Original article

The evaluation of Serum Copper Level in Hemodialysis Patients with and without anemia Compared with Healthy Controls

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Abstract:

Introduction: Anemia is a common complication of ESRD with different etiologies. The most common cause of anemia in such patients is insufficient production of erythropoietin by kidneys. Another possible reason is copper deficiency, thus, this study is aimed to evaluate the serum copper level among hemodialysis patients with and without anemia and to compare them to healthy controls. ***Material and Methods***: A total number of 56 patients who underwent regular hemodialysis due to their ESRD were enrolled and divided into two groups of 28 according to their Hgb results including patients with anemia (Hgb<11 gr/dl) and patients without anemia (Hgb>11 gr/dl). Also, 28 healthy individuals participated as the control group. Venous blood samples were collected from patients prior to hemodialysis. Spectrophotometry employed for copper measurements. Data were analyzed using t-test and Pearson regression analysis. ***Results***: Mean age of patients was 44.36±15.80 years. No significant difference was present among the three groups with regarding to age and gender. Mean and SD of serum copper in patients with anemia, without anemia, and controls were 180.11±54.48, 139.66±35.57 and 155.82±42.06 µg/dl respectively. Serum copper level was significantly higher in hemodialysis patients with anemia compared with those without anemia ($p<0.03$). Also a significant inverse regression between serum copper and Hgb was present in all hemodialysis patients ($p=0.02$, $r=-0.37$). ***Conclusion***: Higher levels of serum copper in anemic patients could be a result of restraining role of high serum copper on iron absorption that has a negative effect on the production of Hgb. However, complementary studies are required.

Key words: ESRD; anemia; serum copper; hemodialysis

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Introduction:

Despite of the recent improvements in defining complications of End Stage Renal Disease (ESRD) as well as recent discoveries in the field of maintenance therapies, recombinant human erythropoietin remained the standard treatment for anemia in hemodialysis patients for nearly two decades[1]. However, sensitivity to this drug has been significantly changed. Nearly 90% of hemodialysis patients with anemia who have been treated by erythropoietin showed appropriate outcomes by increasing Hemoglobin (Hb). The remaining

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patients showed unsatisfactory therapeutic outcomes or decreased response rate after some time [2].

Iron deficiency, infection, inflammation, or insufficient dialysis have and important role in treatment failure. Other reasons for failure in treatment would be blood loss, hyperparathyroidism, aluminum intoxication, vitamin B12 deficiency, carnitine deficiency, and having antibodies against erythropoietin. Even after discarding all these elements still sensitivity to erythropoietin happens [3]. It seems that other factors might play roles in the failure of response to erythropoietin treatment.

Copper deficiency is an uncommon cause for anemia that is being forgotten in most cases[4]. Copper deficiency has been reported in uncommon mechanisms of feeding such as enteral feeding and total parental nutrition results in anemia and neutropenia[5]. The mechanism in which copper deficiency causes anemia and decrease in other blood cells has not been well studied yet.

One possible hypothesis would be the reduced function of copper dependent enzymes such as ceruloplasmin ferroxidase, and cytochrome oxylase that may potentially cause anemia [6]. Previous studies showed that copper deficiency induced anemia in hemodialysis patients would gradually be treated after three months and neutrophil count becomes normal after 2-4 weeks by administration of sufficient copper complementary nutrients [7]. Although there are some reports of decreased copper level in hemodialysis children with anemia such condition is not well studied in adults [8]. Thus the present study is aimed to evaluate serum copper level in hemodialysis patients with and without anemia and compare them to healthy participants.

Material and Methods:

In this descriptive two-group cross-sectional study three groups including hemodialysis patients with and without anemia and healthy participants who were selected from healthy nonrelative companions of patients were enrolled.

Overall 56 hemodialysis patients and 24 healthy individuals were categorized into 3 groups. Groups were normalized according to age and gender parameters. Patients were selected from those who were referred to Imam Reza and Hasheminejad Hospitals, located in Mashhad Northeast of Iran and underwent regular hemodialysis for four hours three times a week during January 2010 to January 2011.

All patients were over 15 years old and at least 6 months past from their first hemodialysis and were on their regular hemodialysis program. Their serum albumin was >4 while adequacy of hemodialysis was defined as KT/V>1.2.

All patients who had the history of blood transfusion in the previous month, primary blood disease, approved active infection or inflammation based on clinical and paraclinical evaluations, active blood loss, and insufficient hemodialysis according to clinical parameters such as presence of edema, pericardial effusion, pleural effusion, and ascites as well as paraclinical findings were excluded from the study.

