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Original Article

Analysis of patients with erythrocytosis in a single center: comparison between polycythemia vera and non-polycythemia vera

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Abstract: We analyzed the clinical features and complications of patients with erythrocytosis, to find differences according to subtypes. The data of patients with erythrocytosis, who were diagnosed at Hallym Medical Center between January 2007 and March 2014, were retrospectively reviewed. A total of 89 erythrocytosis patients, 38 with polycythemia vera (PV) and 51 with non-PV, were enrolled. There were significant differences between the groups in terms of disease prevalence, age, sex, smoking history, body mass index, cell counts, LAP score, erythropoietin, and total cholesterol. Among 38 patients who were diagnosed as PV, both bone marrow examinations and *JAK2* mutation analysis were performed in 32 patients. The remaining PV patients were evaluated with a genetic analysis only (n=3) or marrow examinations only (n=3). Treatments for erythrocytosis were chosen in the following order: phlebotomy, hydroxyurea, anti-platelet agents, and anti-coagulants. The erythrocytosis was less controlled in non-PV patients, despite the absence of a significant difference in complications between the 2 groups. In conclusion, a thorough clinical and laboratory work-up is essential for diagnosing erythrocytosis and for the selection of appropriate further exams and treatment option.

Keywords: Polycythemia vera, secondary erythrocytosis, thrombosis, complication

Introduction

Erythrocytosis is a condition in which a person's red blood cell count is elevated. Counts must be at least 125% of the normal range according to sex and body mass. This condition is suspected when the hemoglobin levels and packed cell volumes are above 18.5 g/dL and 0.52, respectively, in men, or above 16.5 g/dL and 0.48, respectively, in women [1]. Erythrocytosis is classified into 1) primary erythrocytosis consisting of an acquired form, known as polycythemia vera (PV) and congenital erythrocytosis, and 2) secondary erythrocytosis reacting to hypoxemia or excessive erythropoietin stimulus, and 3) idiopathic erythrocytosis.

The prevalence of PV is 44-57 per 100,000 in the United States [2], and 3 per 100,000 in Korea, according to the Korean Health Insurance Review and Assessment Service [3]. The prevalence of secondary erythrocytosis is believed to be higher than that of the primary form, but this is difficult to quantify because of the paucity of data and the variety of causes of secondary erythrocytosis. Idiopathic erythrocytosis is currently applied in clinical practice without precise criteria. In most cases, patients are diagnosed with the idiopathic form if they cannot be classified into one of the other 2 categories. According to a study of evaluating the isolated erythrocytosis by red cell mass (RCM) measurement, 49% of patients with apparent erythrocytosis were diagnosed with secondary erythrocytosis, 13% with PV, and 31% with the idiopathic form [4]. Despite the discovery of *Janus Kinase 2* (*JAK2*) gene-related abnormalities in myeloproliferative neoplasms (MPN), further research is needed in identifying the different causes of erythrocytosis, especially with regard to the idiopathic form. A thorough analysis of the clinical features of erythrocytosis, not

Table 1. Patients' characteristics

	PV (n=38)	Non-PV (n=51)	*P*-value
Age, median (range)	61 (19-79)	45 (19-92)	< 0.0001
Gender (male/female)	14/24	48/3	0.002
Main symptoms			0.233
Asymptomatic	9	17	
Headache	3	2	
Dizziness	1	3	
Chest tightness	0	3	
Facial flushing	3	1	
Fatigue	1	0	
Weakness	2	0	
Blurred vision	0	1	
Snoring	0	3	
Sleep apneic events	0	1	
Abdominal pain/discomfort	0	1	
Erythromelalgia	0	1	
Other symptoms	7	8	
Multiple symptoms	8	3	
Past medical history			0.460
None	7	11	
Hypertension	10	6	
Diabetes mellitus	3	2	
Hepatic disease	0	3	
Renal disease	1	0	
Acquired heart disease	0	1	
Congenital heart disease	0	1	
Chronic pulmonary disease	0	1	
Benign solid tumor	1	0	
Malignant solid tumor	1	0	
Others	3	5	
More than 1 disease	11	20	
Smoking			< 0.0001
Non-smoker	22	14	
Current smoker	4	23	
Ex-smoker	4	11	

Abbreviations: PV, polycythemia vera.

just for PV but for all forms, is crucial for choosing the right candidates to be investigated.

In Korea, there had been few studies about clinical characteristics of erythrocytosis, if any, they were published before recognizing *JAK2* mutation [5-7]. Furthermore, very few reports about the clinical features of erythrocytosis-related complications, especially vascular events, have been published [8, 9]. Herein, we analyzed the clinical features, including complications, of patients with erythrocytosis who were diagnosed with the PV form (according to the WHO criteria of MPN in 2008 [10]) and other forms of the disease.

Materials and methods

Study design

All participants with documented hemoglobin levels > 17 g/dL (males) or > 16 g/dL (females) or hematocrit values > 0.52 (males) or 0.48 (females) on at least 2 separate exams at the Hallym University Medical Center between January 2007 and March 2014 were included in this retrospective study. This study protocol was approved by the Committee for the Protection of Human Subjects of this institution and was conducted in accordance with the principles of the Declaration of Helsinki.

The electronic medical records for each participant were reviewed to gather information about the epidemiologic characteristics, the results of diagnostic work-up, especially *JAK2* gene mutation or bone marrow studies, erythrocytosis risk factors, treatments received for erythrocytosis, and erythrocytosis-related complications. In terms of risk factors of erythrocytosis, BMI, underlying comorbid conditions, such as chronic obstructive pulmonary disease or cardiac disease, and history about smoking and occupation. In order to be adjudicated as a cerebrovascular disease, we required documentation of deep vein thrombosis, pulmonary embolism, or other hemorrhagic disease events in the medical record (progress notes, history, and physical or discharge summaries), with the diagnosis confirmed by an appropriate diagnostic test (e.g., lower extremity venous duplex studies, computed tomography of the chest, pulmonary angiography, or ventilation-perfusion scanning).

Table 2. Laboratory findings (mean value with standard deviation)

	PV	Non-PV	Total	*P*-value
BMI	22.6 ± 2.9	26.7 ± 3.8	24.7 ± 3.9	< 0.0001
Hemoglobin	17.9 ± 3.3	18.7 ± 1.3	18.4 ± 2.4	0.141
RBC counts	6.9 ± 1.4	5.9 ± 0.7	6.3 ± 1.1	< 0.0001
Platelets	664.2 ± 372.0	217.3 ± 70.3	408.1 ± 332.3	< 0.0001
WBC counts	15094 ± 7571	7644 ± 3123	10825 ± 6586	< 0.0001
LAP score	130.3 ± 69.3	46.5 ± 22.9	77.2 ± 60.6	< 0.0001
Erythropoietin	6.6 ± 6.6	11.0 ± 6.2	9.3 ± 7.0	0.005
GFR	79.5 ± 23.5	87.6 ± 27.3	84.2 ± 25.9	0.156
Uric acid	5.8 ± 1.6	6.7 ± 1.9	6.4 ± 1.9	0.03
LDH	420.4 ± 242.1	403.7 ± 868.1	411.0 ± 667.1	0.912
Total cholesterol	157.4 ± 32.7	198.4 ± 47.0	180.8 ± 46.0	< 0.0001
Triglyceride	131.0 ± 58.2	249.9 ± 199.3	205.9 ± 171.2	0.012
LDL-cholesterol	88.9 ± 31.7	116.6 ± 41.5	107.2 ± 40.1	0.026
HDL-cholesterol	43.2 ± 17.5	45.9 ± 14.4	44.9 ± 15.5	0.545

Abbreviations: PV, polycythemia vera; BMI, body mass index; RBC, red blood cell; WBC, white blood cell; LAP, leukocyte alkaline phosphatase; GFR, glomerular filtration rate; LDH, lactate dehydrogenase; LDL. Low-density lipid; HDL, high-density lipid.

Table 3. Diagnostic role of *JAK2* mutation and bone marrow exam in patients with polycythemia vera (n=38)

		Bone marrow exam			
		Done	Not done	Inadequate	Total
JAK2 mutation analysis	Done	30	0	2	35
	No mutation	5	0	0	5
	V617F mutation	16	0	2	18
	Exon 12 mutation	0	0	0	0
	GT heterozygote	7	2	0	9
	TT homozygote	2	1	0	3
	Not done	3	0	0	3
	Total	33	3	2	38

Statistical analysis

Clinical characteristics of participants were analyzed using chi-square and t-test analysis to evaluate the significant differences between PV and non-PV patients. Statistical significance was reached when $P < 0.05$.

Results

Patient characteristics

A total of 89 patients with erythrocytosis met the criteria for enrollment in this study. Patient characteristics are summarized in **Table 1**. Thirty-eight (43%) were diagnosed with PV, with a female predominance (63%). Of the remaining 51 non-PV patients, most patients (94%) were male. Median ages of PV and non-PV patients were 61 and 45 years, respectively ($P < 0.0001$). The major accompanying symptoms in PV patients were headache and facial flushing. In the non-PV group, patients complained of snoring, dizziness, and tightness of the chest. Asymptomatic patients accounted for 24% and 33% of PV and non-PV patients, respectively.

