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The Clinical Course and Prognosis of Patients With Essential Thrombocythemia Treated With Hydroxyurea and Low-Dose Aspirin in an 11-Year Follow-Up

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Abstract

Background: The main objective of this study was to determine the clinical course and prognosis in patients with essential thrombocythemia, treated with hydroxyurea and low-dose aspirin, and complications of treatment in a 12-year follow-up with a precise and regular control.

Methods: In this study, 76 patients with high-risk essential thrombocytosis were retrospectively studied. Data obtained from initial examinations, sonography, complications, and treatment were analyzed.

Results: The mean follow-up was 66.8 months (minimum, 3 months; maximum, 137 months). The mean age of patients was 46.9 years. The mean platelet count of patients at diagnosis and the last follow-up were 818,000/mm^3 and 422,000/mm^3, respectively. In 60 patients, a gene mutation test for JAK2 V617F was performed and was reported as 36.7% positive. Nine patients had bleeding, two of whom were reported after treatment, and eight cases had thrombosis. Macrocytosis and myelofibrosis were seen in 32 and one cases, respectively. Moreover, no cases of leukemia or malignancy due to cytoreduction therapy were seen. One case of skin lesion was seen.

Conclusion: A rapid diagnosis, accurate and regular control, and timely treatment can improve the quality of life and reduce morbidity and threatening complications. This study showed that complications using aspirin and hydroxyurea treatment are at the minimum level.

Keywords: Essential thrombocythemia; Hydroxyurea; Low-dose aspirin; Clinical course

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Introduction

Essential thrombocythemia (ET) is a clonal stem cell disorder characterized by an unknown cause, in which a multipotential precursor cell is involved. The estimated incidence of ET was between 0.38 and 1.7 per 100,000 per year, and the prevalence in the general population is approximately 30 per 100,000 [1, 2]. It can be diagnosed by the increased number of platelets, more than 450,000/mm^3, and by excluding other etiology thrombocytosis [3]. In routine clinical practice, thrombocytosis is more likely to be reactive than primary. Reactive thrombocytosis may be responsible for more than 85% of cases of thrombocytosis seen in routine clinical practice. The main clinical features of ET are splenomegaly, myelofibrosis with varying degrees in the bone marrow, developing into acute leukemia, and increased risk of thrombotic and bleeding events [4-7]. The risk of venous and arterial thrombosis in patients with ET is thought to be high. These patients are at a greater risk of bleeding that can lead to death, particularly in major surgeries [8]. ET can also lead to spontaneous abortion and the loss of a fetus in the first trimester of pregnancy [9]. Because no special clonal index is available for ET, clinical criteria are provided for the differentiation of ET from other chronic myeloproliferative diseases that may also be manifested with thrombocytosis. The revised diagnostic criteria for ET were proposed in 2005 [10]. In more than 50% of patients with ET, JAK2 V617F gene mutation has been observed [11] and can occur either in a homozygous or heterozygous manner. The homozygous type is less common and can occur in the elderly due to a higher leukocyte count and hematocrit. Therefore, patients who have larger spleens are thought to be at a more significant risk of cardiovascular diseases [12]. The main objective of this study was to determine the clinical course and prognosis of ET in patients admitted to the Mahdieh Clinic, Thalaghani and Imam Reza Hospital in Kermanshah, Iran, from 2005 to 2016. This study also aimed to investigate whether ET complications and problems, particularly fibrosis, thrombosis, acute leukemia, and bleeding would be reduced or not. It is thought, while platelets are kept under 450,000/mm^3, aspirin is taken, and accurate medical monitoring, and regular control can be completed to reduce and prevent complications. Hydroxyurea is a human carcinogen. We examined whether hydroxyurea has an acceptable toxicity profile in a long-term follow-up and whether it increased the risk of malignancy in these patients.