This study was approved by the research deputyship of Mashhad University of Medical Sciences regarding ethical and methodological issues. A written consent was obtained from each individual prior to the study and they were all informed about the study aims and anonymousity of the results and all their questions were answered.

A complete medical history obtained from each individual and a detailed physical examination was performed by a physician. Data including age, gender, race, drug history, previous diseases, duration of hemodialysis, and reason for ESRD were registered. Hemodialysis patients were divided into two groups according to Hgb measurements. Patients who had Hgb?11 were regarded as anemic. To perform further analyses iron level, ferritin, TIBC, calcium, phosphorus, and serum PTH were measured for all patients. Moreover vitamin B12 and folic acid were evaluated for anemic patients to study the possible relation between copper deficiency and the kind of anemia. To perform serum copper level comparisons among participants fasting serum copper of 28 healthy individuals similarly measured. Also Hgb, urea, and creatinine were measured for the control group to approve their health. Fasting serum copper level for all participants was measured using a spectrophotometry commercial kit (RANDOX) and calorimetry technique. To perform this test 5ml venous blood obtained from each individual and stored in tubes without any metallic contaminations as well as no antithrombotic agents. After 15 minutes centrifuge in 250 rpm serum was separated and kept in -8ºC till the time of the evaluations.

Normal serum copper level is 100 (80-155) µg/dl and copper levels < 80 µg/dl were considered as copper deficiency. Individuals who had copper levels higher than the normal value were reevaluated about the existence of active infection. To minimize laboratory bias all paraclinical evaluations were performed in a single standard laboratory.

Data were registered and analyzed using SPSS V.16.0. Frequency tables were produced using Chi-squared statistics. To compare mean levels of copper among the three groups, t-test and ANOVA were employed. Pearson regression was applied whenever possible. To discuss the results relative tables and figures were produced. P-values <0.05 were considered as statistically significant.

Results:

A total number of 74 participants including 28 hemodialysis patients with anemia, 28 hemodialysis patients without anemia, and 28 healthy controls were evaluated.

The frequency of males was higher among all the three groups while no significant difference was present among them regarding gender distribution (p=0.99). The average age of patients was 44.36±15.80 years. No significant mean age difference was present in the three groups (p=0.41).

Average time past since the first hemodialysis for patients with and without anemia was 27±14.73 months and 46.85±41.85 months respectively. a slight relation was present between the two groups regarding the average duration of hemodialysis using t-test (p=0.05).

The most common causes for ESRD among patients were diabetes and hypertension respectively (Table 1).

Table 2 summarized the mean and standard deviation of iron, ferritin, TIBC, calcium, phosphorus, and PTH values for hemodialysis patients and vitamin B12 and folic acid values for hemodialysis patients with anemia. T-test results showed no significant differences regarding the paraclinical evaluations between the two groups (p>0.05).

Pair comparisons using Tukey test showed that serum copper level of hemodialysis patients with

Table 1: The frequency of different etiologies for ESRD in hemodialysis patients with and without anemia (p=0.69)

Causes of ESRD	Hemodialysis with anemia		Hemodialysis without anemia	
	NO	%	NO	%
Diabetes	12	42.85	12	42.85
Hypertension	8	28.57	7	25.00
Urinary tract infection and reflux	3	10.71	1	2.50
Glomerulonephritis	2	7.14	3	10.71
Unknown and other	3	10.71	4	14.28
Total	28	100	28	100

No significant difference between both hemodialysis groups regarding the cause of ESRD was observed (p=0.69).

Table 2: Mean values for iron, TIBC, ferritin, calcium, phosphorus, and PTH in hemodialysis patients and folic acid and vitamin B12 in patients with anemia

	Hemodialysis with anemia	Hemodialysis without anemia	p-value
Iron	93.45±97.20	99.68±84.4	0.840
TIBC	294.100±174.95	292.75±156.01	0.981
Ferritin	429.52±166.71	306.20±189.56	0.062
Calcium	9.32±0.54	9.51±0.45	0.278
Phosphorus	6.34±1.81	6.20±1.35	0.801
PTH	171.55±115.76	351±481.04	0.164
Folic acid	17.87±4.34	-	-
Vitamin B12	808.47±657.64	-	-

Table 3: Mean and SD of serum copper in the three groups (p=0.01)

Group	Mean	SD	Minimum	Maximum
Hemodialysis with anemia	180.11	54.48	105	261
Hemodialysis without anemia	139.66	35.57	63	197
Healthy controls	155.82	42.06	129	200

Mean serum copper level was compared among the three groups and revealed significant differences (p=0.01).

anemia is significantly higher than those without anemia (p<0.03). pair comparisons between control group and both hemodialysis groups showed no significant difference (p>0.05) (Figure 1).

