



Vitamin d supplementation explodes the triangle of danger "iron deficiency anemia, inflammation and hypovitaminosis D" in pediatric patients on hemodialysis

¹Soha Abdelhady Ibrahim, ²Eman Ramadan Abdel Gawad, ³Omima Mohamed Abdel Haie, ⁴Amira Ibrahim Mansour, ⁵Akram Elshafey Elsadek

¹Pediatric department, Faculty of Medicine, Benha University

²Clinical and Chemical Pathology department, Faculty of Medicine, Benha University

³Pediatric department, Faculty of Medicine, Benha University

⁴Clinical and Chemical Pathology department, Faculty of Medicine, Benha University

⁵Pediatric department, Faculty of Medicine, Benha University

Corresponding author's E-mail: Amiraww2005@yahoo.com, AMIRA.MANSOUR@fmed.bu.edu.eg

Abstract

Vitamin D deficiency is extremely frequent in chronic kidney disease (CKD) and is associated with erythropoietin hypo-responsiveness. Heparin, the primary regulator of iron homeostasis, may play a critical role in the response of patients with anemia to iron and erythropoiesis-stimulating agent therapy; however, the participation of heparin to anemia in hemodialysis (HD) patients had not been completely characterized. To evaluate the relationship between serum heparin, indicators of anemia, iron status, inflammation and 25-hydroxy vitamin D (25-OH D) levels in children with CKD on HD and the impact of vitamin D therapy on these parameters. This analytical case-control, a double-center study was carried out on CKD patients attending the Nephrology Unit of the pediatric department at Benha and El Menofeya University Hospitals. Participants were classified into two groups: Group I Forty patients with end-stage kidney disease (ESKD) on HD. Group II Thirty healthy children of matched age and sex were included as a control group. All participants were subjected to full medical history, thorough clinical examination and laboratory evaluation in the form of complete blood count (CBC), kidney functions, liver functions, IL-6, serum levels of iron, ferritin, heparin and 25-OH D levels by ELISA. All patients received ergocalciferol as intensive replacement therapy depending on baseline 25-OH D levels for 3 months followed by maintenance therapy. Pre-treatment levels of study parameters were significantly disturbed compared to control measures. Heparin was significantly increased in pediatric HD patients (272.7 ± 152.6 ng/ml) when compared with their respective control subjects (39.1 ± 21.8 ng/ml). A significant positive correlation was demonstrated between serum heparin levels and both IL-6 and serum ferritin, while a significant negative correlation was revealed between serum heparin and, Hb, serum 25-OH D and iron. Post-treatment with ergocalciferol, the serum ferritin, heparin and IL-6 levels were significantly decreased, while Hb level and Hct value were significantly increased compared to pre-treatment levels. These findings suggest that heparin may mediate the negative effects on both disordered iron metabolism and erythropoiesis in HD patients and that Ergocalciferol could be used therapeutically to reduce heparin concentrations and thereby improve erythropoiesis-stimulating agent responsiveness and reduce inflammatory mediators.

Keywords: Chronic kidney disease, Vitamin D therapy, Iron deficiency anemias

INTRODUCTION

Anemia is a common complication in the maintenance of hemodialysis patients and contributes to reduced quality

of life (Eleftheriadis et al., 2009). Erythropoiesis-stimulating agents (ESA), such as recombinant human erythropoietin (EPO) have allowed effective treatment of anemia in patients with chronic kidney disease (CKD). However, the optimal target level of hemoglobin is debated and many patients are resistant to ESA (Swinkels and Wetzels 2008). Iron deficiency is a frequent cause of EPO resistance. Defining an iron-deficient state in maintenance haemodialysis patients is, however, more complex than in the general population and, to date, no reliable marker of iron status has been agreed (Weiss et al., 2009). Hepcidin is a low-molecular-weight protein that plays an important role in iron metabolism. (Swinkels and Wetzels 2008) Hepcidin is produced primarily by hepatocytes, and also by other cells, including macrophages. In addition to hepcidin's antimicrobial properties, it is the main regulator of iron metabolism and controls both the amount of dietary iron absorbed in the duodenum and the iron release by reticuloendothelial cells (Tsuchiya and Kther 2013).

Hepcidin binds to the major cellular iron exporter ferroportin, causing its internalization (Nemeth et al., 2004b). This, in turn, prevents internal iron absorption as well as iron release from the liver and the reticuloendothelial system (Nemeth et al., 2004a and Vokurka et al., 2006). There are four main active regulation pathways (erythroid, iron store, inflammatory and hypoxia-mediated regulation) that control hepcidin production through different signaling pathways. These pathways must be closely coordinated to match iron supply to erythropoietic demand and, in turn, to maintain adequate plasma iron concentrations (Piperno et al., 2009).

Bacchetta and coworkers (2014) found a direct transcriptional suppression of hepcidin gene (HAMP) expression mediated by 1,25-dihydroxyvitamin D binding to the vitamin D receptor caused the decrease in hepcidin mRNA levels. Suppression of HAMP expression was associated with a concomitant increase in expression of the cellular target for hepcidin, ferroportin protein, and decreased expression of the intracellular iron marker ferritin. (Bacchetta et al., 2014).

Iron balance is tightly linked to inflammation and it has been demonstrated that many proteins involved in cellular iron management are up- or down-regulated by inflammatory stimuli, ultimately leading to iron retention in the reticuloendothelial system as evidenced in vitro by incubation of monocytes/ macrophages with lactoferrin prevents the lipo-polysaccharide (LPS) -induced decrease of ferroportin by reducing secretion of IL-6 (Cutone et al., 2014). In previous studies, Ashby and his colleagues, (2009) demonstrated an inverse correlation between serum hepcidin and GFR in adults with CKD, with serum hepcidin levels being highest in dialysis-dependent patients (Ashby et al., 2009). In addition Zaritsky and his associate (2009) using multivariate analysis, they found that hepcidin levels correlated with

markers of iron status and inflammation. Although parenteral iron supplementation can bypass some of the iron-blocking effects of hepcidin in CKD patients with anemia, and free iron and iron stores increase, as a result, the anemia is only partially corrected, and the ESA dose requirements remain significantly higher than needed for physiological replacement (Tsuchiya and Nitta 2013). Vitamin D insufficiency is common in patients with chronic kidney disease (CKD) (Mallbris et al., 2002), with a prevalence rate of up to 80% of all patients with CKD stage 3 or worse (LaClair et al., 2005). Optimal vitamin D status is important in patients with CKD to regulate parathyroid hormone (PTH) concentrations (Chandra et al., 2008, Alvarez et al., 2012 and Wasse et al., 2014) for optimal bone health and prevention of osteomalacia and for potential cardioprotective effects (Ullah et al., 2010, Judd et al., 2011 and Alvarez et al., 2012). Recent reports have established an association between vitamin D insufficiency and anemia in patients with CKD (Carvalho et al., 2011, Perlstein et al., 2011 and Icardi et al., 2013); however, the role for vitamin D in the regulation of anemia has not been fully explained. Additionally, reduced kidney function likely prevents efficient hepcidin clearance from the plasma (Carvalho et al., 2011, Tsuchiya and Nitta 2013).

Treatment with agents that lower serum hepcidin levels or inhibit its actions may be an effective strategy for restoring normal iron homeostasis and improving anemia in CKD patients (Tsuchiya and Nitta 2013).

The purpose of this study was to evaluate the relationship between serum hepcidin, estimated blood indices, Iron status, IL-6 and 25-OH D levels in pediatric patients on regular HD and the impact of vitamin D supplemental therapy on these parameters.

SUBJECTS AND METHODS

This study was carried out on CKD patients attending the Nephrology Unit of the pediatric department at Benha and EL-Menofeya University Hospitals from January 2013 to June 2014. Participants were classified into two groups: Group I included 40 patients on HD, 21 males and 19 females, and their age ranged from 8.6 to 18 years. Dialysis was performed by Fresenius 2008K machines and hollow fiber polysulfone dialyzers (Fresenius, Bad Homburg, Germany) using the standard citrate dialysate solution. The dialysis prescription was as follows: Three times a week, 3-5 hours per session, blood flow 300mL/min, with urea reduction ratio URR >65%. Group II included 30 age and sex matched healthy children, 16 males and 14 females, and their age ranged from 8 to 16 years as a control group.

All participants were subjected to full medical history regarding the age of onset of ESKD, etiology and duration of their disease, duration of dialysis, age at which dialysis began, CKD-underlying pathology, history

of drug administration either in the form of iron therapy or chelation, the need of repeated blood transfusion and the dose of administered recombinant human erythropoietin (rHEPO). Thorough clinical examination as regards anthropometric measures, pallor, organomegaly.

Patients with the following were excluded from the study: previously diagnosed non-renal cause of anemia other than iron deficiency, evidence of active or occult bleeding, blood transfusion within the past 4 months, history of malignancy, end-stage liver disease, or chronic hypoxia and recent hospitalization or infection that required antibiotics within the past 4 weeks, preexisting hyperparathyroidism, co-morbid conditions that may interfere with the absorption or metabolism of ergocalciferol such as malabsorption syndromes and use of glucocorticoids or anticonvulsant therapy. Patients who were receiving recombinant erythropoietin (rhEPO) and iron supplementation were enrolled provided that the dosages of each had been stable for at least 4 weeks. All rhEPO supplements were in the form of recombinant epoetin alfa (Amgen). Parenteral iron supplementation was withheld for 1 week before the measurement of hepcidin.

After approval of the study protocol by the Local, Ethical Committee and obtaining parents' written fully informed consent.

Ergocalciferol was prescribed according to the KDOQI clinical practice guidelines for nutrition in CKD (Zughaier et al., 2014) as an intensive replacement therapy depending on baseline 25-OH D levels for 3 months followed by maintenance therapy. Children older than one year with a baseline 25-OH D level in the range of 40-75 nmol/L was given 1 ml (2000 IU/day), children with baseline 25-OH D in the range of 12.5-40 nmol/L were given 2 ml (4000 IU/day) and children with baseline 25-OH D <12.5 nmol/L were given 4 ml (8000 IU/day) as intensive replacement therapy for 3 months. At the end of 3-months course baseline investigations were repeated. Thereafter, children were given 1 ml (2000 IU) daily as a maintenance therapy.

