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Correlation of FLT3 Mutation in Acute Myeloid Leukemia with Morphology and Cytogenetics

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Abstract

Objective: To investigate the correlation of FLT3 mutation in acute myeloid leukemia with morphology and cytogenetics in the Pakistani population.

Methodology: The study was conducted at Chughtai Institute of Pathology from January 2022 to July 2023. Fifty newly diagnosed cases of AML were included, excluding patients who had received chemotherapy. FLT3 mutations were identified using the Imegen-FLT3 kit through PCR, while cytogenetic analysis was performed using FISH. Data were analyzed using SPSS version 23.00. Normality of data was assessed using the Kolmogorov-Smirnov test, and the Mann-Whitney U test was utilized to assess the correlation of FLT3 mutation.

Results: Of the fifty patients included, 29 (58%) were males and 21 (42%) were females, with a mean age of 41.7 years. FLT3 mutation was present in 29 (58%) patients and absent in 21 (42%) patients. Morphologic analysis did not reveal a correlation with any specific FAB subtype. Cytogenetic analysis was successful in 35 patients, with FLT3 mutation being randomly distributed among different cytogenetic subgroups, showing no specific correlation with any subgroup. The most common cytogenetic chromosomal abnormality detected was gain of chromosome 8. Blast percentage was significantly higher in FLT3 mutation-positive patients (72.3%) compared to FLT3 mutation-negative patients (56.76%) (p=0.02).

Conclusion: Although FLT3 mutation was prevalent among the patients studied, no significant correlation could be established between morphology and cytogenetics.

Keywords: Acute myeloid leukemia (AML), FLT3 mutation, cytogenetics.

Introduction

Acute lymphoblastic leukaemia (ALL) is the most common childhood neoplastic disease, representing approximately a quarter of all paediatric cancer diagnoses.[1] Within this heterogeneous disorder, the presence of specific genetic abnormalities plays a pivotal role in determining clinical behavior and treatment outcomes.[2] One such genetic alteration is the ETV6::RUNX1 gene rearrangement, resulting from the reciprocal t(12;21)(p13;q22) translocation.[3] This rearrangement leads to the formation of a fusion transcript involving the ETV6 (TEL) and RUNX1 (AML1) genes and is found in approximately one-fifth of paediatric ALL patients, specifically those with B-cell ALL (B-ALL).[3]

This translocation is known to develop during intrauterine life, and is thought to be associated with the presence of a pre-leukemic phase.[4] Its activity results in the over-expressions of Recombination-Activating Gene-1 (RAG-1) and RAG recombinase activity.[5] This causes modifications of the process of differentiation of hematopoietic progenitor cells as well as an enhancement of their ability for Acute myeloid leukemia (AML) is the disorder caused by uncontrolled proliferation of neoplastic myeloid stem cells in the bone marrow. Although it mainly involves the hematological cells but extra medullary presentation of AML can also occur.[1] Some of the cases of AML are caused by DNA damaging agents like therapeutic chemotherapy or hazardous agents in the environment but till date, the exact etiology of AML remains unclear.[2] Much work has been done at identifying the exact molecular pathogenesis that leads to leukemogenesis and several mutations have been identified which include *FLT3*, *NPM1*, *KIT*, *CEBPA* and *TET2*.[3]

Authorship Contribution: [1,3,5,6]Conceived and planned the idea of the study, [7]final approval of the version to be published, [2,4]drafting the work or revising it critically for important intellectual content, [8]Collecting the data,

Funding Source: none
Conflict of Interest: none
Received: Oct 29, 2023
Accepted: Jan 08, 2024

FMS-like tyrosine kinase (FLT3) encodes for a tyrosine kinase receptor that plays a significant role in controlling hematopoietic cell proliferation and differentiation and mutation of this gene leads to disruption of this delicate balance of cell survival. [4] *There are two main types of FLT3 mutations. One is* internal tandem duplication mutations in the juxta membrane domain (*FLT3*-ITD) and point mutations or deletion in the tyrosine kinase domain (*FLT3*-TKD). Both these mutations cause uncontrolled cell proliferation and stops apoptosis by effecting the intracellular signaling pathways. [5]