Table 1. Characteristic of High Risk Patient With ET

	High risk	Low risk
Platelet count	Platelet count in excess of $1,500 \times 10^9$/L	Platelet count less than $1,500 \times 10^9$/L
Cardiovascular risk factors: smoking, hypertension and hypercholesterolemia, obese	Yes	No
Markers of hypercoagulability: factor V Leiden and antiphospholipid antibodies	Yes	No
Age	Over 60 years	Under 60 years
Past medical history of thrombosis	Yes	No

Materials and Methods

The standard to diagnose ET is considered as a platelet count over 450,000/mm^3. Reactive thrombocytosis was ruled out and ET was confirmed in the patients using tests and follow-ups. A whole-blood test, a chest X-ray, urinalysis, and sonography of the abdomen and pelvic were carried out for the accuracy of diagnostics to rule out reactive thrombosis. The primary step of evaluation performed to rule out cases of reactive thrombocytosis included: serum iron, total iron-binding capacity, serum ferritin, CRP, ESR, RF test, antinuclear antibody and peripheral blood smear review for presence of macrocytosis or leukoerythroblastic blood picture smear, or abnormal platelet morphology was consistent with clonal thrombocytosis. The second step in evaluating a patient with thrombocytosis was to confirm the diagnosis of ET with the detection of the JAK2 V617F gene mutation, as well as the exclusion of other MPNs with a bone marrow examination, or chronic myeloid leukemia by looking for a BCR/ABL transcript. Furthermore, it is important to identify patients at higher risk of ET due to the odds of complications (Table 1). We investigated only mutation JAK2, but calreticulin and MPL mutations were not performed due to their high costs and lack of funds. Therapy with hydroxyurea at a dose of 1 g daily was initiated to decrease platelet count in high-risk patients. Due to the lack of response to the 1 g/day dose of hydroxyurea, we increased the dose to 1.5 g daily. The dose was adjusted to achieve a level of 450,000/mm^3 platelets gradually. There are a few options to minimize the side effects. For example, protection from sun exposure was advised to decrease the possibility of skin cancer. The patients' response to the cytoreductive therapy was evaluated based on the white blood cell count, platelet count, spleen size, symptoms and complications. Criteria of complete response included: a platelet count less than 400,000/mm^3, a normal spleen size, white blood cells less than or equal to 100,000 and the absence of any signs ET [13]. A partial response was used for patients who had platelets less than 6,000,000/mm^3 or 50% less than the base level (those who did not meet the mentioned criteria for patients with a complete response) [13]. Particularly, in this study, data including age, gender, patients' platelet count, leukocyte count, hemoglobin level, presence or absence of the JAK2 V617F gene mutation, thrombosis or bleeding, the risk of bone marrow fibrosis, the associated symptoms, acute leukemia and other variables were entered into a form and were analyzed and recorded regarding a follow-up examination which occurred every 3 months. The development of secondary malignancies was monitored.

Results

Of the total of 402 patients, 107 had ET and 295 had reactive thrombocytosis. Median follow-up was 66.8 months. Thirty-one patients were low-risk patients. The total number of eligible patients elected as candidates for cytoreductive therapy was 76, who all displayed a high risk for ET, so they were studied retrospectively. Six patients who were enrolled after the date of March 21, 2015 were excluded. The minimum follow-up time in the 70 remaining patients was 16 months until July 2, 2016. Twenty-two patients were male, and 54 patients were female. The age range of patients was 20 - 75 years, 26 patients were younger than 40 years, 26 patients were 40 - 60 years old and 24 patients were older. The mean age of patients

Table 2. Frequency of Co-Morbidities and the Main Clinical Presentation of ET in Patients With ET

	Frequency	Percent
Co-morbidities		
Hypertension	15	19.7
Diabetes	1	1.3
Hypertension+ diabetes	1	1.3
Hypothyroidism	3	3.9
Portal hypertension	1	1.3
COPD	2	2.6
No second disease	53	69.7
Total	76	100.0
The main clinical presentation of ET		
Refer with thrombocytosis	40	52.6
Vertigo and headache	14	18.4
Hemorrhage	7	9.2
Thrombosis	6	7.9
Itching	3	3.9
Other (include functional symptom)	6	7.9
Total	76	100.0

