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Ferric Reducing Ability of Plasma and Lipid Peroxidation in Hemodialysis Patients: Intradialytic Changes

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Abstract

Background and Aims: Patients on maintenance hemodialysis are at an increased risk of cardiovascular disease. Oxidative stress has a negative impact on endothelial functions producing endothelial dysfunction which contributes to cardiovascular risk. FRAP assay has been shown to be a simple cost effective tool for estimating antioxidant capacity. Hence the present study was taken up to evaluate total antioxidant capacity as ferric reducing ability of plasma (FRAP) and malondialdehyde (MDA), in patients during hourly intervals of a single hemodialysis session. During hemodialysis, FRAP is subjected to alterations, due to intradialytic changes in various molecules that contribute FRAP. This study was aimed to assess the utility of FRAP as a measure of the antioxidant capacity during hemodialysis.

Methods: Twenty seven patients with end stage renal disease on maintenance hemodialysis were recruited into the study. Time course changes in plasma MDA, FRAP, uric acid, total bilirubin, vitamin C and vitamin E were evaluated. Statistical evaluation of changes in the biochemical parameters during the whole period of dialysis was done using Friedman's test. Linear regression using generalized estimating equations (GEE) model for repeated measures was applied to study the association between parameters on intradialytic FRAP.

Results: Intradialytic increase in plasma MDA ($p<0.01$), decrease in FRAP (<0.01) and uric acid ($p<0.001$) levels were found. Uric acid was found to have significant association with FRAP as found by GEE ($p<0.001$).

Conclusions: A single session of hemodialysis contributes substantially to oxidative stress. Decreased intradialytic FRAP levels can be due to a decrease in uric acid levels. Assessment of antioxidant status in hemodialysis patients by FRAP method may not truly represent the actual antioxidant status, as changes in uric acid levels are reflected in FRAP levels. Hence measurement of individual antioxidants would give a better picture of the antioxidant status during hemodialysis.

Keywords: Oxidative stress, Hemodialysis, Malondialdehyde, Total Antioxidant Capacity

Introduction

End stage renal disease (ESRD) patients have a well recognized risk of cardiovascular disease that begins early in the course of chronic kidney disease (CKD) and results in a tenfold or higher cardiovascular mortality rates after the start of renal replacement

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therapy (1). Endothelial dysfunction is the potential mechanism that contributes to this cardiovascular risk (2). A proinflammatory state and oxidative stress seen in patients on haemodialysis (3). mediated through the production of reactive oxygen species and free radicals can produce deleterious effect on the endothelium. Oxidative stress is combated effectively by a number of low molecular weight antioxidant molecules, which are either generated during normal metabolism (uric acid, bilirubin, albumin, thiols) or obtained from exogenous sources by the consumption of dietary products rich in antioxidants. ROS cause oxidative damage to a large number of biomolecules resulting in functional derailment. A lot of emphasis has been laid on the pivotal role played by oxidized LDL (low density lipoprotein cholesterol) in atherogenesis (4). Oxidation of polyunsaturated fatty acids within LDL cholesterol leads to the release of short chain aldehydes such as malondialdehyde (MDA) (5). Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and chain breaking antioxidants like vitamin C and vitamin E help prevent LDL oxidation (5).

Chronic renal failure (CRF) is a pro-oxidant state and the degree of intracellular and extracellular oxidative stress is related to the severity of renal failure (6). Chronic kidney disease (CKD) is a major condition in which oxidative processes are implicated by the amplification of inflammatory responses (7). Hemodialysis (HD) represents a state of chronic stress for the patient where the oxidative reactions are mainly due to bioincompatibility of components of dialysis apparatus leading to the production of ROS by inflammatory cells (8). Lipid peroxidation leads to alterations in the biological membranes and is involved in the progression of renal injury.

Because of the difficulty in measuring each antioxidant component of plasma individually and of the interactions that take place among components, Benzie and his research group in 1996, for the first time described a method to measure the total antioxidant capacity (TAC) known as the ferric reducing ability of plasma (FRAP) (9). This is a measure of the antioxidant power, based on the reduction of ferrous ions by the effect of the reducing power of plasma constituents, and contributed by low molecular weight antioxidants of a hydrophilic and hydrophobic character. The low molecular weight compounds are Vitamin C, Vitamin E, bilirubin and uric acid. FRAP is said to give more biologically relevant information than that provided by individual antioxidant measurements and which may describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma (10).