Cytogenetics is the study of chromosomes and is a very important diagnostic tool in assessing the prognosis of patients with AML[6] Fluorescent in situ hybridization (FISH) is the technique that uses fluorescent-labelled probes to analyze chromosomes and detect chromosomal abnormalities like translocations and other complex karyotypes that are present in acute leukemia([7]). The common cytogenetic abnormalities commonly seen in AML are translocations, insertions, or inversions along with monosomies, deletions, and unbalanced translocations.[8]

AML is often associated with old age and poor prognosis.[9] *In United States AML remains the most common blood cancer in adults and usually presents in the 6th decade of life([10]). However, in Pakistan, the prevalence of AML is on the rise and is occurring in younger age groups and has slightly male predominance*.[11] *The knowledge about cytogenetics and molecular mutations helps in stratifying patients and help plan their treatment regimens*[12] *Thus aim of this study is to* establish a correlation of FLT3 positive acute myeloid leukemia with morphology and cytogenetics in Pakistan's population.

Methodology

This study was conducted at the Chughtai Institute of Pathology from January 2022 to July 2023, following approval from the Institutional Review Board (IRB). A total of 50 diagnosed cases of Acute Myeloid Leukemia (AML) were included in the study after obtaining informed consent. Diagnosis of AML was established based on morphology, cytochemical stain, and immunophenotyping (using either Immunohistochemical stains or Flow cytometry). Both male and female patients of all age groups were eligible for inclusion. Patients who had undergone chemotherapy or were diagnosed with any other form of leukemia were excluded from the study.

Bone marrow biopsies were performed under aseptic conditions using a Salah trephine needle. A 2ml bone marrow aspirate was collected in EDTA and Sodium/Lithium heparin vials for cytogenetic testing and molecular analysis. The trephine samples were processed, and immunohistochemical markers were applied to diagnose and subtype AML. Sudan Black B stain was utilized on bone marrow aspirates to confirm the diagnosis of AML.

DNA extracted from white blood cells underwent amplification using gene-specific PCR fluorescently-labeled primers, followed by fragment analysis through Capillary Electrophoresis. The Imegen-FLT3 kit was employed to identify Internal Tandem Duplications (ITDs) in the FLT3 gene via PCR, and subsequently Capillary electrophoresis was utilized. PCR products were separated by capillary electrophoresis and detected using 6-Carboxyfluorescein. For cytogenetics, Sodium/Lithium Heparin samples were used for Fluorescent In Situ Hybridization (FISH), with interpretation of results performed using Cytovision software.

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 23.0. Mean and median were calculated for continuous variables, while frequency and percentages were calculated for categorical variables.

Results

The study comprised fifty patients, with 29 (58%) being male and 21 (42%) female, with a mean age of 41.7 years, ranging from 1 to 73 years. The demographic details are summarized in Table I.

The mean hematologic indices of the patients were as follows: hemoglobin 7.89 g/dL, white blood cell count: 56.39×10^3/µL, platelet count: 47.4×10^3/µL, and blast count: 65.78%.

Table I: Demographic Characteristics of the patients. (n=50)

Parameters	n (%)
Gender	
Male	29 (58%)
Female	21 (42%)
Age in Years	
Mean Age	41.7 years

Table II: Cytogenetic analysis of patients.	
Parameters	n (%)
Normal karyotype	16 (32)
Gain of chromosome 8	5 (10)
Tetrahyperdiploidy	3 (6)
del(7q)	1 (2)
Hyperdiploidy	2 (4)
Philadelphia positive	2 (4)
t(15:17)	1 (2)
47 XY 8[20]	1 (2)
del(5q)	1 (2)
t8:21	3 (6)
Culture failure	15 (30)

All fifty patients were diagnosed with AML, with 5 (10%) having AML M1, 2 (4%) with M2, 7 (14%) with M3, 4 (8%) with M4, 7 (14%) with M5, 1 (2%) with M0, and 24 (48%) patients not categorized into any specific AML subtype.

