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ORIGINAL ARTICLE

Morphological, cytogenetic and molecular characterisation of FLT3 mutations in Pakistani patients with *de novo* acute myeloid leukaemia: A single centre experience

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Abstract

Introduction: Mutations in *FLT3* are the most commonly reported genetic changes in AML patients. These mutations are normally identified in approximately one third of newly diagnosed patients and are reported to have prognostic significance. *Materials and Methods:* Peripheral blood samples was collected from 63 AML patients to study their morphological, cytogenetic and molecular features. PCR was used to determine the prevalence of *FLT3* mutations; internal tandem duplication (ITD) and tyrosine kinase domain (TKD) in AML patients. *Results*: Among 63 AML patients, 42 were males and 21 were females with male to female ratio 2:1with median age of 32 years. AML-M2 was the predominant French-American-British (FAB) subtype (42%) followed by M4 (27%), M3 (8%), M1 (8%), M0 (8%) and M5 (7%) respectively. Cytogenetic analysis of 60 patients showed 58% as cytogenetically normal (CN) whereas 42% had aberrant karyotype.The most frequent aberrations were trisomy8, t(8;21), t(15;17) (8.3%) each, inversion16 (5%), and different deletions (12%) respectively. FAB-M4 subtype showed most of the chromosomal anomalies. Among 63 AML patients, 22% showed *FLT3/ITD* while 6.4% had D835 mutation after molecular analysis. *FLT3* mutations were found in most of the FAB subtypes and cytogenetic groups. *FLT3/ITD* mutations were more common in patients with normal karyotype (26%) and usually present with hyperleukocytosis but association between two was not significant. *Conclusion:* The cytogenetic data of adult AML from Pakistan showed presence of favourable prognostic karyotype with comparable prevalence as reported in international data. Moreover, *FLT3/ITD* mutations are commonly found in our patients as determined by molecular analysis. Therefore, inclusion of this unfavourable prognostic marker should be routine in molecular diagnostic testing of AML.

Keywords: FLT3 mutations, Pakistan, AML, PCR, prognosis

INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive malignancy that is characterized as having heterogenous genetic makeup as well as complex clonal evolution.[1] It can be subtyped by morphology, immunophenotyping, cytogenetic, and molecular genetic features.[1] Clonal cytogenetic abnormalities that are either structural or numerical are usually found in 50-60% AML cases. Some chromosome aberrations in AML cases are nonrandom and are associated with specific cytomorphological subtypes according to French American British (FAB).[2-5] In the remaining cases, gene mutations are not identifiable by cytogenetics. These mutations play a major role in pathogenesis and prognosis of different cytogenetic subgroups.[6] Cytogenetic analysis at diagnosis is now considered the most important prognostic factor to predict clinical outcomes in AML patients.[1,7] Cytogenetic results have also been integrated into the World Health Organization (WHO) classification of AML.[8] Moreover, *FLT3* genetic testing including ITD (internal tandem duplication) and TKD (Tyrosine kinase Domain) mutations is now recommended by the National Comprehensive Cancer Network and European Leukemia Net[8,9] for diagnostic workup of patients with AML to identify patients who may benefit from different targeted treatment options.

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The heterogeneity of chromosome abnormalities in haematological malignancies in different geographic regions is reportedly well known. It has been reported in some published studies from Western and Asian countries that variable frequencies of recurrent cytogenetic markers exist due to ethical/and racial differences.[10-15] Differences in frequencies from similar or varied populations may give a better understanding of the role of ethnic, environmental, and geographical factors while studying the natural history of AML-associated chromosome abnormalities.[15]

The extensive data on the incidence of cytogenetic and genetic mutations in patients of AML from Pakistan is lacking. Limited data is available on the prevalence of these mutations in Pakistan. Therefore, it is important to diagnose and study these mutations in our AML patients with different subtypes. The study was designed to achieve the following objectives; 1) To determine the morphological, cytogenetic and molecular groups in our AML patients. 2) To analyse the association of *FLT3/ITD and FLT3/D835* mutationsin patients with cytogenetically normal and aberrant karyotypes along with clinical and laboratory parameters.

MATERIALS AND METHODS

Patient samples

Peripheral blood samples from 63 adult AML patients were collected from the Institute of Nuclear Medicine and Oncology, Lahore, Pakistan visiting for treatment. The diagnosis of AML was based on morphology and FAB classification as per WHO criteria.[3] Treatment outcomes were not part of study and were not analysed. The cytogenetic and immunophenotypic data for these patients were collected from medical records of the patients. Informed consent was obtained from the patients before the start of therapy.

Immunophenotype analyses

Immunophenotyping was performed by flow cytometry (BD FACSCanto™ II BD, USA). The acute leukaemia panel of monoclonal antibodies used were; CD2, CD3, CD4, CD5, CD7 (T-cell lineage), CD10 (CALLA), CD19, CD20, CD22 (B-cell lineage), cytoplasmic IgM heavy chain, (pre-B ALL), CD 11, CD14, CD15, CD33, CD34 (myeloid markers), CD117 (c-kit), and TdT, used to characterise the phenotypes of the leukaemia cells.