A significant increase in serum MDA and a significant decrease in total antioxidant capacity (TAC) of plasma in CRF patients have been reported (11). CKD patients undergoing HD were found to have enhanced levels of lipid peroxidation, detected as malondialdehyde (MDA)/thiobarbituric acid-reactive substances (TBARS) (12), F2-isoprostanes (13) and lipid hydroperoxides in the plasma (14). In HD patients a pre HD increase in MDA, uric acid and total antioxidant capacity (TAC) and a decrease in vitamin C levels were observed which were decreased following HD (5, 15). Further studies on intradialytic changes revealed oxidative stress due to a single HD session in the form of an increase in plasma and erythrocyte lipid peroxides, plasma SOD and erythrocyte GPX, and a decrease in plasma vitamin E (16). Recently it has been reported that there is an impairment of the antioxidant defenses in HD patients caused by diffusion and loss of hydrophilic antioxidants during the dialysis session (17).

In view of the greatly increased risk of atherosclerosis in hemodialysis patients and the potential effects of HD on free radical production, we undertook the present study to assess the intradialytic changes with regard to lipid peroxidation and total antioxidant We also wanted to assess the utility of FRAP as an indicator of the antioxidant status during HD.

Materials and Methods

Twenty seven patients with end stage renal disease (ESRD) undergoing maintenance hemodialysis in the Department of Nephrology were recruited in the study after informed consent. Exclusion criteria were intravenous iron therapy at the time of collection, active infection, other complications of dialysis and smoking. Inclusion criterion was a minimum duration of three months of HD. The average duration of dialysis procedure the patients in our study group were undergoing was three years (ranging from 2 – 6 years). The study was approved by the institutional ethical committee.

The dialysis program consisted of four hour dialysis sessions three times a week. Dialysis was performed using fresh, first use polysulfone membrane and bicarbonate dialysate. Dialysate flow rate was 500 ml per min and a blood flow rate of 200-250 ml per min. Water for the dialysate was purified by reverse osmosis. Anticoagulation was done with 2000 IU of heparin at the start of dialysis followed by continuous administration at a rate of 500-1000 IU per hour.

For each patient, blood samples were collected serially from the arterial end of the dialyzer at 0 (pre HD), 1st, 2nd, 3rd and 4th hour (immediately after dialysis- post HD) during the dialysis session. The blood samples were collected in heparinized tubes, and centrifuged immediately at 3000rpm for 15 min. The plasma was separated and stored at -80 ^{o}C until further analysis.

MDA was measured as thiobarbituric acid reactive substances (TBARS) (18). TAC was determined by FRAP method in which a colorless ferric tripyridyltriazine complex is reduced to a blue ferrous complex by the antioxidants in the plasma. The change in absorbance at 593nm is directly related to the total reducing power of electron donating antioxidants present in the plasma (9). Vtamin E was estimated using HPLC, vitamin C was estimated colorimetrically, uric acid, bilirubin, albumin, cholesterol, and triglycerides were estimated by standard methods using commercial kits on Beckman Synchron CX9 fully automated analyzer.

Statistics

The data were presented as mean ± standard error of mean. The results obtained at each hour of HD were compared with pre-HD values. The data was converted to percentages with predialytic value taken as 100%. Statistical evaluation of intradialytic changes in the various biochemical parameters during the HD session was done using Friedman's test. A p value of < 0.05 was considered statistically significant. Statistical analysis was performed using Microsoft Excel spread sheets and SPSS for Windows version 16.0.

Results

The mean age (SEM) of the patients was 50.4±2.06 years. The causes of ESRD were diabetic nephropathy (40.7%), hypertensive nephropathy (29.6%), chronic glomerulonephritis (11.11%), ischemic nephropathy (3.73%), chronic interstitial nephritis (3.73%), and unknown etiology (11.11%). Friedman's test showing significance of changes in the parameters during HD is presented in Table 1. Time course of changes observed in MDA and FRAP levels during a HD session are shown in Figures 1 and 2.MDA levels (MDA corrected for creatinine) were found to increase significantly during the dialysis session ($p<0.01$) when compared to the pre-HD levels. There was a significant decrease in FRAP levels during the dialysis session ($p<0.01$) when compared to the pre-HD levels. In GEE analysis significant associations were found between intradialytic uric acid andFRAP levels, when uric acid was introduced alone (B[regression coefficient] of 39.358; CI [Confidence interval] 17.755-60.960; $p<0.001$) and also when introduced in combination with vitamin C, vitamin

Table 1. Intradialytic changes in the biochemical parameters during a single HD session

