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Original Article *Özgün Araştırma*

ASSOCIATION OF *JAK2* F617 ALLELE BURDEN WITH HEMATOLOGICAL PARAMETERS IN MPD PATIENTS

MPH Hastalarında JAK2 F617 Allel Yükü ile Hematolojik Parametrelerin İlişkisi

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ABSTRACT

Objective: A point mutation in the *JAK2* gene, resulting in the substitution of valine for phenylalanine (JAK2 V617F), has been associated with myeloproliferative disorders such as polycythemia vera, essential thrombocythemia, idiopathic myelofibrosis, myelodysplastic syndromes, chronic myelomonocytic leukemia, systemic mastocytosis, chronic neutrophilic leukemia, and eosinophilic disorders.

Material and Methods: The relation of *JAK2* V617F mutation has been studied in myeloproliferative disorder patients by qPCR. The F617 allele was calculated using a standard calibration curve, including less and more than 50% mutational load groups.

Results: A significant relation was found among wild type (wt), less and more than 50% groups, Regarding erythrocyte, hematocrit, platelet, and leukocyte levels. A statistically significant relation was found between wt and more than 50% of groups regarding hemoglobin levels. The mutational load increase has been shown to induce erythrocyte, hematocrit, and leukocyte levels, except platelet levels.

Conclusion: In patients with myeloproliferative disorders, qPCR screening of *JAK2* gene mutation is indicated.

Keywords: *JAK2, myeloproliferative disorders, qPCR, V617F*

ÖZ

Amaç: *JAK2* genindeki bir nokta mutasyonu, fenilalanin yerine valinin geçmesi (*JAK2* V617F) ile sonuçlanmakla beraber, polisitemi vera, esansiyel trombositemi, idiyopatik miyelofibroz, miyelodisplastik sendromlar, kronik miyelomonositik lösemi, sistemik mastositoz, kronik nötrofilik lösemi ve eozinofilik bozukluklar gibi miyeloproliferatif bozukluklarla ilişkilendirilmiştir.

Gereç ve Yöntemler: Miyeloproliferatif bozukluk tanılı hastalarda JAK2 V617F mutasyonu alel yükü qPCR yöntemi ile incelenmiştir. F617 aleli, %50'den az ve fazla mutasyon yükü gruplarını içeren standart bir kalibrasyon eğrisi kullanılarak hesaplanmıştır.

Bulgular: Yabanıl tip (wt), %50'den fazla ve düşük gruplar arasında eritrosit, hematokrit, trombosit ve lökosit düzeyleri açısından anlamlı bir ilişki bulundu. Wt ve %50'den fazla gruplar arasında hemoglobin düzeyleri açısından istatistiksel olarak anlamlı bir ilişki bulunmuştur. Mutasyonel yük artışının trombosit düzeyleri hariç eritrosit, hematokrit ve lökosit düzeylerini indüklediği gösterilmiştir.

Sonuç: Miyeloproliferatif bozukluğu olan hastalarda *JAK2* gen mutasyonunun rutin taraması endike olarak görünmektedir.

Anahtar Kelimeler: *JAK2, miyeloproliferatif hastalıklar, qPCR, V617F*

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INTRODUCTION

A missense mutation in the Janus tyrosine kinase 2 (*JAK2*) gene, results in a change of valine for phenylalanine (*JAK2* V617F). Polycythemia vera (PV), myeloproliferative disorders (MPD) and essential thrombocythemia (ET) are related to this mutation.[1,2] This mutation has been demonstrated to be in 65-97% of PV patients; 23-57% of ET patients and 35-57% of myelofibrosis patients.[2-4]

It has been reported that the conformational changes in JAK2 protein phosphorylate specific tyrosine residues on the intracellular domain of the receptor.[5] The intrinsic inhibitory mechanism is inhibited by *JAK2* gene V617F mutation and it overactivates the *JAK2* protein. This may cause constitutional activation of its receptors, aberrant downstream signaling, and an increase in hematopoiesis.[6]

JAK2 V617F mutation is a major diagnostic criterion according to the 2008 WHO classification. The patients with ET carrying this mutation have been reported to have an increased risk of arterial and venous thrombosis, also patients with PV have been reported to have an increased risk of thrombosis.[7,8] Besides, *JAK2* is also helpful to distinguish between primary and secondary thrombocytosis regarding long-term follow-up.[9]

