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Determination of Resistin and Several Antioxidants in Sera of Patients with Chronic Renal Failure

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Abstract: Background: Chronic kidney disease is a progressive loss of renal function over a period of months or years through five stages. Free radicals are formed in all living organisms during normal cell metabolism. Patients with chronic renal failure who are regulary dialysed are candidates for free radical damage. The aim of this study was to explore the role of decreased renal function on resistin and several Antioxidants. The current study investigate possible links with resistin, several Antioxidants and an increased risk of coronary heart disease present in patients with chronic kidney disease (CKD). The present study is also to compare the plasma ceruloplasmin levels among patients [chronic renal failure] undergoing haemodialysis (before and after haemodialysis) and in control (age and sex matched). Methods: The glomerular filtration rate (GFR) has been measure in 35 patients with CKD using haemodialysis method. Laboratory investigations including kidney function, serum urea, creatinine, albumin, lipid profile [cholesterol, triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL)], in addition to serum total antioxidant capacity (TAC), lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde (MDA)), uric acid, Ceruloplasmin, vitamin A, vitamin E, vitamin C and resistin had been measured in CKD patients. Blood samples were obtained from the patients just before and after the dialysis process of dialysis and the control group consisted of 24 age and sex matched normal healthy individuals who came to the hospital for health checkup. Results: Plasma urea, creatinine, GFR, Resistin, TAC, MDA, uric acid, Ceruloplasmin, vitamin A, vitamin E, vitamin C showed significant difference between the predialysis and control group ($P<0.001$). GFR, LDL, MDA, vitamin A, vitamin E and Ceruloplasmin were increased in the post dialysis group when compared with predialysis ($P<0.001$). Urea, creatinine, resistin, TAC, uric acid and vitamin C were decreased in post dialysis group when compared with predialysis ($P<0.001$). There was also significant difference between control and pre dialysis group ($P<0.001$). TAC was lower than control group in post dialysis ($P<0.001$). Conclusions: Hemodialysis leads to significant changes in the antioxidant system of the blood of patients with chronic renal failure. Despite an adverse metabolic environment in chronic renal insufficiency, serum resistin increases in pre dialysis and post dialysis in patients when renal function deteriorates. resistin is not only affected by renal function per se, but appears influenced by proteinuria, and more significantly. the change in serum anti oxidants that accompanies decline in renal function.

Key words: Haemodialysis, lipid peroxidation, antioxidants, vitamin A, vitamin E and Ceruloplasmin, Chronic kidney disease and resistin.

INTRODUCTION

Chronic Renal Failure (CRF) is defined as progressive and irreversible loss of renal function (Bullock, 2000). It is a major public health problem, with increasing incidence and prevalence, poor outcomes, and high costs (Stevens, 2004). CRF frequently leads to end stage renal disease (ESRD), which without renal replacement therapy would lead to death (Bullock, 2000; Longmore, 2004).

The CRF may be caused by any condition which destroys the normal structure and function of the kidney (Davidson's, 2002). Wide geographical variations in the incidence of disorders causing CRF exist. For example, the most common cause of glomerulonephrities in sub-Saharan Africa is malaria. In part of the Middle East, including southern Iraq, Schistosomiasis is a common cause of renal failure due to urinary tract obstruction (Kumar and Clank., 2002). Cigarette smoking has also been linked with the development of chronic kidney disease, as has dyslipidemia (Chuahirun, 2003; Ejerblad, *et al*., 2004) and elevated homocystein levels (Sjobery

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B., 2006). Hypertension and proteinuria occur in most patients with chronic kidney disease and are risk factors for faster progression of this disease (Jafar T.H., 2003).

The lifespan of patients with CRF disease is markedly reduced due to premature cardiovascular death in more than 50% of this population. Traditional risk factors cannot explain the high prevalence and incidence of cardiovascular disease in patients with CRF disease. Therefore non-traditional factors are taken into account, such as oxidative stress, endothelial dysfunction or insulin resistance(Sarnak MJ, 2003).

