

Serum Hcpidin and Ferritin Have Not Correlation With Inflammatory Markers in Kidney Transplant Patients

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Abstract- In renal transplant patients, increased ferritin is associated with an increased risk of cardiovascular disease, transplant rejection, and mortality. Serum ferritin elevates in renal transplant patients due to blood product transfusion, inflammation, and malignancies. Hcpidin is also a peptide hormone produced in the liver in response to anemia, hypoxia, or inflammation. The aim of this study was to investigate the relationship between ferritin, hcpidin, iron, TIBC, and serum inflammatory markers in renal transplant patients. The cross-sectional descriptive-analytical study was conducted on 60 renal transplant patients referred to Hazrat-Rasool and Imam-Ali clinics and Al-Mahdi Laboratory of Shahrekord selected by convenience sampling method. Serum ferritin, hcpidin, iron, TIBC, and inflammatory markers levels were determined by standard kits by ELISA. Data were analyzed by SPSS software. Serum iron had a significant negative correlation with erythrocyte sedimentation rate (ESR) ($r = -0.418$, $P = 0.001$) and a negative correlation with C reactive protein (CRP) ($r = -0.243$, $P = 0.061$). TIBC had a significantly negative association with ferritin ($r = -0.27$, $P = 0.037$). Ferritin, hcpidin, and TIBC were not significantly correlated with inflammatory factors. The results of the study showed no significant relationship between ferritin, hcpidin, and TIBC with inflammatory factors in renal transplant patients.

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Introduction

End-stage renal disease (ESRD) is an incurable condition associated with irreversible renal failure (1). When more than 95% of kidney tissue is destroyed for various reasons, the accumulation of toxins in the body reaches such a level that the permanent use of the alternative kidney therapies, including hemodialysis, peritoneal dialysis, or kidney transplant, must be prescribed to avoid uremia and its life-threatening complications (2). Currently, there are about 3 million patients undergoing kidney replacement therapy worldwide, and this number is expected to increase to 5 -

10 million by 2030 (3). The increased prevalence of obesity, diabetes, and hypertension can face the populations with even much worse situations (1). Kidney transplantation is the most important treatment for ESRD patients, which has significantly improved the life expectancy and quality of life of these patients (4). With advances in transplantation methods, acute rejection of the transplant has decreased to 15 percent, and one-year survival of individuals has increased to more than 90 percent (5).

Anemia is considered a common problem in kidney transplant patients (6) and a risk factor for cardiovascular disease (7). Anemia may be occurred in kidney transplant

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patients due to renal dysfunction, iron deficiency due to drugs such as angiotensin-converting enzyme inhibitors (ACEI), mycophenolate mofetil, and azathioprine, hemolysis, erythropoiesis inhibition with uremic toxins, aluminum poisoning and bleeding (8). Human recombinant erythropoietin (rh EOP) is used for the treatment of anemia in these patients. Moreover, iron prescribing plays a key role in increasing the EOP response in kidney transplant patients (9). However, the prescribed iron should be in such a way that the serum level of ferritin does not exceed 800 ng/mL (10). Recently, ferritin levels and iron overload have been identified as predictive factors in the development and progression of atherosclerosis (11).

Hepcidin is a small peptide produced in the liver in response to anemia, hypoxia, or inflammation, which is considered to be a key regulator of iron use. The potential of measuring hepcidin in biological fluids has made serum hepcidin act as a marker of iron status better than other traditional iron indicators such as serum ferritin and transferrin saturation (12). Previous studies have shown significant correlations between high levels of hepcidin and allograft disorder, increased inflammatory markers, and higher transferrin saturation (13). Also, it is reported that in kidney transplant recipients, hepcidin has been significantly associated with ferritin, creatinine (Cr), and glomerular filtration rate (GFR) (12).

In kidney transplant patients, immune system activity increases due to the reception of allografts. In the next steps, inflammation and activation of the immune system that are directly related to atherogenesis can lead to transplant rejection (14). In this regard, previous studies have shown that inflammatory markers (including C reactive protein (CRP), tumor necrosis factor- α (TNF- α), and interleukin 6 (IL-6)) are associated with the occurrence of acute rejection periods (15). Given the kidney damage caused by iron overload and increased inflammatory markers, which can eventually lead to chronic kidney disease (CKD) and transplant rejection, this study was designed to examine the relationship between hematological factors and inflammatory markers in kidney transplant patients.

Materials and Methods

The study was a cross-sectional descriptive-analytical study. The study population included 60 kidney transplant patients referring to Hazrat Rasool and Imam Ali Clinics and Al-Mahdi Laboratory in Shahrekord, Iran.

Inclusion criteria were kidney transplantation duration greater than one year, the age range of 18 to 70

years, $Cr \leq 1.5$ mg/mL or $GRF \geq 50$ mL/min/1.73 m² and written consent to participate in the study. Exclusion criteria were malignancy and active infection, clinical evidence of heart failure, a history of iron deficiency anemia, and a reluctance to cooperate and use iron supplements or blood transfusions over the past three months.

