

Impact of Kidney Transplantation on Biomarkers of Oxidative Stress and Inflammation

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Introduction. Hemodialysis patients face oxidative stress and inflammation induced by both kidney dysfunction and hemodialysis procedure. These are supposed to be partly responsible for the excessive cardiovascular morbidity and mortality in hemodialysis patients. We investigated the impact of kidney transplantation on the biomarkers of oxidative stress and inflammation.

Materials and Methods. In a prospective cohort study on 32 kidney transplant candidates on hemodialysis, biomarkers of oxidative stress and inflammation were compared before and 3 months after kidney transplantation and were compared with each other as well as their values in the kidney allograft donors as the controls. These biomarkers included total antioxidant capacity, total thiol molecules, lipid peroxidation, plasma catalase, superoxide dismutase, glutathione peroxidase, and C-reactive protein.

Results. The mean age of the patients was 38.0 ± 15.5 years. The levels of total antioxidant capacity, total thiol molecules, and activity of glutathione peroxidase were significantly lower and the level of activity of plasma superoxide dismutase was significantly higher in the hemodialysis patients before transplantation as compared to the values for the controls and after transplantation. Lipid peroxidation was significantly higher in the patients before transplantation compared to the controls. A significantly higher level of C-reactive protein was noted in the hemodialysis patients as compared to their levels after transplantation and also C-reactive protein in the controls.

Conclusions. These results suggest that oxidative stress and inflammation are elevated in hemodialysis patients which could be improved partly and significantly by restoration of kidney function after kidney transplantation.

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INTRODUCTION

Oxidative stress is characterized by an imbalance between an excessive formation of oxidant agents and inadequate antioxidant defense systems, leading to tissue damage.¹ In addition to traditional risk factors, elevated oxidative stress is recognized as one of the main components of extreme cardiovascular morbidity and mortality in patients with end-

stage renal disease (ESRD).^{2,3} It is well established that inflammation and oxidative stress increase with gradual deterioration of kidney function.^{4,5} Heightened oxidative stress in hemodialysis patients is owing to kidney failure and also hemodialysis procedure, which provokes oxidative imbalance via loss of antioxidants during dialysis, contacts between blood and incompatible dialysis membrane,

and bacterial endotoxins in dialysis solution passing the dialysis membrane.^{6,7}

There are a few reports indicating that chronic inflammation and increased oxidative stress in hemodialysis patients can be partly improved by recovery of kidney function early after kidney transplantation.^{8,9} As a result, transplantation may correct the ominous prognosis of ESRD patients by reversing cardiovascular sequelae. In our country few studies have been carried out regarding oxidative stress in hemodialysis or kidney transplant patients,¹⁰⁻¹³ particularly for evaluating the impact of reversal of kidney function by successful transplantation on inflammatory and oxidative markers. We aimed to explore biomarkers of inflammation and oxidative stress in a cohort of hemodialysis patients before kidney transplantation and 3 months after transplantation, and to compare them with their healthy living donors.

MATERIALS AND METHODS

Participants

The study population cohort involved 35 consecutive nondiabetic patients on maintenance hemodialysis for at least 6 months who were candidates for their 1st living donor transplantation in our center. They had no underlying inflammatory diseases (such as rheumatologic diseases and vasculitis) and no viral infection (hepatitis B, hepatitis C, and acquired immunodeficiency syndrome). All of the patients were on dialysis with polysulfone dialysis membranes. The control population was the healthy donors of each hemodialysis patient. Both patients and control donors were nonsmokers. Informed consent was obtained prior to taking blood samples.

Based on previous studies, in order to reduce oxidative stress parameters by 30% posttransplant and considering a standard deviation of 30% and a type 1 error of 5% and a power of 95%, the required sample was 15. Given that for all antioxidant parameters data was not sufficient in the literature, we considered 35 patients to evaluate reduction in antioxidant parameters by more than 30%. Three of the 35 patients were excluded from the study owing to recurrent rejection or infection and surgical complications after kidney transplantation.

