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Assessment of Iron Status in Anemic Children with Chronic Kidney Disease

Ali K Alzahrani[1, 2], Abdrahman Alzahrani[3], Nihad A Al Nashar[4] , Hala A Mohamed[4]
[1]Department of Pediatrics, Taif University, Taif, Saudi Arabia
[2]Department of Pediatrics and Neonatology, King Abdul Aziz Specialist Hospital, Taif
[3]Pediatrics' Hospital, Taif, [4]Department of Pathology, Taif University

Abstract

Background: Iron deficiency is the commonest cause of resistance to erythropoiesis stimulating agents (ESAs) in dialyzed children treated from anemia of chronic kidney disease (CKD).

Aim of the work: This study was conducted in order to evaluate the significance of different biomarkers in assessment of iron status during management of anemic children with CKD.

Patients and method: Twenty five children with diagnosis of anemia of chronic kidney disease were enrolled for the study. They were classified into two groups according to their stage of the kidney disease. Group I; included 15 children with anemia of CKD and their Glomerular Filtration Rate (GFR) was 15.5 – 29.6 ml/min/1.73m^2 (stages; III & IV CKD) and they were managed conservatively. Group II; It included 10 anemic children with end stage renal disease (Stage V CKD, GFR was 6.1 – 13.7 ml/min/1.73m^2) and they were under regular hemodialysis. Another 10 healthy children with matched age and gender served as control group (group III).

Results: The study showed that the hypochromic cell percentage was significantly higher in both groups I and II before treatment when compared to controls ($p < 0.0001$). Serum ferritin showed very high significant elevation in all the studied groups as compared to controls, also group II was highly significant when compared with group I before treatment. Improvement of iron mobilization and metabolism after 8 weeks of therapy with intravenous iron and erythropoietin was evidenced by significant increase in hemoglobin (Hb) level, RBCs and HCT % when comparing the group II patients before and after treatment. Also significant decrease in hypochromic cell percentage and increase in serum ferritin were proved. The sTfR and sTfR/ F indices showed elevation in the post-treatment group.

Conclusion: No single biomarker is reliable alone in the assessment and monitoring the iron status in anemic patients with CKD under ESAs therapy. Measurement of hypochromic cell percentage may be simple and reliable method, and sTfR represents a valuable quantitative assay of marrow erythropoietic activity as well as a marker of tissue iron deficiency. However, the sTfR / Ferritin index is considered to be more efficient in anemic patients with CKD for early prediction of functional iron deficiency and is a sensitive tool for follow up of iron status during ESAs therapy.

Keywords: Anemia of chronic kidney disease, iron status, erythropoiesis stimulating agents, serum soluble transferrin.

Introduction

Chronic kidney disease is the gradual, progressive reduction in the glomerular filtration rate (GFR) with deterioration of kidney function and the disease is classified to five stages where the 5th stage represents the end stage renal failure (1) The cause of anemia of chronic kidney disease (CKD) is mainly due to a decrease in the response to endogenous erythropoietin (EPO) which is produced in the kidney and the liver and stimulates erythropoiesis (2). The erythropoietic stimulating agents (ESAs) as, recombinant human erythropoietin (rHuEPO), epoetinalfa, and darbepoetin alpha; are analogues of the natural hormone erythropoietin (2-6). These agents are used to manage the anemia of CKD instead of the blood transfusion to decrease the risk of transfusion-related complications with marked improvement in the outcome especially in the end stage of the disease (6, 7). However; ESAs mobilize iron stores to promote erythropoiesis; with subsequent decrease in these stores representing the most common reasons for

