Blood Cells, Molecules and Diseases 59 (2016) 100–105

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Assessment of serum bioactive hepcidin-25, soluble transferrin receptor and their ratio in predialysis patients: Correlation with the response to intravenous ferric carboxymaltose

Athina Drakou [a], Alexandra Margeli [b], Stamatia Theodorakopoulou [a], Ioannis Agrogiannis [a], Christos Poziopoulos [b], Ioannis Papassotiriou [b,*], Demetrios V. Vlahakos [a]

[a] Renal Unit, Attikon General University Hospital, University of Athens, School of Medicine, Athens, Greece
[b] Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece

ARTICLE INFO

Article history:
Submitted 4 March 2016
Revised 9 May 2016
Accepted 9 May 2016
Available online 11 May 2016

Editor: Mohandas Narla

Keywords:
Chronic kidney disease
Intravenous iron administration
Hepcidin-25
sTfR
Ferric carboxymaltose

ABSTRACT

Background: No reliable biomarker exists to predict responsiveness to intravenous (IV) iron (Fe) in iron deficient patients with CKD. We aimed to investigate the clinical value of bioactive Hepcidin-25 and soluble Transferrin Receptor (sTfR) levels in predialysis patients.
Patients and methods: In this prospective study 78 stable stage III-IV CKD predialysis patients with (responders) (40 patients) and without (non-responders) (38 patients) adequate erythropoiesis after IV administration of ferric-carboxymaltose (FCM). Patients were divided in two groups according to their response to IV administration of ferric-carboxymaltose (FCM). Along with measurements of common hematologic and blood chemistry parameters, determinations of sTfR and bioactive Hepcidin-25 were performed.
Results: Hepcidin-25 levels were lower in the responders ($p = 0.025$), while sTfR and sTfR/Hepcidin-25 ratio were higher ($p < 0.01$ and $p = 0.002$ respectively). Diagnostic efficacy indicated cut off point of 1.49 for Hepcidin-25 had sensitivity 84% and specificity 48%, while cut off point of 1.21 for sTfR/Hepcidin-25 ratio had sensitivity 82% and specificity 52% to predict correctly response to iron supplementation therapy. Furthermore, log sTfR/Hepcidin-25 correlated negatively with hs-CRP ($p = 0.005$) and IL-6 ($p < 0.04$) in non-responders, while such correlations were not found in responders ($p > 0.05$).
Conclusions: These results suggest that lower Hepcidin-25, as well as higher sTfR and sTfR/Hepcidin-25 ratio were significant predictors of favorable hemoglobin response within a month after IV administration of FCM in patients with CKD. Further experiments and clinical studies in other groups of patients are needed to better elucidate the role of Hepcidin-25 and sTfR/Hepcidin-25 ratio as predictors of response to intravenous iron administration.

1. Introduction

Chronic kidney disease (CKD) is a worldwide public health issue, with increasing incidence and prevalence, poor outcome and high cost [1]. Anemia is a common complication of CKD and its management has been revolutionized since the discovery of recombinant human erythropoietin (rhEPO) >30 years ago [2]. Iron is essential for hemoglobin (Hb) production, and the absolute or relative iron deficiency has emerged, as the major cause of rhEPO resistance in patients with CKD [3]. Iron is also an essential nutrient for microorganisms and its administration could increase the risk for infections [4]. Therefore, the need to correctly assess iron status is considered to be very important in order to avoid useless, costly and potentially dangerous iron administration [5–7]. Classical laboratory biomarkers of iron deficiency, such as ferritin levels and transferrin saturation (TSAT), exhibit a wide biological variability, especially when inflammation is present, and do not reliably identify iron-restricted erythropoiesis or predict the efficacy of iron supplementation. In response, newer markers have been proposed including soluble transferrin receptor (sTfR), the sTfR/log ferritin index and the reticulocyte hemoglobin content [8,9].

Hepcidin is a 25 amino acid peptide that was first identified in urine and plasma, as an antimicrobial agent [10,11]. It is synthesized in hepatocytes, as the main regulator of iron metabolism and controls both the

* Corresponding author at: Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens 11527, Greece.
E-mail addresses: biochem@paidon-agiasofia.gr, ipapassotiriou@gmail.com (I. Papassotiriou).

http://dx.doi.org/10.1016/j.bcmd.2016.05.006
1079-9796/

amount of dietary iron absorbed in the duodenum and the iron release by reticuloendothelial cells. Hepcidin's expression is up-regulated by inflammation and iron overload, and down-regulated by anemia, hypoxia and iron deficiency. For these reasons, hepcidin levels have been evaluated, as predictors of functional iron deficiency in a variety of clinical conditions, including hospitalized adult patients, anemic children, early and late stages of CKD, metabolic syndrome, obesity, etc. [12–17].

Although the oral iron supplements are widely available, inexpensive and easy to administer, they are less effective compared to intravenous (IV) iron therapy in patients with CKD. In order to overcome the disadvantages of oral therapy, parenteral intravenous preparations have been developed, including ferric carboxymaltose (FCM), a non-dextran iron preparation, which can be rapidly administered in high doses [18]. Nevertheless, a substantial subset of patients with CKD fails to respond to IV iron.

