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Role of Hepcidin in Pediatric Chronic Kidney Disease with Anemia

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ABSTRACT

Introduction: Anemia is a frequent complication of chronic kidney disease (CKD) in children and it causes an increase in morbidity, mortality and accelerates the rate of progression of CKD. Inflammation and impaired kidney clearance increase plasma hepcidin, inhibiting duodenal iron absorption and sequestering iron in macrophages. However, the role of hepcidin in increasing the risk of anemia in children with CKD is still uncertain. This study aimed to investigate the association between hepcidin levels and anemia in children with pre-dialysis CKD.

Methods: A cross-sectional study was conducted at Dr. Soetomo Academic Hospital from December 2018 to February 2019. Children with pre-dialysis CKD were enrolled in this study. The subject had no history of erythropoietin administration and blood transfusion 3 months before the blood sample were withdrawn. A complete blood count, ferritin serum, transferrin saturation (TSAT) and hepcidin serum were performed. The correlations between Hepcidin and ferritin level, between ferritin level and anemia, and between TSAT and anemia were analyzed using Spearman correlation and the Mann-Whitney test.

Results: A total of 47 children, 27 boys and 20 girls, ranged in age from 3 months to 18 years old. There was a significant correlation between hepcidin and ferritin levels (p=0.006) and the value of the Spearman correlation was r=0.392. While the correlation between ferritin level and anemia showed a significant result, p=0.001. However, TSAT did not show any significant correlation with anemia (p=0.230).

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Conclusion: There was an indirect association between hepcidin level and anemia by increasing ferritin level that induces anemia in pre-dialysis CKD children.

Keywords: Hepcidin; anemia; children; chronic kidney disease

Introduction

Anemia is highly prevalent in CKD and the severity increases as the disease advances and is associated with poor prognosis and increased mortality[1]. Data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) revealed the prevalence of anemia in children with CKD, ranging from 73 to 93%, depending on the CKD stages[1], [2]. While data from Dr. Soetomo Academic Hospital, Surabaya, Indonesia from January 2010 to February 2014 revealed the prevalence of anemia was 73.2%[3]. Anemia is an important risk factor for the development and progression to end-stage renal disease[1], [2].

Hepcidin is being extensively studied for its association with anemia in CKD where it has also been associated with inflammation. Hepcidin is thought to be the major regulator of dietary iron absorption and cellular iron release from macrophages, and it exerts its regulatory by function contracting the function of ferroportin, the major cellular iron exporter. Hepcidin induces internalization and degradation of ferroportin which results in increased intracellular iron stores, decreased dietary iron absorption and decreased circulating iron levels which may be the cause of functional iron deficiency (FID)[4]–[6]. Inflammation is a characteristic feature of CKD and is caused by multiple factors, such as the toxic uremic milieu and the dialysis procedure itself. The interpretation of iron biomarkers is hindered by inflammation, which can directly affect the concentrations of most iron biomarkers[7], [8], including ferritin and hepcidin[8]–[10]. Inflammation in CKD increases ferritin and hepcidin independent of iron status which reduces iron availability[9]. High hepcidin levels inhibit iron absorption from the gut and release from iron-storing cells, thus restricting erythropoiesis and leading to anemia[10], [11]. However, the role of hepcidin in increasing the risk of anemia in children with CKD is still uncertain. This study aimed to investigate the association hepcidin levels and anemia in children with pre-dialysis chronic kidney disease.

Methods

A cross-sectional study was conducted in Nephrology Division, Department of Child Health, Dr. Soetomo Academic Hospital, Faculty of Medicine Airlangga University, Surabaya-Indonesia from December 2018 to February 2019. Pre-dialysis patients with CKD from the age of 3 months to 18 years old were enrolled after obtaining informed consent from their parents. Patients having erythropoietin, blood transfusion, supplementation of iron and acute infection within 3 months before the blood sample was withdrawn and

excluded from the study.

