Oxidative stress and serum paraoxonase activity in patients on maintenance hemodialysis

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Citation

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Abstract

Incidence of atherosclerosis is high in hemodialysis (HD) patients. Paraoxonase may have protective effects against atherosclerosis as it prevents oxidative modification of LDL. Hence, the aim of the present study was to assess the lipid peroxidation and the antioxidant status including paraoxonase activity in HD patients. Thirty HD patients and thirty controls were included in the study. Serum malondialdehyde (MDA) was assessed as a marker of oxidative stress, while antioxidant status was assessed in terms of whole blood reduced glutathione, erythrocyte catalase and serum paraoxonase activity. A statistically significant ($p < 0.001$) rise of 196 % in serum MDA levels and decline of 36% in erythrocyte catalase activity was noted in HD patients as compared to controls. However, reduced glutathione (GSH) level in whole blood was not altered. Both basal and salt stimulated serum paraoxonase activities were reduced significantly ($p < 0.001$) as compared to controls with percentage decline of 53% and 37% in basal and salt stimulated paraoxonase activities respectively. These findings indicate that in addition to increased oxidative stress, reduced serum paraoxonase activity could also contribute to development of atherosclerosis in HD through increased oxidative modification of LDL.

INTRODUCTION

Chronic renal failure (CRF) patients have high prevalence of atherosclerotic cardiovascular complications. This is true for patients who are not yet on hemodialysis (HD) and remains so after the initiation of maintenance hemodialysis (1). A tendency to atherosclerosis in these patients may be due to the enhanced oxidative stress resulting from an imbalance between free radicals formation and antioxidant defense mechanism.

It has been suggested that oxidative stress is one of the most important “non-conventional” risk factors for atherosclerotic cardiovascular disease (2). According to “oxidative theory” of atherosclerosis, oxidative modification of lipoproteins is considered a key pathological step, with low density lipoprotein (LDL) oxidation being an early event in the development of atherosclerosis. Oxidized LDL (ox-LDL), which is formed as a result of oxidative stress, is taken up by the scavenger receptors of macrophages leading to foam-cell formation. In addition to its pivotal role in foam-cell formation, oxidized-LDL possesses additional atherogenic properties, which include cytotoxicity and the stimulation of thrombotic and inflammatory events (3).

High density lipoprotein has a protective effect against atherosclerosis. This effect is due not only to the reverse cholesterol transport activity but is also partly enzymatic. Paraoxonase, a serum esterase – synthesized and secreted by the liver and associated with specific high density lipoprotein (HDL) particle, might be involved in the mechanism (4). Paraoxonase possesses peroxidase like activity that can contribute to protective effect of paraoxonase against LDL oxidation (5).

In view of these assumptions, the present study aimed to investigate, in HD patients, the level of oxidative stress and the enzymatic antioxidant status including paraoxonase, an enzyme that prevents oxidative modification of LDL.

MATERIALS AND METHODS

Thirty CRF patients (16 males and 14 females) on maintenance HD and thirty age matched healthy controls (18 males and 12 females) were included in the study after informed consent had been obtained. Patients with diabetes, inflammatory or malignant diseases were excluded. Primary renal diseases included chronic glomerulonephritis (n = 20), nephroangiosclerosis (n = 4) and chronic interstitial nephritis (n = 6).The mean ages of patient and control groups were 40.56 ± 14.38 years and 43.33 ± 10.8 years respectively. All (CRF) patients included in the study were receiving regular

bicarbonate hemodialysis therapy (2-3 hours three times weekly) using high-flux polysulphone hollow fiber dialysers for 2 to 3 years. None of the patients was receiving any antioxidant therapy.

COLLECTION AND PREPARATION OF BLOOD SAMPLES

Twelve hours fasting venous blood samples were drawn from patients and control subjects. Blood was collected in EDTA containers and in plain bulbs. Blood collected in EDTA was used immediately for the assay of reduced glutathione and also for the preparation of erythrocyte hemolysate. Serum was separated by centrifuging the blood collected in the plain bulb at 600 g for 10 minutes and was used immediately for the estimation of urea nitrogen and creatinine by kit methods. The remaining serum sample was used immediately for estimations of paraoxonase and malondialdehyde (MDA).

PREPARATION OF ERYTHROCYTE HEMOLYSATE

Erythrocytes were isolated from blood collected in EDTA by centrifugation. The erythrocytes were washed thrice with ice-cold normal saline and then lysed by adding ice-cold distilled water. The hemolysate was used for the assay of catalase.

