Oxidative DNA Damage Is Increased in Living Kidney Donors

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ABSTRACT

Background. Long-term consequences of donor nephrectomy might be reduced kidney function, increased risk for cardiovascular disease, and impaired quality of life. The purpose of the current cross-sectional study was to evaluate the relationship between clinical, laboratory, and donation-specific outcomes of living kidney donors and systemic oxidative DNA damage.

Methods. We conducted a cross-sectional study and assessed retrospectively pre- and postdonation data from 60 donors who donated between 2010 and 2015. Plasma malondialdehyde levels and 8-hydroxy-2′-deoxyguanosine/deoxyguanosine ratio (8-OHdG/dG ratio) were determined as oxidative stress markers. Catalase, carbonic anhydrase, and paraoxonase (PON) activities were measured as antioxidants.

Results. Approximately 3 years after donation, the hypertensive donor ratio was 12%, and 11% of the donors had glomerular filtration rate <60 mL/min/1.73 m^2. Mean serum urea ($P = .001$) and serum creatinine levels ($P = .001$) were increased; creatinine clearance level (126.2 ± 35.5 vs 94.6 ± 26.8, $P = .001$) was decreased in the postdonation period. There was a significant positive correlation between predonation serum urea and 8-0HdG/dG ratio ($r = 0.338$, $P = .016$) and predonation serum creatinine and 8-0HdG/dG ratio ($r = 0.442$, $P = .001$), while there was a significant negative correlation between serum creatinine and PON activity ($r = -0.545$, $P < .001$).

Conclusion. Our data have demonstrated that kidney donors exhibit increased oxidative DNA damage and decreased antioxidant activity. We propose that predonation serum creatinine is positively correlated with 8-0HdG/dG ratio and negatively correlated with antioxidant PON activity. This is the first study to demonstrate that plasma oxidative DNA damage increases in healthy kidney donors.

PREVIOUS studies have suggested that living kidney donors maintain long-term renal function and experience no increase in cardiovascular or all-cause mortality [1]. However, considerable attention has recently been paid to the possibility of an increased risk of chronic kidney disease (CKD) in living kidney donors [2]. Studies have emerged that demonstrated unfavorable outcomes after living kidney donation with regard to the long-term risk of CKD and increased risk of mortality [3].

Evidence shows that there is an increased incidence of atherosclerosis in CKD patients [4]. Although traditional risk factors such as hypertension, diabetes, and hyperlipidemia are highly prevalent in CKD patients, these factors cannot fully explain the increased cardiovascular morbidity in the CKD patient population [5]. There has been ample evidence to support the role of nontraditional risk factors

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for cardiovascular events in CKD patients such as oxidative stress and endothelial dysfunction [6].

Reactive oxygen species are a group of small reactive molecules that play critical roles in the regulation of various cell functions and biological processes. Endogenous antioxidants function as checkpoints to avoid untoward consequences of reactive oxygen species, and an imbalance in the oxidant/antioxidant mechanisms leads to a state of oxidative stress [7]. Reduced antioxidant capacity might play a major role in the initiation of DNA damage [8]. DNA oxidative damage has been identified as a useful index of oxidative stress and a possible indicator of cardiovascular disease and cancer risk. 8-hydroxy-2′-deoxyguanosine (8-OHdG) is considered to be a sensitive DNA damage marker [9].

The aim of the present study was to investigate the relationship between pre- and postdonation renal functions, oxidative DNA damage, and antioxidant capacity in living kidney donors.

METHODS

Study Population

We conducted a cross-sectional study and assessed retrospectively pre- and postdonation data from 60 living kidney donors who donated between 2010 and 2015. The study was approved by the local ethics committee, and all participants gave written informed consent. All study subjects were nonsmokers and did not consume alcohol. None of the subjects received antibiotics, corticosteroids, anti-inflammatory drugs, cytotoxic drugs, or vitamins during the study period.

Data Collection

Medical records and laboratory data were reviewed. Patient characteristics including age, gender, body mass index, and medical history including systolic and diastolic blood pressure and the time of nephrectomy were recorded. Laboratory data including serum renal function tests, hemoglobin, 24-hour albuminuria, 24-hour proteinuria, and creatinine clearance were also recorded.

Determination of DNA Damage

Fasting blood samples were obtained from all subjects and collected into tubes without coagulant. Serum was obtained by centrifugation at 2500 rpm for 15 minutes and stored at −80°C until assayed.

