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**Original Article****Oral Lactoferrin as a Source of Iron Supplementation in Pediatric Hemodialysis Patients.****Mohamed S. El-Farsy<sup>1</sup>, Ihab Z. El-Hakim<sup>1</sup>, Hend K. Rabie<sup>2</sup>**

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**Abstract:**

**Introduction:** Iron deficiency (ID) and iron deficiency anemia (IDA) are the most common iron disorders worldwide. Children with chronic kidney disease (CKD) may suffer from anemia due to a multi-factorial process, one of which, is disturbance in iron homeostasis.

**Aim of the study:** To assess the effect of oral lactoferrin in treatment of iron deficiency anemia in children with end stage renal disease (ESRD) on regular hemodialysis.

**Methods:** Sixty pediatric patients with end stage renal disease (ESRD) on regular hemodialysis in Pediatric Dialysis Unit, Children's Hospital, at Ain Shams University, were included in the study to evaluate the effect of oral lactoferrin on treating iron deficiency anemia instead of IV iron sulphate. This is a follow up case study in which all the included patients were on erythropoietin therapy ranging (150-300 IU/Kg) once per week. The patients were subjected to washout period of two weeks in which they were not given IV iron, and that was followed by the initiation of treatment with oral lactoferrin (100 mg bovine lactoferrin) twice daily for 6 months consecutively. Clinical and laboratory evaluation was done at 0 and 6 months after initiation of treatment.

**Results:** In the current study out of the total valid cases 56.5% (26/46) of the patients had a drop of blood hemoglobin (Hb) level at the end of the six month, 26% (12/46) had a stable Hb level, and 17.4% (8/46) had an elevated Hb level. In this study on the valid cases, it was noted that the mean change of Hb level between 0 month and 6 months had a highly significant decrease in Hb level with (p=0.000) while mean iron and ferritin levels showed non-significant changes with (p=0.125) and (p=0.239) respectively. While TIBC level showed a highly significant decrease with (p=0.001).

**Conclusion:** Oral lactoferrin can be used as adjuvant therapy for anemia in children on hemodialysis, not the replacing for the IV iron therapy.

**Keywords:** Oral Lactoferrin, Iron Supplementation, Pediatric Hemodialysis

**Running title:** Oral Lactoferrin as a Source of Iron Supplementation in Pediatric Hemodialysis Patients

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## INTRODUCTION

Iron deficiency (ID) and iron deficiency anemia (IDA) are the most common iron disorders worldwide. When iron requirement is higher than that absorbed, a negative iron balance occurs and iron storage decreases [1].

Children with chronic kidney disease (CKD) may suffer from anemia due to a multi-factorial process, one of which, is disturbance in iron homeostasis. Iron homeostasis is tightly regulated through iron absorption, storage, and transport [2].

Anemia in CKD can be due to combination of multiple factors as: erythropoietin production is inappropriately low. The mechanism for this decreased production could be a result of progressive loss of the erythropoietin-producing peritubular fibroblast-like interstitial cells in the kidney [3]. Iron deficiency anemia in CKD children might be a result of nutritional deficiency, poor enteral absorption of iron supplements, or chronic blood loss from frequent laboratory testing or in the context of chronic hemodialysis [2].

Chronic inflammation is common in patients with CKD and is associated with an increased risk of anemia. Elevated levels of cytokines induce production of hepcidin, which is an antimicrobial and iron-regulatory peptide. Hepcidin controls blood iron levels both by down-regulating the absorption of iron in the intestine and by inhibiting the release of iron from iron storing reticuloendothelial cells [4] hence causing anaemia. Chronic uremia may also contribute to anemia. Uremic serum has been shown to shorten the survival of erythrocytes and might also stimulate hemolysis [5].

Hyperparathyroidism has been associated with a poor response to erythropoietin and might be associated with decreased bone marrow production of red blood cells as a result of myelofibrosis [6].

Iron absorption occurs in the proximal duodenum. In the apical site of enterocytes, the reduction of ferric ions occurs by a ferrereductase, then apical uptake and the transcellular trafficking occurs via divalent metal transporter 1 (DMT1), the storage into ferritin and finally, the basolateral efflux by the iron transporter ferroportin. Ferroportin is the only known cellular iron exporter from tissues into blood [7].

Disorders in iron hemostasis results in iron deficiency anemia which is mostly treated by iron supplementation. Recently, approaches about the use of lactoferrin are introduced for managing anemia. Lactoferrin, is a non-haem iron binding protein that is structurally and chemically similar to serum transferrin, whose function is to transport iron in blood serum. This glycoprotein is produced by mucosal epithelial cells and found in mucosal secretions as saliva, tears, nasal, bronchial secretions and most highly in milk making it the second abundant protein after casein [8].