Table 3. Laboratory Findings at Diagnosis

	No. of patients	Percent
Platelets ($\times 10^9$/L) The mean platelets 818,000		
450,000 - 1,000,000/μL	61	80.3
> 1,500,000/μL	15	19.7
Leukocytes The mean white blood cells (9,300)		
> 4,500/μL	3	3.9
4,500 - 11,000/μL	59	77.6
> 11,000/μL	14	18.4
Hemoglobin (g/dL) Male: 13 - 16 g/dL Female: 12 - 15 g/dL		
< normal	20	26.3
Normal	53	69.7
> normal	3	3.9
Spleen	10	13.2
> 135 mm		
< 135 mm	66	86.8

was 46.9 years, the mean age of men was 61.7, and the mean age of women was 40.9 years. The patients' chief complaint and frequency of comorbidities are shown in Tables 2 and 3. Four patients reported weight loss, three chest pain, and five bone pain as the associated symptoms which were recorded as incidental findings. The portion the functional symptoms include vasomotor disturbance, dizziness, headache and visual disorders, all of which were manifested in some patients at diagnosis of ET. According to the sonography results, the spleen size of patients was recorded in their documents. Among 76 studied patients, 66 patients had a spleen size less than 135 mm and 10 patients had a spleen size larger than 135 mm. The average size of the spleen was 123 mm. The counts of white blood cells, platelets, and the concentration of hemoglobin are shown in Table 3. CRP test was also carried out for all patients, whereby 12 cases were positive and 64 cases were negative. In this study, nine patients had bleeding, of whom seven cases occurred before presenting to the clinic, and two cases occurred during the therapy. The most common bleeding reported was gastrointestinal bleeding. Among the 76 studied patients, 68 patients did not mention any history of thrombosis. Table 4 presents the frequency of thrombosis in different areas. The JAK2 V617F gene mutation test was done for 60 patients to help for definitive diagnosis. Among 60 patients, 38 were negative, and 22 were positive. RT-PCR JAK2 mutation positive was heterozygote, and the only patient was homozygote. In this study, the response to the therapy according to the patient's records was also evaluated. For this reason, the blood platelets were recorded in the last blood test. Among patients, 54 patients had a platelet count less than 450,000/mm^3, 21 patients had a platelet count of 450,000 - 1,000,000/mm^3, and one patient had a platelet count over a 1,000,000/mm^3. The mean platelet count

Table 4. Frequency of Thrombosis in Different Areas

Thrombosis	Frequency	Percent
No thrombosis	68	89.5
Portal vein	2	2.6
Lung embolism	1	1.3
Brain	5	6.5
Total	76	100.0

in the last follow-up was 422,000/mm^3. Furthermore, one patient after 6 years of follow-up had myelofibrosis, while there were no patients with leukemia or other malignancies related to treatment in our follow-up. The primary toxicity observed in our patients was mild and reversible (Table 5). Usually, mild neutropenia and thrombocytopenia were observed. No renal or hepatic toxicity was found based on serum creatinine, whereby the liver function test measurement was noted. The skin changes were mild. Skin and subcutaneous tissue disorders including maculopapular rash, hyperpigmentation, and atrophies of skin were observed in only one patient. One of the most important complications reported was macrocytosis, which was observed in 32 patients. In this study, most of the patients did not experience all of the side effects. Side effects were almost always reversible but were not reversible for one patient with skin side effects. Hence, the drug was discontinued.

Discussion

From the analysis of the results, the differences and similarities with other studies can be found. A limitation of this study was its small sample size. This study revealed the mean white cell count was 9,300/mm^3, which is consistent with the results of Vannucci [12] (9,000/mm^3) and Passamonti study [14] (8,900/mm^3). The hemoglobin level was evaluated and described considering the normal hemoglobin level as 12 - 15 g/L in women and 13 - 16 g/L in men. Among 76 studied patients, 20 patients had a below normal hemoglobin level (anemia) and at follow-up 26 patients for anemia. The more likely anemia of chronic disease caused by ET was considered, 53 patients had a normal hemoglobin level, and three patients had a hemoglobin level higher than the normal level (erythrocytosis). In Fenaux's study [15], without gender segregation, 9% of the patients had a hemoglobin level less than 12 g/L, 91% had 12 - 16 g/L, and none of the patients had a hemoglobin level over 16 g/L. The mean hemoglobin level was 13.2 g/L, which is reported as 14 and 14.2 g/L in the Vannucci and Pasamounti studies, respectively. The difference can be due to the low hemoglobin level in Iran compared with other countries, owing to the Iranian nutritional habits. Likewise, the CRP test was reported as negative for 64 patients. If interleukin (IL) levels increased, the level of CRP was also found to be increased. Measurement of the CRP level may be used as a substitute for measurement of IL-6. An increase in the level of IL-6 is rare in uncomplicated in ET and this is seen in reactive thrombocytosis [16]. Repeatedly low levels of both IL-6 and CRP are most consistent with clonal thrombocytosis. In patients with ET, the elevated CRP levels

