Changes in Biomarkers Associated with Living Kidney Donation

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Key Words

Biomarkers · Cardiovascular disease · Transplantation · Risk factors

Abstract

Living donor kidneys have been associated with better graft and overall survival in kidney transplant recipients. