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Evaluation of oxidant and antioxidant status in living donor renal allograft transplant recipients

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Abstract The objective of this study was to evaluate the oxidant and antioxidant status in living donor renal allograft transplant recipients. Ninety-two renal transplant recipients with mean age of 34.75 ± 11.22 years were included in the present study. Venous samples of the recipients were drawn: before the transplant (baseline), 5 min after reperfusion, and 2 weeks after transplant. Samples were processed for the measurement of markers of oxidant and antioxidant status viz. malondialdehyde, catalase, glutathione peroxidase, reduced glutathione, ascorbic acid, and total antioxidant system. The mean baseline levels of reduced glutathione, ascorbic acid, and total antioxidant system were 1.61 ± 0.84 mg/g hemoglobin, 3.64 ± 1.49 mg/dL, and 1.42 ± 0.14 mmol/L which decreased at 5 min after reperfusion to 1.32 ± 0.72 ($p = 0.010$), 2.96 ± 1.25 ($p = 0.002$), and 1.36 ± 0.12 ($p = 0.042$), respectively. The malondialdehyde levels increased from a baseline value of 3.11 ± 1.02 µmol/mL to 3.32 ± 1.09 at 5 min after reperfusion ($p = 0.344$) and 4.01 ± 1.21 ($p = 0.000$) at 2 weeks. Glutathione peroxidase level decreased from 68.59 ± 32.79 units/g hemoglobin (baseline) to 63.65 ± 32.92 at 5 min after reperfusion ($p = 0.530$) and increased significantly at 2 weeks to 86.38 ± 37.18 ($p = 0.00$). There was no significant change in the catalase level. In living donor renal transplantation, oxidative stress starts after reperfusion and is reflected by fall in antioxidant factors and enzymes in the early period. Over the next 2 weeks, there is increased oxidative stress and simultaneous strengthening of antioxidant system which is implied by increase in malondialdehyde and improvement in the markers of antioxidant status.

Keywords Renal transplant · Oxidative stress · Lipid peroxidation · Malondialdehyde · Antioxidants

Introduction

Renal transplantation is inevitably associated with a period of ischemia starting at the recovery of organ from the donor until its reperfusion in the recipient [1]. This ischemia reperfusion injury of the graft kidney induces oxidative stress in the recipient which is defined as an imbalance between the formation of reactive oxygen species and the total antioxidant capacity of the individual [2]. Oxidative stress is further associated with an increased rate of acute rejection, primary non-function of the graft, delayed graft function or initial poor graft function, decreased creatinine clearance, proteinuria, and graft loss [3–6]. Also the long-term effects of oxidative stress have been of special interest in renal transplantation [7, 8]. The measurement of oxidant activity and antioxidant system function may indicate the individual's liability to oxidant induced diseases and the degree of oxidant damage occurring at a certain time. It may also lead to development of clinical strategies to combat oxidative injuries to the graft kidney. In this study, the oxidant and antioxidant status of living donor renal

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allograft transplant recipients was evaluated by measurement of plasma malondialdehyde (MDA), as marker of lipid peroxidation, and by measurement of antioxidant enzymes like glutathione peroxidase (G-Px), catalase (CAT), and the non-enzymatic antioxidant factors like reduced glutathione (GSH), ascorbic acid (AA), and total antioxidant system (TAS). These markers of oxidant and antioxidant status were measured before, during, and after the renal transplant, and results were statistically analyzed.

