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Original Research Article

C-Kit and Flt3 mutation status in acute myeloid leukaemia with cytogenetic correlation and prognosis: A series of 75 cases

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ABSTRACT

Introduction: Recent WHO classification (5^{th} edition) has included Acute Myeloid Leukaemia (AML) with mutations in NPM1, CEBPA as separate entities along with AML with cytogenetic abnormalities in AML with recurrent genetic abnormalities. Even though C-kit and FLT3 mutations in AML have no diagnostic importance, prognostic significance in different subtypes of AML for the presence of these mutations are not the same.

Aims & Objectives: To assess the correlation between French American British (FAB) AML classification, specific cytogenetic abnormalities with C-kit, and FLT3 mutation in AML and to evaluate the prognosis and survival of AML patients with respect to cytogenetic abnormalities and C-kit and FLT3 mutation status.

Materials and Methods: Retrospectively all AML cases in which C-kit and FLT3 mutation status was assessed were retrieved; C-kit D816V mutation status had been assessed by Real time PCR; For FLT3 mutation, both Internal tandem duplication (ITD) and D835V mutation status had been assessed using PCR and gel electrophoresis; The data regarding morphological with immunophenotypic diagnosis, conventional karyotyping, FISH for translocation 8;21 (t (8;21)) and inversion16 / translocation16;16 (t (16;16) were also retrieved in all these cases along with follow-up from hospital records.

Results : Total 75 cases were included; Male/ Female ratio was 1.21:1 (41/34); Median age was 31 (Range: 2 - 64); 18 cases had translocation 8;21 (t (8;21)); 3 cases showed inversion16 / translocation16;16 (t (16;16); Out of the 18 cases which showed t (8;21), 10 cases had associated loss of sex chromosome. Eight cases had C-kit D816V mutation; three of which had t (8;21) while two had inversion 16. 12 cases had FLT3 mutations among which nine were ITD while three had D835V mutation. On karyotyping, one of these cases showed hyperdiploidy while the majority had normal karyotype. A single case had both C-kit D816V mutation and FLT3 D835V mutation with inversion 16 on karyotyping;

Discussion : Most common type of AML in both cases with FLT3 mutation and C-kit mutation was AML-M2 (FAB); Commonest karyotyping abnormality for cases with C-kit mutation was t(8;21); while for FLT3 mutation, the majority had normal karyotype; The single case which had both C-kit D816V mutation and FLT3 D835V mutation was alive event-free at three-year follow-up. Both FLT3 ITD and TKD mutations had a worse prognosis in our study. However, AML cases with C-kit mutation had a similar prognosis comparable to C kit negative cases. A large-scale study is required to elucidate the significance of this.

1. Introduction

World Health Organisation (WHO) classification (5^{th} edition) for Acute myeloid leukemia (AML) have AML

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with mutations in NPM1, CEBPA and RUNX1 and AML with cytogenetic abnormalities (eg. t (8;21), inv (16)) as separate entities.[1] This stratification was done as each of these have their unique genetic profile with a varied prognosis.[2] C-kit and FLT3 mutations in AML have no diagnostic importance; however, they have prognostic significance. FLT3 mutation including both ITD and TKD has an overall bad prognosis across various cytogenetic abnormalities.[1,3–8] Even though C-kit mutations has no stand-alone prognostic significance in inv(16), it has a worse prognosis in t(8;21). Multiple studies show contradictory results for C-kit mutation in relation to prognosis. While most of the studies concentrate on C- kit mutation status in core-binding factor (CBF) AML which includes AML with t(8;21) and AML with inv(16), it was not elucidated in AML patients with other cytogenetic abnormality and in AML with normal karyotype. Also, literature is lacking in terms of C-kit and FLT3 mutation status in Indian patients and their prognosis.[4,9] We carried out our study to assess the correlation between French American British (FAB) AML classification and specific cytogenetic abnormalities with C-kit and FLT3 mutation in AML. Also, we wanted to evaluate the prognosis and survival of AML patients with respect to cytogenetic abnormalities and C-kit and FLT3 mutation status.

2. Materials and Methods

Retrospectively all AML cases in which C-kit and FLT3 mutation status was assessed were retrieved from hospital case records. C-kit D816V (Exon 17) mutation status was assessed by Real-time PCR; For FLT3 mutation, both Internal tandem duplication (ITD) and D835V mutation status were assessed using PCR and subsequent Gel electrophoresis (Figures 1 and 2).