FLT3 mutation was detected in 29 (58%) of the patients, with 3 (6%) having both FLT3 ITD and TKD mutations, 6 (12%) having ITD alone, and 20 (40%) having TKD alone. Cytogenetic analysis was performed on all 50 patients but was successful in only 35 cases, as 15 samples experienced culture failure, as detailed in Table II.

On the basis of cytogenetic analysis, the patients were grouped into 10 categories, the details of which are given in table II. 15 (30%) out of all samples had culture failure. 16(32%) of the patients had a normal karyotype. The most common cytogenetic abnormality encountered was gain of chromosome 8 encountered in 5 (10%) of the patients. Ordinal $\chi 2$ analysis showed that the FLT3 mutation was randomly distributed amongst different cytogenetic subgroups and there was no specific correlation with any cytogenetic subgroup (Table III).

Out of 29 FLT3 positive patients 9(31%) had a normal karyotype. The least encountered abnormalities were t(15:17), 47 XY [8:20] and del(5q) , found in 1 patient each. Both the patients with 47 XY [8:20] and del(5q) were positive for FLT3 mutation while the only patient with t(15:17) was negative for FLT3 mutation.

Morphologic analysis was available for all 50 patients. No correlation with any single specific FAB subtype was found. FLT3 mutation was equally distributed in AML-uncategorized subtype (12 patients positive for FLT3 and 12 negative) and AML M2 (1 positive and 1 negative). All 4 (100 %) patients of AML M4 were positive for FLT3. Only one out of the 50 patients were diagnosed as AML M0 and was found to be negative for FLT3 (Table III).

Table III: Correlation of FLT 3 mutation with AML subtype and Cytogenetics.			
	FLT 3 Positive	FLT3 Negative	p-value
Normal karyotype	9	7	0.863
Gain of chromosome 8	4	1	0.293
Tetrahyperdiploidy	2	1	0.754
del(7q)	0	1	0.235
Hyperdiploidy	1	1	0.815
Philadelphia positive	1	1	0.815
t(15:17)	0	1	0.235
47 XY [8:20]	1	0	0.390
del(5q)	1	0	0.390
t8:21	3	0	0.128
Culture failure	7	8	0.288
AML M0	0	1	0.235
AML M1	3	2	0.924
AML M2	1	1	0.815
AML M3	5	2	0.438
AML M4	4	0	0.076
AML M5	4	3	0.960
AML – Non categorized	12	12	0.271

Table IV: Correlation with Age, TLC, Hb, platelet count and blast percentage.		
Parameter	FLT Positive	FLT Negative
Mean age	40.86 years	42.86 years
Median TLC	45 x 10^9	31 x 10^9
Mean Hb	7.8 g/dl	7.9 g/dl
Median Platelet	21 x 10^9 /L	39 x 10^9/L
Mean Blast percentage	72.3 %	56.76%

Normality of data was assessed using Kolmogorov-Smirnov test. Means were calculated for normally distributed data and median was calculated for data which was not normally distributed. Mann whitney U test was used to assess correlation of FLT3 mutation with age, TLC, Platelet count and blast percentage. The analysis showed that there was no significant difference in the Age, Hemoglobin levels, Total leukocyte count and platelet counts between the patients who were positive for FLT3 mutation and those who were negative for FLT3 mutation. However, we observed that the Blast percentage was significantly higher in patients who were positive for FLT3 mutation (72.3%) as compared to those who were negative for FLT3 mutation (56.76%) ($p=0.02$). (Table IV)

FLT3 mutation was almost equally distributed in both genders. 15(30%) of the malesu included in study were positive for FLT3 while 14(28%) of the females were positive for the mutation ($p = 0.291$).

