

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
 Open Access Macedonian Journal of Medical Sciences. 2020 Jun 20; 8(B):524-529.
<https://doi.org/10.3889/oamjms.2020.4268>
 eISSN: 1857-9655
 Category: B - Clinical Sciences
 Section: Pediatrics



Hepcidin and its Related Hematological Biomarkers of Anemia in Children on Hemodialysis: Role of Carnitine Deficiency

Safinaz E. El-Toukhy¹, Fatina Fadel², Manal F. Elshamaa^{3*}, Gamila S. M. El-Saeed¹, Hanan Abdelaziz², Marwa Elsonbaty^{4,5}, Eman A. Elghouroury⁶, Eman.Hamza⁶, Soha Atef⁶

¹Department of Medical Biochemistry, National Research Centre, Cairo, Egypt; ²Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt; ³Department of Pediatrics, National Research Centre, Cairo, Egypt; ⁴Department of Child Health, Medical Research Division, National Research Centre (Affiliation ID: 60014618), Cairo, Egypt; ⁵Department of Pediatrics, College of Medicine, Taibah University, Al-Madinah Al-Munawwarah, Kingdom of Saudi Arabia; ⁶Department of Clinical and Chemical Pathology, National Research Centre, Cairo, Egypt

Abstract

BACKGROUND: Anemia is one of the most common complications in end-stage renal disease (ESRD) patients. Hepcidin is a hormone that regulates iron homeostasis in patients with ESRD. Carnitine deficiency is commonly seen in hemodialysis (HD) patients.

AIM: This study aimed to investigate the relationship between hepcidin and inflammatory and other anemia markers in children with ESRD and to evaluate the association of carnitine deficiency with anemia in these patients.

SUBJECTS AND METHODS: Thirty pediatric patients with ESRD undergoing HD, and thirty healthy, age- and sex-matched children served as controls were included in the study. Serum levels hepcidin, iron status, high-sensitivity C-reactive protein, and total carnitine were measured.

RESULTS: Statistically significant increases in serum levels of hepcidin (100.7 ± 0.99 ng/ml vs. 77.43 ± 0.8 ng/ml, $p = 0.000$), was found in HD children as compared to healthy controls. Statistically significant increase in serum levels of hs-CRP (3.94 ± 0.19 mg/l vs. 1.36 ± 0.07 mg/l, $p = 0.04$) was found in HD children as compared to healthy controls. However, serum levels of carnitine (29.59 ± 2.46 μ mol/L vs. 36 ± 2.39 μ mol/L, $p = 0.000$) showed statistically significant decreases in HD children as compared to healthy controls positive correlation was found between hepcidin and hs-CRP ($r = 0.059$, $p = 0.042$). Furthermore, a positive correlation was present between serum carnitine levels and serum iron levels ($r = 0.651$, $p = 0.042$).

CONCLUSION: Serum hepcidin may be a more useful biomarker of functional iron deficiency in children on HD. The efficacy of carnitine treatment for children on HD with carnitine deficiency and its effect on anemia needs to be studied.

Edited by: Mirko Spiroski
Citation: El-Toukhy SE, Fadel F, Elshamaa MF, El-Saeed GSM, Abdelaziz H, Elsonbaty M, Elghouroury EA, Hamza E, Atef S. Hepcidin and its Related Hematological Biomarkers of Anemia in Children on Hemodialysis: Role of Carnitine Deficiency. Open Access Maced J Med Sci. 2020 Jun 20; 8(B):524-529. <https://doi.org/10.3889/oamjms.2020.4268>
Keywords: Hepcidin; High-sensitivity C-reactive protein; Carnitine; Anemia; Inflammation; Children; Hemodialysis
***Correspondence:** Manal F. Elshamaa, Department of Pediatrics, National Research Centre, Cairo, Egypt. E-mail: manal_elshamaa@hotmail.com
Received: 06-Jan-2020
Revised: 14-Apr-2020
Accepted: 15-Apr-2020
Copyright: © 2020 El-Toukhy SE, Fadel F, Elshamaa MF, El-Saeed GSM, Abdelaziz H, Elsonbaty M, Elghouroury EA, Hamza E, Atef S.
Funding: This study was supported by the National Research Centre, Cairo, Egypt
Competing Interests: The authors have declared that no competing interests exist
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Introduction

Anemia is one of the most significant leading factors of progression of chronic kidney disease (CKD) to end-stage renal disease (ESRD) [1], [2]. Active blood loss, hemoglobinopathies, aluminum overload, and hypothyroidism are leading factors of anemia in patients with ESRD [3]. In addition, anemia is exaggerated by oxidative damage of RBCs membrane in chronic hemodialysis (HD) patients which shorten erythrocytes lifespan [4]. The inflammatory mechanisms result in oxidative stress which in turn participates in anemia of ESRD patients. Depletion of redox capacity with membrane structural deformity and decreased life span of erythrocytes is tagged as mechanisms. This, in turn, increases the production of hepcidin [5].

