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Frequency of FLT3–ITD Mutation in Newly Diagnosed Acute Myeloid Leukemia: A Single-Center Study in Pakistan

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Abstract

Objectives: To determine the frequency of FLT3-ITD mutation in newly diagnosed cases of acute myeloid leukemia.
Methodology: The cross-sectional study was carried out over a period of one year from January to December in the department of hematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi Total 85 patients were included in this study. After obtaining 3 ml of venous blood in EDTA tubes, DNA was extracted using QIAamp DNA kit. Conventional PCR was performed for detection of FLT3-ITD mutation with positive and negative controls.
Results: FLT3-ITD mutations were detected in 15 cases (17.6%). Out of 85 cases, 56 (65.9%) were males while remaining 29 patients (34.1%) were females. Most common AML type according to French American British (FAB) classification was AML-M2. 33 cases (38.8%). Mean values of different hematological variables were TLC 51.8 x10^9/L (±80.98), Hb9.16 g/dl (±8.15), Platelets 59.25 x10^9/L (±60.49) and Myeloblasts 47.57% (±27.93) in bone marrow aspirates.
Conclusion: It is concluded that FLT3-ITD mutations are quite frequent in our AML patients with different age specificity. Frequency of FLT3-ITD mutation in our study was lower (17.6%) than other international studies. The detection of these mutations would be helpful in risk stratification and selection of appropriate management of the patients.

Key Words: FLT3 Mutation, Acute myeloid leukemia, PCR

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Introduction

Acute Myeloid leukemia (AML) is a malignant disorder that evolve from clonal transformation of immature hematopoietic cells.[1] Overproduction of immature cells (myeloblasts) decreases the efficiency of the hematopoiesis leading to anemia, thrombocytopenia and abnormal leucocyte count.[2]

For survival analysis, AML is separated into three cytogenetic subcategories: good risk [t (8;21), t (15;17), Inversion (16)]; intermediate risk (Normal Karyotype, numerical and other structural abnormalities); and poor risk (>3 clonal abnormalities).[3] FMS like tyrosine kinase 3 (FLT3) is a class III tyrosine kinase receptor with significant roles in hematopoietic stem cell differentiation and propagation.[4] FLT3 is positioned on chromosome 13q12 and comprises of 24 exons. FLT3 mutation can be separated into two groups. Internal tandem duplication (FLT3-ITD+ mutations) in juxta-membrane domain of receptor and point mutations leading to single amino acid changes arising within activation loop of tyrosine kinase domain (FLT3-TKD mutations).[5]

FLT3-ITD is the most frequently mutated gene in AML and seems to be triggered in one third of AML cases.[6] Patients with FLT3-ITD AML tend to have a poor prognosis, very aggressive course and high incidence of relapse after remission. It usually presents with high TLC and normal karyotype on cytogenetics thus shifting the intermediate risk to poor risk.[7] International literature reports the frequency of FLT3-ITD and FLT-TDK mutation in20% and 10% cases of newly diagnosed AML patients regardless of age.[8] According to a local study from Lahore, the frequency of FLT3 mutation was witnessed in 17% of AML patients.[2] This study will provide us with local magnitude of the problem. Early detection of FLT3 mutation shall thus be useful for patients to overcome adverse prognosis by selecting most effective available treatment option.

Methodology

The cross sectional study was carried out over a period of one year from January to December in the department of hematology, Armed Forces Institute of Pathology

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(AFIP), Rawalpindi Newly diagnosed cases of AML irrespective of age and gender were included in the study. Patients already on treatment for AML or with incomplete data were excluded from the study.

Total 85 newly diagnosed patients were included in this study. Peripheral blood and bone marrow samples were obtained from all the patients belonging to different ethnic groups. Samples were collected over a period of one year including both male and female patients between the ages of 15-75 years. Consecutive non-probability sampling was done. After taking 3ml of venous blood in EDTA tubes, DNA was extracted using QIAamp DNA kit. Conventional PCR for detection of FLT3-ITD mutation was used.

