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Frequency of FLT3/ITD Mutation in Patients with Acute Myeloid Leukaemia presenting at Combined Military Hospital, Rawalpindi

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ABSTRACT

Objective: To assess the frequency of FLT3/ITD mutation in patients of Acute myeloid leukaemia presenting at Combined Military Hospital, Rawalpindi Pakistan.
Study Design: Cross-sectional study.
Place and Duration of Study: Oncology Department, Combined Military Hospital, Rawalpindi Pakistan, from Jan to Jun 2020.
Methodology: A sample of 46 patients with acute myeloid leukaemia was included using a non-probability, consecutive sampling technique. Genetic testing was done on all blood samples to identify FLT3-ITD mutation.
Results: Out of 46, 10(21.7%) were females and 36(78.3%) were males. The FLT3/ITD mutation frequency in our study sample was 10(21.7%), with all in de-novo AML patients and none in secondary AML. White cell count (55x109), bone marrow blasts (85%) and peripheral smear blasts (75%) were high in the FLT3-ITD mutated group in comparison to the non-mutated group.
Conclusion: Timely detection of FLT3/ITD mutation and an amplification of induction therapy would benefit this group of patients.

Keywords: Acute myeloid leukemia, AML prognosis, FLT3/ITD mutation.

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INTRODUCTION

Acute Myeloid Leukemia is a disorder of clonal hematopoietic stem cells and is a heterogeneous group of disorders which shows variable response to the therapy.[1] Even though most AML patients show complete morphological remission after intensive induction chemotherapy, the relapse rate is still significantly high.[2] To reduce the incidence of relapse, the decision regarding the options of post-remission therapy is dependent on the detection and confirmation of a particular set of genetic indicators at the time of diagnosis and evaluation of the residual disease by using the "multi-parameter flow cytometry.[3,4] The trend of the somatic mutations detected at the time of diagnosing acute myeloid leukaemia has been described in the literature. However, the pre-malignant mutational landscapes of AML and its influence on the risk and time to diagnosis are still not known.[5]

Mutations of the FMS, like tyrosine kinase mutation (FLT3), are generally observed mutations in AML and are frequently expressed in almost 30% of AML patients globally. This high frequency of mutation confers a poor prognosis of disease.[8] However, no national figures are available for the frequency of FLT3 mutation. FLT3, also known as FLK2 (fetal liver kinase-2) and STK1 (human stem cell kinase-1), was initially isolated as a hematopoietic progenitor cell-specific kinase and belongs to Class-III receptor tyrosine kinase (RTK) family.[6] The two key types of mutation that arise are internal tandem duplication (ITD) mutations of the juxta-membrane region and point mutations in the tyrosine kinase domain (TKD).[7] Normal expression of FLT3 is limited to hematopoietic progenitor cells in specific tissues like bone marrow (BM), thymus, and lymph nodes. However, this gene plays a strategic role in hematopoietic cells regarding cell survival, proliferation, and differentiation.[8] Many studies reported poor cure rates and treatment failure in AML patients, including FLT3- ITD mutations. Inhibitors of FLT3 mutations are in active clinical development. Midostaurin is the approved first-in-class FLT3 inhibitor used to treat these patients.[9,10] This study was planned to determine the frequency of FLT3/ITD mutation in AML patients at the regional level so that future research could be carried out regarding disease burden in our population and appropriate treatment therapies could be included right at the time of diagnosis to prevent relapse and determine poor prognosis.

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METHODOLOGY

The cross-sectional study was performed at the Oncology Department, Combined Military Hospital Rawalpindi Pakistan, from January to June 2020 after formal approval from the Ethical Review Board (104/08/20). The sample size was calculated by the WHO calculator taking frequency of FLT3 mutation from the previous study, i.e. 17%.[10]

Inclusion Criterion: Patients of either gender, aged 15-65 years who were diagnosed with AML a year ago and under treatment at CMH Rawalpindi were included in the study

Exclusion Criterion: Patients who developed metastatic disease or any malignancy other than acute myeloid leukaemia were excluded.