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resistance to its effect (7). Other factors which lead to iron deprivation in dialyzed patients aggravating the resistance to ESAs include; Inadequate diet, repeated laboratory testing, blood retention in the dialyzer and tubing during dialysis (2).Therefore; to ensure an adequate response to ESAs, anemic patients with CKD have to be supplemented with oral or intravenous iron (2, 7). For long time the stainable iron from a bone marrow biopsy was used as the best indicator for the bone marrow iron stores, however; this method carried the risks of infection or bleeding at the biopsy site (8). The ferritin and percent saturation of transferrin (TSAT) levels were used also to monitor the iron status during therapy, but they are less reliable for the diagnosis of iron deficiency in CKD which is a pro-inflammatory state and in the presence of an inflammatory condition, transferrin concentration decreases and ferritin concentration increases (8, 9). However; the newer laboratory biomarkers are less influenced by the underlying state of inflammation in CKD and can accurately reflect the state of iron stores and its requirements (10). These biomarkers include; the paramagnetic assessment of iron in the liver using Superconducting QUantum Interference Device (SQUID), measuring hepcidin level which is a peptide produced by the liver that regulates the absorption of iron in the intestine and its release from macrophages, and it was found that increased levels of hepcidin are associated with a decrease in available iron (10-12). Soluble transferrin receptor (sTfR) is another important biomarker which measures the availability of iron in the bone marrow (10, 13). Transferrin receptor (TfR) is the iron gateway to cells and the soluble transferrin receptor (sTfR) is a truncated form of the tissue transferrin receptor that has probably been proteolytically released from the cell membrane to circulate in plasma (13). Iron deficiency is one of the principal causes of elevated concentrations of sTfR, because with intracellular lack of iron, the iron regulatory proteins stimulate the synthesis of transferrin receptor, which is eventually shed, into the plasma (14). As the concentration of sTfR is proportional to the total concentration of cellular TfR, therefore; an increase in concentration of plasma transferrin receptor provides a sensitive quantitative measure of iron deficiency (10, 13, 14). However it was found that early after treatment with ESAs and iron there is initial increase in sTfR to be followed by gradual decrease (5, 6). Because serum ferritin reflects the storage iron compartment, and sTfR reflects the functional iron compartment, the sTfR/log ferritin index (sTfR/F index) based on these two values has been suggested as a good estimate of body iron (13-15). This study was conducted in order to evaluate the significance of different biomarkers in assessment of iron status during management of anemic children with chronic kidney disease.

Patients and Methods: This was a prospective controlled clinical trial conducted at the King Abdul Aziz Specialist Hospital in Taif, Saudi Arabia, after approval of the ethical committee of the hospital. Twenty five children with diagnosis of anemia of CKD (stages, III-V), were enrolled for the study from January 2012 to January 2013 and they were classified into two groups according to their stage of the kidney disease. Patients with stages I and II "with normal or mildly impaired kidney functions" were excluded from the study. Another 10 healthy children with matched age and sex served as control group. Informed consents for undertaking the research were obtained for all subjects.

Group I: included 15 children with anemia of chronic kidney disease and their Glomerular Filtration Rate (GFR) was 15.5 – 29.6 ml/min/1.73m^2 (stages; III & IV CKD) and they were managed conservatively and they were received oral iron and other hematinics for treatment of anemia and they were evaluated before treatment and 8 weeks after treatment. They were 10 males and 5 females. Their ages ranged between 2-15 years with a mean age of 9.3$\pm$ 3.79 years.

Group II: It included 10 anemic children with end stage renal disease (Stage V CKD, GFR was 6.1 – 13.7 ml/min/1.73m^2) and they were under regular hemodialysis. They were 7 males and 3 females. Their ages ranged between 4 - 10 years with a

mean of 8.1± 1.79 years. The therapeutic protocol for each patient in this group included intravenous recombinant human erythropoietin (r-HuEPO) in a dose of 150 IU/kg body weight three times weekly for 8 weeks, in addition to intravenous iron supplementation according to the weight of the patients and their hemoglobin levels. They were evaluated before treatment and at the end of this phase.

Group III: It comprised 10 apparently healthy children. They were 5 males and 5 females. Their ages ranged between 6-13 years with mean value of 10 ± 2.88 years. All patients and control were subjected to; Clinical evaluation comprising full medical history laying stress on manifestations of anemia, frequency of bleeding and frequency of blood transfusion. Laboratory analysis; included complete blood counts, renal and electrolytes profiles, serum iron, serum ferritin, total iron binding capacity (TIBC), and the serum soluble transferrin receptors (sTfR).

Statistical analysis: Results were expressed as mean ± standard deviation and the analyses were performed using SPSS version 15. Pearson and spearman's correlation test were used to correlate each parameter with different variants in the same group to differentiate between positive and negative correlations and to find significant difference. Comparison of clinical characteristics of the groups was done using ANOVA test.

Results: The results of this study have been demonstrated through tables (1-7) and figure (1-3).Table (I) showed the mean ± SD values of the clinical characteristics in the studied groups and the p-values between them. Statistical analysis of this data revealed that the duration of illness in group II was significantly more than group I ($p<0.05$). Also, the mean value of body mass index (BMI) showed a very high significant reduction in groups I and II when compared to the control group ($p<0.001$). Moreover, on comparing group II to group I, a significant reduction was noticed ($p< 0.05$).

