DOI:10.31557/APJCB.2019.4.4.81

RESEARCH ARTICLE

Molecular Study of *FLT3* Gene Mutations in Acute Myeloid Leukemia from Pakistan: Correlation with Clinicopathological Parameters

Mariam Faiz[1], Farzana Rashid[2]

[1]Institute of Nuclear Medicine and Oncology, P.O Box 10068, New Campus Road, Lahore, Pakistan. [2]Lahore College for Women University, Lahore, Pakistan.

Abstract

Introduction: *FLT3* mutations are common genetic changes reported to have prognostic significance in acute myeloid leukemia (AML). Bone marrow/peripheral blood samples of 63 AML Pakistani patients were collected and DNA was isolated. **Materials and Methods:** The *FLT3* internal tandem duplication (ITD) and the D835 activating mutation in the tyrosine kinase domain (*TKD*) were analyzed by polymerase chain reaction (PCR). **Results:** Among 63 AML patients, 42 were males and 21 were females with male to female ratio 2.1:1. The age ranged between 15 to 75 years with a median age of 32 years. AML-M2 was the predominant French-American-British (FAB) subtype (32%) followed by M3 (27%), M4 (19%), M5 (6.3%) and M1 (6.3%). The incidence of *FLT3/ITD* and *TKD* was 22% and 6.3% respectively. Majority of the *FLT3/ITD* mutation were detected in AML-M4 (38%) patients while *D835* mutation was common in both FAB M1, M2. Presence of mutation was significantly associated with age but significance was not achieved for hyperleukocytosis. **Conclusion:** This study constitutes the first report from Pakistan reporting significant presence of *FLT3/ITD* mutations in our adult AML patients with different FAB subtypes Molecular mutation analysis in different cytogenetic groups with follow-up is required to understand the pathogenesis of leukemias and their role as a valuable prognostic marker in our patients.

Keywords: *FLT3* mutations- Pakistan- AML- PCR

Asian Pac J Cancer Biol, **4 (4)**, 81-84

Submission Date: 08/29/2019 Acceptance Date: 11/25/2019

Introduction

In acute myeloid leukaemia (AML), mutations for cell differentiation and proliferation are considered to be effective factors amongst various factors for its development. Fms-like tyrosine kinase3 (*FLT3*) genes belong to the family of tyrosine kinase class III receptors that induce signals for cell proliferation. Mutations of the genes in the form of internal tandem duplication in the juxtamembrane region (*FLT3/ITD*) has been described as single most common molecular genetic abnormality in acute myeloid leukemia with direct clinical impact on the disease outcome [1-2]. Another mutation called D835 activation loop domain mutation has also been reported. In literature, an average frequency of approximately 20% for *FLT3/ITD* and 7% for D835 has been reported [3]. The *FLT3/ITD* has been reported to occur in 16.5% of paediatric AML patients [3]. The occurrence increases with age, i.e 20% of adult AML patients [3] and 34% of elderly AML patients [4]. These mutations are associated more frequently with standard risk cytogenetics, *PML/RARα* rearrangement [4-5]. However, they are less frequent with core binding factor leukemia, secondary or pediatric AML [6]. This mutation is thought to be associated with leukaemia progression and a poorer clinical outcome in both paediatric and adult patients. These mutations are recommended in international clinical guidelines as for estimating prognosis and deciding treatment after complete remission (CR), particularly in cytogenetically-normal patients with acute leukaemia [7]. In Pakistan, cases of Acute leukaemia have been defined solely by the French– American–British (FAB) classification system [8]. As such, little is known about the prevalence of mutations and their prognostic importance in terms of

Corresponding Author:
Dr. Mariam Faiz
Institute of Nuclear Medicine and Oncology, P.O Box 10068, New Campus road, Lahore, Pakistan.
Email: mariamfaiz@gmail.com

Acute leukaemia classified according to the World Health Organization (WHO) classification system in Pakistan. In the present study, we have examined the cohort of 63 AML patients for mutation and determined its correlation to basic hematological data in them. Due to limited data on these mutations in Pakistan, the diagnosis and frequency of these mutations with different FAB subtypes in Pakistani AML patients is an important concern.

Materials and Methods

Patient samples

Blood samples from 63 adult AML patients with various French-American-British (FAB) classifications were collected from different Haematology departments of Lahore, Pakistan. The diagnosis of AML was based on morphology and FAB classification. The cytogenetic and immunophenotypic data for these patients were not available due to lack of such facilities in these departments. Informed consent was obtained from the patients before start of therapy.

Molecular studies

DNA extraction from blood samples of 63 AML patients was performed by the proteinase K and Phenol methods [9]. The patients DNA was isolated and stored at -20°C for further analysis.

