Available online at www.medicinescience.org

ORIGINAL RESEARCH

Medicine Science 2017;6(2):285-98

Medicine Science International Medical Journal

Erythropoietin hyporesponsiveness among egyptian hemodialysis patients

Salah Eldin Khedr[1] PhD, Alaa E. Saad[2] PhD, Mohamed M. Abdo[3] PhD, Heba M. Zaki, Mohamed[4] MSc

[1] *Professor of Internal Medicine, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*
[2] *Professor of Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*
[3] *Assistant Professor of Internal Medicine, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*
[4] *Assistant Lecture of Internal Medicine, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*

Received 12 October 2016; Accepted 12 December 2016
Available online 15.12.2016 with doi: 10.5455/medscience.2016.05.8559

Abstract
Almost all ESRD patients on HD receive iron and EPO to treat anemia, however some patients do not respond sufficiently to the therapy. Response to EPO influenced by various factors, such as malnutrition, vitamin deficiencies, chronic blood loss, secondary hyperparathyroidism, chronic inflammation and decrease hepcidin excretion. This study included 80 anemic patients who had been on regular HD for more than 3 months. Patients received adequate doses of ESA and iron for 3 months, after that they were classified into two groups ESA responders and ESA hyporesponders. Laboratory investigations were done to assess some of risk factors which may lead to ESA hyporesponsiveness as Iron profile, IL-6, CRP, anti-EPO antibody, hepcidin level and PTH. Sixty percent of the patients were EPO hypo-responsive; ferritin has higher level in hypo-responders. Hepcidin and IL-6 markedly elevated among ESRD patients also were markedly elevated among hypo-responders. Hepcidin did not show any correlations with IL-6 or CRP but has strong correlation with ferritin. Prevalence of anti-EPO antibody was 7.5 % of total population. Parathyroid hormone had no statistically significant association with HB or reticulocytes. Hepcidin was higher among EPO-hyporesponders and had strong correlation with ferritin, so we can consider hepcidin as an important biomarker for iron stores utilization in anemic patients and to predict patient response to EPO therapy. Hepcidin did not show any correlations with IL-6 or CRP, so inflammatory mediators did not influence its high level, but mostly due to its retention in ESRD patients.

Keywords: Erythropoietin hyporesponsiveness, hemodialysis patients, hepcidin

Introduction

Anemia is considered as a prevalent problem in CKD patients, and its incidence has a significant negative correlation with GFR. The frequency of anemia in CKD stages 1 and 2 is less than 10%, in CKD stage 3 20–40%, in CKD stage 4 50–60% and in CKD stage 5 more than 70% [1-2].

A study was done at Manipal University, India to assess the prevalence of anemia in hemodialysis (HD) patients, showed that the prevalence of anemia was 85.85% of the patients of the study [3]. Another study done by Cairo University, Egypt; the results were that 98% of studied HD patients have anemia [4].

Anemia markedly affects the quality of life (QoL) of the patients & leads to fatigue, reduced exercise capacity, impaired immunity, decreased cognition, & increased load on the heart, which result in left ventricular hypertrophy and cardiomyopathy, increase the risk of death from heart failure or ischemic heart disease [5].

***Coresponding Author:** Dr. Mohamed M. Abdo, Assistant professor of internal Medicine, Suez Canal University, Ismailia, Egypt
E-mail: elbaihzico@yahoo.com

Causes highly contribute to anemia among patients with CKD: 90 percent caused by erythropoietin (EPO) insufficiency [6], uremia that resulted in suppression of erythropoiesis, shortened red blood cell (RBCs) survival and nutritional deficiencies [7].

The majority of CKD patients need both ESA and iron supplementation to manage anemia associated CKD but a lot of CKD patients who have anemia may do not respond sufficiently to anti-anemic drugs or need higher doses of iron and ESA. Hyporesponsiveness to ESA is defined as patients who don't have an increase in levels of Hb from baseline following the first month of ESA therapy according to adjusted weight-based dosing [8]. ESAs Hyporesponsiveness can have a lot of underlying causes. The main causes are inflammation and iron deficiency (absolute or functional).

Causes of Erythropoietin Resistance

1. **Iron deficiency:** It may occur because of absolute or functional iron deficiency. The second appears to be the main cause of ESAs hyporesponsiveness among HD patients [9].

2. **Inflammation:** Typically, HD patients have elevated levels of inflammatory markers as TNF-α, INF-γ, IL-

6, CRP, and reduced albumin levels [10]. These cytokines suppress EPO production and prompt apoptosis in CFU-E, which result in arresting the process of maturation of CFU-E into RBC.

A poor response to ESA in addition seems to be related with increase T cell ability to express IL-10, IL-13, TNF-α and IFN-γ [11].

3. **Decreased hepcidin excretion:** Hepcidin is considered as the chief regulator of systemic iron homeostasis **[12]**. It developed via the liver and transferred to circulation [13]*;* hepcidin attaches to iron exporters (ferroportin) and promotes its degradation; ferroportin expressed on hepatocytes, macrophages and duodenal enterocytes to prevent iron entrance into the plasma [14]. Hepcidin is elevated among HD patients [15-16] and its production controlled by inflammation and is associated with ESA resistance [17-18], and also related with ferritin which an indicator for marked inflammation and increased iron stores.

4. **Anti-erythropoietin antibodies** [8].

5. **Secondary hyperparathyroidism:** PTH is believed by EUTox Work Group **[**19]*,* as a middle molecule uremic toxin with multiple biological effects. PTH initiates bone marrow fibrosis, has an inhibitory impact on BFU-E and interferes with endogenous production of EPO [20]*;* interfering with RBC survival since PTH rises erythrocytes osmotic fragility [20].

6. **Aluminium toxicity:** Though the current advance in the procedures of dialysis, a few patients have high AL levels **[21]**. Commonly, elevated AL levels cause a microcytic, hypochromic or normochromic anemia which shows ESA hyporesponsive, as it affects the enzymes required for the synthesis of heme [22].

7. **Inadequate dialysis:** Dialysis adequacy (measured by Kt/V) may alter the response to ESA treatment. OL-HDF and High flux HD (HF-HD) enhance the response to ESAs compared with low flux HD (LF-HD), possibly owing to a better elimination of middle and large molecules which affect erythropoiesis [23]. But, a few studies did not succeed to attain that conclusion [24].

8. **ACEi and ARBs:** Hyporesponsiveness to EPO occurs due to prevention of angiotensin provoked the release of EPO and raised levels of plasma N-acetyl-serylaspartyl-lysil-proline which affects the induction of pluripotent hemopoietic stem cells [9].

9. **Chronic blood loss**.

10. **Vitamin deficiencies** [9-25].

11. **Malnutrition** [26].

Our study is designed to identify the main risk factors that are assumed to be the causes of hypo-responsiveness to treatment by ESAs as inflammatory status of the patients, presence of anti-erythropoietin antibody and hepcidin level, it is very helpful as we can treat the patients who have hyporesponsiveness to ESA by management of the underlying cause and modifying the patient treatment.

Material and Methods

A cross–sectional–analytic study was done at 80 anemic ESRD patients on regular HD for more than 3 months using bicarbonate dialysate and polysulphone synthetic membranes, the patients on regular HD at Units in Ismailia - Egypt Hospitals. Laboratory investigations of the study were done in Clinical Pathology Department in Suez Canal University Hospital and the samples were withdrawn from the patients before the HD session. Ten apparently healthy individuals with normal kidney function and not anemic were included in the study to derive a reference range to Hepcidin and IL-6.

Excluded from our study patients who have anemia due to known secondary cause as malignancy, bone marrow disease, history of chronic blood loss, Patient received blood transfusion during follow up course or patients who have severe infection.

Methods of Data Collection

In person interview questionnaire was done by the researcher and the following data were collected: Socio-demographic data of the patient, body weight, Duration of dialysis in months, Co-morbid illness. Also Lab investigations were done.

The following histogram demonstrates the initial investigations that have been done to the patient and the follow up course for 3 months.

** EPO (erythropoietin Alfa [Epoetin SEDICO] 4000 IU S.C.)
** Iron (5 ml ampoule contain 100 mg iron sucrose)

After 3 months the following lab tests were done to evaluate response of the patient to TTT and to assess the risk factors which may affect it.

