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The impact of the FLT3/ITD mutation on the remission induction outcome in Iraqi adult de-novo acute myeloid leukemia patients

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Abstract— **Background:** AML is a heterogeneous haematological malignancy , it is the most common acute leukemia in adults. FLT3 is the most common molecular aberration in AML , belongs to the group of class III receptor tyrosine kinases (RTKs) which also include the receptors c-kit and c-fms and PDGFR, mutated in more than one third of the AML cases, subdivided into two major classes :internal tandem duplication(ITD) and tyrosine kinase domain(TKD).FLT3/ITD is associated with higher probability of relapse and worse outcome, and however, there is controversy about its impact on the induction outcome. **Objectives:** 1.The prevalence of the FLT3/ITD mutation in Iraqi adult de-novo AML patients. **2**. The effect of the FLT3/ITD mutation on the remission induction outcome (CR rate) . **3**. The association of this gene with the laboratory tests(WBC, Hb ,PLT ,BM blast%). **Patients and methods:** This Cohort study comprised 41 adult de-novo AML patients, admitted in Baghdad medical city complex from Jan.2018 to Dec.2019, Each patient was investigated by complete blood count and differential, bone marrow aspiration and biopsy as well as immunophenotyping to confirm the diagnosis of AML.All of them had received the standard (3+7) protocol.FLT3 gene was screened by expert genetist using conventional PCR technology with special primers in teaching laboratories/molecular unit in Baghdad medical city complex, just at time of diagnosis . The post-induction assessment was done by examination of bone marrow aspirate smears. **Results:** FLT3/ITD gene mutation is present in 6(14.6%) from the total 41 AML cases .4 of the 6 positive cases enter CR while 2 don't enter CR where P-value =0.66 ,the results of the laboratory parameters (WBC, Hb ,PLT ,BM blast %) are comparable in relation to the presence or absence of this gene , where the P-value (0.701 ,0.72 ,0.94 ,0.63) respectively. **Conclusions:** the FLT3/ITD is mutated in 14.6% in this study ,this mutation has no adverse impact on the induction outcome(CR rate) as well as the laboratory parameters (WBC, Hb ,PLT , and BM blast %) .

Keywords: heterogeneous haematological malignancy, FLT3/ITD mutation.

Introduction

Overview

Acute myeloid leukemia (AML) is a heterogeneous haematologic malignancy characterized by the clonal expansion of myeloid blasts in the peripheral blood, bone marrow, and/or other tissues. It is the most common form of acute leukemia among adults and accounts for the largest number of annual deaths from leukemias in the United States. An estimated 21,450 people had been diagnosed with AML in 2019, and 10,920 patients had been died of the disease (1). The median age at diagnosis is 67 years; with 54% of patients diagnosed at 65 years or older (and approximately a third diagnosed at ≥75 years of age)(2).

Environmental factors that have long been established to increase the risks of MDS and AML include prolonged exposure to petrochemicals; solvents such as benzene; pesticides; ionizing radiation ,and chemoimmunoradiotherapy(3)..

Initial Evaluation and work-up

The evaluation and initial workup for suspected AML consists of a comprehensive medical history and physical examination and laboratory evaluations which include a comprehensive metabolic panel and a complete blood count, bone marrow core biopsy and aspirate analysis (including morphological examination ,immunophenotyping and cytochemistry) and cytogenetic analysis and molecular genetic tests.

Identification of mutations that carry prognostic and therapeutic impactis rendering molecular profiling for all AML cases a standard part of the diagnostic workup

New molecular markers can help refine prognostics groups, particularly in patients with a normal karyotype. These markers include NPM1, FMS-like tyrosine kinase 3 (FLT3), CEBPA, isocitrate dehydrogenase 1 and 2 (IDH1/2), DNA (cytosine-5)-methyltransferase 3A (DNMT3A), and KIT, TP53, RUNX1, and ASXL1 gene mutations(4)

FLT3 mutation status ideally should be obtained rapidly to allow for addition of FLT3 inhibitor (midostaurin) on day 8 of upfront intensive chemotherapy(5)