Analysis of ITD/FLT3 and D835 Mutations

PCR amplification of *FLT3/ITDs* exons 14 and 15 were amplified using primers as described elsewhere [4]. *D835* and I836 amino acids are encoded by GATATC, which is the recognition sequence for *EcoRV*. PCR product was digested in a reaction volume of 15 ml, with 5U of *EcoRV* (New England BioLabs), at 37°C for 3 hours. The digestion products were separated on a 3.5% agarose gel, and mutants were detected by the loss of GATATC site.

Statistical analysis

Chi square χ^2 and Fisher's exact tests were used to analyze differences in the distribution of variables among subsets of patients using SPSS 16.0.

Results

Clinical characteristics of Patients

Among 63 AML patients, 42 (67%) were males and 21 (33%) were females with male to female ratio 2:1. The age ranged between 15 to 75 years with a median age of 32 years. Only six patients were above the age of 50 years. Among 63 patients, AML-M2 was the predominant French-American-British (FAB) subtype (32%) followed by M3 (27%), M4 (19%), M1 (9.5%), M1 (6.3%) and M0 (6.3%). Details of clinical characteristics at diagnosis of the 63 de novo AML patients were given in Table 1.

Clinical Characteristics of the AML patients harboring the FLT3/ITD⁺ and D835mutations

Of the 63 AML patients studied for mutations, 14 (22%) had *FLT3/ITD,* indicated by presence of longer PCR fragments. While 4 AML patients (6.3%) were found to contain the *D835* mutation. None of the patients

Table 1. Clinical Features of AML Patients Classified According to *FLT3/ITD* Mutations Status

Characteristic	Wild Type FLT3		Mutant FLT3		
	No	%	No	%	p
Cases	49	78	14	22	
Age (median= 32 years)					
≥15- 20	15	31	3	21	
21-35	21	43	7	50	
36-50	9	18	7	14	0.03
>50	4	8	2	14	
Gender					
Male	32	65	10	71	
Female	17	35	4	29	0.75
Haemoglobin g/dl					
≤10	36	73	11	79	0.3
> 10	13	27	3	21	
WBC Count x 10^9/L					
≤10	9	18	2	14	
>10-50	16	33	4	29	0.14
>50	24	49	8	57	
Platelet count x10^9/L					
≤50	36	73	10	71	0.3
>50	13	27	4	29	

Abbreviations, FLT3, fms-like tyrosine kinase 3; AML, acute myeloid leukemia; ITD, internal tandem duplication; WBC, white blood count; FAB, French American British.

Table 2. Frequency of the *FLT3/ITD* Gene Mutation in Patients with Acute Myeloid Leukaemia Classified According to the French–American–British (FAB) Classification System (n= 63) Compared with International Data (n=863)

†FAB Subtype	*Current study data	International data [11]
M0 4/63 (6.3%)	1/4 (25)	0/14
M1 6/63 (9.5%)	1/6 (17)	40/148 (27.0)
M2 20/63 (32%)	3/17 (18)	49/210 (23.3)
M3 17/63 (27%)	5/20 (25)	58/159 (36.5)
M4 12/63 (19%)	3/12 (25)	51/172 (29.7)
M5 4/63 (6.3%)	1/4 (25)	20/81 (24.7)
M6 0/63	0/2	1/15 (6.7)
Total	14/63 (22.2)	219/810 (27.0)

*Data are shown as *FLT3/ITD* mutation-positive cases/ tested cases (%); † Data shown as prevalence (%) of FAB subtypes in AML patients

had a combination of *FLT3/ITD* and *D835* mutation in the *FLT3* gene. The frequency of mutations in different FAB subtypes in present study was compared with international data as given in Table 2. The data revealed *ITD* mutation was found in all FAB subgroups among the studied patients. M4 subtype showed most of mutations as compared to other subtypes (Table 2) whereas majority of the D835 mutant patients (14%) were M1 and M2 type each. *ITD* mutation was not confined to any gender or age as these were found in all age groups (Table 1). However, statistical significance was achieved in different age groups. Presence of *FLT3/ ITD* was clearly associated with hyperleukocytosis where WBC counts (mean $150x10^9$/L) were higher in ITD^+ patients than in *FLT3/WT* patients (mean $65x10^9$/L) but significance was not achieved. Association of clinical variables with *D835* mutations could not be studied due to limited samples though WBC counts were higher (mean $34.8x10^9$/L), but not statistically significant when compared with wild type patients.