Discussion

FLT3 mutation specially FLT3-ITD is positive in around 25-30% of the newly diagnosed cases and is associated with poor prognosis, high TLC and greater number of blast cells at the time of diagnosis.[13] The same was observed in our study where FLT3 positive patients had greater number of blast cells and TLC at the time of diagnosis.

FLT3 mutation was detected in 58% AML patients in our study group. It is slightly higher as compared to 24% patients in Syrian study conducted by Moualla et al. They attributed these variations to differences in sample size, geographical and racial diversities of the study group.[14]

AML is known to have poor outcome in elderly patients as they usually have a myelodysplastic phase before its transformation into acute leukemia and also have unfavorable cytogenetics.[15] Likewise, according to Pemmaraju et al young adults and adolescents have better outcome as compared to older patients.[16] Contrary to this in our study FLT3 mutation which is a bad prognostic factor was detected comparatively more in younger patients.

According to a study conducted in Iran, FLT3 mutation is commonly associated with AML M3, M4 and M5 and there is no significant difference in prevalence of FLT3 mutation in both genders. The same is supported by our study in which FLT3 was commonly seen in AML M3, M4 and M5 and no significant difference was observed in prevalence of FLT3 mutation in both genders.[17]

According to a study conducted by Santos et al patients with FLT3 mutation commonly had inv [16] abnormality as compared to t(8;21).[18] However, in our study none of patient was detected to have inv [16] abnormality but 6% of patients had t(8;21) and all of them had FLT3 mutation.

In a study conducted at NIBD, Karachi, significant contributing factors responsible for the failure of cytogenetic culture growth were identified. These included poor bone marrow sample quality, such as samples with high total leukocyte count (TLC) leading to clot formation, and patients presenting immediately after undergoing chemotherapy induction cycles. Other contributing factors to culture failure included improper sampling techniques, delays in sample transit to the laboratory, internal laboratory errors, specimen type, and suppression due to the patient's disease condition.[19]

Similarly, in our study, the rate of culture failure for cytogenetic studies was notably high. This was attributed to the low cellularity of bone marrow aspirate samples, which were hemodiluted, and delays in the transportation of samples to the cytogenetic department.

Limitations of the study: The biggest limitation of this study was that it was conducted on only 50 patients due to lack of funding and non-availability testing kits. Mutational analysis and karyotyping are only available in large tertiary care laboratories and are very expensive which most of the patients are unable to afford.

Conclusion

Although FLT3 mutation was detected in most of our patients and proved to be a poor prognostic factor for AML patients but no significant correlation could be established between FLT3 mutation, morphology and cytogenetics of these patients. This could probably be attributed to the smaller sample size and set back we faced due to culture failure of some samples. But we strongly recommend that such studies must be carried out on larger scale so that we can establish our own population's prognostic factors as most of the data that is available is of the western population who vary markedly from our genetic make-up and demography. Thus, we can modify treatment regimens according to our population and give AML patients a better fighting chance.

References

1. Pelcovits A, Niroula R. Acute Myeloid Leukemia: A Review. R I Med J (2013). 2020 Apr 1;103(3):38-40.
2. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. Blood Rev. 2019;36:70-87. https://doi.org/10.1016/j.blre.2019.04.005
3. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The Origin and Evolution of Mutations in Acute Myeloid Leukemia. Cell. 2012;150(2):264-78. https://doi.org/10.1016/j.cell.2012.06.023
4. Grafone T, Palmisano M, Nicci C, Storti S. An overview on the role of FLT3-tyrosine kinase receptor in acute myeloid leukemia: biology and treatment. Oncol Rev. 2012;6(1):8. https://doi.org/10.4081/oncol.2012.e8
5. Kiyoi H, Kawashima N, Ishikawa Y. FLT3 mutations in acute myeloid leukemia: Therapeutic paradigm beyond inhibitor development. Cancer Sci. 2020;111(2):312-22. https://doi.org/10.1111/cas.14274
6. Schoch C, Haferlach T. Cytogenetics in acute myeloid leukemia. Curr Oncol Rep. 2002;4(5):390-7. https://doi.org/10.1007/s11912-002-0032-0