Table (2) clarifies the renal functions profiles. The mean values of serum creatinine showed a high significant increase on comparing groups I and II with group III ($p <0.0001$), also a highly significant increase was found on comparing group II with group I ($p <0.0001$). As regards the blood urea nitrogen (BUN), the mean values of BUN in groups I and II showed highly significant increase when compared with group III ($p <0.0001$). Moreover, on comparing group II with group I, a statistically highly significant increase was evident ($p <0.0001$). The mean glomerular filtration rate (GFR) was 22.47± 5.05 and 10.26± 2.41 ml/min/1.73m^2 in group I and II respectively indicated a significant decrease in GFR in group II than group I.

Regarding the serum electrolytes; no statistical difference was found between the mean values of the three studied groups.

Table (3): displays the hematopoietic parameters. The red blood corpuscular indices (RBCs counts, Hb, HCT%, and MCH) all were significantly decreased in both groups I and II before treatment, as compared to the control group. On comparing group I before and after treatment there was no significant change, whereas, on comparing group II after treatment to group II before treatment, a significant increase was noticed for RBCs count, Hb and MCV ($p< 0.05$). Moreover, a high significant increase was evident for HCT% ($p< 0.001$) and a very high significant increase for MCH ($p<0.0001$) was detected showing the effect of treatment on these indices.

Looking upon reticular cell percentage, there was a very high significant increase in reticular cell percentage on comparing group I with group III ($p<0.0001$).Moreover, on comparing group II after treatment to group II before treatment, a significant increase can be pointed ($p< 0.05$).

Table (4) demonstrates the ferrokinetic data of the studied groups. There was a very highly significant increase in the mean hypochromic cell percentage on comparing groups I, II before treatment and II after treatment with group III ($p< 0.0001$). A very high significant decrease in the hypochromic cell percentage was noticed in group II

after treatment than II before treatment; however, there were no significant changes in group I before and after treatment.

Regarding the serum iron, no statistically significant changes could be seen on comparing group I and II before treatment to group III. But a significant increase was found after therapy on comparing group II before treatment and group II after treatment ($p < 0.05$) with non significant increase in group I.

Serum ferritin showed a very high significant increase in groups II before, and after treatment when compared to control ($p < 0.0001$). Group II after treatment showed a very high significant increase in serum ferritin than before treatment ($p < 0.0001$).

Statistical significant differences were proved between the levels of serum soluble transferrin receptors (sTfR) of the control group and group II before treatment and after treatment. A higher significant increase was found in sTfR levels in patients of group II after treatment than before treatment.

As regards the transferrin / log ferritin index in group II before treatment and II after treatment, there was a significant increase when compared to the controls. On comparing group II after treatment with group II before treatment, there was a high significant increase ($p < 0.0001$).

There were no significant changes in group I before and after treatment in the sTfR and transferrin / log ferritin index,

Tables (5), (6), and (7) showed the correlation coefficient between sTfR and different hematological parameters in group I and II before treatment and after treatment. There was a significant negative correlation between sTfR and both ferritin and TIBC in group II before and after treatment. Meanwhile, a significant positive correlation was observed between sTfR and each of Hb, HCT% and reticulocytes % ($p < 0.05$) in all studied groups.

DISCUSSION: All children who were enrolled for this study in group I & II were anemic with a significant reduction in RBCs counts, Hb levels and HCT values if compared with group III (control group). Many authors proved that anemia of chronic kidney disease is primarily due to erythropoietin deficiency declaring that Physiologic response to anemia is usually preserved early in the course of CKD (with GFR >30 ml/min/1.73 m^2), however; with more advanced disease (GFR <30 ml/ ml/min/1.73m^2), anemic patients will show EPO deficiency which is likely to explain a major part of CKD anemia (3, 7, 16, 17). In the present study; all patients of group I & II had GFR < 30 ml ml/min/1.73m^2.

The mean hemoglobin reported by Gupta et al. (16), was 7.27 ± 1.26 g% which was in agreement with our findings where we recorded that, the mean hemoglobin was 8.49 $\pm$ 1.99gm% in group I and 7.9 $\pm$ 1.24gm% in group II which were significantly lower if compared with group III (mean was 13.17 $\pm$ 0.63, $P < 0.0001$). However; the findings of Singh et al (6), was 10.5 ± 1.4 g% and Fusaro et al. (18), was 11.4 ± 1.2 g% which were higher than that in the present study and this may be related to difference in methodology.

