Original Research Article

Effect of Lactoferrin Administration on Serum Iron Status of Children on Regular Hemodialysis

Abstract

Background: This study aimed to evaluate the effect of lactoferrin administration on serum iron status of children on regular hemodialysis.

Methods: This case-controlled study was carried on 60 children with end stage renal disease on regular hemodialysis and 30 healthy children served as a control group. Patients were classified into 2 groups, group A: 30 patients received oral lactoferrin supplementation 100 mg for 3 months and group B: 30 patients received oral lactoferrin supplementation plus oral iron 5mg/kg/day for 3 months. Serum ferritin, transferrin saturation (TSAT), serum hepcidin and IL6 were measured before and after supplementation

Results: Serum ferritin was significantly higher in patient groups compared to controls before the supplementation (P <0.001) but significantly decreased after supplementation (P= <0.001). No significance difference between patient groups in serum ferritin levels before or after the supplementation (P=0.778, P=0.763) respectively. TSAT was significantly lower in patients groups compared to controls before the supplementation (p<0.001) but showed significant decrease after supplementation in group A (P=0.036) and significant increase in group B after the supplementation (P<0.001). TSAT was significantly increased in group B compared to group A (P<0.001). Serum hepcidin and serum interleukin 6 levels were higher in patients than controls before supplementation but significantly decreased at the end of the study in patient groups. . There were positive correlations between serum hepcidin and interleukin 6, CRP, and ferritin levels but a negative correlation with hemoglobin and TSAT. There was a positive correlation between serum IL-6 and both CRP and ferritin but a negative correlation between IL-6 and both Hb and TSAT.

Conclusions: Oral lactoferrin was not able to maintain iron status in HD children while Lactoferrin plus oral iron was effective in treating iron deficiency in HD patients.

Keywords: Ferritin, Hepcidin, Interleukin 6, Hemodialysis.

Introduction:

"Anemia is one of the most common and clinically significant complications of chronic kidney disease (CKD) in children and is associated with increase in mortality, the development, and the progression of cardiovascular diseases".[1]Anemia of CKD is a

multifactorial process; Erythropoietin deficiency, uremic toxins, shortened red cell survival, lack of essential nutrients like folic acid and vitamin B12 and iron deficiency are involved in the development of anemia of CKD.[2,3] "Iron deficiency may be secondary to absolute iron deficiency in which accessible iron stores are depleted or functional iron deficiency due to impaired iron trafficking in the setting of inflammation". [4] "Absolute iron deficiency may be due to decreased nutritional intake, poor enteral absorption or blood loss via the gastrointestinal tract menstruation, frequent phlebotomy, and hemodialysis". [1]

"Functional iron deficiency occurs due to changes in iron homeostasis causing shift in iron from the circulation to deposits (macrophages and reticulo-endothelial cells) and limited availability to erythroid progenitors, thereby reducing erythropoiesis". [5]

During inflammation, there is increase in serum interleukin 6 which induce synthesis of hepcidin by hepatocytes. Hepcidin is specifically involved in the diversion of iron traffic through duodenal absorption and blocks its release from macrophages. "Hepcidin regulates intestinal iron absorption and body iron distribution through its post translational suppression of cell membrane ferroportin expression". [6] "When bound by hepcidin, ferroportin is internalized and degraded leading to down regulation of dietary iron absorption by intestinal enterocytes and inhibition of the release of intracellular iron stored in ferritin for utilization in erythropoiesis". *[7]*

"The primary mechanism for clearance of circulating hepcidin is glomerular filtration and proteolysis in the proximal tubule, which is decreased in the setting of reduced glomerular filtration rate (GFR).So, hepcidin levels are elevated in children with CKD and those on dialysis". [8]

"Lactoferrin (Lf) has long been recognized as a member of the transferrin family of proteins and an important regulator of the levels of free iron in the body fluids of mammals.[5] Its ability to bind ferric iron with high affinity and to retain it to low PH gives the protein bacteriostatic and antioxidant properties".[9]—"Lf exhibits other functions besides iron sequestration, such as a strong capacity to modulate the inflammatory response by its capacity to reduce proinflammatory cytokine expression including IL-6". [10]

Oral lactoferrin administration was found to be helpful in reducing serum level of IL-6 and hepcidin in pregnant females suffering functional iron deficiency anemia and in adults with colorectal cancer receiving chemotherapy. **[9, 11)]**Thus, oral lactoferrin administration may be useful in treating Iron deficiency anemia in hemodialysis children.

Patients and Methods:

This case-controlled study was carried on 60 children with End stage renal disease on regular hemodialysis attending Nephrology Unit at Tanta University Hospitals, Pediatric Department, their ages ranged from 5 to 18 year. They were undergoing hemodialysis three times per week, with each dialysis session lasting for three to four hours. End stage renal disease was considered when GFR is equal or less than 15 ml/min./1.73m^2 for more than 3 months.

Estimated glomerular filtration (eGFR) was calculated individually by modified Schwartz formula. [12]

Patients were dialysed on Fresenius 4008 B or Fresenius 4008 S dialysis machine (Germany) at blood flow rate equal 5-7ml/kg/min., using polysulphane hollow fiber dialysers suitable for the surface area of patients (Fresenius F3 = 0.4 m^2, F4 = 0.7m^2, F5 = 1.0m^2 and F6=1.2m^2). Bicarbonate dialysis solutions were used.