Chronic Renal Failure (CRF) is accompanied by oxidative stress (Himmelfarb, 2003; Galle J., 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 (Stenvinkel, 1999; Locatelli, 2003) malnutrition, presence of endogenous stable oxidants in the uremic plasma (Hellman, and Gitlin J.D., 2002).

Ceruloplasmin is a member of the highly conserved family of blue multi copper oxidase. It is an enzyme (E.C. 1.16.3.1) which is synthesized in the liver as a single polypeptide chain (Panichi, 2004). Ceruloplasmin is also endogenous modulation of the inflammatory response (Erel O.,1998) and probably transports copper to the tissues which have separate membrane receptors for Ceruloplasmin and albumin-bound copper (Percival, 1999). The active holoprotein has its ferroxidase activity conferred through the incorporation of six copper atoms (T L Ortel, 1984). In addition, Ceruloplasmin is an effective antioxidant because of its ability to oxidize highly toxic ferrous iron to the relatively non toxic ferric form and helps to prevent oxidative damage of proteins, lipids, and DNA(Wiggins, 2006).

Resistin, a 12.5-kDa cysteine-rich protein secreted mainly by adipocytes and apparently inhibiting insulin action in vitro, (Steppan, 2001; Holcomb, 2000; Kim KH, 2001) has generated much interest. Resistin, like adiponectin, circulates in serum in at least two distinct dimeric assembly forms that appear to have different levels of bioactivity. (Patel SD, 2004) Resistin has been characterized as insulin resistance-induced adipokines that is primarily involved in modulation of insulin sensitivity and adipocyte differentiation and thus increases the risk of cardiovascular disease (Steppan, 2001).

Few studies (Janke, 2002; Heilbronn, 2004; Pfutzner, 2003) that have so far been performed in non-renal patients have been unable to find a significant relationship between serum glucose levels or markers of insulin resistance and circulating resistin or resistin expression by subcutaneous adipocytes. Similar results were observed in a small study of 30 CKD
patients by Kielstein *et al*., (2004)

This study was designed to determine the effect of haemodialysis on resistin and several serum antioxidant before and after the dialysis process and compared with control group, investigate possible links with resistin, several antioxidants present in patients with chronic kidney disease (CKD)

MATERIAL AND METHODS

The sampling procedure was done in 35 haemodialysis patients with average age (38.53±6.32) years (range 23- 49). None of these patients received antioxidant medicines or foods. Patients were chosen from the patients referred to the Medical City -Kidney Transplant Center, Iraq. Patients were compared with 24 healthy control subjects were included (mean age 39.27±5.53). All patients were subjected to a detailed history taking, thorough clinical examination, and laboratory investigations including kidney function, lipid profile [cholesterol, triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL)], in addition to serum total antioxidant capacity (TAC), lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde (MDA)), uric acid, Ceruloplasmin, vitamin A, vitamin E, vitamin C and resistin had been measured in CKD patients. Blood samples were obtained from the patients just before and after the dialysis process of dialysis and control group, Five ml were collected from each subject by vein puncture, centrifuged at 3000 rpm for 5 min after allowing the blood to clot at room temperature. The serum urea, creatinine, Uric acid, Albumin, lipid profile levels were measured by spectrophotometeric methods supplied by Giesse Diagnostic. Plasma malondialdehyde was determined according to the modified method of Satoh (Satoh K., 1978). Total antioxidant capacity (TAC) in serum samples was carried out according to Rice -Evans and Miller (Ric e -Evans C, 1994). Ascorbic acid levels were estimated by the method of Tietz (Tietz, NW., 1986). vitamin E levels were determined according to a modified of Hashim and Schuttringer (Hashim, 1966). The concentration of vitamin A in serum was determined according to a modified method of Neeld and pearson (NEELD J.B. Jr., 1963). The ceruloplasmin levels in human serum by turbidimetric method. supplied by Fortress Diagnostics Limited. The serum resistin, insulin were measured by Enzyme Linked Immunosorbent Assay (ELISA) (Biovender Laboratory Medicine, Brno, Czech Republic).

