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Original Research Article

Evaluation of the Role of Serum Hepcidin Predicting the Response to Erythropoietin Therapy in Children with Chronic Kidney Disease on Regular Hemodialysis

Enas Tawfek[1], Marwa Elhady[1*], Walaa Mohammed El Said Shebl[2], Hanan Medany[1]
[1]Department of pediatrics, Faculty of Medicine (for girls), Al-Azhar University, Cairo, Egypt
[2]Department of biochemistry, Faculty of medicine (for girls), Al-Azhar University, Cairo, Egypt

***Corresponding author:** Marwa Elhady
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Abstract

Background and Aim: Anemia is a common and serious complication of chronic kidney disease (CKD). Despite the use of erythropoietin, anemia of CKD can be resistant to therapy. Hepcidin is the main iron regulatory hormone; increased hepcidin production during chronic inflammation interferes with iron absorption, prevents iron recycling leading to hypoferremia and iron-restricted erythropoiesis. Our study aims to evaluate the role of serum hepcidin in predicting the response to erythropoietin (EPO) therapy in children with CKD on regular hemodialysis. ***Subjects and methods:*** a cross sectional comparative study included 40 children with CKD on regular hemodialysis who received EPO therapy for at least 3 months and 40 age and sex matched healthy children. Assessment of anemia and iron profile before and after 3 months of EPO therapy and was correlated to the serum level of hepcidin. ***Results:*** in comparison to healthy controls, CKD children have significant anemia and high hepcidin level. CKD children have CKD children with high hepcidin level have significant lower hemoglobin and impaired response to EPO therapy in comparison to those with normal hepcidin level (P-value <0.005). There was significant negative correlation between serum hepcidin level and HB, HCT and iron level in children with CKD. ***Conclusion:*** children with CKD on regular dialysis have higher hepcidin level that interferes with the response of anemia to EPO therapy suggesting functional defect in iron utilization among those children.

Keywords: Hepcidin, erythropoietin, children, chronic kidney Disease.

INTRODUCTION

Chronic kidney disease (CKD) represents a progressive irreversible loss of kidney function that associated with high mortality and morbidity [1]. Anemia is a common serious multifactorial complication among those children that is challenging for management. Anemia adds further risk for cardiovascular disease and expose patients to transfusion related complication that significantly impairs their quality of such children [2]. Several mechanisms can contribute to the development of anemia in CKD subjects, including iron deficiency, blood loss, erythropoietin deficiency, and chronic inflammation [3].

According to guidelines, hematocrit should be maintained at a range of 33 to 36 and the hemoglobin at 11.0 to 12.0 g/dl in children with CKD to improve their cognitive performance, cardiac function, exercise tolerance, as well as decreased mortality [4].

Several modalities are used for treatment of anemia in CKD children including administration of recombinant erythropoietin therapy and iron supplementation. Prior to the use of erythropoiesis stimulating agents, blood transfusions way the only available method to correct anemia in CKD children [5].

However, EPO resistant anemia has been emerged as a major problem in CKD subjects. Both absolute and functional iron deficiency along with inflammation can contribute to EPO resistance [6].

Hepcidin is a 25 amino acid peptide that synthesized in hepatocytes and act as the main iron homeostasis regulator. Hypoxia, anemia and low iron stores decrease hepcidin production while systemic inflammation and elevated iron stores stimulate hepcidin production. Circulating hepcidin binds to ferroportin iron transporter on enterocytes and macrophages, leading to receptor internalization and lysosomal degradation. Thus, in chronic inflammation,

excess hepcidin production decreases iron absorption, reduce iron release from its stores and interfere with iron utilization, leading to iron-restricted erythropoiesis, despite normal iron stores (functional iron deficiency) [7].

CKD is characterized by steady state of low–grade inflammation especially in dialysis patients that may contribute to over expression of hepcidin among those children [8]. Our study aims to evaluate the role of serum hepcidin in predicting the response to erythropoietin therapy in children with CKD on regular hemodialysis.

