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Decreased serum prohepcidin concentration in patients with polycythemia vera*

Justyna KWAPISZ†, Ewa ŻEKANOWSKA, Joanna JASINIEWSKA

(*Department of Pathophysiology, Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Poland*)

†E-mail: justyna.kwapisz@gmail.com

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Abstract: Objective: Iron deficiency is a common complication in patients with polycythemia vera (PV). Hepcidin is a principal regulator of iron homeostasis. The aim of our study was to assess prohepcidin, a hepcidin precursor, and other iron status parameters in the serum of PV patients. Methods: The study was performed in 60 patients (F/M 26/34) aged 38~84 (66±10) years. The control group consisted of 20 healthy volunteers, age and sex matched. The following parameters were determined in blood serum samples: prohepcidin concentration, iron content, unsaturated iron binding capacity (UIBC), total iron binding capacity (TIBC), transferrin saturation (TfS), and concentrations of ferritin and soluble transferrin receptor (sTfR). Results: All PV patients showed significantly lower levels of prohepcidin, higher levels of sTfR and TIBC compared to the control group. 40% of the patients from the study group showed concentrations of ferritin below the normal range and significantly lower levels of serum iron and TfS, and significantly higher levels of sTfR, UIBC and TIBC in comparison with the rest of the study group. In this group of patients, prohepcidin concentrations were significantly lower than those in other patients. Conclusion: The results indicate that PV patients suffer from iron metabolism disorders. The decreased serum level of prohepcidin in PV patients may be a result of iron deficiency.

Key words: Polycythemia vera (PV), Iron metabolism, Prohepcidin, Hepcidin
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INTRODUCTION

Polycythemia vera (PV) is currently classified as one of the myeloproliferative neoplasms (MPNs) and is characterized by marrow hyperplasia with an increased number of erythrocytes as well as leukocytes and platelets in peripheral blood (Tefferi and Vardiman, 2008). Several studies have shown that iron deficiency is common in PV patients and can significantly influence the quality of their life (Hutton, 1979; Pearson *et al*., 1981; Rector *et al*., 1982; Birgegard *et al*., 1984). These complications are a result of expansive erythropoiesis, in addition to phlebotomy and/or bleedings (Hutton, 1979; Pearson *et al*., 1981; Spivak, 2002). There was no data in the last decade documenting iron disorders in PV despite progress in the understanding of iron metabolism and new laboratory techniques measuring iron parameters. For example, prohepcidin (hepcidin prohormone) expression in PV has not been evaluated yet. Hepcidin is a peptide shown to be a key regulator of iron metabolism. It acts by binding and down-regulating the expression of the iron transporter ferroportin. In consequence, hepcidin decreases iron absorption in the intestine and blocks iron release from its stores (Ganz and Nemeth, 2006; Nemeth and Ganz, 2006). Numerous studies have consistently shown that production of hepcidin is down-regulated by anemia and hypoxia (Benedict *et al*., 2007; Ganz *et al*., 2008), which in turn allows an increase in iron store mobilization and an increased absorption from diet, making it bioavailable for erythropoiesis and other

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physiological processes. In the present study, we analyzed prohepcidin and other iron metabolism parameters in PV patients.

MATERIALS AND METHODS

Patients

The study group comprised 60 PV patients (34 males, 26 females). The patients ranged in age from 38 to 84 years old [(66±10) years old]. The diagnosis of PV was based on the Polycythemia Vera Study Group Diagnostic Criteria for Polycythemia Vera and confirmed by trepanobiopsy. All patients were followed in the hematology outpatient clinic. Physical and diagnostic examinations consisting of complete blood cell count, uric acid concentration, and lactate dehydrogenase concentration were performed at regular intervals. The maximum patient follow-up period was 27 years, and the mean observation period was 7.5 years. Every patient from the study group was treated with hydroxyurea (HU) alone or HU with supplemental phlebotomy. The control group was age [(63±11) years old] and sex (10 males, 10 females) matched and consisted of 20 healthy volunteers. The biochemical analyses were performed with blood serum samples. All procedures in the study were approved by the local ethics committee. Informed written consent was obtained after the purpose, nature, and potential risks were explained to the subjects.

Analytical methods

Levels of prohepcidin were determined by a stable enzyme-linked immunosorbent assay (ELISA) (Hepcidin Prohormone ELISA, DRG Instruments GmbH, Germany).

