TURKISH JOURNAL OF INTERNAL MEDICINE

ISSN:2687-4245

Original Article

Comparison of Clinical Data and Treatment Responses of Patients With Essential Thrombocythemia Using Anagrelide by JAK2 Gene Mutation Status

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ABSTRACT

Background Essential Thrombocytemia (ET) is a clonal stem cell disease that manifests itself with proliferation in the megakaryocytic lineage in the bone marrow, with clinical presentations ranging from asymptomatic to bleeding and thrombosis spectrum. In medical treatment, aspirin and/or monitoring are recommended for low-risk patients, while cytoreductive therapy is recommended for high-risk patients. Cytoreductive therapy is often used in patients with a very high platelet count (>1,000,000/mm^3). The first choice in cytoreductive treatment is hydroxyurea, and anagrelid treatment is preferred in the young patient group and patients with hydroxyurea resistance/intolerance. This study aimed to evaluate the effects of the JAK2 mutation, which is associated with high risk, in the patient group receiving anagrelide therapy.

Material and Methods The files of patients diagnosed with ET according to 2016 WHO criteria and followed up under anagrelide therapy in our center between January 2002 and December 2021 were reviewed retrospectively. In addition to the demographic data of the patients, diagnostic tests, bone marrow evaluations, JAK2 mutation status, and laboratory data were noted. Patients were divided into two groups according to JAK2 mutation positivity and negativity. The obtained data were compared between the two groups.

Results Thirty-three patients (male/female: 20/13) treated with Anagrelide for the diagnosis of ET were included in the study. It was observed that 14 (42%) of the patients were positive for JAK2 mutation. There was no significant difference between the groups regarding age at diagnosis, gender, duration of anagrelide use, and bone marrow fibrosis degrees. When the laboratory tests were compared at the time of diagnosis, the WBC count was significantly higher in the JAK2 positive group; other series were similar. When the last control laboratory data of the patients were compared, leukocyte, neutrophil, and hemoglobin levels were observed to be significantly higher in JAK2 positive patients, while LDH levels were significantly lower.

Conclusions It was observed that JAK2 mutation positivity, which is associated with high risk in ET risk staging, did not negatively affect anagrelide treatment response. In ET patients, leukocytosis (>11.000/mm^3) has been identified as a risk factor for the whole lifespan. It was observed that the WBC counts of the patients who were positive for JAK2 were significantly higher at the time of diagnosis and during the treatment process. Since the LDH level after treatment is higher in patients with positive JAK2 mutation, it has been evaluated that JAK2 mutation may play a role in resistance to cytoreductive therapy.

Turk J Int Med 2022;4(Supplement 1):S7-S11
DOI: 10.46310/tjim.1080749

Keywords: *JAK2, essential thrombocytosis, anagrelide.*

Received: February 28, 2021; Accepted: March 09, 2021; Published Online: March 14, 2022

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Introduction

Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are included in the BCR-ABL negative myeloproliferative neoplasms (MPN) group.[1] Clonal proliferation and cytokine hypersensitivity are essential in distinguishing them from reactive states.[2] Mutations that occur directly or indirectly in the Janus Kinase (JAK2) signal are essential in the pathogenesis of MPNs. The JAK2 mutation was positive in >95% of PV patients, while positive in 60% of ET and PMF patients. JAK2 is a non-receptor tyrosine kinase responsible for converting signals from class 1 cytokine receptors required for erythropoiesis, thrombocytopoiesis, myelopoiesis, and granulocyte-colony stimulating factor.[3] JAK2 is inactive, bound to clas1 cytokine receptors in the unstimulated state. The two most common mutations in JAK2 (V617F and exon 12 mutations) cause JAK2 activation without class 1 receptor stimulation.[1] In medical treatment, aspirin and/or monitoring are recommended for low-risk patients, while cytoreductive therapy is used for high-risk patients. Cytoreductive treatment is often recommended in patients with a very high platelet count (>1,000,000/mm^3). The first choice in cytoreductive treatment is hydroxyurea, and anagrelid treatment is preferred in the young patient group and patients with hydroxyurea resistance/intolerance. Our study aimed to evaluate the effects of JAK2 mutation on clinical and laboratory data before and after treatment with anagrelide.