The current study showed a significant decrease in MCH in the dialyzed group of patients when compared to control group but no significant changes could be elicited on comparing MCH of patients, on conservative treatment, to the control group. This could be explained by the fact that in early iron deficiency, no changes could be observed in the mean corpuscular hemoglobin but changes occur later in the course of the disease, however; the red cell distribution width (RDW) which is a recent parameter in fully automated hematology analyzer was found to be more reliable before other RBC indices in such situations (19).

This study also showed a significant negative correlation between BUN and MCH in group II (before therapy) ($p < 0.05$) indicating the inhibitory effect of the pro-inflammatory and inflammatory state of CKD on erythropoiesis. This was in accordance with other studies concerning the pathogenesis of anemia in chronic kidney disease (20, 21).

Increased percentage of hypochromic cells in groups I and II was clarified in our study showing a very high statistical

increase when compared to control group ($P < 0.0001$) suggesting iron deficiency. Confirming our finding, Hasegawa et al. (22), reported that the baseline percentage of hypochromic red cells was significantly higher in functional iron deficiency state. Similar data were reported in other studies (2, 7, 8)Schaefer and Schaefer (23, 24) declared that hypochromic cell percentage more than 2.5% is considered as a sensitive tool for iron deficiency in CRF and values more than 10% are strongly suggestive for functional iron deficiency.

In group I and II before treatment, although iron was present in sufficient quantities in the serum and in storage iron pool evidenced by increased serum ferritin and normal serum iron, yet it was not available for the erythropoietic tissues. Also, the precursor cells were unable to use the excess iron. These findings are in agreement with the study of Fishbane et al (3) Mercadal et al (7) and Buttarello, et al (8), who found that patients with anemia of CKD despite having normal or increased reserve iron but iron mobilization is typically disturbed due to erythropoietin deficiency.

The transit iron pool can be measured directly as serum iron, serum transferrin and soluble transferrin receptors (8-10). This study showed that sTfR was significantly higher in group I and II before treatment ($p < 0.05$ and $p< 0.0001$ respectively) when compared to control group. It was also significantly higher in group II before treatment than group I ($p < 0.001$). This could be explained by erythropoietin deficiency and the presence of functional iron deficiency. This finding is in accordance with the study of Margetic et al., and Gupta et al. (16), who proved that elevated sTfR levels is a characteristic feature of functional iron deficiency, a situation defined by tissue iron deficiency despite adequate iron stores.

No significant changes were detected in all hematopoietic parameters in group I before and after treatment with oral iron and other hematinics. Whereas, after administration of r-HuEPO and intravenous iron therapy for 8 weeks in the dialyzed group of patients (group II after treatment) an improvement in different hematopoietic parameters was noticed indicating the efficacy and superiority of therapy by ESAs in anemic children with CKD. There was a significant increase in mean RBCs count, hemoglobin level, MCH and MCV. This is in agreement with the finding of Mizuguchi *et al.* (25), who claimed that HCT%, Hb levels and RBCs counts showed significant increase at 8 weeks after initiating r-HuEPO treatment. Compatible results were reported in other clinical trials performed by studying the effect of combining r-HuEPO or other ESAs as epoetin alfa and darbepoetin alfa with intravenous iron therapy in dialyzed patients with anemia of CKD (2-8). Meanwhile, the results showed a very high significant decrease in the hypochromic cell percentage after therapy ($p < 0.0001$). Thus, hypochromic cell percentage can be used as a tool for early prediction for response to r-HuEPO. This finding is in harmony with the study of Schaefer and Schaefer (23, 24) and Mac-Dougall, (26), who proposed that measurement of hypochromic cell percentage, is simple and reliable method for detecting functional iron deficiency during r-HuEPO therapy.

Looking upon serum ferritin value after therapy in group II, a very high significant increase was observed ($p < 0.0001$). This could be explained by the fact that the intravenous iron therapy provides sufficient iron to avoid iron deficiency during r-HuEPO and other ESAs treatment as these agents mobilize iron stores to promote erythropoiesis; with subsequent decrease in the iron stores representing the most common reasons for resistance to its effect (2, 4-10). Another mechanism which reduces these stores may be mediated by the reduction of hepcidin level which regulates the absorption of iron in the intestine and its release from macrophages (11, 12).

