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Serum iron metabolism and erythropoiesis in patients with myelodysplastic syndrome not receiving RBC transfusions

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ABSTRACT

Dysregulation of hepcidin, a key iron regulating hormone, is important in the pathogenesis of iron overload in patients with myelodysplatic syndrome (MDS). However, most studies of hepcidin levels are complicated by concomitant RBC transfusions. To evaluate the relationship between iron metabolism and erythropoiesis, we measured serum levels of hepcidin, growth-differentiation factor-15 (GDF15) and other markers of erythropoiesis in 107 subjects with MDS not receiving RBC transfusions. Patients with MDS had significantly higher levels of hepcidin than normals. However, their hepcidin–ferritin ratio was markedly decreased compared to normals ($P < 0.001$) and varied substantially between MDS subtypes ($P = 0.011$). GDF15 levels positively correlated with percent of bone marrow erythroblasts ($P < 0.001$), soluble transferrin receptor (sTfR) ($P = 0.018$), and also with transferrin saturation (ISAT) ($P = 0.038$). The hepcidin–ferritin ratio negatively correlated with serum erythropoietin (EPO) levels ($P < 0.001$), and also with GDF15 levels ($P = 0.014$). Colony forming cells (CFC) were evaluated in 70 subjects. Those with serum ferritin (SF) levels <500 ng/ml had significantly more BFU-E than subjects with SF ≥ 500 ng/L ($P = 0.007$), but numbers of granulocyte/macrophage-colony-forming cells (CFU-GM) were similar ($P = 0.190$). Our data indicate serum hepcidin levels are inappropriately low in patients MDS not receiving RBC transfusions. GDF15 levels correlated with low hepcidin levels and may contribute to iron overload in this setting. Iron overload may in turn suppress erythropoiesis by imparing the proliferative capacity of the erythroid progenitor cells.

1. Introduction

Myelodysplastic syndromes (MDS) are heterogeneous clonal hematopoietic stem cell disorders characterized by dysplastic and ineffective hematopoiesis, blood cytopenias, severe anemia and a variable risk of progression to acute myleoid leukemia (AML) [1]. Eighty percent of patients with MDS have hemoglobin levels less than 100 g/L at diagnosis and most of them will become RBC transfusion-dependent [2]. Although extensive RBC transfusions are thought to be the dominant cause of iron overload in MDS patients, many patients have iron overload soon after diagnosis and even before receiving RBC transfusions [3].

Most information regarding iron homeostasis in patients with MDS is from patients receiving RBC transfusions [4]. In contrast, iron homeostasis in patients with MDS not receiving RBC transfusions is not well-studied. We took advantage of the more conservative RBC transfusions policy in China to study this issue.

Previous data suggest that dysregulation of hepcidin, a key regulator of iron absorption and recycling, may play an important role in the pathogenesis of iron overload in patients with MDS.

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Hepcidin binds to ferroportin causing internalization and degradation of iron, which results in cessation of iron release from cells [5]. Hepcidin production is regulated by iron and erythropoiesis. Iron excess stimulates hepcidin production, whereas iron deficiency and increased erythropoiesis suppresses hepcidin production [6].

Iron accumulation in patients with MDS is self-sustaining because ineffective erythropoiesis stimulates intestinal iron absorption. The molecular nature of signal underlying this process is thought to be secretion of growth-differentiation factor-15 (GDF15) by mature erythroblasts, which results in suppression of hepcidin production by liver cells [7]. GDF-15, a member of the transforming growth factor-β super-family [8], was produced by erythroblasts in diseases with ineffective erythropoiesis such as congenital dyserythropoietic anemia, pyruvate kinase deficiency and thalassemias [7,9–11]. However, GDF15 levels in patients with MDS are not extensively studied and its role in regulating hepcidin is controversial [7].

To evaluate the relationship between iron burden and erythropoiesis in patients with MDS not receiving RBC transfusions, we studied levels of hepcidin, GDF15, soluble transferrin receptor [sTfR], erythropoietin [EPO] and serum ferritin (SF) in this population.

2. Subjects and methods

2.1. Subjects

The study was approved by the Ethics Committee of the Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College according to the guidelines of the Declaration of Helsinki. 107 adults with MDS diagnosed between April, 2011 and March, 2013 at the Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences (CAMS) were included. To be enrolled in this study, patients had to be untreated and previously without any RBC transfusions. Individuals with any hematological and malignant diseases other than MDS, those with hepatic and/or renal diseases, decompensated heart disease and active infection were excluded. MDS subtypes were classified according to World Health Organization (WHO) and stratified for prognosis according to International Prognostic Scoring System (IPSS) [12,13].There were 66 males. Median age was 50 years (range, 16–96 years). Forty healthy individuals with a rigorous definition of normal iron state as previously described were used as controls [14,15].