- Complete blood count and Reticulocytic count
- Total iron profile (iron, ferritin, TSAT and TIBC)
- Blood chemistry (ALT, AST, albumin, Ca, phosphorus, urea and Zinc)
- Parathyroid hormone and HCV Ab
- Interleukien-6 (was measured by ELLISA) and quantitative C-reactive protein

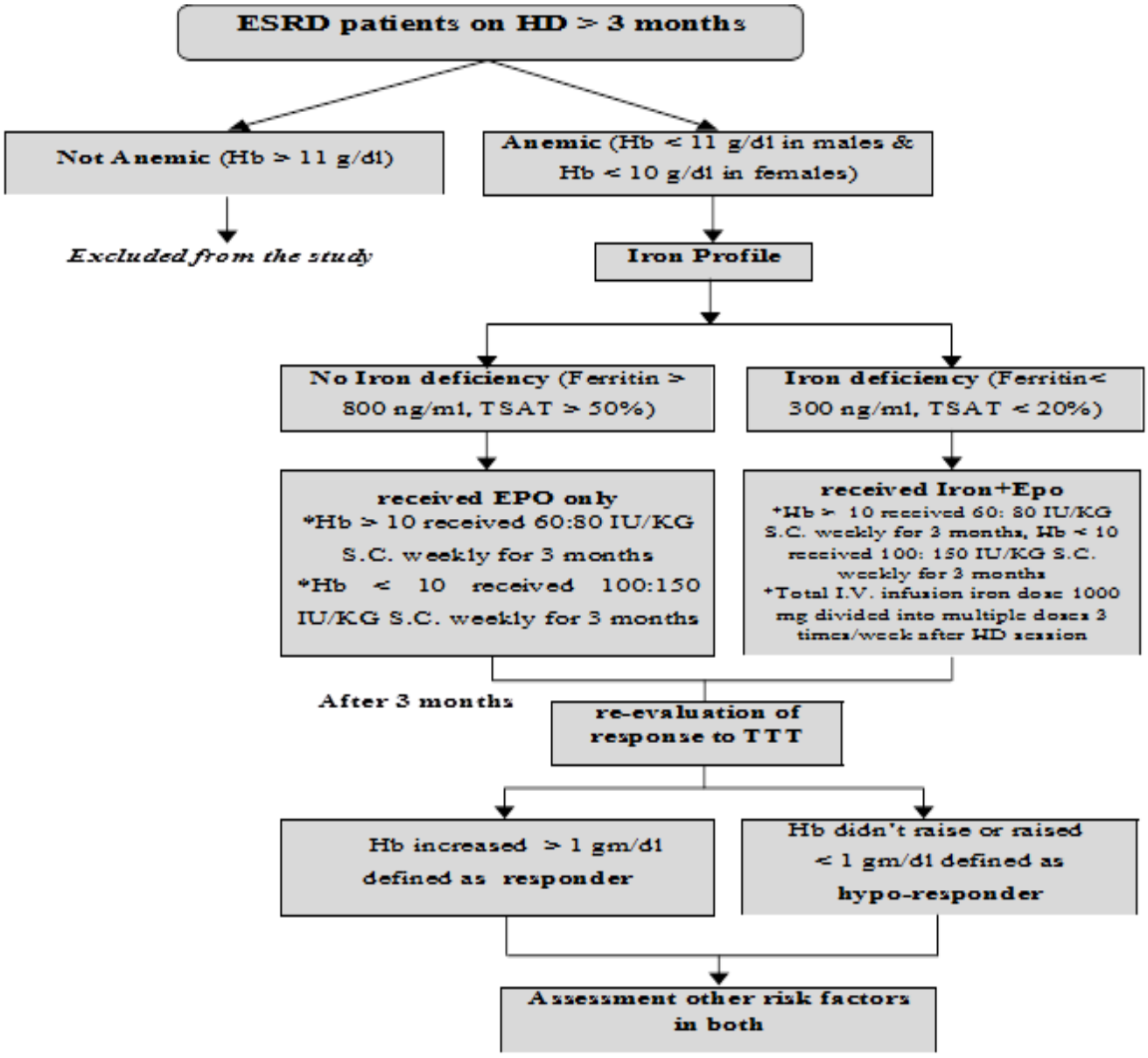
- Hepcidin-25 bioactive form (was measured by DRG Hepcidin-25 ELISA kits)
- Anti-erythropoietin antibody (was measured by ELLISA)

Then patients divided into 2 groups EPO responder & EPO hypo-responder.

**** The following equations were done in the study:**

- **Reticulocyte Production Index** (RI) = **Corrected Reticulocyte Count / maturation correction** [27].
- **Erythropoietin resistance index** (ERI) was established as the weekly adjusted dose of EPO according to body weight (U/kg/week) divided by Hb level (g/dl) [28].
- **TSAT** = S. iron / TIBC *100.

Data Management: Data were processed using Stata software 12 for windows, Quantitative data were presented as mean ± SD and qualitative data were presented as numbers and percentage, T-test and analysis of variance test were used to test significance of difference for quantitative data & Chi-square test was used to test significance of difference for qualitative data. Multiple regression analysis was used to assess the association between response to therapy and multiple laboratory factors. Probability value (p-value) < 0.05 was considered statistically significant.



Results

80 patients included in our study, 55% of the patients were males & 45% were females, with mean age (46.25 ± 17.7) (Table 1). 90% of the patients received dialysis from AV fistula, and 10% from central catheter. According to duration of dialysis, 38.75% of the patients were on regular HD for < 3 years, 21.25% for ≥ 10 years & 40% for 3-10 years. 66.25% of the patients made HD session for 3 – 3½ hours & 33.75% for 4 hours (Table 2). Most common chronic disease among our patients is hypertension (51.25%) while 36.25% of patients were found to be HCV positive (Table 3).

EPO responsive was 40% of the studied patients & EPO hypo-responsive was 60 % of the patients. The following factors showed no statistically significant difference between both responders and hypo-responders regarding age, sex, access, duration of dialysis in years, duration of each dialysis session and associated co morbidities.

Mean baseline ferritin level in both our studied groups before & after TTT was high as: before TTT ferritin level was 1187.9 ± 1140.5 & after TTT ferritin level was 1130.13 ± 1044.06. Each of iron, TIBC & TSAT levels were higher in responders than hypo-responders group. Whilst, ferritin level was lower in responders than non-responders without statistically significant (Table 4,5).

Regarding blood picture it showed that Hb level, Hct and RBC count after three months of TTT are significantly higher among responders compared to hypo-responders. MCV and RDW are higher among our ESRD patients than in control but without any statistically significant Ttable 6).

Blood chemistry revealed that albumin was significantly higher in responders, but other parameters as Ca, Ph, PTH, CRP and urea were higher in hyporesponder group without statistically significant. Zinc was higher in responder group but without statistically significant (Table 7)

RI was higher in responder group and ERI was higher in hyporesponder group, but both without statistically significant (Table 8).

Both hepcidin and IL-6 were markedly elevated among our studied ESRD patients than in control, mean level of hepcidin was (83.39 ± 13.69 Vs 17.88 ± 9.88) and IL-6 was (17.29 ± 13.03 Vs 11.19 ± 4.04). Also hepcidin and IL-6 were higher in hyporesponder than in responder group. The anti-EP antibody was positive among 12.5% of hypo-responders versus none of responders (Table 9).

Hepcidin has a statistically significant positive correlation with ferritin, statistically significant negative correlation between TIBC and no correlations with Il-6 or CRP. IL-6 has significant positive relation with CRP and negative relation with iron, albumin and zinc (Table 10).

Hb level, RBCs, iron and ferritin were higher in patients negative to anti-EPO antibody, while hepcidin was higher in patients positive to anti-EPO antibody (Table 11).

Our studied ESRD patients made HD at different times; we did not observe any significant diurnal variations in levels of hepcidin and IL-6 as present in normal populations (Tables 2 & 12).

Parathyroid hormone did not show any statistically significant correlation with HB, Hct, RI, CRC, calcium or phosphorus (table 13).

Hct has a statistically significant positive correlation with Hb, iron, ferritin, TSAT and negative relation with ERI (Table 14).

Mean level of ERI among our studied patients was 11.58 ± 4.38 and it has higher level among hyporesponders. ERI has a statistically significant negative correlation with Hb, MCV and RBC (Table 8 & 15).