FLT3 Mutations:

The FLT3 protein is expressed on early hematopoietic and lymphoid progenitors(6) and seems to play an important role in early stem cell survival and myeloid differentiation(7). FLT3-ITD mutation occurs in approximately 30% of AML cases and are more common than FLT3-TKD mutation, which occurs in approximately 10% of patients(8).These mutations cluster in exons 11 and 12 of the human FLT3 gene on chromosome 13q12, a part that codes for the juxtamembrane domain of the FLT3 protein. In AML patients, the FLT3-ITD mutations were found to be associated with increased leukocyte counts and were frequent in patients lacking other cytogenetic aberrations. The presence of FLT3 aberrations appears to be associated with an unfavorable clinical response(9).

Controversy exists to the prognostic impact of FLT3/ITD mutation on the remission induction outcome. In the Japanese study, the presence of this mutation had been shown no influence on the CR rate but predicted the RR and adversely affect the OS(10),a subsequent relatively small Dutch study showed that the presence of this mutation is associated with lower CR rate and high relapse rate(9).Another German study have also shown no prognostic significance of this mutation.

Numerous studies have shown the negative prognostic influence of FLT3-ITD in patients with AML, resulting in shorter remission durations and poorer survival outcomes compared with patients who have wild-type FLT3. Among patients with FLT3-ITD and NK-AML, median OS from the time of diagnosis ranged from 6 to 12 months(5).

The objectives of the study

1. The incidence of FLT3/ITD mutation in Iraqi adult de-novo AML patients.

2. The effect of this mutation on the remission induction outcome(CR rate).

3. The effect of this mutation on the laboratory hematological parameters including(WBC , Hb , PLT and BM blast percent).

Patients and methods

The data collection was conducted during the period of the first of January/2018 through the first of December/2019 among the diagnosed adult de-novo acute myeloid leukemia patients whowere admitted to Baghdad medical citycomplex/Baghdad/Iraq who where in young and middle age ,good performance score and fit to receive the intensive chemotherapy protocols that consisted from 3days

of anthracycline plus 7 days of cytosine arabinoside (3&7 protocol)
Forty one patients were included in the study their data were analysed by Cohort study with analytical elements.
Bone marrow aspiration was done on day(14-28) of induction to assess remission status ,Complete remission(CR) was defined as a normocellular BM containing less than 5% blasts and showing evidence of normal maturation of other BM elements.
This study has taken approval of the ethical committee from the Iraqi board for medical specialization in medical college/university of Baghdad.

Results

3.1 Molecular findings (FLT3/ITD):

The molecular tests have shown that only 14.6%(6) of patients had positive FLT3/ITD mutation and 85.4%(35) of patients had negative FLT3/ITD, table 3-1.

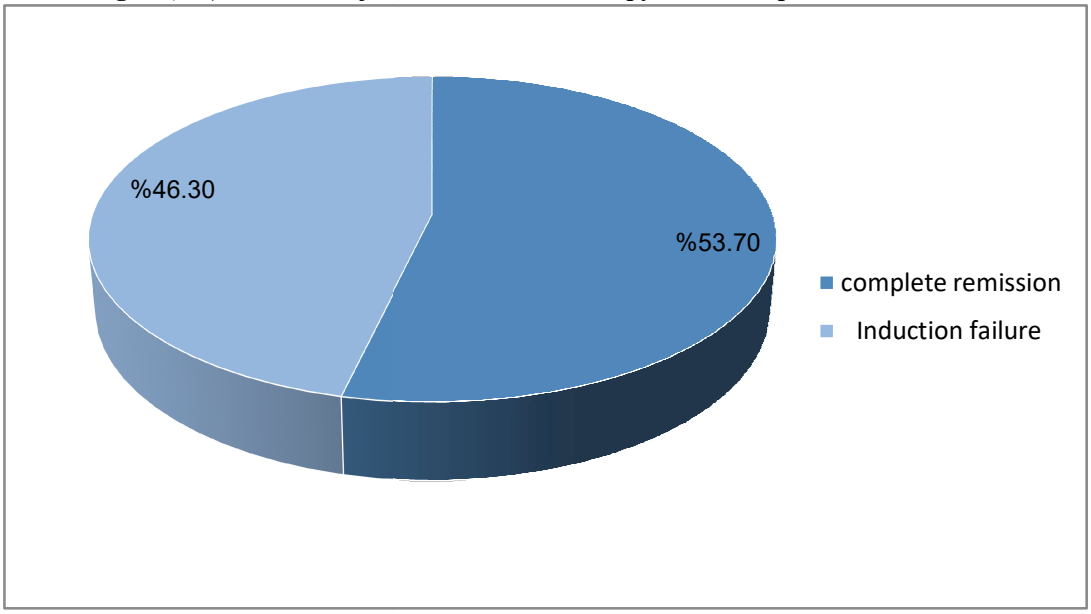
Table(3-1): the frequency of molecular markers of studied patients