Table 5. Toxicity in Our Patients

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4
Palmar-plantar erythrodysesthesia syndrome*	1	1	0	-
Skin hyperpigmentation**	1	0	-	-
Leukocytes (total WBC), mL***	21	0	0	0
Platelets, $\times 10^3$/mL****	11	1	0	0
Hb, g/dL******	26	0	0	0
Febrile neutropenia******	0	0	0	0
Hepatic toxicity	0	0	0	0
Renal toxicity	0	0	0	0
Leukemia secondary to oncology hemotherapy	-	-	-	0

*Grade 1: minimal skin changes or dermatitis (e.g., erythema, edema, or hyperkeratosis) without pain. Grade 2: skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting instrumental ADL. Grade 3: severe skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting self care ADL. **Grade 1: hyperpigmentation covering < 10% BSA; no psychosocial impact. Grade 2: hyperpigmentation covering > 10% BSA; associated psychosocial impact. ***Grade 1 :< LLN - 3,000/mm^3; Grade 2: > 2000 - < 3000/mm^3; Grade 3: > 1,000 - < 2,000/mm^3; Grade 4: < 1,000/mm^3. ****Grade 1: < LLN - 75,000/mm^3; Grade 2: > 50,000 - < 75,000/mm^3; Grade 3: > 10,000 - <50,000/mm^3; Grade 4: < 10,000/mm^3. *****Hemoglobin (Hgb). Grade 1: < LLN - 10.0 g/dL; Grade 2: 8.0 - <10.0 g/dL; Grade 3: 6.5 - < 8.0 g/dL; Grade 4: < 6.5 g/dL. ******ANC < 1,000/mm^3 with a single temperature of > 38.3 °C (101 °F) or a sustained temperature of ≥ 38 °C (100.4 °F) for more than 1 h. Life-threatening consequences; urgent intervention indicated.