The V617F mutation in the *JAK2* gene has been accepted as a characteristic of myeloproliferative diseases.[8] In patients with PV, a significant correlation has been indicated between V617F mutation and leukocytosis, high hematocrit values and thrombosis.[8] Also, this mutation has been reported to be both a reliable and noninvasive molecular marker for myeloproliferative diseases, so it can be recommended as a first test for diagnosing. myeloproliferative diseases.[10] Clonally increasing of the cells carrying V617F mutations tend to turn into cells carrying the heterozygous mutation in the first place and then turn into the cells carrying the homozygous mutation. This increases the F617 allele ratio from 0-50% to 50-100%. Also, the *JAK2* allele burden of more than 50% has been reported to be a risk factor for progression to myelofibrosis in PV.[3,11,12] The higher *JAK2* allele burden has also been related to more advanced myelofibrosis, greater splenomegaly, and higher white blood cell count in polycythemia vera.[8]

In a recent study, the genetic test results of *JAK2* V617 mutation in Philadelphia negative myeloproliferative neoplasm patients revealed 67%, 33% and 25% of incidence in polycythemia vera, essential thrombocytosis and primary myelofibrosis groups, respectively.[13]

This mutation is reported to be present in 95% of patients with PV and 50–60% of patients with essential thrombocythemia or primary myelofibrosis.[14]

In this study; we investigated, the relation between *JAK2* V617F mutation allele burden and hematological parameters of myeloproliferative disorder patients.

MATERIALS AND METHODS

Patients

A total of 196 myeloproliferative disorder patients (female=75, male=121) were studied retrospectively and 111 of them (female=32; male=79; mean age=46.5) were diagnosed as PV; 43 of them (female=20; male=23; mean age=49.7) were diagnosed as MPD and 42 of them (female=23; male=19; mean age=45.2) were diagnosed as thrombocytosis. Written informed consent form was obtained from the patients. We took venous blood samples (5 ml in tubes containing EDTA) of patients were stored at -20 °C until DNA isolation.

Hematological parameters including; red blood cell (Rbc), hemoglobin (Hgb), hematocrit (Hct), leucocyte count (Wbc), and platelet (Plt) levels were analyzed in PV and other MPD groups (Tables 1, 2).

An informed consent form had been obtained and this study was approved by the Clinical Researches Ethics Committee of the Ankara Etlik City Hospital (Protocol no: AEŞH-BADEK-2024; Date:22.05.2024)

qPCR

The genomic DNAs were extracted and *JAK2* V617F target was PCR amplified and detected by TaqMan probes using an ABI real-time PCR instrument. Allelic discrimination is facilitated by software analysis of the fluorescence data. The F617 allele was calculated using a standard calibration curve, revealing less and more than 50% mutational load groups.

Statistical analysis

Statistical analyses were performed with SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Distribution of variables was investigated using Kolmogorov-Simirnov test. Significance of differences for mean values was determined by the independent T-test. Data were presented as mean. The one-way analysis of variance test was used to compare the hematological parameters between groups. A p value below 0.05 was accepted as significant.

RESULTS

Our results revealed no significant relationship between gender and *JAK2* V617F mutation ($p > 0.05$). At the same time, no relation was found between indication and *JAK2* mutation ($p > 0.05$).

In all of the patients, a significant increase in Rbc, Hct, and Wbc values between less and more than 50% mutational load groups with respect to wild-type group was found ($p < 0.05$). Also, the increase in Rbc, Hct, and Wbc values and decrease in Plt values was also detected in sperate groups of PV, MPD and thrombocytosis

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patients. Besides, a significant correlation was found between more than 50% mutational load and wild-type groups regarding Hgb values (p <0.05). Finally, a significant decrease in Plt values was found between less and more than 50% mutational load groups (Table 1,2).

Table 1: The Rbc, Hgb, Hct, Plt and Wbc mean values in PV group (n=111), MPD(n=43), and thrombocytosis (n=42) groups with respect to wt, *JAK2* allele burden <%50 and *JAK2* allele burden >%50 values