Isolation and hydrolyzation of DNA. DNA isolation from blood was performed according to Miller et al with some modifications [10]. Two mL of blood with ethylenediaminetetraacetic acid (EDTA) was mixed with 3 mL of erythrocyte lysis buffer and incubated for 10 minutes in ice, which was followed by centrifugation (10 minutes at 3500 rpm). The supernatant was decanted, and the pellet was thoroughly resuspended in sodium dodecyl sulfate (10%, v/v), proteinase K (20 mg/mL), and 1.9 mL leukocyte lysis buffer. The mixture was incubated at 65°C for 1 hour and then mixed with 0.8 mL of 9.5 M ammonium acetate. After centrifugation at 3500 rpm for 25 minutes, the clear supernatant (2 mL) was transferred to a new sterile tube, and the DNA was precipitated by the addition of 4 mL of ice-cold absolute ethanol. DNA samples were dissolved in Tris EDTA buffer (10 mM, pH 7.4) and then were hydrolyzed according to Shinenaga's method.

Analysis of 8-OHdG and dG by the high-performance liquid chromatography method. In the hydrolyzed DNA samples, 8-OHdG and dG levels were measured respectively by high-performance liquid chromatography (HPLC) with electrochemical (HPLC-ECD) and variable wavelength detector (HPLC-UV) systems as previously described [11]. A total of 20 µL of the final hydrolysate were analyzed by HPLC-ECD (HP, Agilent 1100 modular systems with HP 1049A ECD detector, Boblingen, Germany): column, reverse phase-C18 (RP-C18) analytical column (250 mm × 4.6 mm × 4.0 µm, Phenomenex, Torrance, Calif, United States). The mobile phase consisted of 0.05 M potassium phosphate buffer [pH 5.5] containing acetonitrile (97:3, v/v) with a flow rate of 1 mL/min. The dG and 8-OHdG concentrations were monitored based on absorbance (245 nm) and electrochemical reading (600 mV), respectively. Levels of dG and 8-OHdG were quantified using the standards of dG (Sigma Chemical Co., St. Louis, Mo, United States) and 8-OHdG (Cayman, Ann Arbor, Mi, United States); the level of 8-OHdG was expressed as the number of 8-OHdG molecules per 10^6 dG. The intra-assay coefficients of variation (CV) ranged from 3% to 7%, and interassay CV ranged from 4% to 9%; the lower numbers refer to the CV for the high standard, and the higher numbers refer to the CV for the low standard.

Determination of the Oxidative Stress Markers

Measurement of plasma malondialdehyde (MDA) concentration was performed according to Khoschsorur et al [12]. Briefly, 50 µL of plasma sample was mixed with 0.44 M H_3PO_4 and 42 mM tiobarbituric acid and incubated for 30 minutes in a boiling water bath. After rapidly cooling on ice, an equal volume of alkaline methanol was added to the sample, vigorously shaken, and centrifuged (3000 rpm for 3 minutes), and the aqueous layer was removed. Then, 20 µL of supernatant was analyzed by HPLC (HP, Agilent 1100 modular systems with fluorescence detector, Germany): column, RP-C18 (5 µm, 4.6 × 150 mm, Eclipse VDB-C18. Agilent); elution, methanol (40:60, v/v) containing 50 mM KH_2PO_4 buffer (pH 6.8); flow rate, 0.8 mL/min. Fluorometric detection was performed with excitation at 527 nm and emission at 551 nm. The peak of the MDA-tiobarbituric acid adduct was calibrated as a 1,1,3,3-tetraethoxypropane standard solution carried out in exactly the same process as with the plasma sample. The intra-assay and interassay CV were 3.5% and 5.2%, respectively.

Determination of Antioxidant Activity

The blood samples were centrifuged at 1,500 rpm for 20 minutes, and the plasma and buff coat were removed. After washing the paced red cells twice with NaCl (0.9%), the erythrocytes were hemolyzed with distilled water, and this hemolysate was used to determine antioxidant enzyme activity.

Carbonic anhydrase activity was assayed by hydration of carbon dioxide, which was measured by the method of Rickli and Wilbur-Anderson with bromothymol blue as an indicator [13]. The intra-assay and interassay CV for carbonic anhydrase were 4.8 and 7.3, respectively. Biochemical analysis of catalase (CAT) activity in erythrocytes was performed with a method described by Aebi [14]. The intra-assay and interassay CV for CAT were 2.3 and 3.9, respectively.

Serum paraoxonase (PON) assay was estimated spectrophotometrically using 5.5 mM 4-nitrophenylacetate as the substrate in 20 mM Tris–HCl buffer at a pH of 8.0. The increase in absorbance due