Blood Sampling

Blood samples were taken during the two days

before kidney transplantation and after 3 months (mean, 3.86 ± 1.45 months), when the patients had a stable kidney function with no infection or rejection since 1 month prior to samplings. The time lag between the last hemodialysis and the first sampling was at least 24 hours. Blood samples were drawn with ethylenediaminetetraacetic acid anticoagulant and immediately centrifuged at 1000 g for 15 minutes, and then serum samples were stored at -80°C until analysis. The following biomarkers of oxidative stress and inflammation were measured in the blood samples: total antioxidant capacity (TAC), total thiol molecules (TTMs), lipid peroxidation, plasma catalase, superoxide dismutase (SOD), glutathione peroxidase, and high-sensitivity C-reactive protein (CRP).

Measurement of Oxidative Stress and Inflammation

Total antioxidant capacity. Antioxidant capacity of plasma (TAC) was determined by measuring the ability of plasma to reduce ferric to ferrous. The complex between ferrous and 2,4,6-tripyridyl-S-triazine gives a blue color with absorbance at 593 nm.¹⁴

Total thiol molecules. To evaluate the plasma TTMs, 5, 5'-dithiobis-2-nitrobenzoic acid was used as a reagent, which reacts with thiol molecules and creates a yellow complex which has good absorbance at 412 nm in spectrophotometer.¹⁵

Lipid peroxidation. Plasma samples were mixed with trichloroacetic acid (20%) and the precipitate was dispersed in sulfuric acid (0.05 M). 2-thiobarbituric acid (0.2% in sodium sulfate 2 M) was added and heated for 30 minutes in boiling water bath. Thiobarbituric acid-reactive substances adducts were extracted by n-butanol and absorbance was measured at 532 nm.¹⁶

Catalase activity. Catalase activity was assayed in the samples by measuring the absorbance decrease at 240 nm in a reaction medium containing hydrogen peroxide (10 mM), sodium phosphate buffer (50 mM; pH, 7.0). One unit of the enzyme was defined as 1 mol of hydrogen peroxide as substrate consumed per minute, and the specific activity was reported as U/mL plasma.¹⁷

Superoxide dismutase activity. The activity of copper and zinc SOD was measured using a commercial kit (Ransod, Randox Laboratories Ltd, Crumlin, UK). Measurement of the enzyme was

based on the generation of superoxide radicals produced by xanthine and xanthine oxidase and reacted with 2-(4-iodophenyl)-3-(4-nitrophenol) 5-phenyltetrazolium chloride to form a red formazan dye.

Glutathione peroxidase activity. The amount of glutathione peroxidase was determined using a commercially available kit (Ransel, Randox Laboratories Ltd, Crumlin, UK) by measuring the rate of oxidation of nicotinamide adenine dinucleotide phosphate at 340 nm. A unit of enzyme was expressed as the amount of enzyme needed to oxidize 1 nmol of nicotinamide adenine dinucleotide phosphate oxidase per minute.

High-sensitivity C-reactive protein. Concentration of CRP was measured by enzyme-linked immunosorbent assay, with a low detection limit of 0.1 µg/ml (high sensitivity).

Statistical Analysis

Numerical data were presented as mean ± standard deviation or median and range based on normality of distribution. Variables were compared between hemodialysis patients before and after transplantation with the control group using the Student *t* test and the Mann-Whitney U test, as appropriate. Hemodialysis patients before and after transplantation were compared using the paired *t* test and the Wilcoxon test. The Pearson correlation coefficient was used to evaluate the association between variables and glomerular filtration rate (GFR) after transplantation. Analyses were carried out with the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA). Significance level was considered as a *P* value less than .05.

RESULTS

The age and sex distribution of the patients and their controls (donors) are summarized in Table 1. The Etiology of ESRD consisted of diabetic glomerulosclerosis (4 patients), glomerulonephritis (6 patients), polycystic kidney disease (3 patients), urologic problems (5 patients), nephronophthisis (1 patient), hypertensive nephrosclerosis and unknown causes (12 patients).