Although there were no remarkable differences between 2 groups in terms of past medical history, the proportion of patients with a past or current history of smoking was higher in the non-PV group compared to the PV group (73% versus 42%, $P < 0.0001$).

Comparison of laboratory findings of the 2 groups revealed some differences (**Table 2**). Although the mean value of serum hemoglobin of the PV group was slightly lower than that of the non-PV group (17.9 g/dL vs. 18.7 g/dL, P=0.141), mean red blood cell count (6.85×10^6/μL vs. 5.90×10^6/μL, $P < 0.0001$) of the PV group was significantly greater than that of the non-PV group. Mean white blood cell count (15,094/μL vs. 7,644/μL, $P < 0.0001$) and platelet count (664×10^3/μL vs. 217×10^3/μL, $P < 0.0001$) of the PV group was also significantly greater than that of the non-PV group patients. Otherwise mean erythropoietin level (6.6 mIU/mL vs. 11.0 mIU/mL, P=0.005) and total cholesterol (157 mg/dL vs. 198 mg/dL, $P < 0.0001$) of the PV group was significantly lower than that of the non-PV group.

Diagnosis of erythrocytosis

A total of 38 patients met the WHO diagnostic criteria for PV published in 2008. For the diagnosis, a bone marrow examination was per-

Table 4. Treatment of erythrocytosis

	PV	Non-PV	Total	*p*-value
Phlebotomy				
Yes	27	14	41	< 0.0001
No	10	37	47	
Hydroxyurea	31	0	31	< 0.0001
Interferon-alpha	0	0	0	-
Anti-platelet agents				
Yes	29	13	42	< 0.0001
Aspirin	29	10	39	
Plavix	0	3	3	
No	8	36	44	
Anti-coagulants				
Yes	2	4	6	0.304
No	34	47	81	

Abbreviations: PV, polycythemia vera.

Table 5. Clinical courses

	PV	Non-PV	Total	*p*-value
Complications				0.078
None	32	36	68	
Cerebrovascular disease	2	3	5	
Deep vein thrombosis	1	0	1	
Hemorrhagic disease	1	0	1	
Others	0	2	2	
Unknown	2	10	12	
Improving erythrocytosis				0.001
Yes	30	23	53	
No	5	26	31	
Unknown	3	2	5	

Abbreviations: PV, polycythemia vera.

formed in 35 patients and a test for *JAK2* mutation was done in 30 patients (**Table 3**). Of 30 patients who had a *JAK2* mutation test, a V617F mutation was observed in 16 patients, a *GT* heterozygote in 7 patients, and *TT* heterozygote in 2 patients. Five patients had no *JAK2* mutation.

Treatment and clinical courses

Erythrocytosis treatment included phlebotomy, hydroxyurea, anti-platelet agents, or anti-coagulants (**Table 4**). Phlebotomy was used in 27 PV patients and 14 non-PV patients. Hydroxyurea was prescribed to 31 PV patients and no non-PV patients. Antiplatelet agents such as aspirin and Plavix were used in 29 PV patients and 13 non-PV patients, while a very small number of patients in each group received anti-coagulation therapy.

There was no significant difference between these groups in terms of the development of complications during follow-up (**Table 5**). Cerebrovascular disease occurred in 2 PV patients and 3 non-PV patients, 1 PV patient developed deep vein thrombosis, and finally a hemorrhagic event was observed in 1 PV patient.

Discussion

In the current study, we retrospectively analyzed the clinical and laboratory data of 89 patients with erythrocytosis at our institution. Considerable changes have been made in the diagnostic criteria used for PV. The older criteria according to the Polycythemia Vera Study Group (PVSG) consisted of 3 major factors (total RBC volume, arterial oxygen saturation, and presence of splenomegaly) and 4 minor factors (thrombocytosis, leukocytosis, elevated LAP, and elevated serum vitamin B12 level). The current criteria come from the WHO classification, which adopted a presence of *JAK2* mutation, bone marrow findings, serum EPO level and other diagnostic elements. The latter criteria put emphasis on the *JAK2* mutation as a clonal marker and on the importance of precise pathologic findings on bone marrow exams. This study based on the WHO criteria for the diagnosis of PV showed significant differences were observed in the median age, the distribution of sex and smoking status between PV and non-PV groups. With respect to the measurements, we also observed significant differences of the mean value of BMI, RBC counts, platelets, WBC counts, LAP score, erythropoietin, and total cholesterol between the 2 groups. These results are similar to those of previous studies published before the publication of WHO criteria in 2008 [5-7]. We presumed that the causes of erythrocytosis affected the clinico-laboratory features of the patients. Furthermore, we can suggest that the candidates for *JAK2* mutational tests and/or bone marrow examinations could be determined by taking thorough medical history and running an adequate number of laboratory tests.