Informed written consent was obtained from the participating patients. After a thorough history and examination, blood samples were taken and sent to assess FLT3 / ITD mutations. PCR was applied on 20µl mixture that contained 1 x PCR buffer (Fermentas), 1.0 mM MgCl2, 0.2 mM dNTPs, 0.5 µM each of the primers, 0.5 units Taq polymerase (Fermentas) & 30ng DNA. Forward (11F) 5'-GCAATTTAGGTATGAA-AGCCAGC-3' and Reverse (12R) 5'-CTTTCAGCATT-TTGACGGCAACC-3' primers,

The PCR products were run on 2% agarose gel, and the findings were recorded.

Statistical Package for Social Sciences (SPSS) version 22.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages.

RESULTS

In our study, a total of 46 acute myeloid leukaemia patients were studied. The age range in our study was 15-65 years, with a mean age of 35±9 years. Out of 46,(36)78.3% were males and (10)21.7% were females (Figure-1). A total of 10 patients had FT3-ITD mutation, making an overall percentage of 21.7%, as shown in Figure-2. There were two secondary AML patients, and 44 were de-novo AML with respective percentages of 4.3% and 95.7%. In the case of secondary AML patients, none showed FLT3-ITD mutation.

In FLT3-positive patients, the mean WCC was 55x109/l compared to 12.5x109/l in the negative group. The mean platelet count and haemoglobin level in FLT3 mutated subjects were 53x109/L and 10g/L, respectively, compared to 71x109/l and 11.5 in the negative group. Peripheral blood blast count was raised in the FLT3 mutated group compared to the negative group (75% versus 57%). A similar pattern of bone marrow blasts was observed with a raised mean level of 85% in FLT3 mutated and a relatively decreased count (73%) in FLT3-ITD negative patients (Table-I).

Table-I: Comparison of Study Parameters among FLT3/ITD Mutated and Non-Mutated Groups (n=46)

Parameters	FLT3/ITD Positive	FLT3-ITD Negative
Frequency	10	36
Male: Female	8:2	28:8
WBC count	55x109/L	12.5x109/L
Platelet Count	53x109/L	71x109/L
Hemoglobin	10g/L	11.5 g/L
History of Acute Myeloid Leukemia		
De-Novo AML	10/46	34/46
Secondary AML	0/46	2/46
Blasts		
Bone marrow Blasts	85%	73%
Peripheral smear Blasts	75%	57%

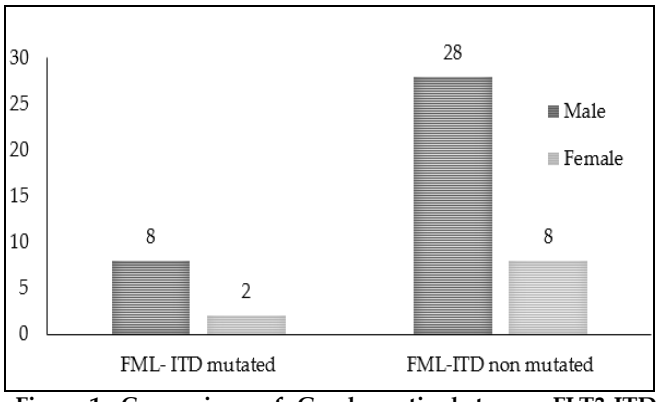

Figure-1: Comparison of Gender ratio between FLT3-ITD Positive and Negative Group(n=46)

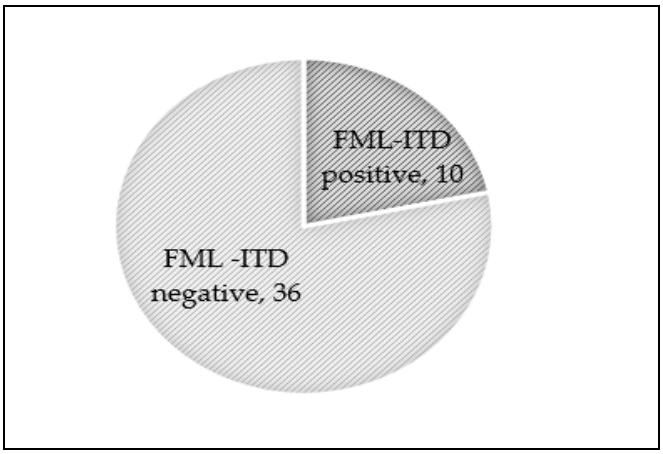

Figure-2: Frequency FLT3-ITD Mutation (n=46)

