in men than women. [2]

AML is of great variability in the pathogenesis, clinical features, and treatment consequences. Progresses in molecular research have greatly enhanced understanding of the leukemogenesis in AML. [3]

FMS-like tyrosine kinase 3 (FLT3) diagramed at 13q12,

codes a receptor tyrosine kinase. [4] Fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutation produced by doubling of the exon 14 sequence at juxtamembrane domain, leading to constitutive activation of downstream signaling. [5] It occurs in about 25% of adult AML and displays association with normal cytogenetic and NPM1 mutation. The patients with this mutation have elevated white blood cell (WBC) counts, shorter disease-free survival and overall survival, and increased relapse rate. [6] Absence of FLT3-ITD combined with NPM1 mutation is considered as a favorable prognostic genotype. [7]

2. Patients, Materials and Methods

It is a cross-sectional study; included collection of bone

marrow aspirate (BMA) samples of 53 patients who were recently diagnosed as AML at the Hematology Ward of Baghdad Teaching Hospital in Medical City from April 2015 to September 2016. BMA samples of 53 control individuals (age and sex matched) were also collected. All the control bone marrows were negative for infiltrative lesions and obtained from patients with anemia and idiopathic thrombocytopenic purpura. Patients with AML were diagnosed according to the FAB classification. [8] Immunophenotyping was done where it was deemed necessary, in patients with diagnostic uncertainty.

From each patient; 0.5 ml of BMA was taken and equivalently separated into 2 eppendorff tubes each contain (1.5 ml) RBC lysis buffer mixed well and incubated for 15 minutes then centrifuged at 1800 rpm for 10 min at 4°C. The supernatant was removed and discarded. New RBC lysis buffer (0.5 ml) was added to the cell pellet. Tubes centrifuged at 1800 rpm for 10 min at 4°C, supernatant was removed and discarded. Buffer RLT (600 µl) was added to pelleted leukocytes. The samples were frozen and stored at -40°C until the day of RNA extraction.

RNA was extracted with QIAamp® RNA Blood Mini kit (Qiagen, Germany). The isolated RNA was reverse transcribed into cDNA using QuantiTect® Reverse Transcription (QIAGEN, Germany). (42°C for 30 minutes,

followed 95°C for 3 minutes). The final volume for PCR reaction was 20 µL containing 100 ng cDNA, 10 µL of QuantiTect® SYBR® Green PCR kit from QIAGEN (Germany), 600 nmol/L of forward primer and 600 for reverse primers, using the primers: FLT3- Reverse, (5'-CACCTGTACCATCTGTAGCTGGCT-3') FLT3-Forward, (5'-TCAAGTGCTGTGCATACAATTCCC-3'). GAPDH housekeeping gene used as internal control, using the primers: GAPDH- Reverse (5'-AAAGGTGGAGGAGTGGGTGTCG-3') GAPDH-Forward (5'-CCAAAATCAAGTGGGGCGATG-3'). All the primers were designed using Primer Express Software (Applied Biosystem, Foster City, CA, USA). Real-time polymerase chain reaction (PCR) assays using Mx3000PTM real time PCR System sequence Detection System (Stratagene, USA) was done by heating at 95°C for 15 minutes, followed by 40 cycles of 94°C for 15 seconds, 55°C for 30seconds and 72°C for 30 seconds. After PCR procedure had been done amplified FLT3 PCR products of AML and control samples were separated on agarose gels and stained with nucleic acid diamond dye. The amplified product of the wild type presented as 2 bands; While FLT3-ITD mutation showed additional 2 bands as shown in figure (1). All the control cases enrolled in this study showed wild type FLT3 gene.

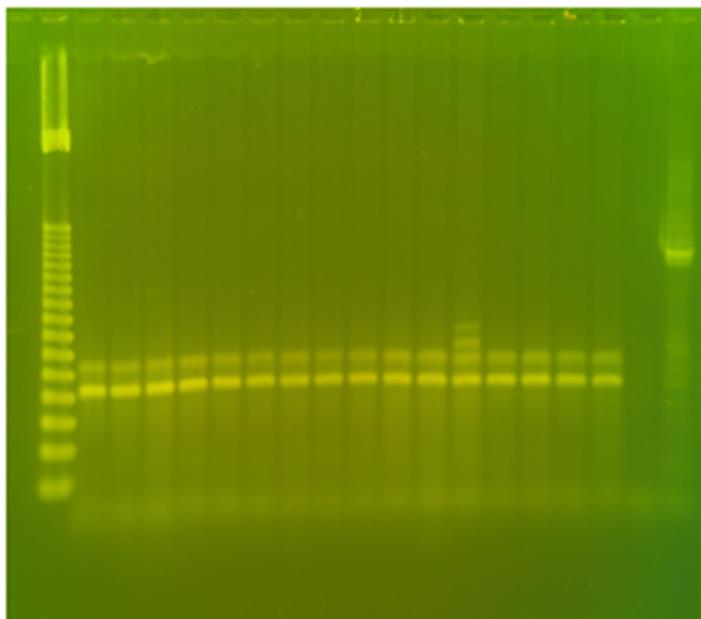


Figure 1. PCR detected FLT3-ITD mutation.