All patients were receiving supportive therapy in the form of SC erythropoietin in a dose of 50 -250 IU/Kg/session, oral folic acid 1 mg/day, oral calcium-based binders 50mg/kg/day, oral vitamin D (one alpha hydroxyl vitamin D) in a dose of 0.01-0.05 µg/Kg/day and oral antihypertensive medications for hypertensive patients. All patients enrolled in the study discontinued iron supplementation two weeks before the study.

Patients were classified into 2 groups:

Group A: 30 patients received oral lactoferrin supplementation 100 mg bovine lactoferrin (Pravotin, HYGINT Pharmaceuticals) r daily for 3 months.

Group B: 30 patients received oral lactoferrin supplementation Plus oral iron 5mg/kg/day elemental iron for 3 months. (Haemojet syrup, 161.25mg ferric hydroxide polymaltose/5ml equal 50 mg elemental iron "European Egyptian pharm, Egypt" or Haemojet capsules, 322.5mg ferric hydroxide polymaltose equal 100 mg elemental iron taken two hours after meal).

Exclusion criteria: Patients with acute infection **,**hepatic diseases**,** malabsorption syndrome **,**genetic types of anemia (thalassemia, sickle cell anemia and G6PD), active bleeding, anemic patients with hemoglobin level < 8 g/dl at start of the study, history of blood transfusion within 2 and patients on hemodialysis for less than three months.

Specimen collection and handling:

Blood samples were taken from all patients before starting supplementation and after completing the course of supplementation. Ten ml of blood were withdrawn aseptically from all patients from arterial blood line at initiation of hemodialysis.

Blood samples were divided into:

- **Sample (1):** 6 ml of them were used for routine investigations.
- **Sample (2):** 4 ml were collected without additives then stored at room temperature until coagulation occurred (usually 15-45 minutes), then centrifuged for 20 minutes at the speed of 2000-3000 run per minute to obtain the serum specimen for assay of serum ferritin, serum iron, total iron binding capacity, serum hepcidin and serum interleukin 6.

These specimens were kept at -20°C till analysis.

Serum hepcidin levels were determined using enzyme-linked immuno-sorbent assay. ***(Shanghai Sunred Biological Technology Co., Ltd)***

Serum interleukin 6 levels were determined using enzyme-linked immuno-sorbent assay. ***(Shanghai Sunred Biological Technology Co., Ltd)***

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level. Chi-squar test for categorical variables, to compare between different groups. Fisher's Exact or Monte Carlo correction, Correction for chi-square when more than 20% of the cells have expected count less than 5. Student t-test for normally distributed quantitative variables, to compare between two studied groups. F-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons. Paired t-test for normally distributed quantitative variables, to compare between two periods. ANOVA with repeated measures for normally distributed quantitative variables, to compare between more than two periods or stages, and Post Hoc test (Bonferroni adjusted) for pairwise comparisons. Mann Whitney test for abnormally distributed quantitative variables, to compare between two studied groups. Kruskal Wallis test for abnormally distributed

quantitative variables, to compare between more than two studied groups and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. Wilcoxon signed ranks test for abnormally distributed quantitative variables, to compare between two.

Results:

Table 1: Demographic data of the studied groups.

	Patients (n = 60)		Controls (n = 30)		Test of Sig.	p
	No.	**%**	**No.**	**%**		
Sex						
Male	31	51.7	14	46.7	χ^2= 0.200	0.655
Female	29	48.3	16	53.3		
Age (years)						
Mean ± SD.	12.27 ± 2.90		11.27 ± 3.20		t=1.488	0.140
Z-score for weight						
Mean ± SD.	**-2.30 ± 0.67**		**-0.12 ± 1.0**		t=6.346*	<0.001*
Z-score for height						
Median (IQR)	**-2.20(-2.90 – -2.0)**		-0.65 (-0.80 – 1.0)		U=38.0*	<0.001*
Systolic blood pressure percentile						
<90th	32	53.3	30	100.0	χ^2= 21.294	FEp <0.001*
≥90 - <95	8	13.3	0	0.0		
95th-99th	14	23.3	0	0.0		
>99th	6	10.0	0	0.0		
Diastolic blood pressure percentile						
<90th	**30**	**50**	**30**	**100.0**	χ^2= 24.918*	MCp<0.001*
≥90 - <95	**7**	**11.7**	**0**	**0.0**		
95th-99th	**16**	**26**	**0**	**0.0**		
>99th	**7**	**11.7**	**0**	**0.0**		

Table 2: Routine laboratory investigations of the studied groups.

	Group A		Group B		Controls		Test of sig.	p
	No.	**%**	**No.**	**%**	**No.**	**%**		
Hb (g/dl)								
Mean ± SD.	9.31 ± 0.93		9.12 ± 0.68		11.35 ± 0.69		F=75.998*	<0.001*
Sig.bt.Grps	p1=0.632,p2<0.001*,p3<0.001*							
MCV(fl/cell)								
Mean ± SD.	87.09 ± 6.04		81.75 ± 3.89		91.20 ± 3.99		F=29.914*	<0.001*
Sig.bt.Grps	p1<0.001*,p2=0.003*,p3<0.001*							
MCH (pg/cell)								
Mean ± SD.	26.06 ± 1.07		27.33 ± 2.65		29.77 ± 1.81		F=27.912*	<0.001*
Sig.bt.Grps	p1=0.037*,p2<0.001*,p3<0.001*							
MCHC(g/dl)								
Mean ± SD.	31.43 ± 1.90		29.92 ± 3.95		34.60 ± 1.77		F=22.920*	<0.001*
Sig.bt.Grps	p1=0.087,p2<0.001*,p3<0.001*							
PLT*10^3 (c/$mm^{3)}$								
Mean ± SD.	259.77 ± 69.55		231.03 ± 48.59		262.13 ± 46.05		F=2.895	0.061
WBCs (c/$mm^{3)}$								
Mean ± SD.	6501.67 ± 1443.21		5703.33 ± 1496.54		6405.0 ± 1186.73		F=2.981	0.056

p: p value for comparing between the studied groups
p_1: p value for comparing between group A and group B
p_2: p value for comparing between group A and control
p_3: p value for comparing between group B and control
*: Statistically significant at $p \leq 0.05$

