ORIGINAL ARTICLE

PREVALENCE OF FLT-3 MUTATION IN ACUTE MYELOID LEUKAEMIA

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Background: FLT-3 mutation is a valuable prognostic marker in patients of AML being related with bad prognosis and poor clinical response to conventional chemotherapeutic agents. Frequency of FLT-3 mutation in AML varies from 25% to 35%. The objective of this study was to determine prevalence of FLT-3 mutation in patients with Acute Myeloid Leukaemia. **Methods:** This observational cross-sectional Study was conducted in Department of Oncology, Jinnah Hospital Lahore from 1st October 2018 to 31st March 2019. Patients with acute myeloid leukaemia, aged 15–60 years**,** of both genders were included. After taking consent, demographic data was noted. Three ml of sample of blood was obtained from each patient and sent for detection of FLT-3 mutation. Data was analysed using SPSS version 20.0. Chi square test was applied, *p*-value <0.05 significant. **Results:** A total of 180 patients were enrolled in this study. The mean±SD age of patients was 34.72±14.3 years, among which 38.3% were female and 61.7% male. The mean±SD duration of disease was 3.39±2.8 months. The mean±SD haemoglobin level, TLC and platelet counts were 7.2±2.3 g/dl, 30,913±63,573 per cm and 58.6±52.3×10^3 per cm. The blast cell (%) count was 69.6±19.8. FLT-3 mutation was present in 18.9%. **Conclusion:** We conclude that FLT-3 mutation to be present in only a minority of patients with Acute Myeloid Leukaemia having no significant association with age, sex, haemoglobin, WBCs and blast counts.

Keywords: FLT-3 mutation; Acute Myeloid Leukaemia

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INTRODUCTION

Acute Myeloid Leukaemia (AML) is a life-threatening hematologic malignancy that is detonated by accumulation of immature myelogenous cells in the blood and bone marrow.[1] It presents usually at a later adulthood stage and has a substantial proportionate mortality in all the cancer related deaths throughout the world.[2] AML is one of the most common form of leukaemia in the Western world and accounts for nearly 25% of all adult leukemia.[3] Affected hematopoietic cells progressively accumulate a variety of molecular alterations during pre-leukemic evolution and disease propagation, which include somatic mutations, epigenetic alterations, cytogenetic abnormalities, and transcriptomic changes.[4] Cytogenetics, together with mutation status, form the basis of risk classification system and provide means for risk stratification in AML patients.[5]

FMS-like tyrosine kinase 3 (FLT-3) is a tyrosine-kinase receptor with vital roles in hematopoietic stem/progenitor cell survival, proliferation and differentiation. Mutations in FLT-3, specifically internal tandem duplication in the juxta-membrane domain (FLT3-ITD), are seen in approximately 20% of AML patients.[6] It is related with bad prognosis of patients and a poor clinical response to conventional chemotherapeutic agents. Specific targeted therapy with tyrosine kinase inhibitors such as ibrutinib, midostaurin, sorafenib and sunitinib along with bone marrow transplantation is needed to improve the outcome in these patients.[6] Literature has shown that the frequency of FLT-3 mutation in AML varies and was reported to range from 25–35%.[7] However the emergence of drug resistance[8] to FLT3-inhibitors has posed a crucial challenge in optimal management of patients, warranting the desire for further comprehending the biology and complexity of FLT-3 in AML. FLT-3 mutation is a valuable prognostic marker in patients of AML. Very limited published data is available regarding presence and prognosis of FLT-3 mutation in AML patients in the Pakistani population. It is not known whether ethnic differences play a role in the progression and prognosis of disease. Relapse of leukaemia and poor response to conventional treatment in our population leads to increased disease burden, in-hospital stay and mortality. It is highly important to have information regarding the magnitude of the disease in Pakistani population, so that early targeted therapy can be given to these patients thereby reducing disease morbidity and

mortality in our country that has limited resources and a significant financial strain.

The objective of this study was to determine frequency of FLT-3 mutation in patients with Acute Myeloid Leukaemia presenting to Department of Oncology, Jinnah Hospital Lahore.

MATERIAL AND METHODS

This observational cross-sectional study was conducted for duration of six months from 1st October 2018 to 31st March 2019 in the Department of Oncology, Jinnah Hospital Lahore. Keeping margin of error of 6% and confidence level of 95%, the sample size was 180 patients (Allahyari *et al*.[9] showed frequency of 21%) using non-probability consecutive sampling technique. Patients of both sexes, aged 15–60 years, diagnosed with Acute Myeloid Leukaemia (presence of at least 20% myeloblast cells in bone marrow biopsy of the patient by microscopic examination) were included. Patients with AML secondary to myelodysplastic syndrome and patients of Chronic Myeloid Leukaemia in acute blast crisis were excluded. Written informed consent was taken. Information regarding demographic data was noted after which 3 ml of blood sample was obtained from each patient using aseptic technique and sent to the pathology laboratory for detection of FLT3 mutation using Polymerase Chain Reaction (PCR) - Qualitative. Presence of FLT3 mutation was labelled and noted. Confidentiality of the data was ensured. Data was entered and analysed using SPSS version 20.0. Stratification of outcome using chi-square test was done, keeping *p*-value <0.05 as statistically significant.

