The Effect of Renal Hemodialysis on Serum Endogenous Antioxidants Defense of Patient with Chronic Renal Failure

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تاثير الغسيل الدموي الكلوي على مضادات الاكسدة داخلية المنشأ المصلية لمرضى الفشل الكلوي المزمن

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الخلاصة:

الغسيل الدموي الكلوي هو عملية إزالة السوائل والفضلات الزائدة من الدم عندما لا تؤدي الكلية عملها بكفاءة وتُجرى عادة ثلاث الى أربع مرات في الأسبوع أو اكثر. تهدف الدراسة الحالية لتقصي تأثير الغسيل الدموي الكلوي على دفاعات مضادات الأكسدة داخلية المنشأ الإنزيمية وغير الإنزيمية المصلية. شملت الدراسة (45) مريضاً (25) منهم من الذكور وبنسبة (55,54%) ومتوسط أعمار يتراوح بين 11-70 سنة و (20) من الإناث بنسبة (44,46%) وبمتوسط أعمار 6-65 سنة ، بينما كانت عينة السيطرة (35) فرد لم يخضعون للغسيل الدموي الكلوي، (23) منها من الذكور بنسبة (65,71%) بمتوسط أعمار 15-65 سنة و(12) أنثىبنسبة (34,29%) وبمتوسط أعمار من 18-62سنة. أظهرت النتائج الحالية إنخفاضا معنويا عاليا($p<0.05$) في مستوى مضادات الأكسدة داخلية المنشأ الإنزيمية (catalase)وغيرالانزيمية(transferrineو bilirubin) بينما لوحظ وجود إرتفاع معنوي عالي ($p<0.05$) في مستوى مضاد الاكسدة غير الانزيمية لحامض اليوريك (uric acid) للمرضى المصابين الفشل الكلوي المزمن. يستنتج من بيانات الدراسة بان مرضى الفشل الكلوي المزمن الذين يخضعون للغسيل الدموي الكلوي أظهرواأضطراب فسيولوجي في مستوى مضادات الاكسدة داخلية المنشأ الانزيمية وغير الانزيمة المصليةقد تكون لها علاقةبالضغوط التاكسدية لمرض الفشل الكلوي.

Abstract

Hemodialysis means removes waste and excess fluid from the blood when thekidneyscannot do so sufficiently usually performed three or more times a week for 4 hours or more.The present work was aimed to investigate the effect of renal hemodialysis on serum anenzymatic and non-enzymatic endogenousantioxidantsdefenses. Forty five patientswere taken for this study, 25males (55.54%) with age range (11-70) years and 20 females (44.46%) with age range (6-65)years, while the subjects of control group was thirty five individuals non-undergoing hemodialysis treatment, 23 males (65.71%) with age range (15-65) years and 12 females (34.29%) with age range (18-62) years. The present results showed highly significantly decrease ($p<0.05$) in serum enzymatic endogenous antioxidant (catalase) andnon-

enzymatic antioxidants (transferrin and bilirubin), while serum non enzymatic antioxidant uric acid showed highly increase ($p<0.05$) in patient with chronic renal failure.In conclusion, the present data showed that patients with chronic renal failure (CRF) undergoing hemodialysis treatmenthave physiological disturbances in both serum enzymatic and non-enzymaticendogenous antioxidants levels in all age ranges may be related to oxidative stress by renal failure disease.

Introduction

Renal failure occur when a sufficient number of nephrons are damaged and therefore the kidneys unable to perform their tasks. Renal failure can be divided into two main types, namely acute and chronic renal failure (Stevens and Lowe, 2000)**.**

Chronic Renal Failure (CRF) is defined as progressive and irreversible loss of renal function. It is a major public health problem, with increasing incidence and prevalence, poor outcomes, and high costs. CRF frequently leads to end stage renal disease (ESRD), which without renal replacement therapy would lead to death (Bullock and Henze 2000; Longmore*et al.*, 2004). Hemodialysis (HD) is a renal replacement therapy. The treatment process in HD consists of circulating the patient's blood through an artificial kidney, a dialyser, to remove waste products, such as potassium and urea, and excess fluids.HD treatment is usually required three times a week. One treatment session usually lasts for 4-5 hours (Smeltzer and Bare,2004).

However, (CRF) is accompanied by oxidative stress (Himmelfarband Hakim 2003; Galle, 2001), which consists in the damage of biological structures by reactive oxygen species due to their excessive generation and impaired efficiency of antioxidant defense mechanisms. In renal failure patients enhanced reactive oxygen species production is underlain mainly by inflammation (Locatelli*et al*., 2003), malnutrition, and presence of endogenous stable oxidants in the uremic plasma (Hellman, and Gitlin, 2002).

