International Blood Research & Reviews
2(1): 1-7, 2014, Article no. IBRR.2014.001

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

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Authors' contributions

This work was carried out in collaboration between both authors. Author HB designed the study, performed the statistical analysis, done the ARMS-PCR, wrote the manuscript. Author AI shared in the design of the study, collected the clinical data. Both authors read and approved the final manuscript.

Research Article

Received 15th June 2013
Accepted 20th September 2013
Published 11th October 2013

ABSTRACT

Aim: To search for JAK2V617F mutation in patients with acute myeloid and acute lymphoblastic leukemia in south Egypt.
Study Design: JAK2V617F mutation detected by amplification refractory mutation system (ARMS) -PCR.
Place and Duration of Study: Department of clinical pathology and department of medical oncology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt, between December 2010 and December 2012.
Method: We included 90 patients (58 men and 32 women; age range 2-67 years) with denovo acute leukemia (30 acute myeloid leukemia and 60 acute lymphoblastic leukemia), JAK-2 V617F mutation using ARMS-PCR was done for all the patients.
Results: JAK-2 V617F mutation was absent in all of the studied patients.
Conclusion: Our results confirm the finding published previously which reported that JAK2 V617F mutation is very rare or absent in acute leukemia.

Keywords: Acute leukemia; JAK-2; ARMS-PCR.

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1. 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), have 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, induces 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].

The role of JAK2 V617F in acute myeloid leukemia (AML) is not clear from the previous 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 [4,5]. 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 Myeloproliferative disorders (MPD), AML, and acute lymphoblastic leukemia (ALL) [6-8] and activation of the JAK–STAT pathway is observed in hematopoietic and nonhematopoietic malignancies [9].

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

2. MATERIALS AND METHODS

In this prospective study conducted at South Egypt Cancer Institute – Assiut University, Assiut, Egypt, bone marrow samples were collected from 90 patients (58 males and 32 females), the age of the patients ranged from 2-67 years, diagnosed as denovo acute leukemia, all of them were Egyptian, 30 of them were AML (19 males and 11 females) and 60 were ALL (39 males and 21 females) .The diagnosis and classification of leukemia were done according to FAB classification which were as follow; two 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, 24common 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.

2.1 Analysis of the JAK2 V617 F Mutation

Genomic DNA was isolated from bone marrow aspirate by QIamp blood mini kit (Qiagen,Germany). the JAK2 V617F mutation was detected by amplification refractory mutation system (ARMS)-PCR, the ARMS-PCR technique uses 4 primers as follows; a forward outer primer, a reverse outer primer, a forward inner wild type specific primer and a reverse inner mutant specific primer as shown in Table 1.

Table 1. The primers used in the ARMS-PCR

Primer name	Sequences (5'-3')
FO	TTG GAT TTT TCC TTT TTG CTT
RO	GGC CTG GAA TCT CCT CTA TCA
FWT	TCC TCA GAA CGT TGA TGG CAG
RMT	GTTTTACTTACTCTCGTCTCCACAAAA

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

PCR reaction was performed on the thermal cycler (PTC-100TM Peltier thermal Cycler, MJ Research) used the following program; 94ºC for 15 minutes, 94ºC for 30 seconds, 58ºC for 30 seconds, 72ºC for 30 seconds and repeat the steps 2-4 for 32 times then 72ºC 10 minutes and finally 15 C pause, according to Jones et al. with some modification [10].

The PCR reagents were as follow; Promega buffer 10 ul, 0.4 ul from each primers (5 uM), 6.9 ul nuclease free water, and 1.5 DNA. Then 10 ul of each reaction were electrophoresed on 2% agrose gel to show the presence of PCR products. Each PCR run contained 2 DNA controls one normal and the other mutant (the normal DNA extracted from healthy person while the mutant one extracted from a known polycythemia Vera patient positive for JAK-2 V617F mutation), a water blank is also included.

2.2 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 shown in Fig. 1.

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

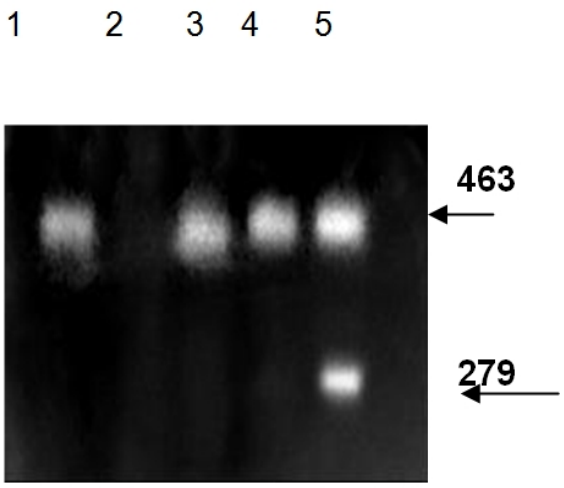

Fig. 1. ARMS-PCR for the V617Fmutation. The 463-bp band serves as an internal control. The 279 -bp band indicates the presence of the mutation. Lane 1; normal DNA, lane 2; negative (Blank), lanes 3 and 4 negative patients, finally lane 5; positive mutant patient (DNA from known polycythemia vera positive for JAK-2 V617F)

3. RESULTS AND DISCUSSION

This study included 30 AML patients, 18 of them had FLT3 mutation, and the other 12 had no FLT3 mutation, as regards ALL 32 of them were positive for Philadelphia chromosome, and 28 were negative. Furthermore 3 of the T ALL were mature and the other 5 were immature T cell linage, in spite of this diversity none of the all studied patients samples were positive for JAK2 V627 F mutation as shown in Table 2.

Table 2. The results of JAK 2 V617 F mutation in the studied patients

Type	Number		JAK 2 V617 F mutation
	Male	Female	
AML			
M0			
M1	2	0	0
M2	1	1	0
M4	6	3	0
M5	6	4	0
M6	2	3	0
	2	0	
Total	**19**	**11**	**30**
ALL			
Pro B	12	6	0
Common B	15	9	0
Mature B	6	4	0
TALL	6	2	0
Total	**39**	**21**	**60**

AML: acute myeloid leukemia - ALL: acute lymphoblastic leukemia

Myeloid neoplasia could be categorized into AML, myelodysplastic syndrome (MDS) and MPD. The common feature of myeloid neoplasm is their origin in a progenitor cells which producing terminally differentiated cells of myeloid series [11]. As JAK2 V617F mutation has been found in MPD [12-14] and MDS [15], 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 [16]. It is hypothesized that JAK2 mutation could partly be responsible for the STAT activation in AML [6]. Some studies showed expression of JAK2 V617F mutation in AML, like Dong et al. [15] 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. [6] 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 V617F mutation in all the studied cases of AML, this results are in line with Steensma et al. [17] 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., studied 222 AML patients, they identified JAK2 V617F mutations in only four cases (2%), three of whom had a preceding MPD and one had AML secondary to essential thrombocythemia [18].

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 [19-21].

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 non of studies cases (128 ALL samples) showed positive V617F mutation by allele-specific polymerase chain reaction [22]. This also has been reported by Ruiz-Argüelles et al. [23] who found no positive cases of JAK 2 V617 F mutation in either AML or ALL cases.

4. CONCLUSION

Our results confirm that JAK-2 V617F mutation 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.

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