There was significantly lower hemoglobin, MCV, MCH, MCHC in patient groups than controls, but there were insignificant differences regarding Hb, MCV, MCH and MCHC between patient groups.

Table 3: Iron indices in studied groups before and after the supplementation:

		Group A (n = 30)	Group B (n = 30)	Control (n = 30)	H	p
	Before					
Serum Ferritin(ng/ml)	Median (IQR).	530.0 (130.0 –700.0)	545.50 (330.0 –700.0)	118.0 (72.0 –139.0)	31.422*	<0.001*
	Sig.bt.Grps	p_1=0.778,p_2<0.001*,p_3<0.001*				
	After					
	Median (IQR)	350.0 (120.0 –600.0)	301.50 (197.0 –348.0)	118.0 (72.0 –139.0)	34.481*	<0.001*
	Sig.bt.Grps	p_1=0.763, P4<0.001*, p5<0.001*				
Transferrin saturation%	**Before**					
	Median (IQR)	15.0 (14.0 – 15.0)	17.50 (17.0 – 18.0)	35.0 (28.0 – 41.0)	H= 66.566*	<0.001*
	Sig.bt.Grps	p_1=0.010*,p_2<0.001*,p_3<0.001*				
	After					
	Median (IQR)	14.0 (13.0 – 15.0)	19.0 (19.0 – 21.0)	35.0 (28.0 – 41.0)	H= 77.602*	<0.001*
	Sig.bt.Grps	p_1<0.001*, $p_4$0.036*, p_5<0.001				

p: p value for comparing between the studied groups
p_1: p value for comparing between **group A** and **group B**
p_2: p value for comparing between **group A** and **control**
p_3: p value for comparing between **group B** and **control**
p_4: p value for comparing between **the before supplementation and after supplementation in group A**
P_5: p value for comparing between **the before supplementation and after supplementation in group B**

Serum ferritin was significantly higher in-patient groups compared to controls before the supplementation (P <0.001). In patient groups, serum ferritin significantly decreased after supplementation in comparison with serum ferritin before supplementation (P= <0.001) .No significance difference between patient groups in serum ferritin before the supplementation or after the supplementation (P=0.778, P=0.763) respectively.

TSAT was significantly lower in patients groups compared to control before the supplementation (p<0.001). TSAT showed significant decrease after supplementation in group A (p=0.036), but there was significant increase in group B after the supplementation. TSAT was significantly higher in group B compared to group A (P<0.001).

Table 4: Serum hepcidin and interleukin 6 in studied patients before and after the supplementation:

		Group A (n = 30)	Group B (n = 30)	Controls (n = 30)	Test of sig.	p
Serum Hepcidin(ng/ml)	**Before**					
	Median (IQR)	624.7 (233.5 – 784.8)	714.1(432.5 – 800.8)	76.37 (67.81 – 84.92)	H= 59.873*	<0.001*
	Sig.bt.Grps	p_1=0.495,p_2<0.001*,p_3<0.001*				
	After					
	Median (IQR)	250.5(120.7 – 339.1)	282.5(197.7 – 365.9)	76.37(67.81 – 84.92)	H= 59.057*	<0.001*
	Sig.bt.Grps	p_1=0.543,p_4<0.001*,p_5<0.001*				
Serum interleukin 6 (pg/ml)	**Before**					
	Median (IQR)	145.62 (124.4 –160.2)	137.74 (110.6 –150.1))	25.71 (13.6- 40.8)	F= 203.852*	<0.001*
	Sig.bt.Grps	p_1=0.249,p_2<0.001*,p_3<0.001*				
	After					
	Median (IQR)	88.94 (79.4 –102.6)	90.62 (69.0 –103.4	25.71 (13.6- 40.8)	F= 86.758*	<0.001*
	Sig.bt.Grps	p_1=0.537,p_2<0.001*,p_3<0.001*				

p: p value for comparing between the studied groups
p_1: p value for comparing between **group A** and **group B**
p_2: p value for comparing between **group A** and **control**
p_3: p value for comparing between **group B** and **control**
p_4: p value for comparing between **the before supplementation and after supplementation in group A**
P_5: p value for comparing between **the before supplementation and after supplementation in group B**

Hepcidin was significantly higher inpatient groups compared to controls before supplementation (P<0.001). There was a significant decrease in serum hepcidin in patient groups after the supplementation (P<0.001). No significant difference between patient groups before the supplementation or after the supplementation (P = 0.495, P=0.543) respectively.

Serum interleukin 6 was significantly higher in-patient groups compared to controls before supplementation (P<0.001) but significantly decreased in patient groups after the supplementation (P<0.001).