The data regarding morphological with immunophenotypic diagnosis, conventional karyotyping, FISH for translocation 8;21 (t (8;21)) and inversion16 / translocation16;16 (t (16;16)) were also retrieved in all these cases along with follow-up from hospital records (Figure 2).

3. Statistical analysis

SPSS software (version 20) was used for statistical analysis; Chi-square test, ANOVA test, and t-test were used wherever necessary depending on the variables. p-value of <0.05 was taken as significant

4. Results

A total of 75 AML cases on which C-kit and FLT3 mutation was done were included in the study. The median age of the patients was 31 years with an age range of 2-64 years. Male-female (M:F) Ratio was 1.21:1 (41:34).

AML- M2 (34/75) was the commonest in the French American British classification (FAB) of AML followed by M1 (20/75) and M4 (12/75). There were eight cases of M5 and a single case of M0.

Among the Chromosomal abnormalities, excluding the single case in which karyotyping and fluorescent in-situ hybridization (FISH) was not done, t(8;21) was the commonest cytogenetic abnormality(18/74) with additional abnormalities of t(8;12;21) in one case and deletion of sex chromosome in 9 cases. Hyperdiploidy and trisomy 8 were the next common (both - 4/74) followed by inv(16) (3/74). Inv(16) had associated trisomy 22 in 2 cases. Other than this, two cases had monosomy 7, and one case each had t(5;11) and complex karyotype. Predominant cases were of normal karyotype (NK) (41/74) (Table 1).

Follow-up data was available for 43(57.3%) cases while 32 cases (42.7%) cases were lost to follow-up. Among these, 19 cases expired while on follow-up (26.3%) and 4 cases relapsed (5.3%) on follow-up. The Median follow-up period was 2.5 months.

Among the 18 cases of t(8;21), AML-M2 was the commonest(14/18), while for inv(16), there were 2 cases of M4 and a single case of M5a.

Two cases each of hyperdiploidy and trisomy 8 expired and none of these 8 cases attained remission post-induction therapy.

Both the cases with monosomy 7 were of AML- M2. One among them expired at 10-month follow-up and one other case which attained remission post induction was lost to follow-up. A single case of complex translocation had AML- M1 on morphology. This case did not attain remission post-induction therapy and expired at 10 months follow-up.

4.1. Ckit-D816V mutation

There were eight cases that were positive for C-kit – D816V mutation. AML- M2 was the commonest morphology identified (3/8) followed by M4(2/8). C-kit D816V mutation was associated equally with t(8;21) and NK (3 cases each). Two other cases had inv(16) with trisomy 22 as an additional abnormality in both cases. 62.5% of AML with C-kit mutation had associated cytogenetic abnormalities, while 37.5% of cases were associated with normal karyotype.

Mean WBC count was in 30000/mm3 in C-kit positive cases while it was 45000/mm3 in negative cases. Mean Blast percentage in positive cases were 61%, while in negative cases was 56%. Both of these findings were not statistically significant. Remission post-induction therapy was attained in 37.5% (3/8) of C-kit positive cases which was not statistically significant from the overall remission rate post-induction (29.3%) (22/75 cases). While 50% of cases (4/8) did not attain remission in C-kit positive cases, 57.3% (43/75 cases) did not attain remission overall.

A single case of AML with C-kit mutation had relapse which had associated inv(16) with trisomy 22. Two cases of C-kit mutation are still on follow-up, one each with associated inv16+tri(22) and normal karyotype. No adverse events were noted in both these cases even at 3 years follow-up (Table 2).

4.2. FLT3 mutation

There were a total of 12 cases with FLT3 mutation, among which FLT3-TKD(D835) mutation was seen in 3 cases and FLT3-ITD mutation was seen in 9 cases. Mean WBC count and Blast percentage were not statistically significant. No specific association was seen with morphological FAB classification. AML-M1(4/9) is the commonest in cases with FLT3-ITD mutation and AML-M2(2/3) is the commonest in cases with FLT3-D835 mutation. The commonest karyotyping finding in these cases was NK (10/12) with one case each of hyperdiploidy and inv(16) with trisomy(22). Excluding 4 cases that were lost to follow-up, remission post-induction therapy was not attained in 80% (4/5) of FLT-3 ITD positive cases which was statistically significant (p-0.03) compared to the overall remission rate post-induction (29.3%) (22/75 cases). The one case which attained remission also relapsed during follow-up. Two of these cases expired (one each with hyperdiploidy and normal karyotype).

