

Received: December 2019

Accepted: March 2020

PCR-HRM for Detecting *JAK2*V617F Gene Mutation: Is It a Sensitive Assay?Mitra Rezaei^{1,2}, Mihan PourAbdollah Toutkaboni³, Babak Salimi⁴, Sharareh Seifi⁴, Fatemeh Maryam Sheikholeslami^{3,5*}

A B S T R A C T

173

1. Virology Research Center, National Research Institute of Tuberculosis and Lung diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Department of Pathology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shaheed Beheshti University of Medical Sciences; Tehran, Iran.
5. Department of Molecular Biology, Dr. Khosroshahi's Pathobiology Laboratory, Tehran, Iran

***Corresponding Authors:**

Fatemeh Maryam Sheikholeslami,
Pediatric Respiratory Diseases
Research Center, National Research
Institute of Tuberculosis and Lung
Disease (NRITLD), Shahid Beheshti
University of Medical Sciences,
Tehran, Iran.

Phone: (+98)9109820034

Email: m.sheikholslami@gmail.com



2019; 11(4):173-181

www.bccrjournal.com

Background: A substitution of G to T at nucleotide 1849 in exon 14 of the Janus kinase2 (*JAK2*) gene is well recognized in myeloproliferative neoplastic disorders (MPNs). Based on WHO guidelines, detection of the mutation is very important to confirm the disease in suspected patients.

Methods: Eighty-seven patients with different background diseases were tested for *JAK2*V617F mutation by four different methods, including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), amplification refractory mutation system (ARMS), polymerase chain reaction-high resolution melting (PCR-HRM), and two different commercial kits.

Results: The mean age of patients was 53.38±17.43 years, 72.4% were males, and 37.6% were females. *JAK2* mutation was detected in 16 patients (18.3%). Of those, 7 (43.75%) suffered from PV, 5 (31.25%) from ET, 3 (18.75%) from PMF, and 1 (6.15%) from unclassified neoplastic disorders. The frequency of *JAK2* mutation was 71.4% (5/7) in PV, 80% (4/5) in ET, and 66.7% (2/3) in PMF patients. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and GE of PCR-HRM for detection of the *JAK2* mutation was 86.7%, 100%, 100%, 97.3%, and 97.7%, respectively. While the sensitivity, specificity, PPV, NPV, and GE of PCR-RFLP were 93.3%, 80.5%, 50%, 98.3%, and 82.7%, respectively. On the other hand, the sensitivity, specificity, PPV, NPV, and GE of ARMS assays were evaluated by about 80%, 96%, 100%, 96%, and 96.5%, respectively.

Conclusion: This study showed that PCR-HRM was a more sensitive assay to detect the *JAK2*V617F mutation than the other assays. So, it can be used as a quick, easy, and effective method for screening the *JAK2*V617F mutation in patients with MPNs disorders. PCR-RFLP must accompany it as a gold standard method for confirmation of the mutation of *JAK2*V617F.

Keywords: *JAK2*V617F Mutation, PCR-HRM, PCR-RFLP, Myeloproliferative Neoplastic Disorders



INTRODUCTION:

The Janus kinase 2 (*JAK2*) is a critical component of diverse signal-transduction pathways. These molecules are involved in cellular survival, proliferation, differentiation, apoptosis, and cytokine signaling in normal and neoplastic cells through tyrosine kinase activity^{1,2}. The substitution of valine for phenylalanine at amino acid position 617 (V617F) of the *JAK2* protein results in the activation of the *JAK/STAT* signaling pathway. Mutations in genes encoding *JAK2* are, to some extent, responsible for tumorigenesis. Nowadays, the incidence of the *JAK2* V617F mutation is well established in chronic myeloproliferative neoplastic disorders (MPNs)². The prevalence of the mutation is 65%–97% in polycythemia vera (PV), about 23%–57% in essential thrombocythemia (ET), and 35%–57% in primary myelofibrosis with myeloid metaplasia³⁻⁷. One of the significant WHO criteria for the diagnosis of PV is the detection of *JAK2* mutations⁸. Therefore, reliable and sensitive methods are needed for the detection of these mutations.