So, the Antioxidants are the first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells (Percival, 1996). These antioxidants act as free radical scavengers by preventing and repairing damages caused by reactive oxygen species (ROS), and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases, (Ebrahimzadeh*et al*., 2010).

Endogenous antioxidants that are synthesized in the human body include antioxidant enzymes, metal binding proteins and other small molecule antioxidants (Evans and Halliwell, 2001). Antioxidant enzymes which include superoxide dismutase (SOD), glutathione reductase and catalase defend the host against the damaging effects of the free radical species (Nagaraju and Belur, 2008).

Additionally, the non-enzymatic antioxidants are also divided into metabolic antioxidant and nutrient antioxidants. Metabolic antioxidants belong to endogenous antioxidants, are produced by metabolism in the body, such as lipoid acid, glutathione, L-ariginine, , melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc. While nutrient antioxidants belong to exogenous antioxidants, are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc. (Droge, 2002; Willcox, 2004;)The present study was aimed to investigate the effect of renal hemodialysis on serum anenzymatic and non-enzymatic endogenousantioxidants defensesof chronic renal failure patients.

Material and Methods

Experimental Design

Patients and Control subjects:

Patients

The subjects for this study consist of CRF patients undergoing hemodialysis (thrice a week) in the unit of artificial kidney in AL-Kut hospital of Wasit health directorate in AL-Kut city, Wasit province, Iraq induration from November 15, 2011 to March 15,

2012. Forty five subjects were taken for this study, 25males (55.54%) with age range (11-70) years and 20 females (44.46%) with age range (6-65) years. The patients were diagnosed as chronic renal failure for both sexes based on the age, gender, race, habitation and clinical status.

Control Subjects

The subjects of control group in current study was thirty five individuals non-undergoing hemodialysis treatment who were free from any signs and symptoms of chronic renal disease,

liver disease, lipid disorders, diabetes mellitus, hypertension and other, 23 males (65.71%) with age range (15-65) years and 12 females (34.29%) with age range (18-62) years.

Blood Sample Collection and Antioxidant Assay:

Collection of blood and separation of blood serum

Five milliliters of venous blood were drawn from control subjects and patients with chronic renal failure under fasting condition immediately before hemodialysis session by using disposable syringe of (5 ml) for one time per month, their blood samples were collected in a centrifuge tubes for maximum coagulation and separation of serum, after centrifuged at 3000 rpm/10minutes, serum was isolated, frozen at (-20°C) and then processed for antioxidant assays.

Antioxidant assay

The catalase, serum enzymatic endogenous antioxidant was assayed and performed depends oncatalase kit assay prepared manually in Iraq, its absorbance read at (240) nm, while transferrin was performed by percentage Iron /Total Iron Binding Capacity (TIBC) depend on Randox (UK) kitassay at (595) nm. Bilirubin and uric acid were performed depend on kit assay procedure Biolabo laboratories (France) and the absorbance were measured at (550) nm and at (520) nm respectively.

Statistical Analysis:

The data of present study was made with using SPSS 16 (statistical software package) and analyzed by ANOVA (one way analysis). The results were expressed as mean ± standard error of the mean (M±S.E.M.). LSD andDunnetts were used for comparisons between the hemodialysis patients and control group. $P<0.05$ was considered to be statistically significant.

Results and Discussion

Enzymatic Endogenous Antioxidant
Serum Catalase Activity (U/I)

Catalase is a common enzyme found in the blood and in most living cells that catalyzes the decomposition of hydrogen peroxide into water and oxygen. It is a tetramer of four polypeptide chains, each over 500 amino acids long (Reddy *et.al*, 2011).

The current study showed a highly significant decrease ($p<0.05$) in serum catalase activity in CRF patients undergoing hemodialysis treatment in the all months of dialysis and age ranges as compared with control subjects. Nevertheless, the fourth months of hemodialysistreatment was no showed any significant differences of serum catalase level in CRF patients undergoing hemodialysistreatment (table 1).

