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Erythropoietic drive is the strongest predictor of hepcidin level in adults with sickle cell disease

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ABSTRACT

Levels of hepcidin, a key modulator of iron metabolism, are influenced by erythropoiesis, iron, and inflammation, all of which may be increased in patients with sickle cell disease (SCD). The objectives of this study were to determine: 1) the variation in hepcidin level, and 2) the relative contribution of erythropoietic drive, iron, and inflammation to differences in hepcidin level in an adult cohort with SCD. In a prospective study, cross-sectional measurements of hepcidin, reticulocyte percentage, erythropoietin, ferritin, and high-sensitivity CRP were obtained. A regression tree analysis was used to measure the association between these interacting factors and hepcidin level. The cohort was comprised of 40 adults with SCD. Median age was 26 years, 68% were female, and all had HbSS. Hepcidin values ranged from 30 ng/ml to 326 ng/ml, with a median of 87 ng/ml. Regression tree analysis demonstrated that reticulocyte percentage, erythropoietin, ferritin and hs-CRP all were associated with hepcidin. The highest hepcidin values were found in subjects with low reticulocyte percentage and erythropoietin. In conclusion, erythropoietic drive, iron status, and inflammation all contribute to variation in hepcidin level. The strongest contributor is erythropoietic drive. Future studies could determine whether suppression of erythropoiesis with chronic transfusion influences hepcidin level.

1. Introduction

In patients with sickle cell disease (SCD), frequent red cell transfusion often leads to iron overload [1–3]. Despite the risks of excess iron, including liver and heart failure, patients with SCD and iron overload have less end-organ damage than other patient populations with similar iron burden [4–10]. This difference suggests that patients with SCD may manage excess iron in a more organ-protective manner than other transfused populations, such as those with β-thalassemia [4].

Hepcidin, the key regulator of iron metabolism, may modulate the risk of end-organ damage from transfusion-related iron toxicity. A negative regulator of iron homeostasis, hepcidin decreases intestinal absorption and cellular release of iron [11–13]. Higher levels of hepcidin, therefore, may limit tissue injury through a reduction of iron in circulation and sequestration of iron within cells, including toxic non-transferrin bound iron (NTBI) [4]. One potential explanation for the lower incidence of iron-related end-organ disease in patients with SCD compared to other transfused populations would be higher levels of hepcidin and lower levels of NTBI. Although lower levels of NTBI have been reported in patients with SCD compared to other transfused populations [6,14], studies of hepcidin level have yielded inconsistent results [15–18].

Selection bias may have limited the results of prior studies. Iron and inflammation are positive regulators of hepcidin, whereas erythropoietic drive (as defined by markers such as erythropoietin) is a negative regulator [11–13,19]. Since iron, inflammation and erythropoietic drive can all be increased in patients with SCD to varying degrees, hepcidin levels likely differ significantly from patient-to-patient based on their levels of positive and negative regulators. Prior studies largely reported lower levels of hepcidin in patients than controls [17,18]. These studies were limited, however, by narrow patient selection, mostly children without iron excess. In a cohort with more iron excess or inflammation, or less erythropoietic drive, hepcidin levels may be higher. To address this limitation, we examined hepcidin levels in a cohort of adults with SCD with a significant history of transfusion and iron overload. Our objectives were two-fold: 1) to determine variation in hepcidin levels, and 2) to elucidate the contribution of erythropoietic drive, iron burden, and inflammation to the observed variation. Further insight into the regulation of hepcidin may lead to strategies to modulate hepcidin levels in order to maximize organ-protective effects.

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2. Methods

2.1. Patients and data collection

Patients with HbSS who were greater than 18 years of age were eligible for this study. The Medical College of Wisconsin institutional review board approved this study. All patients provided written consent prior to participation.

Blood was collected from adult patients with SCD at steady state, defined as patient report of baseline symptoms and without admission to the emergency department (ED) or hospital in the previous 4 weeks. Patients on a chronic transfusion regimen had samples collected up to 72 h prior to the transfusion. Samples were tested for erythropoietin (EPO) by immunoassay (Dynacare Laboratories, Milwaukee, WI), high sensitivity C-reactive protein (hs-CRP) (Dynacare Laboratories, Milwaukee, WI), and plasma hepcidin (Intrinsic LifeSciences, La Jolla, CA). Patient demographics were obtained from the electronic medical record. Chronic transfusions, simple or exchange, were administered on a 4–8 week schedule as part of their routine, non-acute care. The most recent available hemoglobin, alanine aminotransferase (ALT), glomerular filtration rate (GFR), hemoglobin S percent (%), reticulocyte percent (%), and ferritin were also obtained. When possible, the result was obtained at the same time as the hepcidin sample.

2.2. Statistical analysis

The primary outcome of interest in this study was plasma hepcidin (ng/ml) and its dependence on other patient characteristics. Descriptive statistics were used to summarize participant characteristics. Patient-related factors were compared using the Kruskal Wallis test for continuous variables and the Fisher's Exact test for categorical variables. Potential factors to predict hepcidin included age, gender, days from last transfusion, number of transfusions in the last 12 months, EPO, hs-CRP, ALT, GFR, hemoglobin, ferritin, hemoglobin S %, and reticulocyte %, each of which were examined with a Spearman correlation.

