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Impact of ferritin level on testicular size in hemodialysis patients

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ABSTRACT

Background: Iron deficiency anemia is common among ESRD patients on regular hemodialysis. Upon that erythropoiesis-stimulating agents and iron supplementation are essential. Being a good marker that reflect body iron stores, ferritin is used to evaluate the iron overload status to monitor iron therapy in chronic kidney disease patients.

Aim of work: To determine the effect of ferritin level on the testicular size in ESRD patients on regular hemodialysis.

Patients and Methods: We recruited 150 male ESRD on regular HD three times per week divided into 3 groups according to hemodialysis duration (group A: those on HD for at least 3 years, group B: those on HD for at least 6 years and group C: those on HD for at least 9 years) and 50 healthy controls. Scrotal ultrasound to assess testicular volume and ferritin level as a part of iron profile were done.

Results: Our study demonstrates that the testicular size was inversely correlated to ferritin level and duration of hemodialysis in the 3 HD groups when compared to controls (p-value: <0.0001).

Conclusion: ferritin level can be used as a diagnostic test to predict the testicular volume with sensitivity 91% and specificity 97% (p-value: <0.0001).

Keywords: *ferritin- testes-testicular size-testicular volume- hemodialysis-ESRD*

INTRODUCTION

Iron deficiency anemia is common among patients with advanced chronic kidney disease, particularly those on regular hemodialysis (1). Common contributing cause of Iron deficiency anemia are; frequent blood drawing for laboratory tests, surgical procedures for vascular access and blood loss into the hemodialyzer and tubing (2).

To manage the anemic status in hemodialysis (HD) patients, a well-balanced combination therapy of erythropoiesis-stimulating agents (ESAs) and iron supplementation is essential (3). Intravenous (IV) iron is a convenient treatment to supplement iron and is widely used among patients on hemodialysis (4).

Intravenous iron is beneficial in both dialysis dependent(DD)-CKD and recently in non-dialysis dependent -CKD, also it was proven to be more efficient in rising hemoglobin level, beside reducing ESA and transfusion requirements (5). Some concerns raised about IV iron such as enhanced oxidative stress and endothelial dysfunction. Further, IV iron has been associated with an increased risk of hypotension, headaches or hypersensitivity. Labile iron, which is the free iron in circulation after administration and non-bound to transferrin, is an important cause of such adverse reactions (6).

Excess iron can lead to toxicity because it catalyzes the conversion of hydrogen peroxide into free radicals that cause damage to the cell

membranes, proteins, and DNA. Beside, free iron accumulation in various tissue leading to hazardous effect (7).

Serum ferritin is a good marker used to reflect body iron stores and conventional tool to evaluate the iron overload status to monitor iron therapy in chronic kidney disease patients (8). Ferritin levels are influenced by both endogenous and exogenous iron as well as inflammatory conditions such as chronic diseases, infection, and cancer. An iron-mediated rise of ferritin stems from dietary iron absorption, macrophage recycling, and oral or intravenous iron administration (9,10).

PATIENT AND METHODS

This study included 150 male ESRD on regular HD three times per week divided into three groups according to hemodialysis duration (group A: those on HD for at least 3 years, group B: those on HD for at least 6 years and group C: those on HD for at least 9 years) and 50 healthy volunteers as control. Inclusion criteria: ESRD males on regular HD without other comorbidities were included in the study. Exclusion criteria: liver disease, congestive heart failure, malignancy, sepsis, autoimmune disease, varicocele, alcoholic patients.

All were subjected to the following: (1) History taking (age, alcohol consumption, sexual, medical and drug history, hemodialysis duration). (2) Clinical examination including: general examination with emphasis on signs of liver disease, signs of autoimmune diseases, signs of heart failure (3) laboratory tests including: A) 4 ml were delivered in K3 ethylene diamine tetra-acetic acid tube for complete blood count (CBC) and HbA1c%. B) Another 4 ml was collected in a clean dry tube for ferritin level, serum iron, total iron binding capacity, liver function tests (AST, ALT, serum albumin and total and direct bilirubin), kiney function test (serum creatinine and urea), fasting and 2hours postprandial blood sugar and inflammatory markers (CRP and ESR). (C) Another 2 ml blood was collected in a clean dry tube to asses HDL. (D) LDL: it could be calculated by the following equation: LDL = total cholesterol- triglycerides/5 – HDL (4) Scrotal ultrasound using Hitashi Hi vision avius with Using Hitachi EUP-L65 Small Parts. Length and thickness of the testis were measured in maximal longitudinal plane and width in axial plane. Testicular volumes were calculated using the approximation for a prolapsed ellipsoid: V =0.523× length ×thickness× width. (5) Echocardiography to exclude heart failure