Table 5: Correlation between serum hepcidin, serum interleukin 6, iron indices and hemoglobin of the patients:

	Hepcidin		Interleukin 6	
	r_s	**p**	**r**	**p**
Interleukin 6	0.825	<0.001*	–	–
S ferrtitin	0.625	<0.001*	0.676	<0.001*
Transferrin saturation	-0.708	<0.001*	-0.823	<0.001*
Hb	-0.808	<0.001*	-0.794	<0.001*
CRP	0.670	<0.001*	0.521	<0.001*

r: Pearson coefficient **r_s: Spearman coefficient**

There was a positive correlation between serum hepcidin and both serum interleukin 6 and serum ferritin. Negative correlation between serum hepcidin and both TSAT and Hb. There was a negative correlation between serum interleukin 6 and both TSAT and Hb. Negative correlation was observed between CRP and both hepcidin and interleukin 6.

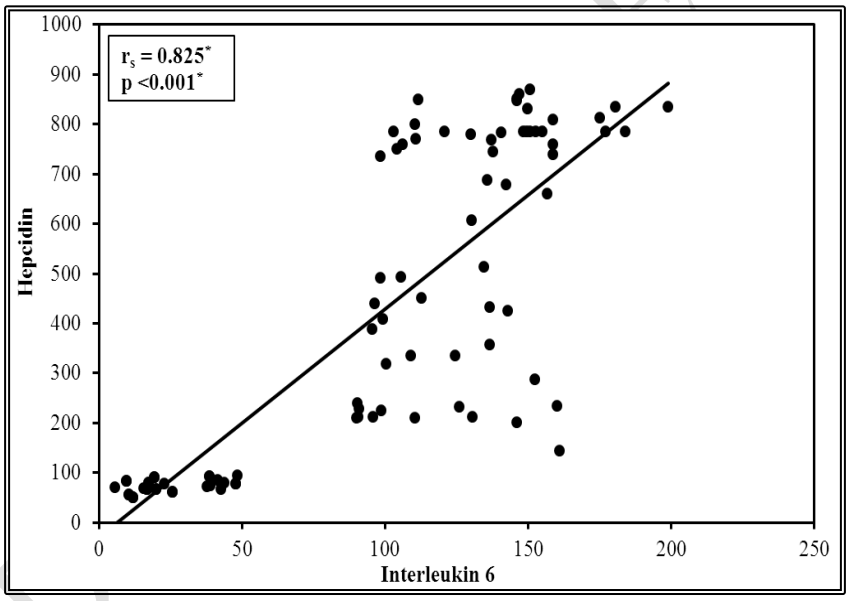

Figure (1): Correlation between serum interleukin 6 and Hepcidin.

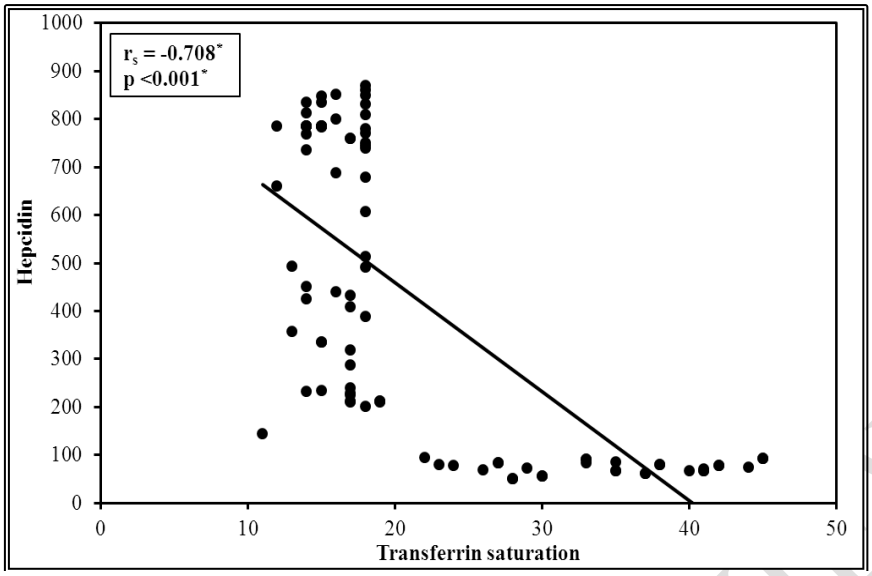

Figure (2): Correlation between serum hepcidin and transferrin saturation.

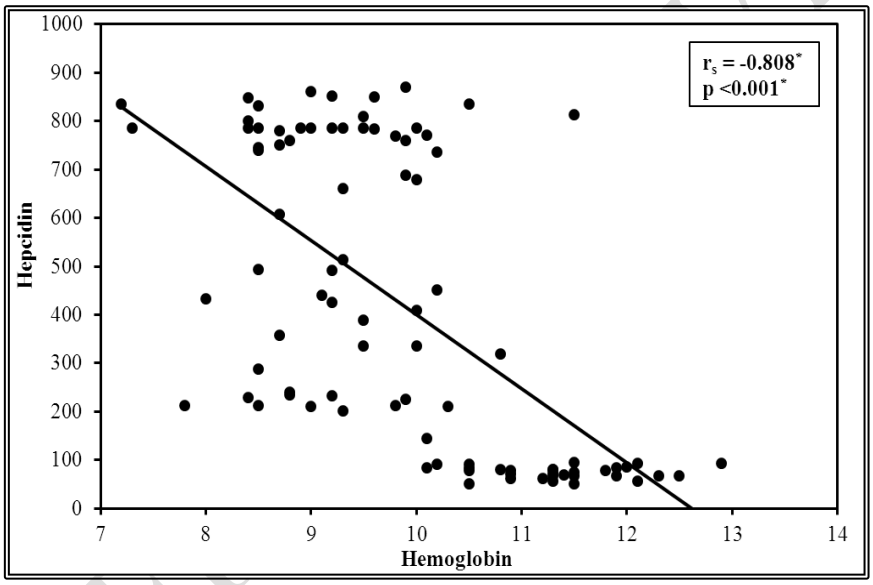

Figure (3): Correlation between serum hepcidin and Hb.