Multiple linear regression of factors affecting response to erythropoietin therapy among studied patients showed that baseline Hb level, ERI, hepcidin and plasma albumin are significant determinants of response to erythropoietin therapy (Table 16).

Table 1. Personal characteristics among the studied patients according to response to erythropoietin therapy

Characteristics		Total (n=80)		Hypo-responders (n=48)		Responders (n=32)		p-values
Age	Mean ± SD	46.25 ± 17.7		46.75 ± 17.8		45.5 ± 17.9		0.8 (NS)
Sex	Male	44	55%	25	52.08%	19	59.38%	0.5 (NS)
	Female	36	45%	23	47.92%	13	40.63%	
Weight (Kg)	Mean ± SD	64.8 ± 19.1		66.9 ± 17.5		61.8 ± 21.2		0.3 (NS)

NS: no statistically significant difference

Table 1. shows that there is no statistically significant difference between both responders and hypo-responders regarding age, sex and weight. Most of the studied patients are males (55%).

Table 2. Dialysis characteristics among the studied patients according to response to erythropoietin therapy.

Characteristics		Total (n=80)		Hypo-responders (n=48)		Responders (n=32)		p-values
Access	AV fistula	72	90%	43	89.58%	29	90.63%	0.9 (NS)
	Catheter	8	10%	5	10.42%	3	9.38%	
Duration of dialysis (years)	< 3 years	31	38.75%	16	33.33%	15	46.88%	0.6 (NS)
	3 – 5	18	22.5%	11	22.92%	7	21.88%	
	6 – 9	14	17.5%	9	18.75%	5	15.63%	
	≥ 10 years	17	21.25%	12	25%	5	15.63%	
	Mean ± SD	5.5 ± 4.9		5.9 ± 5.1		4.9 ± 4.7		0.4 (NS)
Time of dialysis	6:30 am	34	42.5%	22	45.83%	12	37.5%	0.5 (NS)
	12 pm	36	45%	19	39.58%	17	53.13%	
	4 pm	10	12.5%	7	14.58%	3	9.38%	
Duration of dialysis session (hours)	3 – 3½ hours	53	66.25%	32	66.67%	21	65.63%	0.9 (NS)
	4 hours	27	33.75%	16	33.33%	11	34.38%	
	Mean ± SD	3.65 ± 0.25		3.65 ± 0.26		3.65 ± 0.27		0.9 (NS)

NS: no statistically significant difference
All patients are subjected to 3 dialysis session per week

Table 2 shows that there is no statistically significant difference between both responders and hypo-responders regarding access, duration of dialysis in years and duration of each dialysis session (hours). It appears that most of patients have AV fistula access (90%). Mean duration of dialysis is 5.5 years. Also, there is no statistically significant difference between responders and hypo-responders regarding time of dialysis.

Table 3. Medical disorders among the studied patients according to response to erythropoietin therapy.

Co morbidities		Total (n=80)		Hypo-responders (n=48)		Responders (n=32)		p-values
Autoimmune disorders	No	77	96.25%	45	93.75%	32	100%	0.1 (NS)
	Yes	3	3.75%	3	6.25%	0	0%	
DM	No	68	85%	43	89.58%	25	78.13%	0.2 (NS)
	Yes	12	15%	5	10.42%	7	21.88%	
Hypertension	No	39	48.75%	21	43.75%	18	56.25%	0.3 (NS)
	Yes	41	51.25%	27	56.25%	14	43.75%	
Heart diseases	No	73	91.25%	45	93.75%	28	87.5%	0.3 (NS)
	Yes	7	8.75%	3	6.25%	4	12.5%	
HCV	-ve	51	63.75%	31	64.58%	20	62.5%	0.8 (NS)
	+ve	29	36.25%	17	35.42%	12	37.5%	

NS: no statistically significant difference

Table 3 shows that only 3.75% of patients have autoimmune disorders. Most common chronic disease is hypertension (51.25%). 36.25% of patients are found to be HCV positive. There is no statistically significant difference between responders and hypo-responders.

Table 4. Hemoglobin, serum iron, ferritin, TIBC and transferrin saturation (after 3 months of treatment) among the studied patients according to response to erythropoietin therapy.

		Total (n=80)	Non-responders (n=48)	Responders (n=32)	p-values
Iron (after treatment)	Mean ± SD	76.93 ± 36.23	73.6 ± 36.1	80.58 ± 38.5	0.4 (NS)
Ferritin (after treatment)	Mean ± SD	1130.13 ± 1044.06 - 33.75% < 500 -17.5% ≥500<800 - 48.75% ≥ 800	1176.26 ± 1049.18	1060.9 ± 1049.17	0.6 (NS)
TIBC	Mean ± SD	204.26 ± 36.9	201.02 ± 39.4	209.13 ± 32.75	0.3 (NS)
TSAT	Mean ± SD	39.25 ± 20.3	38.55 ± 20.9	40.29 ± 19.6	0.7 (NS)

NS: no statistically significant difference

Table 4 shows that there is no statistically significant difference between both responders and non-responders regarding iron, serum ferritin, TIBC and TSAT after 3 months of treatment.

Table 5. Hemoglobin, serum iron and ferritin (Before and after treatment) among the studied patients according to response to erythropoietin therapy.

Characteristics		Non-responders (n=48)			Responders (n=32)		
		Before	After	p-value	Before	After	p-value
Hb	Mean ± SD	8.9 ± 1.1	8.87 ± 1.14	0.8 (NS)	8.3 ± 1.1	9.9 ± 1.16	0.001*
Iron	Mean ± SD	65.8 ± 39.9	73.6 ± 36.1	0.3 (NS)	71.9 ± 30.3	80.58 ± 38.5	0.3 (NS)
Ferritin	Mean ± SD	1189.1 ± 1126.8	1176.26 ± 1049.18	0.9 (NS)	1186.1 ± 1178.9	1060.9 ± 1049.17	0.7 (NS)

NS: no statistically significant difference
*Statistically significant difference

Table 5 shows that Hb level increases significantly among responders while there is no statistically significant change among non-responders. Iron and ferritin levels don't change significantly among both groups.

Table 6. CBC parameters (after 3 months of treatment) among the studied patients according to response to erythropoietin therapy.

CBC parameter		Total (n=80)	Hypo-responders (n=48)	Responders (n=32)	p-values
Hb	Mean ± SD	9.29 ± 1.25	8.87 ± 1.14	9.9 ± 1.16	0.001*
MCV	Mean ± SD	83.15 ± 3.41in control 86.1 ± 8.09 p-value 0.07 (NS)	85.98 ± 8.37	86.27 ± 7.79	0.8 (NS)
MCH	Mean ± SD	27.21 ± 4.29	27.35 ± 0.73	27.01 ± 2.78	0.7 (NS)
MCHC	Mean ± SD	30.9 ± 1.64	30.7 ± 1.77	31.3 ± 1.4	0.09 (NS)
Hematocrit	Mean ± SD	29.8 ± 4.04	28.7 ± 3.8	31.7 ± 3.7	0.001*
Reticulocytes	Mean ± SD	2.16 ± 1.18	2.23 ± 1.27	2.04 ± 1.03	0.5 (NS)
WBC ($\times 10^3$)	Mean ± SD	6.18 ± 1.66	6.17 ± 1.8	6.2 ± 1.5	0.9 (NS)
Platelet ($\times 10^3$)	Mean ± SD	189.9 ± 57.95	192.08 ± 62.2	186.8 ± 51.7	0.7 (NS)
RDW	Mean ± SD	15.58 ± 1.7	15.9 ± 1.7	15.2 ± 1.59	0.08 (NS)
RBC ($\times 10^6$)	Mean ± SD	3.47 ± 0.59	3.32 ± 0.57	3.69 ± 0.55	0.005*

NS: no statistically significant difference
*Statistically significant difference

As presents in *table 6*, after three months of treatment only Hb, Hct and RBC count are significantly higher among responders compared to hypo-responders.

Table 7 shows that there is no statistically significant difference between both responders and hypo-responders regarding all parameters of blood chemistry after 3 months of treatment except for plasma albumin that is significantly higher among responders.

Table 7. Blood chemistry and other laboratory parameters (after 3 months of treatment) among the studied patients according to response to erythropoietin therapy.

