Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014; 39(4):529–533
doi: 10.5505/tjb.2014.19480

Research Article [Araştırma Makalesi]

Yayın tarihi 5 Aralık 2014 © TurkJBiochem.com
[Published online December 05, 2014]

Serum hepcidin, iron metabolism and infection parameters in children with anemia of inflammation and with iron deficiency anemia

[Demir eksikliği ve inflamasyon anemili çocuklarda serum hepsidini, demir metabolizması ve enfeksiyon parametreleri]

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Registered: 06 September 2013; Accepted: 12 July 2014
[Kayıt Tarihi: 06 Eylül 2013; Kabul Tarihi: 12 Temmuz 2014]

ABSTRACT

Objective: Hepcidin is a key regulator of iron homeostasis. Increased hepcidin concentrations cause iron sequestration in enterocytes and macrophages. The role of hepcidin in children with iron-deficiency anemia and anemia of inflammation is unclear. In this study, we aimed to evaluate the use of serum hepcidin level as an index for iron deficiency and inflammation anemia in pediatric population. Furthermore, hepcidin is also known to be an acute-phase reactant induced by interleukin-6 (IL-6) during infection. Therefore, we investigated the relationships between hepcidin and inflammatory markers.

Methods: This study included 50 children with anemia (13 with iron deficiency and 37 with inflammation) and 17 age- and gender-matched healthy children (control group). Parameters related to iron metabolism (ferritin, serum iron and total iron binding capacity), infection (C-reactive protein, white blood cell count and neutrophil percentage) and hepcidin levels were measured.

Results: Serum hepcidin levels in patients with anemia of inflammation (362.63±132.27 ng/mL) were significantly higher than in patients with iron-deficiency anemia (234.10±93.59 ng/mL) and healthy controls (220.44±49.52 ng/mL) ($p=0.002$, $p<0.001$, respectively). Serum hepcidin levels were positively correlated with ferritin ($r=0.246$, $p=0.045$), leucocytes ($r=0.259$, $p=0.034$) and CRP ($r=0.426$, $p<0.001$) levels in all children.

Conclusion: This study suggested that hepcidin may have the potential advantage of being able to distinguish between anemia of inflammation and iron-deficiency anemia. In addition, serum hepcidin levels are significantly correlated with acute phase reactants and can be more useful marker than CRP which decreases quickly after the onset of an infection.

Key Words: Anemia, ferritin, hepcidin, infection, inflammation, iron deficiency

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Hepsidin demir metabolizmasının düzenlenmesinde anahtar rol oynar ve enterositlerde ve makrofajlarda demirin sekestrasyonuna yol açar. Demir eksikliği ve inflamasyon anemisi olan çocuklarda hepsidinin rolü açık değildir. Bu çalışmada serum hepsidin düzeylerinin demir eksikliği ve inflamasyon anemisinde bir belirleyici olarak kullanımı pediyatrik popülasyonda çalışıldı. Ayrıca hepsidinin infeksiyonlarda interlökin-6 (IL-6) tarafından indüklenen bir akut faz reaktanı olduğu bilinir. Bu nedenle serum hepsidin düzeyleri ile inflamatuar markerlar arasındaki ilişkiyi araştırdık.

Metod: Bu çalışmaya 50 anemili (37'si inflamasyon, 13'ü demir eksikliği) çocuk ve onlarla benzer yaş grubunda ve cinsiyette 17 sağlıklı çocuk (kontrol grubu) alındı. Serum hepsidin düzeyleri, demir metabolizması ile ilgili olarak ferritin, demir ve demir bağlama kapasitesi parametreleri ve infeksiyon belirteçleri olarak da CRP, lökosit sayısı ve nötrofil oranları belirlendi.

Bulgular: Serum hepsidin düzeyleri inflamasyon anemili grupta (362,63±132,27 ng/mL) demir eksikliği anemisi (234,10±93,59 ng/mL) ve sağlıklı kontrol grubundan (220,44±49,52 ng/mL) anlamlı olarak daha yüksekti ($p=0,002$, $p<0,001$,sırasıyla). Serum hepsidin düzeyleri ferritin ($r=0,246$, $p=0,045$), lökosit sayısı ($r=0,259$, $p=0,034$) ve CRP ($r=0,426$, $p<0,001$) düzeyleri ile pozitif olarak ilişkili bulundu.