Discussion

A number of reports have shown that *FLT3/ITD* is associated with a poor prognosis in AML patients [7-8-10]. However, in Pakistan, no data exists that investigated the prevalence and prognostic value of the *D835* and *FLT3* mutations in AML patients with different FAB subtypes. Of the 63 AML patients examined, fourteen patients (22%) showed *FLT3/ITD* and four patients (6%) showed D835 mutations. None of the patients showed a combination of both *FLT3/ITD* and D835 mutations. In this study, FAB-M2 was most commonly seen (32%) that has also been reported from another study from Pakistan (32.26%) followed by M1 and M4 (22.58% each) [8]. In contrast, another study from different centre reported AML-M4 as most common FAB subtype in 116 patients studied [11-12]. In this study, the highest rate of *FLT3/ITD* mutations was detected in FAB-M4 patients while *D835* mutations were equally detected in FAB-M1, M2 patients. The incidence of *ITD* mutations detected in our cohort of AML patients fall within the range of reported international studies [7-8-10]. Among FAB subtypes, *FLT3* mutations were more commonly reported within the M2, M3 subtype [13]. However, fairly even distribution of *FLT3/ITD* mutations across all other FAB subtypes has also been reported [14]. This study is the first attempt to determine the incidence of these mutations in our patients in the absence of any cytogenetic data. It is important to note that incidence of mutation varies significantly between AML subtypes particularly in those subgroups defined by cytogenetic abnormalities. Variation in the frequency of *ITD* mutations reported (13–27%) may be because of the differences in the size of cohorts or subgroups of cohorts examined in the various studies. Therefore this limitation will be addressed and incidence of these mutation in different cytogenetic groups of AML will further be investigated in future studies.

In AML patients, the *FLT3-ITD* mutations were reported to be associated with increased leukocyte counts at diagnosis. This was observed in our cases harbouring *FLT3/ITD* mutations as compared with those experiencing wild type *FLT3* that is consistent with other reported studies [4]. In this study, significant association between incidence of mutations and patient age was found. Whereas no such correlation between patient age and *FLT3* mutation status has been reported in other studies [4].

In conclusion, our results confirmed the significant presence of *FLT3/ITD* mutations in our adult AML patients. The biology of AMLs is very diverse and varies in different populations Therefore, extensive mutation analysis in different cytogenetic groups with follow-up durations is required to understand the pathogenesis of leukemias and their role as a valuable prognostic marker in our patients.

Acknowledgements

The authors would like to acknowledge Director, INMOL for permission to use technical facilities and collection of patient data.

Conflict of interest

The authors declare no conflicts of interest.

Source of Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Stirewalt DL, Radich JP., The role of *FLT3* in haematopoietic malignancies. Nat Rev Cancer 2003; 3: 650 – 655.
2. Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K et al., Internal tandem duplication of the *FLT3*gene found in acute myeloid leukemia. Leukemia 1996; 10: 1911–1918.
3. Yokota S, Kiyoi H, Nakao M, Iwai T, Misawa S, Okuda T et al. Internal tandem duplication of the *FLT3*gene is preferentially seen in acute myeloid leukemia and myelodysplastic syndrome among various hematological malignancies. A study on a large series of patients and cell lines. Leukemia1997; 11: 1605–1609.
4. Kiyoi H, Naoe T, Nakano Y, et al. Prognostic implication of *FLT3* and N-RAS gene mutations in acute myeloid leukemia. Blood 1997; 93:3074-3080.
5. Levis M and Small D. *FLT3*: ITDoes matter in leukemia. Leukemia. 2003; 17: 1738–1752
6. Abu-Duhier FM, Goodeve AC, Wilson GA, Gari MA, Peake IR, Rees DC et al. *FLT3* internal tandem duplication mutations in adult acute myeloid leukaemia define a high risk group. Br J Haematol. 2000 111: 190–195.
7. Rombouts WJ, Blokland I, Lowenberg B, Ploemacher RE.. Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the *FLT3*gene. Leukemia 2000; 14: 675–683.
8. Hassan K, Qureshi M, Shafi S, Ikram N, Akhtar MJ. Acute myeloid leukemia-FAB classification and its correlation with clinico-haematological features. J Pak Med Assoc. 1993; 43:200-203.
9. Sambrook J, Russell DW. 2001.Molecular Cloning, A Laboratory Manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press.
10. Kiyoi H, Towatari M, Yokota S, Hamaguchi M, Ohno R, Saito H et al. Internal tandem duplication of the *FLT3* gene is a novel modality of elongation mutation which causes constitutive activation of the product. Leukemia 1998; 12: 1333–1337.
11. Harani MS, Adil SN, Shaikh MU, Kakepoto GN, Khurshid M. Frequency of FAB subtypes in acute myeloid leukemia patients at Aga Khan University Hospital Karachi. J Ayub Med Coll Abbottabad. 2005;17: 26-29
12. Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA et al. The presence of a *FLT3* internal tandem duplication in patients with acute myeloid leukaemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 2001; 98: 1752–1759.
13. Schnittger S, Schoch C, Kern W, et al. *FLT3* length mutations in AML: correlation to cytogenetics, FAB-subtype, and prognosis in 652 patients. Blood 2000;. 96:826a.
14. Thiede C. Steudel C. Mohr B. Schaich M. Scha-kel, U. Uwe, P, Wermke, M. Bornha¨user, M et al. Analysis of *FLT3*-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis Blood 2002; 99: 4326-4335.