Many molecular assays, including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and amplification refractory mutation system-polymerase chain reaction (ARMS-PCR), have been previously described for the detection of *JAK2* V617F mutations. High resolution melting (HRM) is a rapid and cost-effective methodology for mutation scanning and genotyping. Its mechanism based on the chelating of dsDNA dye with double-strand DNA. PCR-HRM assay is a suitable and specific closed-tube technique for mutation scanning and makes it more convenient than other scanning methods². Our study aimed to evaluate the diagnostic value of the PCR-HRM analysis for identifying the *JAK2* V617F missense mutation. Therefore, PCR-HRM analysis was compared with ARMS, PCR-RFLP, and commercial kits, as well as the clinical background of the patients. Then, we reported the fre-

quency of *JAK2* V617F missense mutation in Iranian patients with PV, ET, and PMF.

METHODS:

Patient samples and DNA extraction

Peripheral blood samples were obtained from 16 patients with MPD and 71 control subjects. Control subjects consisted of 27 patients (38.03%) with chronic diseases such as hypertension, 31 patients (43.66%) with lung disorders such as Asthma and COPD, 8 (11.27%) patients with infectious diseases such as tuberculosis and 5 normal subjects (7.04%) without any signs and symptoms of diseases. The demographic data of the subjects are summarized in **Table 1**. The study population consisted of 7 PV patients (43.75%), 5 ET patients (31.25%), 3 myelofibrosis patients (18.75%), and a patient with an undetermined myeloproliferative disorder (6.25%). DNAs of the blood leucocytes were extracted using the silica-based kit (Yekta Tajhiz, Iran) according to the manufacturer's instructions. This study was approved by the National Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Iran.

The HRM technique and PCR conditions

A PCR reactions were carried out in 20 µl final volume using 1× buffer, containing Taq DNA polymerase, nucleotides, and the SYBR green dye, 2.5 mM of each primer (forward (F): 5'-AAGCTTTCTACAAGCAT-TTGTTT-3' and reverse (R): 5'-AGAAAGGCATT-AGAAAGCCTGTAGTT-3') as well as MgCl₂ and 30 ng DNA to amplify 155 bp of *JAK2* gene harboring probably V617F mutation. The PCR program consisted of an initial denaturation-activation step at 95 °C for 15 min, followed by a 45-cycle program (denaturation at 95 °C for 15 s, annealing at 58 °C for 15 s and elongation at 72 °C for 10 s with the acquisition of the fluorescence dye at 533 nm at the end of the elongation step).

Table 1. Demographic and blood characteristics of the patients

Variables	Minimum	Maximum	Mean	N (%/%)
Age (y)	17	84	53.38±17.43	
Male/female gender				63/24(72.4%/27.6%)
Hemoglobin	8	24	15.64±4.07	
Platelet count	32000	1588000	418045.9±364649.4	
Hematocrit	23.40	76.30	47.58±11.74	
Mean corpuscular hemoglobin concentration	12.70	38.80	32.51±3.33	
Red Blood Cells	2.81	9.63	5.61±1.47	
Red blood cell distribution width	12.00	33.80	16.88±4.29	
White Blood Cells	4300	74980	13157.01±11605.58	

The melting program included a rising melting ramp by 0.1 degrees each level from 65 to 95°C that consists of a continuous fluorescent reading of fluorescence per °C. The PCR-HRM reactions were performed and analyzed with the Rotor-Gene real-time analyzer 6000™ (Corbett Life Sciences, Mortlake, Australia). Negative controls and positive controls were used as a reference curve to generate the difference plot (**Fig 1**).

