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# Assessment of Pro Hepcidin and Related with Iron Profile on Hemodialysis Men Patients

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## Abstract:

Patients with renal failure in the final stages undergo the treatment by hemodialysis. Hemodialysis is used to reinstate the intracellular and extracellular fluid environment, by propagation of molecules in solution through a semipermeable membrane along an electrochemical concentration gradient. Blood catching in the dialysis machine and the recurrent phlebotomy may lead to losing about 1-3 g of iron per year. Prohepcidin hormone is an acute phase protein (type II) that plays a major role in the systemic iron irregularities as it is a mediator of anemia in inflammation and regulator of iron metabolism. This study aims to evaluate the effect of hemodialysis on iron hemostasis and its relationship with prohepcidin as an inflammatory marker. This study includes forty four adult male patients with end-stage renal failure (in pre and post –treated) by means of chronic hemodialysis-HD with mean age (53.27 ± 13.76 years). The following biochemical investigations have been studied: Prohepcidin, Iron, Ferritin, Transferrin, Total Iron-Binding Capacity (TIBC), The Unsaturated Total Iron Binding Capacity (UIBC), and transferrin saturation (TAST).Decrement of Prohepcidin level on hemodialysis patients in post dialysis with non-significantly compared to pre dialysis, while iron and ferritin was increment in post treated than pre- treated with non-significantly.
Hemodialysis affects Prohepcidin levels as it was long duration and Glomerular Filtration rate GFR (cock croft equation) and prohepcidin level affect the iron profile related with the iron store depletion.

**Key words**: Prohepcidin, Hemodialysis, Transferrin.

## Introduction:

Hemodialysis is a process including the perfusion of blood and dialysate on a reverse of a semipermeable membrane. Substances are detached from the blood by diffusion and convection. Excess plasma water is removed by Ultrafiltrating a regular occurrence among patients getting long-term hemodialysis [1].

Acute phase protein, as a type II, is a prohepcidin released as a small peptide mainly from the liver. It acts as a regulator metabolism of iron and an

arbitrator of anemia in inflammation. [2]. The hepatocyteacrossa basolateral membrane is released from pro-hepcidin into the blood and is visible to renal elimination.[3] Prohormone prohepcidin produces Hepcidin which originates from extra hepatic enzymatic cleavage . [4]Hepcidin-mediated decrement in extracellular iron levels through infections and inflammation, [5] its unusual disulfide motifs, seems to be preserved among species, because it, antimicrobial peptides may be characterized as a new class of it. [6]A Hepcidin(hep) is released into plasma and excreted in urine. It regulates iron absorption (homeostatic iron) in the intestinal mucosa, iron recycling by macrophages, and hepatic storage of iron mobilization. [6,7]On the cell surface of macrophages and enterocytes act as hepcidin which leads iron exporter ferroportin to internalization and degradation. [8]Anemia of chronic disease is addressed by treating the underlying state [9].

Iron is initially stored as a protein-iron complex ferritin, but ferritin can be incorporated by phagolysosomes to create hemosiderin granules [10] Ferritin is an acute phase protein whereby concentrations increase during inflammation and thereby no longer reflect the level of the store. [11]The present study aims to evaluate the effect of hemodialysis on iron hemostasis and its relationship with prohepcidin as an inflammatory marker

## Materials and Methods:

**Subjects:**

This study includs forty four adult male patients with end-stage renal failure (in pre and post –treated) by means of chronic hemodialysis-HD Excluded from the study are men who have viral infection (hepatitis and HIV), kidney transplant, and malignant. Patients are divided into two groups with {Diabetes Mellitus (nephropathy) and Hypertension} as a reason of renal failure. In AL- Yurmok Teaching Hospital located in the city of Bagdad, Iraq during January ,2014 up to April,2014.Those patients require a regular hemodialysis for 3 hr a day 2-3 times per week. Patients are selected, having mean Hb values <10g/dl .Blood specimens are obtained before the patient starts the hemodialysis.