Serum iron concentrations and values of unsaturated iron capacity (UIBC), total iron capacity (TIBC) and transferrin saturation (TfS) were measured on the Architect c800 System (Abbott Laboratories, USA).

Serum ferritin was quantified using the DRG Ferritin kit (EIA-1872, DRG International Inc., USA). Serum soluble transferrin receptor (sTfR) concentrations were examined by an ELISA (the Human sTfR ELISA, BioVendor Laboratory Medicine Inc., Czech Republic).

Data analysis

All statistical analyses were performed using the software Statistica 6.0 (Stat-Soft, Cracow, Poland). Results are presented as mean±standard deviation (SD) when the data demonstrated a normal distribution, or as medians [Q_1 (lower quartile) to Q_3 (upper quartile)] due to their being abnormally skew of the data. An independent sample t test for parametric continuous variables and the Mann Whitney U test for nonparametric continuous variables were used to compare the difference between the two subject groups. The relationships between the examined parameters were determined by Spearman's rank-order correlation. A P value of <0.05 was considered statistically significant.

RESULTS

In this work, we demonstrated that prohepcidin concentrations were decreased in PV patients in comparison with the control subjects ($P<0.05$) (Table 1).

The group of PV patients revealed significantly higher levels of two iron status parameters in comparison with the control group: TIBC ($P<0.05$) and sTfR ($P<0.05$) (Table 1).

Table 1 Iron metabolism parameters in polycythemia vera patients and the control group

	PV patients	Control group	P
No. patients	60	20	
Age (year)	65.63±10.46	63.3±11.05	
Sex, M/F	34/26	10/10	
Parameter*			
Prohepcidin (ng/ml)	93.61 (80.64~105.67)	293.67 (172.81~353.25)	<0.0000001
Serum iron (μg/dl)	85.00 (37.00~116.00)	84.20 (69.70~94.00)	0.811
UIBC (μg/dl)	251.23±113.36	200.35±60.42	0.060
TIBC (μg/dl)	338.65±75.79	285.94±60.61	0.006
TfS (%)	26.90 (8.98~41.37)	28.46 (24.19~38.79)	0.267
Ferritin (ng/ml)	27.99 (5.81~64.62)	35.79 (23.43~73.21)	0.243
sTfR (μg/ml)	2.27 (1.48~4.66)	1.48 (1.07~2.01)	0.008

* Results are presented as mean±SD or medians (Q_1~Q_3)

PV patients and the control group did not differ significantly regarding serum iron, UIBC, TfS and ferritin (Table 1).

In our study we have also observed that 40% of the 60 PV patients showed concentrations of ferritin below the normal range (male: <20 ng/ml, female: <10 ng/ml) concomitant with lower levels of serum iron (P<0.05) and TfS (P<0.05), and significantly higher levels of sTfR (P<0.05), UIBC (P<0.05), and TIBC (P<0.05) in comparison with the rest of the study group. In this group of patients prohepcidin concentrations (P<0.05) were significantly lower than those in other patients (Table 2).

Table 2 Iron metabolism parameters in polycythemia vera patients with ferritin concentration within the normal range, in comparison with polycythemia vera patients with ferritin concentration below the normal range

	PV patients 1	PV patients 2	P
No. patients	36	24	
Age (year)	64.64±10.73	67.92±9.44	
Sex, M/F	23/13	13/11	
Parameter*			
Prohepcidin (ng/ml)	99.26 (85.85~113.05)	88.09 (80.21~97.80)	0.03
Serum iron (μg/dl)	100.50 (68.50~121.50)	33.00 (23.00~82.00)	0.000008
UIBC (μg/dl)	177.36±65.92	362.04±70.69	<0.0000001
TIBC (μg/dl)	288.39±42.88	414.04±45.29	<0.0000001
TfS (%)	34.04 (25.74~47.59)	8.19 (5.14~20.68)	<0.0000001
sTfR (μg/ml)	1.77 (1.04~2.27)	5.15 (2.86~7.52)	0.000002

Group: PV patients 1, PV patients with ferritin concentration within the normal range; PV patients 2, PV patients with ferritin concentration below the normal range. * Results are presented as mean±SD or medians (Q_1~Q_3)