Conventional PCR reaction was done using 500 ng of DNA, 50 mmol of KCL, 67 mmol/ L, Tris HCL, PH 8.8, 1.0 mmol/ L of MgCl2, 0.001% (w/v) of gelatin, 200 mmol/L of dNTPs, 300ng of each published primer, and 1U of gold Taq polymerase in a volume of 50µL. The PCR reaction was started with incubation step at 94°C for four minutes followed by 35 cycles at 94°C for one minute, 52°C for 30 seconds, and 72°C for 30 seconds and final extension step at 72°C for 10 minutes on a GeneAmp PCR system 9700. PCR products were examined on standard 4% polyacrylamide gels stained with ethidium bromide and photographed under U/V light. 100 base pair ladder was used for revealing of FLT3-ITD mutation. Wild type FLT3-ITD mutation appeared at 325 base pair and was run as negative control. Mutant FLT3-ITD mutation appeared above 325 base pair and was run as positive control. Samples presenting added longer PCR products above 325 base pair were considered FLT3-ITD positive.

Collected data was analyzed using SPSS version 20. Mean and standard deviation were calculated for quantitative variables like age and TLC (Total leucocyte count). Frequencies and percentages were calculated for qualitative variables like gender and FLT3-ITD mutation. Data has been stratified for age and gender.

Results

Total 85 patients were included in this study during the period of 12 months. Age-wise 63 patients (74.1%) were in 15-45 years age group and 22 (25.9%) were between 46-75 years. Mean age of the patients was 33.99±18.37 years. Out of 85 patients, 56 (65.9%) were males while remaining 29 patients (34.1%) were females (Table I). Distribution of cases according to FAB type revealed AML-M2as the most common subtype with 33(38.8%) cases. FLT3-ITD mutation was identified in 15 patients (17.6%). In both age groups males (65.9%) were more affected than females (34.1%). Mean values of different hematological variables were as follows: TLC 51.8 x10^9/L (±80.98), Hb8.32g/dL (±2.45), Platelets 59x10^9/L (±60.49) and blasts 47.59% (±27.93%) and 73.28% (±20.08%) in peripheral blood and bone marrow respectively (Table II). Stratification of cases with respect to age and gender was also carried out. No significant difference was noted in the presence of FLT3-ITD mutation with respect to age or gender. (Chi square= 0.329, p value = 0.567) and (Chi square = 0.005, p value = 0.944) respectively.

Table I: Patients' stratification as per age and sex.

		N (%)
Age (years) (Mean=33.99± 18.37)	15-45	63 (74.1%
	46-75	22 (25.9)
Sex	Male	56 (65.9)
	Female	29 (34.1)

Table II :AML – Haematological Values

Parameter	Mean	Range
TLC (X 10 ^{9}/l)	51.8	68-489
Hb (g/dl)	8.32	3.9-15.0
Platelets (X10 ^{9} /l	59.25	4-311
Blasts in Peripheral Blood (%)	47.59	2-99
Blasts in Bone Marrow	73.28	20-97

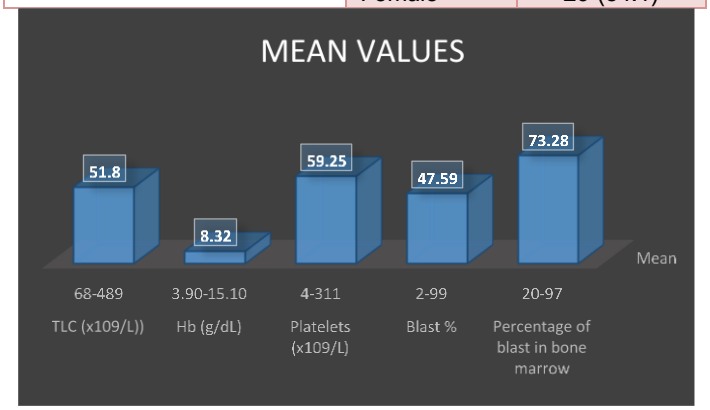

Figure 1. Mean values of hematological variables. (n=85)