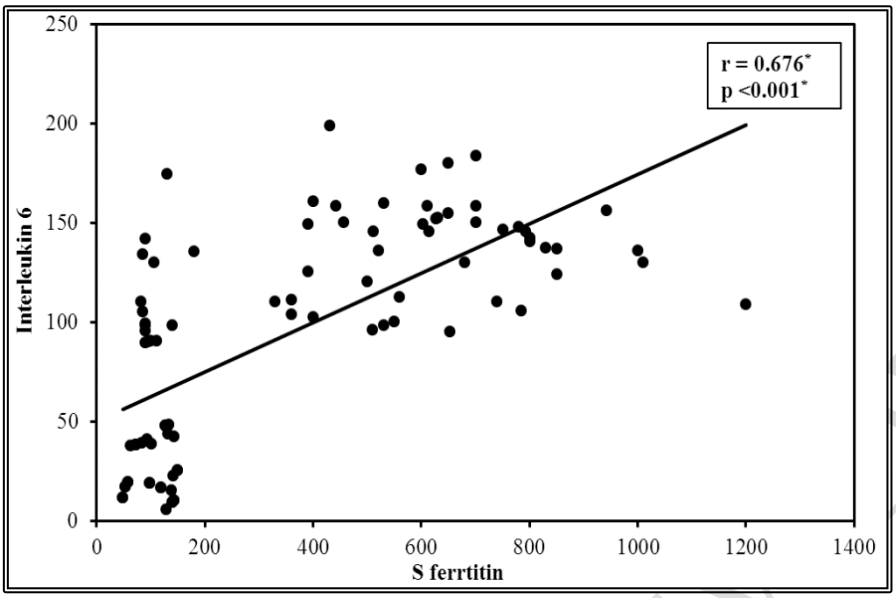

Figure (4): Correlation between serum interleukin 6 and ferritin.

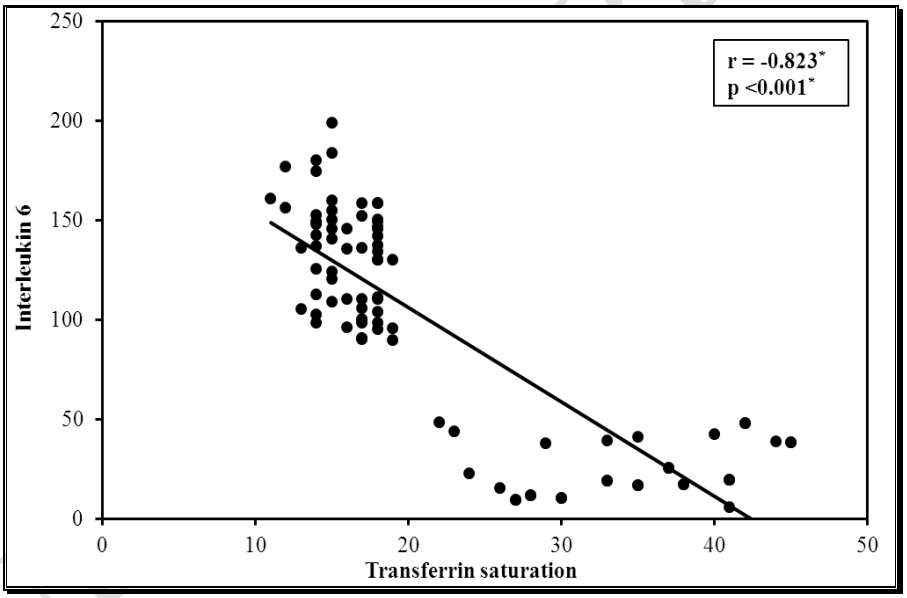

Figure (5): Correlation between serum interleukin 6 and transferrin saturation.