	PV			MPD			Thrombocytosis			
	wt	*JAK2* allele burden <50%	*JAK2* allele burden >50%	wt	*JAK2* allele burden <50%	*JAK2* allele burden <50%	wt	*JAK2* allele burden <50%	*JAK2* allele burden <50%	p
Rbc (x10/u)	4.27	4.93	6.99	4.66	4.91	6.12	4.42	4.74	6.37	<0.05[1,2,3]
Hgb (g/dL)	12.3	14.02	16.53	14.38	13.66	18.60	12.72	13.53	17.30	<0.05[1,2,3]
Hct (%)	36.07	41.74	51.76	41.9	41.21	54.7	37.41	40.12	51.7	<0.05[1,2,3]
Plt (x10/ul)	387.99	656	519.51	390.23	579.26	348	452.97	579.86	1081	<0.05[1,2,3]
Wbc (x10/ul)	8.13	10.79	15.18	9.307	10.29	12.4	8.043	9.76	13.2	<0.05[1,2,3]

wt: wild type; Rbc: Red blood cell; Hgb: Hemoglobin,Hct: Hematocrit; Plt: Platelet; Wbc: White blood cell [1]PV, [2]MPD, [3]Thrombocytosis
p<0.05; between wt and *JAK2* allele burden <50% for PV, MPD and thrombocytosis groups
p<0.05 between wt and *JAK2* allele burden 50% for PV, MPD and thrombocytosis groups
p<0.05 between *JAK2* allele burden<50% and *JAK2* allele burden>50% for PV, MPD and thrombocytosis groups

Table 2: The Rbc, Hgb, Hct, Plt ve Wbc mean values in total (n=196) with respect to wt, *JAK2* allele burden <%50 and *JAK2* allele burden >%50 values

	wt	*JAK2* allele burden <50%	*JAK2* allele burden >50%	p
Rbc (x10/u)	4.51	4.92	6.93	<0.05
Hgb (g/dL)	13.64	13.80	16.66	<0.05
Hct (%)	39.79	41.42	51.95	<0.05
Plt (x10/ul)	393.05	608.78	508.79	<0.05
Wbc (x10/ul)	8.95	10.49	15	<0.05

wt: wild type; Rbc: Red blood cell; Hgb: Hemoglobin,Hct: Hematocrit; Plt: Platelet; Wbc: White blood cell
p<0.05; between wt and *JAK2* allele burden <50% for total groups
p<0.05 between wt and *JAK2* allele burden 50% for total groups
p<0.05 between *JAK2* allele burden<50% and *JAK2* allele burden>50% for total groups

DISCUSSION

Polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis have been reported to be clonal myeloproliferative disorders. Patients with the V617F mutation have been reported to have a significantly longer duration of disease and a higher rate of complications in myeloproliferative disorders.[12] Also, *JAK2* mutation has been reported to be a reliable marker for myeloproliferative disorders.[10,11]

An association between *JAK2* V617F allele burden and WBC in PV patients had been reported, but relationships with RBC count, Hct, and Hb levels could not be demonstrated.[15] Also, an increased allele burden has been reported to be correlated with increases in thrombosis and disease transformation.[16] Besides, Vannucchi et al. categorized their patients into four distinct groups according to their *JAK2* V617F allele burden. They reported that patients with an allele burden of 75% or higher exhibited a significantly elevated risk of thrombosis during the follow-up period.[17] There is evidence showing that *JAK2* V617F allele burden may progressively increase with age.[18] Although the majority of studies divided the patients using a 50% *JAK2* V617F allele burden as a cut-off, other studies revealed 58% and 70%.[19,20]

In previous studies, *JAK2* V617F mutation in PV has been reported to be between 69.6-94.7%. It also has a great importance for the diagnosis of patients.[3,4,13] The expression of *JAK2* V617F mutation in PV results in erythrocytosis, which involves the JAK2-signaling pathway.[2] *JAK2* mutation also correlated to the severity of the clinical phenotype.[8] In this study, the *JAK2* mutation rate in PV patients was found to be 24.2%.

The *JAK2* gene mutation in myeloproliferative disease has been reported to be 70% and is also reported to be a reliable molecular marker.[10] Also, differences in Hgb, Hct, and neutrophil percentages between V617F positive and negative patients with ET have been reported.[4] Our study revealed the ratio for MPD to be 23.5%. The difference between the literature and our values may be related to the limited number of patients in our study and inadequate exclusion of secondary polycythemia.

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JAK2 V617F mutation is a major diagnostic criterion and the *JAK2* mutation rate due to early treatment is of great importance.[7,8] Recent studies indicate direct relationships between F617 allele burden and hemoglobin concentration, white blood cell count, spleen size, and age-adjusted bone marrow cellularity. Also, an inverse relationship was found between F617 allele burden and platelet count.[3,8] Regarding our study, we found that regardless of the indication, *JAK2* expression affects blood parameters. Also, we detected that the increase in the *JAK2* V617F mutation rate leads to an increase in Rbc, Hct, Hgb, and Wbc levels whereas a decrease in Plt levels.