The decreaseof the activity of catalase could be due to less availability of NADPH. The hexosemonophsphate pathway is the principal source of NADPH in RBCs and it has been suggested that chronic acidosis in uremia leads to inhibition of glucose–6–phosphate dehydrogenase activity, the key enzyme of this pathway (Chauhan*et al*., 1982). The second reason which cause decreased the activity of catalase may be due to increase in themalondialdehyde (MDA), which can cross link with amino group of protein to form intermolecular cross links thereby inactivating several membrane bound enzymes (Kiklugawa*et al*., 1984). Increased serum MDA levels in CRF patients in prehemodialysis indicate that indeed there is oxidative stress. Further increase in serum MDA levels in posthemodialysis shows that the oxidative stress has increased in these patients. This could be due to the fact that hemodialysis by the application of a modified circulation and forced passage of blood through a number of filters activates endogenous inflammatory mechanisms and induces chronic release of molecules resulting in an increased production of reactive oxygen species (Dakshinamurty*et al*., 2002).

On the other hand, there is a negative relationship between catalase activity and (MDA), many cells in kidney like vascular cells, glomerular and tubular cells may produce ROS in response to the inflammation process. In addition, many circulating infiltrating cells like platelets, granulocytesand macrophages may produce ROS as a part of inflammatory process (Nath *et al*., 1994).

This finding is in accordance with result of Bhogade*et al*., (2008) who showeddeficiency of antioxidants like catalase, erythrocytic superoxide dismutase, vitamin E or increased generation of free radicals like superoxide anions in chronic renal failure patients undergoing hemodialysis

treatment. Also, Rico *et al*., (2006) who reported activity of enzymatic antioxidant such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), decreased in chronic renal failure patient and HD treatment results ina significant recovery of these enzyme activities but remain lower as compared with control values, but thisfinding is not accordance with study of Reddy*et al*., (2011) who showed an increase in catalase activity ($p<0.05$) as a result of dialysis.

Non Enzymatic Endogenous Antioxidant

Serum Transferrin Concentration (mg/dl)

The results obtained from table (2) indicate a significant decrease($p<0.05$) in serum transferrin concentration in hemodialysis patients inthe all months of dialysis session in two moderate age ranges (25-44) and (45-64) years but this decrease in the 2nd, 3rd and 4th months of dialysis treatment was not showed any significant differences during the last three months in age range(25-44) years in comparisonwith control subjects. But, these present data showed non-significant differences ($p>0.05$) in serum transferrin activityin CRF patients during all months in first and fourth age ranges (5-24) and (65-74) yearsrespectively.

The decrease of serum transferrin may result in low availability of iron for the erythron. It may be caused by three factors: high iron requirements as a result of an enhanced erythropoiesis, an insufficient release of iron from the body iron stores, and insufficient iron absorption. The first factor, a high demand for iron, exists when erythropoiesis is enhanced by recombinant human erythropoietin (rHuEpo) treatment. The second factor, a disturbed release of iron from ferritin and haemosiderin molecules in the mononuclear phagocytic system and from hepatocytes, is known to occur in patients with the anemia of inflammation (Brock,1994). Such a mechanismmay playa role in dialysis patients also, for instance since the dialysis procedure itself is proinflammatory. The third factor, insufficient iron absorption, can be suspected in dialysis patients, since the rate of iron absorption, even when iron is supplemented orally, is apparently not sufficient to saturate transferrin when erythropoiesis is enhanced. However, when iron is administered intravenously, transferrin saturation improves dramatically (Macdougall*et al*.,

1989;Van Wijck, 1989; Sunder-Plassmann and Horl,1995).This finding is in accordance with Kooistra*et al*., (1998) who reported that serum transferrin receptor concentration reflects erythron transferrin uptake; low levels suggest a low rate of erythropoiesis alsolow transferrin saturation reflect a low availability of iron at the level of serum and erythron in hemodialysis patients. Similarly, Yoong*et al*., (2005) who found significant association between serum albumin and transferrin. However, albumin and TIBC revealed suboptimal nutritionalstatus in significant numbers in chronic renal failure patients.

Serum Bilirubin and Uric Acid (mg/dl) Concentration

Statistical data of serum bilirubin anduric acid (mg/dl) as non-enzymatic antioxidant in CRF patients undergoing hemodialysis treatment were listed in table (3). The level of serum bilirubin considerably significant decreased ($p<0.05$) in CRF patientsundergoing HD inall age ranges during the 2nd dialysis month and also in the rest of HD times as compared with control group, but mostly, with no significant differences noted in serum bilirubin inCRF patients undergoing hemodialysisduring in all month of dialysis.

The considerably decrease in serum Bilirubin in CRF patients during dialysis treatment as compared with control subjects may be due tothe capacity of plasma to bind bilirubinin patients with renal failure was less than that of normal plasma. The present results is in accordance with Pasternack and Tenhunen (1976) whoreported that serum bilirubin values in renal failure patients are lower than normal and when renal failure is moderate this decrease is not due to a reduced red-cell mass or to low levels of serum albumin. While,Marjani, (2006) reported that no significant differences in serum bilirubin between control group and patients with (pre-,post-) dialysis process.