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 20 (IBM, USA). The parametric data expressed as mean ± SD (Range) or number (%) for categorical data. Comparisons for parametric data were carried out using One-way ANOVA test followed by Turkey's test for multiple comparisons. Chi square test was carried out for categorical data. The correlation between variables was evaluated using Pearson correlation coefficient test (2- tailed). The level of significance will be identified at $P<0.05$. Receiver operating characteristic (ROC) curve was constructed and the area under curve (AUC) was used to assess specificity and sensitivity of the predictive power of ferritin in predicting testicular dysfunction.

RESULTS

The study was conducted on 150 HD patients and 50 healthy volunteers as control.

The patients were further divided into three groups according to hemodialysis duration:

Group A: 50 patients on HD for at least 3 years
Group B: A: 50 patients on HD for at least 6 years
Group C: A: 50 patients on HD for at least 9 years

There were no statistical significant differences as regard age and gender among the four studied groups as shown in table 1.

TABLE 1: Demographic and clinical data of the studied groups

	CKD stage 5 on regular hemodialysis for				
	Group A (n=50)	Group B (n=50)	Group C (n=50)	Healthy controls (n=50)	P value
Age	42.5± 8.5 (24 – 59)	42.4 ± 8.9 (22- 57)	44.2 ± 9.1 (24 – 58)	42.3 ± 8.8 (22 – 56)	0.65
Sex; Male	50 (100%)	50 (100%)	50 (100%)	50 (100%)	-

TABLE 2: Complete blood picture and inflammatory markers of the studied groups

	CKD stage 5 on regular hemodialysis for			Healthy controls (n=50)	P value
	Group A (n=50)	Group B (n=50)	Group C (n=50)		
Hb	10.1 ± 1.5 (6.5 – 12.9)	9.7 ± 1.2 (6.5 – 12.6)	10.05 ± 1.3 (6.5 -13.1)	12.6 ± 1.07 abc (6.5 – 14.8)	<0.0001
TLC	6.9 ± 2.3 (3.2 – 14.8)	6.7 ± 2.2 (3 – 15)	7.2 ± 2.4 (3.2 – 15)	7.3 ± 1.6 (4.4 – 10.5)	0.48
Platelets	224.5 ± 72.6 (65 -490)	241.1 ± 87.5 (117 – 440)	230.5 ± 84.3 (82 – 422)	266.1 ± 55.8 abc (174 – 397)	0.03
ESR	19.2 ± 6.3 (10 -32)	21.1 ± 5.7 (12 -36)	18.6 ± 5.9 (10 -32)	18.6 ± 6.3 (12 – 43)	0.153
C-Reactive protein	9.02 ± 2.1 (5-14)	9.1 ± 2.1 (5-14)	8.9 ± 2.1 (5 – 14)	2.6 ± 1.2 abc (1-5)	<0.0001

a significant difference from Group A
b significant difference from Group B
c significant difference from Group C

The study showed that there were high statistical significant differences in HB level, platelets and CRP between the three HD groups and the control group (P value: <0.0001, 0.03 and <0.0001 respectively, otherwise no statistical significant differences between the groups as regard CBC, ESR and CRP as seen in table 2