Blood chemistry		Total (n=80)	Hypo-responders (n=48)	Responders (n=32)	p-values
Creatinine	Mean ± SD	9.37 ± 7.59	9.85 ± 9.7	8.66 ± 2.02	0.4 (NS)
Urea	Mean ± SD	129.9 ± 36.45	133.3 ± 35.2	124.8 ± 38.2	0.3 (NS)
Calcium	Mean ± SD	8.99 ±8.56	9.4 ± 11.1	8.4 ± 1.01	0.6 (NS)
Phosphorus	Mean ± SD	4.63 ± 1.27	4.65 ± 1.4	4.58 ± 0.9	0.8 (NS)
Parathyroid hormone	Mean ± SD	522.1 ± 534.1	596.6 ± 608.8	410.3 ± 378.8	0.09 (NS)
ALT	Mean ± SD	19.2 ± 19.4	18.5 ± 14.5	20.2 ± 25.3	0.7 (NS)
AST	Mean ± SD	17.7 ± 10.5	18.1 ± 10.9	17.1 ± 10.1	0.7 (NS)
Albumin	Mean ± SD	3.97 ± 0.48	3.84 ± 0.44	4.16 ± 0.48	0.002*
CRP	Mean ± SD	7.65 ± 9.89	8.4 ± 11.7	6.5 ± 6.2	0.4 (NS)
Zinc	Mean ± SD	109.9 ± 68.9	104.2 ± 73.2	118.5 ± 62.1	0.4 (NS)
Rheumatoid factor	Mean ± SD	11.07 ± 32.48	13.3 ± 41.5	7.7 ± 8.7	0.4 (NS)

NS: no statistically significant difference
*Statistically significant difference

Table 8. Laboratory indices (after 3 months of treatment) among the studied patients according to response to erythropoietin therapy.

Laboratory indices		Total (n=80)	Hypo-responders (n=48)	Responders (n=32)	p-values
ARC	Mean ± SD	81587.57 ± 35496.04	83885.24 ± 39386.58	78141.07 ± 29046.65	0.5 (NS)
CRC	Mean ± SD	1.35 ± 0.72	1.34 ± 0.79	1.36 ± 0.61	0.9 (NS)
RI	Mean ± SD	0.83 ± 0.43	0.75 ± 0.43	0.94 ± 0.41	0.06 (NS)
ERI	Mean ± SD	11.58 ± 4.38	11.93 ± 5.09	11.07 ± 3.01	0.3 (NS)

NS: no statistically significant difference
ARC: Absolute reticulocyte count **CRC:** corrected reticulocyte count
RI: Reticulocyte production index **ERI:** Erythropoietin resistance index

Table 8 shows that there is no statistically significant difference between both responders and hypo-responders regarding laboratory indices include ARC, CRC, RI, and ERI.

Table 9. Hepcidin, IL-6, and anti- erythropoietin antibody among the studied patients according to response to erythropoietin therapy.

Characteristics		Total (n=80)		Hypo-responders (n=48)		Responders (n=32)		p-values
Anti-EP antibody	-ve	74	92.5%	42	87.5%	32	100%	0.03*
	+ve	6	7.5%	6	12.5%	0	0%	
Hepcidin	Mean ± SD	83.39 ± 13.69		86.09 ± 10.05		79.59 ± 17.05		0.04*
IL-6	Mean ± SD	17.29 ± 13.03		19.46 ± 15.55		13.94 ± 6.58		0.03*

*Statistically significant difference

Table 9 shows that anti-EP antibody is positive among 12.5% of hypo-responders versus none of responders with statistically significant difference. There is statistically significant difference between both groups regarding hepcidin and IL-6 levels that are higher among hypo-responders.

Table 10. Correlation between Hepcidin, IL-6, and CRP with other parameters among the studied patients.

	Hepcidin		IL-6		CRP	
	r	**p-value**	**r**	**p-value**	**r**	**p-value**
Hemoglobin after treatment	-0.08	0.5 (NS)	-0.07	0.6 (NS)	-0.1	0.2 (NS)
Iron after treatment	0.08	0.5 (NS)	**-0.3**	**0.007***	-0.1	0.3 (NS)
Ferritin after treatment	**0.5**	**0.001***	-0.09	0.4 (NS)	0.2	0.07 (NS)
TIBC	**-0.5**	**0.001***	0.05	0.7 (NS)	-0.2	0.1 (NS)
Transferrin saturation ratio	0.2	0.05 (NS)	-0.2	0.08 (NS)	-0.04	0.7 (NS)
Hematocrit	-0.03	0.8 (NS)	-0.1	0.3 (NS)	-0.07	0.5 (NS)
Albumin	0.07	0.5 (NS)	**-0.3**	**0.002***	-0.2	0.2 (NS)
CRP	0.2	0.1 (NS)	**0.5**	**0.001***	-	-
IL-6	-0.09	0.4 (NS)	-	-	**0.5**	**0.001***
ARC	0.07	0.5 (NS)	-0.1	0.4 (NS)	0.01	0.9 (NS)
CRC	-0.06	0.6 (NS)	-0.09	0.4 (NS)	0.01	0.9 (NS)
RI	0.06	0.6 (NS)	-0.01	0.9 (NS)	-0.03	0.8 (NS)
ERI	-0.1	0.3 (NS)	-0.09	0.4 (NS)	0.09	0.4 (NS)
Zinc	-0.1	0.3 (NS)	**-0.2**	**0.03***	-0.2	0.1 (NS)
Parathyroid hormone	0.02	0.8 (NS)	-0.09	0.4 (NS)	0.02	0.8 (NS)

NS: not statistically significant
*Statistically significant

Table 10 shows that there is statistically significant positive correlation between ferritin after treatment and hepcidin level and statistically significant negative correlation between TIBC and hepcidin level. IL-6 level is significantly correlated with CRP, iron after treatment, albumin and zinc.

Table 11. Comparison between different lab parameters according to anti- erythropoietin antibody:

		Anti-EPO antibody -ve (n=74)	Anti-EPO antibody +ve (n=6)	p-values
Hb after treatment	Mean ± SD	9.4 ± 1.23	8.01 ± 0.67	0.009*
Reticulocytes	Mean ± SD	2.18 ± 1.19	1.8 ± 0.9	0.4 (NS)
Hematocrit	Mean ± SD	30.1 ± 4.02	26.97 ± 3.34	0.07(NS)
RBCs	Mean ± SD	3.52 ± 0.58	2.9 ± 0.33	0.009*
Iron before treatment	Mean ± SD	69.47 ± 36.8	52 ± 26.05	0.1 (NS)
Iron after treatment	Mean ± SD	78.69 ± 37.2	48.17 ± 18.79	0.008*
Ferritin before treatment	Mean ± SD	1225.7 ± 1175.5	721.77 ±307.8	0.01*
Ferritin after treatment	Mean ± SD	1139.17 ± 1080.7	1018.6 ±392.4	0.5 (NS)
TIBC	Mean ± SD	204.6 ± 38.12	199.67 ± 16.8	0.6 (NS)
TSAT	Mean ± SD	39.82 ± 20.89	32.3 ± 9.25	0.1 (NS)
Hepcidin	Mean ± SD	82.7 ± 14.01	91.33 ± 4.46	0.003*
IL-6	Mean ± SD	16.39 ± 11.39	28.17 ± 25.06	0.3 (NS)
CRP	Mean ± SD	7.4 ± 9.8	10.3 ± 10.8	0.5 (NS)

NS: no statistically significant difference

Table 11 shows that there is statistically significant difference between both anti-EPO antibody -ve and anti-EPO antibody +ve groups regarding Hb after treatment, RBCs, iron after treatment, ferritin before treatment and hepcidin.

Table 12. Diurnal variation of hepcidin and Il-6 among ESRD patients.

Characteristics		Time of dialysis			p-values
		6:30 am	**12 pm**	**4 pm**	
Hepcidin	Total	86.9 ± 13.23	80.31 ± 14.8	83.6 ± 8.1	0.1 (NS)
	Responders	80.5 ± 17.87	78.53 ± 18.29	82.69 ± 6.93	0.9 (NS)
	Hypo- responders	90.95 ± 7.21[a]	81.89 ± 11.08[b]	84.29 ± 9.03[ab]	0.02*
	p-value	0.03*	0.5 (NS)	0.6 (NS)	
IL-6	Total	14.7 ± 11.6	18.42 ± 12.61	22.15 ± 17.8	0.2 (NS)
	Responders	12.09 ± 6.93	15.9 ± 6.37	10.67 ± 3.62	0.2 (NS)
	Hypo- responders	16.13 ± 13.47	20.52 ± 16.01	27.07 ± 19.46	0.3 (NS)
	p-value	0.3 (NS)	0.2 (NS)	0.03*	

*Statistically significant difference NS: no statistically significant difference
a, b indicate statistically significant difference with in different times of dialysis (post hoc test)

Table 12 shows that hepcidin level is lower among hypo-responder patients whom have their dialysis at 12 pm compared to patients have their dialysis session at 6:30 am. There is no statistically significant in diurnal variation of IL-6 in responders or hypo-responders groups.