The purpose of this study was to evaluate serum ferritin, hepcidin-25, sTfR and their ratios, as biomarkers for true iron deficiency and responsiveness to infusion of FCM in a cohort of elderly patients with moderate-to-severe CKD and iron deficiency. The sTfR/hepcidin-25 index was formulated in view of the fact that in true iron deficiency its nominator and denominator move to opposite directions maximizing their ratio. Indeed, in true iron deficiency sTfR is expected to increase and hepcidin-25 to decrease. By contrast, in functional iron deficiency during inflammation this index is expected to decrease, since sTfR, its nominator, tends to remain stable, while hepcidin-25, its denominator, is expected to increase.

1. Patients and methods

1.1. Study design

After having obtained informed consent and approval from the Hospital Ethics Committee Review Board for Human Studies, 78 elderly men and women with stable stage III-IV CKD, anemia (defined by WHO criteria, as Hg level <12 g/dl in women and <13 g/dl in men) and serum ferritin levels <100 ng/ml were enrolled in this prospective study. Patient data, clinical information and blood samples were collected prospectively. In order to achieve sufficient iron stores and maintain serum ferritin levels >100 ng/ml, patients received 1 g of FCM dissolved in 100 ml of normal saline, as an IV infusion over a period of 20 min. The clinical course was closely monitored and according to the Hg level attained at the end of the first month of observation, patients were divided in two groups: Group R (responders) included 40 patients who had true iron deficiency and increased their Hg concentration by >1 g/dl from baseline. Group NR (non-responders) included 38 patients, who failed to respond. Exclusion criteria were active infection, acute illness within the last month, history of chronic inflammatory disease or treatment with immunosuppressant agents and anti-inflammatory drugs, acute bleeding, malignancy, renal replacement therapy, red blood cell transfusion or medical therapy with iron within 3 months of enrollment.

1.2. Laboratory analyses

Since hepcidin-25 levels show diurnal changes and may be affected by exogenous erythropoietin administration, all blood specimens were collected early in the morning after 12 h of fasting, prior to the administration of any medication and at least two weeks after the last darboepoetin injection. In particular, 4 mL of whole blood was drawn in serum isolation tubes and centrifuged at 3700 rpm for 7 min and the serum was collected in cryovials and stored in −80 °C until further analysis.

Hematological parameters were measured using the Sysmex XE-2100 automated hematology system (Sysmex Corporation, Kobe japan). Blood chemistry including measurements of parameters of renal and nutrition function was performed using the ILAB 600 chemistry analyzer (Instrumentation Laboratory, Bedford, MA, USA). The MDRD Study equation was used for GFR estimates in all patients. Ferritin was quantitatively determined using the Roche E411 Cobas immunoassay analyzer (Roche Diagnostics, Mannheim, Germany), using an electrochemiluminescence technique. sTfR and hs-CRP concentrations were measured by means of immunonephelometric techniques using the BN Prospec nephelometer (Siemens Healthcare Diagnostics, Liederbach, Germany). sTfR values expressed as mg/L (1 mg/L = 0.085 (nmol)/L). Serum concentrations of IL-6 were measured by the quantitative sandwich enzyme immunoassay technique (Quantikine, R&D Systems, Minneapolis, MN, USA). The mean detectable concentration was 0.039 pg/mL. The intra-assay coefficient of variation ranges from 6.9 to 7.4%, and the inter-assay coefficient of variation ranges from 6.5 to 9.6%. Serum hepcidin-25 isoform was detected by using a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit (Hepcidin-25 ELISA; DRG Instruments GmbH, Marburg, Germany) according to the manufacturer's instructions. The method is a solid-phase ELISA, based on the principle of competitive binding. The microtiter wells were coated with a monoclonal antibody directed toward the antigenic site of the bioactive hepcidin-25 molecule. Endogenous hepcidin-25 of a patient sample competes with the added hepcidin-biotin conjugate for binding to the coated antibody. After incubation, the unbounded conjugate is washed off. Incubation with a streptavidin peroxidase enzyme complex and a second wash step follows. The addition of substrate solution results in a color development, which is stopped after a short incubation. The intensity of color developed is reverse proportional to the concentration of hepcidin-25 in the patient sample. The minimum detectable concentration was 0.6 ng/mL while inter- and intraassay coefficients of variation were 7.37 and 4.1%, respectively. Hepcidin-25 levels were expressed ng/mL (1 ng/mL = 0.358 nmol/L).

1.3. Statistical analysis

Data are expressed as mean ± standard deviation (S.D.) for quantitative variables and as percentages for categorical variables. The Kolmogorov—Smirnov test was utilized for normality analysis of the quantitative variables. In case of violation of normality of data, we use logarithmic transformation and non-parametric analysis. Comparisons between two groups of biochemical markers were performed using the independent sample's *t*-test, Welch–test (in case of unequal S.D.) and Mann-Whitney test (in case of violation of normality). Pearson's correlations between hepcidin and markers of iron deficiency, inflammation and renal function were used in the entire study population, as well as in Group R and Group NR.

A receiver operating curve (ROC) analysis was conducted to determine the diagnostic ability and obtain cut off levels of biochemical markers for the classification of patients, as responders and non-responders, by calculating the respective areas under the curve (AUC). The AUC with their standard error and 95%CI were calculated using the maximum likelihood estimation method, which has the advantage of being free of assumption about the Gaussian distribution of underlying variables.

All biochemical markers, whether or not they demonstrated significant associations with outcome variable (responders vs non-responders) in univariate analysis were included in the multiple logistic regression model, and forward elimination Wald method was used to arrive at the final model. Goodness of fit was evaluated using the Hosmer-Lemeshow statistic. All tests are two-sided, a p-value of <0.05 was used to denote statistical significance. All analyses were carried out using the statistical package SPSS vr 17.00 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA).