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FLT3-ITD Incidence and FLT-D835 Mutations in Acute Myeloid Leukemia Patients with Normal Karyotype in Morocco: A Preliminary Study

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Abstract

Background: According to numerous studies, FMS-like tyrosine kinase 3, internal tandem duplication, and the D835 mutation are associated with a poor prognostic clinical outcome in acute myeloid leukemia patients. Detection of the FMS-like tyrosine kinase 3 mutation in patients who present with normal karyotype acute myeloid leukemia helps in both the understanding of the disease and the treatment of patients. This study evaluates the incidence of FMS-like tyrosine kinase 3-internal tandem duplication and FMS-like tyrosine kinase 3-D835 mutation in newly diagnosed patients with normal karyotype acute myeloid leukemia.

Methods: This study looked at 33 new cases who presented with normal karyotype acute myeloid leukemia at diagnosis. We collected peripheral blood samples from patients at diagnosis. FMS-like tyrosine kinase 3-internal tandem duplication mutation was detected using polymerase chain reaction and FMS-like tyrosine kinase 3-D835 mutation by restriction fragment length polymorphism after polymerase chain reaction.

Results: FMS-like tyrosine kinase 3-internal tandem duplication mutation was found in 18% (6/33) of all patients and in 30% (6/20) of patients over 30 years of age. FMS-like tyrosine kinase 3-internal tandem duplication mutation was most common with M2 (50%), M5 (33.3%), and M0 (16.7%). FMS-like tyrosine kinase 3-D835 mutation was detected in one patient (3%) that had M2. No significant association was found between FMS-like tyrosine kinase 3 mutation and age, sex, white blood cell count, platelets, or blasts percentage.

Conclusion: Most of FMS-like tyrosine kinase 3 mutations were found in patients older than 30 years. The frequency observed in this work is comparable with that observed in the literature. No pediatric case of FMS-like tyrosine kinase 3 mutation was found in this study. A large scale study is needed to confirm our findings and to further appreciate the prognostic value of FMS-like tyrosine kinase 3 mutation among Moroccan patients.

Keywords: FMS-like tyrosine kinase 3, FLT3-ITD, FLT3-D835, mutations, NK-AML

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Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy characterized by bone marrow infiltration with blast cells, particularly myeloblasts. AML is divided into seven groups: the first represents the AML with recurrent genetic abnormalities, including translocations, inversions and gene mutations. This group is heterogeneous in its prognosis. Currently the karyotype remains the most important prognostic factor.[1] However AML with normal karyotypes (NK-AML), which represent 40%-50% of AMLs, vary in term of prognosis. In recent years somatic mutations in genes involved in cell proliferation, differentiation, survival, and the cell cycle have been discovered. These findings have helped clarify the prognosis of this group. Some of these abnormalities have a good prognosis value, such as mutations in the nucleophosmin1 gene (NPM1), while others confer a poor prognosis, particularly mutations of the FMS-like tyrosine kinase 3 gene (FLT3).[2] FLT3 is a tyrosine kinase receptor normally expressed on early myeloid cell precursors. The gene encoding FLT3 is located on chromosome 13q12. In-frame internal tandem duplication (ITD) mutations in the juxta-membrane region or point mutations in the tyrosine kinase domain may occur that result in constitutive tyrosine kinase activity.[3] Many studies have shown that mutations in FLT3 gene are associated with a worse prognostic value. Elsewhere, an increased risk of relapse and reduced overall survival are observed among patients carrying the FLT3 mutation, but the prognostic value of FLT3-D835 mutation is not very clear.[4,5] Recently, there is hope for AML management, with the development of specific drug FLT3 tyrosine kinase inhibitor.[4]

To our knowledge there is no data in Morocco concerning the incidence and type of FLT3 mutation. Genotypic analysis of this gene can help in the understanding of the pathogenesis of AML and as an important tool for clinical decision-making. This study explores the frequency of the FLT3 mutation in Moroccan patients with NK-AML.