Patients with inclusion criteria entered the study by easy and available sampling method after signing the consent form. Patients' information and clinical history were collected based on medical history and with the help of a questionnaire. Age, sex, duration of transplantation, and type of transplant donor (live/quadruped) were recorded. All patients underwent a standardized immunosuppressive suppression three-drug protocol, including prednisolone, cyclosporine, and mycophenolate mofetil or azathioprine.

Five milliliters of blood were taken from patients in the morning and after 12 hours of fasting. Blood samples were centrifuged at 4000 rpm for 5 min in heparin tubes, and the serum was collected and kept at -20° C until experiments.

The levels of serum ferritin, iron, total iron-binding capacity (TIBC), and CRP were measured by the BIO SYSTEM (282AA) kit (USA) using the ELISA method. Hepcidin was measured using Bioassay Technology Laboratory Kit by the ELISA method.

Results

In the present study, 60 patients with kidney transplantation aged 47.67 ± 13.85 years entered the study. 19 (31.7%) patients were female, and the other 41 (68.3%) patients were male. The characteristics of the studied patients are shown in Table 1. The mean of inflammatory factors, iron, TIBC, ferritin, and hepcidin in kidney transplant patients are shown in Table 2.

The mean of inflammatory factors, iron, TIBC, ferritin, and hepcidin, according to the type of donor, are shown in Table 3. According to the results, ESR and CRP levels were higher in patients with live donors, while hepcidin and ferritin were higher in brain death donors. However, none of the inflammatory factors, iron, TIBC, ferritin, and hepcidin, showed a significant difference in terms of donor type ($P > 0.05$).

The results of the Spearman correlation coefficient of inflammatory factors with iron, ferritin, TIBC, and hepcidin are presented in Table 4. Based on the results, no significant relationship was observed between ESR and CRP ($r = 0.086$, $P = 0.516$). Serum iron had a significant negative relationship with ESR ($r = -0.418$,

$P=0.001$); however, the relationship between serum iron and CRP was not significant ($r=-0.243$, $P=0.061$). The TIBC factor was negatively associated with ferritin ($r=-$

0.27 , $P=0.037$). Ferritin, hepcidin, and TIBC had no significant association with inflammatory factors ($P>0.05$).

Table 1. The characteristics of the studied patients

Variable	Minimum	Maximum	Mean±standard deviation
Age (year)	20	70	47.67 ± 13.85
Weight (kg)	41	93	69.90 ± 12.95
Height (cm)	145	185	167.87 ± 8.50
BMI (kg/m ²)	17.53	34.34	24.73 ± 3.95
Duration of transplantation (year)	1	26	6.53 ± 5.35

Table 2. The mean of inflammatory factors, iron, TIBC, ferritin, and hepcidin

Variable	Minimum	Maximum	Mean±standard deviation
ESR (mm/h)	2	26	8.05±6.01
CRP (mg/l)	1	49	6.97±8.27
Iron (mg/ml)	21	152	82.70±29.53
TIBC (mg/ml)	231	429	342.20±46.73
Ferritin (ng/ml)	13.20	810	149.05±176.82
Hepcidin (pg/ml)	326	4850	1501.28±1584.33

Table 3. The mean of inflammatory factors, iron, TIBC, ferritin, and hepcidin, depending on the type of donor

Group Variable	Transplantation from live donor (n=24)			Transplantation from brain death donor (n=36)			P
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD	
ESR (mm/h)	2	26	9.17±7.08	2	24	7.31±5.14	0.346
CRP (mg/l)	3	49	7.29±9.06	1	48	6.75±7.82	0.225
Iron (mg/ml)	34	149	83.08±29.49	21	152	82.44±7.08	0.809
TIBC (mg/ml)	258	417	338.88±46.07	131	429	344.42±47.68	0.506
Ferritin (ng/ml)	17.40	810	123.52±155.91	13.20	675.3	166.06±189.70	0.809
Hepcidin (pg/ml)	336	4850	1079.3±1216.8	326	4850	1782.6±1747.7	0.141

Table 4. The results of the Spearman correlation coefficient of inflammatory factors with iron, ferritin, TIBC, and hepcidin

Variable	ESR	CRP	Iron	TIBC	Ferritin	Hipsidine
CRP	$r = 0.086$ $P = 0.516$	1				
Iron	$r = -0.418$ $P = 0.001^{**}$	$r = -0.243$ $P = 0.061$	1			
TIBC	$r = -0.115$ $P = 0.381$	$r = -0.038$ $P = 0.775$	$r = -0.026$ $P = 0.845$	1		
Ferritin	$r = -0.112$ $P = 0.393$	$r = 0.031$ $P = 0.815$	$r = 0.185$ $P = 0.156$	$r = -0.27$ $P = 0.037^{*}$	1	
Hepcidin	$R = -0.108$ $P = 0.411$	$R = -0.034$ $P = 0.796$	$R = -0.163$ $P = 0.214$	$R = 0.032$ $P = 0.807$	$R = -0.205$ $P = 0.116$	1