TABLE 3: Liver and kidney functions of the studied groups

	CKD stage 5 on regular hemodialysis for			Healthy controls (n=50)	P value
	Group A (n=50)	Group B (n=50)	Group C (n=50)		
AST	23.3 ± 12.1 (12 – 61)	26.3 ± 14.7 (12 – 61)	25.6 ± 14.5 (12 – 61)	23.8 ± 13.1 (12 – 61)	0.64
ALT	17.2 ± 6.3 (11 – 40)	17.1 ± 5.8 (11 – 27)	16.7 ± 5.8 (11- 27)	16.9 ± 6.3 (11-40)	0.98
Serum albumin	4.1 ± 0.27 (3.2 – 4.9)	4.02 ± 0.34 (3.1- 4.9)	4.1 ± 0.34 (3.2 – 4.9)	4.1 ± 0.29 (3.2 – 4.9)	0.39
Serum creatinine	7.7 ± 2.1 (4.2 – 13.2)	7.8 ± 2.1 (4.2 – 13.2)	7.9 ± 2.1 (4.2- 13.2)	0.7 ± 0.13 abc (0.53 – 1.1)	<0.0001
urea	140.5 ± 35.7 (54 – 211)	148.2 ± 36.2 (68 – 235)	141.4 ± 37.6 (54 – 211)	32.1 ± 3.2 abc (27 – 39)	<0.0001

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a significant difference from Group A
b significant difference from Group B
c significant difference from Group C

There were significant differences in serum creatinine and urea between the three HD groups and the control group (P value: <0.0001 and <0.0001 respectively) as shown in table 3.

TABLE 4: Blood glucose levels and lipid profile of the studied groups

	CKD stage 5 on regular hemodialysis for			Healthy controls (n=50)	P value
	Group A (n=50)	Group 8 (n=50)	Group C (n=50)		
Fasting blood glucose level	82.8 ± 9.5 (67 – 101)	81.7 ± 8.7 (67 – 95)	84.1 ± 9.3 (67 – 101)	84.1 ± 9.7 (67 – 101)	0.53
Blood glucose post prandial	117.6 ± 13.1 (93 – 139)	118.2 ± 13.8 (93 – 139)	118.5 ± 12.5 (93- 139)	118.5 ± 12.4 (93 – 139)	0.97
HbA1C%	4.5 ± 0.45 (3.8 – 5.5)	4.6 ± 0.41 (3.9 – 5.5)	4.6 ± 0.41 (3.9 – 5.5)	4.5 ± 0.45 (3.8 – 5.5)	0.74
TC	197 ± 50.2 (109 – 326)	195.6 ± 45.1 (121 – 312)	186.8 ± 38.6 (129 – 312)	185.2 ± 34.7 (129 – 276)	0.39
LDL-C	100.1 ± 64.1 (47 – 467)	93.1 ± 60.1 (52- 467)	102.3 ± 95.4 (52- 467)	99.3 ± 95.3 (52- 467)	0.94
HDL-C	44.9 ± 7.2 (31-59)	45.5 ± 7.7 (31-59)	45.4 ± 7.6 (31-59)	45 ± 7.7 (31-59)	0.97
TG	198.6 ± 107.1 (72 – 498)	194.2 ± 104.8 (78 – 498)	169.7 ± 91.4 (79 -498)	181.1 ± 102.8 (79 -498)	0.49

As regard the lipid profile, fasting blood sugar, 2-hour post prandial and glycated hemoglobin; there were no statistical significant differences as regard age and gender among the four studied groups as seen in table 4.

TABLE 5: Ferritin, serum iron and TIBC of the studied groups

	CKD stage 5 on regular hemodialysis for			Healthy controls (n=50)	P value
	Group A (n=50)	Group B (n=50)	Group C (n=50)		
Ferritin	318.5 ± 83.2 (136 – 456)	395.5 ± 113.6 a (176 – 621)	512.2 ± 174.6 ab (188 – 844)	170.2 ± 38.4 abc (86 – 243)	<0.0001
Serum iron	88.5 ± 19.9 (58 -132)	88.8 ± 19.7 (58 -132)	88.2 ± 19.8 (58 -132)	87.9 ± 19.5 (58 -132)	0.99
TIBC	325.7 ± 61.4 (243 -442)	324.6 ± 61.3 (243 -442)	324.7 ± 60.4 (243 -442)	327.8 ±60.6 (243 -442)	0.99

a significant difference from Group A
b significant difference from Group B
c significant difference from Group C

The study showed that ferritin level was highly statistically significantly different among the HD groups when compared to controls (p-value = <0.0001). Also, it was highly statistically significant in GROUP B when compared to GROUP A (p-value = <0.0001). In addition to, GROUP C showed highly statistically

significance in comparison to GROUP A and B (p-value = <0.0001) as seen in table 5.