Direct DNA sequencing

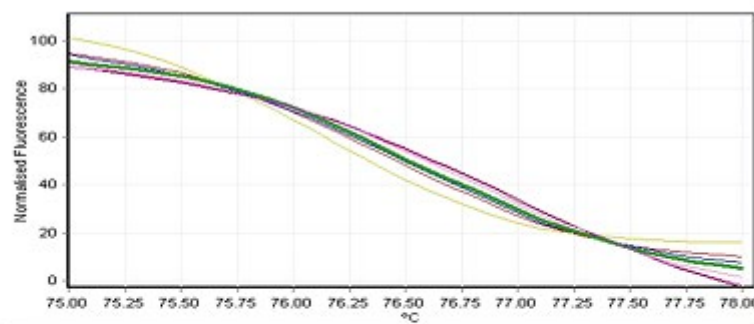
To confirm the results of the HRM analysis, sequencing analysis was carried out for 5 positive samples. The sequence detection was conducted using the ABI Prism 310 Genetic Analyzer (Applied Biosystems). The graphs were analyzed by BioEdit v7.0.5.

JAK2V617F genotyping by ARMS

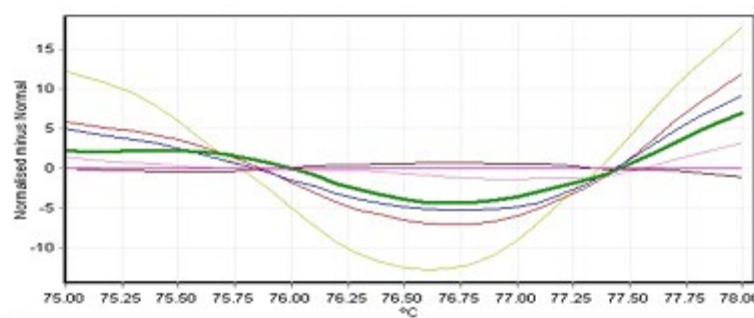
ARMS-PCR was carried out by tetra primers earlier

described⁹: G49-*JAK2*-FO, 5'-TCCTCAGAACGTTGATGGCAG-3'; G50-*JAK2*-RO, 5'-ATTGCTTTCCTTTTTCACAAGAT-3'; G51-*JAK2*-FWT, 5'-GCATTTGGTTTTAAATTATGGAGTATaTG-3'; G52-*JAK2*-RMT, 5'-GTTTTACTTACTCTCGTCTCCACAaAA-3'. Also, multiplex PCR was using three primers: G46-*JAK2*-AS-R, 5'-CTgAATAgTCCTACAgTgTTTTCAgTTTCA-3'; G47-*JAK2*-AS-mtf, 5'-AGCATTTggTTTTAAATTATggAgTATATT-3'; G48-*JAK2*-AS-CF, 5'-ATCTATAgTCATgCTgAAAgTAggAgAAAg-3' described by Baxter⁷ previously. Amplifications were performed for 30 cycles with 2X master mix (PARS Tous co, IRI) (consisting of 1X buffer, 0.2 uM of each dNTP, 1.5 µM MgCl₂, and 1 U Taq DNA polymerase), 25 ng genomic DNA, and standard amplification conditions with an annealing tempera-

A-Normalized Graph for HRM A-HRMA-Normalized Graph for HRM A.HRM



B-Normalized minus Normal



C- The final result of HRM

No.	Colour Name	Genotype	Confidence %
1	■	P.C JAK2	Mutant 83.15
2	■	JAK2	Mutant 25.79
3	■	97-9-6	Mutant 94.48
4	■	98-3-4	Normal 96.17
5	■	98-3-25	Normal 99.31
6	■	97-3-4	Mutant 100.00
7	■	97-2-19	Normal 100.00

Figure 1. HRM curves of *JAK2* exon 14 V617F missense mutation. A-A melting curve profile plot which synchronization samples in maximum and minimum fluorescence emission. B- Difference plot showed a characteristic melting curve and temperature shifted plot of mutant (T allele) and wild type (G allele). C- The result of HRM generated by Rotor-Gene 6000 Series Software 1.7- the mutant allele was confirmed by confidence more than 80%.