R=spearman correlation coefficient

P=P value

* Indicates significant difference at $P<0.05$; ** indicates significant difference at $P<0.001$

Discussion

In our study, the mean CRP was 6.97 ± 8.27 mg/l, and the mean ESR was 8.05 ± 6.01 . In a study conducted by Malyszko *et al.*, (2006), the mean serum CRP of kidney transplant patients was significantly higher than that of the control group (16). Decreased renal function may exacerbate inflammatory responses due to decreased renal clearance of inflammatory factors. For example, the serum half-lives of pro-inflammatory cytokines, tumor

necrosis factor- β (TNF- β), and interleukin-1 (IL-1) have been reported to be higher in mice with impaired renal function than in healthy mice (17). In the present study, the mean serum iron, TIBC, and ferritin content of kidney transplant patients were 82.70 ± 29.53 , 342.20 ± 46.73 , and 149.05 ± 176.82 , respectively. In a study by Malyszko *et al.*, (2006), man serum iron and TIBC did not show any significant difference between control and kidney transplant groups. However, the mean ferritin was significantly higher in the kidney transplant group than in

the control group (16). Also, in a study by Jairam *et al.*, (2010) on ESR patients, a significant increase in ferritin was observed compared to the control group. However, there was no significant difference between serum iron in ESRD patients and the control group (18). The increased ferritin in ESRD and kidney transplant patients may be due to iron administration without an accurate assessment of iron status and repeated blood transfusions.

In our study, the mean hepcidin of kidney transplant patients was 1501.28 ± 1584.33 pg/ml. Serum hepcidin had no significant relationship with inflammatory factors (CRP and ESR) as well as iron, ferritin, and TIBC. In this regard, paradoxical and sometimes similar findings have been reported for the relationship between hepcidin and inflammatory and hematological factors. Malyszko *et al.*, (2006) studied patients with renal transplantation and reported that the mean serum hepcidin of patients was greater than the control group. Also, serum hepcidin was significantly associated with inflammatory factors (IL- β , TNF- α , and CRP), neutrophil count, and renal function (16). An experimental study indicated that IL-6 increased hepcidin expression (19). In a study conducted by Małyszko *et al.*, (2005), it was observed that serum iron levels, TIBC, TSAT, red blood cell counts, hemoglobin, hematocrit, and platelet counts in hemodialysis patients were lower, but ferritin and hepcidin were higher than those of control group (20). Hemodialysis patients with erythropoietin-resistant anemia have also shown an increase in the activity of inflammatory markers, hepcidin, and ferritin (21). In another study conducted on ESRD patients, an increase in inflammatory markers (CRP, IL-6, and TNF- α), transferrin saturation (TSAT), ferritin, and hepcidin were observed compared to the control group. Also, there was a significant relationship between inflammatory markers, TSAT, hepcidin, and ferritin (18). In the study of Detivaus *et al.*, (2013) on patients undergoing liver surgery, serum hepcidin levels had a significant association with hemoglobin and liver iron stores, but it had no significant association with CRP levels (22). In the study by Aoki *et al.*, (2005), it was observed that the expression of hepcidin mRNA had no significant relationship with the level of inflammatory markers of the liver in HVC patients (23). In a study by Kato *et al.*, (2008) on CKD patients, hepcidin levels had a significant relationship with ferritin, but it did not have a significant relationship with CRP (24). Dallalio *et al.*, (2003) reported a significant positive association between hepcidin and ferritin in patients with bone marrow anemia (25). In a study conducted by Kulaksiz *et al.*, (2004), an increase in pro-hepcidin levels was found in CKD patients despite their normal hemoglobin levels (26). In

the present study, serum hepcidin levels in kidney transplant patients did not have a significant relationship with CRP and ESR. These results could be due to the small sample size, few positive CRP cases, and low CRP value in determining inflammation.

In the present study, serum hepcidin levels in kidney transplant patients were not significantly associated with ferritin, iron, and TIBC. However, serum hepcidin showed a weak negative relationship with iron ($r=-0.163$) and ferritin ($r=-0.205$), and patients with less ferritin and iron had higher hepcidin, which may indicate the relationship between anemia and hepcidin. In line with these results, in the study of Małyszko *et al.*, (2005), in hemodialysis patients, the levels of iron, red blood cells, hemoglobin, and hematocrit decreased, while ferritin and hepcidin increased compared to the control group. Hpcidin showed a positive relationship with ferritin and a negative relationship with the number of red blood cells, hemoglobin, and hematocrit (20).

In our study, contradictory to some previous studies (16,20), no positive relationship was observed between hepcidin and ferritin. We believe that the result may be due to the lack of patients with high ferritin levels (in this study, all patients had ferritin levels below 600 ng/mL).

The results did not show any significant relationship between ferritin, hepcidin, and TIBC with inflammatory factors in kidney transplant patients. Serum iron levels were negatively associated with ESR and CRP. Serum hepcidin levels showed a weak negative relationship with iron and ferritin, and patients with less ferritin and iron level had higher hepcidin, which may indicate a link between anemia and hepcidin.

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