As mentioned above, discovery of *JAK2* V617F and exon 12 mutations enabled a more precise

diagnosis and adequate therapeutic approaches as well as a better understanding of the pathophysiology of MPNs including PV. Furthermore, it changed how physicians in clinical practice approach patient treatment. Frequency of a test for the analysis of *JAK2* mutations, one of the essential components of diagnostic criteria for PV, had increased across the world, including in Korea. In comparison, the bone marrow examination, which is still an important diagnostic test for PV, is less recommended than prepared with in pre-WHO criteria era. Actually, many guidelines about PV recommend that patients harboring the *JAK2* mutated gene mutated do not necessarily require an invasive, stressful work-up such as this one. In the present study, 30 patients had both marrow examinations and *JAK2* mutational analysis for the diagnosis of PV. Among 33 patients with PV who underwent adequate marrow examination, 5 patients had no genetic mutations and in 3 patients, *JAK2* mutational status was not evaluated. In comparison, 3 patients were assessed for the *JAK2* genetic mutation without undergoing marrow examinations. The discrepancies between the guidelines and the clinical practice are related to the accuracy of genetic analysis, the physician's preference, and the patient's aversion to genetic analysis.

There are many therapeutic approaches for erythrocytosis, including phlebotomy, cytoreductive chemotherapy, interferon-alpha, antiplatelet agents and anti-coagulants. Phlebotomy is one of the most commonly used 'erythrocytosis' controlling modalities, and in the present study, the number of phlebotomy-treated patients in the PV group was significantly higher than that of the non-PV group. In terms of anti-platelet agents, the majority of both PV and non-PV patients received aspirin and not Plavix. Furthermore, patients in the PV group were more commonly treated with antiplatelet agents than in the non-PV group. These findings can be explained by physician preferences and the differences of red blood cell counts between 2 groups. In terms of anticoagulants, there were no significant differences in the number of treated patients between PV and non-PV groups. We assumed that the underlying, co-incidental diseases of non-PV patients, such as coronary artery diseases and cerebrovascular diseases, need the use of anti-coagulants.

The clinical course of PV is characterized by 3 main complications: vascular complications, an evolution to myelofibrosis, and transformation to acute myeloid leukemia [11, 12]. Patients with PV have longer survival times than those of patients with other hematologic malignancies, but only if their red and white blood cell counts are controlled.

To date we are not aware of any reports that evaluate the incidence of vascular complications, myelofibrosis, and acute myeloid leukemia between PV and non-PV. With respect to thrombosis, in a laboratory study using peripheral blood mononuclear cells (PBMC) of patients with erythrocytosis, the degree to which laboratory markers of coagulation activation are elevated is substantially less in secondary polycythemia than in PV. There was no clear clinical evidence that secondary polycythemia poses an elevated thrombosis risk [8]. One case-control study showed that patients with secondary erythrocytosis did not have elevated venous thromboembolism (VTE) risks compared with control group [9].

In terms of treatment of erythrocytosis, both PV and non-PV patients should be treated aggressively to prevent complications. Furthermore, erythrocytosis owing to non-PV can be improved by addressing underlying factors, such as diet, exercise, and smoking status. In the current study, there were no significant differences in the number of complications between PV and non-PV patients although the erythrocytosis was less improved in non-PV than in PV patients. We assumed that the cause of erythrocytosis were intractable and the compliance of non-PV patients to the non-medical treatments, including weight control, exercise and quitting smoking, was poorer than medical treatment. Therefore, aggressive, thorough therapeutic approaches are necessary for erythrocytosis independent of etiology.

This study has limitations, including the relatively small patient sample size. In addition, none of the enrolled patients underwent Cr-51 Red Cell Mass (RCM) testing for the diagnosis of erythrocytosis because this test is unavailable at our center. Cr-51 RCM is considered a highly precise test because using surrogate markers to measure RCV increases are inadequate, especially in early cases of PV [13]. However, only a limited number of centers have

continued employing this technique despite its diagnostic usefulness. Furthermore, Cr-51 RCM testing is not necessary for men with a hemoglobin > 18.5 g/dL or a hematocrit > 60% and for women with a hemoglobin > 16.5 g/dL or a hematocrit > 56% [13]. Despite these limitations, our study is -to the best of our knowledge- the first study in Korea to evaluate the clinical features of erythrocytosis according to the 2008 WHO criteria for the diagnosis of MPN.

Conclusion

The precise evaluation of the cause of erythrocytosis by means of a thorough medical history and laboratory findings is essential in the diagnostic approach and choice of treatment in clinical practice.

Disclosure of conflict interest

None.

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