Research Article

Study of Serum Oxidant-Antioxidants Status in Patients With Chronic Renal Failure

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ABSTRACT

Renal failure is a situation in which kidney fails to function adequately.The aim of this study was to investigate changes relevant to oxidative stress in CRF patient, using the lipid peroxidation marker, Malondialdehyde (MDA) and preventative antioxidants ceruloplasmin (Cp), transferrin (Tf) and albumin (Alb), in serum of patients with chronic renal failure.Blood samples were obtained from (100) patients with [chronic renal failure] undergoing haemodialysis (HD) just before and after the dialysis process as well as (35) healthy subjects as a control group. The patients divided into fourgroups according to type of accompanied diseases (Non): without accompanied disease,(DM): with Diabetes Mellitus, (HT): with hypertension,(DM+HT): with Diabetes Mellitus and hypertension.**Results**:The results show a presence of a significant increasein MDA, Ceruloplasminand transferrinlevels in all groups of patients in pre-HD in comparison with control group.But,Albumin showed a significant decreasein all groups of patients pre- and post-HD in comparison with control group. Also, MDA and transferrin showed a significant increase in all patients groups in post-HD in comparison with control group.while, CP showedinsignificant increasein all groups of patients in post-HD as compared to the control, with the exception of HT patients group that showed a significant increaseas compared to the control. MDA was decrease in the post dialysis groups when compared with predialysis, with the exception of HT+DM patients group that showed insignificant decrease in MDA level in the post dialysis groups when compared with predialysis.But, albumin showed insignificant increase in patients groups in the post dialysis groups when compared with predialysis .ceruloplasmin showed insignificant decrease in the post dialysis groups when compared with predialysis,with the exception of HT+DM patients group that showed significant decrease in CP level in the post dialysis groups when compared with predialysis.Transferrin showed significant increase in Non and HT patients groups when compared between them before and after haemodialysis.While, serum transferrin levels demonstrated no significant differences in DM and HT+DM patients group when compared between thembefore and after haemodialysis.The correlation coefficient (r) test is used to describe the association between lipid peroxidation products and different antioxidants.MDA was negatively correlated with some serum antioxidant (Tf and Alb), while correlated with Cp levels positively assuring their function as antioxidant.

Key Words: Chronic Renal Failure, Haemodialysis , lipid peroxidation, antioxidants and Ceruloplasmin.

INTRODUCTION

Chronic Renal Failure (CRF) is defined as progressive and irreversible loss of renal function (1). It is a major public health problem, with increasing incidence and prevalence, poor outcomes, and high costs (2). CRF frequently leads to end stage renal disease (ESRD), which without renal replacement therapy would lead to death (1,3). The CRF may be caused by any condition which destroys the normal structure and function of the kidney (4). 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 (5). Cigarette smoking has also been linked with the development of Chronic Renal Failure, as has dyslipidemia (6,7). Hypertension and proteinuria occur in most patients with Chronic Renal Failure and are risk factors for faster progression of this disease (8).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 incidenceof cardiovascular disease in patients with CRF disease. Therefore non-traditional factors are taken into account, such as oxidative stress, endothelial dysfunction or insulin resistance (9).Chronic Renal Failure (CRF) is accompanied by oxidative stress (10,11), which involves in the damage of biological structures by reactive oxygen species due to their excessive

generationand impaired efficiency of antioxidant defense mechanisms. In renal failure patients enhanced reactive oxygen species production is underlain mainly by inflammation (12,13) malnutrition,presence of endogenous stable oxidants in the uremic plasma (14).

Lipid peroxidation (LPO) is one of the major interrelated derangements of cell metabolism which is produced by oxidative stress. Therefore, it is a well-established mechanism of cellular injury in human and is used as an indicator of oxidative stress in cell and tissues(15). Malondialdehyde (MDA), a stable secondary product of lipidperoxidation and can be measured after oxidant stress, is used asan in vivo marker to assess LPO in diseases such as renal failure(16). Oxidative stress is results from an imbalance between oxidative reaction and antioxidant defenses(17).the antioxidant are classified into three types:(a) Antioxidant Enzymes catalyzing the breakdown of FR ; (b)Chain Breaking Antioxidants (Scavengers), those which are FR scavengers ; and (c) Preventative Antioxidants (sequestration of metal ions) these which prevent the participation of transition metal ions in FR generation such as transferrin (Tf), albumin (Alb) and ceruloplasmin–Cu containing has ferroxidaseactivity"prevents Fe2+ from reacting with H2O2"(18).

