

Research Paper

JAK-2V617F Mutation In Acute Leukemia (South Egypt Experience)

Abstract

Background / Aim: Mutation of JAK2 (Janus kinase 2) tyrosine kinase was described in chronic myeloproliferative disorders (MPDs). Its role in other hematologic neoplasms is still unclear; some studies suggest a possible role of the JAK2V617F mutation in the pathogenesis of some specific acute myeloid leukemia . This led us to search for JAK2V617F mutations in our patients with acute myeloid and acute lymphoblastic leukemia.

Study design: we screened 90 acute leukemia (30 AML and 60 ALL) for JAK-2 V617F mutation

Place and Duration of Study: Department of medical oncology and department of clinical pathology – South Egypt Cancer Institute, Assiut University, Egypt. Between June 2010 and July 2012.

Methodology: We included 90 patients (56 men, 34 women; their ages were 28.33 ± 2.1 SE years all the patients diagnosed acute leukemia (30 AML and 60 ALL) and screened for JAK-2 V617F mutation by Amplification Refractory Mutation System (ARMS)- PCR

Results: In all cases of AML and ALL the mutant JAK-2 V617F was absent.

Conclusion: Our data add to the observation that the JAK2 V617F mutation seems to be rather rare in acute leukemia either myeloid or lymphoid .

Introduction

Protein kinases (PK) are enzymes that catalyze protein phosphorylation, whereas protein phosphatases do the opposite; regulate PK activity through protein dephosphorylation. Protein-tyrosine kinases (PTK) are PK that catalyze the transfer of the γ -phosphate group of adenosine triphosphate (ATP) to the hydroxyl groups of specific tyrosine residues in signal transduction molecules. In humans, the Janus PTK family (JAKs), has two similar domains facing in opposite directions –hence its name of roman origin–and contains four members: JAK1, JAK2, JAK3 and TYK2. JAKs phosphorylate signal transducers and activators of transcription (STATs) simultaneously with other phosphorylations required for activation [1].

Upon activation by an associated receptor, JAKs phosphorylate cytoplasmic STATs, inducing a conformational change that allows STATs to dimerize, translocate to the nucleus, and alter expression of target genes [2]. JAK/STAT-mediated signal transduction is felt to play a critical role in the proliferative and anti-apoptotic effects of multiple cytokines that activate this pathway; and alterations in JAK/STAT pathways have been associated with diverse human diseases [3]. Myeloproliferative disorders (MPD) include four main types of diseases: chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and myeloid metaplasia with myelofibrosis (MMM) [4]. The target of neoplastic transformation in MPD is a multipotent hematopoietic stem cell [5]. However, except for CML, the molecular mechanisms underlying the MPD neoplastic transformation were largely unknown. Recently, several research groups reported a somatic missense mutation in the pseudokinase domain of

JAK2 gene (V617F) in most PV (up to 97%) as well as in about one-third of both ET and MMM [6-9]. Many of the effects of JAK2 activation in the cells are mediated by the STAT, and JAK2-STAT signaling is frequently activated both in hematologic malignancies and solid tumors [10,11].

The role of JAK2 V617F in AML is not clear from the preceding studies. On one hand, it is known that multiple pathways besides JAKs can lead to STAT activation, On the other hand, the association of the JAK2 V617F mutation with multiple chronic myeloproliferative syndromes that display diverse pathologies and natural histories raises the possibility that this mutation might be associated with additional neoplastic lymphohematopoietic conditions, including AML [7,12]. Also, the identification of a single disease allele in the three related myeloid diseases suggests that the JAK-2V617F mutation may be important in the pathogenesis of additional hematopoietic malignancies. In addition, the TEL-JAK2 and PCM1-JAK2 fusions have been identified in patients with MPD, AML, and acute lymphoblastic leukemia (ALL) [13-15] and activation of the JAK-STAT pathway is observed in hematopoietic and nonhematopoietic malignancies [16].

This led us to search for JAK2V617F mutations in our patients with acute myeloid and acute lymphoblastic leukemia.

Patients

This is a prospective study was done at south Egypt cancer institute – Assiut university and included Ninety patients (56 males and 34 females) with acute leukemia, 30 AML and 60 ALL. The diagnosis and classification of leukemia were done according to conventional criteria [17] which Were as follow; two acute myelogenous leukemias (AMLs) minimally differentiated, 2 AMLs without maturation, 9 AMLs with maturation, 10 acute myelomonocytic leukemias, 5 acute monoblastic and monocytic leukemias and 2 acute erythroid leukemias, and as regards ALL (18 proB ALL, 24 common B ALL and 10 mature B ALL , 8 T ALL) and the ages of the patients

were (28.33 ± 2.1 SE). . The study was performed after obtaining approval from the local Institutional Review Board committee and in accordance with the Declaration of Helsinki, the Good Clinical Practices, and local ethical and legal requirements. All patients provided informed consent

Analysis of the JAK2 V617 F mutation

Genomic DNA was isolated from bone marrow aspirate by QIamp blood mini kit (Qiagen, Germany) and then amplification refractory mutation system (ARMS) -PCR was done using the primers which showed in table 1 and the program cycles using the thermal cycler (PTC-100TM Peltier thermal Cycler, MJ Research) were as follow ; 94C for 15 minutes, 94C for 30 seconds, 58C for 30 seconds, 72C for 30 seconds and repeat the steps 2-4 for 32 times then 72C 10 minutes and finally 15 C pause, according to Jones et al with some modification [18]

The PCR reagents were as follow; Promega buffer 10 ul ,0.4 ul from each primers (5 uM) and 6.9 ul nuclease free water ,then add 1.5 DNA .Then electrophorese 10 ul of each reaction on 2% agrose gel to show the presence of PCR products. Each PCR run contained 2 DNA controls one normal and the other heterozygous, a water blank is also included. Interpretation of the results : PCR products should be observed as bands in the well tracks of the gel .

