Effect of hemodialysis on total antioxidant status of chronic renal failure patients in government hospitals in Lagos Nigeria

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Abstract

Background: Renal failure is accompanied by oxidative stress, which is caused by enhanced production of reactive oxygen species and impaired antioxidant defense.
Aim: To assess the effect of hemodialysis (by cellulose membrane dialyzer) on plasma total antioxidant status and lipid peroxidation of patients in chronic renal failure before and after dialysis.
Objective: The finding would serve as guide to administration or otherwise of supplementary therapeutic antioxidant before or after hemodialysis. Also, it will assist in the choice of antioxidant impregnated over the conventional non-impregnated dialyzer membrane.
Materials and Methods: Twenty-five patients (14 men and 11 women, aged 24-75 years; median 61) with end-stage renal failure who were undergoing hemodialysis for the first time were recruited. Plasma level of potassium (K⁺), sodium (Na⁺), blood urea nitrogen (BUN), creatinine, total antioxidant status (TAS), and lipid peroxidation (MDA) were measured, before and after hemodialysis.
Results: The mean ± SD of plasma level of TAS (1.10 ± 0.3 mmol/L trolox Eq) for males and (1.09 ± 0.2 mmol/L trolox Eq) for females postdialysis were significantly reduced (P < 0.05) in comparison with (1.72 ± 0.4 mmol/L trolox Eq) for males and (1.83 ± 0.7 mmol/L trolox Eq) for females predialysis, respectively. However, the mean ± SD plasma level of MDA (6.03 ± 0.4 nmol/ml) for males and (6.71 ± 0.7 nmol/ml) for females were significantly increased postdialysis (P < 0.01) compared to predialysis (3.98 ± 0.8 nmol/ml) for males and (4.05 ± 0.9 nmol/ml) for females, respectively.
Conclusions: Based on the outcome of this study, it is suggested that antioxidant-impregnated dialysis membranes and/or exogenous supplementary antioxidant would be beneficial to patients with chronic renal failure. Removal of reactive oxygen species could improve the health and general quality of life of uremic patients.

Key words: Chronic renal failure, hemodialysis, total antioxidant status, lipid peroxidation

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Introduction

Numerous reasons have been advanced for the increased oxidative stress in patients with chronic renal failure and hemodialysis. These include retention of phenol indoxyl carboxyl, methylguanidine metals, and oxidized lipids, which are oxidants or pro-oxidants and should be excreted in urine or eliminated.[¹,²] Generalized deteriorations in renal metabolic activities, for instance, with reduction in glutathione disulfide to glutathione (GSH) and cystine to cysteine and decreased arginine synthesis with resultant decreased bioavailability of nitric oxide and reactive oxygen as a scavenger.[¹,²]

Endogenous production of free radicals during cellular metabolism may be greatly enhanced by exogenous
Increased concentrations of malondialdehyde (MDA)—intermediate product of oxidation of polyunsaturated fatty acids—have been reported in the plasma and erythrocytes, as well as in platelets and mononuclear cells of hemodialyzed patients. Chronic renal failure is a pro-oxidant state, characterized by increased levels of free radical oxidants relative to antioxidants. However, so far, it is not clear whether increased MDA is caused by the dialysis procedure or by the kidney disease itself. Some studies in hemodialyzed patients have investigated markers of oxidative stress such as MDA, or antioxidant defense systems such as SOD, GPx activity in erythrocytes, or free radical scavengers, eg, ascorbic acid, β-carotene, and tocopherol. There are conflicting reports on a deficit in serum antioxidant vitamins and a decreased activity of erythrocyte antioxidant enzymes. 

Experimental and clinical studies have shown that in chronic renal failure, oxidative stress is a potentially important source of patient morbidity and mortality. It has also been implicated in the pathogenesis of atherosclerosis, malnutrition, anemia, dialysis-induced amyloidosis, and, possibly, an increased risk of cancerogenesis, in these patients. Various therapeutic interventions have attempted to reduce oxidative stress in chronic renal failure so as to improve patient outcome. Therapeutic approaches to reduce oxidative stress in patients with chronic renal failure are focused on reduction of inflammatory cell activation, removal of inflammatory mediators, and the use of antioxidants.

Hemodialysis has been suggested to induce oxidative stress, with reactive oxygen species being generated on the surface of dialysis membranes by activation of polymorphonuclear leukocytes. Indeed, some studies have shown that a single session of hemodialysis significantly increases lipid peroxides and decreases antioxidants. Some investigators have suggested that in order to attenuate the activation of polymorphonuclear leukocytes on the surface of dialysis membranes, the recently invented vitamin E-coated multilayer hemodialysis filter could be beneficial. This device attenuates consumption of blood antioxidants, oxidative demolition of lipids, and activation of leukocytes. The aim of this study was to assess the effect of hemodialysis on plasma lipid peroxidation and antioxidant status before and after hemodialysis using the conventional cellulose membrane dialyzer. The observe effect of hemodialysis therapy on the oxidative markers may guide us in the use of alternative dialyzer membrane for a better result for patients undergoing dialysis.

