Original Article

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Quick Response Code:

Website: https://journals.lww.com/ijhm

DOI: 10.4103/ijh.ijh_66_23

FMS-like tyrosine kinase 3 internal tandem duplication mutation in patients with acute myeloid leukemia in Kurdistan region/Iraq

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Abstract:

BACKGROUND: Mutations in the FLT3 gene are very common in acute myeloid leukemia (AML). These mutations are linked to a high chance of the disease relapse and decrease overall survival. This study aimed to investigate this mutation in AML cases and to correlate it with the clinicopathological presentation and their response to induction therapy.

OBJECTIVES: This study aimed to investigate this mutation in AML cases and to correlate it with the clinicopathological presentation and their response to induction therapy.

METHODS: This study was done on 63 AML cases; for each patient, the clinical presentation and the hematological lab parameters were recorded. The results of FLT3 internal tandem duplication (ITD) mutation were recorded and the mutation was detected by conventional polymerase chain reaction technology. Postinduction assessment was recorded for each patient.

RESULTS: The mean age of the studied group was 36.06 (22.42), with a male to female ratio of 1:1.6. Out of 63 AML patients, 16 (25.4%) had FLT3-ITD mutation. The highest incidence of the mutation was found among the age group of 10–19 years (40%). The highest incidence of FLT3 mutation was among M3 (45.5%). The blast count was significantly higher in patients with than without the mutation ($P = 0.04$). The remission rate was significantly lower in FLT3 AML patients than in those lacking the mutation ($P = 0.45$).

CONCLUSION: FLT3-ITD mutation was common in our AML patients. This mutation was associated with significantly higher counts of blast and poorer response to induction therapy; thus, it is considered one of the poor prognostic factors.

Keywords:

Acute myeloid leukemia, FMS-like tyrosine kinase 3 internal tandem duplication mutation, remission rate

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Submission: 03-09-2023
Accepted: 23-09-2023
Published: 07-12-2023

Introduction

Acute myeloid leukemia (AML) is a genetically diverse hematological malignancy characterized by the accumulation of acquired genetic abnormalities in hematopoietic progenitor cells caused by a complicated network of cytogenetic and molecular changes that disrupt normal cell development, proliferation, and differentiation mechanisms, resulting in abnormal accumulation of immature precursor cells in the bone marrow and/or peripheral blood. As a result, genetic characterization of all AML patients is now regarded mandatory to make therapy decisions.[1-3]

About 50% of AML patients exhibit a normal karyotype and no detectable cytogenetic alterations, making them part of an

How to cite this article: Rasool SS, Mohammad NS, Hamza HH, Alnuaimy SL. FMS-like tyrosine kinase 3 internal tandem duplication mutation in patients with acute myeloid leukemia in Kurdistan region/Iraq. Iraqi J Hematol 2023;12:159-63.

intermediate-risk category with unpredictable clinical outcomes. Therefore, more prognostically significant markers are needed to identify clinically meaningful subgroups in AML patients with normal karyotypes.[4-6]

FMS-like tyrosine kinase 3 (FLT3) is a type III tyrosine kinase receptor preferentially expressed in hematopoietic progenitor cells and has an important role in cell survival and proliferation.[7] This gene is highly mutated in acute leukemia, present in around one-third of all cases of AML. The main type of mutation, called internal tandem duplication (ITD), happens in the FLT3 gene's juxtamembrane domain and accounts for 20%-25% of all AML cases. Furthermore, 7% of AML patients have point mutations in the tyrosine kinase domain.[8] FLT3-ITD has been regarded as an adverse prognostic factor in AML, as it is related to increased blast counts in peripheral blood and bone marrow, increased risk of relapse, and lower survival rates. Thus, this mutation plays an important role in the development of leukemia, rather than initiation.[9,10]

Several FLT3 inhibitors have been developed to target these mutations; however, it took more than two decades from the initial identification of this mutation until FLT3 inhibitors become clinically available as a targeted therapy. Currently, three FLT3 inhibitors are approved in clinical practice as monotherapy or in combination with conventional chemotherapeutic treatments.[11]

The aim of this study is to investigate the FLT3-ITD mutation in patients with AML and correlate the presence of this mutation with the clinicopathological features and response to induction therapy.

Methods

This was a retrospective study that extended from 2014 to 2022. During this period, 63 AML patients were collected from Nanakali Hospital for blood diseases and cancer. The selection of the cases was based on the availability of FLT3 mutation testing. Fifty-five patients were newly diagnosed, while 8 patients were in relapse. This study has been approved by the Ethical Committee of College of Medicine, HMU.