Materials and methods

Study design and protocol

This was a prospective study conducted between May 2012 and May 2014 in Department of Renal Transplant Surgery and Department of Biochemistry of Post Graduate Institute of Medical Education and Research, Chandigarh, India. The inclusion criteria for the study were end-stage renal disease patients planned for living donor renal allograft transplant with no prior positive crossmatch and donor's body mass index $\leq$30 kg/m^2, whereas patients on drugs like allopurinol, desferroxamine, and antioxidants and patients infected with Hepatitis B, Hepatitis C, or human immunodeficiency virus (HIV) were excluded from this study. According to the inclusion and exclusion criteria, 92 subjects were included in this study and three subjects died during the study and were later excluded from the statistical analysis of the results. The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration, and the study (project number P-107) was approved by the Institute Ethics Committee of PGIMER, Chandigarh (India) vide letter number PGI/IEC/2011/709-10, dated 16/01/2012. All the subjects included in this study were identified by numbers and written informed consent was obtained. First blood samples (5.0 mL venous, baseline samples) were drawn from the patients before the transplant surgery and were quickly aliquoted into vacutainers containing heparin to prevent coagulation for the antioxidant studies. The samples were kept in ice during transportation to the laboratory and were processed immediately. Induction immunosuppression comprised either antithymocyte globulin or basiliximab and maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil/sodium and steroids. Immunosuppressant drugs were started at 48 h prior to surgery as per the standard protocol of the department. All donor nephrectomies were done laparoscopically using the conventional 3–4 port technique. Custodiol® HTK solution (DR. FRANZ KOHLER CHEMIE GMBH, Neue Bergstra βe 3-7, 64665 Alsbach-Hahnlein, Germany) was used as preservation solution, and static cold storage technique of perfusing and cooling the graft kidney was followed. Recipient surgery was performed as per the standard procedure for renal transplant. Injection hydrocortisone 500 mg, injection furosemide 100 mg, and injection mannitol 20 % were administered intravenously just before reperfusion of the graft kidney. Second patient sampling was done during surgery, at 5 min after reperfusion of the graft kidney from the graft renal vein. Third blood samples were drawn at 2 weeks after the transplant surgery. These first (baseline), second, and third blood samples were used for the assessment of markers of oxidant and antioxidant status like MDA, CAT, G-Px, GSH, AA, and TAS.

Assessment of oxidant and antioxidant status

The reagents used for this study were of analytical grade from commercially available company. Whole blood was processed for measurement of GSH and G-Px, and the maximum storage time for whole blood was 1 week at 4 °C. Rest of the blood was centrifuged at 3000 radian per minute (rpm) for 10 min to separate the erythrocytes from the plasma. Plasma samples were stored at −70 °C for measurement of MDA, AA and TAS. Erythrocytes were processed to prepare hemolysate, stored at −70 °C for measurement of CAT. Hemolysates were prepared as follows: The packed red cells were washed three times with 0.9 % sodium chloride. The buffy coat was removed and the cells hemolyzed by adding an equal volume of double distilled water and subsequent centrifugation for 10 min at 3000 rpm. The cell stroma was discarded and supernatant was collected as hemolysate [9]. Hemoglobin content of the whole blood and hemolysate was estimated using the Drabkins method [10].

Measurement of oxidant status (MDA)

MDA was determined according to the method of Ohkawa et al. [11]. Sample was homogenized with aqueous trichloroacetic acid and thiobarbituric acid, boiled for 15 min, and then cooled down to room temperature. After addition of pyridine and butanol (3:1) and sodium hydroxide, the homogenate was centrifuged. MDA was directly quantified on the basis of third-derivative absorption spectrum of the pink complex formed which was measured spectrophotometrically at 535 nm, and results were expressed as μmole/mL of plasma [11].

Measurement of antioxidants status

CAT activity

The CAT activity in hemolysate samples was measured by the method as described by Aebi et al. [9]. CAT catalyzes

the breakdown of hydrogen peroxide which was measured spectrophotometrically at 240 nm and expressed in units/g hemoglobin (Hb) [9].

G-Px activity

G-Px activity was assayed in whole blood as per the manufacturer's instruction provided with RANDOX Ransel kit (Randox Laboratories Limited, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom). G-Px catalyzed the oxidation of GSH by cumene hydroperoxide, and in the presence of glutathione reductase and NADPH, the oxidized glutathione was immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm was measured, and results were expressed in units/g Hb.

GSH

Total GSH in red blood cells was determined according to the procedure described by Beutler et al. [12]. The protein-free filtrate obtained after precipitating the blood sample with metaphosphoric acid was used to react with 5,50-dithiobis-nitrobenzoic acid. This reaction produced a relatively stable yellow-colored solution in proportion to concentration of GSH in the sample, which was measured spectrophotometrically at 412 nm. This method provided an accurate estimation of GSH in the sample, and the values were expressed in mg/g Hb [12].