Table 13. Correlation between parathyroid hormone with reticulocytes, RI, CRC, calcium, phosphorus, and hemoglobin after treatment among the studied patients.

	Parathyroid hormone	
	r	**p-value**
Reticulocyte	-0.2	0.08 (NS)
Hematocrit	0.08	0.5 (NS)
RI	0.01	0.9 (NS)
CRC	-0.07	0.5 (NS)
Calcium	-0.04	0.7 (NS)
Phosphorus	0.2	0.05 (NS)
Hb after treatment	0.06	0.6 (NS)
RDW	-0.03	0.8 (NS)

NS: not statistically significant
*Statistically significant

Table 13 shows that there is no statistically significant correlation between parathyroid hormone and reticulocytes, Hct, RI, CRC, calcium, phosphorus, RDW and Hb after treatment.

Table 14. Correlation between hematocrit with other parameters among the studied patients.

	Hematocrit	
	r	**p-value**
Hb after treatment	**0.9**	**0.001***
Iron after treatment	**0.3**	**0.02***
Ferritin after treatment	0.2	0.1 (NS)
TIBC	-0.02	0.8 (NS)
TSAT	**0.2**	**0.04***
Albumin	**0.3**	**0.01***
Hepcidin	-0.03	0.8(NS)
CRP	-0.07	0.5 (NS)
IL-6	-0.02	0.9 (NS)
ARC	0.06	0.6 (NS)
CRC	-0.03	0.8 (NS)
RI	-0.03	0.8 (NS)
ERI	**-0.5**	**0.001***

NS: not statistically significant
*Statistically significant

Table 15. Correlation between ERI with Hb after treatment, MCV, RBC & TIBC among the studied patients:

	ERI	
	r	p-value
Hb after treatment	**-0.5**	**0.001***
Hematocrit	**-0.5**	**0.001***
MCV	**-0.2**	**0.03***
RBC	**-0.2**	**0.03***
TIBC	**0.2**	**0.04***

*Statistically significant difference

Table 14 shows that there is statistically significant positive correlation between Hct and each of Hb after treatment, iron, ferritin, TSAT and ERI.

Table 15 shows that there is statistically significant negative correlation between ERI and Hb after treatment, MCV and RBC. Whereas, there is statistically significant positive correlation between ERI and TIBC.

Table 16. Multiple linear regression of factors affecting response to erythropoietin therapy among studied patients.

	Coefficient	Standard error	t	p-value	95% CI		
Constant	2.001	0.723	2.77	0.007	0.56	-	3.44
Il-6	-0.005	0.004	-1.16	0.3 (NS)	-0.012	-	0.003
Hepcidin	-0.01	0.004	-2.88	0.005*	-0.017	-	-0.003
Anti-EP antibody	-0.239	0.183	-1.31	0.2 (NS)	-0.6	-	0.12
Hb before treatment	-0.186	0.044	-4.19	0.001*	-0.27	-	-0.09
ERI	-0.039	0.011	-3.4	0.001*	-0.06	-	-0.019
Serum albumin	0.358	0.108	3.3	0.002*	0.14	-	0.57

*Statistically significant
NS: no statistically significant

Table 16 shows that baseline Hb level, ERI, hepcidin and plasma albumin are significant determinants of response to erythropoietin therapy.

Discussion

A study in 2003 displayed that the incidence of EPO hypo-responsiveness was approximately 5–10 percent of the patients due to immunological abnormalities and chronic inflammation [29]. But in our study EPO hypo-responsive were 60 % of the patients. The following factors showed no statistically significant difference between both responders and hypo-responders regarding age, access, duration of dialysis in years, duration of each dialysis session and associated co-morbidities.

In CBC parameters among our studied patients, only Hb, Hct (responders 31.7 ± 3.7: hypo-responders 28.7 ± 3.8) and RBC count (responders 3.69 ± 0.55 $\times 10^6$: hypo-responders 3.32 ± 0.57$\times 10^6$) were significantly higher among responders compared to hypo-responders. However, there was no statistically significant difference between both groups regarding reticulocytes, TIBC and TSAT.

Mean baseline Hb level in responders group was 8.3 ± 1.1 g/dl and in hypo-responders group it was 8.9 ± 1.1 g/dl. Hb level after treatment in responders showed marked improvement to be 9.9 ± 1.16 g/dl, but in hypo-responders showed no change in Hb level as it was still 8.87 ± 1.14 g/dl.

Regarding MCV in our studied population, it was 86.1 ± 8.09, while in control group it was 83.15 ± 3.41, however it did not differ statistically. This agreed with the study of *Ahmed M H et al* emphasized that MCV was significantly higher in CKD patients compared with control group (88.14 fl vs. 84.12 fl) [30]. This may appear as a result of multivitamins and EPO insufficiency [31].

Normal level of RDW in normal population ranges from 11.6 to 14.6 percent [32], in our study the mean level of RDW in the studied patients was 15.58 ± 1.7 % which was markedly elevated and there was no statistically significant difference between responders and hypo-responders. That may be explained by raised erythropoietic activity, increased RBCs destruction and reduced half life of RBCs as these mechanisms affect distribution of red cell size. This finding has been supported by a study by *Tekce et al* proved that RDW among studied population ranged from 14.7 ± 1.6 percent to 18.5 ± 2 percent [33], and also a study by *Emans et al* revealed that 26 percent of the patients had RDW more than 15 percent [34].

In our study Hct had statistically significant difference between responders and hypo-responders (31.7 ± 3.7 vs. 28.7 ± 3.8), Hct had statistically significant positive correlation with Hb, iron, albumin, TSAT and negative correlation with ERI and no correlation with urea, hepcidin, CRP, IL-6, ferritin, PTH. There was statistically significant difference between patients with duration of dialysis less than and more than 12 years regarding Hct (32.1 ± 3.6 pg/dl vs. 29.5 ± 4.01 pg/dl). *Rubab Z et al* described the same finding of our study regarding Hct: lower Hct level was significantly found in studied patients with ESRD than in control (30.9 ± 4.5 vs. 39.5 ± 3.1); responders were significantly higher (33.7 ± 3.0) compared with non-responders (26.8 ± 3.6) [35].

Mean baseline ferritin level in both of our studied groups before and after TTT was high (1187.9 ± 1140.5: 1130.13 ± 1044.06).High level of ferritin in nearly half of the studied patients was iatrogenic induced by the patients as

they used iron in excess doses as they falsely believed that iron can treat their anemia instead of EPO (due to financial causes) without consulting their doctor.

Each of iron, TIBC and TSAT levels were higher in responders than hypo-responders group (80.58 ± 38.5 vs. 73.6 ± 36.1), (209.13 ± 32.75 vs. 201.02 ± 39.4) and (40.29 ± 19.6 vs. 38.55 ± 20.9), respectively. Whilst, ferritin level was lower in responders than hypo-responders (1060.9 ± 1049.17 vs.1176.26 ± 1049.18) without statistically significant. These results were supported by *Rubab Z et al* who summarized the levels of iron, TIBC and ferritin in responder and non-responder groups (37.9 ± 12.5 vs. 33.0 ± 11.0: 278.9 ± 120 vs. 237.6 ± 93.0: 332.6 ± 264 vs. 378.7 ± 346.7), like our results: iron and TIBC higher in responders while ferritin higher in non-responders, but no significant difference was found [35]. High levels of ferritin might be noticed among patients with CKD, which caused by reticulo-endothelial blockade or functional iron deficiency [36].

Change in serum iron and ferritin after treatment with EPO among the studied patients:

- Change in iron level: increased in responders 8.69 ± 34.1 ng/ml and in hypo-responders raised about 7.93 ± 31.8 ng/ml.
- Change in ferritin level: decreased in responders 125.2 ± 593.9 ng/ml and in hypo-responders reduced 12.86 ± 683.2 ng/ml.