In this study, the mean sTfR concentrations were increased rather than declined (from 506.87 ± 109.23 to 679.63 ± 119.7) after 8 weeks of iron and r-HuEPO therapy, this is because the erythropoietic effect of r-HuEPO and its effect on the iron stores could mask the effect of the iron status on the sTfR

concentrations. This finding is in line to the work done by Chiang et al. (27), who mentioned that the use of r-HuEPO therapy leads to increased erythropoiesis with early rise in sTfR. However; Tarng and Huang (28), found that the maintenance of r-HuEPO therapy in hemodialyzed patients would lead to significant lower levels of sTfR than before the treatment and early after it. Similar results were recorded in other studies (3, 4, 6, 7, 18).

The current study showed a positive correlation between sTfR levels and parameters that evaluate efficient erythropoiesis (Hb, HCT% and reticulocytes percentage) in group II before and after treatment. This is in accordance with the work performed by Lorenzo et al. (14), Margetic et al. (15), Gupta et al.(16), and Park et al. (29), who found that sTfR levels correlated positively with Hb, HCT % and reticulocytes % in hemodialyzed patients one week and 5 weeks after treatment with r-HuEPO and intravenous iron.

As regards sTfR /Log ferritin index, (sTfR/F index), the values were high but, statistically insignificant in groups I and II (before treatment), whereas a very high significant increase was observed on comparing group II (after treatment) with II (before treatment) ($P< 0.0001$), indicating its efficacy as a monitoring tool of ESAs and iron therapy in those patients. In accordance to this study Matsuda *et al.* (30), proved that in chronic renal failure patients with rHuEPO treatment, the sTfR/logF index showed marked elevation compared to serum sTfR. Park *et al*. (29)**,** concluded that sTfR/log ferritin has a higher discriminating power than the sTfR alone in the assessment of the iron status of patients with anemia of CKD and they added that; combined measurements of ferritin and sTfR concentrations with sTfR/log ferritin index calculation could improve the accuracy and diagnostic reliability particularly in anemic patients with chronic kidney disease with concomitant inflammatory or infective conditions. In conclusion, no single biomarker is reliable alone in the assessment and monitoring the iron status in anemic patients with CKD under ESAs therapy. Measurement of hypochromic cell percentage may be simple and reliable method, and sTfR represents a valuable quantitative assay of marrow erythropoietic activity as well as a marker of tissue iron deficiency. However, the sTfR / Ferritin index is considered to be more efficient in anemic patients with CKD for early prediction of functional iron deficiency and is a sensitive tool for follow up of iron status during ESAs therapy.

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Table (1): Comparison of the clinical data between the three studied groups

		Group I n=15	Group II n=10	Group III n=10
Age (years)	Range Mean±SD P- value	2 - 15 9.3 ± 3.79 >0.05[a]	4-10 8.1± 1.79 >0.05[a] >0.05[b]	6 – 14 10 ± 2.8
Duration of illness (years)	Range Mean±SD P-value	0.6 – 6 2.3 ± 1.7	1.6 – 6.5 4.01 ± 1.66 <0.05[b]	
BMI (kg/m^2)	Range Mean±SD	13.2 -20 16.15 ± 2.11 P < 0.0001[a]	13.2 – 18.3 15.9 ± 1.47 P < 0.0001[a] P < 0.05[b]	17 – 29 23.85 ± 4.76

(a) : variance analysis compared to group III. **P** < 0.05 is considered significant.
(b) : variance analysis compared to group **I**

Table (2): Comparative study of the renal function indices among the three studied groups

		Group (I)	Group II	Group III
S. creatinine (mg/dl)	Range Mean±SD P-value	1.3 – 5.2 2.98 ± 0.91 < 0.0001[a]	4.0 – 10.5 5.59 ± 1.95 < 0.0001[a] < 0.0001[b]	0.6 – 1.2 0.89 ± 0.19
Serum BUN (mg/dl)	Range Mean±SD P-value	36 – 98.5 71.83± 16.13 < 0.0001[a]	61.3 – 176 121.1± 38.62 < 0.0001[a] < 0.0001[b]	8.5 - 10.9 9.58 ± 0.77
GFR ml/min/1.73m^2	Range Mean±SD P-value	15.5 – 29,6 22.47 ± 5.05	6.1 – 13.7 10.26 ± 2.41 < 0.0001[b]	
S. calcium (mg/dl)	Range Mean±SD P-value	7.6 – 10.5 9.45 ± 0.85 > 0.05[a]	7.0 – 9.8 9.15 ± 0.84 > 0.05[a] > 0.05[b]	9.1 – 10.4 9.75 ± 0.48
s.phosphorus (mg/dl)	Range Mean±SD P- value	2.9 – 7.2 5.55 ± 0.94 > 0.05[a]	4.0 – 8.0 6.64 ± 0.63 > 0.05[a] >0.05[b]	4.7 – 6.9 5.72 ± 0.6