Discussion

FLT3 (Fms-like tyrosine kinase-3), also known as FLK2 (fetal liver kinase-2) and STK1 (human stem cell kinase-1), was originally secluded as a hematopoietic progenitor cell-specific kinase and belongs to Class-III receptor tyrosine kinase (RTK) family.[9]Normal expression of FLT3 is limited to hematopoietic progenitor cells in the bone

marrow, thymus, and lymph nodes but is also found in other tissues such as placenta, brain, cerebellum, and gonads.[10]

FLT3 is frequently overexpressed in Acute Leukemia. FLT3 mutations occur in approximately 30% of acute myeloid leukemia (AML) patients and confer an adverse prognosis.[11] Many studies have shown that AML patients with FLT3-ITD mutations have poor clinical outcome due to high relapse rate. This has led to the development of a number of small molecule tyrosine kinase inhibitors (TKI) with activity against FLT3. Adult patients usually have a higher incidence of FLT3-ITD (24%) than pediatric AML patients.[12]

Moreover, the clinical impact of FLT3-TKD mutation which is found in approximately 5–10% of AML patients is not clear yet, but several studies have shown that it is also an adverse prognostic indicator.[13] Clinically AML patients with FLT3-ITD tend to have high WBC counts and an increased percentage of blast cells.[14] FLT3 mutations have also been seen in myelodysplastic syndrome (MDS) in about 3–5% of newly diagnosed patients and occasionally negative MDS patients also develop FLT3 mutations when evolved to AML.[15]

Kennedy VE et al in their review article describes frequency of FLT 3 mutation in one third cases of AML (25%).[16] The overall 17.6% frequency of FLT3 mutations in our study is relatively less than stated in other International studies. Elyamany G et al study in Saudi Arabia showed FLT-ITD mutation's frequency of 14.4%.[17] Similarly, another study carried out by Nadyme S et al from Lahore reported FLT3 mutations frequency 17% in AML patients which is consistent with our figures.[2] The explanations of lower frequency of FLT3 mutations in our study from other several studies may be explained by differences in the sizes of examined groups or might be due to population genetics, environmental factors, selected patient population studies, or because of differences of age.

Short, N.J studied 3525 adults with newly diagnosed AML, the incidence of FLT3-ITD mutations ranged from 23% in patients <45 years of age to 15% in those >70 years of age.[18] In the present study the mean age of the patients was 33.99±18.37 years with a male predominance and according to FAB type, AML-M2 as the most common subtype with 33(38.8%) cases. Faiz M et al in 2019 collected data from multiple hospitals in Lahore. Their results showed that 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.[19]

In the present study the hematological parameters in peripheral blood and bone marrow showed high TLC with low hemoglobin and platelets. Blasts were numerous (TLC 51.8 x 109/L (±80.98), Hb 8.32g/dL (±2.45), Platelets 59x109/L (±60.49) and blasts 47.59% (±27.93%) and 73.28% (±20.08%). A four year study in Western India by Shankaralingappa S. et al on 424 cases of AML showed FLT3 ITD mutations in 70 cases. They compared various hematological parameters in FLT3-ITD positive and negative cases. They found that FLT3-ITD mutation cases showed high tumor burden characterized by higher total leucocyte count ($p < 0.001$), higher blast percentages in peripheral blood ($p = 0.01$) as well as in bone marrow ($p = 0.03$), and higher serum LDH levels ($p = 0.10$) compared with FLT3-negative patients.[20] Similar findings are reported by Fan Y et al who analyzed seventeen trials involving 2252 patients. Their results suggested that FLT3 ITD mutations could become an indicator of poor prognosis of AML, and these patients should receive more intensive therapy according to current guidelines.[21]

Conclusion

The frequency of FLT3 - ITD mutation in our study is 17.6%, which is lower than other international studies. It was closely associated with male gender and relatively younger age group. Further large studies are needed to provide epidemiological data and clinical datato device optimal therapeutic strategies for better clinical outcome.

Limitations of Study: The generalizability of the results might have been affected by the convenient purposive sampling. Study population mainly represents Khyber PakhtunKhawah and Central Punjab of Pakistan.

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