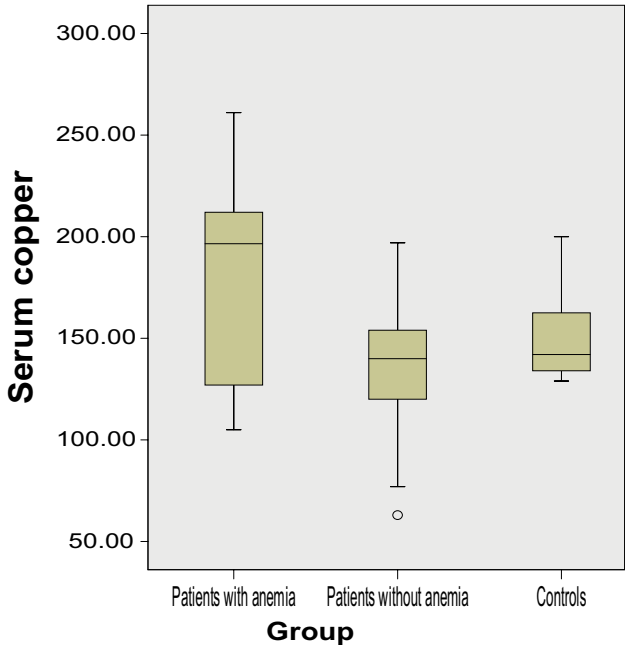

Figure 1: comparison of mean serum copper in the three groups

Also mean copper level of all hemodialysis patients was compared with healthy controls which revealed no significant relation (p=0.42). Mean serum copper level of hemodialysis patients and healthy controls was 158.33±49.10 and 150.68±21.74 respectively (Figure 2).

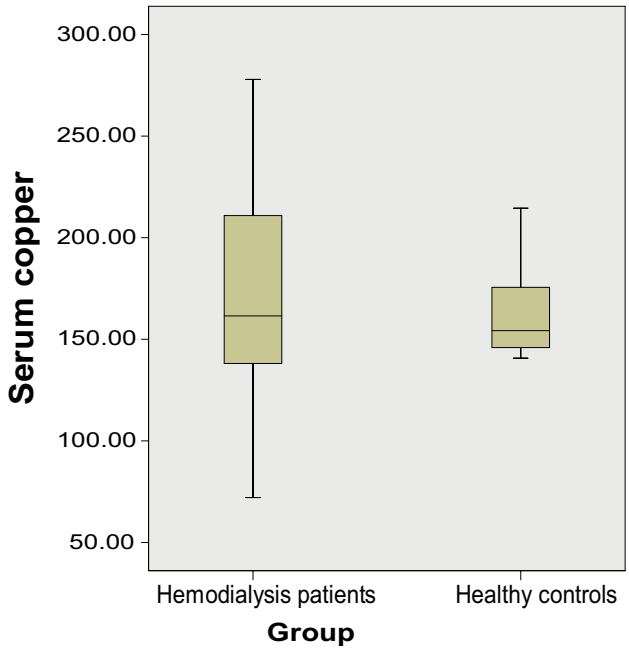

Figure 2: comparison of mean serum copper in all hemodialysis patients and healthy controls

Pearson regression analysis showed a significant reverse regression between serum copper and Hgb among hemodialysis patients (p=0.02, r=-0.37) (Figure 3).