While in FLT-TKD mutated cases, 33.3% cases (1/3) did not attain remission in comparing 57.3% (43/75 cases) cases overall. This was not statistically significant. Among these three cases, one case relapsed on follow-up, while one case expired during induction chemotherapy; both of these cases had a normal karyotype (Table 3).

There was a single case with both C-Kit D816V mutation and FLT3 D835 mutation. Morphologically it was AML-M4. It had associated inv 16 with trisomy 22. This case attained complete remission post induction and the patient is event free at 3-year follow-up.

5. Discussion

Correlating with the general trend, AML occurs in adults (median age-31 years) with a wide age range (2-64 years).[10–12] AML- M2 was the commonest followed by AML M1 and M4 with a slight male preponderance (M:F ratio: 1.21:1). t(8;21) was the commonest cytogenetic abnormality accounting for 24%(18/75) of cases which was relatively higher than in published literature(5%).[13,14] Hyperdiploidy and trisomy 8 are both the second most common cytogenetic abnormalities with 4 cases each (5.3%) followed by inv16 (3/75) (4%).[15,16]

Coming to additional cytogenetic abnormalities, t(8;21) was commonly associated with loss of sex chromosomes(50%) and inv(16) had additional trisomy 22 in 66% cases. Both of these findings were concordant with the literature. Both hyperdiploidy and isolated Trisomy 8 had a bad prognosis showing a low complete remission rate and poor survival in our study similar to other studies.[15,16]

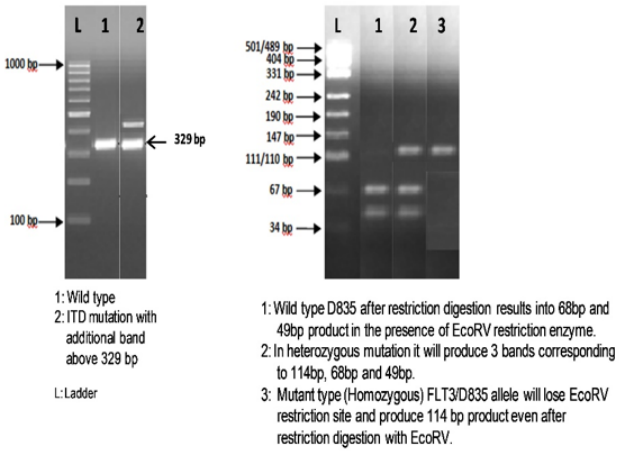

Figure 1: Guidelines for FLT3 gel electrophoresis

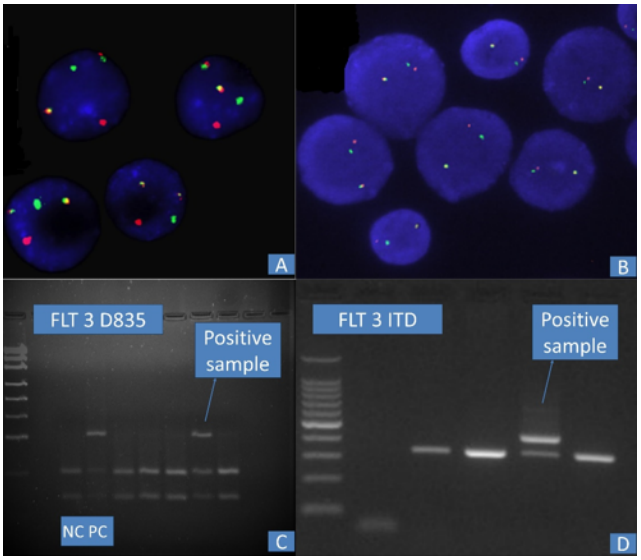

Figure 2: A: Cells show positivity for t(8;21) – FFOG (Dual colour dual fusion probe); **B:** Cells show positivity for inv (16)-FOG (Break apart probe for CBFB); **C:** Gel electrophoresis post restrictive endonuclease digestion showing one sample with FLT3 D835 haterozygous mutation; D: Gel electrophoresis showing one sample with FLT3 ITD mutation.