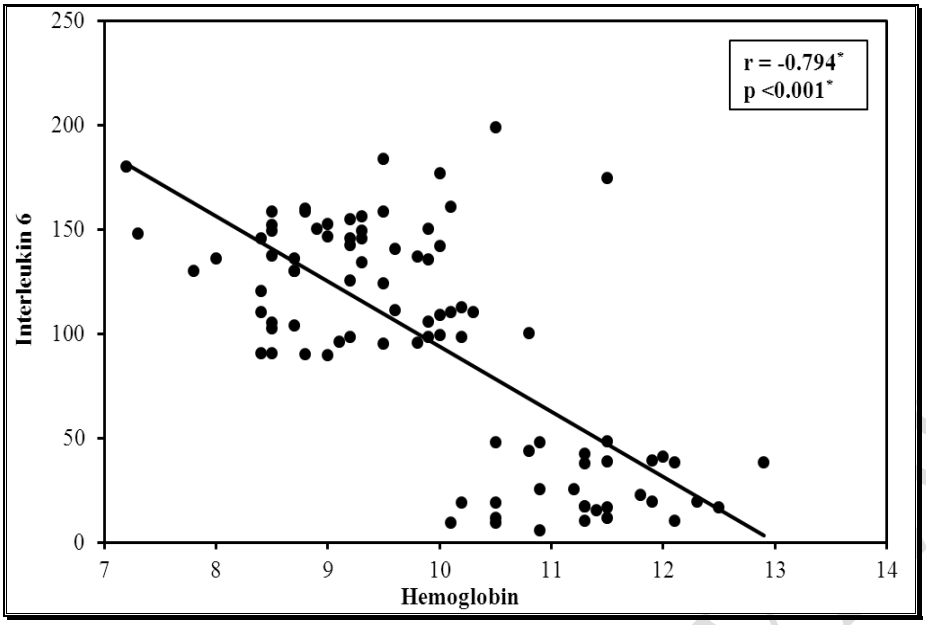

Figure (6): Correlation between serum interleukin 6 and hemoglobin.

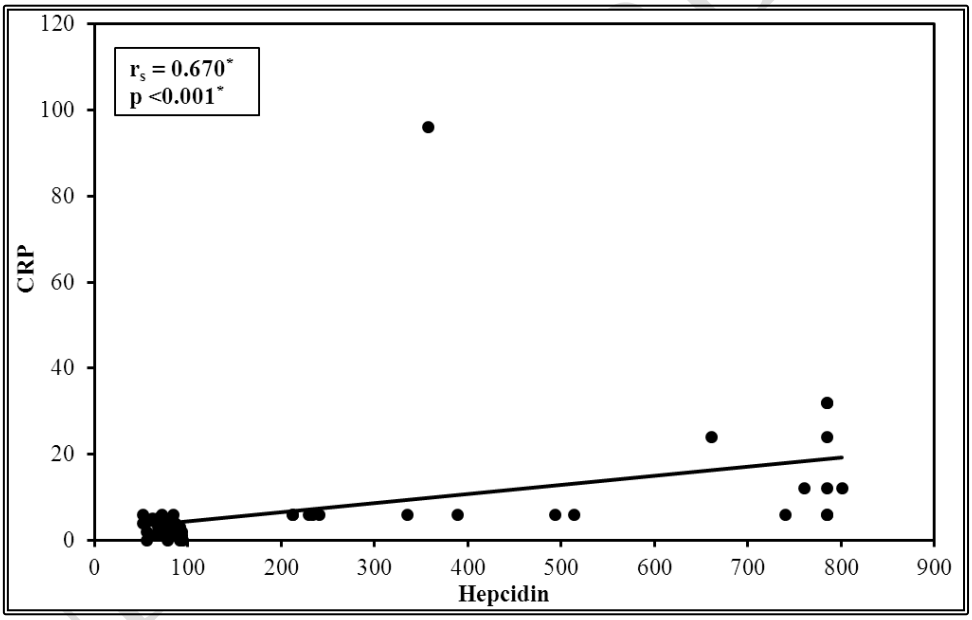

Figure (7): Correlation between serum hepcidin and CRP.

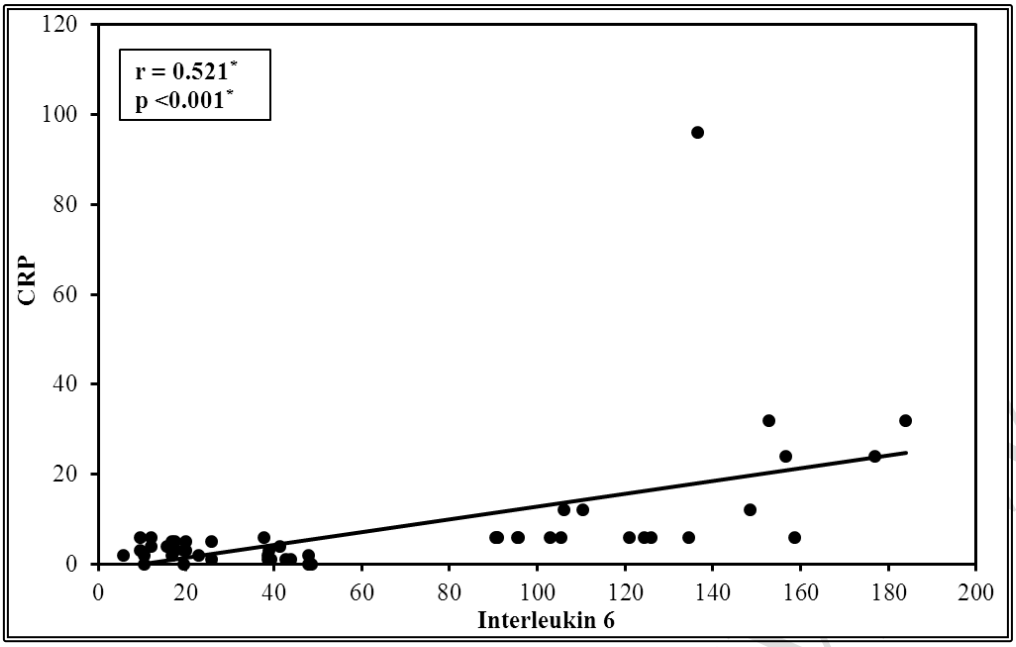

Figure (8): Correlation between serum interleukin 6 and CRP.