DISCUSSION

In the present work, the concentrations of prohepcidin and other iron status parameters were determined in the serum of PV patients. Our data show that serum prohepcidin concentrations in PV patients were significantly lower than those in the control group. Moreover, prohepcidin concentrations were significantly lower in patients with ferritin concentrations below the normal range in comparison with patients with normal ferritin levels. In our opinion, reduced prohepcidin concentration in PV patients probably represents an adaptive change secondary to iron deficiency and, eventually, to expansion of erythropoiesis. It may be the result of organism compensation, which increases iron absorption in the intestine. This idea has been confirmed in experiments on mice, which demonstrated that anemia induced by phenylhydrazine or phlebotomies triggered a considerable decrease in hepcidin mRNA (Nicolas *et al*., 2002). In addition, some authors claimed that in response to low serum iron level, a soluble form of hemojuvelin (s-HJV) binds to bone morphogenetic proteins (BMPs) and inhibits BMP/SMAD signaling pathway that normally exists as a positive regulator of hepcidin expression (Piperno *et al*., 2009; Fleming, 2008).

Since the discovery of hepcidin in 2000, its role in pathomechanisms of many diseases was studied. For example, Kulaksiz *et al*.(2004) reported significantly lower serum prohepcidin concentrations in hemochromatosis patients than in hemodialysis patients with renal anemia or in healthy volunteers. Shinzato *et al*.(2008) examined hemodialysis patients and showed that patients with iron deficiency anemia had significantly lower prohepcidin levels than patients with erythropoietin (EPO)-resistant anemia, as well as patients who had no iron deficiency and anemia, and healthy volunteers. These findings are in agreement with our data and support the theory concerning a potential diagnostic and therapeutic utility of inappropriately raised or lowered prohepcidin concentrations in many diseases. Accordingly, reduced prohepcidin levels may explain some inherited forms of iron loading (hemochromatosis) or help to identify iron deficient states. On the contrary, it is also well known that serum prohepcidin concentration might not reflect changes in iron metabolism as well as hepcidin determinations. It has been confirmed that prohepcidin levels do not correlate with urinary and serum hepcidin, nor do they respond to relevant physiological stimuli (Frazer and Anderson, 2009; Kemna *et al*., 2008; Roe *et al*., 2007; Kemna *et al*., 2005; Brookes *et al*., 2005). Thus, further studies are required to examine serum hepcidin levels and their mutual relationships with prohepcidin in PV and other diseases.

All the parameters of the iron status in the group of PV patients with low ferritin in our study and earlier published data (Hutton, 1979; Pearson *et al*., 1981;

Rector *et al*., 1982; Birgegard *et al*., 1984) confirm that many PV patients suffer from iron disorders. The question arises: what are the reasons for iron deficiency in PV patients? We are convinced that iron deficiency in examined PV patients is not a consequence of phlebotomy treatment. In our study, most patients were on maintenance therapy with hydroxyurea, only some of them had been previously treated by phlebotomy. We assume that, in this case, iron deficiency not only originates from massive erythropoiesis, but could also be caused by high-dose long-term hydroxyurea treatment. Some side effects of hydroxyurea on the digestive system were described in patients taking HU, e.g., nausea, vomiting, diarrhea, stomatitis, loss of appetite, flatulence and constipation (Mleczko, 2004). These complications may negatively influence the iron absorption from food and finally cause iron depletion.

The role of $JAK2^{V617F}$ in pathogenesis of iron deficiency in PV is also very intriguing. Kinase JAK2 is involved in signal transduction via the erythropoietin receptor, and as we know, EPO is one of the hepcidin synthesis regulators (Spivak, 2002; Pinto *et al*., 2008). Some of the data has confirmed that JAK2 mutation may be involved in the regulation of the iron status in myeloproliferative disorders. In murine transplant models, JAK2 mutation induced PV-like phenotype, which includes low serum EPO level and anemia (Tefferi, 2008). What is interesting is that patients who were essential thrombocythemia $JAK2^{V617F}$-positive had lower serum ferritin and serum EPO levels than those who were mutation-negative (Agarwal, 2007).

CONCLUSION

Summing up, the results of studies on the serum concentration values of prohepcidin and other iron metabolism parameters in PV patients indicate that many PV patients suffer from iron deficiency. In conclusion, this preliminary study is the first clinical report relating to serum prohepcidin concentrations in PV patients. Further studies are required to precisely define the role of prohepcidin and hepcidin in iron deficiency appearing in PV.

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