Original Research Paper

Volume - 14 | Issue - 03 | March - 2024 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Human Genetics

EXPLORING FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA

Pankaj Gadhia* Ph.D., Molecular cytogenetic Laboratory, S.N. Gene Laboratory Pvt. Ltd. President Plaza A Ring Road, Nanpura, Surat 395007, India *Corresponding Author

Parth Shroff M.Sc., Molecular cytogenetic Laboratory, S.N. Gene Laboratory Pvt. Ltd. President Plaza A Ring Road, Nanpura, Surat 395007, India

Monika Patel Ph.D., Molecular cytogenetic Laboratory, S.N. Gene Laboratory Pvt. Ltd. President Plaza A Ring Road, Nanpura, Surat 395007, India

Salil Vaniawala Ph.D., Molecular cytogenetic Laboratory, S.N. Gene Laboratory Pvt. Ltd. President Plaza A Ring Road, Nanpura, Surat 395007, India

ABSTRACT Investigation of molecular evidence of FLT3 mutations in acute myeloid leukemia (AML) was carried out. Mutation analysis was conducted on 146 patients diagnosed with acute myeloid leukemia (AML) utilizing polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. Among the 146 AML patients analysed, 33 (22.60%) exhibited FLT3 mutations. Specifically, 27 patients harboured FLT3-ITD mutations, 3 presented FLT3 D835 mutations, and 3 showed both FLT3-ITD and FLT3 D835Y mutations. Notably, all patients with FLT3-D835Y or both FLT3-ITD and FLT3-D835Y mutations displayed heterozygous mutations. The present study did not identify a significant difference in the incidence of various types of FLT3 mutations concerning age and sex. Nevertheless, the study offers valuable insights into FLT3 mutation analysis among AML patients.

KEYWORDS : FLT3, AML, ITD, TKD, Mutation

INTRODUCTION

Acute Myelogenous Leukemia (AML) is a heterogeneous disease that encompasses diverse subtypes with distinct clinical and prognostic features. The heterogeneity arises from variations in genetic and molecular abnormalities present in leukemic cells.[1] Prognostic factors are crucial in determining the outcome of AML patients. Age, cytogenetic (chromosomal changes in leukemic cells), performance status (general health and functioning ability of the patient), and response to treatments are among the most important prognostic factors in AML. [2,3]

Recent research has focused on identifying molecular abnormalities that play a significant role in AML prognosis. These abnormalities include mutations in genes such as NPM1, FLT3, WT1, and CEBPA. [4]. The presence or absence of these mutations can provide valuable prognostic information. Recognizing the impact of genetic and molecular abnormalities, the World Health Organization (WHO) has revised the classification of AML to incorporate these factors. The latest WHO classification reflects a shift from a purely morphological classification to a more comprehensive classification that incorporates genetic and molecular characteristics.[5]

AML is indeed an aggressive hematologic malignancy. It is characterized by the abnormal proliferation and accumulation of immature myeloid cells, known as blasts, in the bone marrow and bloodstream. These blasts fail to mature into normal blood cells, leading to a disruption in the production of healthy red blood cells, white blood cells, and Platelets.[6] The rapid growth of immature cells in the bone marrow interferes with the normal functioning of the hematopoietic system. As a result, individuals with AML often experience symptoms such as fatigue, weakness, shortness of breath, frequent infections, easy bruising or bleeding, and anaemia's[6]

FLT3 (FMS-like tyrosine kinase 3) is a receptor tyrosine kinase (RTK) belonging to class III of the RTK family, which also includes KIT. [7] FLT3 is expressed in early hematopoietic stem cells and a subset of dendritic cell progenitors. [8] The deregulation of FLT3 signalling, often caused by genetic mutations, can result in the aberrant activation of these pathways, leading to uncontrolled cell proliferation and decreased apoptosis. Mutations in FLT3, particularly internal tandem duplications (ITD) and point mutations in the tyrosine kinase domain (TKD), are commonly observed in AML and are associated with poor prognosis [8].

Indeed, the most common FLT3 mutation observed in acute myeloid leukemia (AML) is the internal tandem duplication (ITD) mutation within the juxtamembrane (JM) segment [4,7]. FLT3-ITD mutations are found in approximately 20-30% of AML patients and are more commonly observed in specific AML subtypes, such as normal karyotype (NK)-AML, acute promyelocytic leukemia, and AML with t(6;9)(p23;q34) translocation [9-11]. In NK-AML patients with FLT3-ITD, higher leukocyte counts are often observed. While the complete remission (CR) rates may be similar to FLT3-ITD negative patients, the presence of FLT3-ITD is associated with lower disease-free survival (DFS) and overall survival (OS), primarily due to a higher incidence of relapse [10-12]. It is worth noting that the prognosis is influenced by the allele burden of FLT3-ITD, with patients having a higher mutation burden generally experiencing a worse prognosis [13].