Laboratory investigations

Sampling

- Five ml of venous blood samples were taken at baseline (pre-treatment) and at the end of treatment. Each blood sample was rapidly and gently divided into 2 tubes. The first one contains 2 ml with anti-coagulant (EDTA) for complete blood count. The second tube was plain tube. Serum was separated and divided into 2 aliquots:
 - The 1st was placed in pyrogen-free Eppendorf tubes and stored at -70°C until assayed for estimation of serum 25-OH D, hepcidin and IL-6.
 - The 2nd was used for estimation of serum urea,

creatinine, iron and ferritin.

Estimated glomerular filtration rate (eGFR) was calculated by the modification of Diet in Renal Disease equation (Wan et al., 2012) as follows: $eGFR (ml/min/1.73 m^2) = 186.3 \times (\text{serum creatinine in mg/dl})^{-1.154} \times (\text{age in years})^{-0.203}$ (x 0.742 for females). Patients were categorized according to baseline estimated serum 25-OH D levels, according to the KDIGO guidelines of the 2012 (Levey et al., 2009 and The International Society of Nephrology 2012) as normal serum level (≥ 50 nmol/l), mild deficiency (25-50 nmol/l), moderate deficiency (12.5-25 nmol/l) and severe deficiency (<12.5 nmol/l).

Statistical Analysis

Study variables were summarized using mean and SD. Because of its non-normal distribution, hepcidin values were presented as medians and means. The differences in biochemical and HD clearance measurements between patient groups were compared using the Mann-Whitney rank sum test for non-parametric continuous data or independent t test for parametric data. For univariate and multivariate analysis, log transformation was applied to variables with non-normal distribution. Univariate correlations between biochemical measurements and hepcidin were calculated using the Pearson correlation test. All tests are two-sided with significance level of 0.05, and all analyses were performed using SAS statistical software (SAS Institute).

RESULTS

The study was conducted in 40 maintenance HD patients (21 males and 19 females; mean \pm SD of age was 8.67 ± 4.09 years). All patients were maintained on HD since a mean duration of 3.4 ± 1.4 ; range: 1-6 years and their mean eGFR was 8.4 ± 2.2 ; range: 10.2-7.2 ml/min/1.73m². Thirty age-matched healthy control subjects (16 males and 14 females; mean \pm SD of age was 8.52 ± 3.86 years). There was a statistically significant decreasing difference between cases and control, as regards to some demographic characteristics; body weight, height and BMI; ($P < 0.05$).

Baseline levels of study parameters were significantly disturbed in children on regular hemodialysis therapy compared with healthy controls and this was manifested as disturbed iron indices with significantly lower Hb conc., MCV, MCH, MCHC, Hct value and serum iron levels, while serum ferritin levels were significantly higher in comparison to healthy controls. Moreover, pre-treatment serum levels of inflammatory mediators; hepcidin and IL-6 were significantly higher in children on regular hemodialysis therapy compared to control children. However, serum 25-OH D levels were significantly lower in HD patients compared to control children (table 1)

Table 1: Baseline levels of estimated parameters in studied patients compared to control levels

		Control Mean \pm SD	Patients Mean \pm SD	Pvalue
Iron indices	Hb conc. (gm/dl)	11.8 \pm 0.9	8.78 \pm 1.51	0.0003
	Hct value (%)	38 \pm 2.1	27.9 \pm 3.3	0.0001
	Serum iron (μ g/dl)	77.5 \pm 10.2	42.5 \pm 8.3	0.0001
	Serum ferritin (ng/ml)	85.2 \pm 16.1	175.3 \pm 50.2	0.0001
Inflammatory mediators	Serum hepcidin (ng/ml)	39.1 \pm 21.8	272.7 \pm 152.6	0.0001
	Serum IL-6 (ng/ml)	7.1 \pm 1.16	13.3 \pm 2.1	0.0001
25-OH D level (nmol/L)		65 \pm 7.2	39 \pm 16	0.0001

Hb conc.: Hemoglobin concentration; Hct value: Hematocrite value; IL-6: interleukin-6; 25 OH D; 25-hydroxy vitamin D; p<0.05: significant difference.