**Specimens, Collection, and Evaluation.**

Venous blood samples are collected from each subject in the morning (5-12am), 5 ml of blood is obtained by vein puncture using a 5 ml disposable syringes. About 3.5 ml in tube with clotting jell and 1.5 ml in another tube anticoagulant. It is left for 15 minutes to clot at room temperature, while the tube with anticoagulant is dispensed at once and then separated by centrifugation at (3000 rpm) for (5 min) to collect plasma. Serum is divided into 2 aliquots; 0.5ml in each eppendorff tubes, each one of them is frozen under (-20) $C^0$ until being used for assays. Serum prohepcidin is measured by using commercially available ELISA kits, Demeditec Diagnostics (Germany), whilst ferritin DRG Diagnostics (Germany) ELISA-Kit is also used to measure serum ferritin, and Iron Cromazurol (Linear, Spain) by using spectrophotometer, while transferrin, transferrin saturation (TAST), UIBC was calculated by the following equation: [12]

Transferrin = 0.7 * TIBC.

%TAST=(serum iron conc.)/(TIBC) *100.

UIBC = TIBC- Iron concentration.

**Statistical Analysis**

The Statistical Analysis System-SAS (2012) uses different factors in studying parameters. Least significant

difference –LSD test is used to significantly compare between means. Estimate of correlation coefficient between difference parameters in this study has been done.Cary. N. C. (2012). Statistical Analysis System, User's Guide. Statistical version 9.1th ed. SAS. Inst. Inc. USA.

## Results:

Number of patients with the age ≥ 60 years is significantly higher than the other age groups (43.3%). The mean ± SD of hemoglobin is (10.43 ± 0.28 g/dl) and some characteristics of patients are shown on Table (1), (2).

**Table (1): Distribution of Sample Study according to the Age Groups**

| Age in( year) | Patients no (%) |
|---|---|
| 20-29 | 4(9.01%) |
| 30-39 | 3(6.8%) |
| 40-49 | 7(15.9%) |
| 50-59 | 11(25%) |
| ≥ 60 | 19(43.1%) |
| Chi-square – $\chi^2$ | 9.819 ** |
| ** (P<0.01). | |

**Table(2): Baseline Characteristics of Patients**

| *Variables* | HDpre – treated | HD post treated |
|---|---|---|
| Age (years) | 53.27 ± 13.76 | - |
| BMI Kg/m$^2$ | 26.64 ± 0.30 | 25.80 ± 0.29 * |
| HD Duration (month) | 17.4 ± 13.5 | - |
| Hb g/dl | 10.43±0.28 | 10.43±0.28 |
| * (P≤0.05), NS: Non-significant. | | |

HD=hemodialysis, BMI =Body mass index, Hb= Hemoglobin.

Pro-Hepcidin level of hemodialysis patients decreases non-significantly in post comparison with pre-treated (154.10 ± 13.01), (175.15 ± 14.32) respectively. Serum iron level in patients on hemodialysis shows decrement in pre –treated (mean ± SD) (56.30 ± 4.08) and to be in normal range in post –treated (64.68 ± 33.72). However, Serum ferritin is different in pre and post -treated (344.35 ± 80.2), (336.24 ± 74.51), while serum transferrin increases (154.18 ± 30.42)in post compared to pre -treated(143.13 ± 24.56) respectively.Serum TBIC increases significantly in post –treated (221.12 ± 33.61) , (204.47 ± 24.0) compared with pre –treated patients as shown in Table (3), and Figure (1, 2, 3, 4, 5)