7. Kannan TP, Zilfalil BA. Cytogenetics: past, present and future. Malays J Med Sci. 2009;16(2):4-9.
8. Mrózek K. Cytogenetic, Molecular Genetic, and Clinical Characteristics of Acute Myeloid Leukemia with a Complex Karyotype. Semin Oncol. 2008;35(4):365-77. https://doi.org/10.1053/j.seminoncol.2008.04.007
9. Lai C, Doucette K, Norsworthy K. Recent drug approvals for acute myeloid leukemia. J Hematol Oncol. 2019;12(1):100. https://doi.org/10.1186/s13045-019-0774-x
10. Siegel RL, Miller. Cancer Statistics. A Cancer J Clin 2020;0:1-24 8;70(1):7-30. https://doi.org/10.3322/caac.21590
11. Mahmood Q, Noor B, Arshad S. Acute Myeloid Leukemia; Experience at a Resource Limited Health Care Facility of Pakistan. J. Haematol Stem Cell Res. 2022; 2(2):
12. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and panel. Blood. 2017;129(4):424-47. https://doi.org/10.1182/blood-2016-08-733196
13. Bazarbachi A, Bug G, Baron F, Brissot E, Ciceri F, Dalle IA, et al. Clinical practice recommendation on hematopoietic stem cell transplantation for acute myeloid leukemia patients with FLT3 - internal tandem duplication: a position statement from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Haematologica. 2020;105(6):1507-16. https://doi.org/10.3324/haematol.2019.243410
14. Moualla Y, Moassass F, AL-Halabi B, Al-achkar W, Georgeos M, Yazigi H, et al. Evaluating the clinical significance of FLT3 mutation status in Syrian newly diagnosed acute myeloid leukemia patients with normal karyotype. Heliyon. 2022;8(11): https://doi.org/10.1016/j.heliyon.2022.e11858
15. Appelbaum FR, Gundacker H, Head DR, Slovak ML, Willman CL, Godwin JE, et al. Age and acute myeloid leukemia. Blood. 2006 ;107(9):3481-5. https://doi.org/10.1182/blood-2005-09-3724
16. Pemmaraju N, Kantarjian H, Ravandi F, Nogueras-Gonzalez GM, Huang X, O'Brien S, et al. Patient Characteristics and Outcomes in Adolescents and Young Adults (AYA) With Acute Myeloid Leukemia (AML). Clin Lymphoma Myeloma Leuk. 2016 ;16(4):213-222.e2. https://doi.org/10.1016/j.clml.2015.12.010
17. Yazdandoust E, Sadeghian MH, Shams SF, Ayatollahi H, Saadatpour Y, Siyadat P, et al. Evaluation of FLT3-ITD Mutations and MDR1 Gene Expression in AML Patients. Iran J Pathol. 2022 ;17(4):419-26. https://doi.org/10.30699/ijp.2022.543485.2776
18. Santos FPS, Jones D, Qiao W, Cortes JE, Ravandi F, Estey EE, et al. Prognostic value of FLT3 mutations among different cytogenetic subgroups in acute myeloid leukemia. Cancer. 2011;117(10):2145-55. https://doi.org/10.1002/cncr.25670
19. Javed S, Hassan J, Naz M, Shan S, Abid M, Shamsi TS. Cytogenetic culture failure and its causes in hematological disorders; a single centre perspective. Mol Cytogenet. 2023 Dec 1;16(1). https://doi.org/10.1186/s13039-022-00635-9