Table 2: Correlation coefficient between estimated baseline parameters of study patients

	25-OH D level (nmol/L)		Hepcidine(ng/ml)	
	r	P	r	P
Hbconc.(gm\dl)	0.284	0.028	-0.445	<0.001
Serum iron (μ g/dl)	-0.302	0.019	0.381	0.003
Hepcidine (ng/ml)	-0.393	0.002		
IL-6 (ng/ml)	-0.176	>0.05	0.326	0.011
Ferritine	0.63	<0.05	0.080	<0.001

IL-6: interleukin-6; 25-OH D: 25-hydroxy vitamin D; r: Pearson's correlation coefficient; p<0.05: significant; p>0.05 non-significant

Table 3: Post-treatment levels of estimated parameters in studied patients compared to pre-treatment Levels

Parameters		Pre-ttt Mean \pm SD	Post-ttt Mean \pm SD	P value
Iron indices	Hb conc. (gm/dl)	8.78 \pm 1.51	9.24 \pm 1.86	0.040
	Ht value (%)	27.9 \pm 3.3	30.4 \pm 3.6	0.018
	Serum iron (μ g/dl)	42.5 \pm 8.3	44.3 \pm 10.4	>0.05
	Serum ferritin (ng/ml)	175.3 \pm 50.2	126.2 \pm 36.2	0.0008
Inflammatory mediators	Serum hepcidin (ng/ml)	272.7 \pm 152.6	239.9 \pm 144.5	0.034
	Serum IL-6 (ng/ml)	13.3 \pm 2.1	12.9 \pm 2.4	0.002
25-OH D level (nmol/L)		39 \pm 16	43.6 \pm 18.1	>0.05

Hb conc.: hemoglobin concentration; Hct value, Haematocrite value, IL-6: interleukin-6, 25-OH D : 25- hydroxyvitD

This study revealed positive significant correlations between serum 25-OH vitamin D and Hb concentration and ferritine and negative significant correlations with serum iron and hepcidin. Also, revealed positive significant correlations between serum hepcidin and serum iron, IL-6 and ferritine, while showed a negative significant correlation with Hb conc (Table 2).

The applied treatment policy significantly improved iron indices manifested as significantly lower post-

treatment ferritin levels compared to pre-treatment levels, non-significant increase of serum iron, but significant increase of Hb conc. and Ht value. Moreover, post-treatment levels of hepcidin and IL-6 were significantly lower compared to pre-treatment levels. Concerning serum 25-OH D levels, the applied therapeutic regimen increased its level, despite being non-significant, (table 3).

Verification of estimated parameters using regression

Table 4: Regression analysis of estimated pre-treatment levels as predictors for pre-treatment Hb conc. of studied patients

	Hb conc.	t	P
eGFR	0.129	0.327	>0.05
Hepcidin	-0.445	3.787	0.0003
IL-6	-0.062	0.496	>0.05
Serum iron	-0.014	0.108	>0.05
Serum 25-OH D	0.129	1.011	>0.05

eGFR: estimated glomerular filtration rate; IL-6: interleukin-6; 25-OH D: 25-hydroxy vitamin D

analysis as dependent predictors for hemoglobin concentration as a measure for iron indices status defined high serum hepcidin level as the significant predictor for Hb conc., (Table 4)

DISCUSSION

Anemia is common in patients with renal insufficiency. EPO deficiency is by far the major cause, although shortened erythrocyte survival due to haemolysis, bleeding and oxidative stress may contribute. Most patients with CKD and anemia can be effectively treated with ESA. However, 10% of the patients are hypo- or non-responsive to ESA. Several cohort studies have reported an association between higher doses of ESA and mortality (Zhang et al., 2004 and Kaysen et al., 2006). Identification of these ESA-resistant patients is thus pivotal in the proper management of anemia in patients with CKD. Iron deficiency contributes to ESA resistance. Indeed, adequate iron availability is critical in HD patients, since it appears to influence the response to recombinant erythropoietin (EPO), the mainstay of treatment of anemia in this condition (Hamada and Fukagawa 2009).

Hepcidin has been object of intense investigation as a potential biomarker of iron status in patients with CKD (Campostrini et al., 2010). In HD patients, various factors may modulate serum hepcidin levels with opposing influence (Hamada and Fukagawa 2009).

For example, hepcidin may increase because of reduced glomerular filtration, iron therapy, and inflammation, with interleukin-6 as a well-known stimulus for its production (Nemeth et al., 2004b, Malyszko and Mysliwiec 2007). On the other hand, hepcidin may be reduced by hypoxia, iron deficiency and EPO therapy by itself (Robach et al., 2009).

Thus, in the setting of CKD, increased serum hepcidin and the resulting iron restriction could play a major role in disordered iron homeostasis and resistance to erythropoiesis-stimulating agents (ESA) (Zaritsky et al., 2010). The removal of hepcidin via hemodialysis (HD)

has been demonstrated in adult patients using mass spectrometry (MS)-based assays, with varying degrees of efficacy seen (Peters et al., 2009 and Weiss et al., 2009). Definitive resolution of this issue is needed, because increased removal of hepcidin by intensified HD could provide a much needed therapeutic intervention in cases of functional iron deficiency by relieving reticuloendothelial blockade as a result of inflammation-induced hepcidin overproduction. The improvement in ESA responsiveness reported in prolonged dialysis regimens supports the potential utility of this intervention (Ting et al., 2003 and Schwartz et al., 2005). Based on the role of hepcidin in iron metabolism and anemia

In human cells, the suppression of HAMP expression by 1,25D or 25D appears to be due to direct inhibition of HAMP transcription, the effects of vitamin D in suppressing hepcidin and promoting ferroportin are consistent with its established intracellular antibacterial activity. Therefore, regulation of the hepcidin-ferroportin axis is another key facet of vitamin D-mediated innate immune function, complementary to its reported effects on antibacterial proteins and autophagy. (Bacchetta, et al., 2014)