Lactoferrin has proved to have 300 times higher affinity to iron as compared to serum transferrin and an ability to retain iron over a broad PH range. Also, it influenced iron homeostasis by increasing iron export from GIT and enhancing iron storage in ferritin. These mechanisms have proved to give better results with patients who are using lactoferrin as compared with those using ferrous sulphate in terms of red cell count,

hemoglobin level, serum ferritin and serum iron [9].

**Aim of the study:** To assess the effect of oral lactoferrin in treatment of iron deficiency anemia in children with end stage renal disease (ESRD) on regular hemodialysis.

## METHODS

This follow up case study was conducted on children and adolescents following in Pediatric Dialysis Unit, Children's Hospital, at Ain Shams University. It is a non-randomized trial. It was conducted on 60 pediatric patients with end-stage renal disease (ESRD) on regular hemodialysis. It is included pediatric patients with end-stage renal disease on regular hemodialysis with iron deficiency anemia for at least 6 months. It is a pilot study; we are the first to study the effect of lactoferrin on ESRD pediatric patients on HD with iron deficiency anemia.

The following patients were excluded from the study: patients who received transfusion of PRBCs during the study (5/60), who referred for transplantation (1/60), who refused to continue the study despite absence of clinical symptoms (1/60), who experienced side effects with the drug (nausea and vomiting more than once) (5/60), who were referred to adult dialysis unit (1/60), who passed away during the study (1/60), patients with other causes of anemia (hemoglobinopathy, acute blood loss, acute infection, etc.) (0/60). Valid cases after exclusion were 46 out of 60.

**Study Design:** Washout period of two weeks [10] was assigned, at which the patients were not given any form of iron

supplementation neither IV nor oral, but any other medications such as calcium, phosphate binders or erythropoietin continued to be given. A 5 ml blood sample was collected from the patients, included in the study, prior to receiving lactoferrin as an initial record of hemoglobin level, TIBC, serum iron and serum ferritin. Lactoferrin sachets were given twice daily for six months (a sachet of 100 mg bovine lactoferrin on 1/4 cup of water before meals) with the dose of 200 mg daily [9]. During the treatment period hemoglobin level was assayed monthly. After six months' period of the study, hemoglobin level, TIBC, serum iron, and serum ferritin were assayed again. The study was carried out from the first of July 2018 to the end of December 2018.

All patients included in the study were subjected to the following: History was collected from patients on hemodialysis with stress on age, gender, duration of dialysis, drug history regarding type, route, dose, duration, and date of last dose of the drug, compliance to treatment, blood transfusion history. General examination with stress on heart rate, blood pressure, clinical signs of anemia and any signs of iron overload. Measuring hemoglobin level, serum iron, TIBC and serum ferritin before and after 6 months of receiving 100 mg lactoferrin twice daily. CBC was done to know the type of anemia. During the study a 3 ml sample was collected monthly for measuring hemoglobin level.

**Statistical Analysis:** Data was collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 23. Interpretation of

probability values was as follows:  $p > 0.05$ : non-significant  $< 0.05$ : significant.

## RESULTS

This study included 46 valid pediatric patients with age ranges from 11 to 17 years (Mean  $14.37 \pm 1.78$  years) **Table 1**, which included 26 (56%) male and 20 (44%) female.

**Table 2** shows that there is a non-significant change in serum iron level at

zero month and after lactoferrin administration for 6 months.

**Table 3** shows a comparison between serum ferritin level at 0 month and 6 months which shows a non-significant difference.

**Table 4** shows a highly significant decrease in TIBC level after 6 months of lactoferrin administration.

**Table 5** shows a significant decrease in blood Hb level after 6 months' administration of lactoferrin sachets twice daily.

**Table 1:** Descriptive data of age.

Age	(years)
Mean $\pm$ SD	14.37 $\pm$ 1.78
Minimum	11
Maximum	17

**Table 2:** Comparison between serum iron level at zero month and at 6 months.

Iron ( $\mu$ g/dl)	Iron-0	Iron-6
Mean $\pm$ SD	106.91 $\pm$ 50.061	95.93 $\pm$ 47.761
Minimum	14	20
Maximum	179	178
<b>P value</b>	<b>0.125</b>	

**Table 3:** Comparison between serum ferritin level at 0 month and 6 months.

Ferritin (ng/ml)	Ferritin-0	Ferritin-6
Mean $\pm$ SD	1854.17 $\pm$ 246.54	1863.04 $\pm$ 238.84
Minimum	1300	1200
Maximum	2000	2000
<b>P value</b>	<b>0.239</b>	