**Table3: The Comparison between Pre and Post Treated in Pro hepcidin Fe, Ferritin, Transferrin and TIBC.**

| Parameters | Mean ± SD | | T-test | P-value |
|---|---|---|---|---|
| | Pre-treated | Post-treated | | |
| ProHepcidin ng/mL | 175.15 ± 14.32 | 154.10 ± 13.01 | 38.861 NS | 0.284 |
| Iron ug/dl | 56.30 ± 4.08 | 64.68 ± 33.72 | 11.798 NS | 0.161 |
| Ferritin ng/ml | 344.35 ± 80.2 | 336.24 ± 74.51 | 84.430 NS | 0.849 |
| Transferrin ng/dl | 143.13 ± 24.56 | 154.18 ± 30.42 | 13.196 NS | 0.066 |
| TIBC ug/dl | 204.47 ± 24.0 | 221.12 ± 33.61 | 16.854 * | 0.05 |
| TSAT | 0.278 ± 0.016 | 0.297 ± 0.024 | 0.0579 NS | 0.520 |
| UIBC | 145.81 ± 6.20 | 153.29 ± 7.63 | 109.541 NS | 0.449 |
| * (P≤0.05), NS: Non-significant. | | | | |

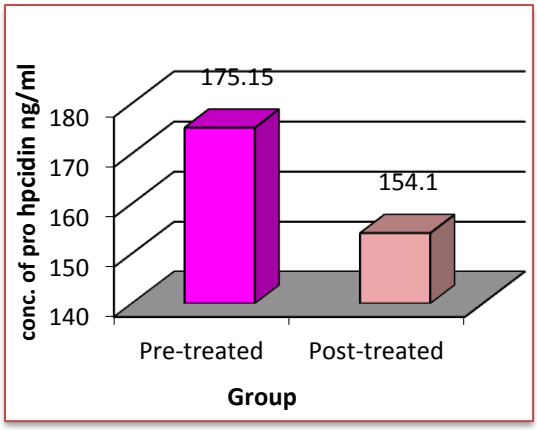


**Fig.(1) Comparison between Pre and Post Treated in Prohepcidin**

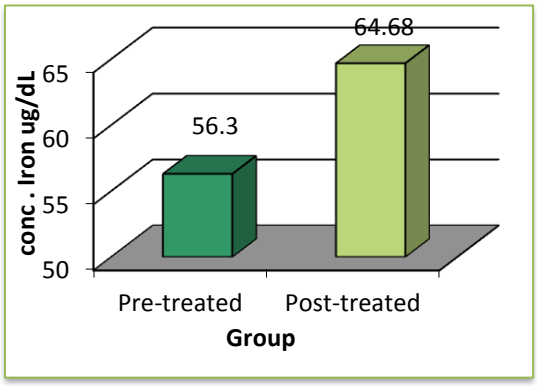


**Fig. (2) Comparison between Pre and Post Treated in Iron**

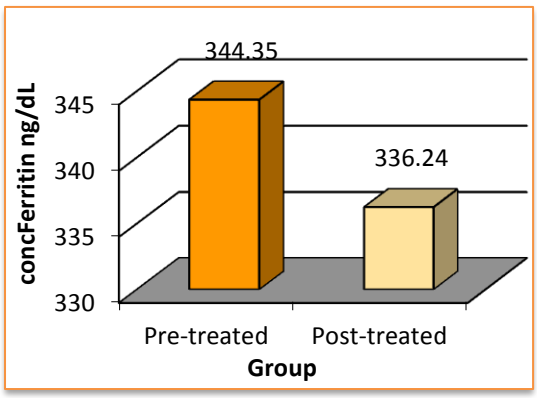


**Fig. (3) Comparison between Pre and Post Treated in Feritin**

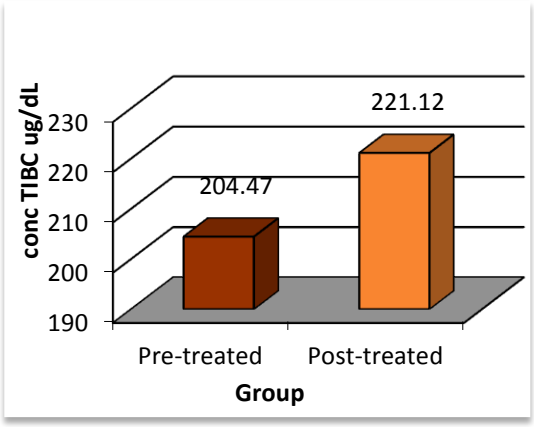


**Fig. (4) Comparison between Pre and Post Treated in TIBC**

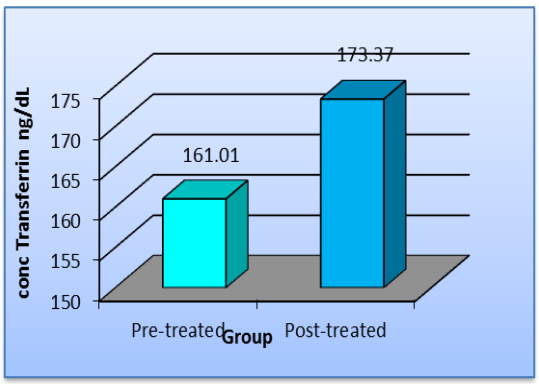


**Fig. (5) Comparison between Pre and Post Treated Transferrin.**

## Correlation

The association between the studied variables is tested by Pearson's correlation coefficient, where the (p-values <0.05) is considered as statistically significant (Table 3). Studying the correlation between serum pro- hepcidin and tested of iron, ferritin, transferrin, TIBC. There is no correlations between serum pro-hepcidin and parameters as shown in Table (4):

**Table (4) Correlation Coefficient between Prohepcidin with Other Parameters**

| Parameters | Iron Ug/dL | | Ferritin ng/ml | | Transferrin ng/dL | | TIBC ug/dL | |
|---|---|---|---|---|---|---|---|---|
| | r | P value | r | P value | r | P value | r | P value |
| **Pro-hepcidin** | -0.03 | NS | 0.02 | NS | 0.08 | NS | 0.07 | NS |

## Discussion:

Some patients with renal failure are reported to show abnormality regulation of iron metabolism and promote anemia of chronic disease (ACD). [13]In the present study the serum prohepcidin is high in hemodialysis patients before dialysis, and becomes within normal range after dialysis. (Yasar et al) [14] suggest that:

hepcidin production may be suppressed under iron deficient conditions because of the soluble transferrin (sTfR), the production of which is enhanced by the decreased intracellular iron. However, ferritin production is increased under inflammatory conditions and the serum ferritin concentration does not always reflect the intracellular iron concentration[15].Hepcidin production in liver cells is suppressed by iron deficiency and consequently absorption of iron in food is promoted in the duodenal intestine. [16]Serum pro-hepcidin in iron deficient anemia patients is significantly lower than that in controls. [17] (Kattamis et al) [18]report that liver hepcidin mRNA expression is positively correlated with Hb, suggesting that the effect of anemia on hepcidin production is greater than that of iron load.

Recombinant human Erythropoietin (rHuEPO) administration may have suppressed hepcidin production, based on a report that hepcidin production is reduced by erythropoietin [19]. (Kulaksiz, et al) [20] find that serum pro-hepcidin concentrations in patients with chronic renal disorder treated with rHuEPO are higher than those in healthy volunteers. The possibility is that hepcidin is removed by hemodialysis due to its small molecular weight [21, 22]. Others have reported significantly higher levels in HD patients.(Eleftheriadis, et al) [23]increment prohepcidin level due to inflammation with deepening anemia. Thus therapeutic interventions play a role in the treatment of anemia [24]. In another study (Faruk, et al) [25] is similar to the present study.

Iron decrement is a common complication in patients on hemodialysis and this may be due to blood loss during this procedure. Serum ferritin measurement allows easier quantitation of iron stores in dialysis patients[14].

Patients who are on hemodialysis have a lower iron level because of the increased blood loss from: the blood left in the dialyzer circuit, the frequent blood sampling, the low-grade gastrointestinal bleeding, multiple vascular access surgeries, *etc*. This also may be compounded by decreased oral iron absorption because of dietary restrictions, loss of taste for iron-rich foods, and hepcidinin. Another study (Jairam, el al) [26] is similar to present study.

Treating anemia in hemodialysis patients may correct hemoglobin and aggravates iron deficiency due to the increased iron utilization. For good response, iron should be given by i.v. route for patients with real anemia[27].