Sonuç: Çalışma hepsidinin inflamasyon ve demir eksikliği anemisini ayırt etmede uygun olacağını düşündürdü. İlave olarak serum hepsidin düzeyleri akut faz reaktanları ile anlamlı olarak ilişkili olup infeksiyonların başlangıcında hızla azalmaya başlayan CRP'den daha yararlı bir marker olacağı kanısına vardık.

Anahtar Kelimeler: anemi, ferritin, hepsidin, enfeksiyon, inflamasyon, demir eksikliği

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Iron is essential to most living organisms and it has vital importance. It takes place in oxygen transport, protein synthesis, and function of enzymes. Adequate iron stores are necessary for normal childhood growth and development [1,2]. Although the biological importance of iron has been known since ancient times, there have been new developments in the field of absorption and storage of the iron during the the last decade, especially in the last 2-3 years. One of the new developments is increasing recognition of the importance of hepcidin, a type II acute phase peptide [3].

Hepcidin plays a major role in the regulation of iron homeostasis [4]. Iron overload is toxic to the organism. The organism is programmed to protect itself against harmful effects of iron. Once iron is absorbed, there is no physiological mechanism for excretion of excess iron from the body. Systemic iron balance is provided by inhibition of the absorption of dietary iron from the intestine. Hepcidin inhibits movement of iron into the plasma by binding to ferroportin that functions to export iron from cells. Ferroportin exports iron into plasma from duodenocytes, hepatocytes and erythrophagocytosing macrophages [5]. Measurement of hepcidin concentrations in serum or urine is thought to be useful to differentiate iron-deficiency anemia from anemia of infection [2]. Ferroportin is the sole known iron exporter. Hepcidin inhibits the cellular outflow of iron by binding to ferroportin. Thus, hepcidin provides inhibition of iron absorption. Hypoferremia produced by hepcidin is one of the defense mechanisms against infection. Hepcidin synthesis is repressed by both iron deficiency anemia and hypoxia. Conversely, hepcidin synthesis is increased during inflammation, decreasing plasma iron concentrations and causing anemia of inflammation. The molecular mechanisms implicated in these complex regulations are not fully understood, but the induction of hepcidin synthesis by inflammation has been shown to be interleukin (IL)-6 dependent [4].

Recent studies have discussed the role of hepcidin on iron regulation and inflammation status in adult patients [6,7]. However, there are not enough studies investigating the effectiveness of serum hepcidin levels in iron-deficiency anemia and anemia of inflammation in children [1,2,8-11].

We evaluated the relationship of serum hepcidin levels with iron deficieny and infection/inflammation status in children. This study is different from previous studies which included patients with chronic inflammatory conditions for being performed on patients with acute infections. Our results support a feedback mechanism between iron deficiency anemia and/or low ferritin with hepcidin secretion. Furthermore, since hepcidin could be classified as an acute phase reactant, we investigated its relationship with WBC count and CRP.

Material and Methods

The sudy was approved by the Patients and Methods Ethics Committee of Haseki Educational and Research Hospital (Protocol No: 30/06/2009-2009/56). Our examinations of the patients conformed to good medical and laboratory practices and the recommendations of the Declaration of Helsinki on Biomedical Research involving Human Subjects.

Patients

A total of 67 children (26 males and 41 females) were enrolled in this study. The age of the patients ranged from 15 months to 17 years.

The following patients were excluded from the study: (1) patients who have taken iron suplementation therapy within the past 4 weeks (2) patients with an evidence of active or occult bleeding (3) patients who received blood transfusion within the past 3 months (4) patients with a history of malignancy, liver disease, renal failure or chronic hypoxia.

