Frequency of FLT3-Internal Tandem Duplication (FLT3 ITD) Mutation in Sudanese Acute Myeloid Leukemia Patients

Eman Ali Elzain[1], Mudathir Abdelshafea Abdelkareem[2], and Hiba Khalil[3,4]

[1]Department of Haematology, Faculty of medical laboratory Sciences, International University of Africa, Khartoum, Sudan; [2]Department of Microbiology and Immunology, Faculty of Medical Laboratory Sciences, Alzaiem Alazhari University; [3]Department of Hematology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan. [4]Al Neelain Stem Cell Center, Al Neelain University, Khartoum, Sudan

Abstract

Aim: Acute myeloid leukemia (AML) is a malignancy of proliferative, clonal, abnormally, or poorly differentiated cells of the hematopoietic system, characterized by clonal evolution and genetic heterogeneity. The Fms-like tyrosine kinase-3 (FLT3) is a receptor tyrosine kinase that plays a key role in cell survival, proliferation, and differentiation of hematopoietic stem cells. Mutations of FLT3 were first described in 1997 and account for the most frequent molecular mutations in AML. The aim of this study was to determine the frequency of FLT3 ITD mutation among Sudanese patients with acute myeloid leukemia and explore if it has a correlation with FAB subtypes of acute Myeloblastic leukemia or not. **Material and Methods:** One hundred patients diagnosed based on morphological, immunophenotype, and molecular genetics with acute myeloblastic leukemia were enrolled in this study; blood samples or bone marrow samples were collected from all patients in ethylene diamine tetra acetic acid; genomic DNA was extracted from all samples using guanidine method, and analyzed for FLT3- internal tandem duplication mutation using conventional polymerase chain reaction. **Results**: One hundred acute myeloblastic leukemic patients already diagnosed with acute myeloblastic leukemia, grouped as 54 were newly diagnosed and 46 were under treatment, also 46 were males and 54 were females; the range of age was one-year-old to eighty years. FLT3 ITD mutation was positive in 8 (8%) of the patients, two (2%) of them were male and six (6%) were female; the mean age of the patients with the mutation was 32.9 years. Four (50%) of the cases with the mutation were FAB sub-type M2 and two (25%) was M5b. **Conclusion**: The frequency of FLT3 ITD mutation among Sudanese patients with acute myeloid leukemia participated in this study was found lower than other populations, and there was no correlation between the mutation and FAB subtypes.

Keywords: FLT3-ITD, Acute Myeloid Leukemia, Sudanese

Introduction

Acute myeloid leukemia (AML) is the most common hematologic malignancy, characterized by uncontrolled production of hematopoietic stem cells resulting in abnormal accumulation of myeloblasts. Generally, based on the cytogenetic abnormalities, the prognosis of AML patients is grouped into three risk groups: good, intermediate, and poor (Rezaei et al, 2017).

Acute myeloid leukemia is the result of more than one mutation; the most common of these is the hematopoietic receptor tyrosine kinase FLT3. There are internal tandem duplications (ITDs) in the juxta membrane domain of FLT3, ranging in size from several to greater than 50 amino acids. FLT3 mutations are an independent indicator of a poor prognosis in AML patients under the age of 65 in most studies (Daver et al, 2019). It is associated with high leukemia cell count in patients with AML, and are thus an attractive target for therapeutic intervention (Mohamed et al, 2014).

Genomic investigations of acute myeloid leukemia (AML) have confirmed that more than a few genes are recurrently mutated, leading to new genomic classifications, predictive biomarkers, and new therapeutic targets. Mutations of the FMS-like tyrosine kinase 3 (FLT3) gene occur in approximately 30% of all AML cases, with the internal tandem duplication (ITD) representing the most common type of FLT3 mutation (FLT3-ITD; approximately 25% of all AML cases). FLT3-ITD is a common mutation that presents with a high leukemic load and confers a poor prognosis in patients with AML (Daver et al, 2019).

FLT3 is a member of class III receptor tyrosine kinase family (RTK), in general expressed in early bone marrow precursors that and plays a central role in ruling of hematopoietic cell proliferation and differentiation. Binding of the FLT3 ligand (FL) to its receptor, recruits and activates several signaling molecules affecting cell proliferation, differentiation and survival (Rezaei et al, 2017).