From left to Right; Molecular weight marker (DNA ladder). Lanes P1-16 except 12: amplified PCR product from patients 'with normal FLT3 gene (wild type). Lane 12: amplified products from patients show extra-mutated bands of FLT3-ITD. Lane 17: negative control (no template). Lane 18: amplified PCR product of GAPDH gene. Electrophoresis was carried in 2.5% agarose gel at (6V/cm) for 60 min using nucleic acid diamond dye. Stepladder: 50 bp DNA ladder ranging from 800bp to 50 increments.

3. Statistical Analysis

Data were analyzed using statistical software program SPSS version 22 (SPSS Inc. Chicago, IL, USA). Continuous

data were expressed as mean \pm standard deviation (SD); frequency was used to express categorical data. Student t-test was used to analyze continuous data while fisher exact test was used to analyze categorical data. Values were considered statically significant when P value (<0.05).

4. Results

The study was focused on 53 newly diagnosed Iraqi AML patients (Age range 15-83 years; median 42.00). In this study FLT3-ITD mutation was detected in only 1 (1.88%) out of 53 AML patients (Table 1). FLT3-ITD mutation was not related to age, gender, total WBC count (Table 2 & 3) and FAB subtypes with statistical significance ($P>0.05$).

Table 1. Clinicopathological features of AML patient with positive FLT3-ITD mutation.

Total number: 1/53 (1.88%)
Gender: Male
Age: 34 years
Clinical features: Pallor, HSM, LAP
Hb: 10.7 g/dl
WBC count: 24.5 X10 ⁹
Platelet count: 13.1 X10 ⁹
FAB classification: AML-M5

Table 2. FLT3-ITD mutation according to gender in AML patients.

FLT3-ITD	Gender		Total
	Male	Female	
Positive	1	0	1 (1.88%)
Negative	31	21	52 (98.12%)
Total	32	21	53 (100%)
Fisher exact test ($P>0.05$)			

Table 3. Age and WBC count Associations with FLT3-ITD mutation in AML patients.

	FLT3-ITD		P value
	Positive (1 case)	Negative (52 cases)	
Mean Age (\pm SD) years	34.00 (\pm 00.00)	44.00 (\pm 19.07)	> 0.05
Total WBC count (\pm SD) x10 ⁹	24.50 (\pm 00.00)	33.97 (\pm 51.98)	> 0.05

5. Discussion

The frequency of FLT3-ITD mutation in AML patients was 1.88%. Although it was lower than Dhahir et al study⁹ (An Iraqi study on AML patients showed frequency of ITD mutation 14.54%), both of them were lower than most of the reported studies worldwide; which range between 20%–25% in Japanese, [10] Thai, [11] Chinese, [12] or German [13] populations. Therefore, the information till now showed that the rate of the FLT3-ITD was low in Iraqi AML patients in the adult age groups. Some reports from Saudi Arabia, Iran and Korea agree with our result. [14, 15, 16]

Fenski et al. [21] have revealed that FLT3 oncogene is constitutively activated in 3/18 cases of AML patients and only 1/3 of those cases showed the FLT3-ITD mutation. [17]

Lower occurrence of FLT3 mutations in this study from several reported studies may be explained by small size of our AML samples or might be due to environmental factors, population genetics or because of differences of age as the

median age of AML patients in this study was low in contrast to comparative studies or a combination of the all. However, these results should be treated with reservation, due to the relatively small sample size and further research and investigation are required.

In this study FLT3-ITD mutation was not correlated to any clinical variables with statistical significance. Because the small sized samples included here and there were only one patient had FLT3-ITD mutation in this study, larger gauge analysis is required to clarify the clinical significance of this mutation in Iraqi patient. However; although we could not identify any baseline characteristic related with FLT3-ITD. It was noteworthy that only patient with FLT3-ITD mutation had a high WBC count and belong to M5 FAB subtype. Kottaridis et al stated that the clinical significance of FLT3 mutations in AML was established from the correlation between the presences of ITD correlated with leukocytosis, high blasts percentage and unfavorable response to therapy. [18] Thiede et al. reported that FLT3 mutations including ITD were significantly increased in AML patients with FAB M5 subtype. [13]

6. Conclusion

The rate of FLT3-ITD mutation in Iraqi adult patients with AML reported in this study is lower than expected.

No correlations are established between FLT3-ITD mutation and age, gender, total WBC count and FAB classification.

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