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# The Role of JAK2 Mutation in Polycythaemia Vera in Some Iraqi Patients

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## Abstract :

Polycythaemia vera (PV) is a myeloproliferative disease that arises in a clonal haematopoietic stem cell and is characterized by increased red blood cell production that is independent of the mechanisms that normally regulate erythropoiesis. Evaluation of the role of JAK2 mutation had been carried out in polycythaemia vera in some Iraqi patients, from beginning June 2008 up to the 30 of November 2009,attending national center of hematology for research and treatment . Thirty two patients have been included with age range of 30-66years and ten apparently healthy control group with age range of 32-55 years. Results revealed that polycythemic patients had mean age of (50.41 ±1.71). The molecular study using amplification refractory mutation ,has clarified that (90.6%) of patients with polycythaemia vera had JAK2 mutation .According to the above results to introduce JAK2 mutation test as part of routine work in suspected patients with Polycythaemia vera.

***Key Words:***

*JAK2 , polycythaemia vera , Amplification Refractory Mutation System Analysis.*

## Introduction:

Janus kinase 2 (JAK2) is a cytoplasmic (i.e. nonreceptor) protein kinase and its structure is uniquely characterized by the presence of 2 homologous kinase domains: JAK homology (JH) 1, which is functional, and JH2, which lacks kinase activity (i.e., pseudokinase) (1) . The Janus cytoplasmic protein tyrosine kinase mediate signaling downstream of cytokine receptors, via distinct type I and II cytokine receptors, as well as receptor protein tyrosine kinase (2 ). JAK/STAT signaling is regulated at multiple levels by distinct mechanisms including, for example direct dephosphorylation of JAK2 by specific protein-tyrosine phosphatase (PTP), proteolytic degradation of JAK2 through binding with a family of suppressors of cytokine signaling , and inhibition of DNA binding of STAT by protein inhibitors of activated STAT) (3 ). The JAK/STAT signal transduction pathway plays a major role in both cellular proliferation and cell surviva1(4). In hematopoiesis, for example, definitive erythropoiesis and cytokine response by myeloid progenitors have been shown to be absent in JAK2 knockout mice (5 ). The kinase domain of JAK2 mediates antiapoptotic signals in hematopoietic cells by inducing bcl-2 production (6).

Abnormalities affecting either members of the JAK/STAT signaling pathway or its regulatory elements have been associated with various tumor phenotypes including hematologic malignancies. For example, germline JAK3 mutations have been associated with certain forms of autosomal recessive severe combined immunodeficiency syndrome (7).

The mutation in JAK2 is a guanine to thymidine substitution that results in a valine to phenylalanine substitution at codon 617 of JAK2. Genetic and biochemical data demonstrate that JAK kinase activation is required for cytokine receptor signaling, and that the disparate physiologic effectsof different cytokine receptors is in part due to the ability of these cytokine receptors to engage different JAK kinases after cytokine stimulation (8 ). Most cytokine receptors can associate with more than one JAK kinase, but it has been shown that Jak2-deficient myeloid progenitors fail to respond to EPO, thrombopoietin, or granulocyte-monocyte colony stimulating factor, and that Jak2 deficiency results in an absence of definitive erythropoiesis (9). These data suggest that JAK2 is the predominant JAK kinase involved in myeloid cell proliferation and differentiation. It is perhaps not surprising, therefore, that a gain-of-function mutation in JAK2 is observed in a spectrum of myeloid malignancies and that JAK2V617F+ cells are hypersensitive to cytokine stimulation (10 ).This study was carried out to detect the role of JAK2 mutation in diagnosis polycythaemia vera in some Iraqi patients

## Material and Methods:

A total of ( 32) patients suspected with polycythaemia

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vera who were attending to Iraqi national center of hematology for research and treatment with age ranged from (30 - 66) years with 24 males and 8 females.

History was taken in regards of headache , malaise , vertigo ,history of arterial and venous thrombosis (from patients medical record), physical examination include abdominal examination for palpable spleen and liver .On other hand a total of (10) of apparently healthy individuals were included in this study as control group with age ranged from (32-55) years from both sex , the number of males was 6 while female was 4.

**Sample collection**

The present study was conducted from 11th June 2008 to the 30 of Nov. 2009, from each individual 3-5 ml of blood was obtained by venapuncture using disposable syringes . The blood was placed in EDTA plastic disposable tubes.

**Molecular investigation**

***Granulocyte Preparation***

Granulocytes was separated by double-density sedimentation according to (11).

***Genomic DNA isolation***

The genomic DNA isolated from the whole fresh blood collected in EDTA tubes for molecular studies using Promega genomic DNA purification kits.

The isolation of DNA was based on five steps process using salting out Methods according to (12).

***Genomic DNA Isolation protocol***

The protocol supplied by Promega Company was used for DNA isolation .

Amplification Refractory Mutation System Analysis

The Amplification Refractory Mutation System(ARMS) method which is based on PCR amplification using allele-specific oligonucleotides.