The patients were classified according to hemoglobin (Hb) levels, iron and infection parameters as follows: Group 1: iron-deficiency anemia (n=13); Group 2: infection anemia (n=37) and Group 3: controls (n=17) with normal iron levels and without signs of infection and anemia.

Grup 1 patients were considered to have iron-deficiency anemia when (1) they had not an infection or autoimmune disease, (2) they were anemic with low Hb levels than expected for their age and gender norms used in pediatric population and, (3) they had a transferrin saturation (TSAT) less than 16%, or a serum ferritin concentrations <12 ng/mL in children aged ≤5 years or <15 ng/mL in children aged >5 years,

Grup 2 patients were accepted to have anemia of inflammation when (1) they had an infectious disease, (2) they were anemic with a Hb concentration of less than age and gender-adjusted norms used in the pediatric population.

Hematological analyses

Complete blood count, blood film, iron and total iron binding capacity (TIBC) studies were performed on each child. Complete blood count was conducted using the fully automated Pentra DX 120 blood cell counter. Serum ferritin concentrations were measured by chemiluminescent immunoassay (Advia Centaur® XP). Iron and TIBC were measured by Cobas Mire autoanalyzer. TSAT was calculated as serum iron/total iron binding capacity.

Serum hepcidin levels were determined by using a validated enzyme-linked immunosorbent assay (ELISA) kit [DRG® Hepcidin Prohormon ELISA (EIA-4015)" (Enzyme-Linked Immunosorbent Assay) International Inc. (USA)]. It is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of the competitive binding. Peripheral blood samples were centrifuged for hepcidin analyses. Serum samples were snap-frozen at −80°C and stored for one month. Reference values for serum hepcidin are between 58.9 and 158.1 ng/ml.

Statistical analyses

All data were analyzed using SPSS version 15.0 for Win-

Table 1. Demographic features and laboratory data of of the study groups

	Grup I Iron Deficiency Anemia (n=13)	Grup II Inflammation Anemia (n=37)	Grup III Healthy Control (n=17)
Age (year) (Mean.±SD)	10.44±5.24	8.36±3.87	10.48±3.71
Male, n (%)	4 (30.77)	15 (40.54)	7 (41.18)
Female, n (%)	9 (69.23)	22 (59.46)	10 (58.82)
Iron (Mean±SD)	19.35±16.09	39.24±31.74	89.53±38.81
TIBC (Mean±SD)	443.46±43.96	310.30±94.7	318.00±83.97
TSAT (Mean±SD)	4.59±4.26	12.84±10.44	37.91±47.84
Ferritin (Mean±SD)	4.10±3.29	111.88±158.2	36.93±12.8
Hemoglobin (Mean±SD)	8.39±2.20	10.35±1.71	12.88±0.77
HCT (Mean±SD)	26.91±6.18	31.41±4.99	38.38±2.90
MCV (Mean±SD)	63.30±5.99	75.32±8.50	82.88±4.15
MCH (Mean±SD)	19.70±2.90	24.94±3.78	27.72±1.64
MCHC (Mean±SD)	31.11±3.12	33.05±2.78	33.37±0.78
RDW (Mean±SD)	20.04±3.51	17.49±4.00	14.48±2.25
PLT (Mean±SD)	328.38±81.77	395.57±185.24	307.65±70.49
MPV (Mean±SD)	8.34±0.89	8.28±1.48	8.02±0.61
Leucocyte (Mean±SD)	6726.15±1838.27	8100.07±4877.93	6700.00±1367.94
CRP (Mean±SD)	0.09±0.18	4.20±7.16	0.10±0.22
Hepcidin (Mean±SD)	234.10±93.59	362.63±132.27	220.44±49.52

