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Prognostic Significance of FLT-3-ITD Mutations in Adult Patients with Acute Myeloid Leukemia (AML)

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ABSTRACT

Background: FLT3 is a receptor tyrosine kinase with important roles in hematopoietic stem/progenitor cell survival and proliferation. Recently, it emerged as a possible prognostic factors in AML patients especially those with normal cytogenetics who constitute a heterogeneous group of patients requiring individualization of treatment.

The Aim of the Study: Is to test for the presence of FLT3-ITD mutation in exon 11 and to correlate it with other prognostic factors.

Patients and Methods: A total of 75 patients with newly diagnosed AML were included in this study between January 2004 and January 2006. Diagnosis was established by bone marrow examination, and immunophenotyping. Mononuclear cells (MN) were obtained from bone marrow samples at presentation by Ficoll-Hypaque density gradient centrifugation method and stored at –80ºC until use. All samples were analyzed for ITD mutation in exon 11 of the FLT 3 gene after extraction of genomic DNA from MN cells using PCR technique.

Results: Our patient group included 37 females (49.3%) and 38 males (50.7%) with a median age of 33 years. Blood indices analysis revealed a mean total leucocytic count of 43.42 x 10^9/L ±53.45, mean hemoglobin level of 6.7gm/dL ±2 and mean platelet count of 39.24 x 10^9/L ±31.46. The median percentage of blasts in peripheral blood was 46% and in marrow was 63%. The most commonly encountered FAB subgroup was M2 (44%), followed by M1 (36%), M4 (12%), M5 (6.7%) and finally M0 (1.3%). The FLT3-ITD mutation was tested for the 75 patients; 17 were found to be positive (22.7%) and 58 (77.3%) were negative. An attempt to correlate the clinical, hematological and immunophenotypic findings with the likelihood of positive FLT3/ITD mutation failed to find a correlation between the possibility of FLT3/ITD mutation and any of these variables except high percentage of blasts cells ≥50% in bone marrow. Molecular genetics testing for inv 16 and t(8,21) was available in 47 patients. Six/47 patients (12.7%) were positive, however, there was no correlation with FLT3/ITD status. Complete remission was achieved in 54/75 of patients (72%). Forty out of the 75 patients (53.3%) achieved CR after one course of induction, while 14 out of the 20 patients who received a second induction achieved CR (18.7%). Among those who were FLT3/ITD+ve, 10/17 (59%) achieved CR compared to 44/58 (76%) among those who were FLT3/ITD –ve (p=0.22). Three out of seventeen patients (17.64%) with FLT3/ITD +ve required a second course of induction to achieve CR compared to 17/58 (29.3%) with FLT3/ITD –ve. After a minimum follow up period of 12 months, the overall median duration of complete remission was 8.59 months (95% confidence interval 6.64, 10.55), [8.13] months for FLT3/ITD –ve patients (95% confidence interval 7.13, 11.69) and 3.93 months for patients who are FLT3/ITD +ve (95% confidence interval 2.51, 7.69 p0.0258)]. After a follow up period of 38 months the median survival was 7.4 month with a mean of 4.8 month (95% confidence interval 2.7-6.8). (4.1 month FLT3/ITD +ve with a mean of 10.1 months (95% confidence interval 7.9-12.3,8.9 month FLT3/ITD –ve p=0.0064).

Statistical analysis of the possible prognostic factors showed that only high TLC and age showed statistically significant influence on incidence of CR rate. Whereas age and FLT3/ITD demonstrated statistically significant longer duration of CR and survival (p=0.000 and 0.025) respectively.

Conclusion: FLT-3 ITD mutations are correlated with adverse prognosis particularly in patients with AML. This genetic marker either alone or in combination with others might serve to tailor treatment for some heterogenous AML patient population like those with normal cytogenetics.