Table-II: Summary of Frequency of FLT3-ITD mutation in Previous Studies

Reference	FLT3-ITD Mutation Frequency
Frohling *et al*.[15]	32.0%
Wang *et al*. [16]	25.9%
Auewarakul *et al*.[17]	27.3%
Gari *et al*.[18]	11.6%
Ishfaq *et al*.[19]	13.3%
Zaker *et al*.[20]	18.0%
Sheikhha *et al*.[21]	10.0%
Xu *et al*.[22]	20.8%
Al-Tonbary *et al*.[23]	20.0%
Thiede *et al*.[13]	20.4%

DISCUSSION

This study showed a frequency of 10(21.7%) FLT3-ITD mutations in 46 patients. Research studies reported FLT3 mutations in approximately 25–35% of patients while sub-type FLT3-ITD embodies a frequency of 20-27% in adult AML patients13 and 10-16% in childhood cases.[11-14] A significant range of results is observed when comparing our study results with other published studies. This figure of 21.7% is much lower than 32%, 25.9%, and 27.3%, respectively, reported in a few studies.[15-17]. On the other hand, the same percentage is significantly higher than described in a few other studies with figures of 11.6%, 13.3%, 18% and 10% respectively (Table-II).[18-21]. However, the finding of FT3-ITD mutation in our study lies close to other studies conducted by Xu *et al*. Al-Tonbary *et al.* and Thiede *et al*. with percentages around the figure 20s.[22,23,13] Considering the literature demonstrated the link of FLT3 mutations with treatment failure in AML, this broad range of results in different studies implies that similar percentages of treatment failure and poor prognosis could be predicted in these populations.

The rate of FLT3-ITD mutations in males (8/10) is higher than in females (2/10), which is in accordance with a small study conducted on a sample of 30 AML patients with a frequency of FLT3-ITD mutation of 4/30(13.3%); three were males, and one was female.[19] These gender differences could be secondary to a small study sample, population genetics and environmental factors. This result needs further research and investigation in a larger sample size.

In our study, high white cell counts, high blast count in bone marrow, and peripheral smear of the FLT3-ITD mutated group in comparison to the non-mutated group confers a poor prognosis in these patients. The accordance of these findings with other published research exhibited FLT3-ITD mutation as a strong prognostic factor in AML patients and its association with disease progression.[24]

Our study demonstrated the burden of mutated disease in our population and the need for genetic testing at the initial diagnosis. This also gives way to individual modified treatment approaches and the possibility of preventing treatment failure in addition to timely management of poor prognostic factors. This will ultimately add to the survival of patients.

LIMITATION OF STUDY

The study limitations include a smaller sample size collected over a short time. More studies in the future incorporating multi-centre data can give us a more in-depth understanding of the frequency of this mutation. Another limitation of this study was the unavailability of testing other mutations like FLT3-TKD, KMT2A, ASXL1, TP53, NPM1, CEBPA, and RUNX1 in our institute, which can give us more understanding of prognostication. In the future, if we include the above markers with a multi-centre study with a larger study population, more valuable prognostic information can be calculated for better care and treatment.

CONCLUSION

There was insufficient data on the frequency of FLT3 mutations in our population. The study was intended to find the frequency of FLT3-ITD mutation in AML patients presented to our tertiary care unit. A significant population showed this mutation and needs further study regarding its importance in prognosis in our population. The study results that need further investigation in a larger multi-centre population is the gender-based high ratio of FLT3/ITD mutation in males compared to females. Timely detection of FLT3/ITD mutation and an amplification of induction therapy would benefit this group of patients.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

AB & RA: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

MNP & ZAA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AR & AZ: Concept, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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