Material and Methods

After obtaining approval from the local ethics committee approval, the files of patients diagnosed with ET according to 2016 WHO criteria[4] and followed up with anagrelide therapy between January 2002 and December 2021 in our center were retrospectively reviewed. Of the 122 patients whose files were evaluated, 33 patients whose JAK2 mutations were examined before treatment and who came to their regular follow-ups were included in the study. In addition to the demographic data of the patients, diagnostic and more recent laboratory values, bone marrow evaluations, and JAK2 mutation status were noted. Bone marrow fibrosis was scored between 0 and 4, with fibrosis four being the most severe. Patients were divided into two groups according to JAK2 mutation existence. The obtained data were compared between the two groups.

Statistical Analysis

The study's statistics were made in SPSS 26.0 (Chicago, IL) for Mac. Continuous variables are given with a mean, standard error, median, minimum and maximum values, while categorical variables are given numbers and percentages. The Shapiro Wilk test was used to determine the normal distribution. Independent-samples t-test was used to compare groups since it was observed to be suitable for normal distribution. Fisher's exact chi-square test was used for intergroup comparisons of categorical variables. $p<0.05$ was considered statistically significant in the study.

Results

Thirty-three patients (male/female: 20/13) who were treated with Anagrelide between 2002-2022 with ET diagnosis in our center were included in the study. The mean age of the patients was 48.96±11.94 years. It was observed that 14 (42%) of the patients were positive for JAK2 mutation. There was no significant difference between the groups regarding age at diagnosis, gender, duration of anagrelide use, and bone marrow fibrosis levels. When the laboratory tests at the time of diagnosis were compared, it was observed that the leukocyte count was significantly higher in the JAK2 positive group (11,940±3,524 vs. 9,313±2,737/mm^3, p=0.024). Although the platelet count was lower in JAK2 positive patients at the time of diagnosis, the difference could not reach statistical significance (879,285±272,731 vs. 1,030,021±424,448/mm^3, p=0.254). It was noted that other parameters were similar.

In the comparison of the data at the last control visit, it was determined that JAK2 positive patients' leukocyte (10,209±2,570 vs. 8,026±1,905/mm^3, p=0.010), neutrophils (6,626±1,977 vs. 5,248±1,356/mm^3, p=0.029) and hemoglobin (13.28±1.55 vs. 11.66±2.22 g/dL, p=0.027) levels were significantly higher, and LDH (216±28.55 vs. 281±95.22 U/L, p=0.049) levels were significantly lower. Although a lesser decrease was observed in the decrease in platelet count with

anagrelide treatment in the JAK2 positive group, the difference between the groups did not reach statistical significance. The number of patients whose bone marrow biopsy was available at diagnosis was 16 (48%). There was no significant difference between the groups regarding the degree of fibrosis (*Table 1*).

Table 1. Comparison of the data according to JAK2 mutation status.