Key Words: *AML – FLT3.*

INTRODUCTION

Karyotyping is still recognized as the most important prognostic factor in patients with AML. However, more than 50% of these patients have normal karyotype and are allocated in the intermediate risk group **[1,2]**. The results of

recent studies showed that they represent a heterogenous group and that molecular differences might help for further prognostication and tailoring of treatment **[3,4]**.

FLT3 (fms-like tyrosine kinase 3) is a class III tyrosine kinase receptor (RTK) involved in signaling pathways regulating the proliferation of hematopoietic stem cells and early progenitor cells. Like other class 3 RTKs (e.g., fms, kit, PDGF), FLT3 consists of 5 extracellular immunoglobulin-like domains, a transmembrane domain, a juxta-membrane (JM) domain, 2 intracellular tyrosine kinase (TK) domains separated by a kinase insert domain and an intracellular C-terminal domain **[5]**. The gene encoding FLT3 maps to chromosome band 13q12 and comprises 24 exons that span a genomic region of approximately 100kb **[6,7]**.

In patients with AML two types of activating FLT3 mutations have been identified in two functional domains of the receptor, the juxtamembrane (JM) domain and the split TKD. The JM domain which is crucial for kinase autoinhibition is disrupted by ITDs of various size and insertion sites in 28% to 34% of cytogenitically normal AML (CN-AML). FLT3-ITDs result in ligand-independent dimerization and tyrosine auto-phosphorylation as well as activation of the RAS/MAPK, STAT5 and PI3K/AKT pathways **[8,9]**. The activation loop (AL) in the carboxy-terminal lobe of the TKD is affected by point mutations, small insertions or deletions mainly involving codon 835 and 836 in 11% to 14% of CN-AML. In vitro studies and results from global gene expression profiling revealed that there are similarities but also important differences in signal transduction properties between FLT3-ITDs and FLT3 TKD mutations that may explain differences in clinical phenotypes **[10]**.

The aim of this study is to estimate the incidence of FLT-3 ITD mutation in Egyptian patients with be novo AML other than M3, and to correlate it with potential prognostic factors.

PATIENTS AND METHODS

This study involved 75 patients with newly diagnosed AML presenting to the Medical Oncology Department, National Cancer Institute, Cairo University, in the period between January 2004 and January 2006. Patients fulfilled the following criteria: Age between 18-60 years, ECOG performance status ≤2, all FAB subtypes except M3, no other malignancy, no prior chemotherapy or radiotherapy, no medical contraindications, normal ejection fraction (as assessed by echocardiography). Pretreatment evaluation: All patients underwent the following: Full history and physical examination, complete and differential blood count, bone marrow analysis including cellularity, morphology, cytochemistry. Flowcytometry for immunophenotyping, cytogenetics, serum chemistry including hepatic and renal profiles, calcium level as well as uric acid. All cases were classified according to the French, American, British [FAB] classification. CSF examination was performed to those with symptoms of CNS involvement. Baseline Chest X-ray and abdominal sonar were obtained. All patients signed informed consent before starting treatment.

FLT3 ITD detection: Mononuclear cells were obtained from bone marrow samples at presentation and stored at –80ºC until use. Genomic DNA of all samples were analyzed for mutation of exon 11 of the FLT3 gene using genomic PCR method. The use of exon 11 specific primers allowed covering the whole JM & the first part of TK-1 domain where most of the reported mutations are located.

Fifty µg genomic DNA was amplified in a 50µl reaction containing 10µl TisHcl (PH 8.3), 50µl Kcl, 1.5µl Mgcl, 200µl of each d NTP, 2.5U Tag polymerase, 40pmol of each primer and 6% dimethylsulphate. Amplification process consisted of 40 cycles of 4ºc for 30 seconds (denaturation), 50ºc for 45 seconds (annceling) and 72ºc for 1 minute (extension). One more step of final extension at 72ºc for 7 minutes was added.