LABORATORY EVALUATION

ASSESSMENT OF RENAL STATUS

Renal status was assessed by estimating urea nitrogen and creatinine levels in serum sample by kit methods.

Measurements of MDA, reduced glutathione, catalase and paraoxonase.

Serum malondialdehyde (MDA) was estimated by thiobarbituric acid method (6). Reduced glutathione in whole blood was estimated by 5, 5'-dithio bis- (2-nitrobenzoic acid) (DTNB) method (7). Catalase activity in erythrocyte hemolysate was estimated by monitoring the decrease in absorbance at 230 nm, resulting from decomposition of hydrogen peroxide for 10 minutes at 37ºC (8). Serum basal and salt stimulated paraoxonase activities were measured using p – nitrophenyl acetate as a substrate (9).

STATISTICAL ANALYSIS

The results are expressed as Mean ± SD. The results of hemodialysis patients were compared with those of control subjects by performing unpaired student's 't' test. The statistical significance was determined from p value.

Pearson's correlation coefficient was used to determine the correlations among different parameters. A p value < 0.05 was considered as statistically significant.

RESULTS

HD patients showed significant elevations of serum urea nitrogen ($p < 0.001$) and serum creatinine ($p < 0.001$) levels (Table 1).

Figure 1

Table 1: Mean Levels of Serum Urea Nitrogen and Creatinine in CRF patients and in Control Subjects

Group	Urea Nitrogen (mg/dl)	Creatinine (mg/dl)
Controls (n=30)	14.98 ± 7.71	0.48 ± 0.04
HD Patients (n=30)	93.05 ± 44.54 #	12.42 ± 3.82 #

Values are expressed as mean ± SD

#: P < 0.001 as compared to controls.

Figure 2

Table 2 : Mean Levels of Serum MDA, Serum Paraoxonase, Erythrocyte Catalase and Whole Blood Reduced Glutathione in CRF Patients and in Control Subjects

Group	MDA (µmoles/l)	Catalase (U/mg Hb)	GSH (mg/dl)	Paraoxonase (nmoles/min/ml)	
				Basal	Salt Stimulated
Controls (n=30)	3.47 ± 1.77	0.035 ± 0.002	52.818 ± 6.13	53.44 ± 6.13	33.72 ± 7.33
HD Patients (n=30)	10.26 ± 2.98 ##	0.022 ± 0.001 #	52.72 ± 9.14	31.03 ± 13.5##	14.50 ± 6.95##

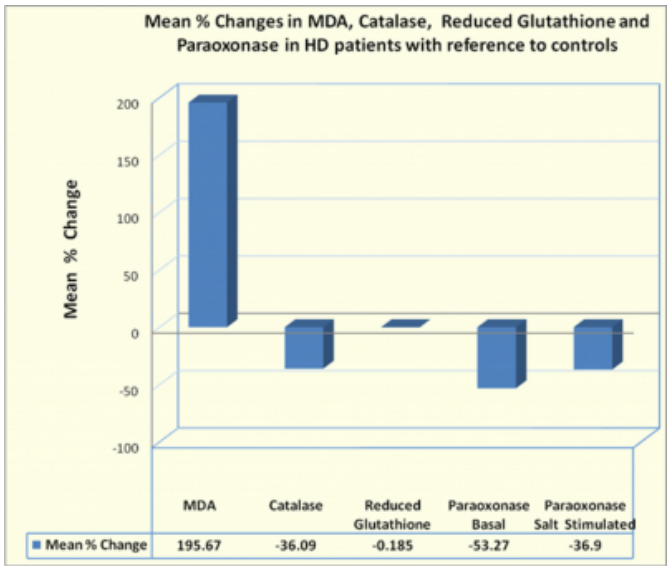
Values are expressed as mean ± SD.

: P < 0.02 as compared to controls.

: P < 0.001 as compared to controls.

Figure 3

Figure 1



The serum levels of MDA, a lipid peroxidation product, were found to be elevated significantly ($p < 0.001$) as compared to the controls (Table 2). The mean percentage rise in serum MDA levels was 196 % (Figure 1). Erythrocyte catalase activity was reduced highly significantly ($p < 0.02$) as compared to controls with percentage decline of 36%. However, reduced glutathione (GSH) level in whole blood was not altered. Both basal and salt stimulated serum paraoxonase activities were reduced highly significantly ($p < 0.001$) in HD patients as compared to controls with percentage decline of 53% and 37% in basal and salt stimulated paraoxonase activity respectively.