AA

AA level in plasma was measured by the method described by Frei et al. [13]. In this method, AA was oxidized to dehydro-ascorbic acid by cupric sulfate. The dehydro-ascorbic acid in a strong acidic solution reacted with 2,4-dinitrophenyl hydrazine to form dinitrophenyl hydrazone. The hydrazone in the presence of strong sulfuric acid solution developed yellowish-orange color which was measured spectrophotometrically at 520 nm, and the concentration of AA was expressed in mg/dL of plasma [13].

TAS

TAS was assayed in plasma as per the manufacturer's instruction provided with RANDOX Ransel kit (Randox Laboratories Limited, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom). This kit enabled assessment of the integrated antioxidant system which encompasses all biological components with antioxidant activity. ABTS® (2, 2′-Azino-di-[3-ethylbenzthiazoline sulfonate]) was incubated with a peroxidase (metmyoglobin) and hydrogen peroxide to produce the radical cation $ABTS^{®*+}$. This had a relatively stable blue-green color, which was measured at 600 nm. Antioxidants in the added sample caused suppression of this color production to a degree which was proportional to their concentration, and the results were expressed in mmol/L of plasma.

Statistical analysis

Statistical analysis was performed with SPSS program (version 20.0; SPSS Inc., Chicago, IL). "One way" analysis of variance (ANOVA) and 2-sided Dunnett test were used to analyze the results of the markers of oxidant and antioxidant status. All data were expressed as mean ± standard deviation (SD), and p values less than 0.05 were considered significant.

Results

Demographic profile of donors and recipients

In this study, ninety-two living donor renal transplant recipients with mean age of 34.75 ± 11.22 years were included and three patients with glomerulonephritis as the basic disease died during this study and were excluded from the statistical analysis of results. Tables 1 and 2 show the demographics of the recipients and donors included in this study. Mean warm ischemia time (WIT) and cold ischemia time (CIT) were 4.57 ± 1.09 and 92.0 ± 63.57 min, respectively. Glomerulonephritis was the basic disease in 64 (69.6 %) patients, and the mean duration of chronic kidney disease (CKD) stage V was 16.17 ± 14.7 months. The mean age and body mass index (BMI) of the donors were 41.72 ± 9.9 years and 24.65 ± 3.70, respectively.

Measurement of markers of oxidant and antioxidant status

Table 3 depicts the measurement of markers of oxidant and antioxidant status. The mean baseline levels of GSH, AA, and TAS were 1.61 ± 0.84 mg/g Hb, 3.64 ± 1.49 mg/dL, and 1.42 ± 0.14 mmol/L which decreased at 5 min after reperfusion to 1.32 ± 0.72 mg/g Hb ($p = 0.010$), 2.96 ± 1.25 mg/dL ($p = 0.002$), and 1.36 ± 0.12 mmol/L ($p = 0.042$), respectively. The mean MDA level increased from a baseline value of 3.11 ± 1.02 μmol/mL to 3.32 ± 1.09 μmol/mL at 5 min after reperfusion ($p = 0.344$). After two weeks of transplant, MDA increased to 4.01 ± 1.21 μmol/mL, which was statistically significant