The sequence of the primer used is:

- 11F (Sense) 5'' CAATTTAGGTATGAAAGCC –3''.
- 11R (Antisense) 5' C AAA CTCT AAATTTT CTCT–3''.

Ten ul of the PCR product were electrophoresed on 2% agarose gel, stained with ethidium bromide and photographed. Wild type band size was 133bp whereas an extra PCR band (mutant) appeared in case of FLT ITD.

Fusion genes detection by RT-PCR:

RNA was extracted from 300µl peripheral blood or bone marrow sample using a salting out procedure (Purescript, Gentra, Minneapolis, MN, USA) according to manufacturer's instructions A visible, translucent RNA pellet was then performed, washed by 70% ethanol and rehydrated for 50min in an ice bath. Reverse transcription was done using Multiscribe Reverse Transcriptase enzyme in a final mix of 40µl volume with a Gold RNA PCR kit (Applied Biosystems, USA). Cyclic conditions consisted of 25ºC for 10min and 42ºC for 1 hour. PCR was performed to detect the core binding factor fusion genes t(8;21) (q22;q22) and inv (16) (p13q22)/t(16;16) (p13;q22).

Primers used for detection of fusion genes were taken from the European BIOMED-1 concerted action Investigation of minimal residual disease in acute leukemia **[11]**.

Five µl c DNA was amplified in a 50µl reaction volume containing 20µml TrisHcl, 50µml Kcl (pH 8.3), 2.5µml Mgcl, 400µml final concentration of each primer, 200µml of each deoxyribonucleotide triphosphate (d NTPs) and 1.5U Tag polymerase.

Cyclic conditions consists of 95ºc initial molting for 10 minutes then 35cycles of 94ºc for 30 seconds (denaturation), 65ºc for 60 seconds (annealing) and 72ºc for 60 seconds (extension). Nested PCR consisted of same volume, reagents and cycle conditions as for first round using internal (nested) primers and using 1µl DNA template from the first round.

Primer sequence for t(8;21) (q22;q22) AML1/ETO:

- AML1-A: CTACCGCAGCCATGAAGAACC.
- ETO-B: AGAGGAAGGCCCATTGCTGAA.
- AML1-C: ATGACCTCAGGTTTGTCGGTCG.
- ETO-D: TGAACTGGTTCTTGGAGCTCCT.

Primer sequence for Inv 16 (p13,q22) CBFB-MYM11:

- CBFB-A: GCAGGCAAGGTATATTTGAAGG.
- MYM11-B2: TCCTCTTCTCCTCATTCTGCTC.
- MYM11-D2: CTTGAGCGCCTGCATGTT.
- CBFB C: GGGCTGTCTGGAGTTTGATG.

Product size for AML1-ETO was 395bp for first round PCR and 260bp for second round whereas amplicans size for CBFB-MYM11 ranged from 418 to 1345bp for first round and from 175-1200bp for second round.

All PCR products obtained through individualized RT-PCR reactions were separated on a 2% ethidium bromide agarose gel for 30 minutes. Fragments size was determined by running a molecular weight marker of known size and comparing the distance of unknown fragment in relation to the ladder (ø, Phi X DNA-HAE III, 500µg/ml; Cat. 302-61, New England Biolab).

Treatment plan:

Patients received standard induction chemotherapy using cytosine arabinoside and anthracycline as 7 and 3 protocol. Patients who achieved 1st CR were consolidated by the same regimen then HLA typing was done for those below 40 years of age with good general condition, –ve inv(16) & t(8,21). Those with HLA identical donor were referred for allogenic BMT as soon as possible. Those with no HLA identical donor were given 4 cycles of HAM regimen, patients who were +ve for inv (16) or t(8,21) i.e. favorable risk or >40 years were given 4 cycles of HAM consolidation.

Intrathecal prophylaxis was given only for cases with AML M5 (high risk of CNS disease) after reaching CR by induction chemotherapy and for patients with CNS disease at presentation. Triple intrathecal prophylaxis was given every 8 weeks for a total of 6 injections using methotrexate 15mg, Ara-C 40mg and dexamethazone 4mg.