PATIENTS AND METHODS

Study Design and Population

This study is a cross sectional comparative study. It was include 40 children with CKD (GFR 15ml/min/1.73) on regular hemodialysis; all of them were receiving at least 4 hours sitting of regular hemodialysis for 3 cessions per week using volumetric controlled machines and low flux polysulfone membrane dialyzer who were selected from pediatric nephrology unit of Al-Zahraa University Hospital during the period from May 2017 to May 2018. Additionally, 40 age and sex matched healthy children with no history of anemia or acute illness and did not receive iron supplementation within the previous 4 weeks were included as a control group. They were randomly selected from outpatient clinic of Al-Zahraa hospital, Al-Azhar University.

Inclusion criteria included children aged 4-16 years with CKD on regular hemodialysis more than three months. All of them had received subcutaneous EPO therapy in a dose of 50 IU/kg/setting for at least 3 months and follow up their heamatological and iron profile before and after treatment.

Children were excluded from the study if they who did not require dialysis, had acute kidney injury on temporary hemodialysis, had chronic systemic illness (hepatic, chest, GIT, endocrinal and heart). Patient received blood transfusion or has evidence of active or occult bleeding during the previous 4 months. Patients previously diagnosed with non-renal cause of anemia e.g. hemolytic anemia or aplastic anemia

An informed written consent was obtained from all patients and control groups before getting them involved in the study.

All studied children were subjected to full medical history taking with stress on: the etiology of chronic kidney disease, age of onset of CKD, duration of dialysis, hematological symptoms, current medication, previous bleeding or blood transfusion. Thorough clinical general and systemic examinations were done.

Laboratory Investigation

5ml of venous blood samples were withdrawn before the dialysis setting and divided into 2 ml for complete blood picture were taken on EDETA solution and 3 ml were left to clot and sera were separated without delay for the biochemical parameters to be done on the same day. Complete blood picture was done automatically by coulter MD 18 automated hematological counter. ESR was estimated by wester green technique. Serum urea and creatinine were done using HITACHII auto analyzer. Serum iron and iron binding capacity were determined by cobas c311 auto analyzer using Roch reagent kits. Serum ferritin was measured using immulite 200 ferrozin analyzer for the quantitative measurement of ferritin in serum no (LZKFE2). Transferrin saturation were calculated as (Serum iron level x 100) / total iron-binding capacity). Transferrin saturations of less than 20% indicate iron deficiency. Quantitative measurement of bioactive hepcidin in serum was carried out using ELIZA kits provided by IBL Hamburg code no. RE 54051.

Statistical Analysis

Data analysis was done using the Statistical Package for Social Sciences (version 21; SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± SD. Differences between 2 groups were analyzed using independent t student test for numerical data and Chi-square test for qualitative data. Correlations were performed using Spearman correlation coefficients. P-value <0.05 were considered significant.

RESULTS

This case control study was conducted on 40 children with CKD on regular hemodialysis and 40 healthy children as control group who were matched for sex and age. Their age ranged between 6-16 years. Both groups included 21 females and 19 males. The mean duration of dialysis is 3.72±3.29 years. The underlying renal pathology included 14(35%) focal segmental glomerulosclerosis followed by 7 (17.5%) atrophic kidney, 6 (15%) obstructive uropathy, while absent kidney, Alport syndrome, vesico-ureteric reflux, interstitial nephritis, polycystic kidney, nephropathesis were 2 (5%) for each and 1(2.5%) was nephrocalcinosis.

Table-1 showed comparison of clinical, hematological data, iron profile and serum hepcidin between healthy and CKD children. No statistically significant differences were found between CKD children and the control group regarding sex and age. CKD children had a significant decrease in hemoglobin level, hematocrit value, mean corpuscular volume. There was statistically significant decrease in serum iron, ferritin and significant increased serum ferritin and hepcidin level in CKD group compared to the controls.

Regarding serum level of hepcidin; 13 out of 40 CKD children had normal hepcidin level while 27 had high hepcidin level. CKD children with high hepcidin have statically significant lower HB, HCT, MCV than those with normal hepcidin level. However, there was no significant difference between patients with normal hepcidin or high hepcidin level regarding iron profile as shown in Table-2.

After 3 months of EPO therapy, there was significant increase in HB and HCT in those with normal hepcidin. Additionally, there was statically significant increase in total iron binding capacity and decrease in transferrin saturations in CKD patients with normal hepcidin level in response to EPO therapy while those with high hepcidin showed no significant response as shown in Table-3.