DISCUSSION

Risk of atherosclerotic cardiovascular disease is high in CRF patients on long term hemodialysis as compared to normal population. Several mechanisms such as hypertension, dyslipidemia and hypoalbuminemia associated with CRF are the contributory factors to the increased risk. Although atherosclerosis is a multifactorial process, oxidatively modified LDL resulting from increased oxidative stress may play an important role in its initiation and progression by activation of foam cells, induction of inflammatory mediators etc.

Hemodialysis is considered to aggravate the uremia related pro-oxidant/antioxidant imbalance. This may result from increased of Reactive Oxygen Species (ROS), generated by circulating neutrophils when they enter into contact with dialysis membranes. The dialysis membrane is subjected to immunologic response by low molecular weight plasma constituents such as IgG and complement components to make the membrane biologically active for granulocytes. Activation of blood granulocytes can increase ROS (10). As malondialdehyde (MDA) is an indicator of increased oxidative stress, the rise in serum MDA levels noted in the present study as well as reported by other studies (11 - 13) indicates increased oxidative stress in these patients.

In addition to increased production of ROS, decreased antioxidant defense mechanism could also contribute to increased oxidative stress in HD patients. Though no change was noted in whole blood reduced glutathione (GSH) levels, low erythrocyte catalase activity was demonstrated in our patients, which was consistent with the findings of Durak et al (14) and Zwolinska D (15). The data regarding GSH is contradictory. Mohamed-Siael S. Alhamdani (16) and Paik-Seong Lim et al (17) reported significant decline in GSH levels in CRF patients, while Lucchi L. (18) reported significantly elevated erythrocyte GSH content in end stage CRF and HD patients as compared to controls. Various studies (19 - 21) have reported rise in erythrocyte GSH levels during hemodialysis with no differences between post dialysis levels and control levels. As our patients were on regular hemodialysis, unaltered levels of whole blood reduced glutathione may be explained on the basis of hemodialysis treatment. These findings suggest that measurement of whole blood GSH level may not be a good indicator of antioxidant status in these patients.

Oxidative stress is responsible for oxidative modification of LDL. This modified LDL is known to accelerate the process of atherosclerosis more than the native LDL. In CRF patients, the susceptibility of LDL to oxidative modification is very high (22) suggesting that the protection of lipoproteins against oxidation might be impaired. In order to find an explanation for this, we assessed serum paraoxonase activity in our HD patients as this HDL – linked enzyme has been shown to decrease LDL peroxidation in vitro and more recently evidence has emerged suggesting protective role of paraoxonase against atherosclerosis.

In the present study, reductions observed in both basal and salt stimulated serum paraoxonase activities are in agreement with the study of Dantoine et al (23) who reported reduced paraoxonase activity in CRF patients who were on conservative treatment and also those who were receiving regular hemodialysis (HD) treatment as compared to controls; however, the reduction was more pronounced in HD group. Biasioli S. et al (24), Gulcin et al (25) and M. Dirican (26) et al also reported lower activity of serum

paraoxonase in uremic predialysis and hemodialysis patients. Further, in the later study basal paraoxonase activity was significantly lower in hemodialysis patients compared to controls, whereas salt stimulated paraoxonase activity was not significantly different. In our HD patients also the decline in basal paraoxonase activity was more profound than salt stimulated paraoxonase activity. High concentrations of HOCl that severely oxidize serum proteins and tryptophan residues in the active site of paraoxonase decrease paraoxonase arylesterase activity in serum. In haemodialysis patients, overproduction of HOCl that leads to high concentrations of severely oxidized proteins and increased oxidants in plasma might also contribute to low serum paraoxonase arylesterase activity (27).

Statistically significant inverse ($r = - 0.33$, $p < 0.01$) correlation was also noted between serum paraoxonase and serum MDA levels. This suggests a possible involvement paraoxonase in antioxidant mechanism. The decreased serum paraoxonase activity found in this these patients may be responsible for increased oxidative modification of LDL that is known to accelerate the process of atherogenesis in HD patients.

In conclusion, in addition to increased oxidative stress, reduced serum paraoxonase activity could also contribute to development of atherosclerosis in HD through increased oxidative modification of LDL, a major risk factor for atherosclerosis.

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