CKD was defined according to the National Kidney Foundation/Kidney Disease Outcome Quality Initiative (NKF/KDOQI, 2003). CKD is defined as abnormalities of kidney structure or function of the kidney, with or without decreased GFR, or GFR < 60 ml/minute/1.73 m^2 for ≥ 3 months, with or without kidney damage.

The pre-dialysis patients that were included in the study had CKD with stage 1-5 pre-dialysis. Anemia was defined according to KDIGO Anemia 2012, diagnosis anemia in children with CKD if Hb concentration is <11.0 g/dL (< 11 g/dL in children 0.5-5 years, <11.5 g/dL in children 5-12 years, and < 12.0 g/dL in children 12-15 years), and anemia in children > 15 years with CKD when the Hb concentration is < 13.0 g/dL in males and < 12.0 g/dL in females.

Six ml of venous blood samples were drawn using fresh Ethylene diamine tetra acetic acid (EDTA). The serum and plasma were obtained after centrifugation for at least 15 minutes at 2200-2500 RPM within one hour of collection and then frozen at -80°C before laboratory analysis was done. Complete blood count, iron profile, creatinine, and urea were measured using standard laboratory methods (automated system) in a central laboratory. While, commercially available kits were used to measure hepcidin-25 (by sandwich enzyme-linked immunosorbent assay (ELISA) methods, human Hepc[Hepcidin] kit e-lab science biotechnology). The study was approved by the institutional Ethical Committee at Dr. Soetomo Academic Hospital (No. 0835/KEPK/XII/2018).

Baseline characteristics were assessed with standard descriptive statistics. The Kolmogorov-Smirnov test was used to determine the normalcy of the data. If normalcy was rejected, the nonparametric test was used. Continuous variables were presented as mean ± standard deviation (SD) and median with interquartile range (as applicable). Quantitative variables were determined using the Mann-Whitney test (for nonparametric data) between ferritin and anemia. The Spearman correlation coefficient was used to find a correlation between Hepcidin with ferritin levels, between ferritin levels with anemia and between TSAT with anemia. The data were entered into SPSS 17, and analysis was performed using statistics softwareStatistical significance was defined as $p<0.05$

Result

A total of 47 patients were enrolled in this study. The majority (38.2%) of the patients had lupus nephritis, nephrotic syndrome (23.4%), urology disorder (23.4%), tubulopathy (10.6%), and others (4.3%). In pre-dialysis CKD stages 1, 2, 3, 4, and 5, there were 16, 6, 12, 7, and 6 patients. Anemia occurred in 26 patients (55.3%) and without anemia in 21 patients (44.7%). Various parameters in pre-dialysis CKD in table 1. There was a significant correlation between hepcidin and ferritin level p=0.006(<0.05), the value of Spearman correlation coefficient was 0.392 (positive correlation). The ferritin level showed a significant correlation with anemia, p=0.001(<0.05). However, TSAT did not show any significant relation with anemia p=0.230(>0.05).

Table 1. Various parameters in pre-dialysis chronic kidney disease

Parameters	CKD anemia n=26	CKD without anemia n=21
	Mean/IQR	Mean/IQR
Age (months)	135.6	163.8
Male:female (%)	53.8:46.2	61.9:38.1
Hemoglobin (g/dL)	9.9 (5.4-13.1)	13.7 (11.1-16.3)
Ureum (mg/dL)	35.0 (9-123)	15.5 (4-44)
Creatinine (mg/dL)	2.65 (0.49-17.18)	0.44 (0.24-11.9)
eGFR (mL/min/1.73 m^2)	84.4	109.8
Iron (ug/dL)	54.8	68.0
TIBC (ug/dL)	195.0	286.0
TSAT (%)***	35.6	25.8
Ferritin (ug/L)**	396.3 (3.17-1825.6)	78.8
Hepcidin (ng/mL)*	32.3 (2.94-137.7)	69.6 (3.0-1192.0)

p<0.05: Statistically significant
*Spearman correlation between hepcidin (ng/mL) and ferritin (ug/L) level in pre-dialysis CKD (*p*=0.006; r=0.392)
** Spearman correlation between ferritin level and anemia, (*p*=0.001)
***Spearman correlation between TSAT and anemia (*p*=0.230)
CKD chronic kidney disease, IQR interquartile range, eGFR estimated glomerular filtration rate, TIBC total iron-binding capacity, TSAT transferrin saturation.