Table 1 Recipient demographics

Number of subjects (recipients)	92
Age (years)	34.75 ± 11.22 (range 13–63)
Gender (male:female)	79:13 (85.9:14.1 %)
Basic disease	Glomerulonephritis 64 (69.6 %)
	Hypertension 11 (12 %)
	Chronic interstitial nephritis 3 (3.3 %)
	Autosomal dominant polycystic kidney disease 3 (3.3 %)
	Diabetes mellitus 8 (8.7 %)
	Obstructive uropathy 3 (3.3 %)
Human leucocyte antigen matching for Class I antigens	0/4 match 45 (48.9 %)
	2/4 match 40 (43.5 %)
	3/4 match 5 (5.4 %)
	4/4 match 2 (2.2 %)
Immunosuppression	Tacrolimus + mycophenolate mofetil/sodium + prednisolone 65 (70.7 %)
	Antithymocyte globulin + tacrolimus + mycophenolate mofetil/sodium + Prednisolone 16 (17.4 %)
	Basiliximab + tacrolimus + mycophenolate mofetil/sodium + prednisolone 11 (12 %)
Warm ischemia time (min)	4.57 ± 1.09 (range 2–10)
Cold ischemia time (min)	92 ± 63.5 (range 43–540)
Duration of chronic kidney disease stage V (months)	16.17 ± 14.7 (range 3–72)

All values for age, warm ischemia time, cold ischemia time, and duration of chronic kidney disease stage V are expressed as mean ± SD

Out of 92 subjects included in this study, three subjects with glomerulonephritis as basic disease died and were excluded from the statistical analysis of the results

Table 2 Donor demographics

Age (years)	41.72 ± 9.9 (range 22–63)
Body mass index	24.65 ± 3.70 (range 16.03–37.46)
Relation to the recipient	Parents 35 (38 %)
	Spouses 33 (35.9 %)
	Siblings 13 (14.1 %)
	Unrelated 10 (11.4 %)
	Offspring 1 (1.1 %)
Comorbidity	No comorbidity 77 (83.7 %)
	Hypertension alone 6 (6.5 %)
	Hypertension + asthma 1 (1.1 %)
	Hypothyroidism 1 (1.1 %)
	Left nephrolithiasis + generalized dystonia 1 (1.1 %)
	Paraaortic mass 1 (1.1 %)
	Treated carcinoma cervix 1 (1.1 %)
	Dyslipidemia 1 (1.1 %)
	Scarred left kidney 1 (1.1 %)
	Non alcoholic fatty liver disease + cholelithiasis + dyslipidemia 1 (1.1 %)
	Lichen planus + hypothyroidism 1 (1.1 %)

All values for age and body mass index are expressed as mean ± SD

when compared to the baseline ($p = 0.000$). The G-Px levels decreased from 68.59 ± 32.79 units/g Hb (baseline) to 63.65 ± 32.92 units/g Hb at 5 min after reperfusion ($p = 0.530$) and then increased significantly at 2 weeks to 86.38 ± 37.18 units/g Hb ($p = 0.001$). There was no significant change in the CAT levels. The blood levels of GSH, AA, and TAS were not statistically significant at 2 weeks after transplant.

Table 3 Measurement of markers of oxidant and antioxidant status before, during and after renal transplant ($n = 89$)

Markers of oxidant and antioxidant status	Before transplant; baseline (mean ± SD)	5 min after reperfusion (mean ± SD)	2 weeks after transplant (mean ± SD)
MDA (μmol/mL)	3.11 ± 1.02	3.32 ± 1.09 ($p = 0.344$)	**4.01 ± 1.21 (p = 0.000)**
GSH (mg/g Hb)	1.61 ± 0.84	**1.32 ± 0.72 (p = 0.010)**	1.40 ± 0.58 ($p = 0.089$)
AA (mg/dL)	3.64 ± 1.49	**2.96 ± 1.25 (p = 0.002)**	3.25 ± 1.23 ($p = 0.094$)
CAT (units/g Hb)	1297.44 ± 784.31	1155.56 ± 780.21 ($p = 0.402$)	1263.15 ± 875.90 ($p = 0.944$)
G-Px (units/g Hb)	68.59 ± 32.79	63.65 ± 32.92 ($p = 0.530$)	**86.38 ± 37.18 (p = 0.001)**
TAS (mmol/L)	1.42 ± 0.14	**1.36 ± 0.12 (p = 0.042)**	1.39 ± 0.15 ($p = 0.376$)

All values are expressed as mean ± SD, and p values less than 0.05 were considered significant

The values with significant difference with base line are shown in bold

Eighty subjects had either glomerulonephritis ($n = 61$; 76.25 %) or hypertension ($n = 11$; 13.75 %) or diabetes mellitus ($n = 8$; 10.0 %) as the cause of end stage renal disease. The baseline GSH levels in the diabetics were found to be statistically low as compared to the patients with glomerulonephritis ($p = 0.021$). However, it was insignificant at 5 min after reperfusion and at 2 weeks after transplant. All other oxidative stress markers at baseline, 5 min after reperfusion, and 2 weeks after transplant were comparable among the three groups (Table 4).