**Table 4:** Comparison between TIBC level at 0 month and 6 months.

TIBC ( $\mu$ g/dl)	TIBC-0	TIBC-6
Mean $\pm$ SD	220.78 $\pm$ 77.261	179.93 $\pm$ 35.420
Minimum	122	75
Maximum	416	250
<b>P value</b>	<b>0.001</b>	

**Table 5:** Comparison between blood Hb level at 0 month and 6 months.

Hb (g/dl)	Hb-0	Hb-6
Mean $\pm$ SD	10.843 $\pm$ 1.167	10.215 $\pm$ 1.248
Minimum	8.4	7
Maximum	10.9	10.6
<b>P value</b>	<b>0.000</b>	

## DISCUSSION

Iron deficiency (ID) and iron deficiency anemia (IDA) are the most common iron disorders worldwide. When iron requirement is higher than that absorbed, a negative iron balance occurs and iron storage decreases [1].

Bovine lactoferrin represents an attractive and promising alternative to ferrous sulphate oral administration. Studies has showed that oral administration of bovine lactoferrin, 30% iron saturated, significantly improved hematological markers, including number of RBCs, hemoglobin, total serum iron, serum ferritin concentrations compared to those treated with ferrous sulphate [9].

Lactoferrin, is a non-haem iron binding protein that is structurally and chemically similar to serum transferrin, whose function is to transport iron in blood serum. This glycoprotein is produced by mucosal epithelial cells and found in mucosal secretions as saliva, tears, nasal, bronchial secretions and most highly in milk making it the second abundant protein after casein [8]. According to KDIGO (2012) [13] definition of anemia from 0.5-5 years; blood hemoglobin is less than 11 g/dl, 5-12 years; blood hemoglobin less than 11.5 g/dl, 12-15 years; blood hemoglobin less than 12 g/dl, more than 15 years old; blood hemoglobin less than 12 g/dl in females and less than 13 g/dl in males.

The patients were subjected to washout period of two weeks [10] at which the patients were not given any form of iron supplementation neither IV nor oral, but any other medications such as calcium, phosphate binders or erythropoietin continued to be given. That

was followed by the initiation of treatment with oral lactoferrin for 6 months consecutively. Where patients received one sachet of lactoferrin (100mg) dissolved in 1/4 cup of water twice daily before meals. Patients were assessed by measuring the serum Hb, iron, TIBC and ferritin levels prior to the trial, as an initial record, and the same parameters were repeated after 6 months of the trial. During these six months Hb levels were assayed monthly.

Out of the 60 patients involved in this trial only 46 were considered valid cases (46/60 =76.6 %). The remaining invalid cases (14/60= 23.3%) were excluded for the following: one patient undergone transplantation, one patients shifted to adults dialysis unit, one patient refused to continue the trial despite absence of clinical symptoms; patient preference, one patient passed away, three patients received packed RBC transfusion after their initial lab results, so, were immediately excluded for the sake of the patients , two patients after receiving the treatment for 3 month had tachycardia and a drop of Hb which required transfusion of packed RBC, five patients complained of nausea and vomiting after the administration of the drug, so, these were excluded for the clinical symptoms. In the current study out of the total valid cases 56.5% (26/46) of the patients had a drop of Hb level at the end of the six months, 26% (12/46) had a stable Hb level and 17.4% (8/46) had an elevated Hb level. In terms of laboratory investigations, the current study on valid cases noted that at 0 month mean Hb level was  $10.843 \pm 1.16$  g/dl while at 6 months mean Hb level was  $10.215 \pm 1.24$  g/dl on a dose of 100mg bovine lactoferrin twice daily with

( $p=0.000$ ) which indicates a highly significant decrease in Hb level.

A suggested explanation of this highly significant decrease in Hb level is the high level of hepcidin in patients with CKD on hemodialysis, this suggestion can be supported by the results done by El-Hakim and El-Masry (2009) [14] which showed higher level of hepcidin in hemodialysis and conservative CKD patients as compared to normal group and higher level of hepcidin in hemodialysis patients as compared to conservative patients. This high level of hepcidin down regulates iron absorption [14].

During the comparison of the study results with other studies, it was found that; this study is a pilot study, we are the first to carry out this study as regard the effect of oral lactoferrin on iron deficiency anemia in pediatric patients with ESRD on regular HD. So, there was no reference in pediatric patients. The available studies were mainly on pregnant women [11]. Rezk et al (2016) [11], stated that that mean increase of Hb level was by ( $2.26 \pm 0.51$  g/dl) in pregnant women with iron deficiency anemia who received bovine lactoferrin for 8 weeks on a dose of 250 mg once daily. Another study was by Paesano et al. (2010) [9], stated that mean increase of Hb level was by ( $1.7 \pm 0.9$  g/dl) in pregnant women with iron deficiency anemia who received bovine lactoferrin for 30 days on a dose of 100mg twice daily.

