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ORIGINAL ARTICLE

Serum prohepcidin level in myelodysplasia

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Abstract

This study highlights the iron profile of myelodysplastic patients in the era of hepcidin and its pro-hormone, pro-hepcidin. Previous studies have focused on the anemia of chronic renal failure, thalassemia, and hemochromatosis. We determined if pro-hepcidin played a role in iron overload in patients with myelodysplasia (MDS). Thirty adult patients with MDS and 20 healthy adults (controls) were selected. Our results revealed a statistically significant difference in pro-hepcidin levels between the two tested groups ($Z=2.9$, $p=0.003$). There was a weak positive correlation between pro-hepcidin and hematocrit (HCT; $r=0.49$, $p=0.02$) in the healthy group only. Neither age, subtypes of MDS, gender, soluble transferrin receptor (sTFR) or ferritin affected the pro-hepcidin level in patients with MDS. The role of ineffective erythropoiesis in the regulation of pro-hepcidin is superior to the role of chronic blood transfusion therapy.

Key Words: *Prohepcidin, myelodysplasia, anemia, iron overload, hepcidin*

Introduction

The myelodysplastic syndromes are heterogeneous hematopoietic diseases associated with bone marrow failure, peripheral cytopenias, and a tendency to progress to acute myeloid leukemia. Clonal cytogenetic abnormalities can be identified in approximately 50% of the cases. Blood transfusion and iron overload are common problems in the course of the disease [1]. Several factors influence the rate of iron absorption, including the body's iron stores, the level of erythropoietic activity in the bone marrow, the blood hemoglobin concentration, the blood oxygen content, and the presence or absence of inflammatory cytokines [2]. Hepcidin is a key iron-regulatory hormone that inhibits intestinal iron absorption and iron release from hepatic stores and from macrophages recycling senescent erythrocytes. It is found in urine and blood samples in three forms (20-, 22-, and 25-amino acid peptides) [3]. The pro-hepcidin molecule is expressed substantially, but not exclusively, in the liver. The pro-hepcidin molecule is further processed through a furin-type pro-peptide cleavage site to generate hepcidin. Furin is considered a trans-Golgi network-spanning membrane protein, and is present on the cell surface of macrophages [4]. It is not clear that analysis of serum pro-hepcidin; the 84-amino-acid reflects mature-active hepcidin. The lack of correlation between the urinary hepcidin and serum pro-hepcidin measurements is not known, whether due to technical limitations of serum assays or because serum pro-hepcidin concentration does not reflect inflammation or iron metabolism changes [5,6]. The chemical synthesis of human hepcidin has been successful, but difficult to achieve due to folding restrictions imposed by the four-disulfide bridges. Other studies have reported the purification of human recombinant hepcidin in the form of fusion proteins, and until very recently, none of the recombinant hepcidins were shown to be bioactive in iron metabolism [7]. Most of the human studies published so far have relied on an immunodot assay for urinary hepcidin based on selective extraction of the peptide from urine by cation-exchange chromatography and its subsequent quantification by chemiluminescence using rabbit anti-human hepcidin primary antibodies. However these methods are quite laborious, and suitable only for relatively small series of patients [8]. In the present study, we assessed the level of serum pro-hepcidin, the pro-hormone form of hepcidin, and determined its relationship with iron status and its role in pathogenesis in patients with MDS.

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Table I. Comparison of demographic and clinical laboratory data between the myelodysplastic group and the control group. N.B. Eight patients didn't receive a blood transfusion at the time of our study.

Parameter	MDS group (30 patients)	Control group (20 ones)
Age in years (mean, SD)	53±17.4	53±12.8
Age (19–49 years)	13 persons	7
Age>50 years	17 persons	13
12/8	11/19	Sex (male/females)
HCT (median, range)	20.5 (9.3–30.6%)	43.2 (36.9–49.8%)
Ferretin (median, range)	715.5 (258–1993) ng/ml	99 (30–220) ng/ml
Stfr sTFR (median, range)	1.3 (0.13–4.08) mg/L	1.23 (0.76–1.77) mg/L
Prohepcidin (median, range)	191.8 (60.6–380.2) ug/L	254.3 (192.7–392.5) ug/L
Blood transfusion	0–90 units (median 4.5)	0

Patient and methods

Thirty adult patients with MDS, either new or follow-up (mean age, 53±17.4 years; 19 females and 11 males), were recruited from the Hematology Clinic of Kaser El Eini Hospital (Cairo University). The diagnosis was based on bone marrow aspiration (BMA) and biopsy with iron and reticulin stains, as well as karyotyping. Sub-classification of MDS was based on the WHO classification, as follows: degree of cellularity, lineages with dyserythropoiesis, presence of ring sideroblasts, and number of blast cells. Accordingly, the patients were classified as follows: 14 patients with refractory cytopenia and multilineage dysplasia (RCMD) (47%), five patients with refractory anemia with excess blasts (RAEB) (17%), four patients with hypoblastic MDS (13%), and seven patients with refractory anemia (RA) or refractory anemia with ring sideroblasts (RARS) (23%).