Cytogenetic analysis

Conventional cytogenetic GTG-banding technique was used to study the metaphase chromosomes of 60 AML patients. A normal karyotype group is classified by analysing a minimum of 20 metaphases in each patient. The patients showing abnormal karyotypes were grouped according to (a) number of single or more than single abnormalities, (b) gain or loss of genetic material, and (c) type of cytogenetic aberration. Karyotypes and chromosomal anomalies were characterised as per ISCN 2005 guidelines.[16]

Molecular studies

DNA extraction from blood samples of 63 AML patients was performed by commercial kit (Genejet, Thermo, USA). The patients DNA was isolated and stored at -20°C for further analysis.

Analysis of FLT3 Mutations

PCR amplification of *FLT3/ITD*and *D835* sequences located in the juxtamembrane and tyrosine kinase domain respectively were amplified by using primers described elsewhere.[17] D835 mutation was detected by RFLP method by using restriction enzyme *EcoRV*. PCR product was digested with 5U of *EcoRV* (NewEngland, BioLabs) at 37°C in a reaction volume of 15 ul. The mutants were detected after separation of digestion products on a 2% agarose gel.[17]

Statistical analysis

Statistical differences in the distribution of variables among subsets of patients was analysed by Chi square χ^2 and Fisher's exact tests using SPSS 16.0. A *p*-value of <0.05 was considered significant.

RESULTS

Clinical characteristics of patients

Among the 63 AML patients, 42 were males and 21 were females with male to female ratio 2:1. The median age of the patients was 32 years with an age range between 15 to 75 years. Only six patients were above the age of 50 years. Among the different age groups, patients having age of 30 years or younger are 33 (52%). While older than 30 years were 30 (48%). Details of clinical characteristics at diagnosis of the 63 *de novo* AML patients were given in Table 1.

Morphological characterisation

AML-M2 was the predominant French-American-British (FAB) subtype (42%) followed

Table 1: Demographic and Clinical features of AML patients (N=63) according to *FLT3/ITD* mutations status

Characteristic Cases	Wild Type 49(N)	*FLT3* 78(%)	Mutant 14(N)	*FLT3* 22(%)	*p*
Age (median= 32 years)					
≥15- 20	15	31	03	21	
21-35	21	43	07	50	
36-50	09	18	02	14	0.03
>50	04	08	02	14	
Male	32	65	10	81	
Female	17	35	04	29	0.75
Haemoglobin (g/dl)					
≤10	36	73	11	09	0.30
>10	13	27	03	21	
WBC Count x 10^9/L					
≤10	09	18	02	14	
≥10-50	16	33	04	29	0.14
>50	24	49	08	57	
Platelet count x10^9/L					
≤50	36	73	10	71	0.30
≥50	13	27	04	29	
BM Blasts					
≤70	15	33	04	29	1.0
>70	11	67	10	71	
PB Blasts					
≤50	28	61	05	36	0.12
>50	28	39	09	64	
LDH (IU/L)					
<500	10	20	04	29	0.49
>500	39	80	10	71	
Cytogenetics (n=60)					
NormalKaryotype	26	57	09	64	
Aberrant Karyotype	20	43	05	36	0.75

Abbreviations: *FLT3*, *fms*-like tyrosine kinase 3; AML, acute myeloid leukemia;ITD, internal tandem duplication; WBC, white blood count; FAB, French American British; BM, Bone marrow; PB, Peripheral Blood

by M4 (27%), M3 (8%), M1 (8%), M0 (8%) and M5 (7%) respectively found in this study. None of the patients had M6 subtype in the present study (Table 2). Among the M2 subtype, (18 of 25 ;72% M2 patients) were below 30 years of age while (7 of 25; 28%) were above 30 years of age. Similarly (4 of 16; 25% of M4 patients were below 30 years of age as compared to (12 of 16; 75%) above 30 years of age. No significant association of age groups and gender was found among the M2 and M4 subtype.

Cytogenetic analysis

Chromosome abnormalities were studied in 60 out of 63 cases, where 35 of the 60 patients (58%) were cytogenetically normal (CN). Karyotypes with abnormalities were observed (Fig. 1a) in 25 of 60 (42%). Male patients (18 of 25; 72%) have more chromosomal anomalies than female patients (7of 25; 28%) and the association was not found significant. Among additional chromosomal karyotypes, trisomy8 was observed in 5 of 60 cases (8.33%). Whereas among deletions, (-7del (7q); -5del(5q); -Y) was observed in 7 of 60 cases (12%). Likewise, different balanced translocations seen were; t(8;21) in 5 of 60 cases (8.3%); t(15;17) in 5 cases (8.3%), inversion 16 was seen in 3 cases (5%). Most of the chromosomal anomalies were found in patients with the FAB-M4 subtype (8 of 25; 32%). Similarly, most patients with normal karyotypes (35 of 60; 58%) were found to