Prognostic risk is clear at diagnosis based on the presence of certain cytogenetic and molecular aberrations (Döhner et al, 2017). Guidelines for AML classification and risk stratification have been recognized by several organizations, including the World Health Organization (WHO), National Comprehensive Cancer Network (NCCN), and European Leukemia Net (ELN) (O'Donnell et al, 2017). The WHO lists FMS like tyrosine kinase 3 internal tandem duplication (FLT3- ITD) as a molecular genetic alteration significantly affecting the clinical outcome in patients with AML (Arber et al, 2016).

2. Materials and Methods

2.1. Patients. One hundred patients already diagnosed with AML which was diagnosed using morphology, cytochemistry and immunophenotyping. Clinical and laboratory data, including French–American–British (FAB) sub class, complete blood count, blast percentage, and hemoglobin (Hb) level were also collected. blood samples or Bone marrow samples were collected from them, grouped as 54 were newly diagnosed recruited at the time of diagnosis prior to chemotherapy achievement and 46 were under treatment who attend the RICK as follow up, accordingly, 34 samples were Bone marrow and 66 samples were peripheral blood sample collected in ethylene diamine tetra acetic acid. All of them were screened for FLT3 mutations.

2.2. Methods. DNA was extracted using manual guanidine technique

*2.2.1. Analysis of the ITD of the FLT3 Gene.*The extracted DNA were analyzed for detection of FLT-ITD mutation on chromosome 13 exon 11 using conventional PCR. 2µl of genomic DNA was amplified in 10µl reaction mixture containing 2µl ready to load mastermix (containingFIRpol DNA polymerase, 5x reaction buffer B, 12.5 Mm MgCl, 1Mm for each deoxyribonucleotidetriphosphate-dNTP) and 1µl of each of the forward and reverse primers. Primers sequences used for FLT-ITD mutations were mentioned in table No (1). PCR amplification was performed using PCR thermal cycler. Amplification process consisted of initial denaturation at $95C^0$ for 2 mins, 30 cycles of 30 sec at $95C^0$for denaturation, $59.5C^0$ for 30 sec for annealing. 50 sec at $72C^0$ for extension and $72C^0$ for 5 mins as final extension. Statistical analysis of data was done using SPSS program version 22.

Table 1: The Primers of FLT3 ITD

NAME	PRIMER
FLT3-ITD F	5'-GCAATTTAGGTATGAAAGCCAGCT -3
FLT3-ITD R	5'-CTTTCAGCATTTTGGACGGACGGCAAC-3'

3. Results

This study included One hundred patients diagnosed based on morphological, immunophenotype, and molecular genetics with acute myeloblastic leukemia ; 54 of them were males and 46 were females; 54 (55.8%) were newly diagnosed and 46 (44.2%) were under treatment, the mean of age is 32.9 years. The size of the amplified DNA fragment of the wild type was approximately 133 bp, whereas the mutated type showed an additional band > 133bp (approximately 180 bp).

Eight (8%) out of 100 Sudanese patients with AML were positive for FLT3-ITD mutation while 92 (92%) were negative. 6 (75%) from the 8 positive for FLT3-ITD mutation were belong to the New diagnosed group, and 2 (25%) from the 8 positive for FLT3 ITD mutation were belong to the treated group. Table (2).

Although two (25%) out of the eight patients with FLT3-ITD mutation were males, and 6 were female (75%), no statistically significant correlation was found between the mutation and gender (P. value=0.282). The mean age of the patients with the mutation was higher than those without the mutation, but the difference was not statistically significant (*P. value*: 0.30). Four of the mutated cases were FAB subtype M2, two were M5b, one was M4 and one was M6. However, no statistically significant correlation was found between the mutation and subtypes of AML (P. value= 0.014). Table (3).

Table 2: Frequencies of Study Group

Study Groups	Frequency	Percent	Positive	Negative
New diagnosed	54	54.0	6	48
Under treatment	46	46.0	2	44
Total	**100**	**100.0**	**8**	**92**

Table 3: Association of Result with FAB Subtypes

Sub-diagnosis	Result				Total
	Positive		Negative		
	count	percent	count	percent	
(M2/M3v)	0	0.0	1	1.0	1
M0	0	0.0	23	23.0	23
M1	0	0.0	16	16.0	16
M1/M2	0	0.0	1	1.0	1
M2	4	4.0	10	10.0	14
M2/M3	0	0.0	1	1.0	1
M3	0	0.0	12	12.0	12
M4	1	1.0	9	9.0	10
M5	0	0.0	9	9.0	9
M5a	0	0.0	1	1.0	1
M5b	2	2.0	2	2.0	4
M6	1	1.0	3	3.0	4
M7	0	0.0	4	4.0	4
Total	**8**	8.0	**92**	92.0	**100**