Another class of FLT3 mutations in AML involves point mutations in the TKD [14-16] with the most common being a mutation at aspartic acid residue 835 (D835) [17]. These point mutations cause a permanent open configuration of the activation loop, resulting in constitutive signalling of the FLT3 receptor[18]. FLT3-TKD mutations are .present in approximately 5-10% of NK-AML patients. The prognostic significance of FLT3-TKD mutations remains controversial and may depend on the presence of other mutations in conjunction with FLT3-TKD.

MATERIALS AND METHODS

The present study was conducted at S N Gene Laboratory and Research Centre, Surat, Gujarat, India. The study focused on Acute Myeloid Leukemia (AML) patients of different age groups. Blood samples were collected from these patients after taking their informed concerned in EDTA vacationers to preserve the blood for molecular studies. To isolate the DNA from the collected blood samples, the Spin column method was employed. The necessary reagents for DNA isolation were provided by the QIAamp blood kit, which is a commonly used for DNA extraction.

Following DNA isolation, genomic DNA was amplified using specific primers. These primers were designed to target and amplify the regions of interest related to the study. The amplification process was carried out using the standard Polymerase Chain Reaction (PCR) technique, which enables the selective amplification of specific DNA sequences. The samples obtained from the patients were subjected to PCR amplification, specifically targeting the FLT3-ITD mutation. The purpose of this step was to determine the presence or absence of FLT3-ITD mutation in the patients' samples.

Furthermore, PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) was performed to investigate the presence of D835Y mutations. PCR-RFLP is a technique used to detect genetic variations

or mutations by analysing the differences in the patterns of DNA fragments resulting from the digestion of amplified DNA with specific restriction enzymes. The PCR conditions included an initial denaturation at 95˚ C for 3 minutes; 28 cycles at 95˚ C for 20 seconds, 52˚ C for 20 seconds and 72˚ C for 30 seconds with final extension at 72˚ C for 3 minutes.

RESULTS

The analysis of samples revealed that the mean age at diagnosis of AML (FLT3) patients was found to be 43.96 years, with a range spanning from 0.583 years to 77 years (Table 1). This suggests that the age of patients diagnosed with AML (FLT3) varied widely, with the youngest patient being 0.583 years and the oldest being 77 years. Regarding the gender distribution among the patients, the study found that 91 patients (62.32%) were males, while 55 patients (37.67%) were females. This indicates that AML (FLT3) affected a higher proportion in male patients as compared to females. Furthermore, out of the 146 patients with AML, 33 patients (22.60%) had FLT3 mutation (Table 2).

Table 1: Baseline Variables Of Patients (n=146)

Variables		No. of Patients	Percentage	Mean ± SD	Range
Age in Years		146	----	43.96 ± 18.08	0.583-77
Sex					
	Male	91	62.32%		
	Female	55	37.67%		
FLT3					
	Mutation	33	22.60%		
	Wild-Type	113	77.39%		

Table 2 : Prevalence Of FLT3 Mutation On The Basis Of Types Of Mutant (n=33)

Mutation	Types of mutant	No. of Patients	Percentage
FLT3 ITD	-	27	81.81%
FLT3-D835Y	Homozygous mutant	0	0
	Heterozygous mutant	3	9.09%
Both FLT3 ITD and FLT3-D835Y	-	3	9.09%
Total		33	

DISCUSSION

FMS like tyrosine kinase III (FLT3) is the most repeatedly mutated gene in AML patients, with a strong disease specificity [19]. FLT3 mutations can lead to the production of an abnormal FLT3 protein that signals cells to divide and grow in an uncontrolled way. This can result in the development and progression of AML, so FLT3 mutations have become an important biomarker in the diagnosis and risk stratification of AML. In this study, we investigated the frequency and types of FLT3 mutations in 146 Indian population with acute myeloid leukemia (AML). FLT3 is a gene that codes for a receptor tyrosine kinase protein involved in cell growth and differentiation, and mutations in this gene are frequently observed in AML. There are two common types of FLT3 such as Internal tandem duplication (ITD) and Tyrosine kinase domain (TKD) mutations [20]. The presence of ITD and TKD mutations in the FLT3 gene is associated with a poor prognosis in patients with AML. FLT3 mutations can cause the FLT3 receptor to be continuously active, leading to the uncontrolled proliferation of leukemic cells.