Materials and Methods

Patients

This study consisted of 33 patients (13 males and 20 females) with a median age of 30 years (range: 3-58) who presented with NK-AML at diagnosis. Samples were collected at the Department of Onco-hematology of the Ibn Rochd University Hospital in Casablanca, Morocco, from 2011 to 2012. Clinical and biological data were collected from patient's data files. The local Ethics Committee approved this study and all participants accepted and signed the informed consent.

Detection of FLT3 mutations

DNA was extracted from peripheral blood

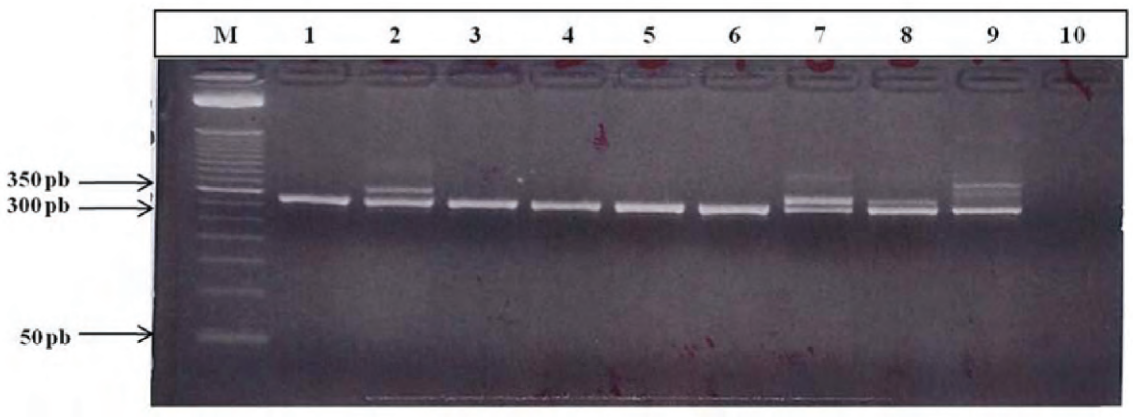

Figure 1. Agarose gel illustrating the profiles obtained following amplification for FLT3-ITD detection. Lane M: 50 bp molecular marker; lanes 1, 3, 4, 5 and 6: normal individual; lanes 2 and 8: patients with single ITD; lanes 7-9: patients with two ITDs; and lane 10: negative control. The wild type of FLT3 is 329 bp; additional larger bands represent ITD.

Table1. Clinical and biological parameters of patients according to the presence or absence of FLT3-ITD and FLT3-D835 mutations.

Parameter	No mutations (n=26)	FLT3/ITD (n=6)	FLT3/D835 (n=1)	Total (n=33)
Age (years)	30 (3-58)	37 (33-47) *P=0.260*	40 *	33 (3-58)
Male:female	1.36:1	4.98:1	*	1.53:1
Hb (g/dl)	7.6 (3.3-12.1)	6.55 (4.8-8.7) *P=0.296*	9.2 *	7.48 (3.3-12.1)
Mean WBC (10^3 /L)	31.519 (1.5-200)	45945 (2.2-151) *P=0.565**	472	47.491 (1.5-472)
Mean platelets (10^3)	80.654 (6-274)	105.5 (17-400) *P=0.575**	27	83.545 (6-400)
Blasts (%)	30.38 (0-96)	14.7 (0-70) *P=0.312**	18	27.06 (0-96)
FAB subtypes n (%)				
M0	2 (7.7)	1 (16.7)	0	3 (9.1)
M1	9 (34.6)	0	0	9 (27.3)
M2	8 (30.8)	3 (50)	1	12 (36.4)
M4	7 (26.9)	0	0	7 (21.2)
M5	0	2 (33.3)	0	2 (6.1)

FAB: French American-British; WBC: white blood cell; Hb: hemoglobin*: No statistical test was performed due to only a single patient. The Mann-Whitney test was used.

using the salting-out method.[6] DNA yields and purity were determined by measuring absorbance at 260/280 nm.