Table-4 demonstrated that serum hepcidin level has significant positive correlation with platelets and significant negative correlation with HB, HCT and iron level.

Table-1: Comparison of clinical, hematological data, iron profile and serum hepcidin between healthy and CKD children

	Control group Mean±SD (N= 40)	Patients group Mean±SD (N= 40)	Chi-square test/ independent t test	
			X^2	P-value
Sex (N,%) Female Male	21 (52.5%) 19 (47.5%)	21 (52.5%) 19 (47.5%)	0.000	1.000
Age(yrs)	12.95 ± 3.36	12.41 ± 3.34	0.893	0.731
WBCs\m³	7.96 ± 1.96	6.39 ± 1.95	1.588	0.071
Platelets\ m³	290.25 ± 51.25	201.15 ± 77.06	6.089	<0.0001*
RBC s\ m³	4.66 ± 0.43	3.42 ± 0.70	9.582	<0.0001*
Hb (g\dL)	12.27 ± 0.87	9.44 ± 1.70	9.385	<0.0001*
HCT%	36.33 ± 2.56	27.58 ± 5.04	9.787	<0.0001*
MCV(fL)	88.70 ± 5.10	80.12 ± 7.75	3.287	0.008*
MCH pg	31.38 ± 3.60	29.83 ± 2.64	2.287	0.012*
MCHC g/dl	34.71 ± 3.61	32.76 ± 4.58	4.287	<0.0001*
RDW%	11.46 ± 1.25	14.43 ± 3.28	5.845	<0.0001*
Serum Fe(µg\dL)	101.40 ± 24.01	79.73 ± 13.60	4.968	<0.0001*
Ferritin(ng\L)	58.85 ± 52.22	125.28 ± 79.65	-4.411	<0.0001*
TIBC (µg\dL)	298.17 ± 64.33	264.68±111.95	1.641	0.105
TS%	34.61 ± 7.29	36.28 ± 18.43	-0.530	0.598
Hepcidin((µg\dL)	2.78 ± 1.69	31.29 ± 10.15	-17.526	<0.0001*

*significant

WBC: Wight blood cells; RBC: red blood corpuscles; HB: hemoglobin; HCT: hematocrit; MCHC: mean corpuscular hemoglobin; MCHC; mean corpuscular hemoglobin content; Fe: iron; TIBC: Total iron binding capacity; TS: Transferrin saturation

Table-2: Comparison of hematological data and iron profile between CKD children with normal and high hepcidin

	Normal hepcidin	High hepcidin	Independent t-test	
	Mean ± SD (N = 13)	Mean ± SD (No. = 27)	t	p-value
WBC\ mm³	5.10 ± 1.85	5.88 ± 1.55	-0.541	0.592
Hb(g\dl)	10.58 ± 1.81	10.03 ± 1.90	2.298	0.027*
Platelet\ mm³	211.15 ± 76.06	206.18 ± 76.09	-1.073	0.290
RBCs\ mm³	3.43 ± 0.76	3.62 ± 0.46	1.169	0.250
HCT%	27.68 ± 5.14	27.38 ± 5.06	2.162	0.037*
MCV(fl)	80.13 ± 4.76	80.13 ± 4.78	2.176	0.036*
MCH(pg)	29.38 ± 2.60	29.83 ± 2.64	1.654	0.106
MCHC(g\dl)	32.71 ± 1.61	32.76 ± 1.58	0.673	0.505
RDW%	14.46 ± 1.25	14.43 ± 1.28	0.426	0.672
Serum Fe(µg\dl)	79.71 ± 13.61	79.72 ± 13.62	-0.799	0.429
Ferritin(ng\dl)	125.88 ± 79.85	125.18 ± 79.63	1.019	0.315
TIBC(µg\dl)	261.68 ± 111.91	264.61 ± 111.93	-0.747	0.460
TS%	36.26 ± 18.45	36.38 ± 18.41	-0.232	0.817

*significant

WBC: wight blood cells; RBC: red blood corpuscles; HB: hemoglobin; HCT: heamatocrite; MCHC: mean corpuscular hemoglobin; MCHC; mean corpuscular hemoglobin content; Fe: iron; TIBC:Total iron binding capacity; TS: Transferrin saturation