Hepcidin (a 2.7-kD peptide) is a hormone that binds to ferroportin on the cell wall and diminishes expression of iron transport protein transferrin and

induces erythropoietin resistance, so it inhibits intestinal absorption and mobilization of iron stores [6], [7]. Induction of ferroportin degradation and increase intracellular iron stores is a known function of hepcidin. Consequently, H-ferritin, an iron-binding ferroxidase with cytoprotective function is induced [8]. Restoration of iron homeostasis induced by hepcidin is accompanied by reduced reactive oxygen species, apoptosis, and inflammation and approximately 70% improvement of renal function after ischemic renal injury [9].

High levels of inflammatory markers are associated with erythropoiesis-stimulating agents (ESAs) resistance in CKD patients [10]. C-reactive protein (CRP) is the gold standard micro-inflammatory marker in HD that predicts mortality. Its level significantly rises in comparison with other acute-phase proteins, considering it a reliable tool in clinical evaluation [11].

Hepcidin levels raise more when patients with HD or peritoneal dialysis, because they are exposed to intermittent infections and exposure of blood to

foreign materials such as catheters and dialysis membranes [12]. The peptide level declines during HD because it is cleared through the membrane [13]. A rebound of hepcidin concentrations happens within an hour after finishing dialysis due to the high rate of its synthesis [14]. Iron requirements increase during HD because of blood loss from discarded residual blood in the dialysis equipment, from the hemorrhagic effects of anticoagulation, and from the blood needed for the frequent laboratory examinations [15], [16].

Carnitine is a low-molecular-weight compound synthesized in the kidney, liver, and brain. It can also be obtained from the diet [17]. Carnitine is an essential amino-acid derivative having vital roles in fatty-acid metabolism in cardiac muscle and skeletal muscle [18]. Carnitine is present in two forms in the body, acylcarnitine, and free carnitine, and the sum of them is defined as total carnitine [19]. Free carnitine binds to an acyl residue and converted to acylcarnitine. In addition, acylcarnitine functions as a transporter of fatty acids to mitochondria and as a scavenger of excess and harmful acyl residues in cells [20]. It has a significant function in organizing energetic substrates flow and their balance through the cell wall. The nutritional status of HD patients is positively affected by carnitine by enhancing a positive protein balance, and by reducing insulin resistance and chronic inflammation [18]. Carnitine is vital for RBCs to perform their metabolic function in renal anemia [19] that can have a negative impact on erythrocyte production and survival [20]. Carnitine deficiency is reported to be associated with several pathological conditions, including cardiac dysfunction, muscle weakness and anemia. Therefore, it is important to promptly detect carnitine deficiency and ensure appropriate carnitine supplementation in patients on HD [20] this study aimed to investigate the relationship between hepcidin and inflammatory and other anemia markers children with ESRD and to evaluate the association of carnitine deficiency with anemia in these patients.

Subjects and Methods

Thirty children with ESRD undergoing HD, at the HD unit of the Centre of Pediatric Nephrology and Transplantation, Children's Hospital, Cairo University, were investigated. Children on regular HD treatment for not <4 months, using bicarbonate dialysate, and free from apparent acute illness were included in the current study. Children with malignancies and active infectious disease, those who had been hospitalized or had undergone surgery or renal transplantation during the 3 months before the study were excluded from the study. All patients were dialyzed using a polysulfone dialyzer, with a bicarbonate dialysate, using a blood flow rate of 80–150 ml/min and a dialysate flow rate of 500 ml/min. Each patient was dialyzed 3 times per-week using

polysulfone membranes. The dialysate fluids were prepared from concentrated salt solutions and from bicarbonate powder in sealed containers.

Thirty healthy, age- and sex-matched children were recruited from the pediatric clinic of the National Research Centre (NRC) to serve as controls. A full history was obtained from all the patients, and all of them underwent a thorough clinical examination. Written consent was obtained from the parents of each participant. The study was approved by the Ethical Committee of the NRC in Egypt (ID = 1001008, 2015).