Molecular markers		Number	Percentage
FLT3 mutation	Positive	6	14.6%
	Negative	35	85.4%

3.2 Outcome:

After remission induction therapy, 53.7% (22) of patients had achieved complete remission (CR) and 46.3% (19) of patients had induction failure(no CR), figure 3-1.

Figure(3-1): Outcome of induction chemotherapy in studied patients



Patients with positive FLT3 mutation who enter complete remission (CR) are 4(66.7%) and patients with positive FLT3 mutation who have induction failure are 2(33.3%) of the total 6 patients and there was no significant association between FLT3 mutational status and remission induction outcome (p=0.66), table 3-2.

Table(3-2): Relation between FLT3 mutation and induction outcome

Variables		FLT3 mutation		Total	P value
		Negative	Positive		
Induction outcome	Complete remission	18(51.4%)	4(66.7%)	22	0.66
	Induction failure	17(48.6%)	2(33.3%)	19	
Total		35	6	41	

*fisher exact test, significant ≤0.05.

FLT3 mutation and initial laboratory parameters:

The mean ±SD of WBC in patients with positive FLT3/ITD was 53.3±78 x10*^{9} while the mean ±SD of WBC in patients with negative FLT3/ITD was 42.8±57.2 x10*^{9} and there was no significant difference in the mean WBC between different FLT3 mutational states (p=0.701), also there was no significant difference in the mean Hb, Platelet and BM Blast percentage (p=0.72, 0.94 and 0.63 respectively), table 3-3.

Table(3-3):Relation between initial laboratory findings and FLT3 status

Lab findings	FLT3 mutational status		P value
	Positive	Negative	
WBC (mean ±SD)	53.3±78 x10*^{9}	42.8±57.2 x10*^{9}	0.701
Hb(mean ±SD)	8.1±3.2g/dl	8.5±2.4g/dl	0.72
Platelet (mean ±SD)	77.8±59.1 x10*^{9}	75.8±68.4 x10*^{9}	0.94
BM Blast %(mean ±SD)	56.6±39.6%	62.6±25.8%	0.63

Discussion

Acute myeloid leukemia(AML) is the most common acute leukemia in adults and responsible for large number of annual deaths in the united states. The FLT3 mutations are the most common molecular aberrations in AML mutated in more than one third of the cases, subdivided into two subtypes either ITD or TKD. In this study ,the FLT3/ITD mutation was detected in 14.6% which is in accordance with previous Iraqi study by Dhahir etal.(10) who found that FLT3 gene was detected in 14.54% of the cases, and the chinese study by WANG etal.(11) as well as the Iranian study by Vahid etal.(12) who found that the FLT3 mutation is present in 15.8% ,16% respectively. The frequency of FLT3/ITD mutation in this study is considered lower than to what reported in other international clinical studies with percentage approaching(20-30%) of the cases(13,14,15). Many studies report that the FLT3/ITD has lower frequencies in the asian countries(19.1%) compared to what is observed in the western countries which is higher reaching(20-30%)(16,17). The difference in the incidence of FLT3 gene mutation in different studies may be attributed to many factors ; racial/geographical factors ,difference in AML subtypes ,difference in age , difference in sample size and different sensitivity of the experiment ,for example, the exclusion of the elderly patients who are unfit for intensive chemotherapy when FLT3 gene is increased with age and the exclusion of the AML M3 subtype which is associated with highest frequency of FLT3 gene among AML subtypes, this partly explains the lower incidence of FLT3 gene in this study .