Those with CNS disease at presentation were given triple intrathecal injection simultaneously with induction treatment until CSF was free. This was followed by craniospinal irradiation 24Gy following recovery then double intrathecal injections in cases of CR with Ara-c 40mg and dexamethazone 4mg every 8 weeks for 7 doses without MTX to avoid leukoencephalopathy.

Statistical analysis:

Statistical analysis was done using IBM compatible computer and according to the following tests:

Descriptive statistics was presented in frequency tables, means, and standard deviations whenever appropriate.

Analytical tests used included:

- Chi-square test for comparing two quantitative variables.
- Survival analysis and analysis of duration of complete remission were done using Kaplan Meier analysis.
- Significance level of 0.05 was used in all statistical tests.
- Disease-free survival (DFS): Included time to an event (death or relapse) measured from the end of induction for patients who achieved CR (induction deaths & non-responders were excluded).
- º Overall survival (OS): Included time from diagnosis to death.

RESULTS

Patient characteristics:

A total of 75 patients were included in this study, 37 females (49.3%) and 38 males (50.7%) with an age range of 18-60 years and a median of 33 years.

The mean total leucocytic count was 43.42 x 10^9/L ±53.45 with a range of 1.7-328 x 10^9/L and a median of 20 x 10^9/L. Blasts in peripheral blood were detected in 62 patients (82.6%) and the mean percentage of blasts in peripheral blood was 49%; while their mean percentage in marrow was 61.96±20.33 with 70.7% of patients having ≥50% blast cells.

All the studied cases were classified according to FAB classification. The most commonly encountered FAB subgroup was M2 (44%), followed by M1 (36%), M4 (12%), M5 (6.7%) and finally M0 (1.3%). The details of patients' characteristics are shown in Tables (1,2).

FLT3-ITD mutation:

The FLT3-ITD mutation was tested for the total 75 patients; 17 were found to be positive (22.7%) and 58 (77.3%) were negative. An attempt to correlate the clinical, hematological and immunophenotypic findings with the mutational status of FLT3/ITD failed to find a correlation between FLT3/ITD mutation with any of theses variables except high total leucocytic count although it did not reach statistical significance, Table (3).

Table (1): Characteristics of adult patients with AML (n=75).

Parameter	Total No. (%)
Age (Y):	
Mean±SD	32.84±10.5
<45	63 (84%)
≥45	12 (16%)
Sex:	
Female	37 (49.3)
Male	38 (50.7)
Symptoms:	
Fatigue	63 (84%)
Fever	21 (28%)
Bone aches	31 (41.3%)
Bleeding	19 (25.3%)
Signs:	
PS	
I	11 (14.6%)
II	64 (85.4%)
Lymphadenopathy	12 (16%)
Splenomegaly	20 (26.7%)
Hepatomegaly	26 (34.7%)
Gum hypertrophy	6 (8%)
Mucositis	18 (24%)
CNS infiltration	3 (4%)

Table (2): Characteristics of 75 adult patients with AML (Hematological and Biological parameters).

Parameter	Total No. (%)
WBCs (x 10^9/L):	
Mean±SD	43.41±53.45
<25	39 (52%)
25-100	28 (37.3%)
>100	8 (10.7%)
HB (gm/dL):	
Mean±SD	6.7±2
<8	58 (77.3%)
≥8	17 (22.7%)
Platelets (x 10^9/L):	
Mean±SD	39.24±31.46
<50	55 (73.3%)
≥50	20 (26.7%)
Peripheral blood blasts:	
+ve	62 (82.6%)
–ve	13 (17.4%)
B.M cellularity:	
Hypercellular	63 (84%)
Normocellular	12 (16%)
FLT3-ITD:	
+ve	17 (22.7%)
–ve	58 (77.3%)
T(8,21):	
+	3 (6.4%)
–	44 (93.7%)
Not done	28 (37.3%)
Inv(16):	
+	3 (6.4%)
–	44 (93.7%)
Not done	28 (37.3%)

Table (3): Correlation of hematological parameters with FLT3/ITD status in 75 adult AML cases.