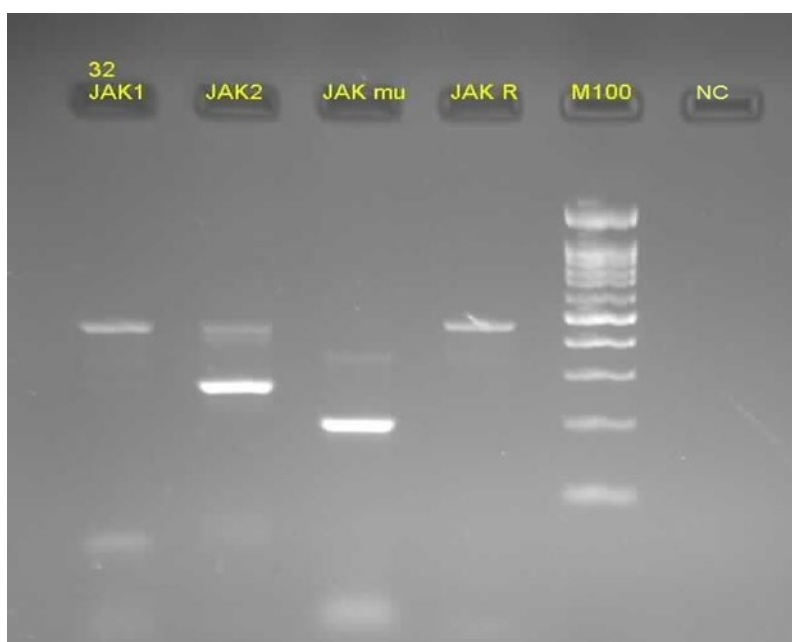


Figure 2. A positive sample with ARMS PCR methods and PCR amplification of *JAK2* for RFLP. (*JAK1*) negative with wild type forward primer, (*JAK2*) positive with mutant forward primer and (*JAK-mu*) positive with multiplex ARMS PCR, (*JAK R*) PCR amplified of *JAK2* for digestion. Lane (M): marker 100bp, NC: negative control.

ture of 58 °C. However, the final concentrations of the outer primers and the mutant/wild-type-specific inner primers were 1 $\mu\text{mol/l}$ and 0.5 $\mu\text{mol/l}$, respectively. Products were resolved on 2% agarose gels and visualized after staining with safe stain (Fig 2).

***JAK2* V617F genotyping by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)**

To detect the *JAK2*V617F mutation by restriction digestion, a portion of the *JAK2* region that acquires this mutation (462 base pairs) was amplified by using two forward and reverse outer primers (G49-*JAK2*-FO and G50-*JAK2*-RO), as described above. The amplified fragment was digested with the BsaXI restriction enzyme (BioLabs™, New England) as the manufacturer's instruction. The final digested products were vis-

ualized on 2% stained agarose gels with safe stain. The undigested fragment implicated *JAK2* V617F missense mutation because of losing the restriction enzyme site but the wild type cases were digested and produced two visible bands (190, 242 and 30bp which is disappear on agarose gel). Logically, existing the undigested bands plus digested one indicated the heterozygosity may be due to the low allele burden of mutation in the background of wild type allele or due to incomplete endonuclease activity (Fig 3).

Statistical analysis

The Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL), version 22.0, and software was used for statistical analysis. Both HRM results and PCR findings were cross compared with the results of the myeloproliferative disease and PCR-RFLP (as the gold standard)

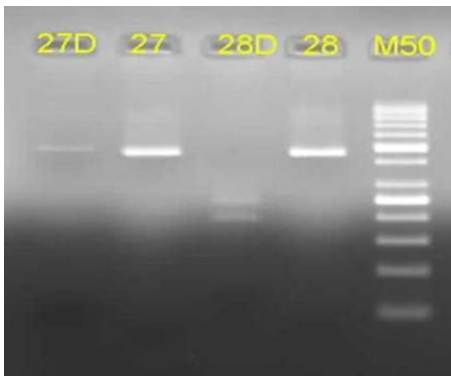


Figure 3. RFLP analysis of *JAK2* V617F mutation: Lane M, 50-bp ladder; Lanes 27 and 28 PCR amplification of *JAK2* fragment. Lanes 27D and 28D *Bsa*XI digestion, two samples from patients with a pattern of mutant and wild-type alleles, respectively.

and accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) of each test are reported.

Ethical Approval

The work has been approved by the ethical committees of National Research Institute of Tuberculosis and Lung Diseases (NRITLD). Written informed consent was obtained from all participants to enter the study.