RESULTS

A total of 180 patients fulfilling the inclusion and exclusion criteria were enrolled in the study after taking informed consent from the patient's attendant. The mean±SD age of patients was 34.72±14.3 years, among which 38.3% (69 patients) were female and 61.7% (111 patients) were male. In this study, 8.3% (15 patients) belonged to high socioeconomic status, 31.7% (57 patients) belonged to middle socioeconomic status, where as 60.0% (108 patients) belonged to low socioeconomic status. The mean±SD duration of disease was 3.39±2.8 months. The mean±SD duration haemoglobin level, TLC and platelet counts were 7.2±2.3 g/dl, 30,913±63,573 per cm and 58.6±52.3 x103 per cm. The blast cell (% in the bone marrow) count was 69.6±19.8. FLT3 mutation was present in 34 patients (18.9%) where as it was absent in 146 patients (81.1%). Stratification for outcome was done as shown in table-1.

Table-1: Stratification of outcome

Variables	Pearson Chi-square coefficient	*p*-value
Age	3.647	.177 (non-significant)
Sex	2.495	.114 (non-significant)
Socioeconomic Status	0.992	.609 (non-significant)
Duration of Disease	2.479	.290 (non-significant)
Haemoglobin level	2.516	.284 (non-significant)
TLC	5.072	.079 (non-significant)
Platelet Count	2.181	.336 (non-significant)
Blast Cell Count	3.132	.209 (non-significant)

DISCUSSION

The presence of FLT-3 aberrancies in high-risk leukaemia patients was identified 2 decades ago and comprehension about the biology, clinical implications, and specific targeting therapies is still on-going. Whether FLT3-ITD is an initiating event in leukaemia genesis or not still remains controversial, but the conspicuous development of intrinsic resistance to FLT-3 inhibitor therapy reinforces its position as one of the crucial co-operating events in the generation of human AML. The FLT-3 mutation is considered a poor prognostic factor as it is associated with higher rates of early disease relapse and overall shorter survival despite chemotherapy. Both paediatric and adult clinical trials are underway to investigate the role of various FLT-3 inhibitors in conjunction with chemotherapy in FLT-3 positive AML patients. In the present study, we analysed 180 patients of AML for presence of FLT-3 mutation. In addition, we studied the clinicopathological features to determine their correlation with FLT-3 mutation as independent prognostic factors. According to the result of this study, FLT-3 mutation was found in 18.9% AML patients and it showed no statistically significant association with age, sex, socioeconomic status, duration of disease, haemoglobin, TLC, platelets and blast cell count. Stratification of this data with FLT-3 mutation showed insignificant association with WBC and blast percentages as in this study 49.4% patients presented with high WBC count >11000 per cm and 84.4% presented with blast counts ≥50%. In this study, 130 patients were aged between 15 to 45 years and among them FLT-3 was positive in 21.8% while 47 patients were aged between 46–60 years and among them 10.6% were positive for FLT-3 mutation.

In the cross-sectional study conducted in Iran on 100 AML patients by Allahyari *et al*[9] PCR for FLT-3 mutation was done. Mean age at diagnosis

was 28.5 years and 52% patients were male. FLT-3 mutation was present in 21% but there were no significant differences between sex, age or visceromegaly and these results were consistent with the findings of our study. Frequency of FLT-3 mutation in our study was also consistent with the study conducted by Ishfaq *et al*[10] in which 55 patients were enrolled. Twenty-five patients had acute lymphoblastic leukaemia (ALL) and 30 had AML. The polymerase chain reaction demonstrated FLT3/ ITD mutations in 4% ALL patients while in AML it was present in 13.3%. In AML, a statistically significant association was found between higher WBC count and FLT-3, and the mutation was more common in elderly patients aged 31–40 years. In ALL, no statistically significant association was found with the clinical features however the mutation was more common in age groups aged 21–30 years. Yunus *et al*[11] in Malaysia studied mutational analysis of exons 14–15 and 20 of the FLT-3 gene in 54 patients utilizing PCR-CSGE (conformational sensitive gel electrophoresis) to characterise FLT-3 mutations in adult AML cases. Mutations in FLT-3 exon 14–15 were seen in 13% and no mutation identified in FLT-3 exon 20. FLT3-ITD mutations were significantly associated with a high blast cell percentage and high white blood cell count however no significant difference was noted in overall median survival within 2 years. Seventy newly diagnosed chemotherapy-naive AML patients were enrolled by Raezei *et al*[12] in Iran for detecting the FLT-3 and NPM-1 gene mutations by PCR followed by direct sequencing. The frequencies of mutations in FLT3-ITD, FLT3-TKD and NPM-1 were 25.9%, 5.9% and 20.8% respectively. The FLT3-ITD mutation was more common in non-M3 subset of AML (FAB classification). No mutation was identified in either FLT3-TKD or NPM-1 genes in patients with M3 AML. No significant correlation was seen between the presence of FLT3-ITD and NPM-1 mutations. Considering the high stability of NPM-1 gene, it may be utilized in conjunction with FLT-3 to monitor patients, especially for detection of minimal residual disease.