Blood sampling

Peripheral venous blood samples were withdrawn from all subjects after overnight fast and in HD patients before the session of dialysis. After centrifugation at 3500 rpm at 4°C for 15 min, sera were separated and stored at –80°C until assayed.

Biochemical analysis

Serum levels of iron, ferritin, total iron-binding capacity (TIBC), hemoglobin, and albumin (ALB) were measured for all participants using an automatic biochemistry analyzer (Olympus America Inc., Center Valley, Pennsylvania, USA). The determination of high sensitivity CRP (hsCRP) in serum was performed by a solid-phase chemiluminescent immunometric assay (Immulite/Immulite 1000; Siemens Medical Solution Diagnostics, Eschborn, Germany). Serum levels of hepcidin prohormone were measured using enzyme-linked immunosorbent assay (Flughafenstrasse 52aD-2233 Hamburg, Germany [ELISA] IBL International GMBH).

Quantitative evaluation of serum total carnitine level by rapid liquid chromatography-electrospray tandem mass spectrometry (MS): To 20 µL of a serum sample, 300 µL of an internal standard solution was added containing 3.3 µmol/L 2H3-L-carnitine in acetonitrile to precipitate the serum protein in an Eppendorf tube. The mixture was vortex-mixed and sonicated for 10 min to improve the precision of the extraction procedure before centrifugation (7000 g for 5 min). The supernatant was then transferred to a sample vial for LC-MS/MS analysis. A series of aqueous calibrators, ranging from 0 to 100 µmol/L was used.

Statistical analysis

Data were analyzed using SPSS version 17. Data were expressed as mean ± standard deviation for quantitative variables, number, and percentage for qualitative ones. Comparisons between the experimental groups were done by the Student's t-test. Pearson's correlation was performed to correlate the individual variables. $p < 0.05$ was considered to be

statistically significant and $p = 0.001$ was considered to be statistically highly significant.

Results

Detailed patient characteristics were given in Table 1.

Comparison between HD patients and controls as regards to serum hepcidin and anemia markers levels. Statistically significant increases in serum levels of hepcidin (100.7 ± 0.99 ng/ml vs. 77.43 ± 0.8 ng/ml, $p = 0.000$) and serum levels of ferritin (968.10 ± 5.3 ng/ml vs. 674.00 ± 4.56 ng/ml, $p = 0.000$) were found in HD children as compared to healthy controls. However, serum iron levels (65.62 ± 0.62 mg/l vs. 90.4 ± 0.73 mg/l, $p = 0.000$) and serum TIBC (234 ± 0.67 mg/l vs. 258 ± 0.89 mg/l, $p = 0.000$) showed statistically significant decrease in HD children as compared to healthy controls Figure 1.

Comparison between HD patients and controls as regards to serum carnitine and inflammatory markers levels

Statistically significant increase in serum levels of hs-CRP (3.94 ± 0.19 mg/l vs. 1.36 ± 0.07 mg/l, $p = 0.04$) was found in HD children as compared to healthy controls. However, serum levels of carnitine (29.59 ± 2.46 μ mol/L vs. 36 ± 2.39 μ mol/L, $p = 0.000$), serum levels of hemoglobin (11.41 ± 0.53 g/dl vs. 14.23 ± 0.75 g/dl, $p = 0.000$), and serum levels of albumin (3.62 ± 0.13 g/dl vs. 4.92 ± 0.39 g/dl, $p = 0.000$) showed statistically significant decreases in HD children as compared to healthy controls Figure 2.

Pearson's correlations between different studied biochemical parameters in HD children were shown in Table 2

A negative correlation was found between serum hepcidin levels and serum albumin levels ($r = -0.371$, $p = 0.011$), but a positive one was found between serum hepcidin levels and serum hs-CRP levels ($r = 0.059$,

$p = 0.042$) in HD children. Positive correlations were present between serum carnitine levels and both of serum ferritin levels and serum iron levels ($r = 0.644$, $p = 0.045$ and $r = 0.651$, $p = 0.042$, respectively). Considering serum ferritin levels, positive correlations were observed with serum albumin levels ($r = 0.682$, $p = 0.03$) and serum TIBC levels ($r = 0.652$, $p = 0.03$).

No correlation was found between serum hepcidin levels and serum carnitine levels ($r = 0.052$, $p = 0.887$).

