Oxidative Stress Modulation Immediately After Hemodialysis

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AIM: The aim of this study was to estimate oxidative stress (OS) before and after dialysis in hemodialysis (HD) patients and correlate this stress with routine biochemical parameters.

METHOD: Sera of patients (n = 21), under regular HD, were collected 5 minutes before and after an HD session. Oxidative stress was estimated using the pro-oxidant-antioxidant assay (PAB), along with routine biochemical parameters in the same sera.

RESULTS: A significant increase of OS value was observed in HD patients 5 minutes after dialysis. Before HD, a significant correlation was established between the PAB values and fasting blood sugar, calcium, and C-reactive protein (CRP); and an inverse correlation between the PAB values and uric acid, serum creatinine, albumin, and albumin/globulin ratio. This correlation was not obvious after HD. PAB value, after dialysis, correlated significantly only with serum iron and inversely with alanine transaminase.

CONCLUSION: This study found that the already-increased OS in HD patients is further increased immediately after dialysis. However, factors influencing post-dialysis OS may be different from pre-dialysis factors.

It is evident that patients undergoing regular dialysis treatment experience increased oxidative stress (OS).[1,2] The lines of evidence range from an increased stimulation of neutrophils,[3,4] elevated serum concentrations of lipid peroxidation products,[5,6] impairment of the antioxidant system,[7-9] endothelial dysfunction,[9] to increased oxidative modification of proteins.[10]

However, controversial results have been published considering the OS status of HD patients. The results depend on the method used for the estimation of OS because of the various serum biochemical parameters used.[7,8,11-13]

Recently, a method has been published that estimates the balance between all major serum pro-oxidants and antioxidants in one sole reaction (PAB).

In the present study, this method was applied to estimate OS 5 minutes before and after hemodialysis. The results of this method were correlated with routine serum biochemical parameters. Different correlations were found before and after dialysis.

Subjects and Methods

Materials

TMB powder (3,3'5,5'-tetramethylbenzidine; Fluka, Buchs, Switzerland), peroxidase enzyme (Applichem: 230 U/mg, order no. A3791,0005; Darmstadt, Germany), chloramine T trihydrate (Applichem: order no. A4331; Darmstadt, Germany), and hydrogen peroxide (30%; Merck, Darmstadt, Germany) were used. All the other reagents used were reagent grade and were prepared in double-distilled water.

Sample Collection and Preparation

All patients and healthy volunteers were recruited at the pediatrics department of the medical school at the University of Tehran, and at the University of Medical Sciences, Mashhad, Iran, and gave both oral and written informed consent.

Blood samples of 21 patients (13 males, 8 females), under regular hemodialysis (duration of dialysis: 15.8 ± 17.4 with a range of 1.5–84 months), with a mean age of 21.3 ± 5.38 years (range, 11–34 years) were collected 5 minutes before and after dialysis.

The etiologies that led the patients to chronic renal insufficiency were diabetic nephropathy (1 patient); left nephrectomy, splenectomy, and right kidney cortical thrombosis (1 patient); posterior urethral valve (1 patient); nephrolithiasis (1 patient); myelomeningocele and paraplegia (1 patient); bilateral hydronephrosis (1 patient); and unknown (15 patients). Patients were treated with the same therapy given to patients with chronic renal failure such as 1,25 dihydroxycholecalciferol, calcium carbonate, folic acid, B6, ferrous sulfate, erythropoietin, antihypertensives

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TABLE I. Clinical and biochemical parameters and their correlation with the PAB values.