FLT3-ITD mutations were identified as described by Haslam et al.[2] The polymerase chain reaction (PCR) reaction was performed in a 50 µl reaction that contained 100 ng of genomic DNA, 1.5 mmol/L $MgCl_2$, 0.2 mM dNTPs, 20 pmol of each oligonucleotide primer (11F: 5'GCA ATT TAG GTA TGA AAG CCA GC 3' and 12R: 5'CTT TCA GCA TTT TGA CGG CAA CC 3'), and 1 unit of ampli-Taq polymerase (Invitrogen Life Technology). The PCR condition consisted of an initial denaturation step at 95°C for 8 min followed by 35cycles at 94°C for 45s, 55°C for 1 min, 72°C for 2 min, and a final step at 72°C for 7 min. Amplified products were visualized on a 3% agarose gel. The duplicated sequence involved exons 11, 12, or the intervening intron. Wild-type FLT3 produced a fragment of 329 bp (Figure1).

Detection of D835 mutation was performed using the PCR-RFLP method as described by Ahmad.[7]

We used a 100 ng sample of each patient's DNA, which was amplified in a 35 cycle PCR reaction at an annealing temperature of 56°C. We used 20 pmol of each primer (20-D835: 5' CCA

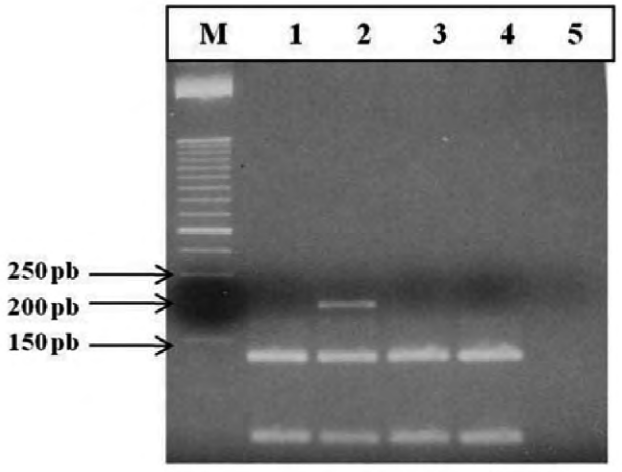

Figure 2. Detection of D835 mutations in the FLT3 gene. PCR amplified products from wild type cases were digested into two bands (130 bp and 65 bp) by EcoRV, while those having D835 mutation showed undigested bands (195 bp), as seen on the agarose gel. Lane M: molecular marker 100 bp; lanes1, 3, and 4: normal patients; lane2: patient positive for D835 mutation; lane5: negative control.

GGA ACG TGC TTG TCA CCC AC 3' and E20TR-D835: 5' TCA AAA ATG CAC CAC AGT GAG 3'). Amplified products were then digested with 10 U of EcoRV for 3 h. The digested PCR products were visualized on a 3% agarose gel (Figure 2).

Statistical analysis

SPSS version 16 was used for data analysis. We used the Mann-Whitney U-test to analyze the differences between age, hemoglobin levels, platelet counts, and white blood cell counts.

Results

This study included 33 AML patients with a normal karyotype; the median age was 33 years (range 3-58). There were 13 males and 20 females, of which 13 patients were less than 30 years. FLT3-ITD and D835 mutations were observed in patients older than 30 years. These mutations were most common with the FAB subtype M2 in 3 (50%) patients and M5 in 2 (33.3%) patients. FLT3-D835 mutation was detected in one patient among the 33. The mean age of the FLT3-ITD group was 37 years against 30 years for the non-mutated group. We did not find any significant association between the FLT3-ITD mutation with age (P=0.26), sex (P=0.217), hemoglobin level (P=0.27), white blood cell count (P=0.85), platelets (P=0.65), and blasts percentage (P=0.22). The FAB classification in addition to clinical and biological parameters of the entire group are summarized in Table 1.