Variable	FLT3/ITD+	FLT3/ITD-	*p*-value
WBCs:			
<25	6 (35.3%)	33 (56.9%)	
25-100	9 (52.9%)	19 (32.8%)	0.23
>100	2 (11.8%)	6 (10.3%)	
HB:			
<8	13 (76.5%)	45 (77.6%)	1.0
≥8	4 (23.5%)	13 (22.4%)	
Platelets:			
<50	13 (76.5%)	42 (72.4%)	1.0
≥50	4 (23.5%)	16 (27.6%)	
% of leukemic cells in marrow:			
<50	9 (52.9%)	13 (22.4%)	0.03
≥50	8 (47.1%)	45 (77.6%)	
FAB:			
M0	8 (47.1%)	1 (5.9%)	
M1	8 (47.1%)	19 (32.8%)	
M2	1 (5.9%)	25 (43.1%)	0.98
M4		6 (10.3%)	
M4eo		2 (3.4%)	
M5		3 (5.1%)	
M5b		2 (3.4%)	

Molecular genetics:

The expression of t(8;21) & inv 16 was tested in 47 patients. Three out of the 47 patients (6.4%) were found to be positive for t(8;21) and 3 patients for inv (16). An attempt to correlate FLT3/ITD with t(8;21) and inv (16) failed to find a statistically significant correlation.

Toxicity of induction:

Scoring of treatment toxicity was done according to WHO criteria.

Hematological toxicity: The mean duration of neutropenia (neutrophils <500mm^3) was 8.1 days ±2.7. On the other hand, the mean duration of neutropenia (neutrophils 500-1000mm^3) was 14.9 days ±5.2. The mean duration of hemoglobin recovery (Hb >8gm/dl) was 12.9 days ±7.2. The mean duration of platelet count recovery (Plt >50 x10^9/L) was 15 days ±6.8. Clinically observed refractoriness to platelet transfusion was encountered in 3 patients.

Non-hematological toxicity: Mucositis was the most common complication, encountered in 65 cases (86.67%) with 38.67% of patients experiencing G3 mucositis. Nausea and vomiting was recorded in 45 cases (60%) with 37.33% having G2. No grade III or IV were encountered.

Other toxicities include diarrhea in 15 cases (20%); mostly GII (10.67%), infectious complications was found in 63 (84%) patients; 38 (50%) had chest infection, while 19 (25.3%) had line related infections, and 4 (5.3%) had perianal infection. Hepatic toxicity was generally mild, with GIII hyperbilirubinemia in 1 patient (1.3%).

Alopecia was observed in 45 (60%) patients with 33.3% developing G2 alopecia.

Response rate:

Complete remission was achieved in 54 out of 75 patients (72%). Forty out of the 75 patients (53.3%) achieved CR after one course of induction, while 14 out of the 20 patients who received a second induction achieved CR (18.7%). Among those who are FLT3/ITD +ve, 10/17 (59%) achieved CR compared to 44/58 (76%) among those who are FLT3/ITD –ve (p=0.22). Three/17 (17.64%) with FLT3/ITD +ve required a second course of induction to achieve CR while 17/58 (29.3%) with FLT3/ITD –ve required a second course of induction to achieve CR. On the other hand, among those who are FLT3/ITD +ve, 2/17 (11.76%) failed to achieve CR compared to 4/58 (6.9%) of the FLT3/ITD –ve patient. Other hematological and clinical factors that might affect CR are shown in Table (4). Only high TLC and age showed statistically significant influence on incidence of CR rate.