Results of the measurement of the biomarkers of oxidative stress and inflammation are summarized in Table 2. Plasma levels of TAC and TTMs were lower in the patients before transplantation as compared

Table 1. Characteristics of 32 Hemodialysis Patients Before Kidney Transplantation*

Characteristic	Value
Mean age, y	38.0 ± 15.5 (12 to 70)
Male sex	17 (53.1)
Median hemodialysis duration, mo	17 (6 to 85)
Mean serum creatinine, mg/dL	
Before transplantation	7.45 ± 2.15
3 months posttransplant	1.22 ± 0.28
Median posttransplant glomerular filtration rate, mL/min/1.73 m ²	60.3 (33 to 118)
Mean plasma hemoglobin, g/dL	
Before transplantation	11.3 ± 1.7
3 months posttransplant	12.6 ± 1.2
Donor male sex	26 (81.2)
Mean donor age, y	27.5 ± 6.6 (21 to 53)

*Values are mean or median (range) or frequency (percentage).

to the donors (*P* = .02 and *P* = .007, respectively) and compared to values after transplantation (*P* = .045 and *P* = .05, respectively). Plasma lipid peroxidation was significantly higher in the patients before transplantation compared to healthy control (*P* = .049), but not after transplantation. Plasma activity of SOD was significantly higher and plasma activity of glutathione peroxidase was significantly lower in the patients before transplantation as compared to the controls (*P* = .006 and *P* = .01, respectively). Plasma activity of SOD was also significantly higher before as compared to after transplantation (*P* = .047). The level of CRP was significantly higher in the patients as compared to the controls before transplantation (*P* = .003) and marginally higher than that after transplantation (*P* = .06). The posttransplant CRP was significantly higher as compared to that in the controls (*P* = .01).

Fifty-eight percent of the transplanted patients were on cyclosporine-based immunosuppression (42% on tacrolimus). There was no significant correlation between oxidative stress biomarkers or CRP and posttransplant GFR or hemoglobin levels (Table 1).

DISCUSSION

There is overwhelming evidence that from early stages of chronic kidney disease (CKD), an imbalance between antioxidant and pro-oxidant activities exists.^{5,18} This pathologic mechanism, by inducing endothelial injury, and as a result, accelerating atherosclerosis as well as by developing of left ventricular hypertrophy, participates in

Table 2. Oxidative Stress and Inflammation Parameters Before and After Kidney Transplantation*

Variable	Patients			P	
	Pretransplant	Posttransplant	Donors	Pretransplant vs Control	Posttransplant vs Control
Total antioxidant capacity, $\mu\text{mol/mL}$	3.83 \pm 1.71	4.75 \pm 2.21	4.84 \pm 1.89	.02	NS
Total thiol molecules, nmol/mL	0.111 \pm 0.065	0.162 \pm 0.107	0.167 \pm 0.087	.007	NS
Lipid peroxidation, nmol/mL	0.513 \pm 0.215	0.459 \pm 0.291	0.406 \pm 0.162	.049	NS
Catalase activity, U/mL	3.24 (0.71 to 17.20)	3.05 (0.23 to 20.10)	2.34 (0.18 to 22.50)	NS	NS
Superoxide dismutase, U/mL	0.409 \pm 0.054	0.382 \pm 0.025	0.377 \pm 0.028	.006	NS
Glutathione peroxidase, U/mL	170.2 \pm 51.2	218.4 \pm 81.3	233.9 \pm 88.3	.02	NS
C-reactive protein, $\mu\text{g/mL}$	2.83 (0.20 to 28.44)	1.96 (0.61 to 14.40)	0.77 (0.15 to 8.31)	.003	.01

*Values are mean \pm standard deviations or median (range). NS indicates not significant.

high cardiovascular morbidity and mortality of CKD and particularly hemodialysis patients (10 to 20 times higher than normal population after adjustment for age and diabetes).¹⁹

The present study was designed to explore biomarkers of oxidative stress status and inflammation in a cohort of 32 hemodialysis patients before and after kidney transplantation, in order to find out the influence of transplantation on these markers in comparison to healthy individuals (here, the allograft donors). Based on the literature, our assumption was that inflammation and oxidative stress are heightened in hemodialysis patients, and transplantation has a significant impact on alleviating this problem by restoration of kidney function.^{8,9} Our results indicated that reformation of GFR by transplantation has a significant effect in lowering CRP level, even though it was still higher compared to healthy controls. This finding is in agreement with other studies which displayed either a significant reduction of CRP level or the inverse correlation that existed between inflammatory markers and GFR level.^{8,20} Given that CRP, as a prototype of inflammation, is a strong predictor of subsequent cardiovascular events and all-cause mortality in the hemodialysis patients,^{21,22} kidney transplantation would potentially improve survival of these individuals.