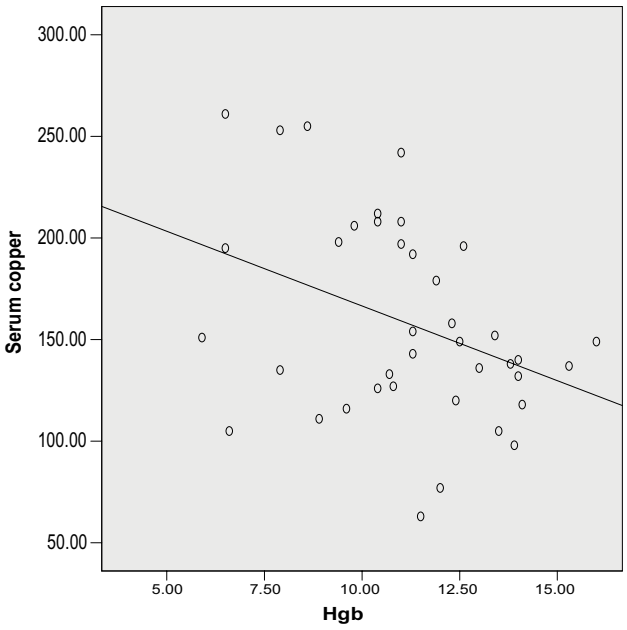

Figure3: Serum copper and Hgb in hemodialysis patients (p=0.02)

However such relation was not observed in pair comparisons between both groups of hemodialysis patients.

Discussion:

In this study three groups of 28 hemodialysis patients with anemia, 28 patients without anemia, and 28 healthy participants were evaluated and compared. No significant relation regarding age and gender distribution was present among the groups. The most common causes of ESRD were diabetes and hypertension among the patients.

We found a significant difference of serum copper among the groups. Serum copper was significantly higher in hemodialysis patients with anemia comparing to patients without anemia. However comparing control group to patients with and without anemia as well as all patients no significant difference in copper level observed.

These results were completely against our initial hypothesis of copper deficiency in hemodialysis patients with anemia. Some previous studies reported normal serum copper and ceruloplasmin in hemodialysis patients while some reported that low [9].

Takashi et al. studied the role of copper deficiency in erythropoietin resistant anemia. Also they evaluated

5 ESRD patients with brain stroke and hypoxic encephalopathy under enteral feeding who had macrocytic anemia and neutropenia but normal vitamin B12 and folic acid. Following administration of copper nutrients to their enteral nutrition their Hgb and Hematocrit raised and successfully their erythropoietin therapeutic dosage reduced [10].

Our study was conducted on hemodialysis patients under regular hemodialysis schedule of 4 hours 3 times a week who had stable clinical status. WaradAy et al. reported 69.1±38.3 as the mean serum copper level in 17 hemodialysis children with erythropoietin resistant anemia which was lower than normal values. They also emphasized on the role of hydrochloride sevelamer in copper deficiency (8). Another study on the relation between serum copper and anemia revealed that in anemic patients with iron deficiency that are resistant to iron treatment, serum copper and ceruloplasmin are in their minimum normal range and concluded that low levels of serum copper have a negative role in the production of Hgb. This relation could be justified by the natural role of copper as an essential micronutrient in the absorption of iron (oxidation of ferous iron to ferric iron by ceruloplasmin and increasing iron absorption capacity) [11].

Similar to our findings some studies indicated that high levels of copper in hemodialysis patients due to releasing copper from cuprophane laminas sterilized by ethylene oxide [12]. Besides, some studies conducted on hemodialysis patients with iron deficiency reported high levels of serum copper. Ece et al. reported high serum copper in children with iron deficiency anemia [13]. Although Hegazy et al. reported higher serum copper in patients with anemia comparing to healthy controls they did not find a significant relation [14]. Regarding normal or high folic acid and vitamin B12 in our patients, megaloblastic anemia was not found that shows the adequate folic acid treatment in the patients. Although some patients did not receive their vitamin B12 therapy during hemodialysis they had high vitamin B12 level that could approve the results of previous studies reporting high vitamin B12 level in hemodialysis patients and safety of routine folic acid administration. However this finding needs more complementary data.

Iron and TIBC were in normal range in our patients which could be a result of receiving intravenous iron along with erythropoietin therefore exact identification of anemia type and its relation to high serum copper was not possible. Moreover regarding that high serum copper could cause anemia through hemolysis admitting anemia as a result of high serum copper requires detailed clinical trials.

Conclusion:

It seems that it is still not possible to find an accurate relation between serum copper level hemodialysis according to the combination of our results and those of previous studies. Very different results of high, normal, and low serum copper in such patients have been reported. These very different results may be due to different physiological effects of uremia, nutrition styles, copper level in drinking water, and medicinal therapies. It is concluded that although serum copper in hemodialysis patients without anemia was in the normal range higher levels in anemic patients would need further investigations. Thus conducting complementary studies with more samples and designing methodologies according to a different hypothesis is recommended to clarify this situation. However it is also suggested that routine evaluation of serum copper in hemodialysis patients unnecessary and it should only applied in the presence of strong clinical suspicions.

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