Table 1. Clinical and Demographic Characteristics of Living Kidney Donors

	Predonation (n = 60)	Postdonation (n = 60)	*P* Value
Age (years)	53.00 ± 12.20		
Sex (male/female)	18/42		
Body mass index (kg/m^2)	26.80 ± 4.90	27.50 ± 3.65	NS
Systolic blood pressure (mm Hg)	121.17 ± 38.56	128.76 ± 28.44	.055
Diastolic blood pressure (mm Hg)	78.43 ± 18.43	83.42 ± 09.20	NS
Serum urea (mg/dL)	28.80 ± 7.40	33.30 ± 8.16	.001
Serum creatinine (mg/dL)	0.74 ± 0.17	0.97 ± 0.22	.001
Hemoglobin (g/dL)	13.20 ± 1.40	13.30 ± 1.30	NS
24-hour albuminuria (mg/day)	8.47 ± 7.28	11.30 ± 8.30	NS
24-hour proteinuria (mg/day)	110.68 ± 48.70	119.52 ± 49.35	NS
Creatinine clearance (mL/min/1.72 m^2)	126.28 ± 34.06	94.60 ± 26.85	.001

Data is presented as mean ± standard deviation.
Abbreviation: NS, not significant.

to the formation of the yellow 4-nitro-phenol was monitored at 412 nm for 3 minutes [15].

Statistical Analysis

Descriptive statistics for continuous variables were expressed as mean ± standard deviation. Continuous measures were compared between the study group at the predonation and postdonation period using the Student *t* test or the Mann-Whitney U test. Dichotomous variables were analyzed with κ^2 test with Fisher's correction. Comparisons between initial and final mean values for the same patient were performed using the paired *t* test or Wilcoxon signed-rank test. Pearson's correlation test was performed to explore the linear relationships between 8-OHdG/dG ratio and pre- and postdonation renal function parameters. Analysis was conducted using SPSS 19.0 for Windows (SPSS Inc., Chicago, Ill, United States). All statistical tests were 2-sided, and a P value $< .05$ was considered statistically significant.

RESULTS

Clinical and demographic characteristics of the living kidney donors are given in Table 1. The mean systolic blood pressure level was inclined to be higher in the postdonation period, although statistically nonsignificant, when compared to the predonation period (Table 1).

Table 1 also shows the renal function parameters of the study population. The mean serum urea level was significantly higher in the postdonation period as compared to the predonation time ($P = .001$). Similarly, the mean serum creatinine level was significantly higher in the postdonation period as compared to the predonation time ($P = .001$). Whereas the mean 24-hour albuminuria levels were similar, 24-hour creatinine clearance was significantly lower in the postdonation period when compared to the predonation period ($P > .05$ and $P = .001$, respectively).

Table 2. Oxidative Stress Parameters of Living Kidney Donors

	Postdonation (n = 60)
Malondialdehyde (μmol/L)	2.44 ± 1.06
8-OHdG/dG	1.24 ± 0.47
Catalase (EU/gHb)-1	28.50 ± 9.08
Carbonic anhydrase (EU/gHb)-1	2.64 ± 1.25
Paraoxonase (EU/mL)	293.95 ± 65.25

Data is presented as mean ± standard deviation.
Abbreviation: 8-OHdG/dG, 8-hydroxy deoxyguanosine/deoxyguanosine ratio.

Table 2 shows the oxidative stress marker MDA, oxidative DNA damage (8-OHdG/dG ratio), and antioxidant parameters CAT, carbonic anhydrase (CAN), and PON activities of the study group in the postdonation period.

When the clinical and laboratory parameters and oxidative and antioxidant markers were pooled for correlation analysis, predonation serum urea was positively correlated with 8-OHdG/dG ratio ($r = 0.338$, $P = .016$). There was also a positive correlation between predonation serum creatinine and 8-0HdG/dG ratio ($r = 0.442$, $P = .001$); while there was a significant negative correlation between predonation serum creatinine and postdonation PON activity ($r = -0.545$, $P < .001$) (Table 3).

DISCUSSION

In the present cross-sectional study, we assessed the relationship between oxidative DNA damage as determined by 8-OHdG/dG ratio, antioxidant enzymes, and pre- and postdonation kidney function parameters in living kidney donors. The main finding of the study is the demonstration of a positive correlation between predonation serum urea and creatinine and postdonation oxidative DNA damage (8-OHdG/dG ratio) in kidney donors.

According to our results, the mean 24-hour creatinine clearance after 3 years follow-up was decreased from the predonation mean level of 126.2 ± 35.5 mL/min to postdonation mean level of 94.6 ± 26.8 mL/min. These results are in line with other studies reporting on a median follow-up of approximately 10 years [16–18]. Najarian et al reported a significant decline in creatinine clearance of living kidney donors after a mean follow-up of 16 years compared with the baseline [19]. Similarly, Muzaale et al showed an increased risk of end-stage renal disease for donors compared with matched healthy nondonors in a cohort of 96.217 donors [20]. Kasiske et al reported that the glomerular filtration rate measured by plasma iohexol clearance increased 1.47 ± 5.02 mL/min per year in kidney donors between 6 and 36 months [21]. The authors also

Table 3. Correlation Analysis Between Predonation Laboratory Parameters and Postdonation Oxidant and Antioxidant Enzymes of Living Kidney Donors