Table 2: Clinico-Biological characteristics of 63 AML patients according to WHO classification with Morphological, Cytogenetic and Molecular information

Cytogenetic/ Molecular Subgroups	Patients N (%)	Gender M/F	Age (years) Med(Range)	Hb (g/dl) Med (Range)	WBC (10^9/L) Med (Range)	Platelets (10^9/L) Med (Range)	BM Blasts (%) Med (Range)	Extramed-dis (Y) Nos	LDH IU/L Med(range)	FAB 0	1	2	3	4	5
Total Cytogenetics	60 (95)	40/20	32 (15-50)	7.8 (3.4-12.6)	23.3 (0.2- 151)	50 (21-189)	70 (55-92)	11	450 (328-5820)	4	5	25	5	16	5
Cytogenetic Groups															
Normal Karyotype	35 (58)	22/13	22 (15- 36)	7.5 (6.5-10.2)	53 (22-119)	1.5 (15-239)	55 (45-92)	2	570 (376-3334)	2	2	20	0	8	3
Abnormal Karyotype	25 (42)	18/7	33 (17-42)	7.3 (7.0-11.5)	55 (15-280)	2.0 (15-250)	65 (40-85)	9	450 (500-1560)	2	3	5	5	8	2
t(8;21)	05 (8.3)	05/0	28 (16-40)	7.5 (5.3-9.2)	6.5 (2.7-16.9)	19 (16-35)	54 (50-70)	4	469 (328-538)	0	1	2	0	2	0
t(15;17)	05 (8.3)	3/2	30 (24-35)	9.0 (9.0-9.5)	10.5 (9.4-62.0)	50 (53-157)	65 (60-80)	0	843 (376-875)	0	0	0	5	0	0
inv(16)	03 (5)	2/1	24 (20-30)	11.0 (10.8-11.0)	41 (35-60)	150 (155-189)	68 (65-90)	1	512 (500 -1118)	1	0	1	0	1	0
-7/7q del	02 (3.3)	0/2	- (9-11)	- (8-12)	- (6.0-.8.0)	- (58-98)	- (80-85)	0	- (1560-1580)	0	0	0	0	1	1
-Y del	03 (5)	3/0	20 (16-24)	9.0 (5.8-9.4)	5 (4.6-16.9)	30 (20-56)	54 (40-65)	1	1284 (1418-2455)	0	0	1	0	1	1
Other deletions	02 (3.3)	2/0	- (35-45)	- (5.4-11.0)	- (4.4-64.4)	- (146-189)	- (80-85)	2	- (3334-4967)	0	1	0	0	1	0
Trisomy	05 (8.3)	3/2	30 (25-50)	7.0 (3.2-9.2)	36 (46-67)	16 (7-53)	75 (65-85)	1	345 (300-570)	1	1	1	0	2	0
Molecular Groups (Cases=63)															
FLT3/ITD	14 (22)	10/4	33 (17-42)	6.9 (1.6-11.2)	50 (13.7-88.1)	35 (8-117)	56 (55-85)	nd	850 (512-875)	1	1	2	2	6	2
FLT3/D835	4 (6.3)	3/1	30 (28-35)	8.5 (7.5- 10.5)	34.8 (20-100)	15 (10-100)	70 (65-75)	nd	500 (450-570)	0	2	2	0	0	0

Med=Median; Extramed-Dis= Extramedullary Disease; FAB=French American British; nd=not determined

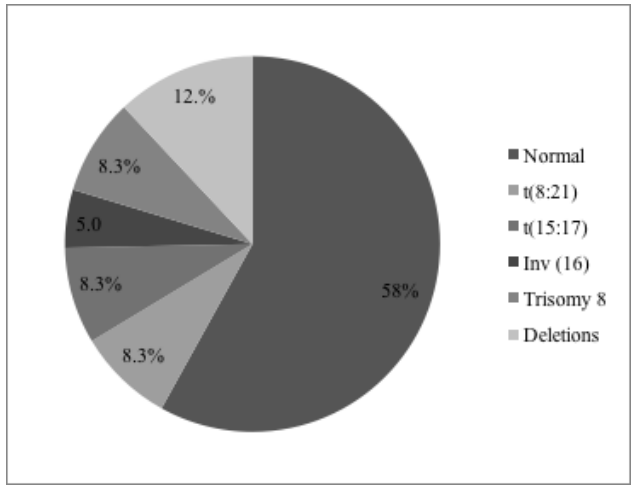

FIG. 1a. Distribution of chromosomal abnormalities in AML patients.

have M2 subtype (20 of 35; 57%). The Clinico-biological characteristics of AML patients according to WHO Classification (Table 2) and distribution of karyotypes in different FAB subtypes was shown (Fig.1b).