Jalalzadeh et al determined that after treatment with EPO and iron: ferritin dropped significantly from 1391 to 938 ng/ml and serum iron from 97.2 to 64.6 [37]. Hence, we recommend that EPO can be used in anemic patient with high serum ferritin to treat the anemia and to decrease iron stores in the body (iron chelating) in EPO responder patients before using iron chelating agent.

Reticulocyte production index appeared to be higher in responders (0.94 ± 0.41 in responders vs. 0.75 ± 0.43 in hypo-responders). Erythropoietin resistance index in total population was 11.58 ± 4.38, it was higher in hypo-responders (11.93 ± 5.09 in hypo-responders vs. 11.07 ± 3.01 in responders), although no significant difference was observed between both groups. Further, in our study ERI did not have statistically significant correlations with IL-6, CRP or hepcidin and had statistically significant negative correlation with each of Hb, MCV, RBCs and Hct and positive correlation with TIBC. Results of a study by *Kudoh Y et al* showed that baseline ERI in total population was 6.1 ± 6.0; ERI was insignificantly higher in non-EPO responder (8.29 ± 6.81) than responder (5.73 ± 3.42) [38]. *Kim et al* determined in their study that ERI had a statistically significant association with each of TSAT, CRP and albumin [39]. So, according to these results we suggest that we can use ERI as a predictive measure for the response to treatment with EPO.

Blood chemistry as (Creatinine, Urea, Calcium, Phosphorus, PTH, ALT, AST, CRP and Zinc) showed no statistically significant difference between both responders and hypo-responders groups except plasma albumin that was significantly higher among responders, hypo-albuminemia in our ESRD patients may due to nutritional factors or due to chronic mild inflammation. A study carried out at *Babol University of Medical Sciences* confirmed that low serum albumin in patients on HD is considered as an indicator of anemia and unresponsiveness to traditional management of anemia [40].

Mean level of hepcidin-25 in control group in our study was (17.88 ± 9.88 ng/mL). While in ESRD studied patients, it was markedly elevated (83.39 ± 13.69 ng/mL), mostly because of impairment in its renal excretion [41-43]. Hepcidin-25 level was significantly higher in hypo-responders (86.09 ± 10.05 ng/mL) compared to responders (79.59 ± 17.05 ng/mL). Similar results were found in a study at Mansoura University, Egypt [44]. Thus, hepcidin level may predict response to EPO treatment as it markedly affects iron metabolism [45].

There was statistically significant positive correlation between hepcidin-25 and ferritin and statistically significant negative correlation between hepcidin and TIBC in the current study. Nevertheless, hepcidin had no statistical correlation with Hb, iron, zinc, TSAT, CRP and IL-6. At Cairo University, a study revealed that hepcidin was significantly higher in HD patients compared with controls. Plasma hepcidin level correlated positively with ferritin, TSAT, CRP, and IL- 6; on the other hand, it negatively correlated with Hb [46], another study done by *Yamaya K et al* found a significant positive relation between hepcidin and iron and a negative relation between hepcidin and zinc [47].

Sedlackova T et al summarized that there was a weak relationship between hepcidin and ferritin and CRP in patients on HD and that the influence of inflammation on levels of hepcidin among HD patients was not pivotal and other factors prompting its retention among ESRD patients [48]. Further, *Ashby DR et al* mentioned in their study that level of hepcidin was considerably high among HD patients, although it did not associate with levels of IL-6, proposing that elevated hepcidin was not as a result of the general inflammatory status of the patients [49].

To evaluate the diurnal variation of hepcidin level among our studied patients, we collected blood samples from different groups of patients at different times; 42.5% of patients had their HD session at 6:30 am, 45% at 12 pm and 12.5% at 4 pm. Hepcidin level was lower among hypo-responder patients whom had their dialysis at 12 pm (81.89 ± 11.08) compared to patients had their dialysis session at 6:30 am (90.95 ± 7.21) with statistically significant difference. But, there was no statistically significant difference among responder patients whom had their

dialysis at 12 pm and at 6:30 am. *Ashby DR et al* documented that control group in their study showed an increase in hepcidin in the afternoon, while in CKD patients and HD patients (on a non-dialysis day), such diurnal variation in hepcidin was not noticed [49]. Another study by *Sedlackova et al* found that morning level of hepcidin was significantly different from the midday and evening levels in the studied patients with ESRD (morning hepcidin 32.1 µg/l: midday 18.8 µg/l: evening 9.6 µg/l) [48]. So, normal diurnal variation of hepcidin-25 disturbed among HD.

Mean level of IL-6 in control group in our study was (11.19 ± 4.04 pg/mL). Whereas, in ESRD studied patients it was significantly higher (17.29 ± 13.03 pg/mL) as most ESRD patients had subclinical chronic inflammation caused by mild chronic activation of complement system as the patient blood chronically exposed to foreign materials as artificial synthetic membranes, non sterile dialysate and other factors as : obesity, uremia, persistent infections as chlamydia pneumonia, oxidative stress, volume overload and genetic factors as single nucleotide polymorphisms [50-51]. Recently, *Alwahaibi NY et al* demonstrated that mean IL‑6 level amongst patients on HD was significantly higher than amongst control group (40.2 ± 41.4 vs.16.4 ± 5.4 pg/mL) and no significant difference was seen between pre- and post- dialysis samples [52].

IL-6 mean level was significantly higher in hypo-responders (19.46 ± 15.55 pg/mL) when compared to responders (13.94 ± 6.58 pg/mL), as the inflammatory cytokines lead to suppression of erythropoiesis and affect response to treatment [53]. This agreed with *Khalil SKM et al* who underlined that mean IL-6 in responders was (18.38 ± 2.20) and in non-responders was (24.06 ± 3.01) [54].

In the present study, IL-6 level had a significant negative correlation with iron, albumin and zinc and had significantly positive correlation with CRP. *Nakagawa H et al* explained in their study that IL-6 level was significantly inversely associated with iron amongst Japanese adults [55].

Anti-EPO antibody was positive in 7.5% of the total population of our study and appeared only in 12.5% of hypo-responder group with statistically significant difference. It is considered as the main risk factor for resistance to EPO [56-57].There was statistically significant difference in our study between anti-EPO antibody negative and anti-EPO antibody positive groups regarding Hb, RBCs, iron, base ferritin and hepcidin, no statistically difference was found between anti-EPO antibody positive and negative patients regarding reticulocytes, Hct, TIBC, TSAT, CRP and IL-6. *Alqahwaji DB et al* affirmed that 22.5 percent of the patients had the anti-EPO antibodies, besides there was statistically significant difference between anti-EPO negative and anti-EPO positive in reticulocytes, but no statistically significant difference regarding albumin, iron, TIBC, TSAT, Hb or RBCs [58].

Mean CRP level in our study was (7.65 ± 9.89) which is slightly elevated from normal population, there was marked difference in its level between responders (6.5 ± 6.2) and hypo-responders (8.4 ± 11.7), but it did not differ statistically. *Khalil SKM et al* asserted that mean CRP in responder group was (16.75 ± 1.54) but in non-responder group it was (30.00 ± 2.17) [54]. A study at Nasr city police hospital in Cairo determined a strong correlation between the response to EPO and levels of CRP [59]. Hence, it can be used in diagnosis and management of EPO hypo-responsiveness. Parathyroid hormone in our study had no statistically significant correlation with reticulocytes, RI, CRC, calcium, phosphorus and Hb or Hct level. This may be explained as most of our patients received regular treatment of calcium and vit D which may influence on function of parathyroid gland and its effect on bone and blood, so no relationships appeared to be statistically significant with PTH. *Adhikary LP et al* demonstrated that there was a weak negative association between Hct and PTH level that might be a cause of unsuspected low level of Hb among CKD patients [60].