RESULTS:

In this study, HRM assay could be able to differentiate the homozygous (T/T) mutant from the wild-type DNA in comparison with the melting profile of wild-type control (G/G). Thirteen out of 16 MPNs patients (81.25%) were positive for the presence of *JAK2*V617F mutation by HRM assay. The prevalence of the mutation in the different subtypes of MPNs was 71.4% in PV (5 of 7 patients), 80% in ET (4 of 5) and 100% in myelofibrosis (3 of 3). The background disease of one patient was not determined. She was suspected of MPN disorders but the WHO criteria were not confirmed in this patient (**Table 2**). This study showed that the sensitivity of HRM analysis to detect the *JAK2*V617F mutation in patients with MPNs and those with suspected MPNs was 86.6% vs 93.3% for PCR-RFLP and 80% for PCR-ARMS assays. The sensitivity of commercial kits (ipsogen® *JAK2* MutaQuant® Kit CE, Qiagen from Germany and *JAK2*

RG version 2, Novin gene from Iran) was lower than homemade assays (66.7%) (**Table 3**).

DISCUSSION

The objective of this study was to evaluate the efficacy of several *JAK2* V617F mutation PCR-based assays. The result should be used in selecting an appropriate method so that both patients and the laboratories are involved in its benefit. Numerous techniques include, allele-specific polymerase chain reaction (AS-PCR)³, genomic DNA-PCR-sequencing⁶, PCR-amplification refractory mutation system⁹, PCR-restriction analysis¹⁰ and DNA-melting curve analysis¹¹, have been developed for the detection of the *JAK2* V617F missense mutation during the last decades. Each of them has its advantages and disadvantages: for example, in spite of the power of DNA sequencing to detect any specific mutation, its sensitivity is low and the cost is high¹². On the other hand, PCR-ARMS, which is introduced as one of the most sensitive (0.01%–5%) techniques needs post-PCR manipulation¹³. The false-positive results of PCR-RFLP are due to incomplete digestion with the restriction enzyme can be complicated the interruptions and on the other hand, this technique is time-consuming and expensive¹⁴. Many important parameters such as sensitivity, specificity, the cost of a test should always be considered to evaluate and select these methods. There are very few studies for evaluat-

Table 2. Comparison of *JAK2* V617F mutation positive samples as assessed by the PCR-based assays.

Disorders	No	PCR-RFLP‡	PCR-ARMS¶	PCR-HRM§	Commercial kit
Polycythemia Vera	7	7 (100%) †	5 (71.4%)	5 (71.4%)	4 (57.1%)
Primary Mielofibroses	3	2 (66.7%)	2 (66.7%)	3 (100%)	2 (66.7%)
Essential Thrombocythemia	5	4 (80%)	4 (80%)	4 (80%)	4 (80%)
Clinically suspected MPNs*	1	1 (100%)	1 (100%)	1 (100%)	0 (0%)
Total	16	14 (87.5%)	12 (75%)	13 (81.25%)	10 (62.5%)

* Myeloproliferative neoplasms.
‡ Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
¶ both multiplex Allele specific and amplification-refractory mutation system
§ Polymerase Chain Reaction-High Resolution Melt analysis
† Two samples were heterozygote (*JAK2* V617F mutation was not confirmed by DNA sequencing)

Table 3. Sensitivity and Specificity of different assays to detect *JAK2* V617F mutation in the confirmed and suspected MPNs patients

Disorders	PCR-HRM§	PCR- RFLP‡	ARMS-PCR¶	Commercial Real-time kit
Sensitivity	86.6%	93.3%	80%	66.7%
Specificity	100%	80.5%	100%	100%
Positive Predictive Value	100%	50%	100%	100%
Negative Predictive Value	97.3%	98.3%	96%	90.9%
General efficacy	97.7%	82.7%	96.5%	92.3%

§ Polymerase Chain Reaction-High Resolution Melt analysis
‡ Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
¶ both multiplex Allele specific and amplification-refractory mutation system

ing the currently available testing assays for detecting the *JAK2* V617F mutation in Iranian MPNs patients. Some studies showed that there is a good concordance among PCR-HRM with three other screening methods (ARMS-PCR, PCR-RFLP, and quantitative method) for *JAK2* V617F detection in MPNs patients especially when there is a higher concentration of the mutant alleles^{2, 15}.