The current study noted that the mean iron level at 0 month was  $106.91 \pm 50.06$  ug/dl while at 6 months mean iron level was  $95.93 \pm 47.761$  ug/dl with ( $p=0.125$ ) which indicates non-significant change between both levels. Paesano et al. (2010) [9], found that the mean iron level

before treatment was  $\leq 30$  mg/dl ( $\leq 30000$  ug/dl) and after treatment for 30 days was  $84 \pm 16$  mg/dl ( $84000$  ug/dl  $\pm 16000$  ug/dl).

In the current study, and after treatment with oral lactoferrin there was no significant increase in the Hb level despite six months of treatment with lactoferrin, and interestingly there is a significant low iron but not surprisingly as the hemodialysis patients had a large iron loss due to blood loss associated with the hemodialysis procedure, gastrointestinal loss, reduced iron absorption and reduced intake due to poor appetite [15].

Moreover, in our study the mean serum ferritin level at 0 month was  $1854.17 \pm 246.54$  ng/ml and at 6 months  $1863.04 \pm 238.848$  ng/ml with a ( $p=0.239$ ) which indicates a non-significant change. But this parameter cannot be much reliable because it is considered an active phase reactant which is by default extremely high in chronic patients as patients on dialysis. In contrast to the serum ferritin level noted by Paesano et al. (2010) [9], which was  $\leq 12$  ng/ml initially while after treatment for 30 days, it was  $29 \pm 7$  ng/ml.

The current study noted the mean TIBC level at 0 month was  $220.78 \pm 77.26$  ug/dl and at 6 months  $179.93 \pm 35.42$  ug/dl with ( $p=0.001$ ) indicating a highly significant decrease in TIBC level.

Both ferritin and transferrin saturation have their shortcomings in assessing iron status and guiding iron therapy in patients with CKD [16]. The diagnosis of absolute iron deficiency is usually based on low serum ferritin concentrations that reflect low body iron stores [16]. In CKD patients, because the presence of inflammation, threshold values indicating iron deficiency are

generally considered to be higher than in those without kidney disease. Serum ferritin levels of 100 or 200 µg/l are frequently cited as a cutoff value in non-dialysis CKD and dialysis patients, respectively [13].

As regards side effects of the drug administered to the studied cases: Constipation and GIT upset were the most frequent side effects, which did not allow compliance on the medication in about 8% of the total number of patients. There were no relevant studies on the side effects of oral lactoferrin in CKD patients. But, Rezk et al. (2016) [11], who studied effect of lactoferrin on pregnant women with IDA over 2 months, found that fewer GIT adverse events and better treatment acceptability. Shepshelovich et al. (2016) [12], had confirmed the superiority of numerous different IV iron formulations to the common oral therapies in both patients who are dialysis dependent and those with CKD stages 3-5, both for

rapidity of improvement in Hb and quality of life.

Finally, we recommend that: Oral iron can be given as a treatment along with oral lactoferrin for a study to control iron deficiency anemia in end stage renal disease (ESRD). The duration of the study can be prolonged for more than six months. The dose of lactoferrin can be increased above 100mg twice daily.

## CONCLUSION

Oral lactoferrin could not be used as a substitute to totally replace IV ferrous sulphate in treating iron deficiency anemia in end stage renal disease (ESRD) on dialysis. Oral lactoferrin could not even control Hb level constant without dropping in patients with end stage renal disease (ESRD). Oral lactoferrin had some gastrointestinal effects on some patients which did not allow compliance on the medication

## ABBREVIATIONS

<b>CKD</b>	Chronic kidney disease
<b>DMT1</b>	Divalent metal transporter 1
<b>ESRD</b>	End stage renal disease
<b>Hb</b>	Hemoglobin
<b>ID</b>	Iron deficiency
<b>IDA</b>	Iron deficiency anemia
<b>TIBC</b>	Total iron binding capacity

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## AUTHORS' CONTRIBUTIONS

All authors have read and approved the manuscript.

Data acquisition: All authors

Analysis and data interpretation: All authors

Drafting of the manuscript: 1<sup>st</sup> author.

Critical revision: 1<sup>st</sup> author.

## STATEMENTS

### Ethics approval and consent to participate:

This study protocol and the consents were approved and deemed sufficient by the Ethical Committee of Faculty of Medicine, Ain Shams University and informed and written consent was obtained in every case from their legal guardians.

### Consents for publication

"Not applicable"

### Availability of data and material

"Not applicable"

### Conflict of interest

The authors declare no conflict of interest.

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