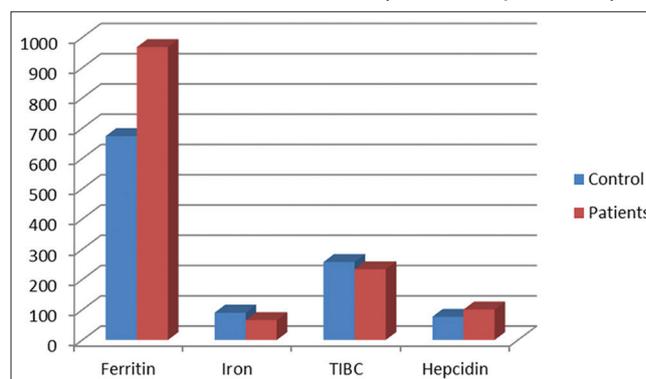


Figure 1: Comparison between hemodialysis patients and controls as regards to serum hepcidin and anemia markers levels. Serum levels of ferritin (968.10 ± 5.3 ng/ml vs. 674.00 ± 4.56 ng/ml, $p = 0.000$), serum iron levels (65.62 ± 0.62 mg/l vs. 90.4 ± 0.73 mg/l, $p = 0.000$), serum levels of TIBC (234 ± 0.67 mg/l vs. 258 ± 0.89 mg/l, $p = 0.000$), and serum levels of hepcidin (100.7 ± 0.99 ng/ml vs. 77.43 ± 0.8 ng/ml, $p = 0.000$). $p < 0.05$ was considered to be statistically significant and $p < 0.001$ was considered to be statistically highly significant. TIBC: Total iron-binding capacity

Discussion

In the present study, hepcidin revealed a statistically significant increase in children on HD

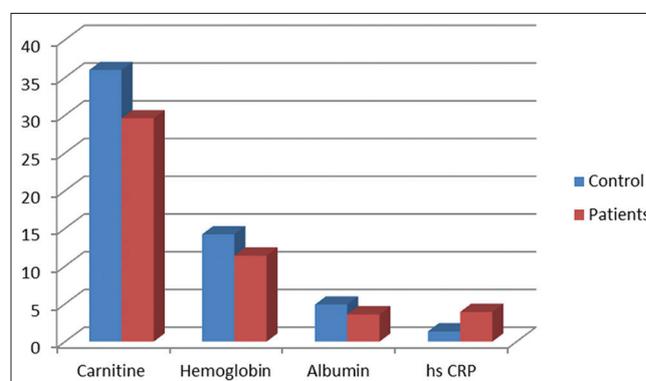


Figure 2: Comparison between hemodialysis patients and controls as regards to serum carnitine and inflammatory markers levels. Serum levels of carnitine (29.59 ± 2.46 μ mol/L vs. 36 ± 2.39 μ mol/L, $p = 0.000$), serum levels of hemoglobin (11.41 ± 0.53 g/dl vs. 14.23 ± 0.75 g/dl, $p = 0.000$), serum levels of albumin (3.62 ± 0.13 g/dl vs. 4.92 ± 0.39 g/dl, $p = 0.000$), and serum levels of hs-CRP (3.94 ± 0.19 mg/l vs. 1.36 ± 0.07 mg/l, $p = 0.04$). $p < 0.05$ was considered to be statistically significant and $p < 0.001$ was considered to be statistically highly significant. hsCRP: High sensitivity C-reactive protein

Table 1: Descriptive and biochemical characteristics of the studied subjects

Parameters	Controls (n=30)	Patients (n=30)	p-value
Age (years)	8.7 \pm 0.51	10.62 \pm 0.49	0.14
BMI (kg/m ²)	20.88 \pm 1.1	18.89 \pm 0.87	0.17
Sex (M/F) %	20/10	23/7	0.31
SBP (mmHg)	94.55 \pm 9.80	125.13 \pm 16.36	0.01*
DBP (mmHg)	60.59 \pm 10.11	83.13 \pm 12.76	0.01*
Urea (mg/dl)	7.80 \pm 2.64	70.56 \pm 19.61	0.02*

Data are mean \pm SD, number (%) or range as applicable. Significance was estimated using independent t-test. BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. * $p < 0.05$, ** $p < 0.001$. $p < 0.05$ was considered to be statistically significant and $p < 0.001$ was considered to be statistically highly significant.