	JAK2 negative (n=19)	JAK2 positive (n=14)	p value
Gender (male/female)	11/8	9/5	0.710
Age (year)	48.05±10.94	50.21±13.51	0.615
Age at onset (year)	38.58±11.12	41.07±11.97	0.542
Anagrelide duration (months)	99.79±69.07	105.21±67.55	0.823
Bone marrow fibrosis degree (n=16)	2.11±0.78	1.71±0.49	0.261
Laboratory values at the onset			
Anagrelide dose (mg)	1.48±0.6	1.23±0.34	0.208
Leukocyte (/mm^3)	9,313±2,737	11,940±3,524	0.024
Neutrophil (/mm^3)	6,388±2,851	8,115±3,699	0.299
Hemoglobin (g/dL)	12.66±2.01	13.83±2.26	0.128
Platelet (/mm^3)	1,030,021±424,448	879,285±272,731	0.254
MPV (fL)	11.96±17.16	8.4±1.64	0.464
BUN (mg/dL)	29.58±8.64	35.11±9.47	0.180
Creatinine (mg/dL)	0.79±0.16	0.82±0.16	0.662
AST (U/L)	23.92±13.09	20.33±8.26	0.481
ALT (U/L)	31.75±38.35	16.89±11.58	0.277
LDH (U/L)	265.57±103.98	247.75±59.17	0.763
Recent laboratory values			
Anagrelide dose (mg)	1.83±0.82	1.92±1.12	0.798
Leukocyte (/mm^3)	8,026±1,905	10,209±2,570	0.010
Neutrophil (/mm^3)	5,248±1,356	6.626±1,977	0.029
Hemoglobin (g/dL)	11.66±2.22	13.28±1.55	0.027
Platelet (/mm^3)	472,277±169,382	507,500±176,120	0.571
MPV (fL)	7.94±1.25	7.77±1.12	0.718
BUN (mg/dL)	35.73±15.16	38.83±28.88	0.722
Creatinine (mg/dL)	0.9±0.28	8.85±27.45	0.271
AST (U/L)	19.57±5.89	24.5±32.75	0.584
ALT (U/L)	20.4±9.88	18.75±22.42	0.800
LDH (U/L)	281±95.22	216±28.55	0.049
Amount of platelet reduction with anagrelide (ΔPLT)	536,577±417,074	371,785±253,847	0.203

MPV: mean platelet volume, BUN: blood urea nitrogen, AST: aspartate aminotransaminase, ALT: alanine aminotransaminase, LDH: lactate dehydrogenase.

Discussion

Our study observed that the JAK2 mutation did not cause a significant difference in terms of age, gender, age at diagnosis, and bone marrow fibrosis, and the effect on myeloid, erythroid, megakaryocytic series was similar at the time of diagnosis. After anagrelide treatment, patients with JAK2 mutation were found to have a significantly higher platelet count and lower LDH levels.

Although the frequency of JAK2 mutations in ET patients was found to be 60% in the literature, JAK2 mutation was found to be positive in 42% of the patients in our study.[5] Rumi et al.[6] reported that they observed a male/female ratio of 167/299 among 466 JAK2 patients and the median age at diagnosis of 50 (15-92). Our current study observed that the JAK2 positive male/female ratio was 9/5 in favor of the male gender, and the median age at diagnosis was 42 (20-59). This may be related to the relatively lower number of patients and the younger patients in the patient group who started on Anagrelide. Younger median age at diagnosis may also be associated with switching to Anagrelide rather than Hydroxyurea at more youthful generations for the concern of leukemogenic effects.

It has been reported that 10-15% of patients with ET and PV progressed to myelofibrosis within a median of 15 years.[7] In our study, no transformation into myelofibrosis was observed in accordance with laboratory data after approximately eight years of follow-up. Among the patients whose bone marrow biopsy was performed at the time of diagnosis, patients with positive JAK2 mutations had lower fibrosis scores, but it was determined that the difference could not reach statistical significance.

Since the JAK2 mutation is a mutation that provides a function (spontaneously active molecule), a significant increase can be expected in all three series.[8]

Conclusions

Our study observed that all three sequences were similar to those without JAK2 mutation in the diagnostic tests and hemogram parameters. After approximately eight years of anagrelide treatment, a more significant decrease in cell counts in the myeloid and erythroid series was observed with anagrelide treatment in those with negative JAK2 mutations. LDH levels typically increase with cellular destruction. Lower LDH levels with lower cellular count change after anagrelide treatment may suggest that JAK2 mutation should play a role in resistance to cytoreduction. Studies with more patients would be necessary to prove this hypothesis.

Acknowledgment

This study has been presented in 18th Uludag Internal Medicine National Winter Congress, 7th Bursa Family Medicine Association National Congress, 12th Uludag Internal Medicine Nursing Congress, 3-6 March 2022, Bursa, Turkey.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contribution

Study Conception: FO, TGK; Study Design: TGK, FO; Supervision: FO, VO; Data Collection and/or Processing: TGK, TE, SE; Statistical Analysis and/or Data Interpretation: TGK, FO; Literature Review: TGK; Manuscript Preparation: TGK; and Critical Review: FO, VO, TE.

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