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 (19). Ceruloplasmin is also endogenous modulation of the inflammatory response(20) and probably transports copperto the tissues which have separate membrane receptors for Ceruloplasmin and albumin-bound copper (21).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 (22).

The aim of this study is to investigate the oxidative stress by measuring the lipid peroxidation marker (MDA)in patients with chronic kidney failure, to evaluate serum antioxidant status in the mentioned disease by measurement (ceruloplasmin (Cp), transferrin (Tf) and albumin (Alb)) before and after the dialysis process and compared with control group, To shed a light on the possible correlation relationships between (MDA and each of Cp, Tf, and Alb),and to evaluate the effect of haemodialysis on oxidant-antioxidant status in the mentioned disease.

MATERIAL AND METHOD

Selection of Subjects

This study was conducted at AL-Hussein teaching hospitalin Thi-Qar and AL-muthanna governorate, in the Artificial Kidney Unit(AKU) for dialysis.It included (135) subjects , control(35) and patients with CRF (100) that were divided into four groups:

-Non group:- include 23 patient withoutaccompaniment disease (Non) [15 male 8 and female] with age range (30-45).

-DM group:- include 22 patient with (Diabetes Mellitus) [15 male and 7 female] with age range (40-65).

-HT group:- include 25 patient with (Hypertension) [16 male and 9 female] with age range (43-65).

-DM+HT group:- include 30 patient with (Diabetes Mellitus and Hypertension) [19 male 11 and female] with age range (40-60).

-CTR group:- control group , consist of 35 Healthy subject [27 male and 8 female] with no history of systematic illness with age range (42-65).

Sample with drawl

From the patients withchronic renal failure,(5mL) Blood sample wastaken from the patients just before and after the dialysis process of dialysis and control group, All patients had been on regular haemodialysis and the dialysis sessions were performed three times a week and each session lasted 4 hours.blood samples 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 was separated and stored at ($-20c^0$) till the time of the biochemical analysis.

Methods

Lipid peroxidation Marker (Serum MDA)

Lipid peroxidation is determined using the thiobarbituric acid method. In this method, MDA level of the serum was measured by the following procedure according to a modified method of Fong *et al*.,(1973)(23).It concentrations were calculated usingthe extinction coefficient of MDA (εMDA) equal to 1.56 x10 mol^{-1}. cm^{-1}(24).

Serum Antioxidants

Serum Cp concentration was measured by the method of Menden *et al*.,(1977)(25) which using the extinction coefficient of Cp (ε Cp) equal to (0.68) to calculate it concentration. The bromocresol green (BCG) method,colorimetric method, is the simplest technique which have been developed to determine Alb concentration(26,27). Also, the Tf concentration was measured by colorimetric method(28), in which an excess of iron is added to the serum to saturate the Tf. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation the iron in the supernatant is determined(29).The concentration of iron remaining is assayed and the result expressed as total iron binding capacity (TIBC). The serum Tfconcentration was calculated from the following equation(28):

(SerumTf (gm/L) = 0.094 × TIBC(µg/dL)–32.8).

Statistical Analysis

Statistical analysis was done using the software SPSS version 15.0, the results were expressed as mean ± standard deviations (mean ± SD). One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant. Person correlation coefficient (r) was used to test the correlation relationship among the different parameters in each patients group(30).

RESULTS AND DISCUSSION

Renal failure refers to a condition where the kidneys lose their normal functionality, which may be due to various factors including infections, auto immune diseases, diabetes and other endocrine disorders, cancer, and toxic chemicals. It is characterized by the reduction in the excretory and regulatory functions of the kidney(31,32). Free radicals and ROS have been well recognized as important intermediates in biological reaction, also as a primary cause of cell injury and cell death in variety of pathophysilogic process. Generally, the increased level of peroxidation products has beenassociated with a variety chronic diseases in both human and animal systems (33).