Early death:

Early death was encountered in 15 out of 75 patients (20%). Early death in the group of FLT3/ITD +ve patients was encountered in 5/17 (29.4%) while it was encountered in 10/58 (17.2%) of the FLT3/ITD-ve patients. Septicemia (39%) was the leading cause of death in our patients followed by bleeding (21%).

Duration of complete remission:

After a minimum follow-up period of 12 months, the overall mean duration of complete remission i.e disease free survival (DFS) was 7.23 months with a mean duration of 8.59 months (95% confidence interval 6.64,10.55). Those who are FLT3/ITD+ve had a shorter DFS [the median duration of DFS is 3.93 months with a mean duration of 5.1 months (95% confidence interval 2.51, 7.69)] compared with FLT3/ITD–ve patients [median duration of DFS is 8.13 months with a mean duration of 9.41

months (95% confidence interval 7.13, 11.69)]. The difference in DFS between the 2 groups was statistically significant (p=0.0258) Figs. (1,2). Other factors that might have had an effect on duration of CR are shown in Table (5). Beside FLT3 status, age was the only variable affecting CR duration.

Table (4): Factors affecting complete remission (CR) rate in 75 adult AML cases.

	Complete remission No. (%)	p value
Age:		
<45	50/63 (79.4%)	0.003
≥45	4/12 (33.3%)	
Sex:		
Females	23/37 (62.2%)	0.075
Males	31/38 (81.6%)	
TLC:		
<25	32/39 (82.1%)	0.004
25-100	20/28 (71.4%)	
>100	2/8 (25.0%)	
HB:		
<8	43/58 (74.1%)	0.45
≥8	11/17 (64.7%)	
PLT:		
<50	40/55 (72.7%)	1.0
≥50	14/20 (70.0%)	
LDH:		
Normal	19/25 (86.4%)	0.09
High	34/50 (65.4%)	
FAB:		
M1,M2	45/60 (75%)	0.34
Others	9/15 (60%)	
FLT3:		
+ve	10/17 (58.8%)	0.22
–ve	44/58 (75.9%)	
T(8;21):		
+ve	3/3 (100%)	1.0
–ve	34/44 (77.3%)	
Inv (16):		
+ve	2/3 (66.7%)	0.49
–ve	36/44 (81.8%)	

Overall survival:

After a follow-up period of 38 months the median survival was 7.4 month with a mean survival of 8.9 months (95% confidence interval 7.0-10.7), with 5 patients (7.8%) remaining alive at 24 month and only 2 (1.6%) remaining alive at 38 months. The median survival was 4.1 months with a mean of 4.8 months (95% confidence interval 2.7-6.8) for FLT3/ITD +ve patients compared with a median survival of 8.9 months and a mean of 10.1 months (95% confidence interval 7.9-12.3) for FLT3/ITD –ve patients and the difference was statistically significant (p=0.0064), Figs. (3,4).

An attempt to find the influence of t(8;21) and inv (16) on overall survival failed to find a significant improvement. Patients positive for t(8;21) had a median overall survival of 12.7 month compared to 8.2 months for those who did not show this expression (p=0.350). The same applied for inv (16). Patients positive for inv (16) had a median survival of 6.8 months, compared to 8.3 months. (p=0.368) in patients who are negative for inv (16).

Prognosis:

Statistical analysis of the possible prognostic factors showed that among the factors listed in Table (4) only high TLC and age showed statistically significant influence on incidence of CR rate. Also the difference in age showed statistically significant influence on CR (p=0.003). CR rate was 79.4% below 45 years and 33.3% in patients aged ≥45 years. On the other hand, age and FLT3/ITD demonstrated statistically significant longer duration of CR (p=0.000 and 0.025, respectively). The latter also affected survival significantly.

Table (5): Factors affecting duration of CR in 75 adult AML cases.