Clinical history with the blood investigations results were retrieved from the patients' files. The demographic data including age and gender were recorded. The clinical presentation and the presence of organomegaly were recorded. Laboratory parameters including the blast counts in peripheral blood and BM, the immunophenotypic markers, and the polymerase chain reaction (PCR) results for FLT3 mutation were all taken. The PCR study was done on ethylenediaminetetraacetic acid blood samples after extraction of the DNA according to the kit protocol (Bioneer) following instruction's manual.

The initial response to induction chemotherapy (3 + 7 protocol) was evaluated for every patient to determine whether they achieved complete remission (CR), treatment failure, or early death. CR was defined as <5% blasts on aspirate and near normal blood counts (Hb >10 g/dl and neutrophil counts >1 × 10^9/L).[12]

Data were analyzed using SPSS v. 25 (SPSS Inc, Chicago IL, USA). Numerical data were expressed as mean (SD) and categorical variables as frequencies and percentages. To compare two numerical variables, the Student's *t*-test was utilized, while the Chi square and Fisher's exact tests were used to compare categorical variables. $P \leq 0.05$ was regarded statistically significant.

Results

A total of 63 AML patients were enrolled in this study: 24 (38.1%) were male and 39 (61.9%) were female. The age range of the patients was between 1 and 97 years with a mean of 36.06 (22.42), and the peak age incidence was in the fifth decade forming 17.5% of the cases [Table 1].

Out of 63 AML patients, 16 (25.4%) were positive for FLT3-ITD mutation, while 47 (74.6%) patients were FLT3-ITD negative.

Table 2 shows the association of FLT3 mutation with clinical presentation and FAB classification of AML. Out of 50 patients who had pallor, 15 (30%) were FLT3 positive and this association was statistical not significant ($P = 155$). Considering the FAB classification, the highest number of FLT3 positive patients were of M3 subtype 5 (45.5%) patients; however, the association was not statistically significant ($P = 0.643$).

Regarding the outcome after induction therapy, total patients who had remission were 39 (61.9%), of whom only 6 (15.6%) were positive for FLT3, while 33 (84.4%) patients were FLT3 negative, and this association was statistically significant ($P = 0.045$) [Table 2].

The blast counts in PB were significantly higher in FLT3-positive patients compared to FLT3-negative patients with $P = 0.04$. The other hematological laboratory parameters did not show any significant differences between FLT3 positive and negative groups [Table 3].

Discussion

AML is a highly malignant neoplasm that generally carries a poor prognosis and is responsible for a large number of cancer related deaths.[13] There are some

Table 1 Association of FLT3-ITD positive mutation with patients' characteristics

Parameters	Positive FLT3-ITD mutation group, *n* (%)	Negative FLT3-ITD mutation group, *n* (%)	Total, *n* (%)	*P*
Gender				
Male	6 (25.0)	18 (75.0)	24 (38.1)	0.955
Female	10 (25.6)	29 (74.4)	39 (61.9)	
Age category				
<10	2 (25.0)	6 (75.0)	8 (12.7)	0.776
10–19	4 (40.0)	6 (60.0)	10 (15.9)	
20–29	1 (10.0)	9 (90.0)	10 (15.9)	
30–39	2 (28.6)	5 (71.4)	7 (11.1)	
40–49	3 (33.3)	6 (66.7)	9 (14.3)	
50–59	3 (27.3)	8 (72.7)	11 (17.5)	
≥60	1 (12.5)	7 (87.5)	8 (12.7)	
Total	16 (25.4)	47 (74.6)	63 (100.0)	

Table 2: Association of FLT3-ITD mutation with some clinical variables

Parameters	Positive FLT3-ITD mutation group (*n*=12), *n* (%)	Negative FLT3-ITD mutation group (*n*=39), *n* (%)	Total, *n* (%)	*P*
Diagnosis condition				
Newly diagnosed	13 (23.6)	42 (76.4)	55 (87.3)	0.407
Relapsed cases	3 (37.5)	5 (62.5)	8 (12.7)	
Clinical presentation				
Pallor	15 (30.0)	35 (70.0)	50 (79.4)	0.155
Fever	11 (26.2)	31 (73.8)	42 (66.7)	0.838
Bleeding	10 (34.5)	19 (65.5)	29 (46.0)	0.126
Lymphadenopathy	4 (36.4)	7 (63.6)	11 (17.5)	0.448
Hepatosplenomegaly	4 (30.8)	9 (69.2)	13 (20.6)	0.723
AML FAB classification				
M0	0	1 (100.0)	1 (1.6)	0.643
M1	3 (27.3)	8 (72.7)	11 (17.5)	
M2	2 (16.7)	10 (83.3)	12 (19.0)	
M3	5 (45.5)	6 (54.5)	11 (17.5)	
M4	3 (33.3)	6 (66.7)	9 (14.3)	
M5	3 (17.6)	14 (82.4)	17 (27.0)	
M6	0	0	0	
M7	0	2	2 (3.2)	
Outcome after induction therapy				
Remission	6 (15.4)	33 (84.6)	39 (61.9)	0.045
Failure	6 (42.9)	8 (57.1)	14 (22.2)	
Death	4 (40.0)	6 (60.0)	10 (15.9)	
Total	16 (25.4)	47 (74.6)	63 (100.0)	