The glomerular filtration rate[GFR] can be estimated using prediction equations that take into account the serum creatinine level and some or all of specific variables (age, sex, race, body size) (Levey, 1999; Manjunath *et al*., 2001). The modification of Diet in Renal Disease (MDRD) study equation was used to estimate the GFR and as follow (Johnson C.A., 2004): GFR (ml/min/1.73 m^2) =186´(Serum creatinine)$^{-1.154}$ ´(Age)$^{-0.203}$´(0.742 if female)´(1.210 if black).

All statistical analyses in studies were performed using SPSS version 15.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability P< 0.05 = significant, P> 0.05 = non-significant.Correlation analysis was used to test the linear relationship between parameters.ANOVA test was used to show the differences between variables of differentiated groups.

Results:

The mean and standard deviation of blood urea and S.creatinine after the dialysis process were 58.57±7.32 mg/dl and 1.85±0.65 mg/dl respectively which showed significant reduction in their concentration after the haemodialysis (P<0.001), as shown in Table 1.

The mean GFR was 6.43±3.56ml/min/1.73m^2 before the haemodialysis while The mean GFR(42.78±8.67) was showed significantly increased after the dialysis process but didn't reach to the control group(81.34±8.88). Chronic renal dysfunction may pass through two main phases: an initially polyuria phase and subsequent oliguria or anuria (Zilva, 2002). In polyuric phase, at first glomerular function may be adequate to maintain plasma urea and creatinine concentration within the reference range. As more glomeruli are involved, the rate of urea excretion falls and cannot balance the rate of its production: as a consequence, the plasma urea and, therefore, glomerular filtrate concentrations through the essentially normal glomeruli rise. This causes an osmotic diuresis in functioning nephrones. In other nephrones, the tubule may be damaged out of the proportion to the glomeruli (Zilva, 2002), Both tubular dysfunction in nephrons with functioning glomeruli and the osmotic diuresis through intact nephrons contribute to the polyuria. In oliguria phase, nephron destruction continues, leading to significant decreases in glomerular filtration and urine output falls which cause a steep rise in serum urea (Zilva, 2002; Johnson, 2004). A high significant elevation in concentration of both serum urea and creatinine were observed in the patients group used throughout this study, and this confirmed the presence of renal failure state. Serum creatinine is widely interpreted as a measure only of renal function, however, the serum level reflects not only renal excretion, but also the generation, intake, and metabolism of creatinine (Levey, 1988; Schemesh, 1985). The clinical utility of the serum creatinine concentration centers on its relation to the glomerular filtration rate (Perrone, 1992). Filtration of creatinine is reduced as a result of a diminished GFR in chronic kidney disease. However, as the GFR falls, the creatinine clearance increases because of an increased tubular secretion of creatinine(Carrie, 1989; Kim K.E., 1969). As a result of the changes in creatinine secretion, changes in creatinine clearance and serum creatinine are blunted (Perrone, 1992). In clinical practice, GFR is usually estimated from the creatinine clearance or from serum creatinine concentration. Measurement of creatinine clearance requires the collection of timed urine sample, which is inconvenient for patient as well as frequently inaccurate (Johnson, 2004).

Descriptive data of patients and control groups are shown in table 1. resistin was significantly elevated in patients before and after haemodialysis compared to control group .Resistin is another peptide secreted from adipose tissue that has generated much interest. Resistin circulates in plasma in two distinct dimeric assembly forms that appear to have different levels of bioactivity (Patel SD, 2004). in humans resistin is expressed primarily in inflammatory cells (Patel L, 2003). Although resistin levels are markedly increased in patients with CKD (Patel SD, 2004; Díez JJ, 2005). a small study of patients with different degrees of renal impairment found no association between elevated resistin levels and surrogate markers of insulin resistance(Kielstein, 2003).