Parameter	Mean duration of CR	95% confidence interval	Significance
Age:			
<45	9.16	(7.12,11.2)	0.000
≥45	1.66	(0.92,2.39)	
TLC:			
25	8.7	(6.23,11.24)	0.994
25-100	8.18	(5.01,11.35)	
>100	8.57	(5.95,11.18)	
HB:			
<8	9.13	(6.76,11.49)	0.238
≥8	6.57	(4.08,9.05)	
Platelets:			
<50	8.01	(6.12, 9.91)	0.446
≥50	9.88	(5.18, 14.57)	
LDH:			
Normal	9.45	(5.53,13.36)	0.513
High	8.21	(6.19,10.23)	
% of leukemic cells in marrow:			
<50	7.63	(4.65,10.62)	0.954
≥50	8.74	(6.49,10.98)	
BM cellularity:			
Hypercellular	9.33	(7.09,11.57)	0.108
Normocellular	5.71	(1.56,9.87)	
Blasts % on D14:			
≤40%	8.6	(6.06,11.15)	0.65
>40%	7.06	(.00,17.07)	
FLT3:			
+ve	5.10	(2.51,7.69)	0.025
–ve	9.41	7.13,11.69)	

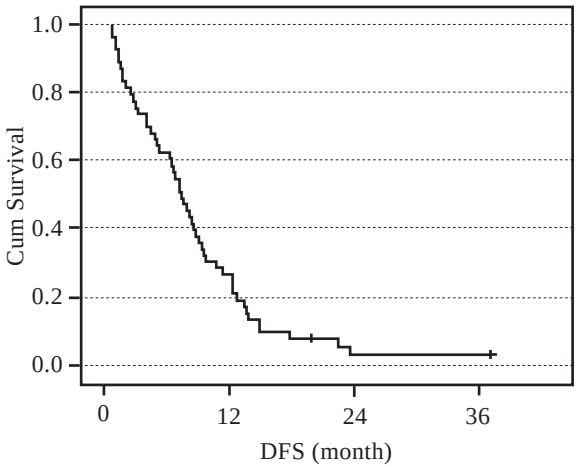

Fig. (1): Duration of complete remission in 75 adult AML cases.

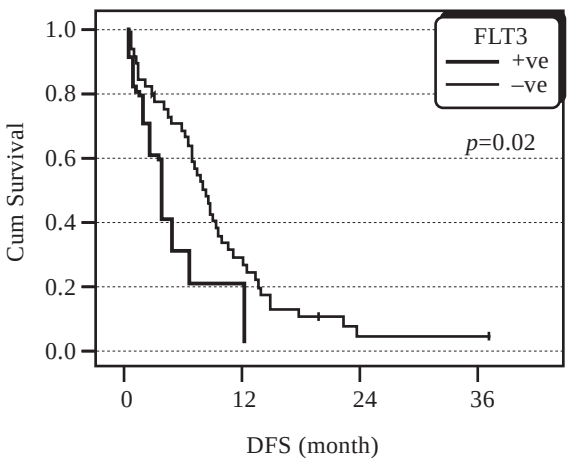

Fig. (2): Duration of complete remission according to FLT3/ITD in 75 adult AML cases.

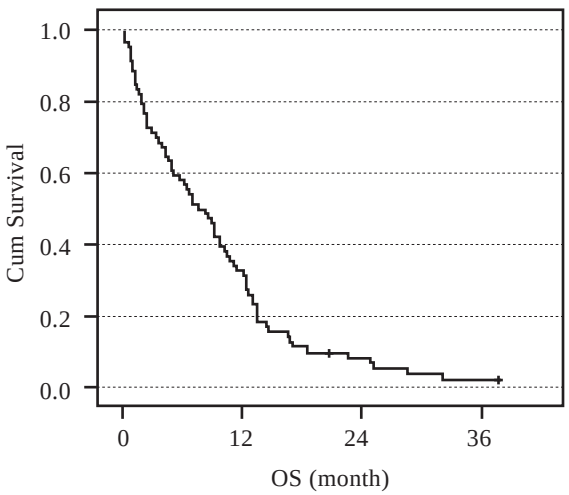

Fig. (3): Overall survival in 75 adult AML cases.