In our study, four different screening methods were assessed using a total of 16 confirmed MPNs out of 87 patients with different symptoms of diseases such as chronic lung disease, infectious and/or solid cancer disorders. The assays were compared with the PCR-RFLP method, a widely used technique, as a reference assay for the detection of the *JAK2* V617F mutation^{16, 17}. The statistical analysis showed a good concordance among all assays ($P=0.000$) but the melting curve assay proved less reliable at lower levels of *JAK2* V617F mutation allele (**Table 2**). A serial dilution of positive samples showed that the PCR-HRM assay could detect the mutant allele by lower than $0.1 \times 10_4$ ng/ μ l of pool genomic DNA. Therefore the burden of the mutant allele in PMF patients who were positive with PCR-HRM probably was lower than the limit of detection of mutation in wild type background by PCR-RFLP assay (**Table 2**). The PCR-HRM test couldn't find the mutation in only 2 out of 7 patients with PV compared with those obtained by the PCR-RFLP. Of course, the PCR-RFLP result in both samples was heterozygote and also not detected by direct sequencing. There is no guarantee that the restriction enzyme can be digesting the restriction site exactly as predicted. Thus, the result of the PCR-RFLP assay was probably false positive due to incomplete digestion. Therefore, the results of the present study indicate that PCR-ARMS and PCR-HRM methods can accurately detect *JAK2* V617F mutation in PV patients. Two PCR-ARMS methods based on a multiplex PCR and Allele-Specific PCR were used. The result of both of them was similar. In the multiplex assay, one intermediate primer generated

a second, smaller fragment in addition to the complete band in mutant allele while in allele specific method two different specific primers could detect the wild type and mutant alleles. The role of the two outer primers was to detect the PCR inhibitor as an internal control. In cases with the presence of *JAK2* mutation, annealing with intermediate primer will happen in multiplex ARMS-PCR assay and consequently, the sideband will be observed. In Allele-specific methods, the mutation was detected if the mutant allele specific primers were annealed and produced a 240bp fragment (**Fig 2**). Both of the ARMS PCR methods need post-amplification manipulation which can cause cross-contamination. It is a major disadvantage of these methods. For this reason and because of the PCR-HRM method's advantages over PCR-ARMS such as its ease of performance and speed of it as well as its high sensitivity compared to other molecular assays, we think PCR-HRM may be a good tool for screening of *JAK2* V617F mutation in clinical suspension patients.

Our evaluation revealed that the two commercial kits for the detection of *JAK2* V617F missense mutation were less sensitive methods. The limit for mutation detection by them was subjective and may depend on the burden level of the allele mutation. Unfortunately, the Iranian manufacturer did not specify the limit allele burden. Maybe the allele burden of the commercial kit was more than 20% mutant DNA in the background of the wild type allele which is higher than the HRM-PCR assay. The limit of detection (LOD) of the ipsogen® *JAK2* MutaQuant® Kit was 0.042% which is lower than ARMS assays. On the other hand, based on ipsogen® *JAK2* MutaQuant® Kit Handbook, in case of additional mutations located in nucleotides 88504 to 88622, the result of test can report false negative. Another study showed the low sensitivity of the ipsogen® *JAK2* MutaQuant® Kit in detection of *JAK2* V617F mutation. Since the accessibility of the ipsogen® *JAK2* MutaQuant® Kit is not easy in our country as well as its lower sensitivity to detect *JAK2* V617F mutation we

recommend the PCR-HRM assay as a substitution assay to detect the mutation.

CONCLUSION

In conclusion, we have compared the results of four PCR-based assays including PCR-RFLP for detection of the *JAK2* V617F mutation in blood samples from clinically confirmed and suspected patients with MPNs disorders. Our results have demonstrated that the PCR-HRM protocol is a quick, easy, and effective method to screen the *JAK2*V617F mutation in patients with MPNs disorders. And for confirmation of the result, it must be accompanied by PCR-RFLP as a gold standard method for the detection of the mutation of *JAK2* V617F.