AML=Acute myeloid leukemia

factors that indicating poorer prognosis according to the 2017 European Leukemia Net recommendations as the complex cytogenetic abnormalities and the association of FLT3-ITD mutation.[14] FLT3-ITD mutations particularly associated with poorer prognosis, higher rate of relapse, and inferior overall survival.[15] This study was employed to investigate FLT3-ITD in a group of AML patients and its association with clinicopathological parameters and the induction outcomes.

In the current cohort, nearly two-thirds of the patients were female which is consistent with some studies;[16-18] however, it is inconsistent with the other studies,[19,20] and this could be due to small sample size.

The frequency of FLT3-ITD mutation in this study was 25.4%, which is in accordance with most of the studies,[5,9,21] while lower than our result reported by other studies in Iraq and India.[15,22,23] In contrary, an Egyptian study found a higher figures than ours (34.6%).[24,25] These variabilities may be due to different subtypes of AML enrolled in these studies.

The current study demonstrates that the incidence of FLT3 mutation in female cases was almost the same as in male and this is inconsistent with a study by Mehta *et al*.,[22] which showed higher incidence in female compared to male cases, while in contrast, an Iraqi study showed higher incidence in male cases;[21] however, this

Table 3: Comparisons of lab parameters between acute myeloid leukemia patients with and without FLT3-ITD mutation

Laboratory parameters	Mean (SD)		*P*
	Positive FLT3-ITD mutation group	Negative FLT3-ITD mutation group	
Hb (g/dL)	9.27 (3.30)	8.42 (2.23)	0.250
WBC count (×10^9/L)	76.25 (50.77)	50.38 (75.30)	0.409
Platelet count (×10^9/L)	89.25 (136.19)	74.21 (67.75)	0.564
Blasts in PB %	60.69 (32.27)	42.40 (29.26)	0.040
Blasts in BM %	61.13 (25.06)	62.11 (22.92)	0.886

SD=Standard deviation, WBC=White blood cell, PB=Peripheral blood, BM=Bone marrow

discrepancy may be due to selection criteria and different patient characteristics.

In this study, the highest age group showing positivity for FLT3 mutation were of the age group (10–19) years; however, the incidence of FLT3 mutation in pediatric was comparable to adult cases (23.1% vs. 26%) and this finding was consistent with an Indian study.[26]

The incidence of FLT3-ITD mutation was more among relapsed than newly diagnosed cases; however, it did not reach a significant level, contradicting the findings of Dhahir *et al*.,[23] but this could be due to the fact that we included very few cases of relapsed AML; thus, we cannot draw a definitive conclusion.

According to the FAB classification, the highest incidence of FLT3-ITD mutation was noted in M3 followed by M4 and this is inconsistent with the study by Mehta *et al*.,[22] which found that the highest was among M6 cases followed by M1.

In this investigation, the hematological parameters exhibited similarities between patients with and without the mutation. Nevertheless, patients with FLT3 mutation showed a notably higher blast count, aligning with findings from Chauhan *et al*.'s study.[26] However, it is noteworthy that Chauhan et al. observed significantly elevated blast counts and total white blood cell levels in patients with FLT3-ITD mutation in their study.

The current study demonstrates that the presence of FLT3 mutation was associated with worse clinical response and the remission for FLT3-positive was lower than FLT3-negative patients (15.4% vs. 84.6%). Similarly worse clinical outcome and decreased remission rate also found in a study achieved by Almudallal *et al*.[21] Thus, this indicates that the presence of this mutation is associated with poorer prognosis and outcome.

Conclusion

The frequency of FLT3-ITD mutation is as high as that of Western countries. This mutation was shown to be associated with a higher number of blasts in peripheral blood and bone marrow and with poorer response to induction therapy. Larger prospective studies are necessary to accurately determine the incidence of FLT3 mutation and subsequently confirm the prognostic significance of this mutation.

Acknowledgment

We thank the staff member of the Department of Hematopathology and the administration of Nanakali Hospital for providing their utmost assistance to carry out this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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