Lipid profile among hemodialysis patients in comparison to control group showed significant increase of triglycerides and LDL; and significant decrease of HDL, as shown in table 1.

Table 2 showed mean and standard deviation of serum, MDA, vitamin E, vitamin A, TAC, uric acid, vitamin C and ceruloplasmin showed significant difference between pre dialysis and control group (P<0.001). It was significantly increased in the post dialysis group when compared with pre dialysis and control group (P<0.001). Serum TAC, uric acid and vitamin C were significantly decreased in the post dialysis group when compared with pre dialysis and control group (P<0.001) as shown in Table 2.

There were a different correlations between resistin MDA,TAC and other parameters in CRF patients before dialysis as shown in table 3

Table 1: The mean and standard deviation of B. urea, S creatinine, Albumin, GFR, Resistin and Lipid profile in [Pre dialysis, Post dialysis] patients group and control group.

Characteristic	Pre dialysis	Post dialysis	Control
Urea [mg/dl/]	135.48±6.42 [a]	58.57±7.32 [a,c]	28.49±4.32
Creatinine[mg/dl/]	16.33±2.98 [a]	1.85±0.65 [a,c]	0.99±0.27
Albumin [g/dl/]	3.60±0.57	3.71±0.62	3.89±0.42
GFR[ml/min/1.73m^2]	6.43±3.56 [a]	42.78±8.67 [a,b]	81.34±8.88
Resistin [ng/ml]	22.66±1.20 [a]	14.45±1.17 [a,b]	7.79±0.83
Total Cholesterol [mg/dl/]	179.68±12.30	175.38±10.41	171.88±35
TG[mg/dl/]	178.22±14.56 [a]	183.12±11.46 [a]	105.65±4.45
LDL Cholesterol[mg/dl/]	153.88±23.67 [a]	162.58±28.44 [c]	109.44±6.89
HDL Cholesterol[mg/dl/]	44.33±4.77 [c]	48.65± 3.97 [c]	60.21.44±3.10
LDL/HDL Ratio	3.42±0.45[c]	3.78±0.55 [c]	2.25±0.34

Results were expressed as the mean±SD.
a P <0.001 compared with control group.
b P <0.001 compared with pre dialysis values.
c P <0.01 compared with control group.

Table 2: Comparison of Different Parameters Related to Oxidative Stress and Antioxidant Defenses Systems in Control and Study Groups in [Pre dialysis, Post dialysis]

Characteristic	Pre dialysis	Post dialysis	Control
TAC [µmol/L]	601.12±41 [a]	381.45±32 [a,b]	417.34±18
MDA [µ mol/L]	2.75±0.29 [a]	3.58±0.29 [a,b]	1.36±0.16
Uric acid [mg/dl/]	7.79±1.23 [a,b]	3.32±1.09 [b,c]	4.98±0.75
Vitamin E [mg/dl]	0.96±0.24 [a]	1.15±0.19 [a,b]	1.36±0.15
Vitamin A [mg/dl]	5.54±0.43 [c]	6.19±0.39 [a,b]	4.89±0.35
Vitamin C [mg/dl]	0.91±0.10 [a]	0.18±0.09 [a,b]	1.67±0.44
Ceruloplasmin [mg/dl]	88.51±6.51 [a]	118.98±7.60 [a,b]	42.31±3.39

Results were expressed as the mean±SD.
a P <0.001 compared with control group.
b P <0.001 compared with pre dialysis values.
c P <0.01 c ompared with control group.

Table 3: correlations between resistin MDA, TAC and other parameters in CRF patients before dialysis.