Discussion

Few studies have been performed for studying the effect of lactoferrin on iron status in anemia of chronic inflammation. Most of these studies were carried out on adult patients. (11,13,14)

Prevalence of anemia in children on regular dialysis ranged from 45% ***(15)*** to 83 %. ***(13)*** In the current study, 65% of patients were considered anemic according to KDIGO guidelines. **(16)**

The disparity in the reported prevalence of anemia in children with ESRD may be due to various definitions of anemia used in the different studies. In ***Davidkova et al., 2016***study fixed value of Hb level <11 g/dl for all ages was used to define anemia, while in ***Mudi and Levy, 2018*** study anemia was defined as hemoglobin below the 5th percentile for age/sex. In addition to different regimens used to treat anemia in these studies including regular EPO dose, iron therapy, control of inflammation and folic acid supplementation. ***(13, 15)***

.

The results obtained in present study detected that serum ferritin was significantly higher in patient groups compared to controls, while serum iron and TSAT were significantly lower in patient groups compared to controls at the start of the study.

Several studies conducted on children on dialysis were compatible with the results of the present study. ***(15, 17, 18, 19)***

In the present study, serum ferritin was negatively correlated with Hb, but a significant positive correlation between serum ferritin and CRP was noticed. An association between high serum ferritin and low Hb has also been observed in an international cohort of 1394 children on dialysis. ***(17)***

Davidkova et al. and ***Atkinson et al.*** also found "a negative correlation between high serum ferritin and Hb but positive correlation between serum ferritin and CRP". ***(6,15)***

The explanation of the negative correlation between serum ferritin and Hb and the positive correlation with CRP is that serum ferritin levels reflect state of chronic inflammation as ferritin is a good marker of chronic inflammation in conditions known to be a state of chronic inflammation like patients on dialysis. "The negative correlation between ferritin and Hb hypothesized that there may be a relationship between reduced hematopoiesis and increased iron storage, suggesting invalid or inefficient iron usage". ***(6)***

In contrast to our results, there was no correlation between Hb level sand serum ferritin in a study conducted on 879 adult HD patients. In these cases, ferritin levels reflect inflammatory state in HD rather than iron stores. ***(20)***

In the current study, there was a significant positive correlation between Hb and both TSAT and serum iron. This is compatible with the results of many studies conducted on pediatric population on regular dialysis. ***(6,17,18,21)***

TSAT reflects the available amount of iron and has been defined as a marker of iron supplementation. ***(6)***

In contrast to our results, there was no correlation between Hb and serum iron or TSAT in a study conducted in 125 adult patients on regular hemodialysis.***(22)***This is probably because "Hb levels in HD patients may not be dependent only on the available iron storage but may be affected by the hematopoietic status under external erythropoietin treatment" [22].

Since iron indices may be misleading in diagnosis of iron deficiency in HD patients, detection of other markers may be of a value. ***Sancho et al*** concluded that "determining hepcidin concentrations together with conventional markers associated with iron metabolism improved the identification of patients with iron deficiency by 26.1%". ***(23)***

Interleukin 6 is one of the main proinflammatory cytokines that induce hepcidin transcription in hepatocytes, so detection of its role may open the door for emerging therapies of anemia in ESRD.

"In the current study, serum IL-6 was significantly higher in patients than controls. There was a significant positive correlation between IL-6 and CRP but a significant negative correlation with Hb was found. The elevated IL-6 level resulting from oxidative stress, chronic inflammation, reduced clearance of IL-6 due to the impaired renal function, therapeutic hemodialysis and exposure of blood to foreign materials such as catheters and dialysis membranes further stimulate inflammatory responses and increase IL-6 production". ***(24)***

"Intravenous iron therapy has been proposed to have superior benefit over oral iron therapy for the management of IDA and efficient maintenance of target hemoglobin in HD patients". ***(25)*** "The majority of HD patients receiving IV iron and ESAs have been shown to have hepatic iron overload evaluated by MRI, with subsequent increased risk of hospitalization, cardiovascular events, infection, and mortality". ***(26)***

In this study we tried to use another modality for facing iron deficiency by using lactoferrin that inhibit IL-6 production and subsequently hepcidin transcription and comparing the effect of oral lactoferrin alone versus oral lactoferrin plus oral iron supplementation on iron status.

Oral iron therapy alone is not effective in correction of iron deficiency in HD patients, lactoferrin alone was not able to treat iron deficiency in our patients**.** Adding lactoferrin to oral iron was able to open ferroportin by decreasing hepcidin and IL-6 levels in addition to the possible increase in iron recycling at macrophage ferroportin levels.

Conclusions

Oral lactoferrin was not able to maintain iron status in HD children while Lactoferrin plus oral iron was effective in treating iron deficiency in HD patients.

Ethical Approval:

Written ethical approval has been collected from the Ethic Committee of Tanta University Hospitals.

Consent

As per international standard or university standard, Parental consent has been collected and preserved by the author(s).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

References

1- **Atkinson MA and Warady BA.** Anemia in chronic kidney disease. *Pediatr Nephrol*. 2018;33(2):227-238.

2- **BabittJLandLinHY.** Mechanisms of anemia in CKD.*JAmSocNephrol*. 2012; 23 (10): 1631-1634.

3- **Zadrazil J and Horak P.** Pathophysiology of anemia in chronic kidney diseases: A review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2015;159(2):197-202.