Molecular analysis

Incidenceand Distribution of *FLT3* mutations in different FAB subtypes

Of the 63 AML patients studied for mutations, 14 (22%) had *FLT3/ITD*, indicated by the presence of longer PCR fragments (Fig. 2) while 4 out of 63 AML patients (6.4%) were found to contain the D835 mutation. A combination of *FLT3/ITD* and D835 mutation in the *FLT3* gene was not found in our patients. *FLT3* mutations were found associated with different FAB subtypes and their distribution in FAB subtypes was shown (Fig. 3). Correlation for a specific FAB subtype with respect to the *FLT3* mutations was

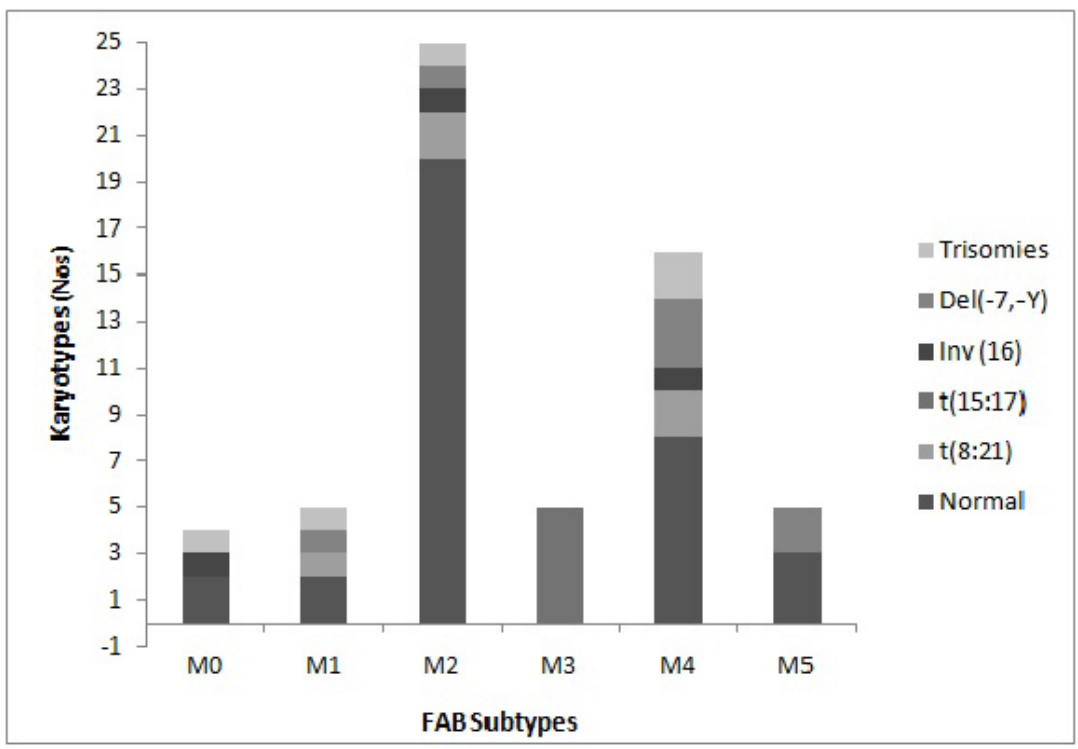

FIG. 1b. Prevalence of different cytogenetic groups according to FAB subtypes.

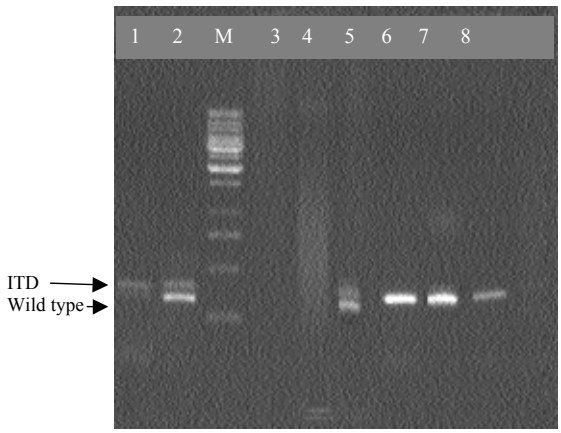

FIG. 2: ITD mutations in the *FLT3* gene. The amplified product of wild type band (329 bp) was shown. When amplified products contained ITD mutations, another larger band (upper) was visualized on agarose gel electrophoresis (lane 1,2, 5,6). Lane M: Molecular size marker (100 bp DNA ladder). Lane3: Blank, negative control with no DNA.

not statistically significant. However, most of the mutations were observed in M4 subtype as compared to other subtypes. While the majority of the patients with M1 and M2 subtype have D835 mutations (14%) each. *ITD* mutation was not confined to any gender or age as these were found in all age groups. Hyperleukocytosis is commonly associated with the presence of *FLT3/ ITD* where WBC counts (mean 68 x10^9/l) were higher in ITD$^+$ patients than in *FLT3*/WT patients (mean 50 x10^9/l) but significance was not achieved (Table 1). No clinical variables were associated with D835 mutations though WBC counts were higher (mean 34.8x10^9/l). Moreover, statistical significance was not achieved when compared with WT patients.