Materials and Methods

The study was carried out at the Renal Unit of the General Hospital, Gbagada, and Lagos Island General Hospital, Lagos State, Nigeria, after the approval of the ethics committee of the state. A total of 25 patients (14 men and 11 women, aged 24-75 years; median 61) with end-stage renal failure who were booked for hemodialysis were recruited in the study. The exclusion criteria were smoking, diabetes mellitus, patients with chronic inflammatory conditions, and hepatic or respiratory diseases. Those on antioxidant vitamin or fish-oil supplements were excluded. All patients were freshly booked for hemodialysis using the conventional cellulose membrane dialyzer.

Patients were fasted overnight, thereafter 10 ml of venous blood was taken at the antecubital fossa without stasis immediately and after hemodialysis. The venous blood was collected in lithium heparinized bottles.

The plasma samples obtained after centrifugation at 3500 rpm for 10 minutes were immediately stored at -20°C until they were analyzed. Sodium, potassium, and bicarbonate were estimated on the Ion Selective Electrode (ISE) equipment supplied by the ISN Nig Ltd. Plasma albumin, blood urea nitrogen (BUN), uric acid, and creatinine were estimated using standard method on Beckman synchron CX5 automation analyzer supplied by Darlez Nig. Ltd. Vitamin E was determined with a fluorescence detector (2475 Multi Wavelength Fluorescence Detector, Waters, Eschborn, Germany) with 293 nm for excitation and 325 nm for emission. Peak areas were analyzed, and quantification was performed by calibration with external standards.

Measurement of MDA as secondary product of lipid peroxidation was employed to assess oxidative stress in this study. Estimation of MDA was done using the method of Satoh, while measurement of total antioxidant status in the plasma was performed by using a method given in commercial kit from Randox Laboratories Ltd, Diamond Road, Crumlin, Co. Antrim, Ireland). Spectrophotometric measurements were done on the Spectronic 21D, the assay was calibrated using 6-hydroxy-2,5,8-tetra-methylchroman-2-carboxylic acid (trolox). Results were expressed as mmol/L of trolox equivalent. All equipment used were routinely standardized. Universal quality control (precinorm and precipath materials were used at level I (normal range) and level II (pathological range).
Statistical analysis
All data were expressed as the mean ± SD. Statistical analysis of the results was performed using SPSS for Windows version. Statistical significance was analyzed with paired Student’s t test. Correlations were performed by Pearson’s method. A value of $P < 0.05$ was considered statistically significant.

Results
A total of 25 patients with renal failure were recruited into the study. Of these, 14 were males, while 11 were female, with a male:female ratio of 1:0.9. [Table 1] shows the measured parameters before and after dialysis. All values were expressed as (mean ± SD). [Table 2] shows correlation values between measured parameters after dialysis with TAS and MDA.

In the male subjects, there was a significant decrease ($P < 0.05$) in plasma K+ level postdialysis (4.0 ± 0.5 mmol/L) compared to predialysis (5.7 ± 1.2 mmol/L). Similar result was seen in female subjects (3.8 ± 0.7 mmol/L) compared to (6.1 ± 1.5 mmol/L). However, in male subjects, there was significant increase ($P < 0.01$) in plasma HCO₃⁻ level postdialysis (22.1 ± 4.7 mmol/L) compared to predialysis (17.1 ± 4.5 mmol/L); similarly, in female subjects (21.9 ± 5.1 mmol/L) compared to (15.5 ± 3.7 mmol/L).

The mean ± SD plasma BUN level postdialysis (17.2 ± 7.2 mmol/L) for male subjects and (19.1 ± 8.1 mmol/L) for female subjects were significantly reduced ($P < 0.001$) compared to predialysis level of (40.6 ± 16.3 mmol/L) and (43.1 ± 15.9 mmol/L), respectively. Similarly, the mean ± SD plasma creatinine level postdialysis (548.1 ± 229.8 umol/L) for male subjects and (572.5 ± 241.3 umol/L) for female subjects were significantly reduced ($P < 0.001$) compared to predialysis level of (1087.3 ± 424.2 umol/L) and (1079.5 ± 381.7 umol/L), respectively.

The mean ± SD plasma TAS level postdialysis (1.10 ± 0.3 mmol/L trolox Eq) for male subjects and (1.09 ± 0.2 mmol/L trolox Eq) for female subjects were significantly reduced ($P < 0.05$) compared to predialysis level of (1.72 ± 0.4 mmol/L trolox Eq) and (1.83 ± 0.7 mmol/L trolox Eq), respectively. However, the mean ± SD plasma MDA level postdialysis (6.03 ± 0.4 nmol/ml) for male subjects and (6.03 ± 0.4 nmol/ml) for female subjects were significantly increased ($P < 0.001$) compared to predialysis level of (3.98 ± 0.8 nmol/ml) and (4.05 ± 0.9 nmol/ml), respectively.