In past, many large and small scale research was conducted for FLT3 mutations yet most of them concentrated on specific aspects such as on juvenile and adult population. However, few studies showed the low prevalence of FLT3 mutation which ranges from 5 to 16% in juvenile community whereas 10 to 45% in adult AML community [22-23]. Hence, a high frequency of FLT3-ITD mutation was shown in juvenile population which was around 40% [10] and 22.5% [24] respectively. Moreover, FLT3-ITD was first described by Nakao and his colleagues [25] and they reported that around 23% were found FLT3-ITD positive in AML. In other studies of Meshinchi et al., (2001) [22] and Ahmad et al., (2010) [23] found ranges from 10 to 45% respectively. In present study, our analysis showed that FLT3-ITD was positive in AML patients was reported around 22.60%.

An investigation by Dehbi et al. (2013)[21] showed that patients with the FLT3-ITD mutation were older than the wild type group (37 years vs. 30 years). We found similar observation (45.13 years vs. 41.25 years) in the present study. There was no significant difference in age between the group of patients with FLT3 mutations and those without FLT3 mutations (29.6 versus 28.2 years). FLT3 gene mutations were equally distributed in both males (49%) and females (51%) [26-27]. In most cases for AML, FLT3 mutation is heterozygous [28]. Similarly, Saroyan et al. (2014)[27] also reported all mutants (ITD and TKD) in their study were heterozygous for FLT3 mutation. Our results are in total agreement with above study.

The frequency of FLT3 mutation in AML patients was found to be 22.60%. Furthermore, the prevalence of FLT3-ITD mutations was higher than that of FLT3-TKD mutations.

The present study did not find any significant differences in prognostic factors such as age and sex between AML patients with FLT3 mutation versus those without FLT3 mutation. Additionally, the prevalence of FLT3 heterozygous mutations was found to be higher than that of homozygous mutations in AML patients. The conclusions suggest that FLT3 mutation, particularly FLT3-ITD, is a relatively common feature of AML and may have implications for prognosis and treatment selection.

CONCLUSION

Consequently, the conclusion drawn is that FLT3 mutations are relatively prevalent in AML patients and can manifest in various forms, with FLT3-ITD being the most frequent. Heterozygous mutations appear to be more prevalent than homozygous mutations. Detecting FLT3 mutations can aid in the diagnosis, prognosis, and treatment of AML. The current study found no significant differences in the incidence of different types of FLT3 mutations between age and sex groups. However, due to the limited sample size or availability of samples, drawing meaningful conclusions is challenging. Therefore, a larger sample size may be necessary to attain more reliable and precise results in such studies. In conclusion, this study offers valuable insights into FLT3 mutations in AML patients and underscores the necessity for more extensive research in this domain.

REFERENCES

1. Gilliland DG, Jordan CT, Felix CA. (2004) The molecular basis of leukemia. Hematology Am Soc Hematol Educ Program :80–97.
2. Avivi I, Rowe JM. (2005) Prognostic factors in acute myeloid leukemia. Curr Opin Hematol; 12(1): 62-7
3. Lowenberg B (2001). Prognostic factors in acute myeloid leukaemia. Best Pract Res Clin Haematol; 14(1):65–75.
4. Scholl S, Fricke HJ, Sayer HG, Hoffken K. (2009) Clinical implications of molecular genetic aberrations in acute myeloid leukemia. J Cancer Res Clin Oncol; 135(4):491–505.
5. Campo, E., Swerdlow, S. H., Harris, N. L., Pileri, S., Stein, H., and Jaffe, E. S. (2011). The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood, 117(19), 5019–5032.
6. Payandeh M, Khodarahmi R, Sadeghi M, Sadeghi E (2015). Appearance of acute myelogenous leukemia (AML) in a patient with breast cancer after adjuvant chemotherapy, case report and review of the literature. Iran J Cancer Prev, 8, 125-8.
7. Gilliland DG and Griffin JD. (2002) The roles of FLT3 in hematopoiesis and leukemia. Blood.; 100(5):
8. Rosnet, O., Bühring, H. J., Marchetto, S., Rappold, I., Lavagna, C., Sainty, D., et al (1996). Human FLT3/FLK2 receptor tyrosine kinase is expressed at the surface of normal and malignant hematopoietic cells. Leukemia, 10(2), 238–248.
9. Fröhling, S., Schlenk, R. F., Breitruck, J., Benner, A., Kreitmeier, S., Tobis, K., et al (2002). Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood, 100(13), 4372–4380.
10. Kottaridis, P. D., Gale, R. E., Frew, M. E., Harrison, G., Langabeer, S. E., Belton, A. A., et al (2001). The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (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, 98(6), 1752–1759.
11. Schnittger, S., Schoch, C., Dugas, M., Kern, W., Staib, P., Wuchter, C., Löffler, H., et al (2002). Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood, 100(1), 59–66.
12. Thiede, C., Steudel, C., Mohr, B., Schaich, M., Schäkel, U., Platzbecker, U., et al (2002). Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood, 99(12), 4326–4335.
13. Gale, R. E., Green, C., Allen, C., Mead, A. J., Burnett, A. K., Hills, R. K., et al (2008). The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood, 111(5), 2776–2784.
14. Abu-Duhier, F. M., Goodeve, A. C., Wilson, G. A., Care, R. S., Peake, I. R., and Reilly, J. T. (2001). Identification of novel FLT-3 Asp835 mutations in adult acute myeloid leukaemia. British journal of haematology, 113(4), 983–988.
15. Bacher, U., Haferlach, C., Kern, W., Haferlach, T., and Schnittger, S. (2008). Prognostic relevance of FLT3-TKD mutations in AML: the combination matters--an analysis of 3082 patients. Blood, 111(5), 2527–2537.
16. Mead, A. J., Linch, D. C., Hills, R. K., Wheatley, K., Burnett, A. K., and Gale, R. E. (2007). FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. Blood, 110(4), 1262–1270.
17. Whitman, S. P., Ruppert, A. S., Radmacher, M. D., Mrózek, K., Paschka, P., Langer, C., et al (2008). FLT3 D835/I836 mutations are associated with poor disease-free survival and a distinct gene-expression signature among younger adults with de novo cytogenetically normal acute myeloid leukemia lacking FLT3 internal tandem