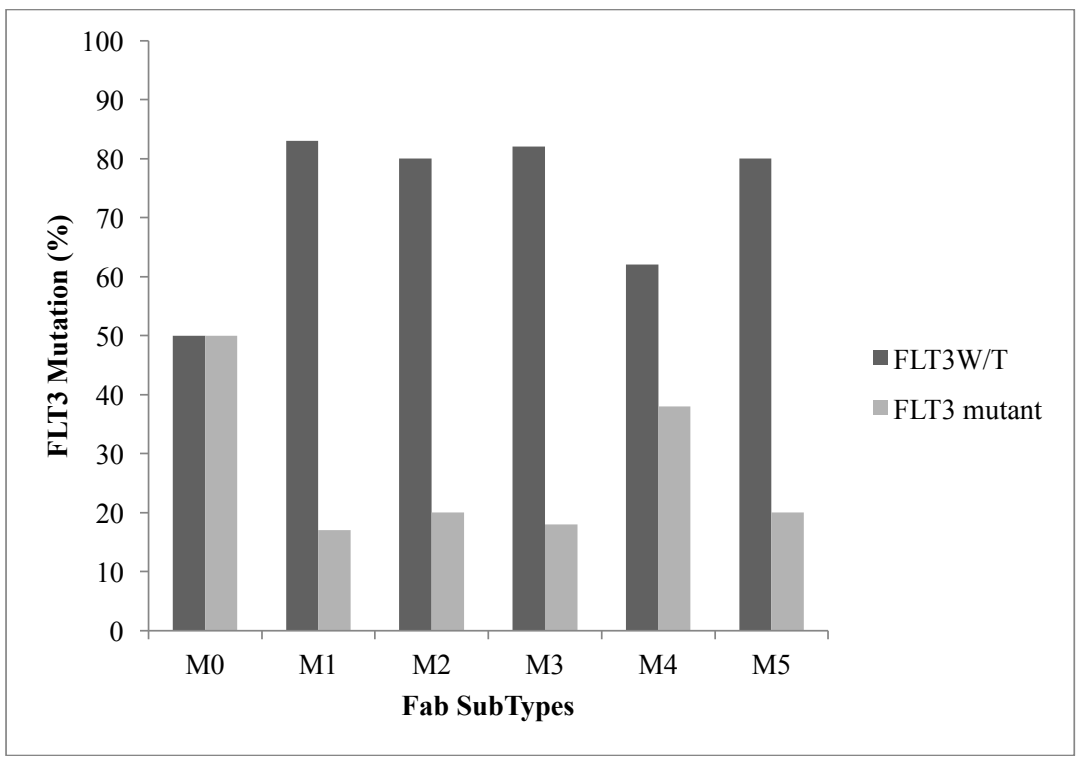

FIG. 3: FLT3/ITD mutations in AML patients with different FAB subtypes.

Association of *FLT3* mutation with cytogenetic findings

FLT3 mutation analysis was performed in 63 cases but successful cytogenetic data was available for 60 cases. The cases included normal Karyotype (NK; n=35), favorable group [t (8;21), t(15;17) and inv (16)] (n=13), numerical and other structural abnormalities (n=12). *FLT3* gene mutations were not equally distributed among the different subtypes and were associated with a wide spectrum of cytogenetic groups (Fig. 4). Among the NK group (9/35) 26% of the cases showed *FLT3* mutation compared to aberrant karyotype (5/25; 20%). Association of *FLT3* mutation status with cytogenetic groups was not observed though increased incidence was found in normal karyotype group (p=0.75; Table 1). Among aberrant karyotypes, 4 cases harboring the mutation were in the favorable karyotype group, (i.e; 2 cases in t(15;17), 1 case in t(8;21), 1 case in inv16) and 1 case in other abnormality group.

DISCUSSION

Adult acute myeloid leukaemia is usually known as disease with diverse, morphologic, immunophenotypic, clinical, cytogenetic and molecular characteristics.[1-2] The heterogeneous distribution of chromosome abnormalities in haematological malignancies among different geographic locations is well known, but limited data is available from different regions of the world.[11] In this study, morphological, cytogenetic and molecular patterns in AML patients from Pakistan was described. The age of our cohort of patients is significantly younger than reported in other studies.[11,12,19-20] The median age of our patients was 33 years whereas median age of 38 years was reported among 702 AML patients in an Indian Study.[21] In other reports from Europe, North America and Asian population, the median age of diagnosis was in the range of 58-63 years.[12,14,20-22] The representation of younger patients in this study may be due to ethnic differences of the population studied or referral cases. Our centre is the tertiary health care hospital and majority of cases are referred here for treatment. In this study, FAB-M2 was the most commonly seen (32%) that has also been reported from another study from Pakistan (32.26%) followed by M1 and M4 (22.58% each).[23] In contrast, another study from different centre reported AML-M4 as most common FAB subtype in 116 patients studied.[24] The distribution of male and females patients in our analysis was comparable with published western data and is twice as common in males than females though significance was not achieved.