There is controversy about the prognostic effect of FLT3 mutation on remission induction outcome(CR rate) in many international studies(13). In this study ,there is no significant association between the presence of the FLT3/ITD mutation and the remission induction outcome(CR) where 4(66.6%) of the total 6 FLT3 positive cases enter complete remission(CR) while 2(33.4%) of the total 6 FLT3 positive cases don't enter CR in which P value =0.66 among both groups ,this study is in accordance with the previous iraqi study Dhahir etal.(10) which had stated that FLT3-ITD mutation had no influence on the CR rate in mutated cases as compared to non-mutated cases , also the present study in agreement with Kiyoi etal.(18) , Schnittger etal.(19) and Moreno etal.(20) which have demonstrated that the presence of the FLT3/ITD mutation had no effect on the CR rate, but that it was associated with adverse LFS and OS.

On the other hand, the present study is in disagreement with the dutch study(9) that associated the FLT3 mutation with the lower CR and increased RR. This study is also in disagreement with the Chinese study(11) and the Serbian study(21) which have shown that there was significant association between the FLT3 mutation and lower CR and overall survival. The lack of effect of FLT3 mutation on the CR rate may be because the FLT3/ITD mutation does not significantly affect chemosensitivity of the majority of blast cells present at diagnosis(13). Additional factors concerning FLT3-ITD need to be identified in order to achieve an even more subtle risk classification of FLT3-ITD-positive AML patients, besides the impact of the FLT3 allelic ratio, the FLT3-ITD diversity (e.g., localization of the start point) should be evaluated to determine the effect on CR and the need for allogenic HSCT after the first CR. It has been clearly demonstrated that localization of the ITD impacts the complete remission rate following AML induction chemotherapy, independently of the mutant allelic burden(22). The lack of these sophisticated details in examining the FLT3 gene mutation may explain the results of this study.

The present study has been shown no significant correlation between the presence of the FLT3/ITD mutation and the hematological parameters and bone marrow blasts percentage at the time of diagnosis which is in accordance with many Turkish studies by Ozbek etal.(23), Karabacak etal.(24) and Dilara etal.(25) which found no correlation between FLT3 mutations and laboratory characteristics of patients (WBC, PLT, Hb, and BM blast %) , while it is in disagreement with Dhahir etal.(10), Kotarridis etal.(13) and Stefan etal.(26) which found that the presence of FLT3 mutation is associated with high WBC and BM blast percent ,this may be due to the constitutive activation of the tyrosine kinase receptor by FLT3 gene mutation leading to autonomous cytokine independent cell proliferation and leucocytosis(27), the sample size and loss of information about the allelic ratio of FLT3/ITD may partly explain the results of this study

Conclusions

I. Lower prevalence of FLT3/ITD gene mutation among Iraqi adult de-novo AML patients than what reported internationally(14.6 % vs 30%).

II. It couldn't be concluded that there is any significant negative impact of this mutation on the (CR rate)

III. It couldn't define any clinical laboratory correlation in relation to its expression.

Recommendations

I. provision of both the FLT3 mutation subtypes(ITD and TKD) and mutation allelic ratio(high vs low) which has prognostic and therapeuticimpact.

II. Application of the full cytogenetic and molecular profiling for precise riskstratification.

III. extending this study by large sample size study comprising multicenter participation

withunified laboratorystandardization

IV. extension of long term follow-up for this study to involve the overall survival(OS), relapse rate(RR), disease free survival(DFS) etc.

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