The statistical data were reported in table (1) , indicated to the present of significant increase in MDA concentrations ($p \leq 0.05$) in all groups of patients pre- HD in comparison with control group CTR. The increase in MDA found in this study is in agreement with the results of other studies (34,35). But, our study demonstrated that the serum MDA concentration showed no significant differences in all groups of patients when compared between them pre-HD. Also, there is no significant differences in serum MDA concentration in HT+DM patients group in comparison with HT patients group post-HD. similarity, serum MDA showed no significant differences in Non patients group when compared with DM patients group. On the other hand, lipid peroxidation was detected in the sera of the patients with CRF and demonstrated that there is a significant increase ($p \leq 0.05$) in serum MDA concentration in HT patients group in comparison with Non patients group post-HD. But, serum MDA demonstrated a significant decrease ($p \leq 0.05$) in renal failure patients post-HD in comparison with pre-HD(36,37), with exception of HT+DM patients group showed no significant decrease in serum MDA concentration in post-HD as compared with pre-HD. MDA is an end product of lipid peroxidation and as an index of oxidative damage, in the current study was significantly increased in renal failure patients before HD than in control. These findings are in agreement of (38,39) They reported that increased MDA level might be a consequence of uremia per se which could prime phagocyte oxidative burst. HD, far from improving the uremic status, results in an enhancement of ROS owing to bioincompatibility of the dialyzer membrane. Since a dialysis membrane is an artificial biomaterial, the leukocytes and complements are activated to produce a variety of ROS, including superoxide, hydrogen peroxide and hypochlorous acid. Furthermore, interleukins and anaphylatoxins produced during HD sessions are potent activators for nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme that is responsible for overproduction of ROS (40). Also, HD could mediate the platelets activation which interact with neutrophils through P-selectin and increasing their production of ROS (41).our study found decreased intradialytic MDA, which is the product of lipid peroxidation, MDA being a small water soluble molecule can diffuse across dialysis membranes. Taking into consideration the clearance of MDA during dialysis (42).

Table 1: Serum malondialdehyde concentration of Non, DM, HT and HT+DM in(pre-HD and post-HD) patient groups and control group

Groups	Number	MDA (nmol/ml) Mean±SD	
		Pre-dialysis	*Post-dialysis*
Non	23	194.70 ± 42.79^{a}*	143.40 ± 41.06^{b}**
DM	22	183.30 ± 53.20^{a}*	135.10 ± 52.38^{b}**
HT	25	195.90 ± 58.52^{a}*	164.30 ± 51.53^{a}**
HT+DM	30	198.40 ± 56.74^{a}*	173.50 ± 49.04^{a}*
Control	35	18.01 ± 3.45^{b}*	18.01 ± 3.45^{c}*

Each value represents mean ± SD values with non-identical superscript (a , b or c … etc.) were considered significantly differences ($p \leq 0.05$). (*) were considered significantly different with (**)($p \leq 0.05$) between pre- and post- haemodialysis groups.

Antioxidants

Albumin

The statistical data were reported in table (2) , indicated to the present of significantdecrease ($p \leq 0.05$) in Serum albuminin all groups of patients pre- HD as compared with control group CTR. This study is in agreement with the other studies done by(43,44).also , there is insignificant decrease ($p \leq 0.05$) in serum albumin concentration in all groups of patients in post-HD in comparison with control group CTR. This study is match with the other studies done by(44,45).However, our study demonstrated that the serum albumin concentration shows no significant differences in all groups of patients when compared between them pre-HD and post-HD.on the other hand , serum albumin concentration showed no significant differences between patients of each group pre-HD when compared to the post-HD, with the exception of HT+DM patients group showed a significant increase ($p \leq 0.05$) in serum albumin concentration post-HD when compared to the pre-HD.

The albumin is a negative acute phase protein. There is an evidence for a significant antioxidant activity of the serum albumin. In fact, this molecule may represent the major and predominant circulating antioxidant in plasma known to be exposed to continuous oxidative stress(46,47) .The depression, which happened in serum albumin levels in case of CRF, may be according to some causes: (1) increasing of the albumin excretion by kidney(48),(2) diffusing of the albumin into damaged tissues by means of increased permeability of blood vessels (49),(3) inflammation is considered the principle cause of a decrease in the serum albumin, however, IL-6 directly decreases the expression of albumin messenger RNA (50), and finally (4) its antioxidant function. According to the points (3) and (4): (stopping for the albumin productionand consumption of the albumin to scavenge free radicals with continuously). Also, hemodilution contribute to the hypoalbuminemia of patients on dialysis (51) or may result from protein restriction ,anorexia , protein-energy malnutrition (52,53).The explained increase of albumin concentration post dialysis belong to haemoconcentration.