In this study, we found that most of our pediatric patients undergoing regular HD have marked growth retardation as regard weight and height centiles for their age. Our results were in agreement with Tom and his colleagues (1999) who reported that growth retardation is an important sequel of childhood end stage renal disease. There was a highly significant reduction in hemoglobin, hematocrit and serum iron levels in the studied diseased group in comparison with the control group. Our results were similar to Youssef and coworkers (2012) as they found a highly significant reduction in serum iron levels in cases compared to control group. While in our study there was a highly significant rise in serum ferritin levels in the studied diseased group in comparison with the control group. These data are consistent with the study by Costa et al., (2009) who showed a significant rise in serum ferritin levels in HD patients to 334.0 mg/ml (174.0-462.9). Also, Kalantar-Zadeh and his associates (2001) in a study on 83

patients, found a significant rise in the serum ferritin levels with a mean concentration of 831 ng/ml and $P = 0.03$, which was significant. These data indicated poor utilization of iron taken, despite its storage in the body.

Contrary to our study Rafi and his associates (2007) showed lower levels of serum ferritin in the 19 HD patients, mostly because of proper iron chelation therapy and better patient compliance. In addition to the usual rationale of anemia in CKD patients, many of our patients are not on the proper dose of erythropoietin hormone due to its high cost, which is not covered by the insurance system, and therefore, they were exposed to frequent blood transfusions due to longer duration of dialysis because of lack of availability of kidney transplantation. Our results were in agreement with Young and Zaritsky, (2009) who reported detailed quantitative measurements of serum hepcidin levels in pediatric patients on regular haemodialysis and found that hepcidin levels were elevated several folds in HD patients, suggesting that hepcidin production is severely altered in CKD. The data of Tomosugi et al., (2008) also indicated that hepcidin-25 levels were approximately two to three folds higher in the patients than in controls.

Ashby et al., (2009) and Zaritsky et al., (2009) also observed a gradual increase of hepcidin across the spectrum of predialysis CKD, suggesting that, hepcidin may increase because of reduced glomerular filtration, iron therapy, and inflammation, with interleukin-6 as a well-known stimulus for its production.

The current study detected only 3 of studied CKD children had normal serum 25-OH D with median level of 62 nmol/L while 37 patients had deficient serum level of 25-OH D with median level of 35.5 nmol/l, we found positive significant correlations between serum 25-OH vitamin D and Hb concentration and ferritin and negative significant correlations with serum iron and hepcidin.

In line with these findings, Kalkwarf and his colleagues (2012) found that nearly half of patients ages 5-21 with kidney disease stages 2-5 were 25-OH D deficient (<20 ng/ml) and the risk of deficiency was significantly greater in advanced disease, 25-OH D levels were inversely related to those of inflammatory markers CRP and IL-6 and concluded that lower 25-OH D may contribute to hyperparathyroidism, inflammation, in children and adolescents, especially those with advanced kidney disease. Satirapoj and coworkers (2013) found that the mean 25-OH D levels were significantly lower, according to severity of renal impairment with the prevalence of vitamin D deficiency/insufficiency was from CKD stage 3a, 3b, 4 to 5, 66.6%, 70.9%, 74.6%, and 84.7% respectively and the odds ratio of vitamin D insufficiency and vitamin D deficiency in developing ESRD, were 2.19 and 16.76, respectively. Denburg and his associates (2013) reported that children with CKD exhibit altered vitamin D metabolism and the concentrations of all vitamin D metabolites were significantly lower with more advanced CKD. Cho et al., (2013) found a high

prevalence of 25-OH D deficiency and insufficiency in children on chronic dialysis and serum 25-OH D was associated with residual renal function in children on PD. Ernst et al., (2015) reported that Patients with 25-OH D values <30 nmol/l and 1,25(OH)₂D values <40 pmol/l had the highest risk for anemia, whereas the risk was lowest in patients with adequate 25-OH D levels (50–125 nmol/l) and 1,25(OH)₂D levels above 70 pmol/l. In patients with deficient 25OHD levels (<30 nmol/l) mean Hb concentrations were 0.5 g/dl lower than in patients with adequate 25OHD levels (50.0–125 nmol/l; $P < 0.001$). Regarding 1,25(OH)₂D, mean Hb concentrations were 1.2 g/dl lower in the lowest 1,25(OH)₂D category (<40 pmol/l) than in the highest 1,25 (OH)₂D category (>70 pmol/l; $P < 0.001$).

In this study, there were positive significant correlations between serum hepcidin and serum iron and ferritin, ($P < 0.001$) while there was a negative significant correlation with Hb conc. Our results were in agreement with Tomosugi et al., (2003) and Kato et al., (2008) they found positive correlation between ferritin and hepcidin in HD patients. Also, Nemeth and coworkers, (2003) previously observing this correlation in populations without CKD and this likely reflects the known regulation of hepcidin by iron stores. Hepcidin may be negatively associated with hemoglobin in CKD patients because, hepcidin inhibits iron absorption from enterocytes and iron recycling from macrophages, leading to limited iron availability for erythropoiesis. In contrast Ashby et al., (2009) using radioimmunoassay, did not observe this correlation in adult HD patients, although target-driven intravenous iron therapy may have confounded those results.