	Before Dialysis $n = 21$	Correlation PAB (Before) $n = 21$, p	After Dialysis $n = 21$	Correlation PAB (After) $n = 21$, p
Age, years	21.3 ± 5.3	.9	...	.7
Duration of dialysis (mo)	15.8 ± 17.4	.9		
Waist/hip ratio	0.81 ± 0.09	.7	...	
BMI, kg/m^2	19.8 ± 3.9	.3	...	
Systolic blood pressure mmHg	145.0 ± 18.4	.6	141.0 ± 17.4*	.7
Diastolic blood pressure mmHg	94.5 ± 10.5	.5	88.0 ± 8.3*	.6
Fasting blood sugar, mg/dL	123.9 ± 125.5	**.04**	110.8 ± 48.6	.42
Triglycerides, mg/dL	127.7 ± 67.2	.6	107.9 ± 50.5*	.6
LDL cholesterol, mg/dL	76.1 ± 25.5	.5	94.6 ± 33.5*	.42
HDL cholesterol, mg/dL	37.9 ± 7.7	.8	48.28 ± 11.1*	.6
LDL/HDL	2.0 ± 0.6	.6	2.0 ± 0.7	.33
Uric acid, mg/dL	6.1 ± 1.2	**.04**	2.2 ± 0.4*	.16
Serum creatinine, mg/dL	8.8 ± 2.4	**.02**	3.9 ± 1.3*	.9
Urea	116.3 ± 22.4	.14	42.2 ± 11.2*	.6
Albumin	4.1 ± 0.5	**.02**	4.6 ± 0.7*	.6
Total protein	7.1 ± 1.0	.7	8.1 ± 1.6*	.4
Albumin/Globulin	1.32 ± 0.22	**.005**	1.30 ± 0.46	.4
Alkaline phosphatase	581.9 ± 449.3	.44	604.6 ± 605.0	.6
Phosphorus	5.1 ± 1.6	.3	3.0 ± 0.7*	.6
Calcium	9.1 ± 0.9	**.04**	9.7 ± 0.73*	.415
Iron	70.0 ± 39.2	**.2**	192.9 ± 273.2*	**.05**
TIBC	231.3 ± 45.4	.5	305.0 ± 105.9*	.2
Ferritin	426.4 ± 690.3	.38	525.2 ± 898.3*	.5
Sodium ion	139.7 ± 3.1	.1	142.5 ± 2.7*	.4
Potassium ion	5.2 ± 0.59	.9	4.0 ± 0.48*	.8
AST	26.0 ± 9.8	.41	26.3 ± 9.8	.4
ALT	18.7 ± 7.3	.5	19.5 ± 7.4	**.01**
CRP	6.8 ± 5.1	**0.003**	7.4 ± 7.0	0.2

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAB, pro-oxidant-antioxidant balance; TIBC, total iron-binding capacity.
**Significant difference before and after dialysis ($p < .05$).*

(amlodipine, captopril, prazosin, losartan) and some sedative drugs. The patient with diabetes was receiving insulin on a regular basis and neutral protamine hagedorn (NPH) in addition to other drugs. Medications and other conditions did not change before or after dialysis and therefore were not expected to play a significant role in the differences observed. However, to eliminate any interference to the therapy, the samples of pre-dialysis and post-dialysis sera were taken immediately (after 5 minutes) in the same session of dialysis. Iron supplementation was administrated by oral route and the patients were followed by determination of iron level, total iron binding capacity, ferritin level, and transferrin saturation. In cases where satisfactory results had not been attained, patients received intravenous iron dextran.

Using Gambro AK 200 machines, patients had 4-hour HD treatments per session. The synthetic filter used was a polysulfone membrane, low flux hollow fiber (R5-R6; Fresenius, Bad Homburg, Germany). Hemodialysis was performed on end-stage renal disease patients 3 times weekly (blood flow: 250 mL/min, dialysate flow: 500 mL/min, bicarbonate buffer, substitution volume: 1.8 L/h). ➲

A total of 22 age-matched volunteers (14 males, 8 females) with a mean age of 23.3 ± 8.1 years old (range, 9–41 y) and with normal clinical and biological parameters and no history of renal disease were studied as controls. Controls were not subjected to dialysis and were used only as baseline references. For serum preparation, the blood samples were centrifuged at 2,000*g* for 15 minutes and the serum aliquots were separated and stored at −80°C until measurement.

Pro-oxidants–Antioxidants Balance Assay

PAB assay was applied based on a previously described method.[14,15] Standard solutions were prepared by mixing varying proportions (0%–100%) of 250 μM hydrogen peroxide with 3 mM uric acid (in 10 mM NaOH). A standard curve was provided from the values relative to the standard samples measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. The values of the PAB are expressed in arbitrary HK units, which are the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

All other parameters were measured by routine clinical tests.

Statistical Analysis

For the statistical analysis, the GraphPad Instant statistical package was used (GraphPad Software, La Jolla, Calif.). All parameters were given as mean ± SD. The group comparisons were assessed by the *t*-test (unpaired *t*-test for control and before HD and paired *t*-test for before and after HD). Parametric and non-parametric correlations were assessed using the Pearson correlation coefficients and the Spearman correlation coefficients, respectively. The level of statistical significance was set to $p < .05$.