Table 2. P values for subgroup analysis

	Iron deficiency anemia and inflammation anemia	Iron deficiency anemia and Healthy control	Inflammation anemia and Healthy control
Iron	0.013	<0.001	<0.001
TIBC	<0.001	<0.001	0.867
TSAT	<0.001	<0.001	<0.001
Ferritin	<0.001	<0.001	0.129
Hemoglobin	0.001	<0.001	<0.001
HCT	0.005	<0.001	<0.001
MCV	<0.001	<0.001	0.001
MCH	<0.001	<0.001	<0.001
MCHC	0.002	<0.001	0.050
RDW	0.012	<0.001	<0.001
CRP	<0.001	0.303	<0.001
Hepcidin	0.002	0.939	<0.001

dows. Numbers and percentages were given for descriptive statistics and categorical variables. The numeric variables were given as mean±SD. Student's t-test, Tukey's test and one-way ANOVA were used in data analyses. Mann-Whitney U test was commented by Bonferroni correction in subgroup analysis. The groups were compared using the Kruskal-Wallis tests. The degree of relationship between the variables was calculated using Pearson's correlation coefficient for numerical data and Spearman's correlation coefficient for non numerical data. Analysis of categorical variables in independent samples was performed by the chi-square test. The factors affecting the level of numeric variable were determined by linear regression analysis. A p value of less than 0.05 was considered statistically significant.

Results

There were no significant differences in the clinical characteristics between the 3 groups with respect to age and gender. In addition, there was no significant difference in WBC count, platelet (PLT) count and mean PLT volume (MPV) between the groups (Table 1, 2).

Hb, mean corpuscular volume (MCV), mean corpuscu-

lar hemoglobin concentration (MCHC), serum iron and TSAT were all significantly lower in children with anemia (iron deficiency and inflammation) compared to controls. As similar, there were significant differences in these parameters between children with iron-deficiency anemia and those with anemia of inflammation. TIBC was significantly higher in children with iron-deficiency anemia compared to children with anemia of inflammation and healthy controls, meanwhile, there was no significant difference in this parameter between children with anemia of inflammation and healthy controls ($p>0.05$). Ferritin was significantly lower in children with iron-deficiency anemia compared to children with anemia of inflammation and healthy controls; meanwhile, there was no significant difference in this parameter between children with anemia of inflammation and healthy controls ($p>0.05$) (Table 1, 2).

Serum hepcidin was significantly increased in children with anemia of inflammation (362.63±132.27) compared to children with iron-deficiency anemia (234.10±93.59) and healthy controls (220.44±49.52) ($p=0.002$, $p<0.001$, respectively); meanwhile, there was no significant difference in serum hepcidin levels between children with iron-deficiency anemia and healthy controls ($p>0.05$) (Table 1, 2).

Serum hepcidin was positively corelated with ferritin ($r=0.246$; $p<0.05$), leukocytes ($r=0.259$; $p<0.05$) and CRP ($r=0.426$; $p<0.001$). There was no association of hepcidin with Hb, MCV, MCHC, serum iron, TIBC and TSAT (Table 3).

Table 3. Factors correlated with serum Hepcidin levels in children (only significant correlations shown)*

	Hepcidin	
	All Groups	
	r	p
Ferritin	0.246	0.045
CRP	0.426	<0.001
Leucocyte	0.259	0.034

*Spearman's correlation analysis.

Discussion

Human hepcidin was defined as an antimicrobial peptide, an acute-phase reactant, and a key regulator of iron homeostasis. In the literature, there have been studies investigating the most important role of hepcidin. First studies have demonstrated that hepcidin has antimicrobial activity against bacteria and fungi [12]. Subsequent studies have demonstrated that hepcidin is an acute-phase reactant and the keystone of iron homoeostasis. Hepcidin expression is regulated by interleukin-6 during bacterial infection and inflammation and it inhibits intestinal iron absorption and iron release from macrophages and hepatocytes in this status [13]. Despite these discoveries about the role of hepcidin on absorption and storage of iron, all of these data cannot be used yet in practice.

Anemia of inflammation, also previously known as anemia of chronic disease, is the most frequent anemia in hospitalized patients and develops in patients with acute or chronic inflammatory conditions, including infections, malignancies, or autoimmune disorders [14]. In this study, we included children with anemia of inflammation due to infection (bacterial pneumonia, empyema, cellulitis, etc.).