Table 2: Serum albumin concentration of Non, DM, HT and HT+DM in(pre-HD and post-HD) patient groups and control group

Groups	Number	Albumin (g/L) Mean±SD	
		Pre-dialysis	*Post-dialysis*
Non	23	$28.69\pm3.67^{b*}$	$29.54\pm3.59^{b*}$
DM	22	$30.35\pm2.94^{b*}$	$31.60\pm1.96^{b*}$
HT	25	$30.26\pm2.48^{b*}$	$31.26\pm3.13^{b*}$
HT+DM	30	$28.50\pm2.70^{b**}$	$30.15\pm2.38^{b*}$
Control	35	$45.02\pm5.67^{a*}$	$45.02\pm5.67^{a*}$

-Legend as intable(1).

Ceruloplasmin

The statistical data were reported in table (3) , indicated to the present of significant increase in Plasma ceruloplasmin levels in cases before haemodialysis($p \leq 0.05$) in all groups of patientsas compared to the controls. Also, serum CP concentration showed insignificant increase($p \leq 0.05$) in groups of patients post-HD in comparison with control group. this is agreement with the other studies done by(54,55), with the exception of HT patients groupsshowed a significant increase in CP levels post-HD in comparison with control group. However, before haemodialysis serum CP concentration demonstrated a significant increase in HT patients group when comparison with HT+DM patients group.while, there is no significant differences in serum CP concentration in HT+DM patients group in comparison with DM patients group . similarity, serum CP level showed no significant differences in DM patients group when compared with Non patients group pre-HD.Plasma ceruloplasmin levels in cases after haemodialysis was significantly increase ($p \leq 0.05$) in HT patients group as compared to the DM patients group. But, serum CP concentration showed no significant differences in DM patients group as compared to the HT+DM patients group . Likewise, there is no significant differences in concentration of CP in HT+DM as compared to the Non patient group.

However, there was no statistically significant change in the plasma ceruloplasmin levels in all groups of patients when compared between them before and after haemodialysis this agreement with the study done by(55).Ceruloplasmin is an α_2-globulin containing copper, an important extracellular antioxidant (56).*Cp* synthesis and /or secretion is altered by inflammation, hormones, and copper. So the physiological factors like cancer, exercise, chronic inflammation, pregnancy increase its level (57) .Here, in this study, a clear and significant rising for *Cp* conc. was gotten in chronic renal failure patients. And the cause behind this result, that ceruloplasmin is an acute phase protein and is synthesized by the liver in response to tissue damage and inflammation (58).

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Table 3: Serum ceruloplasmin concentration of Non, DM, HT and HT+DM in(pre-HD and post-HD) patient groups and control group

Groups	Number	Ceruloplasmin (g/L) Mean±SD	
		Pre-dialysis	*Post-dialysis*
Non	23	$3.80\pm1.18^{b*}$	$3.44\pm1.15^{b*}$
DM	22	$4.38\pm1.32^{b*}$	$3.59\pm1.44^{b*}$
HT	25	$5.44\pm1.71^{a*}$	$4.56\pm1.59^{a*}$
HT+DM	30	$4.83\pm1.43^{b*}$	$3.46\pm1.80^{b**}$
Control	35	$3.16\pm1.12^{c*}$	$3.16\pm1.12^{b*}$

-Legend as in table (1).

Transferrin

The statistical data were reported in table **(4)** , indicated to the present of significant increase in Plasma transferrin concentration in cases before haemodialysis($p \leq 0.05$) in all groups of patients as compared to the controls CTR this in agreement with the study done by**(59,60)**.Also, serum transferrin concentration showed a significant increase ($p \leq 0.05$) in all groups of patients post-HD in comparison with control group CTR. However, before haemodialysis serum transferrin concentration demonstrated a significant increase($p \leq 0.05$) in HT+ DM patients group as compared with the DM patients group**.**Also , serum transferrin concentration demonstrated a significant increase ($p \leq 0.05$) in DM patients group as compared with the HT patients group.while, there is no significant differences in serum transferrin concentration in HT patients group as compared with the Non patients group pre-HD. on the other hand, Plasma transferrin levels in cases after haemodialysis was no significant differences in DM patients group as compared to the HT+ DM patients group.Also, serum transferrin concentration showed no significant differences in HT+ DM patients group as compared to the HT patients group .similarity, there is no significant differences in concentration of transfferin in HT as compared to the Non patients group. On the other hand, our study demonstrated that there was a statistically significant increase ($p \leq 0.05$) in the plasma transfferrin levels in Non and HT patients groups when compared between them before and after haemodialysis. While, serum transferrin levels demonstrated insignificant differences in DM and HT+DM patients group when compared between them post haemodialysis.