Results

Oxidative stress was found to be increased in HD patients and even more 5 minutes after hemodialysis. The PAB values of healthy volunteers and HD patients before and after dialysis were 36.5 ± 20.4, 131.8 ± 46.3 ($p < .0001$) and 155.4 ± 46.2 ($p < .05$), respectively. In addition, a significant correlation of the PAB values before dialysis with the PAB values after dialysis was observed ($p < .05$).

The clinical and biological parameters of the HD patients before and after dialysis and of the control groups are presented in *Table I*, along with the correlations of the clinical and biological parameters with PAB before and after dialysis. As can be observed in *Table I* before dialysis, OS, as estimated by PAB, did correlate with serum uric acid, serum creatinine, serum albumin, C-reactive protein (CRP) levels, and serum calcium. After dialysis, OS, as estimated by PAB, correlated only with total serum iron and serum alanine transaminase activity.

Antioxidant administration during dialysis may be a useful preventive measure for reducing cardiovascular risk during and after dialysis.

Discussion

In the present study, we applied a recently reported method (the PAB assay)[14,15] for estimating OS before and after a hemodialysis session for patients undergoing regular hemodialysis. In previous evaluation studies, the assay showed a linear response against all major serum oxidants and antioxidants.[14,15] In addition, the PAB assay has been previously compared to most of the widely utilized and documented methods that aim to estimate OS and permanent oxidative damage.[14]

A significant increase of OS before dialysis was observed in HD patients in comparison with healthy volunteers. Just after dialysis, PAB value had increased. These results are in accordance with the results of other researchers, indicating that patients undergoing regular hemodialysis manifested a high amount of OS indicators.[18] This result reflects the significant metabolic burden of these patients between hemodialysis sessions that is to be ameliorated with hemodialysis.

It was expected that the reconstitution of metabolic and hemodynamic balance after dialysis would permit OS to fall to levels lower than before dialysis. However a significant increase of the OS value was noted immediately after hemodialysis. These results are in accordance with other researchers[19-22] who reported that OS was exacerbated by the dialysis session. Other researchers reported having the opposite result.[23] These differences may possibly be explained by the different times of sampling after dialysis that were reported.[24]

A more detailed study that would include extensive sampling at various time points during and after dialysis may solve these discrepancies, however this was beyond the scope of the present study, which aimed to establish differences in OS just before and after dialysis.

Another observation of the present study was that OS values after dialysis correlated with OS values before dialysis. This observation may indicate that hemodialysis, as a method, adds to the OS an almost equal amount of stress per patient. It is also possible that antioxidant administration during dialysis would represent a useful preventive measure for the reduction of cardiovascular risk during and immediately after dialysis.[25] Furthermore, it has been suggested[18] that HD patients need new methods aimed to reduce intradialytic OS, such as incorporating antioxidant therapy into the dialysis membrane,[26] hemolipodialysis,[27] using electrolyte-reduced water for dialysate,[28] or using an ultrapure dialysate system to reduce acute phase inflammation.[29] On the other hand, it should be noted that post-dialysis OS may be ameliorated with time and then increased again up to the day of the next dialysis session.[23,24] Accordingly, it has been reported that shorter and more frequent dialysis sessions may better mimic the normal state and lead to less abrupt metabolic changes.[16,30]

Before dialysis, a significant correlation was established between OS and fasting blood sugar, calcium, and CRP; and a significant inverse correlation was established between OS and uric acid, serum creatinine, and albumin. After dialysis, a significant correlation was established ➲

only between the PAB value and serum iron concentration; and a significant inverse correlation was established between the PAB value and alanine transaminase. These observations made for the first time during the present study may reflect the loss of significant antioxidants during dialysis and an increase in iron cations observed immediately after dialysis. However it should be noted that correlation is not causation and that further investigation is needed to attribute OS after dialysis to iron increase.

It has been reported that the passage of blood through dialyzers leads to a decrease in vitamin E and C and glutathione levels.[17,31,32] In addition, HD membrane materials may activate neutrophils[33] and platelets.[34] The abrupt change of hemodynamic and metabolic balance caused by dialysis may also add to the development of OS. Further research should be planned in order to accurately measure OS in time intervals between and during dialysis as well as following various time courses and dialysis therapy schemes.

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