Infection, inflammation and iron overload are the important inducers of hepcidin synthesis. They increase the production of hepcidin in hepatocytes. Nicholas et al. [15], in an animal experiment, produced acute-phase inflammation with a single injection of turpentine (0.1 ml/20 g of body weight) in normal mice. After injection of turpentine, a sixfold increase in liver hepcidin mRNA levels and a twofold decrease in serum iron were observed. However, this effect of turpentine-induced decrease of serum iron was not observed in hepcidin-defficient mice.

We found increased hepcidin concentrations in patients with anemia of inflammation compared with controls and subjects with iron-deficiency anemia. Our results were similar to that of study by Theurl et al. [14]. Unlike our study, they divided the subjects into two groups: those with only anemia of inflammation and those with anemia of infammation/iron-deficiency anemia. Importantly, subjects with anemia of inflammation only had significantly higher serum hepcidin levels than the latter group patients. These results emphasize the role of hepcidin on anemia of inflammation.

A study on anemic Tanzanian children has shown that high concentrations of urine hepcidin were associated with malaria, which could contribute to malarial anemia [8]. In addition, it has been shown that asymptomatic malarial parasitemia was associated with increased serum hepcidin concentrations and anemia in Indonesian children aged 5–15 years [9]. In another study performed on children, the hepcidin levels were found to be low in patients with iron-deficiency anemia. In contrast to the first two studies, this study did not show increased hepcidin levels in children with *Helicobacter pylori* and helminth infection [2].

In a study on elderly patients, any significant difference in prohepcidin levels was not observed between the groups of iron-deficiency anemia and anemia of inflammation [16]. This study suggested that serum prohepcidin does not help distinguish between iron-deficiency anemia and anemia of inflammation in elderly patients.

All these findings show the need for studies on anemia of inflammation due to different causes at different age groups.

In our study, mean serum hepcidin levels were similar in patients with iron-deficiency anemia and in healthy con-

trols (220.44±49.52ng/mL). This result is not compatible with the literature [1]. This finding can be attributed to the small number of patients with iron-deficiency anemia. In addition, serum hepcidin levels in healthy controls and iron deficiency group in our study were higher than the reference values. Although commercial tests are available, the absolute values of hepcidin have not been established. It has been reported that reference values changed according to age, gender and the method used [1,6,11]. Although there are large-scale population studies in adults; there are no studies on children on this scale [6]. Therefore, it is necessary to establish a reference range of serum hepcidin levels for children.

We did not demonstrate a corelation of hepcidin with Hb and MCV. Similar to our results, van Eijk et al. [7] did not find a relationship of hepcidin with Hb and MCV. These findings showed that increased hepcidin levels can not be attributed only to anemia. Serum hepcidin levels were positively correlated with ferritin, leukocytes and CRP levels in our study. Our results show that hepcidin has a role of acute-phase reactant in some cases. In a recent study, serum hepcidin levels were significantly positively correlated with the levels of ferritin, iron, TSAT, and Hb, and negatively correlated with TIBC [1]. In their study, Galesloot et al. [6] showed a strong corelation between ferritin and hepcidin both in men and women and a less strong association of hepcidin with CRP and TIBC was observed in men. This study is significant because it was performed in a large, well-phenotyped sample of the general population and provided age- and sex-specific reference ranges of hepcidin concentration. However, unlike our study, it included only adult subjects. In another study on Kenyan infants aged 6.0±1.1 months, serum hepcidin levels were corelated with CRP and ferritin [4]. Thus, all these results suggest that serum hepcidin levels are linked to both iron status and infection-inflammation status.

A limitation of this study is that the group with anemia of inflammation was not homogenous. Etiologies of infection were different.

In conclusion, we demonstrated high levels of hepcidin in children with anemia of inflammation. Hepcidin may have the potential advantage to distinguish between anemia of inflammation and iron-deficieny anemia in practise. Furthermore, it may be useful as an acute phase reactant indicator in cases with infection and inflammation.

Acknowledgments

We would like to thank all participants in this study. This work was supported, in part, by Turkish Pediatrics Association. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest

There are no conflicts of interest among the authors.

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