On the other hand, the present results listed in table (3) showed highly significant elevation ($p<0.05$) in serum uric acid in CRF patients undergoing hemodialysis in the all dialysis months and age ranges as compared with control group.An increase in serum uric acid in CRF patients may be due to the uric acid is reabsorbed and excreted by the proximal tubular cells. Therefore, hyperuricemia might develop when the production of uric acid increases or the excretion of uric

acid declines, or both(Vaziriet al., 1995). Several studies have shown that an increase in uric acid is associated with hypertension, cerebral vascular disease and chronic kidney disease

Parameter	Catalase U/I	
	Control	Hemodialysis time (month)

(CKD)(Verdecchia*et al.,* 2000; Madsen*et al.,* 2005).It can be concluded thatchronic renal failure patients (CRF) undergoing hemodialysis treatmenthave a physiological disturbance in both enzymatic and non-enzymaticendogenous antioxidantswhich may due to the oxidative stress of chronic renal failure patients.

Table (1): Activity of enzymatic antioxidant (catalase U/I) in CRF patientsundergoing hemodialysis

Age range		1st	2nd	3rd	4th
5-24	47.09±3.58 a	4.63±0.78 b	3.06±0.65 b	2.10±1.05 b	4.61±1.52 b
25-44	50.91±3.19 a	3.00±0.37 b	3.15±0.54 b	3.02±0.54 b	3.73±0.69 b
45-64	45.66±4.14 a	3.06±0.55 b	3.51±0.54 b	2.94±0.45 b	3.14±0.41 b
65-74	57.32±2.97 a	4.35±0.96 b	2.41±0.68 b	2.52±0.43 b	3.56±0.89 b

Data= Mean ± S. E. M.

Means with different subscripts are significantly different from control subjects ($p<0.05$).

Table(2): Activity of enzymatic antioxidant (transferrin mg/dl) in CRF patients undergoing hemodialysis

Parameter	Transferrin (mg/dl)	
	Control	Hemodialysis time (month)

Age Range		1st	2nd	3rd	4th
5-24	0.30±0.03 a	0.20±0.04 a	0.23±0.07 a	0.22±0.09 a	0.19±0.11 a
25-44	0.64±0.03 a	0.31±0.06 b	0.43±0.09 ab	0.47±0.11 ab	0.41±0.09 ab
45-64	0.52±0.05 a	0.27±0.04 b	0.30±0.05 b	0.33±0.07 b	0.30±0.07 b
65-74	0.32±0.04 a	0.37±0.10 a	0.37±0.06 a	0.43±0.08 a	0.69±0.25 a

Data= Mean ± S. E. M.

Means with different subscripts are significantly different from control subjects ($p<0.05$).

Parameter / Age Range	Bilirubin (BIL) mg/dl					Uric acid (UA) mg/dl				
	Control	Hemodialysis time (month)				Control	Hemodialysis time (month)			
		1st	2nd	3rd	4th		1st	2nd	3rd	4th

5-24	0.70±0.12 a	0.37±0.07 ab	0.14±0.02 b	0.27±0.06 ab	0.37±0.05 ab	1.94±0.19 a	8.45±0.58 b	7.62±1.01 bc	7.12±1.08 abd	7.92±0.88 be
25-44	0.84±0.08 a	0.34±0.04 bc	0.20±0.07 b	0.38±0.06 c	0.31±o.04 bc	4.51±0.33 a	7.31±0.51 b	7.42±0.74 b	7.19±0.42 b	7.90±0.38 b
b45-64	0.91±0.08 a	0.34±0.03 bc	0.21±0.04 cd	0.35±0.05 bd	0.42±0.05 be	4.08±0.36 a	7.53±0.57 b	7.37±0.49 b	7.50±0.46 b	8.36±0.46 b
65-74	0.75±0.09 a	0.33±0.05 be	0.16±0.04 c	0.25±0.06 bcd	0.30±0.08 cde	2.82±0.76 a	7.60±1.14 b	8.62±0.66 b	8.27±0.46 b	8.45±0.53 b

Table (3):Activity of non-enzymatic antioxidants(Bilirubin and Uric acid mg/dl)) in CRF patients undergoing hemodialysis

Data= Mean ± S. E. M.
Means with different subscripts are significantly different from control subjects ($p<0.05$).

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