(a) : variance analysis compared to group III. **P** < 0.05 is considered significant.
(b) : variance analysis compared to group I. **P** < 0.001 is considered highly significant
P < 0.0001 is considered very highly significant.

Table (3): Comparison of the hematological data of the studied groups.

		Group I		Group II		Group III
		Before treatment	After treatment	Before treatment	After treatment	
RBCs (10^6/ ul)	Mean±SD P- value	3.13 ± 0.45 < 0.0001[a]	3.18 ± 0.61 NS[d]	2.73 ± 0.38 < 0.0001[a] < 0.05[b]	3.12 ± 0.45 < 0.0001[a] > 0.05[b] < 0.05[c]	4.6 ± 0.61
Hb (g/dl)	Mean±SD P- value	8.49 ± 1.99 < 0.0001[a]	8.6±2.1 NS[d]	7.9 ± 1.24 < 0.0001[a] < 0.05[b]	9.91 ± 1.39 < 0.0001[a] > 0.05[b] <0.05[c]	13.17 ± 0.63
HCT %	Mean±SD P- value	26.68 ± 3.46 < 0.001[a]	27.68 ± 3.46 NS[d]	21.18 ± 2.29 < 0.0001[a] < 0.001[b]	26.33 ± 3.8 < 0.0001[a] > 0.05[b] < 0.001[c]	41.56 ± 0.97
MCV (fl)	Mean±SD P- value	59.2 ± 7.66 < 0.05[a]	62.2 ± 8.4 NS[d]	55.19 ± 3.78 < 0.05[a] > 0.05[b]	67.97 ± 2.02 < 0.05[a] > 0.05[b] <0.05[c]	78.42 ± 12.25
MCH pg/cell	Mean±SD P- value	23.77 ± 3.64 < 0.05[a]	24.91± 4.7 NS[d]	22.49 ± 2.05 < 0.0001[a] > 0.05[b]	27.76 ± 2.53 > 0.05[a] > 0.05[b] < 0.0001[c]	30.46 ± 1.52
Reticulocytes %	Mean±SD P- value	1.59 ± 0.69 < 0.0001[a]	1.56 ± 0.47 NS[d]	0.96 ± 0.71 > 0.05[a] < 0.001[b]	1.7 ± 0.58 < 0.05[a] > 0.05[b] < 0.05[c]	0.97 0.25

(a) : variance analysis compared to group III. . **(b)** : variance analysis compared to group I.

(c) : variance analysis compared to group II before treatment. **(d):** variance analysis comparing group I before and after treatment

P < 0.05 is considered significant.

P < 0.001 is considered highly significant.

P < 0.0001 is considered very highly significant

Table (4):Comparative study of the Ferrokinetic parameters of the three studied groups

		Group I		Group II		
		Before treatment	After treatment	Group II Before treatment	Group II After treatment	Group III
Hypochromic Cell (%)	Mean±SD P- value	3.6 ± 1.4 < 0.0001[a]	3.4 ± 0.91 NS[d]	7.1 ± 1.97 < 0.0001[a] < 0.0001[b] -	3.7 ± 1.49 < 0.0001[a] > 0.05[b] < 0.0001[c]	1.05 ± 0.64
Serum iron (ug/dl)	Mean±SD P- value	65.37 ± 25.42 > 0.05[a]	67.17 ± 18.2 NS[d]	74.12 ± 14.6 > 0.05[a] > 0.05[b]	90.93 ± 19.25 < 0.05[a] < 0.001[b] < 0.05[c]	74.64 ± 8.76
TIBC (ug/dl)	Mean±SD P- value	346.12 ± 94.64 < 0.05[a]	366.12 ± 66.4 NS[d]	308.05 ± 124.71 > 0.05[a] > 0.05[b]	371.04 ± 46.47 < 0.0001[a] > 0.05[b] > 0.05[c]	280.17 ± 28.47
Serum ferritin (ng/ml)	Mean±SD P- value	248.6 ± 70.15 < 0.0001[a]	277.5 ± 73.15 NS[d]	320.4 ± 71.27 < 0.0001[a] < 0.05[b]	437.7 ± 86.21 < 0.0001[a] < 0.0001[b] < 0.0001[c]	45.53 ± 8.05
sTfR (u/ ml)	Mean±SD P- value	494.65 ± 119.3 < 0.05[a]	512.44 ± 126.4 NS[d]	506.87 ±109.23 < 0.0001[a] > 0.05[b]	679.63 ±119.76 < 0.05[a] < 0.001[b] < 0.001[c]	369.81 ± 58.37
Serum transferring/log ferritin	Mean±SD P- value	202.16 ± 49.24 > 0.05[a]	232.14 ± 53.24 NS[d]	205.97 ± 31.73 > 0.05[a] > 0.05[b]	278.75 ± 30.1 < 0.0001[a] < 0.001[b] < 0.0001[c]	178.2 ± 36.14