have been described with *in vivo* leukocytes, platelets and endothelial cells activation [17]. Chronic inflammation triggers *in vivo* activation of platelets, leukocytes, and endothelial cells and the activation of platelets by inflammatory triggers have major importance in coagulation and thrombus formation [18]. Barbui et al [16] showed CRP is a high sensitivity test and major thrombosis rate was higher in the highest CRP tertile. In the study by Barbui et al, levels of hsCRP also correlated significantly with the JAK2 V617F allele burden. The JAK2 V617F gene mutation test was done for 60 patients to help for definitive diagnosis. Among 60 out of 76 patients tested, 38 (63.3%) were negative and 22 were positive. The difference can be due to the different genetics of Iranian people compared with other countries. RT-PCR JAK2 (V617FC 1849G>T) mutation positive was heterozygote in 21 patients and only one patient was a homozygote. In Alshomari's study [19], 73% of the patients were carriers and in Lippert's [20] study, 75% of the patients had the gene. In this study, the blood test results were used to determine the base platelet count. Among 76 studied patients, 17% patients had a platelet count over 1 million; in Pasamounti's study, 211 patients (34.9%) had a platelet count over 1 million. The mean platelet count was 818,000/mm^3, which is consistent with Pasamounti's (811,000/mm^3), Lippert's [20] (812,000/mm^3), and Vannucci's (848,000/mm^3) studies. To assess the response to the therapy, the platelet count of patients was recorded in the last blood test. Fifty-three patients had a platelet count less than 450,000/mm^3, 22 patients had a platelet count between 450,000 and 1,000,000/mm^3, and one patient had a platelet count over 1 million/mm^3. The mean platelet count was 422,000/mm^3 in the last follow-up. The spleen size was recorded in the patients' records. Among 76 studied patients, 66 patients had a spleen size less than 135 mm and 10 patients had a spleen size less 135 mm or larger. In the Vannucci's study, 2% of the patients had a spleen size larger than 150 mm and for the heterozygote patients, regarding the JAK2 V617F gene, had a spleen size larger than other patients. The difference can be due to 150 mm as criteria. Also, in Passamonti's study, splenomegaly was observed in 10.7% of the patients. In this study, the mean spleen size of patients was 123 mm. There was a homozygote patient with a large spleen size, which can be due to portal hypertension. In Passamonti's study, mylofibrosis was reported as 3.5% with the 10 years' risk. Leukemia also occurred in 14 patients, 11 years after the diagnosis of ET, and the 10 years' risk of leukemia was 2.6%. In Fenaux's study, one patient had leukemia. In our study, no case of leukemia was observed in a 10-year follow-up. One patient had mylofibrosis. Macrocytosis was one of the most important complications of hydroxyurea, which was observed in 32 patients. The recommended dose of folic acid is 400 - 800 μg/day; the same recommendation for the prevention of methotrexate-induced side effects for this patient was administrated. In Passamonti's study, 66 patients had a risk of 14% for thrombosis with a 10-year risk. Seventeen patients (3.5%) had progression to myelofibrosis with a 10-year risk. Leukemia occurred in 14 patients with a mean time of 11 years after the diagnosis of ET, and the 10-year risk of developing leukemia was 2.6%. Sixty-four patients died during the study [14]. Compared to the Passamonti study, thrombosis occurred with minimal range and no patient progressed to leukemia. Therapeutic use of cytotoxic drugs had no risk for developing leukemia for our patients and no mortality was observed due to the disease. In Fenaux's [15] study, the underlying characteristics, treatment, and clinical course of 147 patients with ET were studied retrospectively. Twenty-seven patients (18.4%) had large vein thrombosis and 27 patients (18.4%) had bleeding disorders, while only seven patients (4.8%) had both bleeding and thrombotic disorders. During the follow-up period, 14 treated patients and two untreated patients had one or more major thrombotic events, while there was only one major bleed-

ing. The average 7-year survival of the patients was 73%. Only one patient had acute non-lymphocytic leukemia (ANLL) [15]; compared with this study, bleeding problems were less severe and the thrombotic events were lower, as well. Fynazy [21], for instance, studied 1,104 patients with essential thrombocytosis to investigate the risk factors and the incidence of bleeding in these patients. Fifty-five patients (6%) had extensive bleeding during the study, 1.39% of the patients in a year, for which the problems were less intense compared with our study. In our study, the patients were divided into three groups according to the obtained results including 53 controlled patients with complete response, 22 patients with partial response or control, and one uncontrolled patient. In fact, we tried to set platelet levels at a desirable level to prevent complications and reduce clinical signs by more accurate and regular controls and by changing the dose of hydroxyurea. In our study, after a median follow-up of 66.8 months, no patients developed a second cancer during hydroxyurea therapy. Overall survival in our patients was 44 months and no mortality was reported in the follow-up. The incidence of second malignancies during hydroxyurea therapy in other studies was significantly higher compared with our study [22-24]. One reason for the low incidence of secondary cancer is the study by Lanzkron et al [25]. We suggest that hydroxyurea and aspirin, if administered accurately and regularly, can reduce symptoms in high-risk patients. The increased dose of hydroxyurea can be administrated to set the appropriate level of platelets up to 2 g, without worrying about toxicity in patients. There is a very low risk of developing leukemia or skin cancer due to hydroxyurea therapy, which can occur many years after treatment.

Conclusions

However, larger studies of patients treated with hydroxyurea should be performed in order to detect the real incidence of second malignancies.

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Conflicts of Interest

All the authors have reported no conflicts of interest.

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