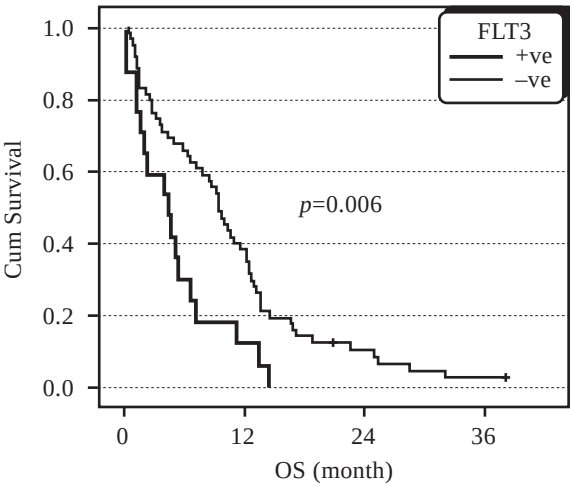

Fig. (4): Overall survival according to FLT3/ITD in 75 adult AML cases.

DISCUSSION

A variety of well-defined factors including age, intensity of post remission therapy (in younger adults), karyotype at diagnosis and P-glycoprotein affect outcome of treatment of adult patients with AML **[12]**.

In this study we investigated mutation of the FLT3-ITD gene by genomic PCR method in 75 newly diagnosed adult AML cases other than M3. The incidence of FLT3/ITD mutation was found to be 22.7% (17/75). This finding is in the range reported by other investigators (17-28%) **[13-17]**.

There was no age or gender preference. Furthermore, patients with FLT3/ITD had higher WBCs at diagnosis, although it did not reach significant value. This concurs with previous studies **[15,18,19]**. The number of bone marrow blast cells, and the presence of peripheral blasts showed no correlation. On the contrary, higher blasts in the bone marrow or peripheral blood were reported in positive cases by some investigators **[18,19]**. Furthermore, Thiede et al. **[20]**, and Munoz et al. **[21]**, showed that FLT3/ITD was significantly increased in patients with the FAB M5, M4, respectively with 40-50% positive cases. More than 60% of our patients were M1, M2, with a 45% incidence of positive cases.

The FLT-3ITD mutation adversely affected the outcome with a significantly shorter disease free survival and overall survival in positive patients confirming the results of previous studies in our patient population **[15,18,19]**. On the

other hand, while some studies **[20,22]** observed no effect of the FLT3/ITD on the overall survival for the whole group of positive patients; yet, survival was significantly affected in those who had both alleles positive for the mutation suggesting that not only the existence of the mutation will affect the bad outcome but this is also related to the level of mutant allele emphasizing the need for use of a'quantitation assay to determine the mutant/wild type ratio.

Complete remission was achieved in 54/75 of patients (72%). Although FLT3/ITD positive cases showed lower response rate, yet it did not reach statistical significance. This concurs the with studies conducted by other investigators **[15,18,19,20,22,23].**

From a clinical perspective, FLT3 mutations are relevant because of their prognostic impact and because constitutively active FLT3 is an attractive target for molecular therapy. Although the value of intensification of treatment (e.g. stem cell transplant) based on the FLT-3 status remains controversial, yet a number of FLT3 inhibitors at various stages of clinical development are available with promising results when combined with conventional chemotherapy **[24].**

In conclusion, there is widespread evidence that the presence of a FLT-3 mutation is a powerful prognostic and potentially predictive factor. Furthermore, it might abolish the good prognostic significance of other genetic markers as NPM1. The biological heterogeneity of AML has started to be unreavled with the wider use of genomic technologies contributing to refined diseases classification and tailoring of therapy.

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