(a) : variance analysis compared to group III. . **(b)** : variance analysis compared to group I.
(c) : variance analysis compared to group II before treatment. **(d):** variance analysis comparing group I before and after treatment
P < 0.05 is considered significant. **P** < 0.001 is considered highly significant. **P** < 0.0001 is considered very highly significant

Table (5) : The correlation between serum transferrin receptors and different biochemical parameters in group I

Parameters	**S. Ferritin**	**TIBC**	**Hb**	**HCT**	**Reticulocytes**
r- value	-0.7411	- 0.652	0.666	0.698	0.685
P - value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

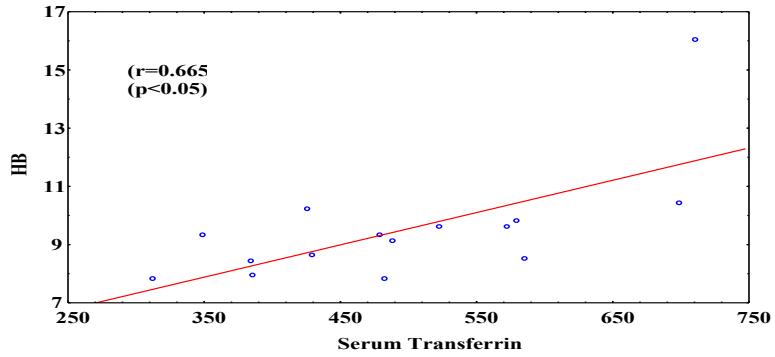
Table (6) : The correlation between serum transferrin receptors and different biochemical parameters in group II before treatment

Parameters	**S. Ferritin**	**TIBC**	**Hb**	**HCT**	**Reticulocytes**
r- value	- 0.886	-0.775	0.812	0.886	0.825
P - value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

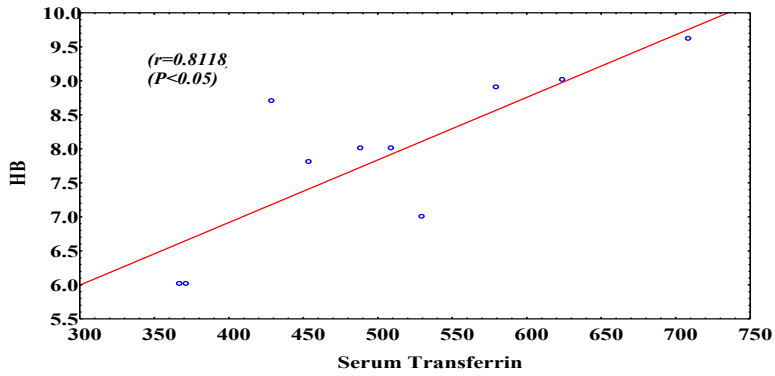
Table (7) : The correlation between serum transferrin receptors and different biochemical parameters in group II after treatment

Parameters	**S. Ferritin**	**TIBC**	**Hb**	**HCT**	**Reticulocytes**
r- value	- 0.778	-0.868	0.869	0.825	0.855
P - value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Fig (1):Correlation between serum transferrin and hemoglobin in patients under conservative treatment



Fig(2):Correlation between serum transferrin and hemoglobin in dialyzed patients before treatment



Fig(3):Correlation between serum transferrin and hemoglobin in dialyzed patients after treatment