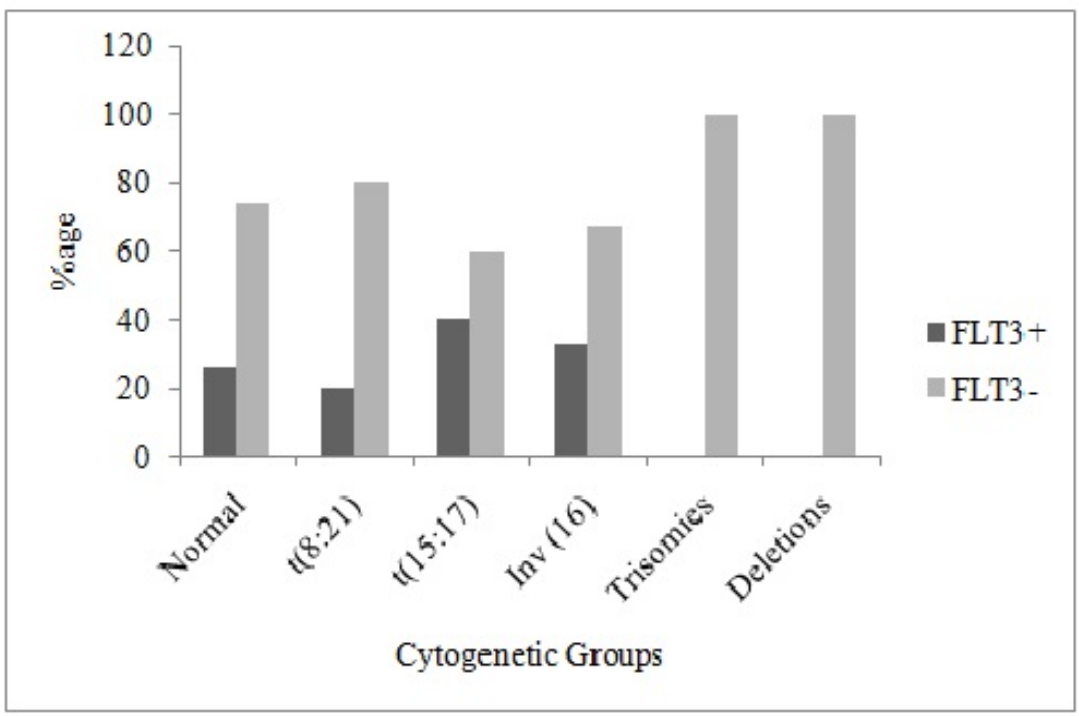

FIG. 4: FLT3/ITD mutations in different cytogenetic groups of AML patients.

About 42% of the patients in this study showed abnormal karyotypes. Reports from other geographic regions showed prevalence of abnormal karyotypes in the range of 52 to 80%.[14,20,25,26] The frequencies of specific karyotypes and specific aberrations observed in our patients were found similar with other reports.[20,21] Among balanced abnormalities, translocation (8;21)(q22;q22), t(15;17)(q22:q12) and inv (16) (p13q22) constituted the only recurring anomalies each with a frequency of 8.3%, 8.3%, 4% respectively. Both translocations comprise 5-8% of AML in other studies.[26-28] The incidence of t(8;21) in our AML patients was found comparable to reports from the West that showed prevalence of 6% - 8%. Higher incidence (15%) was reported in Indian study[21] whereas in the Chinese and Japanese populations, it was found in the range of 8-16%.[11,13]

Of the 63 AML patients examined for the genetic mutations, 14 patients (22%) showed *FLT3/ITD* and four patients (6%) had *D835* mutations. None of our patients showed a combination of both *FLT3/ITD* and *D835* mutations. The incidence of *FLT3* mutation is variably reported as 20%-35% of all AML cases in western countries whereas its incidence increases to 40% in the NK group.[29,30] Among the FAB subtypes, *FLT3* mutations were more commonly reported within the M2 subtype.[29] The *FLT3/ITD* mutations was more commonly observed in FAB-M4 patients in this study. However, cases with M4 or M5 subtypes were also reported to have larger proportion of mutant *FLT3*.[29] In this study, D835 mutations were found equally in both FAB-M1, M2 patients. The incidence of *ITD* mutations were found to be within the similar range as reported in international studies in our cohort of patients.[31-35] However, some studies reported distribution of *FLT3/ITD* mutations across all other FAB subtypes.[36] It has also been observed that the variation in the incidence of these mutations in different FAB subtypes and cytogenetic groups varies significantly. This is particularly more significant in the AML subgroups defined by cytogenetic abnormalities. The differences in the frequency of ITD mutations reported in various studies may be due to differences in the size of cohorts.A large prospective study is therefore required to estimate the incidence of this mutation in our patients.