2.2. Biochemical assays

Serum hepcidin were determined using a commercial ELISA kit following manufacturer's protocol (DRG Instruments, Marburg, Germany). GDF15 serum levels were measured with the DuoSet enzyme-linked immunosorbent assay development kit for human GDF15 (R&D Systems, Minneapolis, MN, USA). SF and EPO were determined by immunoradiometric assay. sTfR was quantified using an immunonephelometric method.

2.3. In vitro colony forming cells (CFC) assay

1 × 10E5 bone marrow mononuclear cells (BMNC) were cultured in semi-solid Methocult GF medium (StemCell Technologies, MethoCult™ GFH4434) and incubated at 37 °C. Each sample was analyzed in triplicate. Erythroid colony forming unit (CFU-E) colonies of at least eight cells were counted on the seventh day of culture, erythroid burst-forming unit (BFU-E) colonies of ≤3 sub-clusters or 1 cluster containing >300 cells and granulocyte–macrophage colony-forming unit (CFU-GM) colonies of >40 cells were scored after 14 days of culture. Normal ranges at our laboratory per 10E5 BMNC were: CFU-GM, 14–29, CFU-E, 68–93 and BFU-E 25–37.

2.4. Statistical methods

Comparison of numerical variables between groups used a nonparametric approach (Mann–Whitney test or Kruskal–Wallis ANOVA). Distribution of categorical variables in different groups was compared with Fisher exact-test (when computationally feasible) or the χ^2 test. Iron parameter correlations were calculated using the Spearman correlation. Results are expressed as means ± SD. *P*-values less than 0.05 (two-sided) were considered statistically significant.

Table 1
Clinical and biochemical characteristics of 107 MDS patients.

	MDS patients (*n* = 107)
WBC (×10^9/L)	2.7(0.6–9.4)
HB (g/dL)	8.1(6.0–10.8)
PLT (×10^9/L)	59(7–395)
ALT (IU/L)	19(1–73)
AST (IU/L)	20(1–71)
Creatinine (μmol/L)	82(49–98)
CRP (mg/L)	3.0 (1.0–8.7)
Cytogenetics	
Low (*N*)	59
Intermediate (*N*)	24
High (*N*)	15
No data (*N*)	9
IPSS	
Low risk (*N*)	8
Int-1 (*N*)	60
Int-2 (*N*)	24
High (*N*)	6
No data (*N*)	9

Abbreviations: WBC, white blood count; HB, hemoglobin; PLT, platelet level; ALT, Alanine Transaminase; AST, Aspartate Transaminase; CRP, C-reactive protein; IPSS, International Prognostic Scoring System.

3. Results

3.1. GDF15 and hepcidin levels in the whole MDS group

Clinical and biochemical characteristics of the whole MDS group including cytogenetic data were summarized in Table 1. Table 2 showed the main characteristics and iron biochemical parameters including serum hepcidin and GDF15 of the whole MDS population as compared with the control group. Patients with MDS had significantly higher levels of SF, GDF-15 and EPO. There was a weak to moderate positive correlation between GDF15 levels and percent of bone marrow erythroblasts ($r = 0.485$; $P < 0.001$), sTfR ($r = 0.285$; $P = 0.018$), and iron saturation ratio (ISAT) ($r = 0.242$; $P = 0.038$) (Fig. 1A–C). In addition, a moderate negative correlation was observed between GDF15 and transferrin levels (TRF) ($r = -0.315$; $P = 0.008$) (Fig. 1D). Unexpectedly, the mean serum hepcidin levels in the MDS cohort were significantly higher than the controls ($P < 0.001$). However, the hepcidin–ferritin ratio was markedly decreased in patients with MDS ($P < 0.001$; Table 2).

3.2. MDS patients stratified according to different WHO subtypes

The hepcidin–ferritin ratio varied substantially among different WHO subgroups ($P = 0.011$; Fig. 2A), with the lowest ratio in patients with refractory anemia with ring sideroblasts (RARS). These patients have the greatest iron overload with the highest SF and ISAT among the MDS subtypes ($P = 0.028$ and $P = 0.004$; Fig. 2B and C). GDF15 levels also varied among MDS categories ($P = 0.005$; Fig. 2D), with the highest levels in subjects with RARS and the

Table 2
Characteristics of persons with MDS and normals.

	MDS (*n* = 107)	Controls (*n* = 40)	*P*
Age (year)	50 (16–96)	40 (16–77)	<0.001
Sex (Male/Female)	66/41	18/22	0.102
SF (ng/ml)	450 (60–3637)	66 (19–115)	<0.001
EPO (mU/ml)	96. 6 (2.2–3784.2)	13.8 (9.9–17.5)	<0.001
Hepcidin (ng/ml)	81.7(4.5–400.4)	55.9(9.0–333.9)	<0.001
GDF15 (pg/ml)	1432 (336–22559)	463 (91–1694)	<0.001
Hepcidin–ferritin ratio	0.18 (0.01–4.90)	0.46 (0.1–14.20)	<0.001

Abbreviations: SF, serum ferritin; EPO, erythropoietin; GDF15, growth-differentiation factor-15.