Characteristic	Resistin [ng/ml]		MDA [µ mol/L]		TAC [µmol/L]	
	r	p	r	p	r	p
Age	0.08	NS	0.09	NS	0.04	NS
GFR[ml/min/1.73m^2]	-0.83	0.01	-0.95	0.001	-0.6	0.01
Uric acid [mg/dl/]	0.59	0.01	0.76	0.01	0.78	0.001
Albumin [g/dl/]	0.37	NS	0.44	NS	0.25	NS
Vitamin E [mg/dl]	-0.65	0.01	-0.85	0.001	-0.79	0.01
Vitamin A [mg/dl]	0.45	NS	0.65	0.01	0.69	0.01
Vitamin C [mg/dl]	-0.75	0.01	-.068	0.01	-0.65	0.01
Total Cholesterol [mg/dl/]	0.65	0.01	0.82	0.01	0.30	NS
TG[mg/dl/]	0.70	0.01	0.50	0.01	0.40	NS
LDL Cholesterol[mg/dl/]	0.77	0.01	0.85	0.01	0.65	0.01
HDL Cholesterol[mg/dl/]	-0.71	0.01	-0.70	0.01	-0.66	0.01
Ceruloplasmin [mg/dl]	00.8	0.001	0.75	0.01	0.70	0.01

Oxidative stress is thought to play an important role in atherosclerosis in patients with CRF because it generates lipid peroxidation and oxidized lipoproteins (Tetta C, 1999; Becker, 1997). The studies looking for oxidative stress in patients with CRF continue to suffer from the same methodologic problems. Whether the study is able to show the presence of oxidative stress depends on the chosen markers (Lim PS, 1999; de Cavanagh EM, 1999). There is currently great interest in the assessment of antioxidant status, as antioxidant depletion may contribute to a number of diseases. Several methods reported in recent years give a single measure of the total antioxidant capacity of serum, and it has been suggested that this may represent a useful way of predicting risk of free-radical-induced tissue damage. If the current study had measured only total antioxidant capacity, it would have concluded that chain-breaking antioxidant capacity was increased in dialysis patients. The results highlight one pitfall of measuring total antioxidant capacity: Changes in one of the major contributors (in this case, uric acid) may mask potentially important changes in other antioxidants. If it had been measured only total antioxidant capacity, it would have concluded that chain-breaking antioxidant capacity was increased in dialysis patients. Uric acid is an efficient antioxidant in some settings, particularly against ozone-derived radicals. However, it is not a good scavenger of some biologically important radical species, and increased uric acid concentrations are unlikely to provide an adequate antioxidant defense in the presence

of deficiencies in other antioxidant systems (Tsuzuki, 2000; Wayner DDM, 1985; Whitehead TP, 1992) This may be particularly the case in the presence of Vitamin C deficiency (as found in these patients), when uric acid -derived radicals may cause tissue damage. Therefore the current study believe that it is important to measure the major chain-breaking antioxidants individually in addition to total antioxidant capacity. The concentrations of Vitamin C were very low, and fell further after hemodialysis. In addition, Vitamin C is a small, water-soluble molecule and is therefore likely to be lost during dialysis. Vitamin C is generally considered to be a key aqueous-phase antioxidant (Frei B, 1989) and Vitamin C deficiency may contribute significantly to oxidative stress in these patients.

Studies using malondialdehyde as an marker of lipid peroxidation have consistently found it to be elevated in patients with CRF (Lim PS, 1999; Tsuzuki D, 2000; Boaz M, 1999; Nguyen-Khoa T, 1999). One of the proposed mechanisms for oxidative stress is the dialysis process itself. Antioxidants such as glutathione, vitaminC, vitaminA, vitaminE, superoxide dismutase and glutathione peroxidase experienced either no change or a further decline in their levels or activity after hemodialysis (de Cavanagh EM, 1999; Tsuzuki D, 2000). These findings would support the theory that dialysis creates oxidative stress by removing antioxidants or inhibiting their action. In contrast, markers of lipid peroxidation such as MDA, 7-ketocholesterol and thiobarbituric acid-reactive substances decreased after hemodialysis in some patients (de Cavanagh EM, 1999; Boaz M, 1999) and increased in others (Tsuzuki D, 2000). Several studies have identified the oxidation of low density lipoprotein (LDL) as a key step in the initiation of atherosclerosis. Increasing evidence also suggests that HDL protects LDL from oxidation. In hemodialysis patients this protective effect is blunted (Morena M, 2000).