The study also included 20 healthy controls (mean age, 53±12.8 years; eight females and 12 males).

Comprehensive histories were obtained, including age, symptoms, and number of units of blood received. The time range for blood transfusion is between 0 and 2 years.

We excluded patients with concomitant hepatorenal disease, recurrent infection, vitamin deficiency or positive C-reactive protein (CRP $\geq$ 0.5 mg/L).

A meticulous physical examination was performed for signs of hemochromatosis, signs of anemia, or bleeding tendency.

The study was approved by the Institutional Ethics Committee, and all participants gave informed consent before entering the study.

Blood samples were drawn from all the participants after an overnight fast. The samples were centrifuged at 3,000 rpm for 10 min to separate sera and stored at −20°C until analysed. Laboratory tests included ferritin, sTFR, and pro-hepcidin.

Serum ferritin was measured by immunoassay (Immulite ferritin: EURO/DPC Limited, Glyn Rhonwy, Llanberis, Caernarfon, Gwynedd LL55 4EL, UK). Serum pro-hepcidin was measured by an immunosorbent assay using antibodies specific for peptides 28–47 of the proregion of the molecule (DRG International Inc., Frauenbergester, USA); and sTFR was measured using polystyrene particles coated with monoclonal antibody specific to human sTfR on a BN II System (Dade Behring Marburg GmbH, Marburg, Germany) according to the manufacturer's instructions.

The hematocrit was determined indirectly from the average size and number of RBCs using Coulter impedance principle by Beckman Coulter.

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 12.0; SPSS, Inc., Chicago, IL, USA). The results are expressed as the median and range. The Mann-Whitney test and the Kruskal-Wallis variance analysis were used to compare group results. Correlation analysis was performed using the Spearman test. Differences and correlation were considered as significant at $p < 0.05$.

Results

Table I summarizes the demographic, clinical, and laboratory data in the patients with MDS and the control group. The pro-hepcidin and hematocrit (HCT) were lower in the MDS group. A statistically significant difference in levels of HCT, ferritin, and pro-hepcidin between the two tested groups was found ($p < 0.05$).

Subclassification of each test group according to age into those less than 50 years and those more than 50 years didn't reveal any statistically significant difference in prohepcidin. There was no statistically significant difference between males and females in prohepcidin level inside each group ($p > 0.05$) (Table II).

Table II. Comparison of prohepcidin between the different age and sex subgroups in both the MDS and control groups.

	Age >50 years mean rank	Age ≤50 years mean rank	*p* value	Males mean rank	Females mean rank	*p* value
MDS group	17.94	12.3	0.08	19.8	13.37	0.08
Control group	11.92	7.86	0.14	11.96	8.3	0.17

Table III. Comparison of prohepcidin between the myelodysplastic and control groups according to age and sex.

	MDS group mean rank	Control group mean rank	*p* value
Age <50 years	8.35	14.5	0.02
Age >50 years	12.8	19	0.057
Females	11.95	18.88	0.03
Males	9.95	14.21	0.1

However, comparison of each age and gender group between cases and control regarding prohepcidin showed a statistically significant difference in females and the age group below 50 years ($p < 0.05$) (Table III).

Table IV illustrates the correlation between prohepcidin and other hematological parameters in the MDS and control groups. There was a statistically significant positive correlation between pro-hepcidin and HCT ($p=0.02$) in the control group while in the MDS group the p value was more than 0.05. No other statistically significant correlation could be established with ferritin or sTFR in either group.

Figures 1 and 2 show the correlation between prohepcidin and HCT in the MDS patients and control.

A comparison between four subtypes of MDS revealed no statistically significant difference among these groups in all tested hematological parameters ($p > 0.05$, Kruskal-Wallis, data not shown).

Our results also revealed that there was no statistically significant correlation between prohepcidin and number of blood units transfused (r=0.021, $p=0.911$). Subclassification of the patients into two groups – those who were heavily transfused with more than 10 units of blood (10 patients) and those who had either not been transfused before or had received less than 10 units of blood (20 patients) however did show a significant difference in ferritin level ($p=0.05$) but there was no statistically significant difference in prohepcidin in either group ($p=0.9$) (data not shown).