Discussion

In renal transplantation, allograft kidneys are prone to oxidative stress by pre and post-transplant conditions that can cause imbalance between oxidants and antioxidants. In the pre-transplant conditions, the inflammatory state associated with diabetes, end-stage renal disease, and hemodialysis plays an important role in causing oxidative stress [14]. During and after renal transplantation, ischemia–reperfusion of the allograft and the immunosuppressive drugs are the main sources of reactive oxygen species generation, leading to oxidative stress [15, 16]. Reperfusion and oxidative injury also occur during kidney preservation and may correlate with the immediate and long-term kidney function [17]. Although some studies on living donor transplant recipients have shown improvement of oxidative stress parameters immediately after kidney transplantation and which continues up to a month [15]. In deceased donor renal transplants, recipients are at greater risk of developing reperfusion injury and oxidative stress-induced kidney injury [17]. The adverse effects of oxidative stress and inflammation on renal transplantation have been shown by experimental studies in animals, observational evidence from population-based studies, and controlled clinical trials. In addition to adversely affecting the allograft, oxidative stress may play a major role in the pathogenesis of systemic inflammation, hypertension, cardiovascular disease, metabolic syndrome, and neoplasms in transplant recipients [18]. In the present study, oxidative stress in renal transplant recipients was evaluated by measurement of markers of oxidant and antioxidant status.

The MDA levels were found to be elevated shortly after transplant although significant increase was seen only at 2 weeks. The initial rise in MDA can be due to oxidative stress induced by surgery and ischemia reperfusion injury of the allograft kidney. Subsequent increase in MDA levels over next 2 weeks could be attributed to lipid peroxidation and oxidative stress secondary to intense immunosuppression with drugs like tacrolimus. In another study, the antioxidant enzymes and degree of lipid peroxidation in renal transplant patients had been studied and compared with a control group [19]. MDA was found to be high in the study group before transplant, decreased slightly at 48 h after transplant, and then at 1 week, there was significant increase which persisted for 2 weeks after transplant. The pre-transplant high MDA was explained with increased lipid peroxidation as a result of bio-incompatibility of the dialyzer membrane during hemodialysis. Although the reason for fall in MDA levels was poorly understood, the later rise in MDA was thought to be because of immunosuppressive therapy with cyclosporine and corticosteroids [19].

Cyclosporine induces production of reactive oxygen species, which increase the oxidative state and also upregulates the nitric oxide (NO) system, thus increasing the endothelial NO synthase and plasma NO metabolite [20]. The relation between oxidative stress and immunosuppressive therapies had been studied in twenty-six renal transplant patients with stable renal function and uneventful postoperative course. Combined immunosuppressive therapy with cyclosporine was associated with high values of MDA at 6 months after transplantation. On the contrary, statistically significant decrease in MDA levels was

Table 4 Measurement of markers of oxidant and antioxidant status in patients with different underlying diseases causing renal failure