Table 4: Serum transferrin concentration of Non, DM, HT and HT+DM in(pre-HD and post-HD) patient groups and control group

Groups	Number	Transferrin (g/L) Mean±SD	
		Pre-dialysis	*Post-dialysis*
Non	23	$3.60\pm1.17^{b**}$	$4.50\pm1.36^{a*}$
DM	22	$4.22\pm1.20^{a*}$	$5.02\pm1.76^{a*}$
HT	25	$3.65\pm1.15^{b**}$	$4.52\pm1.26^{a*}$
HT+DM	30	$4.22\pm1.34^{a*}$	$4.70\pm1.70^{a*}$
Control	35	$2.69\pm0.75^{c*}$	$2.69\pm0.75^{b*}$

-Legend as in table (1).

transferrin family (Trf) constitutes the major iron transport and /or scavenging system in vertebrates and some invertebrates (61). However, Trf can also function as an iron chelator, which contributes to

host defense by limiting iron availability for microbial pathogens. This capacity to bind iron also enables transferrin to protect cells from oxidative damage.Here, in this study, a clear and significant rising for Tfconcentrationwas gotten in CRF patients. the anemia of CRF is normochromic and normocytic it often closely resembles iron-deficiency anemia(62). And the cause behind this result, decrease erythropoietin production by the kidney in CRF patients ,decrease iron level or normal (iron deficiency anemia) therefore the transferrin may be higher or normal.TIBC should be low or normal in anemia of chronic disease (63). The rise in plasma iron after HD might be due to blood transfusion to correct anemia and bleeding tendency in these patients therefore serum transferrin after dialysis may be higher than before dialysis in some groups. an increase in iron stores due to supplementation could also contribute to increased free radical production in HD patients (64).

Correlation

Table (5) shows the negative correlation relationshipbetween MDA and albumin concentration in patients groups with correlation coefficient(r=-0.558) pre-HD and (r=-.011) post-HD in (Non) group, (r=-0.169) pre-HD and (r=-0.159) post-HD in (DM) group, (r=-0.092) pre-HD and (r=-0.296) post-HD in (HT) group, (r=-0.213) pre-HD and (r=-0.546) post-HD in (HT+DM) group. This is in accordance with studies of (44,65). as well as, there is negative correlation relationship betweenMDA and transferrin concentration in patients groups with correlation coefficient (r=-0.370) pre-HD and (r=-0.019) post-HD in (Non) group, (r=-0.117) pre-HD and (r=0-.607) post-HD in (DM) group, (r=-0.025) pre-HD and (r=-0.256) post-HD in (HT) group,(r=-0.194) pre-HD and (r=-0.163) post-HD in (HT+DM) group. But, there is positive correlation relationship between MDA and ceruloplasmin concentration in patients groups with correlation coefficient(r= 0.290) pre-HD and (r=0.190) post-HD in (Non) group,(r=0.240) pre-HD and (r=0.337) post-HD in (DM) group, (r=0.135) pre-HD and (r=0.122) post-HD in (HT) group, (r=0.089) pre-HD and (r=0.373) post-HD in (HT+DM) group. A negative correlation relationship was observed between MDA and albumin table(9).This supports the hypothesis ofSengupta,*et al*., (2001) (66) who suggested that decrease in the levels of this antioxidant accelerate the lipid peroxidation thereby generating more MDA. Proteins may be damaged by specific interactions of oxidants or free radicals with particularly susceptible amino acids. anegative correlation between MDA and transferrin explained that free iron not find transferrin to bind it for transport him therefore the free iron cause oxidation and generation of more free radicals.

Table 5: Correlation between MDA and Antioxidant parameters (Albumin, Transferrin and Ceruloplasmin)

MDA Groups		Albumin	Transferrin	Ceruloplasmin
Non	Pre	-0.56	-0.37	0.29
	Post	-0.01	-0.02	0.19
DM	Pre	-0.17	-0.12	0.24
	Post	-0.16	-0.61	0.34
HT	Pre	-0.09	-0.03	0.14
	Post	-0.29	-0.26	0.12
DM+HT	Pre	-0.21	-0.19	0.09
	Post	-0.55	-0.16	0.37

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