4- **GoodnoughLT, NemethEandGanz T.** Detection, evaluation, and management of iron-restricted erythropoiesis. *Blood*.2010;116 (23):4754-4761.

5- **Macciò A, Madeddu C, Gramignano G, Mulas C, Sanna E, Mantovani G.** Efficacy and safety of oral lactoferrin supplementation in combination with rHuEPO-beta for the treatment of anemia in advanced cancer patients undergoing chemotherapy: open-label, randomized controlled study. *Oncologist*. 2010;15(8):894-902.

6- **Atkinson MA, Kim JY, Roy CN, Warady BA, White CT, Furth SL.** Hepcidin and risk of anemia in CKD: a cross-sectional and longitudinal analysis in the CKiD cohort. *Pediatr Nephrol*. 2015;30(4):635-643.

7- **Babitt JL and Lin HY.** Molecular mechanisms of hepcidin regulation: implications for the anemia of CKD. *Am J Kidney Dis*. 2010;55(4):726-741.

8- **Atkinson MA, White CT.** Hepcidin in anemia of chronic kidney disease: review for the pediatric nephrologist. *Pediatr Nephrol*. 2012;27(1):33-40.

9- **PaesanoR, PietropaoliM, GessaniS and ValentiP.** The influence of lactoferrin,

orally administered, on systemic iron homeostasis in pregnant women suffering of iron deficiency and iron deficiency anemia. *Biochimie*. 2009; 91(1):44-51.

10- **ChattertonDE, NguyenDN, BeringSB and SangildPT.** Anti- inflammatory mechanisms of bioactive milk proteins in the intestine of newborns. *Int JBiochemCell Biol*. 2013;45 (8):1730-1747.

11- **Moastafa TM, El-Sissy Ael-D, El-Saeed GK, Koura MS.** Study on the Therapeutic Benefit on Lactoferrin in Patients with Colorectal Cancer Receiving Chemotherapy. *Int Sch Res Notices*. 2014; 2014:184278.

12- **Schwartz GJ and Furth SL.** Glomerular filtration rate measurement and estimation in chronic kidney disease. *Pediatr Nephrol*. 2007;22(11):1839-1848.

13- **Mudi A and Levy C.**Traditional red cell indices are not suggestive of iron deficiency in children with chronic kidney disease. SAJCH. 2018;12(3):121-126.

14- **Abu Hashim H, Foda O, Ghayaty E.** Lactoferrin or ferrous salts for iron deficiency anemia in pregnancy: A meta-analysis of randomized trials. *Eur J ObstetGynecolReprod Biol*. 2017; 219:45-52.

15- **DavidkovaS, PrestidgeTD, ReedPW, Kara T, WongWandPrestidge C.** Comparison of reticulocyte hemoglobin equivalent with traditional markers of iron and erythropoiesis in pediatric dialysis. Pediatr*Nephrol*. 2016;31(5):819-826.

16- **KDIGO.**Kidney Disease: Improving Global Outcomes(KDIGO)Anemia Work Group.KDIGO clinical practice guideline for anemia in chronic kidney disease. Kidney Int Suppl. 2012; 2:279–335.

17- **Borzych-Duzalka D, Bilginer Y, Ha IS, et al.** Management of anemia in children receiving chronic peritoneal dialysis. *J Am Soc Nephrol*. 2013;24(4):665-676.

18- **Ambarsari CG, Trihono PP, Kadaristiana A, et al.** Low-Dose Maintenance Intravenous Iron Therapy Can Prevent Anemia in Children with End-Stage Renal Disease Undergoing Chronic Hemodialysis. *Int J Nephrol*. 2020; 2020:3067453.

19- **MosselhyA, ZeinM, Abdelsalam M and PessarSA. Association between** GrowthDifferentiationFactor-15, CRP and Iron Parameters in Children on Regular Hemodialysis. *JClinPharmacolTher*. 2020;1(1):1004.

20- **Koo HM, Kim CH, Doh FM, Lee MJ, Kim EJ, Han JH,et al.** The relationship of initial transferrin saturation to cardiovascular parameters and out comes in patients initiating dialysis. *PLoS One*. 2014;9(2):87231.

21- **Jander A, Wierciński R, Bałasz-Chmielewska I, et al.**Anaemia treatment in chronically dialysed children: a multicentre nationwide observational study. *Scand J*

Urol Nephrol. 2012;46(5):375-380.

22- **OgawaC,TsuchiyaK,KandaFandMaedaT.** Low levels of serum ferritin lead to adequate hemoglobin levels and good survival in hemodialysis patients. *Am JNephrol*. 2014;40(6):561-570.

23- **Sancho A, PastorMC,Troya M, et al.**Hepcidin and iron deficiency in pre-kidney transplant patients. *Transplant Proc*. 2009;41(6):2079-2081.

24- **Su H, Lei CT, Zhang C.** Interleukin-6 Signaling Pathway, and Its Role in Kidney Disease: An Update. *Front Immunol*. 2017; 8:405.

25- **Albaramki J, Hodson EM, Craig JC, Webster AC.** Parenteral versus oral iron therapy for adults and children with chronic kidney disease. *Cochrane Database Syst Rev*. 2012;1:CD007857.

26- **Bailie GR, Larkina M, Goodkin DA, et al.** Data from the Dialysis Outcomes and Practice Patterns Study validate an association between high intravenous iron doses and mortality. *Kidney Int*. 2015;87(1):162-168.