Discussion

The main goal of this study was to establish the incidence of FLT3 mutations among NK-AML patients who represent the intermediate prognostic group and to facilitate patient management by clinicians. Generally the frequency of the FLT3 mutation is lower in AML, but its presence can be an important independent prognostic factor among patients aged less than 60 years.[4] Patients who presented with the FLT3-ITD mutation were older than the group with wild type (37 years vs. 30 years); this observation was comparable to that obtained by Auewarakulet al.[8] The frequency of the FLT3-ITD mutation among adults aged over 30 years was 30%, this finding was concordant with the frequency generally observed in the literature (about 25%–30%). We did not find any pediatric FLT3 mutation cases (Figure3); this might have been due to the small size of our sample, but it has been reported that 10%–17% of FLT3 mutations occur in children.[9] In this study, as reported by Sharkawy et al,[10] there was no association between the FLT3 mutation and hemoglobin level, white blood cell count, platelets, and blasts percentage; however, some studies have shown a relationship. Overall the mean white blood cell count and platelets were higher in the FLT3-ITD mutated group. The FLT3-ITD mutation shows a genetic instability due to the presence of a high white blood cell count and platelets; however it is not an important complete remission marker. Some authors have noticed that it is important as a marker of relapse, reduced

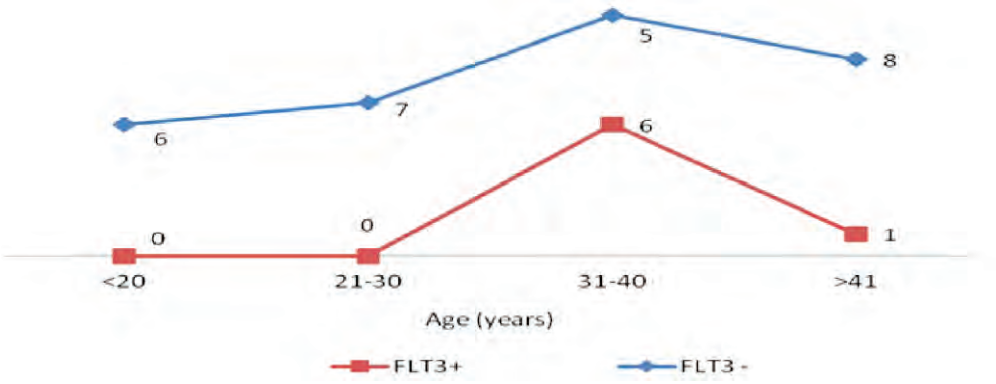

Figure 3. Incidence of FLT3-ITD and FLT3-D835 mutations according to age. The blue curve represents the number of patients without FLT3 mutations and the red curve represents the number of patients with FLT3 mutations.

disease free survival, and overall survival.[3] FLT3-D835 mutation was observed in one (3%) adult patient, which confirmed the rarity of this mutation in AML. According to the literature, the frequency of this mutations is 6%-10%.[13] Although we have not observed any case of double mutation in our patients, it is known that this type of mutation is rare and represents only 1%-2%, with a poor clinical outcome.[14] We have found that FLT3-ITD was more frequent in the FAB type M2 (50%), followed by M5 (33.3%), and M0; this observation might be attributed to our small sample size.

Conclusion

Our study is the first among Moroccan patients with NK-AML. The findings in this study are important for physicians in the management of patients and therapeutic decisions insofar as the FLT3 gene mutation is described as a therapeutic target and also in minimal residual disease monitoring. However, due to the small sample size, a large scale study will be necessary and helpful to understand the genetic changes and the impact of these mutations in AML progression, as well as confirm our observations.

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