Since measures of iron, inflammation, and erythropoietic drive (reticulocyte % and erythropoietin) all interact with each other to influence hepcidin level, we performed a regression tree analysis. A regression tree or recursive partitioning analysis is a nonparametric regression methodology, where the recorded dependent variables noted previously are evaluated and selected to best explain the differences between hepcidin values. The advantages of a tree analysis over, for example, a traditional linear regression, is that highly related variables (reticulocyte % and erythropoietin) can be included as independents. Further, by specifying an optimization function such as least median absolute deviation (least MAD), rather than least squares (used by linear regression), outliers will affect the model less. The tree initially divides the dataset into two groups based on optimizing, by our choice, the MAD, since the data had outliers. It then continues to partition each branch into two sets and so on. Once a cohort of 5 subjects is reached, the partitioning process is halted for that group. A statistical significance (alpha) level of 0.05 was used throughout. SPSS 21 for Windows and Salford Systems CART for the tree analysis were used.

2.3. Sample size calculation

The sample size was chosen to enable investigation of the interrelationships between hepcidin levels and known mediators: EPO, ferritin and CRP. In general, 5 cases per covariate, including any interaction variables, in a model is the accepted minimum needed for appropriate fitting of the model. A sample size of 40 subjects would therefore allow us to consider 3 variables and their 3 interactions. Further, with a sample size of 40 subjects, we would have at least 80% power to detect a significant correlation of ≥0.45 at a Bonferroni corrected alpha of 0.017 (for 3 tests).

3. Results

3.1. Demographics and laboratory values

There were 40 patients evaluated in this study (Table 1). The median age of study participants was 26 years (range: 20–57), all were HbSS, and most were female (68%). These adult patients were all heavily transfused, and no patients were transfusion naïve at the start of the study. Nineteen subjects (48%) were receiving chronic red cell transfusions at the time of the study, 11 (58%) via red cell exchange. Only 5 (12.5%) subjects received no red blood cell transfusions in the previous 12 months. An elevated reticulocyte % (median 8%, range 0.6–23%; reference range 0.5–2%) and EPO (median 57 mIU/ml, range 19–2962 mIU/ml; reference range 3–19 mIU/ml) was observed, consistent with increased erythropoietic drive. The cohort demonstrated iron overload (median ferritin 2,969 ng/ml, range 20–12,300 ng/ml; reference range 13–400 ng/ml), consistent with their transfusion history, and increased inflammation (median hs-CRP 5.6 mg/l, range 0.4–60 mg/l; reference range <1.0 mg/l). Median hepcidin level for the cohort was 87 ng/ml (range: 30–326 ng/ml, reported reference range 17–286 ng/ml female/29–254 ng/ml male).

3.2. Univariate associations with hepcidin

In univariate analyses, hepcidin level had a negative correlation with erythropoietin ($r = -0.34$, $p = 0.03$), and reticulocyte % ($r = -0.47$, $p = 0.002$) (Table 1). A positive correlation was noted between hepcidin and ferritin ($r = 0.56$, $p = 0.0002$). No significant association was found between hepcidin and any other variable, including hs-CRP ($r = 0.25$, $p = 0.11$) or any treatment protocol, including red cell transfusion history ($p > 0.1$).

3.3. Regression tree analysis to predict hepcidin

Significant interactions exist between reticulocyte %, EPO, ferritin, and hs-CRP when univariate analyses are performed to test their association with hepcidin (Supplementary figure). To delineate the relationship between these interacting factors and hepcidin, a regression tree analysis was performed. In the best fit model, markers of erythropoietic drive (reticulocyte % and EPO), iron load (ferritin), and inflammation (hs-CRP) were all significantly associated with hepcidin level (Fig. 1). Of these, erythropoietic drive (reticulocyte % and EPO) was the strongest predictor of hepcidin level. Patients with a low reticulocyte % and EPO

Table 1
Patient characteristics and lab values at steady state and their relationship to hepcidin.

Patient characteristics	N = 40	r	p-Value
Age (median years) (range)	26 (20–57)	−0.001	1.0
BMI (median kg/m^2) (range)	24 (17–35)	0.082	0.61
Female gender (%)	68	NA	0.1[a]
Therapy			
Hydroxyurea (%)	40	NA	1.0[a]
Iron chelation (%)	40	NA	0.2[a]
RBCs in last year	15 (0–78)	−0.1	0.7
Days since last RBC	45 (4–5990)	−0.2	0.2
Laboratory values			
Erythropoietin, ng/dl, median (range)	57 (19–2962)	−0.3	0.03[b]
Hemoglobin, g/dl, median (range)	8.3 (5.2–10.9)	−0.1	0.7
Hemoglobin S percent, median (range)	50.5 (3.3–89.6)	−0.3	0.1
Reticulocyte percent, median (range)	7.9 (0.6–23.0)	−0.5	0.002[b]
Ferritin, ng/dl, median (range)	2969 (20–12300)	0.6	0.0002[b]
Hs-CRP, mIU/ml, median (range)	5.6 (0.4–59.5)	0.3	0.1
ALT, mIU/dl, median (range)	27 (8–147)	0.02	1.0
GFR, % in normal range (>60)	95	NA	0.5
Hepcidin, ng/dl, median (range)	87 (30–326)	NA	NA

[a] Spearman's correlations were evaluated in all cases except for gender, hydroxyurea, iron chelation use, and GFR, where a Kruskal Wallis test was applied.
[b] Significant relationships were defined as $p \leq 0.05$ in all cases.