In our study, 36.25% were HCV positive and 63.75% were HCV negative, with no statistically significant difference between both HCV positive and HCV negative groups regarding Hb, iron, ferritin, TIBC, TSAT, hepcidin, IL-6 and CRP. A study finished in HD units in Mansoura, Egypt, showed that the incidence of HCV was 70.7 percent and level of Hb was comparable in both HCV positive (10.32 ± 2.03 gm/dl) and HCV negative groups (10.22 ± 1.52 gm/dl), hence it did not display statistically significant [61]. Comparable results appeared in our study regarding Hb level, that in HCV positive (9.5 ± 1.1 gm/dl*)* and in HCV negative (9.2 ± 1.3 gm/dl) and also, it failed to show statistically significant. Mean level of CRP was higher in HCV negative studied patients (8.1 ± 10.7) compared to HCV positive patients (6.9 ± 8.3); while, IL-6 level was higher in HCV positive patients (18.43 ± 13.15) compared to HCV negative patients (16.67 ± 13.05) with no significant difference. These results were similar to the findings of *Afzal N et al* which showed that IL-6 levels were higher in HCV positive patients than in HCV negative patients; whereas HCV positive patients had lower CRP levels as compared to HCV negative patients, with no significant difference [62]. The modification in production of CRP among HCV positive patients established that hepatic injury via HCV may cause a deficiency in CRP production.

Conclusion

Hepcidin markedly elevated among our studied ESRD patients, however it did not show any correlations with

inflammatory mediators (IL-6 and CRP), so inflammatory mediators did not influence its high level, but mostly due to its retention as it was not excreted by the kidney.

Hepcidin is strongly correlated with ferritin and is markedly elevated among hypo-responder group to EPO therapy; therefore we may use hepcidin to investigate proper utilization of iron stores in anemic patients and to predict patient response to EPO therapy.

Mean level of IL-6 and CRP among studied ESRD patients was markedly elevated, also both IL-6 and CRP had higher level among hypo-responder group. That high level of inflammatory cytokines consider as an indicator for poor response to EPO treatment.

The prevalence of anti-EPO antibody begins to decrease as it is obvious in our study and other recent studies, which may explained as a result of improvement in its synthetic technique and development of new generations of EPO.

ERI, Hct and albumin are easy and cheap methods, hence we can use them to predict hypo-responsiveness or resistance to treatment.

References

1. Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anemia associated with chronic renal insufficiency among adults in the United States: results from the third national health and nutrition examination survey. J Am Soc Nephrol. 2002;13(2):504-10.
2. Astor BC, Muntner P, Levin A, Eustace JA, Coresh J.Association of kidney function with anemia: the third national health and nutrition examination survey (1988-1994). Arch Intern Med. 2002;162(12):1401-8.
3. Kamath S, Prabhu RA, Patil A. Prevalence of anemia in patients undergoing maintenance hemodialysis. Manipal University, India. Int J Clin Surg Adv. 2013;1(2):16-21.
4. El-Shahed AM, Sharf SA, El-Sebaee HA. Hemoglobin level, associated co-morbidities and quality of life among patients undergoing hemodialysis at one of the university hospitals in cairo governorate. Wasj. 2013;23(1):29-36.
5. Foley RN, Curtis BM, Parfrey PS. Erythropoietin therapy, hemoglobin targets and quality of life in healthy hemodialysis patients: a randomized trial. Clin J Am Soc Nephrol. 2009;4(4):726-33.
6. Hodges VM, Rainey S, Lappin TR, Maxwell AP. Pathophysiology of anemia and erythrocytosis. Crit Rev Oncol Hematol. 2007;64(2):139-58.
7. Anees MF, Hameed A, Mumtaz M Ibrahim M, Saeed Khan MN. Dialysis-related a factors affecting quality of life in patients on. hemodialysis. Iran J Kidney Dis. 2011;5(1): 9-14.
8. National Kidney Foundation. KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease .2012; 2: 288-310.
9. Priyadarshi A, Shapiro JI. Erythropoietin resistance in the treatment of the anemia of chronic renal failure. Semin Dial. 2006;19(4):273-8.
10. De Lurdes Agostinho Cabrita de Lurdes Agostinho Cabrita A, Pinho A, Malho A, Morgado E, Faísca M, Carrasqueira H, Silva AP, Neves PL. Risk factors for high erythropoiesis stimulating agent resistance index in pre-dialysis chronic kidney disease patients, stages 4 and 5. Int Urol Nephrol. 2011;43(3):835-40.
11. Costa E, Lima M, Alves JM, Rocha S, Rocha-Pereira P, Castro E, Miranda V, do SF, Loureiro A, Quintanilha A, Belo L, Santos-Silva A. Inflammation, T-cell phenotype, and inflammatory cytokines in chronic kidney disease patients under hemodialysis and its relationship to resistance to recombinant human erythropoietin therapy. J Clin Immunol. 2008;28(3):268-75.
12. Babitt JL, Lin HY. Molecular mechanisms of hepcidin regulation: Implications for the anemia of CKD. Am J Kidney Dis. 2010;55(4):726-41.
13. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem. 2001;276(11):7806-10.
14. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest. 2004;113(9):1271-6.
15. Zaritsky J, Young B, Gales B, Wang HJ, Rastogi A, Westerman M, Nemeth E, Ganz T, Salusky IB. Reduction of serum hepcidin by hemodialysis in pediatric and adult patients. Clin J Am Soc Nephrol. 2010;5(6):1010-4.
16. Stefansson BV, Abramson M, Nilsson U, Haraldsson B. Hemodiafiltration improves plasma 25-hepcidin levels: a prospective, randomized, blinded, cross-over study comparing hemodialysis and hemodiafiltration. Nephron Extra. 2012;2(1):55-65.
17. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T.Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood. 2003;101(7):2461-3.
18. Song SN, Tomosugi N, Kawabata H, Ishikawa T, Nishikawa T, Yoshizaki. Down-regulation of hepcidin resulting from long-term treatment with an anti-IL-6 receptor antibody (tocilizumab) improves anemia of inflammation in multicentric Castleman disease. Blood. 2010;116(18):3627-34.
19. Duranton F, Cohen G, De Smet R, Rodriguez M, Jankowski J, Vanholder R, Argiles A; European Uremic Toxin Work Group. Normal and Pathologic Concentrations of Uremic Toxins. J Am Soc Nephrol. 2012;23(7):1258-70.
20. Brancaccio D, Cozzolino M, Gallieni M. Hyperparathyroidism and anemia in uremic subjects: a combined therapeutic approach. J Am Soc Nephrol. 2004;1:21-4.
21. Bohrer D, Bertagnolli DC, De Oliveira SM do Nascimento PC, de Carvalho LM, Garcia SC, Arantes LC, Barros EJ. Role of medication in the level of aluminium in the blood of chronic haemodialysis patients. Nephrol Dial Transplant. 2009;24(4):1277-81.
22. Yaqoob M, Ahmad R, Mcclelland P, Shivakumar KA, Sallomi DF, Fahal IH, Roberts NB, Helliwell T. Resistance to recombinant human erythropoietin due to aluminium overload and its reversal by low dose desferrioxamine therapy. Postgrad Med J. 1993;69(808):124-8.
23. Bowry SK, Gatti E. Impact of hemodialysis therapy on anemia of chronic kidney disease: the potential mechanisms. Blood Purif. 2011;32(3):210-9.
24. Locatelli F, Altieri P, Andrulli S, Sau G, Bolasco P, Pedrini LA, Basile C, David S, Feriani M, Nebiolo PE, Ferrara R, Casu D, Logias F, Tarchini R, Cadinu F, Passaghe M, Fundoni G, Villa G, Di Iorio BR, Zoccali C. Predictors of haemoglobin levels and resistance to erythropoiesis-stimulating agents in patients treated with low-flux haemo dialysis, haemofiltration and haemodiafiltration: results of a

multicentre randomized and controlled trial. Nephrol Dial Transplant. 2012;27(9):3594-600

25. Johnson DW, Pollock CA, Macdougall IC. Erythropoiesis-stimulating agent hyporesponsiveness. Nephrology (Carlton). 2007;12(4):321-30.

26. Yen TH, Lin JL, Lintan DT, Hsu CW. Association between body mass and mortality in maintenance hemodialysis patients. Ther Apher Dial. 2010;14(4):400-8.

27. Fishbane S, Shapiro W, Dutka P, Valenzuela OF, Faubert J. A randomized trial of iron deficiency testing strategies in hemodialysis patients. Kidney Int. 2001;60(6):2406-11.

28. Okazaki M, Komatsu M, Kawaguchi H, Tsuchiya K, Nitta K. Erythropoietin resistance index and the all-cause mortality of chronic hemodialysis patients. Blood Purif. 2014;37(2):106-12.