Variable	Pre-HD	1st hr	2nd hr	3rd hr	Post-HD	p
FRAP (µmol/L)	379.5±52.6	292.9±31.8	224.0±31.4	216.5±24.8	237.4±45.5	(↓) 0.000*
Uric acid (mg/dl)	5.4±0.25	3.7±0.17	2.9±0.21	2.5±0.21	2.0±0.11	(↓) 0.000*
Vitamin E (µmol/dl)	10.60±1.58	8.02±1.07	7.11±0.099	7.76±0.088	6.15±0.024	(↓) 0.152
Vitamin E corrected (µmol/mg cholesterol)	0.009±0.001	0.005±0.001	0.004±0.001	0.005±0.001	0.003±0.001	(↓) 0.023*
Vitamin C (mg/dl)	0.38±0.08	0.29±0.06	0.35±0.07	0.33±0.08	0.28±0.04	(↓) 0.173
T. Bilirubin (mg/dl)	1.0±0.06	0.9±0.05	0.9±0.05	0.9±0.05	0.9±0.04	(↓) 0.983
MDA (µmol/L)	5.53±0.71	4.14±0.49	3.76±0.47	2.97±0.37	2.60±0.34	(↓) 0.000*
MDA corrected (µmol/mg creatinine)	0.068±0.008	0.076±0.010	0.076±0.009	0.074±0.009	0.079±0.009	(↑) 0.030*

MDA, Malondialdehyde; **FRAP**, Ferric Reducing Ability of Plasma

*Statistically significant

E and bilirubin as covariates (B 37.418; CI 11.528-63.308; p<0.01).

Discussion

Oxidative stress (OS) poses a serious threat to the cardiovascular outcome in ESRD patients. OS as a result of a single haemodialysis session has been demonstrated previously and was thought to be the principle mediator of endothelial dysfunction in dialysis (3). There have been several studies on oxidative stress in CRF patients, where MDA was found to be significantly increased before initiation of artificial dialysis (19-21). A significant decrease in TAC and a negative correlation between MDA and FRAP levels have been reported in chronic renal failure patients (11).

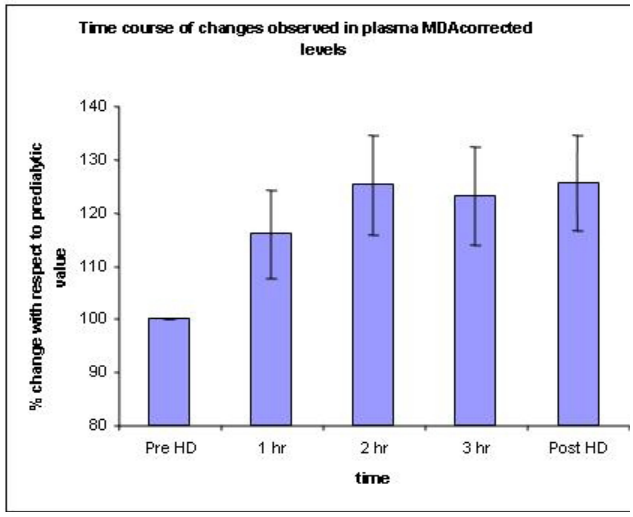

Figure 1. Time course of changes observed in MDA levels during HD. Data collected hourly was corrected for loss through dialysate and converted to percentages with predialytic value taken as 100%.

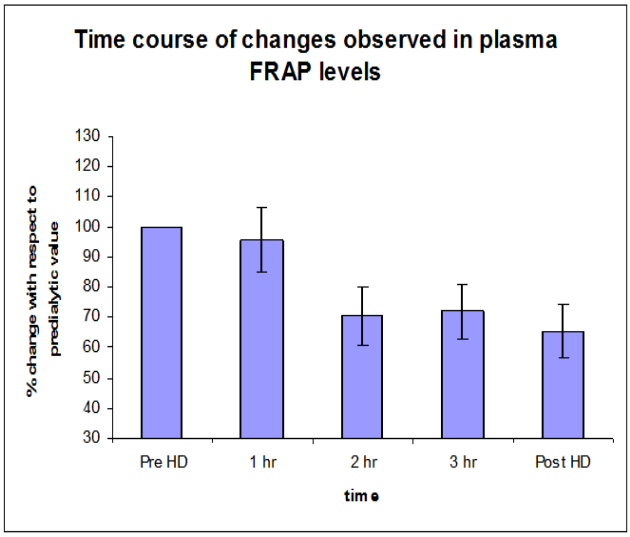

Figure 2. Time course of changes observed in FRAP levels during HD. Data collected hourly was converted to percentages with predialytic value taken as 100%.

The bioincompatibility of dialysis membrane is known to cause generation of superoxide radical due to complement activation followed by respiratory burst in leukocytes (8). The superoxide radical can initiate lipid peroxidation of fatty acids and can react with nitric oxide to form peroxynitrite radicals. The peroxynitrite radical can also get converted to hydroxyl radical and nitrates. The hydroxyl radicals can in turn perpetuate lipid peroxidation (22). In our study, we 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, the ratio of MDA and creatinine is likely to give a better picture about MDA during dialysis. Hence correcting MDA for creatinine we found a significant increase in MDA levels (Table 1; Figure 1) indicating that the increase in MDA during HD is due to its increased production, not withstanding the fact that it is getting cleared by dialysis. This increase in intradialytic MDA levels is an indicator of the presence of oxidative stress during the dialysis session.