The serum Ferritin (SF) reflects storage iron, absolute iron deficiency in healthy and most pathologic conditions, when (SF) is a good indicator of the amount of iron supply. However, erythropoiesis, malnutrition, malignancies, hemolysis and certain inflammatory conditions such as infections, hepatic dysfunction and renal failure may affect SF level. Due to the increase in ferritin activity in acute phase of renal failure, it appears that the SF cut off level for determination of iron deficiency is probably higher in uremic patients.[28]

Transferrin levels in chronic kidney disease are one half to one third of normal levels, diminishing the capacity of the iron-transporting system [29]. This situation is then aggravated by the well-known inability to release stored iron from macrophages and hepatocytes in chronic kidney disease. The concentration of serum iron does not fall until the body`s iron stores are exhausted .As the stores are depleted, the concentration of transferrin rises while the concentration of ferritin falls. Caution is required when assessing patients with inflammatory disease as a

low serum iron may not represent iron deficiency .These patients often have reduced concentration of transferrin [30].

In chronic kidney disease patients, serum is a good indicator of nutritional status also it is clinically related with inflammatory, marker, iron reservoir and survival ratio, though It is changes with time can change the risk of death in HD patients.[31] In another study (Neeta, et al)[32] is similar to present study.

(Taes, et al) [33] correspond to the present study that serum prohepcidin levels are higher than the normal values in the patients on HD, but they are not correlated with the serum iron, TIBC, TSAT, ferritin [34]. Pro-hepcidin is a processing intermediate inflammatory physiology, therefore its assessment is less reflective of iron, because it has eight cysteine that form (4 S-S bridges), resulting in a difficulty to enter a specific antibody toward this body. In another study (Taes, et al) [33] find in HD patients there are no significant correlations between pro-hepcidin levels and age, BMI, duration on dialysis, hemoglobin, serum iron, ferritin, TSAT.

## Conclusions:

Hemodialysis affects Prohepcidin levels as it have a long duration and GFR (cock croft equation), and Prohepcidin level affect iron profile related with iron store depletion.

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# تقييم البروهبسدين وعلاقته مع مجال الحديد في الرجال ا لمرضى الخاضعين للغسيل الكلوي

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**الخلاصة:**

يخضع المرضى المصابين بالفشل الكلوي في المرحلة الاخيرة للمعالجة بالغسل الكلوي .والغسل الكلوي يستخدم (للتخلص من بقية السوائل خارج وداخل الخلية ) عن طريق استعمال غشاء نصف ناضح معتمد على الفرق بتراكيز الجزيئات بين السائل والدم .ان عملية مرور الدم بجهاز الغسل الكلوي المتكرر يؤدي الى فقدان من (1-3) غرام من الدم سنويا .هرمون البروهبسدين :هو هرمون يفرز من الكبد ويعد بروتين دفاعي من النوع الثاني يلعب دوراً رئيسيا في عملية تنظيم الحديد واستقلابه ويسبب فقر الدم الالتهابي في حالة المرض.اهداف الدراسة :تقييم تأثير غسيل الكلى على توازن الحديد وعلاقته مع هرمون البروهبسدين كدالة التهابية.تضمنت الدراسة 44 رجلاً مريضاً يعانون من الفشل الكلوي في المرحلة الاخيرة يتراوح معدل اعمارهم بين (53.27±13.76)سنة والذين هم يعالجون بوساطة الغسيل الكلوي (قبل وبعد الغسيل ) و تم قياس هرمون TAST, ,and TIBCUIBC, ,transferrin ,Fe,Ferritin) البروهبسيدين من مصول مرضى قبل وبعد الغسل الكلوي)وكذلك تتناقص بشكل غير كبير بعد الغسل مقارنة مع قبل الغسل .بينما مستويات الحديد في مصول الدم في مرضى الغسيل الكلوي تعود الى مستواها الطبيعي بعد المعالجة مقارنة مع قبل الغسل الكلوي.

**الكلمات المفتاحية:** البروهبسدين، الغسيل الكلوي، الترانس فرين.