29. Cooper A C, Breen C P, Vyas B, Ochola J, Kemeny DM, Macdougall IC. Poor response to recombinant erythropoietin is associated with loss of T-lymphocyte CD28 expression and altered interleukin-10 production. Nephrol Dial Transplant. 2003;18(1):133–40.

30. Ahmed MH, Khalil H BE, Adam KM. Study of erythrocyte and reticulocyte indices among patients with chronic kidney disease. Journal of Science. 2015;5:1387-90.

31. Arogundade F A, Bappa A, Sanusi A A et al. Haematologic indices and the response to erytheropoeitin in Chronic Renal Failure. Trop. J. Nephrol. 2009; 1:13-20.

32. Miri-Dashe T, Osawe S, Tokdung M, Daniel N, Choji RP, Mamman I, Deme K, Damulak D. Comprehensive reference ranges for hematology and clinical chemistry laboratory parameters derived from normal Nigerian adults. *PLOS One*. 2014;9(5):e93919.

33. Tekce H, Tekce B K, Aktas G, Tanrisev M, Sit M. The Evaluation of Red Cell Distribution Width in Chronic Hemodialysis Patients. Int J Nephrol. 2014;2014:754370

34. Emans ME, van der Putten K, van Rooijen KL, Kraaijenhagen RJ, Swinkels D, van Solinge WW, Cramer MJ, Doevendans PA, Braam B, Gaillard CA. Determinants of Red Cell Distribution Width (RDW) in Cardiorenal Patients: RDW is Not Related to Erythropoietin Resistance. J Cardi Fail. 2011;17(8).626-33.

35. Rubab Z, Amin H, Abbas K, Hussain S, Ullah MI, Mohsin S. Serum hepcidin levels in patients with end-stage renal disease on hemodialysis. Saudi J Kidney Dis Transpl. 2015;26(1):19-25.

36. Wish JB. Assessing iron status: Beyond serum ferritin and transferrin saturation. Clin J Am Soc Nephrol. 2006;1:S4-8.

37. Jalalzadeh M, Shekari E, Mirzamohammadi F, Ghadiani MH. Effect of short-term intravenous ascorbic acid on reducing ferritin in hemodialysis patients. Indian J Nephrol. 2012;22(3):168–73.

38. Kudoh Y, Aoyama S, Torii T, Chen Q, Nagahara D, Sakata H, Nozawa A. Long-term effects of oral L-carnitine supplementation on anemia in chronic hemodialysis. Cardiorenal Med. 2014;4(1):53–9.

39. Kim JK, Park BS, Park MJ, Choi W, Ma SK, Nah MY, Yeum CH, Jung K, Lee SC, Kim SW, Kim NH, Kang YJ, Choi KC. The predictive parameters of erythropoietin hyporesponsiveness in patients on continuous ambulatory peritoneal dialysis. Korean J Intern Med. 2001;16(2):110-7.

40. Heidari B, Taheri H, Hajian-Tilaki K, Yolmeh M[1], Akbari R. Low baseline serum albumin a predictor of anemia in patients on chronic hemodialysis. Caspian J Intern Med. 2015;6(3):161-4.

41. Zaritsky J, Young B, Wang HJ, Westerman M, Olbina G, Nemeth E, Ganz T, Rivera S, Nissenson AR, Salusky IB. Hepcidin a potential novel biomarker for iron status in chronic kidney disease. Clin J Am Soc Nephrol. 2009;4(6):1051-6.

42. Peters HPE, Laarakkers CMM, Swinkels DW, Wetzels JF. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. Nephrol Dial Transplant. 2010;25(3):848-53.

43. Mercadel L, Metzger M, Haymann JP, Thervet E[4], Boffa JJ[5], Flamant M[6], Vrtovsnik F[7], Houillier P[8], Froissart M[9], Stengel B[2]; NephroTest Study Group. The relation of hepcidin to iron disorders, inflammation and hemoglobin in chronic kidney disease. PLOS One. 2014;9(6):e99781.

44. Badawy A, El-Masry E, Hussein A. Role of hepcidin in recombinant human erythropoietin therapy resistance among chronic hemodialysis patients. IOSR-JDMS. 2014;86-92.

45. Sasu BJ, Cooke KS, Arvedson TL, Plewa C, Ellison AR, Sheng J, Winters A, Juan T, Li H, Begley CG, Molineux G. Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammation-induced anemia. Blood. 2010;115(17):3616-24.

46. Ibrahim IA, Mohamad UM, Darweesh HA. Impact of hepcidin, interleukin 6, and other inflammatory markers with respect to erythropoietin on anemia in chronic hemodialysis patients. Egypt J Intern Med. 2014;26:6-14.

47. Yamaya K, Tsuboi S, Tsutaya C. Plasma hepcidin and trace elements in chronic renal failure. NMCC ANNUAL REPORT. 2011.

48. Sedlackova T, Racek J, Eiselt J. Hepcidin and ferritin in hemodialyzed patients. Klin Biochem Metab. 2011;19(40):234–6.

49. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, Taube DH, Bloom SR, Tam FW, Chapman RS, Maxwell PH, Choi P. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. Kidney Int. 2009;75(9):976-81.

50. Cheung WW, Paik KH, Mak RH. Inflammation and cachexia in chronic kidney disease. Pediatric Nephrol. 2010;25(4):711-24.

51. Stenvinkel P, Ketteler M, Johnson RJ, Lindholm B, Pecoits-Filho R, Riella M, Heimbürger O, Cederholm T, Girndt M. Interleukin-10, IL-6 and TNF-a: important factors in the altered cytokine network of end-stage renal disease - the good, the bad and the ugly. Kidney Int. 2005;67(4):1216-33.

52. Alwahaibi NY, Alissaei HK, Alshihi SA. Serum levels of TNF-α, IL-6 and IL-10 in haemodialysis and renal transplant patients and in healthy subjects. Port J Nephrol Hypert. 2016.

53. Jeong JY, Silver M, Parnes A, Nikiforow S, Berliner N, Vanasse GJ. Resveratrol ameliorates TNF alpha-mediated suppression of erythropoiesis in human CD34 (+) cells via modulation of NF-kappaB signalling. Br J Haematol. 2011;155(1):93-101.

54. Khalil SK, Amer HA, El Behairy AM, Warda M. Oxidative stress during erythropoietin hyporesponsiveness anemia at end stage renal disease: Molecular and biochemical studies. J Adv Res. 2016;7(3):348-58.

55. Nakagawa H, Tamura T, Mitsuda Y, Goto Y, Kamiya Y, Kondo T, Wakai K, Hamajima N. Inverse correlation between serum interleukin-6 and iron levels among Japanese adults: a cross-sectional study. BMC Hematol. 2014;14(1):6.

56. Casadevall N, Nataf J, Viron B, Kolta A, Kiladjian JJ, Martin-Dupont P, Michaud P, Papo T, Ugo V, Teyssandier I, Varet B, Mayeux P. Pure red-cell aplasia and anti-erythropoietin antibodies in patients treated with recombinant erythropoietin. N Engl J Med. 2002;346(7):469-75.

57. Eckardt KU, Casadevall N. Pure red-cell aplasia due to anti-erythropoietin antibodies. Nephrol Dial Transplant. 2003;18(5):865-9.

58. Alqahwaji DB, Shubair ME, Sirdah M. Detection of anti-erythropoietin antibodies among hemodialyzed patients treated with recombinant human-erythropoietin. Sky Journal of Medicine and Medical Sciences. 2014;2(4):16–20.

59. El-Ghamry F, Nassar Y, El Sayed MA. The relation between the levels of C-reactive protein and the response to erythropoietin in anemic patients on haemodialysis. Al-Azhar Assiut Medical Journal (AAMJ). 2014.

60. Adhikary LP, Pokhrel A, Yadava SK, Khadka D, Thakur R. Relation between Serum Intact Parathyroid Hormone Level and Hematocrit in Chronic Kidney Disease Patients. Kathmandu Univ Med J. 2015;13(51):220-3.

61. Sabry A, El-Dahshan K, Mahmoud K. Effect of hepatitis c virus infection on haematocrit and haemoglobin levels in Egyptian hemodialysis patients. European Journal of General Medicine. 2007;4:9-15.

62. Afzal N, Abbas S, Ahmed A, Arif M, Javeed K. Effect of Hepatitis C Virus on C - reactive protein and Interleukin-6 in Hemodialysis Patients. Iran J Kidney Dis. 2011;5(3):182-6.