Except for serum TAC, no unique element of serum antioxidants complex could perfectly demonstrate the protective efficacy of blood. In addition, TTMs are important elements in antioxidant defense mechanism with its pertinent cardiovascular disease.²³ For these reasons, we measured TAC and TTMs levels along with lipid peroxidation, and observed a higher lipid peroxidation level in parallel with lower TAC and TTMs before transplantation that significantly reduced after transplantation, albeit they did not reach to a significant level as of healthy individuals. In agreement with our results, the majority of the studies are in favor of increased level of lipid peroxidation in patients on hemodialysis when compared with healthy individuals and after successful kidney transplantation.^{8,9,24,25}

Two lines of antioxidants which antagonize free radicals comprise nonenzymatic antioxidants like vitamin E, vitamin C, β -carotene, and coenzyme Q, and also enzymes such as SOD, glutathione peroxidase, and catalase.² We observed different

patterns in the plasma activity of antioxidant defense enzymes. While activity of SOD was significantly higher before transplantation, the level of glutathione peroxidase was significantly lower and activity of catalase did not differ to a significant level. Various studies reported a considerable variation on the status of these enzymes in hemodialysis patients with both decreased and increased or even no changes in their activities. Indeed, one report has displayed elevated levels of SOD and glutathione peroxidase in patients with CKD, as a defensive antioxidant system, but this was abrogated when they were going to maintenance hemodialysis.²⁶ Other studies have shown a significant defect in glutathione peroxidase scavenging system and also increased or decreased activity of SOD in patients on hemodialysis.²⁷⁻²⁹

In line with other reports, after successful kidney transplantation, a significant improvement, although not normalization, in most inflammatory and oxidative biomarkers was observed. These consequences may contribute to the reduced cardiovascular complications and improved survival provided to hemodialysis patients by recovery of kidney function after transplantation.^{8,9,20,24} It is well appreciated that inflammatory state is increased in kidney function impairment, and factors such as reduced clearance of cytokines, activated neutrophils, malnutrition, hypertension, volume overload, and diabetes mellitus have dominant acts on this state.^{2,30} Reactive oxygen species discharged by activated neutrophils may explain part of the oxidative stress seen in chronic inflammation due to activation of transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells, which controls inflammatory mediator gene expression.³¹ Furthermore, hemodialysis procedure causes further activation of immune cells, owing to incompatibility from membrane and dialysate exposure, and increases production of reactive oxygen radicals, leading to an acute augmentation of inflammation and oxidative stress.³² Therefore, strategies that reverse the harmful effects of these molecules or diminish their production can potentially improve the cardiovascular morbidity and mortality of hemodialysis patients.³³

The median posttransplant GFR (after 3 months) was 60.3 mL/min/1.73 m² in our patients and the mean hemoglobin level was 12.6 mg/dL. There was no significant correlation between oxidative

stress markers or CRP and estimated GFR or hemoglobin levels in this cohort. Other studies demonstrated a strong relationship between GFR and these markers,^{4,5,20} or in some instances no correlation²⁵; however, they included different patients in various stages of CKD.

There were some limitations in the present study. We did not evaluate the effect of some confounding factors such as duration of dialysis before transplantation or impact of immunosuppressive drugs on biomarker levels. Likewise, because of the short duration of the study, we could not assess the impact of oxidative stress on kidney or patient outcome.

CONCLUSIONS

The present study suggests that oxidative stress and inflammation are surged in hemodialysis patients, which could be improved partly and significantly by restoration of kidney function after kidney transplantation. Therefore, successful kidney transplantation particularly in whom with acceptable kidney function and no ongoing inflammation such as rejection or active infection may endeavor an improved survival for ESRD patients in addition to providing better quality of life.

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CONFLICT OF INTEREST

None declared.

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