Markers of oxidant and antioxidant status	Sample collection	Glomerulonephritis (mean ± SD)	Hypertension (mean ± SD)	Diabetes mellitus (mean ± SD)
MDA (μmol/mL)	Baseline	3.14 ± 0.99	3.07 ± 1.13 ($p = 0.98$)	2.66 ± 1.04 ($p = 0.47$)
	5 min after reperfusion	3.33 ± 1.08	3.29 ± 0.92 ($p = 0.99$)	2.68 ± 0.97 ($p = 0.28$)
	2 weeks after transplant	3.92 ± 1.16	4.58 ± 1.25 ($p = 0.20$)	3.95 ± 1.07 ($p = 0.99$)
GSH (mg/g Hb)	Baseline	1.65 ± 0.89	1.69 ± 0.52 ($p = 0.98$)	**0.75 ± 0.39 (p = 0.021)**
	5 min after reperfusion	1.35 ± 0.76	1.24 ± 0.46 ($p = 0.90$)	0.73 ± 0.29 (p = 0.078)
	2 weeks after transplant	1.42 ± 0.56	1.78 ± 0.67 ($p = 0.15$)	1.03 ± 0.57 ($p = 0.21$)
AA (mg/dL)	Baseline	3.73 ± 1.54	3.61 ± 1.23 ($p = 0.96$)	2.71 ± 0.76 ($p = 0.19$)
	5 min after reperfusion	3.07 ± 1.30	2.45 ± 0.84 ($p = 0.27$)	2.50 ± 1.03 ($p = 0.47$)
	2 weeks after transplant	3.46 ± 1.29	2.87 ± 1.08 ($p = 0.31$)	2.58 ± 0.84 ($p = 0.17$)
CAT (units/g Hb)	Baseline	1371.70 ± 885.20	1196.95 ± 347.22 ($p = 0.79$)	1231.07 ± 631.61 (p = 0.90)
	5 min after reperfusion	1195.90 ± 800.70	1177.20 ± 704.13 ($p = 0.99$)	1045.23 ± 1010.24 ($p = 0.88$)
	2 weeks after transplant	1314.93 ± 895.80	1368.81 ± 896.62 ($p = 0.98$)	1028.41 ± 612.74 ($p = 0.69$)
G-Px (units/g Hb)	Baseline	61.20 ± 31.20	76.02 ± 31.51 ($p = 0.32$)	84.33 ± 34.12 ($p = 0.16$)
	5 min after reperfusion	64.31 ± 31.21	51.11 ± 17.23 ($p = 0.35$)	45.42 ± 20.11 ($p = 0.23$)
	2 weeks after transplant	89.62 ± 36.02	65.81 ± 33.61 ($p = 0.10$)	66.83 ± 26.34 ($p = 0.24$)
TAS (mmol/L)	Baseline	1.44 ± 0.15	1.40 ± 0.13 ($p = 0.86$)	1.36 ± 0.12 ($p = 0.74$)
	5 min after reperfusion	1.37 ± 0.12	1.36 ± 0.08 ($p = 0.99$)	1.34 ± 0.06 ($p = 0.96$)
	2 weeks after transplant	1.39 ± 0.16	1.41 ± 0.12 ($p = 0.97$)	1.27 ± 0.11 ($p = 0.55$)

All values are expressed as mean ± SD, and p values less than 0.05 were considered significant

The values with significant difference with glomerulonephritis are shown in bold

observed in the patients receiving tacrolimus-based immunosuppression, and it was concluded that tacrolimus was associated with improved free radical metabolism and decreased oxidative stress [16]. In the present study, all transplant recipients received tacrolimus-based immunosuppression, and the results showed that tacrolimus might contribute to the oxidative stress in early post-transplant period.

In this study, the evaluation of antioxidant factors and enzymes like GSH, AA, and TAS showed an initial fall in the levels which gradually improved significantly over 2 weeks after transplant. Although G-Px levels also fell at 5 min after reperfusion, the rise at 2 weeks of transplant was significant. The level of CAT did not change significantly throughout the study. The initial fall in the antioxidant factors and enzymes might imply that the antioxidant system is depleted by a continuous oxidative stress. The subsequent strengthening of the antioxidant system as suggested by the improvement in markers could be a reflection of the body's natural response to counteract ongoing oxidative stress and lipid peroxidation. CAT activity had been shown to correlate with the concentration of hypoxanthine (ischemia marker) in the graft renal vein and other mediators of oxidative stress [21]. A study on the temporary variation of oxidative stress in renal transplant recipients revealed elevated levels of reactive oxygen species within 48 h after transplantation resulting in high levels of glutathione reductase and a marked decrease in plasma and erythrocyte G-Px. Thereafter, there was a significant improvement in the activity of antioxidant enzymes, but without normalization; the total glutathione levels and the activity of various enzymes approached the average values of the control group. The results of the study showed a high "oxidative stress" rate, resulting from the equilibrium between the production of free radicals and the activity of antioxidants, the former being higher proportionally [22].