1- The blank showed no bands

2- The position of the bands of the control samples should indicate the correct molecular size.

If any of the above points are not observed the results are not valid.

- The presence of a band at 463 bps indicates the sample contains the control band. If absent repeat the sample.
- The presence of a band 463 bps and 279 bps indicate the sample contains the control band and is positive for the V617F JAK-mutation.

- The presences of a band only at 463 bps indicate the sample contains the control band and is negative for JAK2 V716F mutation.

As regards FLT3 mutation and Philadelphia chromosome results were taken from the files of the patients as they have been done routinely

Results

Study were included 30 with AML , 18 had FLT3 mutation ,and 12 had no mutation , as regards B lineage ALL 32 of them had Philadelphia chromosome positive , and 28 had Philadelphia chromosome negative and 3 of the T ALL were mature T cell lineage and 5erer pre T lineage , in spite of this diversity of cases all cases had no mutation of JAK2 V627 F. As shown in Table 2.

Discussion

Myeloid neoplasia could be categorized into AML, MDS and MPD. The common feature of myeloid neoplasm is their origin in a progenitor cells which producing terminally differentiated cells of myeloid series [5]. As JAK2 V617F mutation has been found in MPD [6-9] and MDS [18], it is conceivably possible that AML, as another myeloid stem cell disorder, might also harbor the JAK2 mutation. Although STAT signaling activation has been reported in most AML cases, the cause of constitutive STAT activation in AML is still elusive [10]. It is hypothesized that JAK2 mutation could partly be responsible to the STAT activation in AML ([13].Some studies showed expression of JAK2 V617F mutation in AML, like Dong et al [20] who reported that the incidence of the JAK2 V617F mutation in the AML was 13.3% , on the other hand other studies like Lee et al [21], found that a small percent of the AMLs only 2.7% harbored JAK2 mutation concluded that the incidence of JAK2 mutation in AML is very low.

In this study we noticed absence of the JAK-2 mutation in all the studied cases of AML, this results are in line with Steensma et al. [14] who demonstrated that the JAK2 V617F is frequently observed when MPD transforms to AML but not in de novo AML, and their study

stated that JAK2 V617F is a very rare finding in de novo AML, despite the relatively frequent occurrence of STAT3 phosphorylation in myeloblasts. Moreover, Levine et al., who studied 222 AML patients, they identified JAK2 V617F mutations in only four cases (2%), three of whom had a preceding MPD [22].

Regarding acute lymphoblastic leukemia, the known mechanism of JAK2 activation in ALL is chromosomal translocation with the t(9;12)(p24;p13) yielding a chimaeric Tel-Jak2 fusion protein that has deregulated Jak2 kinase activity, confers cytokine-independent proliferation and results in the formation of ALL in a mouse model system [23-25].

Our results showed also absence of JAK-2 mutation in all the studied cases of ALL, and these finding has been found by Sulong et al., who reported that none of studies cases (128 ALL samples) showed positive V617F mutation by allele-specific polymerase chain reaction [26]. This also has been reported by Ruiz-Argüelles et al [27] who found no positive cases of JAK 2 V617 F mutation in either AML or ALL cases.

Conclusion

Our results confirm that JAK-2 mutations in acute leukemia is very rare or completely absent which indicate multiple pathways other than JAKs can lead to STAT activation in the pathogenesis of acute leukemia.

Competing Interests

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Hosny Badrawy, designed the study, wrote the protocol, and wrote the first draft of the manuscript. And Abeer Ibrahim, sheared in the design of the study, managed the literature searches. All authors read and approved the final manuscript.

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Table 1: The primers used in the ARMS-PCR

Primer name	NO	Sequences(5'-3')	Genbank
FO	L1	TTG GAT TTT TCC TTT TTG CTT	HUMHBB
RO	L2	GGC CTG GAA TCT CCT CTA TCA	HUMHBB
FWT	L3	TCC TCA GAA CGT TGA TGG CAG	HUMHBB 52561-52573
RMT	L4	GTTTTACTTACTCTCGTCTCCACAAAA	HUMHBB 52383-52409

FO: Forward outer, RO: Reverse outer, Fwt: Forward wild-type specific (FWt, RMT: Reverse mutant-specific

Table 2 : The results of JAK 2 V617 F mutation in acute leukemia

Condition	Number	JAK 2 V617 F mutation
AML		
M0	2	0
M1	2	0
M2	9	0
M4	10	0
M5	5	
M6	2	
ALL		
Pro B	18	0
Common	24	0
Mature B	10	0
TALL	8	0

AML : acute myeloid leukemia - ALL : acute lymphoblastic leukemia