TABLE 6: Scrotal ultrasound data of the studied groups

	CKD stage 5 on regular hemodialysis for			Healthy controls (n=50)	P value
	Group A (n=50)	Group B (n=50)	Group C (n=50)		
Right testicle	10.1 ± 1.8 (6.4 – 14.2)	8.1 ± 2.1 a (4.3 – 12.7)	5.8 ± 1.9 ab (2.6 – 10.6)	15.9 ± 1.8 abc (12.8 – 19)	<0.0001
Left testicle	9.9 ± 1.8 (6.1- 13.9)	7.7 ± 1.98 a (3.9 – 13)	5.3 ± 1.93 ab (2.5 -9.8)	15.8 ± 1.7 abc (12.6 – 18)	<0.0001

a significant difference from Group A
b significant difference from Group B
c significant difference from Group C

The study showed that the testicular volume was highly statistically significantly smaller among the HD groups when compared to controls (p-value = <0.0001). Also, it was highly statistically significantly smaller in GROUP B when compared to GROUP A (p-value = <0.0001). Beside, GROUP C showed highly statistically significantly smaller size in comparison to GROUP A and B (p-value = <0.0001) as presented in table 6.

TABLE 7: Pearson correlation between ferritin and Scrotal ultrasound data (testicular volume) (n=150)

Versus ferritin	r	P
Right testicle	-0.84	<0.0001
Left testicle	-0.86	<0.0001

The study showed very strong highly statistical significant negative correlation between ferritin level and testicular volume among the three HD groups (r = -0.84 and -0.86 respectively, p-value: <0.0001) as shown in table 7.

TABLE 8: Pearson correlation between Duration of hemodialysis and Scrotal ultrasound data (testicular volume) (n=150)

Versus duration	r	P
Right testicle	-0.67	<0.0001
Left testicle	-0.7	<0.0001

The study showed strong highly statistical significant negative correlation between duration of hemodialysis and right and left testicular volume among the three HD groups (r = -0.67and -0.7 respectively, p-value: <0.0001) as shown in table 8.

TABLE 9: Diagnostic power of ferritin in predicting testicular dysfunction

AUC	Std. Error	P	Asymptotic 95% Confidence Interval		Cut off Value	Sensitivity	Specificity
			Lower Bound	Upper Bound			
0.969	0.010	<0.0001	.948	.989	234.5	91%	97%

AUC Area under the curve

The study showed that ferritin level has highly statistically significant diagnostic power to predict the testicular volume with sensitivity 91% and specificity 97% (p-value: <0.0001) as seen in table 9 and figure 1.