A total of 113 AML patients were evaluated at diagnosis based on routine morphology and cytochemistry and classified according to the WHO criteria by Sazawal *et al*[13] in India. The distribution of AML subtypes was M1 (1 patient), M2 (32 patients), M3 (57 patients), M4 (14 patients), M5 (1 patient), M6 (1 patient) and 7 patients where morphological subtype could not be classified. RT-PCR was performed to identify PML/RARalpha, AML-1/ETO, CBFbeta/MYH-11 and FLT3/ITD. Of the 57 patients with M3 subtype, 55 had the PML-RARalpha fusion transcript. The prevalence of bcr-3 (short isoform) was 62% and bcr-1 (long isoform) 38% with no correlation with age, sex or white blood cell count. FLT3/ITD mutation was more frequent in APL subtype (17.5%), the frequency greater in patients with bcr3 isoform (70%) than in those with in bcr1 isoform (30%). Patients with FLT3/ITD mutation had a significantly higher median white cell count, short median overall survival. AML1-ETO fusion transcript was detected in 16 of 56 patients with no correlation with clinical or haematological parameters. Patients with APL who have FLT-3 mutation had a poorer prognosis. Therefore, it was concluded that rapid identification of specific translocations at diagnosis is important for prognostic purposes and their detection should be incorporated into routine assessment. In India, Chauhan et al[14] showed FLT3-ITD mutation to be present in 23% with association to young age less than 15 years and high WBC count. However, no significant difference was reported in the response rates to conventional chemotherapy in patients with or without FLT3/ITD mutation.

Kuchenbauer *et al*[15] correlated FLT-3 expression with clinical parameters, FAB types, cytogenetics and flowcytometry in 207 AML patients. FLT-3 levels correlated with high percentages of bone marrow blast cells, high leucocyte counts and M5 FAB subtype. In patients with normal cytogenetics no impact on overall survival was detected regardless of FLT-3 mutation, while in the patients with normal cytogenetics and wild-type FLT3, worse overall survival was noted. Likewise, a study conducted in China by Lee *et al*[16] on 52 AML patients showed FLT3-ITD positivity in 28.8% of AML patients which was associated significantly with absolute leucocyte counts and bone marrow blasts counts. Patients with t (15;17) showed higher prevalence of FLT3-ITD and significantly associated with worse overall survival.

None of WHO FAB subtype is directly associated with FLT-3 mutation in AML as results are variable in different countries according to different citied studies. Further studies are required to validate this to determine any geographic significance. Most of the studies performed proved the association between high WBCs count and blasts percentage with FLT-3 mutation, which was not seen in our study. Limitations of our study include a relatively small sample size and the inability to see association of various FAB subtypes with FLT-3 mutation. FLT3/ITD positive AML patients are often treated by Allogenic Stem Cell Transplant (SCT)[17] and there is an increasing interest to target the FLT-3 receptor tyrosine kinase with specific inhibitors.[18,19] FLT-3 inhibitors such as quizartinib, sorafenib and midostaurin are undergoing clinical

trials, and on 28th April 2017 the FDA approved midostaurin for treating FLT-3 positive adult AML patients.[20]

CONCLUSION

We conclude that this study showed FLT-3 mutation to be present in only a minority of patients with Acute Myeloid Leukaemia with no specific association with age, sex, haemoglobin, WBCs and blast counts. However, further studies with a larger number of patients are necessary to determine the prevalence of FLT-3 mutation in patients with AML and its correlation with severity of disease and prognosis so that appropriate therapeutic advancements can be considered depending upon magnitude and prognosis of the disease.

AUTHORS' CONTRIBUTION

This study was conceived and designed by KS, MA and KB. MA, NIB and JS did the initial literature research and designed the proforma for data collection. KS and JS did the data collection, assembly and patient assessment. Data analysis and interpretation was performed by NIB and FA. KS, NIB and FA were involved in manuscript writing. MA and KB did the final critical review and corrections. NIB is the corresponding author on behalf of all other authors.

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