- *P.* value = .140
- Chi-squire test was used to calculate P value

- P value less than 0.05 considered significant

FAB classification	AML pos for FLT3/ITD, n (%)	AML neg for FLT3/ITD, n (%)
Mo	0 (0)	23 (25)
M1	0 (0)	16 (18)
M2	4 (50)	10(11)
M3	0 (0)	12(13)
M4	1 (12.5)	9 (10)
M5 M5a M5b	0 (0) 0 (0) 2 (25)	4 (15) 1 (1) 0 (0)
M6	1 (12.5)	3 (3)
M7 M1/M2 M2/M3	0 (0) 0 (0) 0 (0)	4 (4) 1 (1) 1 (1)

4. **Discussion**

Patients with FLT3-ITD mutations be inclined to have a particularly unfavorable prognosis, with a bigger risk of relapse and shorter overall survival (OS) compared with patients without the mutation (Lyu *et al*, 2020).

The type III receptor tyrosine kinase "fms-like tyrosine kinase 3" (FLT3) is expressed on normal hematopoietic stem cells and acute myeloid leukemia cells and regulates their proliferation. ITD mutation of FLT3 has been reported to be present in a third of AML cases, results in constitutive activation and aberrant signaling of FLT3, and is associated with adverse treatment outcomes (Mohamed *et al*, 2014).

This study conducted to determine the frequency of FLT3-ITD mutation in Sudanese patients with AML. In this study, FLT3-ITD mutation was detected in 8% of patients with acute myeloid leukemia, and this was lower than that reported in other populations but in comparison with other study done by **Mohamed *et al,* 2014** it is higher, they found (5.8%) of Sudanese AML patients were FLT3 ITD positive and lower when compared with study done by ***Lazenby et al,* 2014** which done on 1312 patients, they found (16%) had an FLT3ITD mutation positive, in addition **Elyamany *et al,* 2014** reported the frequency of FLT3 ITD mutation among Saudi AML patients as (18.55%). **Al-Mawali *et al,* 2013** reported that 31% of adult patients with AML were FLT3-ITD positive in Australia. **Dhahir *et al,* 2012** reported a frequency of 10% for FLT3-ITD mutation in Iraqian pediatric AML patients. The frequency of FLT3 ITD among Japanese AML patients reported to be 23%, in Dutch 22%, United Kingdom 27%, and German 23% (Harada *et al*, 2018).

There is variation in the Incidence rates of FLT3-ITD mutation as find in the previous studies, this variation can be prejudiced by source material (peripheral blood, BM or plasma DNA), or the techniques used to amplify and detect the FLT3 DNA or RNA (Al-Mawali *et al*, 2013*)*. Furthermore, whether these studies were done on samples from newly diagnosed patients or patients treated on chemotherapy or relapsed patients may be a factor results in this variation, as the number of mutated blasts can be so different.

In the present study, FLT3 ITD mutation was not associated with FAB subtypes, 4 of the mutated cases were FAB subtype M2 and 2 was M5b and 1 was M4, 1 was M6. This finding was supported by many studies concerning with the correlation of FLT3-ITD mutation and FAB subtypes**.** In Australia **Al-Mawali *et al,* 2013** reported that half of the FLT3 ITD aberrations were found in patients with FAB M1, and fewer were found in patients with FAB M2, M4, and M5. In Serbia, **Hamed *et al,* 2021** reported that the highest frequency was seen in the M0 subtype, and the lowest in the M1 subtype. In Egypt, Yassin & Sidhom, 2003 reported that in pediatric AML higher frequency of FLT3/ITD was found in M3 and M1, and lesser in M2.

FLT3 mutations are an independent factor of a poor prognosis in AML patients under the age of 65 in most of the studies (Owen *et al*, 2010). In this study, the mean age of the patients with the mutation was 32.9 years.

5. Conclusion

The frequency of FLT3-ITD mutation among Sudanese patients with acute myeloid leukemia involved in this study was lower than other populations, and there was no correlation between FLT3-ITD mutation and FAB subtypes of AML.

6. References

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