Discussion

Anemia guidelines for CKD patients consider that TSAT and ferritin are important markers of anemia in CKD, and iron replacement is based on TSAT and serum ferritin levels[12]. Hepcidin has an important role to identify types of anemia in children. Anemia in CKD showed a high hepcidin level, while, various in TSAT, ferritin, and soluble transferrin receptor. In iron deficiency anemia showed a low level of hepcidin, TSAT, ferritin and an increase of soluble transferrin receptor[13].

Inflammation has been implicated in many complications in CKD including malnutrition, atherosclerosis, and decreased iron utilization[14]. Inflammation stimulates hepcidin expression through various mechanisme[15]. Pro-inflammation cytokines are increased in CKD[8], [9]. Pro-inflammation cytokines

such as IL-Iβ and IL-6 stimulate hepcidin expression via the Janus kinase (JAK)/signal transducer and activator of the transcription 3 (STAT) pathway[15]. Inflammation induces another cytokine active B which stimulates the BMP-6/SMAD pathway synergic ally with IL-6 and STAT3, leading to hepcidin expression[16]. Endoplasmic reticulum (ER) stress associated with inflammation increases hepcidin by activating

SMAD1/5/8[17], IL-6-dependent phosphorylated STAT3 and ER stress-activated transcription factor, cyclic AMP response element-binding protein H (CREBH), which bind and activate hepcidin promoter activity[9], [10]. Inflammation inhibits MT-2 by suppressing STAT5[18] and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) which antagonized LPS-induced hepcidin transcription via the interaction with hepatocyte nuclear factor 4α[10], leading to activation of hepcidin translation. Inflammation-induced IL-1β also activates hepcidin expression by inducing CCAAT enhancer-binding protein (C/EBP)δ in hepatocytes[10], [19]. Hepcidin is the key regulator of systemic iron homeostasis. Hepcidin leads to internalization and degradation of the iron exporter ferroportin, which is present on the cell surface of macrophages and enterocytes. Thus, hepcidin inhibits the release of iron by macrophages and attenuates the iron uptake in the gut[20].

In a study by Zaritsky et al, the median hepcidin level was noted to be 25.3 ng/mL in healthy pediatric control[21]. In another study by Ridha NR *et al.* Makassar, in 35 normal body weight children was obtained the median hepcidin level of about 16.1 ng/mL[22]. This study demonstrated that the hepcidin level increased in CKD with and without anemia (32.3 ng/mL vs 69.6 ng/mL). It is shown that the inflammation occurred in all groups.

This study demonstrates that serum hepcidin levels are positively correlated with serum ferritin. High hepcidin levels increase ferritin levels and induces anemia. Inflammation stimulates hepcidin expression through pro-inflammation cytokines. Furthermore, High hepcidin inhibits the release of iron storage in hepatocytes, macrophages and inhibits iron absorption from the gut, this causes an increase ferritin levels and leading to hypoferremia, that induces anemia [23]. Eleftheriadis et al were found that hepcidin is increased and correlated with ferritin but not with TSAT[24]. Similar results were observed in this study. Higher hepcidin levels were associated with an increased risk for incident anemia in children with CKD.

Conclusion

There was an indirect association between hepcidin level and anemia in which hepcidin increases ferritin level and induces anemia in pre-dialysis CKD children.

Conflict of Interest

Jusli Aras, Astrid Kristina Kardani, Risky Vitria Prasetyo, Ninik Asmaningsih Soemyarso, Mohammad Sjaifullah Noer, and I Dewa Gede Ugrasena declare that they have no conflict of interest this publication.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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