### Table 1: Mean (±SD) plasma concentration of biochemical parameters in the predialysis state and the post dialysis state of the patients

| Biochemical Parameters | Male (n = 14) | | | Female (n=11) | | |
|------------------------|--------------|----------------------|------------------|----------------------|------------------|
|                        | Predialysis  | Postdialysis | $P$ value | Predialysis  | Postdialysis | $P$ value |
| Na+ (mmol/L)           | 132.6 ± 3.8  | 135.1 ± 4.6  | 0.3610 | 138.1 ± 4.1  | 132.9 ± 7.9  | 0.3490 |
| K+ (mmol/L)            | 5.7 ± 1.2    | 4.0 ± 0.5    | 0.0380* | 6.1 ± 1.5    | 3.8 ± 0.7    | 0.0309* |
| HCO₃⁻ (mmol/L)         | 17.1 ± 4.5   | 22.1 ± 4.7   | 0.0096** | 15.5 ± 3.7   | 21.9 ± 5.1   | 0.0072** |
| BUN (mmol/L)           | 40.6 ± 16.3  | 17.2 ± 7.2   | 0.0002*** | 43.1 ± 15.9  | 19.1 ± 8.1   | 0.0003*** |
| Creatinine (umol/L)    | 1087.3 ± 424.2 | 548.1 ± 229.8 | 0.0004*** | 1079.5 ± 381.7 | 572.5 ± 241.3 | 0.0005*** |
| Uric acid (mmol/L)     | 0.51 ± 0.018 | 0.32 ± 0.015 | 0.0071** | 0.49 ± 0.012 | 0.33 ± 0.017 | 0.0083** |
| Albumin (g/L)          | 28.3 ± 5.10  | 43.7 ± 4.90  | 0.0085** | 30.1 ± 4.30  | 44.9 ± 3.80  | 0.0091** |
| α-Tocopherol (µmol/L)  | 24.19 ± 1.78 | 15.37 ± 1.94 | 0.0069** | 23.09 ± 1.51 | 13.97 ± 1.55 | 0.0058** |
| TAS (mmol/L) trolox Equivalent | 1.72 ± 0.4 | 1.10 ± 0.3 | 0.0482* | 1.83 ± 0.7 | 1.09 ± 0.2 | 0.0412* |
| MDA (nmol/ml)          | 3.98 ± 0.8   | 6.03 ± 0.4   | 0.0068** | 4.05 ± 0.9   | 6.71 ± 0.7   | 0.0089** |

Significant level at the $P < 0.001***$, $P < 0.01**$, and $P < 0.05*$

### Table 2: Correlation of plasma measured parameter in postdialysis with total antioxidant status (TAS) and lipid peroxidation (MDA) $r$

|          | Male (n = 14) | | | Female (n = 11) | | |
|----------|--------------|------------------|------------------|------------------|------------------|
|          | TAS          | MDA              | TAS              | MDA              | |
| Na+ (mmol/L) | 0.017        | 0.493            | 0.0094           | 0.735            | 0.010            |
| K+ (mmol/L)  | +0.31        | 0.041*           | +0.42            | 0.025*           | +0.33            |
| HCO₃⁻ (mmol/L)| −0.47        | 0.017*           | +0.49            | 0.015*           | −0.41            |
| BUN (mmol/L) | +0.68        | 0.0095**         | −0.72            | 0.0079**         | +0.69            |
| Creatinine (umol/L) | +0.77 | 0.0064** | −0.79 | 0.0058** | +0.75 |
| Uric acid (mmol/L) | +0.51 | 0.010* | −0.63 | 0.0098** | +0.48 |
| Albumin (g/L) | +0.35 | 0.037* | −0.41 | 0.028* | +0.28 |