The impact of underlying diseases causing renal failure on the oxidant and antioxidant status of the subjects was also studied. As mentioned earlier, diabetes mellitus in association with end-stage renal disease is invariably associated with inflammation and oxidative stress. A study comparing the diabetic and non-diabetic end-stage renal disease patients has shown that although the magnitude of oxidative stress was similar between the two groups before transplantation, it was significantly greater after transplantation in the diabetic patients [23]. In the present study, the low baseline GSH levels in the diabetic group were suggestive of depleted antioxidant system likely due to longstanding oxidative stress. But we did not observe any significant difference in post-transplant oxidative stress in the diabetics.

An interesting observation in this study was significant rise in the G-Px levels at 2 weeks after transplant after a brief initial fall. Apart from being viewed as strengthening of antioxidant system, the result probably correlate with the findings of Yoshimura et al. that extracellular G-Px originates from renal proximal tubular cells [24, 25]. As the graft kidney starts functioning after transplant and returns to normal function at 2 weeks post transplant, synthesis of extracellular G-Px from proximal renal tubules is expected to increase with consequent increase in the blood G-Px levels. The improvement in levels of GSH and AA after transplant could be correlated with hemoglobin. As red blood cells represent an effective antioxidant system, it is possible that long-standing anemia may lead to depletion of the glutathione-mediated antioxidant mechanism, with consequent increased oxidative stress. A study in renal transplant recipients has shown inverse relation of reactive oxygen species formation with hematocrit at multiple regression analysis. They concluded that the relative anemia may favor an imbalance between oxidative stress and the antioxidant barrier [2]. All end-stage renal disease patients tend to have anemia which improves significantly after transplant as the renal function normalizes. Increased hemoglobin after transplant might have contributed to the improvement in antioxidant status.

This study was planned with the intention of evaluating the markers of oxidant and antioxidant status in kidney transplantation. The change in the markers before, during, and after transplant suggested the occurrence of oxidative stress with progressive lipid peroxidation and simultaneous strengthening of antioxidant defense mechanisms. As described earlier oxidative stress is a situation in which an overloading of "oxidants" or free radicals damages or destroys a cell. The natural balance between toxic oxidants and protective antioxidant defenses can jeopardize under certain circumstances. In the present study, we assume that renal transplantation procedure along with intense immunosuppression in the early post operative period could have led to an imbalance between the oxidant stress and antioxidant capacity resulting in the above mentioned findings. We accept the fact that oxidative stress could have been better studied with the use of new and more sensitive markers of oxidant and antioxidant status, which are commercially available. For instance, in the assessment of MDA, the common methods of detection are insufficiently sensitive and perturbed by too much interference coming from MDA-related species or overestimation derived from stressing analysis conditions. With the inclusion of deceased donor renal transplant recipients with longer organ ischemia time, we could have studied the oxidative stress in deceased donor renal transplants as well. Since the use of new and sensitive markers of oxidative stress might not have been cost effective and deceased donor transplants are not frequently done in the institute, the present study was planned in living donor transplant recipients and using conventional markers of oxidative stress. Further randomized studies on markers of oxidant and antioxidant status are required for the assessment of oxidative stress and its impact in renal transplantation.

In conclusion, in renal transplant recipients, oxidative stress starts after reperfusion and is reflected predominantly by fall in antioxidant factors and enzymes in the early period. Over the next 2 weeks, there is increased lipid peroxidation which could be because of both ischemia/reperfusion during surgery and then intense immunosuppression in early post-transplant period. There is simultaneous strengthening of antioxidant system which is implied by improvement in the markers of antioxidant status. Further, randomized studies involving deceased and living donor renal transplant recipients and the use of new and sensitive markers of oxidative stress are required to study oxidative stress and its association with allograft kidney outcome.

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Compliance with ethical standards

Conflict of interest None declared.

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