Ceruloplasmin acts as an antioxidant by removing the free ferrous ion which acts as a major producer of oxidants (superoxide and hydroxyl radicals) (Patel BN, 2002). In addition to this, ceruloplasmin also acts as an antioxidant by catalyzing the destruction of oxygen radicals (Goldstein IM, 1982; Gutteridge JM., 1992; Goldstein IM, 1979) and can bind to and inhibit neutrophil myeloperoxidase oxidant activity (Park YS, 2000). The mean serum ceruloplasmin levels were significantly increased after haemodialysis as compared to the serum ceruloplasmin levels before dialysis in the cases. (table 2). Lughrey *et al*., (1994) studied oxidative stress in haemodialysis and concluded that oxidative stress was more in chronic renal failure cases as compared to controls, but it is further exacerbated by haemodialysis, as is evidenced by increased MDA and low total antioxidant levels. Increased free-radical production leading to oxidative stress may contribute to the development of cardiovascular complications in haemodialysis patients. In addition, the ferroxidase activity of ceruloplasmin forms an important component of antioxidant defense in body fluids. The ferroxidase activity of ceruloplasmin is impaired in renal failure. Inhibition of the ferroxidase activity of ceruloplasmin in dialysis patients may contribute to increased oxidative stress in these patients (Roxborough HE, 2000).

Increased levels of oxidative stress markers are present in the plasma of CKD patients, which indicates that uremia is a prooxidant state (Himmelfarb J, 2002) The presence of numerous defects in the antioxidant defense system, which leads to a decrease in the clearance of reactive oxygen species, can be used as indirect oxidative markers. Increased oxidized to reduced plasma ratio of vitamin C and red blood cell glutathione has been demonstrated in dialysis patients (Nguyen-Khoa, 2000) Moreover, dialysis treatment seems ineffective in the correction of oxidative stress (Pupim LB, 2004). However, several questions related to CRF oxidative stress remain largely unresolved. First, the relative importance of each type of oxidative stress in CRF patients is insufficiently evaluated. The causal relationship between oxidative stress and CVD in CRF patients has not yet been firmly established. The few epidemiological studies available to evaluate the potential association between oxidative markers and CVD are so far inconclusive. Increased oxidative stress, as evaluated by the presence of high serum antibody-titer against oxidized low-density lipoproteins or by low vitamin E levels, was found to be associated with the degree of presence of carotid plaques and intimamedia thickness, respectively, in CKD patients (Stenvinkel, 1999).

Conclusions:

Hemodialysis leads to significant changes in the antioxidant system of the blood of patients with CRF. Abnormality in copper metabolism and their influence on iron handling CRF are intricate .A tentative association exists between increased oxidative stress, which is common features of CRF. This may contribute to endothelial dysfunction and an increased risk of coronary heart disease. Oxidative stress results from an imbalance between anti-oxidant defense mechanisms and excessive generation of oxidants, leading to cell and tissue injury. In CRF patients, there is a deficiency in antioxidant systems (vitamin C deficiency, reduced intracellular vitamin E and may be led to reduce activity of GSH system). At the same time, pro-oxidant activity is increased due to advanced age, diabetes, chronic inflammation and bio incompatibility of dialysis

membranes and solutions. Tissue damage occurs through a number of biochemical mechanisms, all of which have in common the formation of highly reactive intermediate compounds (free radicals) that can oxidize proteins, lipids and nucleic acids.

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