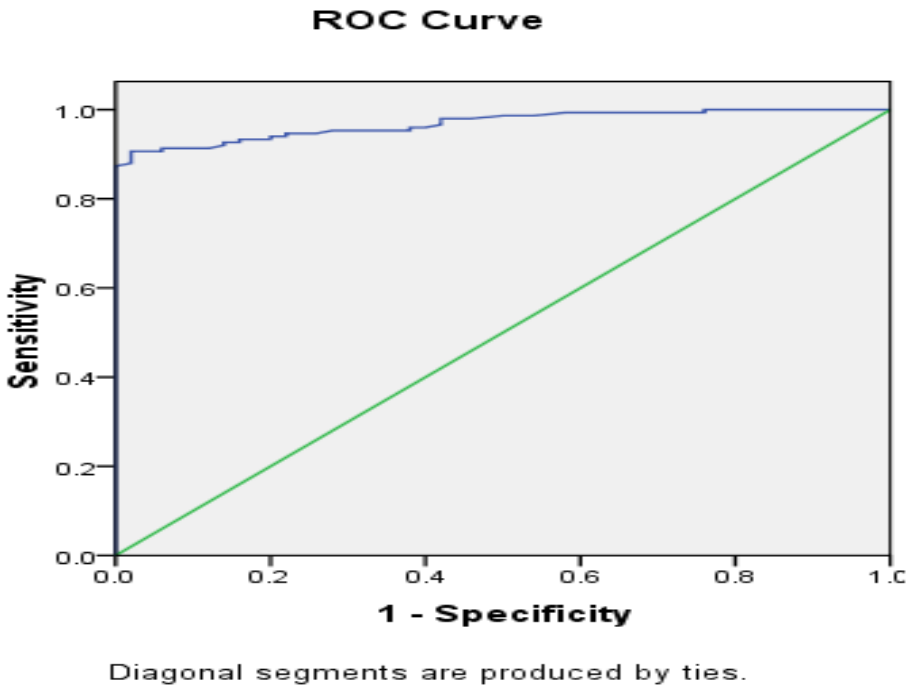

FIGURE 1: Diagnostic power of ferritin in predicting testicular dysfunction

DISCUSSION

Iron deficiency anemia, being one of most common metabolic disorders in ESRD patients on regular hemodialysis, treated using intravenous iron supplementation. Free iron released is considered to be an oxidative stress which can lead to male infertility via peroxidative injury to the sperm and testes (11).

Serum ferritin, a good marker used to reflect body iron stores, does always tend to contain some iron, that may be released and is then not benign. When the iron is varied systematically, it is iron-loaded ferritin that is the most toxic (12).

We conducted a study on 150 HD patients to investigate the effect of serum ferritin and duration of HD on testicular size and its consequences which affect male fertility.

The study showed that ferritin level was significantly higher among all HD patients when compared to controls (p-value = <0.0001). Ford et al, 2009 conducted a study on 60 HD patients to assess ferritin level. It was found that serum ferritin is much higher among them (13).

Also, it got higher as HD duration increase upon that serum ferritin is significantly higher in GROUP B when compared to GROUP A (p-value = <0.0001). Beside, in GROUP C the level

was significantly higher in comparison to GROUP A and B (p-value = <0.0001).

Wibowo et al, 2019 conducted a study on 30 men who were randomly divided into 3 groups: one-month group (1M-G), three-month group (3M-G), and six-month group (6M-G). Each of the included patient undergone hemodialysis for one, three and six months. The levels of ferritin, hemoglobin, and erythrocyte count were analyzed. They concluded that the ferritin levels of 3M-G and 6M-G are significantly higher than that of 1M-G, p< 0.01. The ferritin level of 6M-G is significantly higher than that of 3M-G, p<0.01(14).

However, there were no statistical significant differences between the four groups as regard Serum iron and TIBC.

In addition, the study showed that the testicular volume was significantly smaller among the three HD groups when compared to controls (p-value = <0.0001). And, it was significantly smaller in GROUP B when compared to GROUP A (p-value = <0.0001). Beside, GROUP C showed significantly the smallest size when compared to GROUP A and B (p-value = <0.0001).

In a study conducted on 120 male patients by Shiraishi et al, 2008 both decrease in testis volume and fibrosis were found to be correlated with the duration of hemodialysis (15). In a same manner, a study was conducted by Hekimoglu et al, 2017 including 28 male patients who undergo dialysis three times per week and 25 healthy volunteers. They found that CKD group had lower right, left, and mean testicular volumes (p<0.001) (16).

It was speculated that iron-mediated oxidation of the testicular cells may be involved in triggering changes in testicular morphology and function. However, the underlying mechanisms remain largely still unclear (17).

Our study showed very strong highly statistical significant negative correlation between ferritin level and the right and left mean testicular volume among the three HD groups (r = -0.84 and -0.86 respectively, p-value: <0.0001). Additionally, there was a strong highly statistical significant negative correlation between duration of hemodialysis and right and left mean testicular volume among the three HD groups (r = -0.67and -0.7 respectively, p-value: <0.0001).

In contrast, there was no statistical significant correlation between the both testicular volume and transferrin saturation among the three HD groups.

Based on the above data, ferritin level considered to have highly statistically significant diagnostic power to predict the testicular volume with sensitivity 91% and specificity 97% (p-value: <0.0001).

CONCLUSION

Hemodialysis patients are suffering of infertility due to many causes. One of those can be assessed by measuring serum ferritin as an indicator for testicular size.

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