In the present study, there was a significant positive correlation between serum hepcidin and IL-6 ($P < 0.001$). Our results are in agreement with Zaritsky and colleagues (2013) who provide a relationship between both variables through all stages of CKD, in their pediatric patients using univariate analysis and they found, a positive correlation between hepcidin and hsCRP. Ashby et al., (2009) and Weiss et al., (2009), most groups have found hepcidin levels directly correlate with serum ferritin, and some report a correlation with C-reactive protein.

Hepcidin may contribute indirectly to host defense by reducing iron concentrations. Since iron is required for microbial growth, low iron levels are thought to be bacteriostatic. Hepcidin has also been found to modulate lipopolysaccharide-induced transcription in cultured macrophages and in vivo mouse models. De Domenico et al., (2010), suggesting that hepcidin plays a role in modulating acute inflammatory responses to bacterial infections.

Estimated levels of study parameters after ergocalciferol therapy showed significantly lower serum hepcidin and IL-6 levels with significantly higher Hb concentration compared to pre-treatment concentration. These findings go in hand with Izquierdo et al., (2012)

who reported that treatment with selective vitamin D receptor activators (paricalcitol) significantly reduced serum levels of the inflammatory markers (CRP, TNF- α , IL-6 and IL-18) and serum level of anti-inflammatory cytokine IL-10 was increased and concluded that in renal patients undergoing hemodialysis, paricalcitol treatment significantly reduces oxidative stress and inflammation. Alvarez et al., (2012) studied 46 subjects with early CKD (stages 2 and 3) supplemented with oral cholecalciferol and found by 12 weeks, serum monocyte chemoattractant protein-1 (MCP-1) was significantly decreased in the treatment group compared to the placebo group and concluded that high-dose cholecalciferol decreased serum MCP-1 concentrations by 12 weeks in patients with early CKD. Rianthavorn and Boonyapapong (2013) found administration of ergocalciferol in conjunction with 1,25-dihydroxyvitamin D3 to children with CKD stage 5 and vitamin D insufficiency significantly reduced the required dose of erythrocyte-stimulating agent and may decrease erythropoietin resistance.

CONCLUSION

CKD patients showed significant disturbances in iron indices, inflammatory milieu and vitamin D metabolism and an independent inverse association between vitamin D status and anemia risk. The increased serum hepcidin levels found in pediatric patient on regular hemodialysis could play a major role in disordered iron homeostasis and resistance to ESA.

RECOMMENDATION

Ergocalciferol therapy significantly improved iron indices and ameliorated high inflammatory mediators serum levels. Hepcidin may in the future improve the targeting and timing of iron therapy by identifying patients during periods of reticuloendothelial blockage of iron transport, when they would likely not benefit from iron therapy. The end result of lowering hepcidin levels would be improved erythropoiesis and potentially decreased use and safer dosing of ESA and iron therapies. Administration of vitamin D or activated vitamin D (in case of chronic kidney disease) would be a promising strategy to prevent anemia.

REFERENCES

Alvarez JA, Law J, Coakley KE, Zughair SM, Hao L, Shahid Salles K (2012). High-dose cholecalciferol reduces parathyroid hormone in patients with early chronic kidney disease: a pilot, randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr.* 96(3):672–679.

Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD (2009). Plasma hepcidin levels are elevated but responsive to

erythropoietin therapy in renal disease. *Kidney Int.* 75: 976–981.

Bacchetta J, Zaritsky JJ, Sea JL, Chun RF, Lisse TS, Zavala K (2014). Suppression of Iron-Regulatory Hepcidin by Vitamin D. *J Am Soc Nephrol* 25: doi: 10.1681/ASN.2013040355.

Campostrini N, Castagna A, Zaninotto F, Bedogna V, Tessitore N, Poli A (2010). Evaluation of Hepcidin Isoforms in Hemodialysis Patients by a Proteomic Approach Based on SELDI-TOF MS. *Journal of Biomedicine and Biotechnology Article ID 329646, 7 pages.*

Carvalho C, Isakova T, Collerone G, Olbina G, Wolf M, Westerman M (2011). Hepcidin and disordered mineral metabolism in chronic kidney disease. *Clin Nephrol.* 76(2):90–98.

Chandra P, Binongo JN, Ziegler TR, Schlanger LE, Wang W, Someren JT (2008). Cholecalciferol (vitamin D3) therapy and vitamin D insufficiency in patients with chronic kidney disease: a randomized controlled pilot study. *Endocr Pract.* 14(1):10–17.

Cho HY, Hyun HS, Kang HG, Ha IS and Cheong HI (2013). Prevalence of 25(OH) Vitamin D Insufficiency and Deficiency in Pediatric Patients on Chronic Dialysis. *Perit Dial Int.* Jul-Aug; 33(4): 398–404.