Predonation	MDA (μmol/L)	8-OHdG/dG	Catalase (EU/gHb)	Carbonic Anhydrase (EU/gHb)	Paraoxonase (EU/mL)
Serum urea (mg/dL)	r = 0.127 $P > .05$	r = 0.338 $P = .016$*	r = −0.057 $P > .05$	r = −0.032 $P > .05$	r = −0.331 $P = .019$*
Serum creatinine (mg/dL)	r = 0.324 $P = .022$*	r = 0.442 $P = .001$†	r = −0.086 $P > .05$	r = −0.050 $P > .05$	r = −0.545 $P < .001$†
Hemoglobin (g/dL)	r = −0.089 $P > .05$	r = −0.159 $P > .05$	r = 0.266 $P > .05$	r = 0.104 $P > .05$	r = 0.378 $P = .007$*
Creatinine clearance (mL/min/1.72 m^2)	r = −0.038 $P > .05$	r = −0.014 $P > .05$	r = 0.057 $P > .05$	r = 0.054 $P > .05$	r = 0.101 $P > .05$
24-hour albuminuria (mg/day)	r = 0.182 $P > .05$	r = 0.213 $P > .05$	r = −0.014 $P > .05$	r = −0.027 $P > .05$	r = −0.123 $P > .05$

Abbreviations: 8-OHdG/dG, 8-hydroxy-2′-deoxyguanosine/deoxyguanosine ratio; MDA, malondialdehyde.
*$P < .05$.
†$P < .005$.

reported that serum parathyroid hormone, uric acid, homocysteine, and potassium levels were higher in kidney donors when compared to controls [21]. They concluded that kidney donors manifest several findings of mild CKD. We have interpreted that slightly reduced renal function of kidney donors in our study could be due to the reduction of kidney mass. This study provides, for the first time, evidence that oxidative DNA damage does exist in kidney donors. We have speculated that increased oxidative DNA damage might be a consequence of reduced kidney mass and kidney function.

There is mounting evidence for the presence of disordered oxidative and glycoxidative chemistry in CKD patients that may contribute to poor cardiovascular and global outcome [5]. DNA, in particular, is more susceptible to attack by reactive oxygen species than proteins and membrane lipids, which are protected by antioxidant enzymes. Among many types of oxidative DNA damage, 8-OHdG is one of the most abundant oxidative products of cellular DNA [22]. We have demonstrated that living kidney donors exhibit increased oxidative DNA damage as determined by 8-OHdG/dG. This study is the first to demonstrate increased oxidative DNA damage among kidney donors. We have also shown that predonation renal function parameters are positively correlated with 8-OHdG/dG. Corredor et al have demonstrated recently that kidney transplant recipients exhibit increased DNA damage after transplantation, compared with those observed before transplantation, despite the improvement of their metabolic functions [23]. The mechanism is unknown. Hossain et al reported that healthy adult kidney donors exhibit increased urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine concentration, a sensitive marker of oxidative DNA damage [24]. To the best of our knowledge, no previous report for correlation between predonation renal function and 8-OHdG/dG ratio in kidney donors exists in the literature. We have interpreted that predonation renal reserve might predict postdonation oxidative stress and DNA damage in kidney donors. It is important to consider that oxidative DNA damage may change genes and result in problems for offspring of those with DNA damage. According to our results, we may speculate that increased oxidative stress and DNA damage and decreased antioxidant activity might cause or accompany unfavorable renal functions in our study population.

We have found a decrease in CAT, CAN, and PON activities in donors. Furthermore, we have found that predonation renal function is negatively correlated with postdonation serum PON activity. There is no data in the literature that increased oxidative stress and disturbances in antioxidant enzymes may facilitate the development of any clinical pathology in kidney donors. It is recently reported that in the first decade after donation, the risk of all-cause mortality and cardiovascular events is no higher than in healthy nondonors [25]. Given that reduced creatinine clearance is a risk factor for increased mortality in the general population, and in light of current findings, there are concerns about cardiovascular disease in donors [1]. It would be interesting to assess the evolution of endothelial function abnormalities in a long-term follow-up study and analyze the possible relationship between oxidant/antioxidant status, oxidative DNA damage, renal functions, and the clinical atherosclerosis of kidney donors over time.

This is the first demonstration that living kidney donors exhibit increased oxidative DNA damage and decreased antioxidant CAT, CAN, and PON activity. As there is currently little research on long-term follow-up of living kidney donors, this study may clarify the ambiguities concerning the long-term outcomes and open up new frontiers

concerning cardiovascular outcomes, endothelial dysfunction, and aging in this group.

Due to the cross-sectional design of the study, the results should be interpreted with caution, and causal relationship cannot be suggested. It would be interesting to assess the evolution of oxidative DNA damage and endothelial function abnormalities in a long-term follow-up study and analyze the possible relationship with the clinical atherosclerosis of kidney donors over time.

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