Table-3: Comparison of hematological data and iron profile in response to 3 months of erythropoietin therapy

	Patient with normal hepcidin				Patients with high hepcidin			
	Before ttt	3 months After ttt	Independent t test		Before ttt	3 months After ttt	Independent t test	
	No. = 13	No. = 13	t	p-value	No. = 27	No. = 27	t	p-value
WBC\ m³	5.10 ± 1.85	7.39 ± 2.44	-1.832	0.063	5.88 ± 1.55	7.49 ± 2.31	-1.332	0.066
Hb(g\dL)	10.58 ± 1.81	11.72 ± 0.79	-2.511	0.002*	10.03 ± 1.90	10.12 ± 0.35	-1.801	0.138
Platelet\ m³	211.15 ± 76.06	225.50 ± 83.94	-0.746	0.460	206.18 ± 76.09	213.51 ± 82.96	-0.741	0.464
RBCs\ m³	3.43 ± 0.76	4.31 ± 0.85	3.133	0.001*	3.62 ± 0.46	3.11 ± 0.59	1.836	0.135
HCT%	27.68 ± 5.14	36.61 ± 40.32	-3.083	0.000*	27.38 ± 5.06	31.69 ± 32.32	-1.088	0.283
MCV(fL)	80.13 ± 4.76	85.94 ± 4.15	0.191	0.380	80.13 ± 4.78	81.94 ± 4.98	0.294	0.770
MCH(pg\dL)	29.38 ± 2.60	31.00 ± 1.36	-1.694	0.058	29.83 ± 2.64	30.10 ± 1.38	-1.991	0.054
MCHC(g\dL)	32.71 ± 1.61	33.61 ± 1.35	0.255	0.764	32.76 ± 1.58	34.68 ± 1.38	0.256	0.761
RDW	14.46 ± 1.25	15.36 ± 1.18	0.578	0.446	14.43 ± 1.28	13.76 ± 1.18	0.375	0.650
Serum Fe(µg\dL)	79.71 ± 13.61	67.35 ± 17.38	1.6858	0.559	79.72 ± 13.62	67.25 ± 17.33	1.685	0.561
Ferritin(ng\dL)	125.88 ± 79.85	117.05 ± 47.88	1.426	0.160	125.18 ± 79.63	107.03 ± 47.81	1.420	0.162
TIBC(µg\dL)	261.68 ± 111.91	291.75 ± 25.00	-2.068-	0.044*	264.61 ± 111.93	288.76 ± 27.01	-1.532-	0.146
Transferrin Saturation%	36.26 ± 18.45	36.01 ± 18.41	2.066-	0.045*	36.38 ± 18.41	36.01 ± 18.41	-1.466-	0.153

Table-1: Correlation between clinical variables, hematological data and serum level of hepcidinin patients with CKD on regular hemodialysis

	Hepcidin	
	r	P-value
Age(yrs)	-0.265	0.098
BW	-0.210	0.194
Height	-0.176	0.278
BMI	-0.117	0.474
Duration of dialysis(yrs)	0.91	0.543
Duration of disease(yrs)	0.093	0.569
WBC\ m³	0.131	0.420
Hb(g\dL)	-0.476**	0.002*
HCT%	-0.438**	0.005*
Platelets\ m³	0.330*	0.037*
RBCs \m³	-0.324*	0.041*
MCV(fL\red cell)	-0.165	0.309
MCH(pg\dL)	0.247	0.124
MCHC(g\dL)	-0.091	0.578
RDW FL	-0.027	0.867
Ferritin(µg\dL)	-0.093	0.567
TIBC(µg\dL)	0.142	0.384
Transferrin%	-0.183	0.260
Serum iron(µg\dL)	-0.381*	0.015

DISCUSSION

Anemia affects a substantial proportion of patients with CKD. Renal anemia has been considered as a special form of 'anemia of chronic disease, in which inappropriate levels of erythropoietin is considered as the main cause. Inadequate erythropoietin production by the failing kidneys is a major contributing factor for anemia in patients with CKD [9]. Other possible mechanisms include iron deficiency, hemolysis, and decreased erythrocyte survival [10].