FLT3-ITD mutations associated with increased leukocyte counts in AML patients has been reportedin various studies. Moreover, it is more frequent in patients lacking other cytogenetic aberrations. Similar findings were observed in our cases harbouring *FLT3/ITD* mutation that is consistent with other reported studies.[30] In this study, association between mutations and patient age was not found significant. Likewise, this has not been reported in other studies.[30,36,37] Similarly, lack of significance with regards to haemoglobin level and platelet count was found to have no significance that has also been observed in other reports.[30,36] The correlation between incidence of *FLT3* mutation in different cytogenetic subgroups was also studied in the present study. A non-significant association of *FLT3* mutation with NK(26%) than those with abnormal karyotype(20%, P=0.32) was observed. However, association of this mutation with NK has been reported in other studies.[23,25,29,36]

The major limitation of this study is the sample size. This study included patients referred to a single cancer treatment centre whom complete morphological, cytogenetic and molecular data was available. Most of the patients complete diagnostic workup could not be achieved due to financial constraints of the patients and unavailable diagnostic facilities making the number of studied cases limited. This limitation will be addressed in future work involving collaborative labs/treatment centers. Similarly, *FLT3* mutation is the only gene studied in this study. The mutational spectrum of other genes will be studied in future work.

CONCLUSION

AML patients presented to our tertiary hospital were studied for their morphological, cytogenetic and molecular profile. Our patients were much younger (median age 32 years) as compared to international studies. Recurrent cytogenetic abnormalitieswere observed in 43% ofAML patients in this study. The most common specific chromosomal aberrations include favourable karyotypes, t(8;21)(q22;q22) followed by t(15;17) (q22:q12). However, their prevalence in various age groups was found not significant. *FLT3/ITD* mutations are commonly found in our AML patients who were cytogenetically normal. Therefore, it is important that genetic mutations and cytogenetic alterations are taken into account during the initial diagnostic workup of AML patients for risk stratification and therapeutic decision-making.[37-38] Moreover, with the availability of approved FLT3 inhibitors[39] in new combinations and treatment settings may

help the oncologists to choose patients that could be candidate for FLT3-directed therapy.

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Conflict of interest: The authors declared no conflict of interest.

REFERENCES

1. Döhner H, Estey EH, Amadori S, *et al*. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European Leukemia Net. Blood 2010; 115**:** 453–74.
2. Mrozek K, Heinonen K, Bloomfield CD. Clinical importance of cytogenetics in acute myeloid leukaemia. Best Pract Res Clin Haematol.2001;14:19-47.
3. Hiddemann W, Haferlach T, Schoch C, *et al*. New insights into the biology of acute myeloid leukemia and their impact on treatment. Verh DtschGesPathol.2003; 87:72-8.
4. Bennett JM, Catovsky D, Daniel MT,*et al*. Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML-MO). Br J Haematol. 1991;78:325-9.
5. Bennett JM, Catovsky D, Daniel MT,*et al*. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French American British Cooperative group. Ann Intern Med. 1985;103:620-5.
6. Bennett JM, Catovsky D, Daniel MT,*et al*. Criteria for the diagnosis of acute leukemia of megakaryocyte lineage (M7): a report of the French American British Co-operative Group. Ann Intern Med. 1985;103:460-2.
7. Patnaik MM. The importance of FLT3 mutational analysis in acute myeloid leukemia. Leuk Lymphoma. 2018; 59:2273–86.
8. Döhner H, Estey E, Grimwade D, *et al*. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129: 424–47.
9. Tallman MS, Wang ES, Altman JK, *et al*. Acute Myeloid Leukemia, Version 3.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl ComprCancNetw. 2019;17:721–49.
10. Bacher U, Haferlach T, Schoch C, Kern W, Schnittger S. Implications of NRAS mutations in AML: a studyof 2502 patients. Blood. 2006; 107: 3847-53.
11. Cheng Y, Wang Y, Wang H, *et al*. Cytogenetic Profile of *De Novo* Acute Myeloid Leukemia: A Study Based on 1432 Patients in a Single Institution of China. Leukemia2009;23:1801-6.
12. Lazarevic V, Horstedt AS, Johansson B, *et al*. Incidence and Prognostic Significance of Karyotypic Subgroups in Older Patients with Acute Myeloid Leukemia: The Swedish Population-Based Experience. Blood Cancer Journal2014; 4:188.
13. Wakui M, Kuriyama K, Miyazaki Y, *et al*. Diagnosis of Acute Myeloid Leukemia According to the WHO Classification in the Japan Adult Study Group AML-97 Protocol. International Journal of Hematology 2008; 87:144-51.
14. Preiss BS, Kerndrup GB, Schmidt KG, *et al*. Cytogenetic findings in adult *de novo* acute myeloid leukemia: a population-based study of 303/307 patients. Br J Haematol. 2003;123:219-34.
15. Enjeti AK, Tien SL, Sivaswaren CR. Cytogenetic abnormalities in *de novo* acute myeloid leukemia in adults: relation to morphology, age, sex and ethnicity: a single center study from Singapore. Hematol J. 2004; 5: 419-25.
16. ISCN 2005: an international system for human cytogenetic nomenclature. In: Shaffer LG, Tommerup N, editors. Basel: S. Karger, 2005.
17. Kiyoi H, Towatari M, Yokota S, *et al*. Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. Leukemia1998; 12: 1333–7.
18. Arana-Trejo RM, Go´mez-Morales E, Rubio-Borja ME, *et al*. Cytogenetic findings in 303 Mexican patients with *de novo* acute myeloblastic leukemia. Arch Med Res. 1997; 28:209-14.
19. Roberts GT, Spence DG, Padmos MA, Sheth KV, Clink H, Ernst P. Morphologic immunophenotypic and cytogenetic patterns of adult myeloid leukemia in Saudi Arabia. Leuk Res. 1992; 16: 181-90.
20. Al Bahar S, Zameenikova A, Pandita R, Bavishi K, Kreze O, Nath S. Chromosome aberrations in de novo acute myeloid leukemia patients in Kuwait. Neoplasma 2004;51:22
21. Kadam Amar PS, Jain H, Kabre S, *et al*. Cytogenetic Profile in 7209 Indian Patients with *de novo* Acute Leukemia: A Single Centre Study from India. Journal of Cancer Therapy 2016; **7**: 530-44.
22. Mauritzson N, Johansson B, Albin M, *et al*. A single-center population-based consecutive series of 1500 cytogenetically investigated adult hematological malignancies: Karyotypic features in relation to morphology, age and gender. Eur J Haematol. 1999; 62: 95-102.
23. Hassan K, Qureshi M, Shafi S, Ikram N, Akhtar MJ. Acute myeloid leukemia-FAB classification and its correlation with clinico-haematological features.J Pak Med Assoc.1993; 43:200-3.
24. Harani MS, Adil SN, Shaikh MU, Kakepoto GN, Khurshid M. Frequency of FAB subtypes in acute myeloid leukemia patients at Aga Khan University Hospital Karachi**.** J Ayub Med Coll Abbottabad2005; 17: 26-9.
25. Tien HF, Wang CH, Lin MT, *et al*. Correlation of cytogenetic results with immunophenotype, genotype, clinical features, and ras mutation in acute myeloid leukemias: a study of 235 Chinese