Discussion

To the best of our knowledge, this is the first report of prohepcidin in myelodysplasia. In this study pro-hepcidin was lower in MDS patients in comparison to the control group. This observation may reflect the role of ineffective erythropoiesis in enhancing iron absorption [9] through down regulation of hepcidin and its prohormone so that serum ferritin rises to 500–600 ng/ml but seldom exceeds these values before transfusion begins [10]. Moreover, MDS is characterized by iron overload due to secondary blood transfusion. However its impact on stimulation of pro-hepcidin release to inhibit iron absorption is less than the erythroid drive suppressing its release as shown from the results. Comparing our results to other types of anemia, pro-hepcidin assessment in dialysis patients was similar in the hemodialysis group and the healthy controls [11]. Concentrations of pro-hepcidin were significantly decreased in patients with hereditary hemochromatosis (70.2 ug/L) and the patients with rheumatoid arthritis (115.0 ug/L) [5]. This can illustrate that pro-hepcidin may not play a significant role in the pathogenesis of iron overload in MDS in comparison to hemochromatosis, and rheumatoid factors [6]. However, Erwin et al. [12] reported that serum pro-hepcidin levels were not significantly different between all the groups tested, including the control group and those with

Table IV. Correlation analysis between prohepcidin and other hematological parameters (r correlation, p value <0.05).

	Control prohepcidin		MDS prohepcidin	
	R	P	r	P
sTFR	−0.29	0.21	−0.16	0.38
HCT	0.49	0.026	0.32	0.07
Ferritin	0.24	0.29	−0.015	0.93

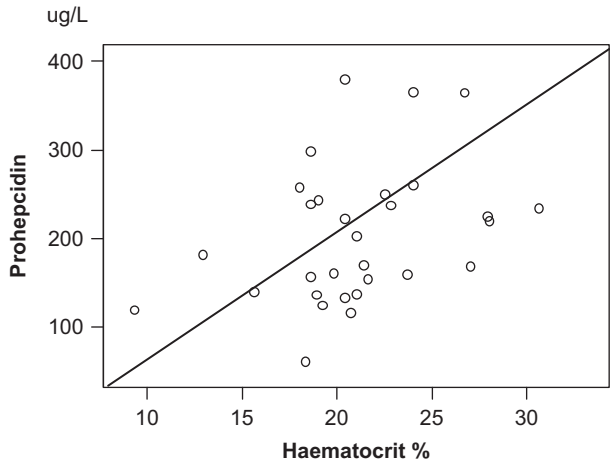

Figure 1. Correlation between haematocrit and prohepcidin in the myelodysplasic group.

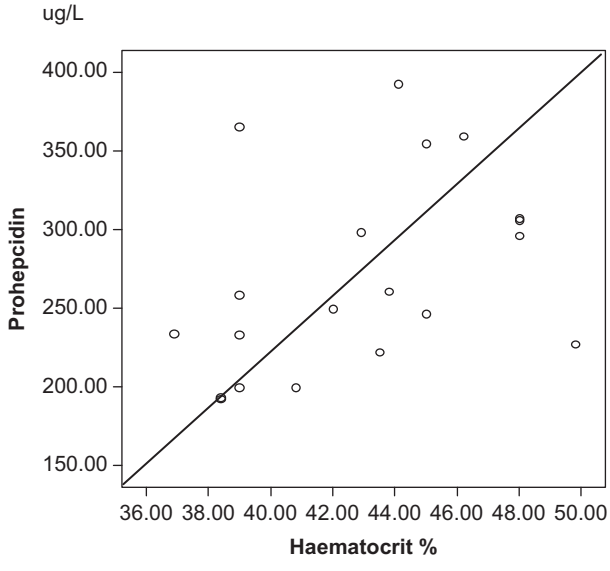

Figure 2. Correlation between haematocrit and prohepcidin in the control group.

thalassemia and iron deficiency, and those injected with lipopolysaccharide, and only the iron deficiency anemia patients showed a tendency to the overall lowest values, and the endotoxin-treated group had the overall highest values.

A positive correlation between pro-hepcidin and HCT was found in the control group only. Pro-hepcidin did not show any correlation with either ferritin or sTFR in our study. Previous studies revealed that pro-hepcidin was positively correlated with only the haematocrit in pregnant mothers and patients with renal failure whether on hemodyalysis or not [5,13,14].

Our results revealed that pro-hepcidin displayed no significant difference in either group with respect to the gender or age subgroups, except between females in both groups and the age group less than 50 years. There is no explanation for this difference, except that our observations need to be verified in larger groups.

In our study no statistically significant difference was found among the different tested groups of MDS ($p > 0.05$). No similar studies were done in MDS, but a direct hepcidin assay in MDS which was conducted on a few patients in association with other types of anemias revealed a variable expression of hepcidin, which was explained by heterogeneity of the disease and variable pathogenesis involving the development of anemia, such as ineffective erythropoiesis and an alteration in the iron state, such as iron deficiency and iron overload with repeated transfusion [15–18].

The pro-hepcidin assay may be a limitation of this study. Hepcidin assay by ELISA is a new hope for easy, rapid assay. However, the specificity of the antibody used with respect to various hepcidin isoforms remains to be verified [19].

We can conclude that MDS is a disease with iron overload and variable degrees of erythroid activity. The pro-hepcidin level in general was less in MDS groups than in healthy subjects. This significant difference in expression was mainly in age subgroups <50 years and between females in both groups. A larger sample size is needed in future studies.

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Declaration of interest: None to declare.

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