Chain breaking antioxidants like vitamin E, thiols, and vitamin C prevent lipid peroxidation (23). Vitamin E being associated with lipoproteins will not be cleared by dialysis as was found in our study, with no significant changes in intradialytic vitamin E levels. However when vitamin E was corrected for cholesterol, taking into account the effect of hemoconcentration, a decrease was found (Table 1). This decrease in intradialytic vitamin E levels may be due to its increased consumption during dialysis. Vitamin E being a lipid soluble antioxidant is more likely to prevent lipid peroxidation by being preferentially oxidized instead of fatty acids. Yet this defense mechanism appears to be defective during HD as evidenced by an increase in MDA levels. Vitamin C being hydrophilic is cleared by dialysis as was found by a decrease in intradialytic vitamin C levels, but which were however not statistically significant. We found a significant decrease in the FRAP levels during dialysis when compared to pre HD levels (Table1; Figure 2). Uric acid which has strong reducing and antioxidant properties is a well known hydrophilic antioxidant and contributes to about 60% of free radical scavenging activity in the blood (24). However, it has been proved that elevated uric acid level is a potential risk factor for CVD. Uric acid at higher concentrations is found to behave as a pro-oxidant under conditions of oxidative stress, especially in the presence of deficiencies in other antioxidant systems (25). Uric acid is known to be cleared by HD as evidenced by a decrease in intradialytic uric acid levels when compared to pre HD (Table 1). In the FRAP assay UA is said to contribute to a major portion of the FRAP values (26) which is about 60% (9). It has been reported that FRAP correlated significantly with serum UA and bilirubin levels in HD patients and controls (27). We studied the association of uric acid with FRAP during HD using generalized estimating equations (GEE) with an independent correlation structure. We found uric acid level to have a significant association ($p<0.001$) with FRAP when introduced alone into the model as a

covariate. The strength of this association was further studied by introducing uric acid along with the total bilirubin, vitamin E and vitamin C as covariates, where uric acid was again found to have a significant association ($p<0.01$) with FRAP. No significant intradialytic associations were found between vitamin E, vitamin C and bilirubin with FRAP. This finding supports uric acid to be a major contributor to FRAP, and whose clearance during dialysis is reflected in the decreased intradialytic FRAP levels when compared to pre HD. This observation is in accordance with a similar study done in HD patients where plasma TAC was found to be lower during and after HD when compared to pre HD (27, 28).

OS is an important contributor to the disease outcome as evidenced in various studies. It is well known that OS is present in CRF patients (11, 20) and in patients undergoing HD (16, 17). This study further strengthens these findings as indicated by the rising MDA levels during HD indicating that OS is present during a single hemodialysis session. The increase in morbidity and mortality in HD patients may be the due to the damage caused by OS, which becomes more pronounced when the antioxidant defense system fails to minimize the damage. Antioxidant capacity of plasma signifies the free radical scavenging ability which is independent of the capacity of any one antioxidant present in the mixture. An increase of TAC may not necessarily be beneficial as various diverse molecules are contributors to the TAC. Certain clinical conditions such as renal failure and jaundice are likely to cause increase in UA, bilirubin and a decrease in albumin, which can modify the TAC in such situations. UA in high concentration has been shown to be a prooxidant, which may itself lead to a further decrease of the plasma antioxidant capacity (29). During HD, the TAC undergoes major variations as the major contributor UA is eliminated by HD, along with bilirubin and vitamin C which are also water soluble molecules. An increased consumption of the other endogenous antioxidants like Vitamins E and C may occur during states of oxidative stress. Though fluctuations in theof MDA and FRAP were found during the time course of HD, the direction of change was not altered when compared to pre HD.

Conclusions

In conclusion, our data suggest the presence of oxidative stress during HD. The TAC assessed by the FRAP assay during dialysis is severely affected by concomitant fluctuations in plasma uric acid levels. Though we did not find statistically significant changes in vitamin C and bilirubin, the dialysis associated fluctuations of these molecules may have an effect on the FRAP assay. The prooxidant effect of uric acid may be lowered during dialysis as a result of its clearance. This may paradoxically improve the antioxidant status during dialysis, the effect of which may however not be reflected in the FRAP assay, which essentially involves uric acid as demonstrated in our study. Additionally, changes in uric acid may mask potentially important changes in other antioxidants. We therefore suggest the measurement of the other chain breaking antioxidants individually to assess the antioxidant status during HD.

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Conflict of Interest

None declared.

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