According to the literature, both C-kit and FLT3 mutation are associated with high tumor burden with more blast percentage and WBC count; However, this was not observed in our study. In our study, 66.6% (2/3) of FLT3 D835 mutation had either relapse or death, highlighting its worse prognosis across varying karyotypes. In addition, FLT3 ITD mutated cases showed a significantly low complete remission rate; Both findings are in concordance with the literature.[2–6]

Table 1: Correlation between FAB classification and karyotyping

		M0	M1	M2	M4	M5A	M5B	Total
Karyotyping	t(5;11)	0	0	1	0	0	0	1
	t(8;21)	0	3	14	1	0	0	18
	Complex	0	1	0	0	0	0	1
	Hyperdiploidy	0	0	2	0	2	0	4
	t (16;16) / inv16	0	0	0	2	1	0	2
	MONO 7	0	0	2	0	0	0	2
	NK	1	14	13	8	3	2	41
	ND	0	1	0	0	0	0	1
	Trisomy 8	0	1	2	1	0	0	4
Total		1	20	34	12	6	2	75

Table 2: Clinicopathological characteristics of C-kit-positive cases

C-kit positive cases	WBC count x 10 ^{3}	blast %	MORPH DX	Event	KARYO	Follow-up days	Remission after induction
1	82	58	M4	FU	N	907	Attained
2	8	80	M1	LFU	N	480	Not Attained
3	10	60	M2	LFU	8;12;21	23	LFU
4 (With Flt-3 D835 mutation)	14	50	M4	FU	INV16+ TRISOMY22	1146	Attained
5	41	29	M2	LFU	8;21	32	Not Attained
6	34	92	M0	LFU	N	49	Not Attained
7	31	89	M5A	Relapse	INV16+ TRISOMY22	393	Attained
8	20	36	M2	LFU	8;21, del-Y	40	Not Attained

Table 3: Clinicopathological characteristics of FLT3 positive cases

		wbc countx103	blast %	MORPH DX	Event	KARYO	Follow-up days	Remission after induction
flt3-D835	1	40	86	M2	Expired	N	18	Not Attained
	2	3	50	M2	Relapse	N	540	Attained
	3 (With C-Kit D816V mutation)	14	50	M4	FU	INV16+ TRISOMY22	1146	Attained
	Total N	3	3	3	3	3	3	3
flt3-ITD	1	64	80	M1	LFU	N	9	LFU
	2	9	28	M2	Expired	Hyperdiploidy	72	Not Attained
	3	185	92	M5B	Expired	N	8	Not Attained
	4	15	82	M2	Relapse	N	144	Attained
	5	24	70	M1	LFU	N	4	LFU
	6	22	80	M4	LFU	N	1097	LFU
	7	112	80	M1	LFU	N	66	Not Attained
	8	27	82	M1	LFU	N	18	LFU
	9	141	90	M2	FU	N	64	Not Attained

The single case of coexistent C-kit D816V mutation and FLT3-D835 mutation in AML with inv(16) has not been previously reported in the literature as far as our knowledge. Further studies are required to elucidate their incidence and prognostic significance which appears to be good in the single case found in our study.

Although C kit mutations are studied in detail in CBF AML in literature,[3,6,15] our study has highlighted the presence of C kit mutated AML cases in normal karyotype; Further studies are required to elucidate their prognosis and survival.

C-kit mutations do not appear to have reduced complete remission rate or poor survival in our study; This could be because of the small sample size; However, a study of large scale including more sample size is required to elucidate the exact survival data across different karyotypes.

Both FLT3 and C-kit mutations provide the potential for exploration of targeted RTK immunotherapy in AML cases in the future.[17–20]

6. Conflict of Interest

None.

7. Source of Funding

None.