Our study demonstrated that CKD children had lower Hb and HCT compared to the healthy controls which agree with previous reports by Locatelli *et al*., [11] and Bhatta *et al*., [12] who reported that CKD patients were significantly anemic than healthy population. Regarding indices or erythrocytes, CKD children had normocytic to microcytic anemia, this together with elevated serum ferritin confirms the state of functional iron deficiency. Functional iron deficiency is characterized by the presence of adequate iron stores with serum ferritin level is either normal or elevated. CKD children especially those on dialysis are at high risk of developing iron restricted erythropoiesis because

the rate at which iron is released from stores and delivered to the bone marrow fails to match the increased iron demand that limited availability of iron to bone marrow [13]. In addition, inflammatory iron block occurs among these patients leading to maintained serum ferritin level with in normal limits [14]. Our patients have high serum ferritin which is typical finding of anemia of chronic illness. Ferritin is the cellular storage protein for iron [15].

In our study, CKD children have higher serum level of hepcidin in comparision to healthy controls. 27 out of 40 (67.5%) had high serum hepcidin level while the remaining has serum hepcidin level within normal limits. Hepcidin concentration was reported to be increased in CKD patients reflecting the state of chronic inflammation which frequently accompanies CKD. Previous studies have reported inverse correlation between glomerular filtration rate and serum hepcidin level [16]. Additionally, progressive loss of renal function decrease hepcidin clearance as kidney is the major route for hepcidin clearance [17].

In agreement with our findings, Samouilidou et al., [18], found that hepcidin levels were elevated several folds in CKD patients. Atkinson et al., [19] found that Hepcidin and ferritin are significantly elevated in CKD subjects especially those on hemodialysis compared to healthy subjects.

In the current study there was significant negative correlation between serum hepcidin and hemoglobin, hematocrit, RBCs with significant positive correlation with platelets. This agree with previous evidence demonstrated that increased hepcidin expression is a major contributor of iron-restrictive anemia. Ganz [20] concluded that elevation of serum hepcidin seems to be multifactorial in this CKD population, with hepcidin correlation with markers of both iron storage and inflammation.

Despite regular EPO and iron administration that keep their serum iron level within normal limits, most of our patients still suffer from anemia (HB<11gm/dl). Macdougal [21] reported that increased hepcidin secretion in CKD patients may explain the experienced unresponsiveness of anemia to oral iron supplementation suggesting that oral iron therapy in CKD patients is of limited value as hepcidin inhibits iron absorption from intestine.

In the present study CKD children with normal limit of hepcidin revealed good improvement in response to EPO; while those with high hepcidin did not improved after 3 months of EPO.

Increased iron utilization for erythropoiesis in response to erythropoietin therapy requires an increased flow of iron to the bone marrow [22]. In patients with systemic inflammation and high hepcidin concentrations, even when iron stores are adequate and sufficient for baseline erythropoiesis, iron cannot be released from stores rapidly enough to meet the needs of pharmacologically stimulated erythropoiesis [23]. Additionally, the bioavailability of this iron for erythropoiesis may by impaired by elevated hepcidin levels, as hepcidin blocks iron release by the liver and macrophages interrupting iron recycling between old senescence red cells and reticuloendothielial system leading to a state of functional iron deficiency [24].

Petrulienė *et al*., [25] reported that hepcidin level was higher in EPO non responders than in EPO responders. Hepcidin correlated directly with ferritin and inversely with Hb and transferrin saturation.

Although iron and EPO doses were similarly administrated, those with higher hepcidin level were more anemic suggesting blunted response to therapy. They also had a significantly higher ferritin level that reflects more sever degree of functional iron deficiency in our included children. This corresponds to the results of other authors comparing EPO responders' and nonresponders' groups [26]. The features of our non-responders reflect reticuloendothelial blockage which can be considered as an extreme form of functional iron deficiency in which iron stores are locked partly due to high hepcidin levels and there is no release of iron to transferrin to be utilized for erythropoiesis [27]. Whether or not evaluation of hepcidin levels may help in driving iron supplementation therapy in CKD patients on dialysis is still matter of debate.

CONCLUSION

Children with CKD on regular dialysis have higher hepcidin level that interferes with the response of anemia to EPO therapy suggesting functional defect in iron utilization among those children.

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