FUNDING

This study was supported by National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Iran, Tehran.

ACKNOWLEDGMENT

We thank Leila Mohammadi Ziazi for her coordination, Mr. Esmaili for registering the data of the patients, Seyed Bagher Aghaii and Jasmin Hassani for their technical supports.

REFERENCES

- Spano JP, Milano G, Rixe C, Fagard R. JAK/STAT signaling pathway in colorectal cancer: a new biological target with therapeutic implications. *European journal of cancer*. 2006;42(16):2668-70.
- Er TK, Lin SF, Chang JG, Hsieh LL, Lin SK, Wang LH, et al. Detection of the *JAK2* V617F missense mutation by high resolution melting analysis and its validation. *Clinica chimica acta; international journal of clinical chemistry*. 2009;408(1-2):39-44.
- Zhao R, Xing S, Li Z, Fu X, Li Q, Krantz SB, et al. Identification of an acquired *JAK2* mutation in polycythemia vera. *The Journal of biological chemistry*. 2005;280(24):22788-92.
- James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal *JAK2* mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005;434(7037):1144-8.
- Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase *JAK2* in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer cell*. 2005;7(4):387-97.
- Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of *JAK2* in myeloproliferative disorders. *The New England journal of medicine*. 2005;352(17):1779-90.
- Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase *JAK2* in human myeloproliferative disorders. *Lancet*. 2005;365(9464):1054-61.
- Tefferi A, Thiele J, Orazi A, Kvasnicka HM, Barbui T, Hanson CA, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. *Blood*. 2007;110(4):1092-7.
- Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the *JAK2* V617F mutation in chronic myeloproliferative disorders. *Blood*. 2005;106(6):2162-8.
- Melzner I, Weniger MA, Menz CK, Moller P. Absence of the *JAK2* V617F activating mutation in classical Hodgkin lymphoma and primary mediastinal B-cell lymphoma. *Leukemia*. 2006;20(1):157-8.
- Olsen RJ, Tang Z, Farkas DH, Bernard DW, Zu Y, Chang CC. Detection of the *JAK2*(V617F) mutation in myeloproliferative disorders by melting curve analysis using the LightCycler system. *Archives of pathology & laboratory medicine*. 2006;130(7):997-1003.
- Frantz C, Sekora DM, Henley DC, Huang CK, Pan Q, Quigley NB, et al. Comparative evaluation of three *JAK2*V617F mutation detection methods. *American journal of clinical pathology*. 2007;128(5):865-74.
- Lay M, Mariappan R, Gotlib J, Dietz L, Sebastian S, Schrijver I, et al. Detection of the *JAK2* V617F mutation by LightCycler PCR and probe dissociation analysis. *The Journal of molecular diagnostics : JMD*. 2006;8(3):330-4.
- Vannucchi AM, Pancrazzi A, Bogani C, Antonioli E, Guglielmelli P. A quantitative assay for *JAK2*(V617F) mutation in myeloproliferative disorders by ARMS-PCR and capillary electrophoresis. *Leukemia*. 2006;20(6):1055-60.
- Moradabadi A, Farsinejad A, Khansarinejad B, Fatemi A. Development of a high resolution melting analysis assay for rapid identification of *JAK2* V617F missense mutation and its validation. *Experimental hematology & oncology*. 2019;8:10.
- Cankovic M, Whiteley L, Hawley RC, Zarbo RJ, Chitale D. Clinical performance of *JAK2* V617F mutation detection assays in a molecular diagnostics laboratory: evaluation of screening and quantitation methods. *American journal of clinical pathology*. 2009;132(5):713-21.
- McClure R, Mai M, Lasho T. Validation of two clinically useful assays for evaluation of *JAK2* V617F mutation in chronic myeloproliferative disorders. *Leukemia*. 2006;20(1):168-71.
- Warshawsky I, Mularo F, Hren C, Jakubowski M. Failure of the Ipsogen MutaScreen kit to detect the *JAK2* 617V>F mutation in samples with additional rare exon 14 mutations: implications for clinical testing and report of a novel 618C>F mutation in addition to 617V>F. *Blood*. 2010;115(15):3175-6.