References

1. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*. 2022;36(7):1703–19.
2. Lagunas-Rangel FA, Chávez-Valencia V, Gómez-Guijosa M, Cortes-Penagos C. Acute Myeloid Leukemia-Genetic Alterations and Their Clinical Prognosis. *Int J Hematol Oncol Stem Cell Res*. 2017;11(4):328–39.
3. Ayatollahi H, Shajiei A, Sadeghian MH, Sheikhi M, Yazdandoust E, Ghazanfarpour M, et al. Prognostic Importance of C-KIT Mutations in Core Binding Factor Acute Myeloid Leukemia: A Systematic Review. *Hematol Oncol Stem Cell Ther*. 2017;10(1):1–7.
4. Hussain SR, Raza ST, Babu SG, Singh P, Naqvi H, Mahdi F, et al. Screening of C-kit gene Mutation in Acute Myeloid Leukaemia in Northern India. *Iran J Cancer Prev*. 2012;5(1):27–32.
5. Marrero RJ, Lamba JK. Current Landscape of Genome-Wide Association Studies in Acute Myeloid Leukemia: A Review. *Cancers (Basel)*. 2023;15(14):3583. doi:10.3390/cancers15143583.
6. Riera L, Marmont F, Toppino D, Frairia C, Sismondi F, Audisio E, et al. Core binding factor acute myeloid leukaemia and c-KIT mutations. *Oncol Rep*. 2013;29(5):1867–72.
7. Shankaralingappa S, Joshi HD, Patel JB, Patel P, Sawhney J. FLT3 Gene Mutation in Acute Myeloid Leukemia: Correlation with Hematological, Immunophenotypic, and Cytogenetic Characteristics. *Asian J Onco*. 2021;8:22–8. doi:10.1055/s-0041-1731091.
8. Whitman SP, Ruppert AS, Radmacher MD, Mrózek K, Paschka P, Langer C, et al. 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*. 2008;111(3):1552–9.
9. Hussain SR, Naqvi H, Mahdi F, Bansal C, Babu SG. KIT proto-oncogene exon 8 deletions at codon 419 are highly frequent in acute myeloid leukaemia with inv(16) in Indian population. *Mol Biotechnol*. 2013;54(2):461–8.
10. Nagar V, Patil N, Gudur A, Gudur R. Clinicopathological study of acute myeloid leukemia in a tertiary care hospital. *Int J Health Sci*. 2022;6(S2):3070–7.
11. You E, Cho YU, Jang S, Seo EJ, Lee JH, Lee JH, et al. Frequency and Clinicopathologic Features of RUNX1 Mutations in Patients With Acute Myeloid Leukemia Not Otherwise Specified. *Am J Clin Pathol*. 2017;148(1):64–72.
12. Shuchismita S, Jamal I, Raman RB, Choudhary V. Clinico-Hematological Profile of Acute Leukemia Cases in Bihar: A Multiparameter Study in a Tertiary-Care Hospital. *J Lab Physicians*. 2022;15(1):84–9.
13. Foucar K, Anastasi J. Acute Myeloid Leukemia With Recurrent Cytogenetic Abnormalities. *Am J Clin Pathol*. 2015;144(1):6–18.
14. Yang JJ, Park TS, Wan TSK. Recurrent Cytogenetic Abnormalities in Acute Myeloid Leukemia. *Methods Mol Biol*. 2017;1541:223–45. doi:10.1007/978-1-4939-6703-2_19.
15. Moarii M, Papaemmanuil E. Classification and risk assessment in AML: integrating cytogenetics and molecular profiling. *Hematology Am Soc Hematol Educ Program*. 2017;(1):37–44.
16. Zheng J, Wang X, Hu Y, Yang J, Liu J, He Y, et al. A correlation study of immunophenotypic, cytogenetic, and clinical features of 180 AML patients in China. *Cytometry B Clin Cytom*. 2008;74(1):25–9.
17. Galanis A, Levis M. Inhibition of c-Kit by tyrosine kinase inhibitors. *Haematologica*. 2015;100(3):77–9.
18. Nieborowska-Skorska M, Paietta EM, Levine RL, Fernandez HF, Tallman MS, Litzow MR, et al. Inhibition of the mutated c-KIT kinase in AML1-ETO-positive leukemia cells restores sensitivity to PARP inhibitor. *Blood Advances*. 2019;3(23):4050–4.
19. Fernandez S, Desplat V, Villacreces A, Guitart AV, Milpied N, Pigneux A, et al. Targeting Tyrosine Kinases in Acute Myeloid Leukemia: Why, Who and How? *Int J Mol Sci*. 2019;20(14):3429. doi:10.3390/ijms20143429.
20. Loschi M, Sammut R, Chiche E, Cluzeau T. FLT3 Tyrosine Kinase Inhibitors for the Treatment of Fit and Unfit Patients with FLT3-Mutated AML: A Systematic Review. *Int J Mol Sci*. 2021;22(11):5873. doi:10.3390/ijms22115873.

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