Significant level at the $P < 0.001***$, $P < 0.01**$, and $P < 0.05*$
In males with chronic renal failure, an inverse correlation was observed between TAS/HCO$_3$ ($r = -0.47, P < 0.05$); however, a direct correlation between TAS/K+ ($r = +0.31, P < 0.05$), TAS/BUN ($r = +0.68, P < 0.01$), and TAS/creatinine ($r = +0.77, P < 0.01$) was observed. On the other hand, a direct correlation was noted between MDA/HCO$_3$ ($r = +0.49, P < 0.05$). However, an inverse correlation between MDA/K+ ($r = -0.42, P < 0.05$), MDA/BUN ($r = -0.72, P < 0.01$), and MDA/creatinine ($r = -0.79, P < 0.01$) was observed. Similarly, in females with chronic renal failure, an inverse correlation was observed between TAS/HCO$_3$ ($r = -0.41, P < 0.5$); however, a direct correlation between TAS/K+ ($r = +0.33, P < 0.5$), TAS/BUN ($r = +0.69, P < 0.01$), and TAS/creatinine ($r = +0.75, P < 0.01$) was observed. On the other hand, a direct correlation was noted between MDA/HCO$_3$ ($r = +0.49, P < 0.05$); however, an inverse correlation was observed between MDA/K+ ($r = -0.38, P < 0.5$), MDA/BUN ($r = -0.70, P < 0.01$), and MDA/creatinine ($r = -0.74, P < 0.01$).

**Discussion**

Under normal conditions, there is a steady state balance between production of oxygen free radicals and their destruction by the cellular antioxidant systems. The oxygen free radicals, which accumulate via an imbalance between generation and scavenging, are behind the pathogenesis of many diseases.\[4\]

In recent years, numerous studies have focused on detection of signs of oxidative stress in renal patients. There is good evidence indicating that uremia is generally associated with enhanced oxidative stress. Treatment of uremic patients with hemodialysis or peritoneal dialysis has been suggested to particularly contribute to oxidative stress and reduced antioxidant levels in such patients. Hemodialysis membrane had been implicated in the induction and activation of macrophages on the surface of dialysis membranes during the dialysis session. This contributes to the oxidative stress. Loss or deficiency of antioxidant activity could also contribute to enhanced oxidative stress in patients with chronic renal failure.\[22\]

In this study, a significant increase in MDA was observed, which is the measurement of secondary products of lipid peroxidation and a significant decrease in total antioxidant status in chronic renal failure patient after dialysis. This finding supports most of the previously published findings that the hemodialysis procedure alters lipid peroxidation and the antioxidant status. Some researchers have reported increased status,\[23\] while some have recorded reduction,\[24\] and others were equivocal\[13\] on MDA levels after hemodialysis. However, using the high-performance liquid chromatography (HPLC) technique, Peuchant et al. reported low post-hemodialysis MDA in contrast to increased MDA reported in studies where less specific analytical techniques were used.\[20\] Virtually all studies yielded increased serum LDL cholesterol levels in patients with chronic renal failure.\[27\]

Our study demonstrated that predialysis patients had low MDA levels and high antioxidant status, but postdialysis (using convective ordinary cellulose membrane dialyzer) samples gave an opposite levels of the analytes. In their study of patients with chronic renal failure that had peritoneal dialysis, Ülver et al.\[28\] observed high MDA levels and low antioxidant capacity. There seems to be no significant difference in the serum level of oxidative stress indices between peritoneal dialysis and hemodialysis patients regardless of the structure of the dialysis membranes used. The use of vitamin E-coated membranes could help minimize the increased oxidative stress and the attendant risk of atherosclerosis in dialysis patients. Mune et al.\[27\] have shown that the use of vitamin E-coated cellulose membrane dialyzers for 6 months resulted in a significant reduction in low density lipoprotein, oxidized-LDL, and eventually low peroxidation compared to the ordinary cellulose membrane dialyzer. In order to decrease membrane bioincompatibility, and thereby minimize oxidative stress in hemodialysis patients, more compatible filters have been elaborated. Preliminary characterization of vitamin E-coated membranes has shown decreased activation of polymorphonuclear cells and monocytes, lower free radical production, and high biocompatibility.

The effect of hemodialysis antioxidant status of patients with chronic renal failure is controversial. One school of thought believes that hemodialysis would aggravate oxidative stress due to activation of inflammatory cells caused by the use of bioincompatible membranes and net losses of soluble antioxidants in water;\[29\] or by generation of free radicals.\[10\] In such an environment, it is necessary to perform prospective studies on hemodialysis-treated patients with various antioxidants-coated membrane rather than the conventional ordinary cellulose membrane dialyzer currently in use.

**Conclusions**

The results of our study show a significant difference in antioxidant capacity between the pre- and postdialysis groups, and sex had no significant influence in our finding. Our study has showed that there is loss of antioxidant during the course of hemodialysis, probably through the dialyzer membranes or generation of free radicals on the surface of dialyzer membranes or both. The decreased antioxidant capacity could be related to increasing lipid peroxidation in haemodialysed patients. The nutritional status of patients going for hemodialysis could be a contributor; further assessment is needed since we are a third world country. This study provides evidence that further studies need to be conducted in the use of other biocompatible
or modify membrane dialyzers, especially antioxidants-coated membrane dialyzers. In addition, exogenous supplementation of antioxidant in pre- and postdialysis may be helpful, but improving our dialysis technique for effective removal of reactive oxygen species is important for successful outcome of hemodialysis therapy.

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References