duplications. Blood, 111(3), 1552–1559.
18. Fenski, R., Flesch, K., Serve, S., Mizuki, M., Oelmann, E., Kratz-Albers, K., et al (2000). Constitutive activation of FLT3 in acute myeloid leukaemia and its consequences for growth of 32D cells. British journal of haematology, 108(2), 322–330.
19. Papaemmanuil, E., Gerstung, M., Bullinger, L., Gaidzik, VI., Paschka, P., Roberts, ND. et al (2016) Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med (2016) 374: 2209–21.
20. Nurul Ain Fathma Abdullah, Rosline Hassan, Abu Dzarr, Ang Cheng Yong, et al (2011). Low incidence of FLT3 gene mutation among Malay patients with Acute Myeloid Leukaemia: A preliminary study 19(2): 57-6
21. Dehbi, H., Kassogue, Y., Nasserddine, S., Quessar, A., Nadifi, S. (2013). FLT3-ITD incidence and FLT-TKD mutations in acute myeloid leukemia patients with normal karyotype in Morocco, a preliminary study. Middle East J Cancer, 4, 1-5
22. Meshinchi, S., Woods, WG., Stirewalt, DL., Sweester, DA., Buckley, JD., et al (2001). Prevalence and prognostic significance of FLT3 internal tandem duplication in pediatric acute myeloid leukemia. Blood, 97: 89-94
23. Ahmad, F., Mandava, S., Das, BR. (2010). Analysis of FLT3-ITD and FLT3-Asp835 mutations in de novo AML: Evaluation of incidence, distribution pattern, correlation with cytogenetics and characterization of internal tandem duplication from Indian population. Cancer investigation, 28(1): 63-73
24. Karabacak, B., Erbey, F., Bayram, I., Yilmaz, S., Acipayam, C., et al (2010) Fms-like tyrosine kinase 3 mutations in childhood acute leukemias and their association with prognosis. Asian Pacific J Cancer Prev., 11: 923-7
25. Nakao, M., Yokota, S., Iwai, T., Kaneko, H., Horiike, S., et al (1996). Internal tandem duplication of the FLT3 gene found in acute myeloid leukemia. Leukemia, 10(12): 1911-1918.
26. Ruan, M., Wang, YQ., and Zhang, L. et al (2011). FLT3 mutations in children with acute myeloid leukemia, a single center study. Zhongguo Dang Dai Er Ke Za Zhi, 13, 863-6
27. Sarojam, S., Vijay, S., Raveendran, S. (2014). FLT3 mutation as a significant prognostic marker in de novo Acute myeloid leukemia patients: Incidence, distribution and association of cytogenetics findings in South India. Middle east journal cancer, 5: 185-96
28. Gari, M., Abuzenadah, A., Chaudhary, A., et al (2008). Detection of FLT3 oncogene mutations in acute myeloid leukemia using conformationsensitive gel electrophoresis. Int J Mol Sci, 9, 2194-204