In conclusion, qPCR screening of *JAK2* V617F mutation in myeloproliferative disorders has a very great importance regarding early diagnosis treatment of the patients.

Conflict of Interest: The authors have no conflicts of interest to declare.

Researchers' Contribution Rate Statement: Concept/Design: MAE, EFP; Analysis/Interpretation: AB; Data Collection: AB, SGE; Writer: SGE, AB; Critical Review: MAE, EFP; Approver: SGE, AB, EFP, MAE.

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REFERENCES

1. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet*. 2005;365(9464):1054-1061.
2. Verstovsek S, Silver RT, Cross NC, et al. JAK2 V617F mutational frequency in polycythemia vera: 100%, >90%, less? *Leukemia*. 2006;20:2067.
3. Passamonti F, Rumi E, Pietra D, et al. A prospective study of 338 patients with polycythemia vera: The impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. *Leukemia*. 2010;24:1574-1579.
4. Zhang S, Qiu H, Fischer BS, et al. JAK2 V617F patients with essential thrombocythemia present with clinical features of polycythemia vera. *Leuk Lymphoma*. 2008;49:696-699.
5. Rane SG, Reddy EP. JAKs, STATs and SRC kinases in hematopoiesis. *Oncogene*. 2002;21(21):3334-3358.
6. Akada H, Yan D, Zou H, et al. Conditional expression of heterozygous or homozygous JAK2 V617F from its endogenous promoter induces a polycythemia vera-like disease. *Blood*. 2010;115(17):3589-3597.
7. Guglielmelli P, Vannucchi AM. Recent advances in diagnosis and treatment of chronic myeloproliferative neoplasms. *F1000 Med Rep*. 2010;2:16.
8. Silver RT, Vandris K, Wang YL, et al. JAK2 (V617F) allele burden in polycythemia vera correlates with grade of myelofibrosis, but is not substantially affected by therapy. *Leuk Res.* 2011;35:177-182.
9. El-Moneim AA, Kratz CP, Böll S, et al. Essential versus reactive thrombocythemia in children: Retrospective analyses of 12 cases. *Pediatr Blood Cancer*. 2007;49:52-55.
10. Primignani M, Barosi G, Bergamaschi G, et al. Role of the JAK2 mutation in the diagnosis of chronic myeloproliferative disorders in splanchnic vein thrombosis. *Hepatology.* 2006;44:1528-1534.
11. Traulsen A, Pacheco JM, Luzzatto L, et al. Somatic mutations and the hierarchy of hematopoiesis. *Bioessays.* 2010;32:1003-1008.
12. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005;352:1779-1790.
13. Bahsi T, Yigenoglu TN. CALR, JAK2 and MPL Genes mutations in myeloproliferative neoplasms, single center experience. *Acta Oncologica Turcica.* 2019;52:388-392.
14. Nangalia J, Green TR. The evolving genomic landscape of myeloproliferative neoplasms. *Hematol Am Soc Hematol Educ Program*. 2014;2014(1):287-296.
15. Chen CC, Chen JL, Lin AJ, et al. Association of JAK2 V617F allele burden and clinical correlates in polycythemia vera: A systematic review and meta-analysis. *Ann Hematol*. 2024;103(6):1947-1965.
16. Alvarez-Larrán A, Bellosillo B, Pereira A, et al. JAK2 V617F monitoring in polycythemia vera and essential thrombocythemia: Clinical usefulness for predicting myelofibrotic transformation and thrombotic events. *Am J Hematol*. 2014;89(5):517-523.
17. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Prospective identification of high-risk polycythemia vera patients based on JAK2 (V617F) allele burden. *Leukemia*. 2007;21(9):1952-1959.
18. Stein BL, Saraf S, Sobol U, et al. Age-related differences in disease characteristics and clinical outcomes in polycythemia vera. *Leuk Lymphoma.* 2013;54(9):1989-1995.
19. Lee AJ, Kim SG, Nam JY, et al. Clinical features and outcomes of JAK2 V617F-positive polycythemia vera and essential thrombocythemia according to the JAK2 V617F allele burden. *Blood Res*. 2021;56(4):259-265.
20. Okabe M, Yamaguchi H, Usuki K, et al. Clinical features of Japanese polycythemia vera and essential thrombocythemia patients harboring CALR, JAK2 V617F, JAK2 Ex12del, and MPL W515L/K mutations. *Leuk Res*. 2016;40:68-76.