*Table 1 :- polymerase chain reaction (PCR) program steps.*

| Cycling Conditions | |
|---|---|
| Denaturing | 94˚c , 30 sec |
| Annealing | 85˚c , 45 sec |
| Extention | 72˚c , 45 sec |
| Cycle number | 40 |

*Table 2 :- primers sequences used in polymerase chain reaction.*

| *Primers* | |
|---|---|
| *Forward Outer (FO)* | *5> TCCTCAGAACGTTGATGGCAG 3>* |
| *Reverse Outer (RO)* | *5> ATTGCTTTC CTTTTTCACAAGAT 3>* |
| *Forward inner Wild Type (Fwt)* | *5> GCATTTGGTTTTAAATTATGGAGTATaTG 3'* |
| *Reverse inner mutant specific primor (Rmt)* | *5> GTTTTACTTACTCTCGTCTCCACAaAA 3'* |

## Result & Disscution:

The results of this study indicate that the age ranged between (30-66 y) (mean of 50.4 + 1.71) among patients with polycythemia Vera and about two third of patients are located within fourth and fifth decades as shown in (table 1), these results coincide with that of Silverstein etal. (13) stated that the male age of onset occurring between 50 and 6o years of age ,also in Europe PV present at median age of 55 years as reported by Marchioli (14) , moreover Zafer etal. (15) confirmed that the mean age of PV patients 59.7 years.

*Table 3: Distribution of the study group according to age and gender.*

| | | PV (n=32) | |
|---|---|---|---|
| | | No | % |
| Age (years) <40 | | 5 | 15.6% |
| 40—59 | | 21 | 65.6% |
| 60-- | | 6 | 18.8% |
| Mean ± SD (Range) | | 50.41±1.71 (30.-66.0) | |
| Gender | Male | 24 | 75.0% |
| | Female | 8 | 25.0% |

*Note :- PV : polycythaemia vera and N:Number*

Our results indicated that JAK2 mutation had been detected in 29 patients out of 32 (90.6%) of the PRV groups( table 1 and figure 1). The result of this study concurrence with that of James et al. (16) and Jelink et at. (17) who found that the percentage of positive JAK2 had been 88% and 86% in PV patients respectively.

*Table 4 : Molecular investigation (JAK2 Mutation) in polycythaemia vera group*

| | | PV (n=32) | |
|---|---|---|---|
| | | **No** | **%** |
| Mutated JAK2 | Yes | **29** | **90.6%** |
| | No | **3** | **9.4%** |

*PV : polycythaemia vera*

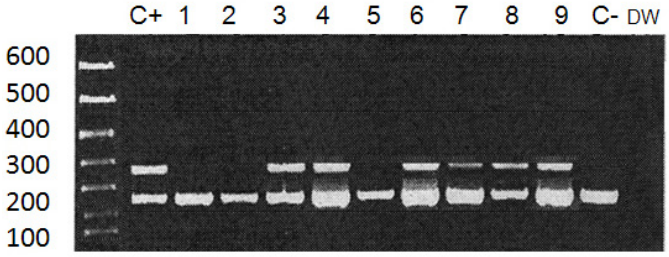


*Figure 1 :- detection of the JAK2V617F mutation in peripheral blood of myeloproliferative disorders . The upper band of 279 bp showing the presence of the mutation. The second band of 229 bp confirms the presence of amplifiable DNA . (C+) positive control, (C-) negative control . The Lane 3,4,6,7,8,9 showed JAK2 mutation. Lane 1,2,5 are non mutated , using 1% agarose for 1.5 hour at 5 volt /cm.*

However, the percentages have been lower as recorded by Kralovics etal.(18) and Levine etal. (19) who has reported the positive JAK2 in PV patients (65%) and (74%).

The prevalence or JAK2 mutation in Taiwanese patients with PV could be detected in 28 out of 33 patients (85%) (20). Besides ,in Pakistan , the JAK2 mutation interestingly had found in all (17 ) patients (100%) of PV patients (21). Finally , Baxter et al. (22) had detected (97%) positive JAK2 (the higher frequency of JAK2 mutation by Baxter etal. is due to the use of a more sensitive technique the amplification refractory mutation(ARMS)) .

Obviously , the results of this study come in agreement with other data stated by from the above data , the findings of James etal. (16) ; Jelinek etal. (17) ; Lieu etal.(20) and Jones (22). However, the others mentioned are not ,this could be explained that it is likely that the technical difference relating to the sensitivity of V617F detection could be contributory factor to the published differences in the proportions of positive cases of PV patients .

One advantage of ARMs is its apparent high sensitivity to small amount of mutant DNA in a wild –type background , at least when hot start Tag DNA polymerases are used to diminish the likelihood of nonspecific primer annealing (22). According to the above results to introduce JAK2 mutation test as part of routine work in suspected patients with Polycythaemia vera.

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# دور طفرة (جاك 2) في مرض زيادة انتاج الكريات الحمراء الاولي لبعض المرضى العراقيين

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**الخلاصة:–**

ينشأ مرض زيادة إنتاج الكريات الحمراء الأولي من نسل الخلايا الجذعية الأولي يمتاز بزيادة إنتاج الكريات الحمراء الغير مسيطر عليه . أجريت هذه الدراسة لتقييم دور طفرة (جاك2) في مرض زيادة إنتاج الكريات الحمراء للفترة من بداية حزيران 2008 الى نهاية تشرين الثاني 2009 لمرضى يراجعون المركز الوطني لبحوث وعلاج إمراض الدم ، ضمت هذه الدراسة اثنان وثلاثون مريضا تتراوح أعمارهم بين (30 – 66 سنة) مع مجموعة السيطرة عشرة أشخاص أصحاء تتراوح أعمارهم بين (32 – 55 سنة) . أظهرت النتائج إن معدل عمر المرضى (50.41 + 1.71) ، بينت الدراسة الجزيئية لطفرة (جاك2) بان (90–61) من المرضى يحملون هذه الطفرة ، ووفقا لهذه النتائج فانه من المهم إدخال هذا الفحص كجزء من الفحص الروتيني لمرضى زيادة إنتاج الكريات الحمراء .