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Original Article

The value of soluble transferrin receptor and hepcidin in the assessment of iron status in children with cystic fibrosis

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Abstract

Background: The value of ferritin in the diagnosis of iron deficiency is limited in patients with CF since it increases in the presence of inflammation. We hypothesized that the soluble transferrin receptor (sTfR) and hepcidin may provide more information than ferritin in assessing iron status in children with CF.
Methods: We analyzed sTfR and hepcidin in relation to conventional iron status indicators in 49 children with CF.
Results: We found no differences in sTfR concentration between children with and those without ID. sTfR concentrations were within the normal range in all children. Hepcidin concentrations were low, and concentrations below the limit of detection were observed in 25% of the clinically stable children.
Conclusion: The sTfR is not useful to determine the iron status in this population, whereas hepcidin might serve as an early indicator of deficient iron stores in children with CF.

Keywords: Children; Anemia; Ferritin; Pseudomonas aeruginosa; Hepcidin; Soluble transferrin receptor; Reticulocyte hemoglobin content

1. Introduction

Iron deficiency (ID) as defined by the criteria of the World Health Organization (WHO) (ferritin < 12 μg/L or < 15 μg/L) is common in patients with cystic fibrosis (CF) [1,2]. Although the exact mechanism is unclear, it has been suggested that ID in adult CF patients is primarily functional due to chronic inflammation [3]. In a recent study on iron status in children with CF we showed that low iron stores (ferritin < 12 μg/L) were common in young children, whereas higher ferritin concentrations were observed in older children with CF [2]. These results suggest that unlike in adult CF patients, ID in young CF patients can be solely attributed to an absolute ID. However, ferritin acts as an acute phase reactant, and is therefore no reliable indicator of ID in the presence of infection or inflammation. We suggested that the

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higher ferritin concentrations observed in older children represent an increased state of inflammation, rather than an improved iron status. The results of this study might therefore underestimate the prevalence of absolute ID in children with CF [2].

The clinical implications of ID in children with CF are unclear. In general, ID in children is associated with negative effects on cognitive and behavioral development later in life (4). However, the use of iron supplementation in CF patients is questionable [5] since increased sputum iron levels are thought to contribute to the persistence of Pseudomonas aeruginosa (PA) infections [6]. To our knowledge, there are no data available on the effect of iron supplementation in children with CF. Intravenous (IV) iron supplementation in adult CF patients colonized with PA resulted in an increase in inflammatory markers and worsening of clinical symptoms [7], whereas no clinical deterioration was observed in CF patients with an absolute ID [5]. The potential negative effects of iron supplementation in CF patients likely depend upon the underlying cause; in absolute ID patients might benefit from iron supplementation whereas in functional ID the underlying inflammation should be treated. The accurate assessment of iron status is therefore highly important in the diagnosis and treatment of ID in CF patients.

The soluble transferrin receptor (sTfR) and hepcidin might provide more information than ferritin in assessing iron status in patients with CF. The sTfR is expressed by iron-requiring cells and reflects cellular iron demands and erythropoietic activity. sTfR is less affected by inflammation than ferritin and is therefore suggested as a biomarker that will aid in distinguishing between absolute and functional ID [8].

Hepcidin is an iron regulatory peptide hormone, mainly produced by the liver. Hepcidin decreases intestinal iron absorption, increases iron retention in the cells of the reticulo-endothelial system, and thereby limits the iron availability in the circulation for red blood cell synthesis [9]. Hepcidin is downregulated by absolute ID, hypoxia and erythropoietic activity, and upregulated by inflammation and infection. A recent study in adult CF patients showed that hepcidin concentrations decrease in response to antibiotic treatment of a pulmonary exacerbation [10]. However, no data are available on hepcidin and sTfR in children with CF.

Since CF is characterized by continuous inflammation from an early age [11], we hypothesized that hepcidin concentrations are increased in children with CF, which may contribute to the development of ID. Furthermore, we hypothesized that sTfR may be a more useful indicator of ID than ferritin in children with CF. We therefore assessed sTfR and hepcidin in addition to conventional iron status indicators in children with CF. Furthermore, we analyzed the association between hepcidin and iron status indicators, erythropoietic activity and markers of CF disease progression such as pulmonary function, PA colonization, pancreas insufficiency and liver function.

2. Methods

All 53 children with CF treated in our hospital were included in the study from January 2012 to May 2013. Cystic fibrosis transmembrane conductance regulator (CFTR) genotype was classified as 'severe' if both mutations were class I, II or III, or 'mild' if at least one mutation was from class IV or V, based on previously published classifications [12,13].

A venous blood sample was taken and analyzed for sTfR, hepcidin-25, other iron status indicators (ferritin, hemoglobin (Hb), mean corpuscular volume (MCV), red cell distribution width (RDW), reticulocyte hemoglobin content (Ret-Hb)), C-reactive protein (CRP) as indicator of infection, reticulocyte count and erythropoietin as indicators of erythropoiesis, and albumin as indicator of liver function. Blood samples were drawn between 8 am and 8 pm. The time of blood sampling was categorized as before or after 12 pm, in line with previously reported serum hepcidin concentration patterns throughout the day [14].

Patients (≥12 years) and parents of patients (<12 years) completed a three-day food record to assess dietary iron intake. They received instructions by a dietician before starting the record, and completed records were reviewed with them. We calculated average daily intake of iron from completed records on a personal computer using the Nutricare program (Mc Kesson, Nieuwegein, The Netherlands). We collected data of pulmonary function tests, results of bacterial sputum culture or throat swab, height and body weight. Assessments were preferably done in a stable clinical condition. However, some children were recovering from a pulmonary exacerbation that occurred shortly before the assessment. Assessments were therefore classified as acute or stable, depending on the presence or absence of an increased CRP concentration (>10 mg/L) [15] or a pulmonary exacerbation in the preceding month. A pulmonary exacerbation was defined as clinical deterioration and/or decrease in pulmonary function requiring antibiotic treatment. Children classified as acute were excluded from further analysis. Pulmonary function test was performed and forced expiratory volume in one second (FEV1) expressed as percentage predicted, was chosen as representative parameters for pulmonary function. Bacterial sputum culture or throat swab were obtained and were sent for routine culture in the Department of Microbiology at our institution. PA colonization was defined as the persistent presence of PA in sputum culture despite antibiotic treatment. Patients were defined as pancreas insufficient when fecal elastase-1 concentration was <200 μg/g stool. Height was measured to the nearest millimeter by using a stadiometer. Weight was measured to the nearest 100 g by using a digital weighting scale. Height and weight were expressed as standard deviation scores (SDS). Body mass index (BMI) (weight divided by height^2) and BMI Z-scores were calculated. All children (≥12 years) and parents of children (<12 years) gave written informed consent. The study was approved by the Medical Ethics Committee of South-West Holland.

3. Laboratory analysis

Venous blood was collected and analyzed for sTfR, hepcidin, ferritin, Hb, MCV, RDW, Ret-Hb, CRP, reticulocytes and albumin. Samples were centrifuged and serum was aliquoted and frozen at −80 °C until measurement of erythropoietin and hepcidin-25. Ferritin assays were determined using a Unicel DxI