Costa E, Swinkels DW, Laarakkers CM, Rocha-Pereira P, Rocha S, Reis F (2009). Hepcidin serum levels and resistance to recombinant human erythropoietin therapy in hemodialysis patients. *Acta Haematol.* 122:226-229.

Cutone A, Frioni A, Berlutti F, Valenti P and Musci G (2014). Lactoferrin prevents LPS-induced decrease of the iron exporter ferroportin in human monocytes/macrophage. *Biometals* 27:807–813.

De Domenico I, Zhang TY, Koenig CL, Branch RW, London N, Lo E (2010). Hepcidin mediates transcriptional changes that modulate acute cytokine-induced inflammatory responses in mice. *J Clin Invest.* 120:2395–2405.

Denburg MR, Kalkwarf HJ, de Boer IH, Hewison M, Shults J (2013). Vitamin D Bioavailability and Catabolism in Pediatric Chronic Kidney Disease. *Pediatr Nephrol.*; Sep; 28(9): 1843–1853.

Eleftheriadis T, Liakopoulos V, Antoniadi G, Kartsios C, Stefanidis I (2009). The role of hepcidin in iron homeostasis and anemia in hemodialysis patients. *Semin Dial.* 22: 70 – 77.

Ernst JB, Becker T, Kuhn J, Gummert JF and Zittermann A (2015). Independent Association of Circulating Vitamin D Metabolites with Anemia Risk in Patients Scheduled for Cardiac Surgery. *10(4): e0124751.*

Hamada Y and Fukagawa M (2009). Is hepcidin the star player in iron metabolism in chronic kidney disease,” *Kidney International.* vol. 75 (9):873–874.

Icardi A, Paoletti E, De Nicola L, Mazzaferro S, Russo R and Cozzolino M (2013). Renal anaemia and EPO hyporesponsiveness associated with vitamin D deficiency: the potential role of inflammation. *Nephrol Dial Transplant.* 28(7):1672–1679.

Izquierdo MJ, Cavia M, Muñoz P, de Francisco ALM, Arias M, Santos J (2012). Paricalcitol reduces oxidative stress and inflammation in hemodialysis patients. *BMC Nephrology.* 13:159.

Judd SE and Tangpricha V (2011). Vitamin D therapy and cardiovascular health. *Curr Hypertens Rep.* 13(3):187–191.

Kalantar-Zadeh K, Don BR, Rodriguez RA and Humphreys MH (2001). Serum ferritin is a marker of morbidity and mortality in hemodialysis patients. *Am J Kidney Dis.* Mar;37(3):564-572.

Kalkwarf HJ, Denburg MR, Strife CF, Zemel BS, Foerster DL, Wetzsteon RJ (2012). Vitamin D deficiency is common in children and adolescents with chronic kidney disease. *Kidney Int.* 81:690–697.

20-Kato A, Tsuji T, Luo J, Sakao Y, Yasuda H and Hishida A (2008). Association of Prohepcidin and Hepcidin-25 with Erythropoietin Response and Ferritin haemodialysis Patients. *American J. of Nephrology.* 28 (1):115-121

Kaysen GA, Müller HG, Ding J, and Chertow GM (2006). Challenging the validity of the EPO. *index.* *Am J Kidney Dis.* 47:166.e1–166.e13.

LaClair RE, Hellman RN, Karp SL, Kraus M, Ofner S and Li Q (2005). Prevalence of calcidiol deficiency in CKD: a cross-sectional study across latitudes in the United States. *Am J Kidney Dis.* 45(6):1026–1033.

Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI (2009). A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 150(9):604-612.

Mallbris L, O'Brien KP, Hulthén A, Sandstedt B, Cowland

- JB, Borregaard N (2002). Neutrophil gelatinase-associated lipocalin is a marker for dysregulated keratinocyte differentiation in human skin. *Exp Dermatol.* 11(6):584–591.
- Malyszko J and Mysliwiec M (2007). Heparin in anemia and inflammation in chronic kidney disease. *Kidney and Blood Pressure Res.* 30(1):15–30.
- Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A and Ganz T (2003). Heparin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood*, 101: 2461-2464.
- Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM (2004b). Heparin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Sci.* 306: 2090–2093.
- Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK (2004a). IL-6 mediates hypoferrremia of inflammation by inducing the synthesis of the iron regulatory hormone heparin. *J Clin Invest.* 113: 1271–1276.
- Perlstein TS, Pande R, Berliner N and Vanasse GJ (2011). Prevalence of 25-hydroxyvitamin D deficiency in subgroups of elderly persons with anemia: association with anemia of inflammation. *Blood.* 117(10):2800–2806.
- Peters HP, Laarakkers CM, Swinkels DW and Wetzels JF (2009). Serum heparin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. *Nephrol Dial Transplant.* 25: 848–853.
- Piperno A, Mariani R, Trombini P, Girelli D (2009). Heparin modulation in human diseases: From research to clinic. *World J Gastroenterol.* 15 (5): 538–551.
- Rafi A, Karkar A and Abdelrahman M (2007). Monitoring iron status in end-stage renal disease patients on hemodialysis. *Saudi J Kidney Dis Transpl.* 18(1)73-78.
- Rianthavorn P and Boonyapapong P (2013). Ergocalciferol decreases erythropoietin resistance in children with chronic kidney disease stage 5. *Pediatr Nephrol.* Aug;28(8):1261-1266.
- Robach P, Recalcati S, Girelli D, Gelfi C, Aachmann-Andersen NJ, Thomsen JJ(2009). Alterations of systemic and muscle iron metabolism in human subjects treated with low-dose recombinant erythropoietin. *Blood*, 113(26):6707–6715.
- Satirapo B, Limwannata P, Chairasert A, Supasynhd O, Choovichian P (2013). Vitamin D insufficiency and deficiency with stages of chronic kidney disease in an Asian population. *BMC Nephrology*; 14:206.
- Schwartz DI, Pierratos A, Richardson RM, Fenton SS, Chan CT (2005). Impact of nocturnal home hemodialysis on anemia management in patients with end-stage renal disease. *Clin Nephrol.* 63: 202–208.
- Swinkels DW and Wetzels JF (2008). Heparin: a new tool in the management of anaemia in patients with chronic kidney disease?. *Nephrol. Dial. Transplant.* , 23(8):2450-2453.
- The International Society of Nephrology, “KDIGO, 2012 clinical practice guideline for the evaluation and management of chronic kidney disease,” Kidney international, Supplement, 2013.
- Ting GO, Kjellstrand C, Freitas T, Carrie BJ, Zarghamee S (2003). Long-term study of high-comorbidity ESRD patients converted from conventional to short daily hemodialysis. *Am J Kidney Dis.* 42: 1020–1035.
- Tom A, McCauley L, Bell L, Rodd C, Espinosa P, Yu G (1999). Growth during maintenance hemodialysis: Impact of enhanced nutrition and clearance. *J. of pediatrics*134(4):464-471.
- Tomosugi N, Kawabata H, Wakatabe R, Higuchi M, Yamaya H, Umehara H (2008). Detection of serum heparin in renal failure and inflammation by using Protein Chip System. *Blood.* 108: 1381–1387.
- Tsuchiya K and Kther N (2013). Heparin is a potential regulator of iron status in chronic kidney disease. *Apher. Dial.*; 17(1):1-8.
- Tsuchiya K and Nitta K (2013). Heparin is a potential regulator of iron status in chronic kidney disease. *Ther Apher Dial. Feb*;17(1):1-8.
- Ullah MI, Uwaifo GI, Nicholas WC, Koch CA (2010). Does Vitamin D deficiency cause hypertension? Current evidence from clinical studies and potential mechanisms. *Int J Endocrinol.* ;2010: 579640.
- Vokurka M, Krijt J, Sulc K and Necas E (2006). Heparin mRNA levels in mouse liver respond to inhibition of erythropoiesis. *Physiol Res.*; 55: 667–674.
- Wan M, Shroff R, Gullett A, Ledermann S, Shute R, Knott C (2012). Ergocalciferol Supplementation in Children with CKD Delays the Onset of Secondary Hyperparathyroidism: A Randomized Trial. *CJASN.*;vol. 7 : 2216-2223.
- Wasse H, Huang R, Long Q, Zhao Y, Singapur S, McKinnon W (2014). Very high-dose cholecalciferol and arteriovenous fistula maturation in ESRD: a randomized, double-blind, placebo-controlled pilot study. *J Vasc Access.* 15(2):88-94.
- Weiss G, Theurl I, Eder S, Koppelstaetter C, Kurz K, Sonnweber T (2009). Serum heparin concentration in chronic haemodialysis patients: Associations and effects of dialysis, iron and erythropoietin therapy. *Eur J Clin Invest.* 39: 883–890.
- Young B and Zaritsky J (2009). Heparin for Clinicians. *clinical J. of American society of nephrology.* 8: 1384-1387.
- Youssef DM, Desoki TA, Tawfeek DM and Khalifa NA (2012). Serum proheparin level in children with chronic kidney disease in relation to iron markers. *Saudi J Health Sci.* 1:126-131.
- Zaritsky J, Young B, Wang HJ, Westerman M, Olbina G, Nemeth E (2009). Heparin: A potential novel biomarker for iron status in chronic kidney disease. *Clin J Am Soc Nephrol.* 4: 1051–1056.
- Zaritsky J, Young B, Gales B, Wang H, Rastogi A, Westerman M (2010). Reduction of Serum Heparin by Hemodialysis in Pediatric and Adult Patients. *Clin J Am Soc Nephrol.* Jun; 5(6): 1010–1014.
- Zhang Y, Thamer M, Stefanik K, Kaufman J, Cotter DJ (2004). Epoetin requirements predict mortality in hemodialysis patients. *Am J Kidney Dis.*;44:866-876.
- Zughaier SM, Alvarez JA, Sloan JH, Konrad RJ and Tangpricha V (2014). The role of vitamin D in regulating the iron-heparin-ferroportin axis in monocytes. *J. of Clinical & Translational Endocrinology* 1; 19-25.