patients in Taiwan. Cancer Genet Cytogenet.1995; 84:60-8.
26. Heim S, Mitelman F. Cancer cytogenetics: chromosomal and molecular genetic aberrations of tumor cells. 2nd ed. New York: Wiley- Liss, 1995. pp. 72-3.
27. Byrd JC, Mrózek K, Dodge RK, *et al*. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with *de novo* acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood2002; 100: 4325-36.
28. Rombouts WJ, Blokland I, Lowenberg B, Ploemacher RE. Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the *FLT3* gene. Leukemia 2000; 14: 675–83.
29. Kiyoi H, Naoe T, Yokota S, *et al*. Internal tandem duplication of FLT3 associated with leukocytosis in acute promyelocytic leukemia. Leukemia Study Group of the Ministry of Health and Welfare (Kohseisho). Leukemia1997; 11:1447-52.
30. Auewarakul CU, Sritana N, Limwongse C, Thongnoppakhun W, Yenchitsomanus PT. Mutations of the *FLT3* gene in adult acute myeloid leukemia: determination of incidence and identification of a novel mutation in a Thai populationCancer Genet Cytogenet. 2005;162:127-34.
31. Su L, Li X, Gao S-J, *et al*. Cytogenetic and genetic mutation features of *de novo* acute myeloid leukemia in elderly Chinese patients. Asian Pac J Cancer Prev. 2014;15:895-8.
32. Bacher U, Haferlach C, Kern W, Haferlach T, Schnittger S. Prognostic relevance of FLT3-TKD mutations in AML: the combination matters—an analysis of 3082 patients. Blood.2008; 111: 2527-37.
33. Kiyoi H, Towatari M, Yokota S, *et al*. Internal tandem duplication of the *FLT3* gene is a novel modality of elongation mutation which causes constitutive activation of the product. Leukemia 1998; 12: 1333–7.
34. Liang DC, Shih LY, Hung IJ, *et al*. FLT3-TKD mutation in child.hood acute myeloid leukemia. Leukemia 2003; 17**:**883-6.
35. Schnittger S, Schoch C, Dugas M, *et al*. 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 2002;100:59-66.
36. Thiede C, Steudel C, Mohr B, *et al*. Analysis of *FLT3*-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis Blood 2002; 99: 4326-35.
37. Metzeler KH, Herold T, Rothenberg-Thurley M, *et al*. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. *Blood*. 2016; 128:686–98.
38. Fatemeh P, Mohamad RZ, Azam SH, Seyed H, Hekmat, I. Genetic Characterization and Risk Stratification of Acute Myeloid Leukemia Cancer Management and Research 2020; 12: 2231-53.
39. Vanessa EK,Catherine CS. *FLT3* Mutations in Acute Myeloid Leukemia: Key Concepts and Emerging Controversies. Front. Oncol. 2020; 10:612880.