Table 2: Pearson's correlations between different studied biochemical parameters in HD children

Parameters	Carnitine		Hepcidin		Hemoglobin		Albumin		hsCRP		Ferritin		Iron		TIBC	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Carnitine	-	-	0.052	0.887	-0.155	0.670	-0.0371	0.291	0.059	0.870	0.644*	0.045	0.651*	0.042	-0.102	0.780
Hepcidin	0.052	0.887	-	-	-0.155	0.836	-0.371	0.011	0.059	0.042	-0.644*	0.363	0.651*	0.869	-0.102	0.443

Pearson's correlation coefficient was used. $p < 0.05$ was considered to be statistically significant and $p < 0.001$ was considered to be statistically highly significant. hsCRP: High sensitivity C-reactive protein, TIBC: Total iron-binding capacity.

when compared to their healthy controls. Moreover, hepcidin levels were positively correlated with hs-CRP and negatively correlated with albumin, which was in agreement with Zaritsky *et al.* [13]. Hepcidin levels are regulated by the erythropoietic activity and iron status [14]. It is now well known that hepcidin levels are increased by inflammation and reduced by anemia and hypoxia. Renal anemia is considered a special form of anemia of inflammation [14]. The decrease in both iron uptake from the small intestine and release of iron from macrophages as well as decreased placental iron transport was recorded to be associated with increased hepcidin levels. Hepcidin levels are predicted to be increased in patients with ESRD due to restricted hepcidin excretion in urine, tissue iron overload, and inflammation [21-24].

In this study, we found decreased levels of serum iron and TIBC in children on HD. Nevertheless, serum ferritin levels were found to be increased in this group. These findings were consistent with previous studies on patients with CKD [14], [18]. Multiple mechanisms are incorporated in the disruption of iron metabolism in advanced kidney diseases [15]. Iron deficiency may be caused by high hepcidin concentrations [12], as a result of decreased iron absorption [25], in addition to increased iron losses, mainly from gastrointestinal losses [26]. Iron binding effects in intra- and extracellular spaces of iron-chelating agents might be of benefit in renal injury [27].

High ferritin levels found in these HD children might be due to functional iron deficiency or reticuloendothelial blockade [14]. Serum ferritin is a good indicator of iron stores when there is no inflammation. In the presence of inflammation, hepcidin stimulates ferritin production and macrophages, proliferative secretors of serum ferritin, trap iron inside it [28]. Many previous studies support the idea that current markers of iron metabolism such as transferrin concentration and ferritin do not predict iron status carefully [14]. These conventional markers had some limitations as it can be affected by age, gender, inflammation, and nutritional factors. It was reported that the determination of hepcidin concentrations together with conventional markers of iron metabolism could improve the identification of patients with iron deficiency by 26.1% [29].

In this study, hsCRP was measured as the most significant marker of inflammation and was found to be higher HD children than in healthy controls, and these levels were found to be significantly correlated with serum hepcidin. It is well known that hepcidin synthesis is induced by inflammation, a process that is mediated by IL-6. CKD patients undergoing HD have high levels of inflammatory markers such as

CRP, IL6, tumor necrosis factor-alpha, and interferon-c accompanied with low serum levels of albumin which was in accordance with the current study [30].

The present study showed decreased serum carnitine levels in children on HD and was found to be positively correlated with serum iron levels. Previous studies had been concluded a high prevalence of carnitine deficiency in patients on HD [17], [18], [19], [20]. Carnitine loss through dialysis machines was concluded to be associated with the deficiency of carnitine in HD patients. Loss of carnitine during dialysis, in addition to the reduced renal synthesis and decreased intake of meat and dairy products (dietary source of carnitine), had been proposed as a cause of carnitine deficiency in the ESRD population [31], [32]. Several studies had concluded that carnitine deficiency was associated with anemia [17], [33], [34]. Carnitine contributes to erythrocyte membranes stabilization, allowing an improvement of their deformability [17], [33], [34]. The National Kidney Foundation had proposed in a conference report that carnitine deficiency in patients on HD was a dialysis-related carnitine disorder [35]. They recommended carnitine supplementation for patients on HD who have erythropoietin-resistant anemia, hypotension during HD sessions, cardiac dysfunction, and muscle weakness [35]. Therefore, evaluation of carnitine deficiency together with appropriate carnitine supplementation is important for treating children on HD.

Conclusions

Serum hepcidin levels were shown to be increased in children on HD. It may be a more useful biomarker of anemia in these patients. Serum hepcidin provides a piece of good utility information about the iron status during inflammation as compared with conventional markers of iron status. Carnitine deficiency was found in children on HD and was significantly correlated with the iron levels. Further studies on the efficacy of carnitine treatment for children on HD with carnitine deficiency and its effect on anemia need to be done.

Acknowledgment

The authors would like to thank NRC, Egypt, for continuous support.

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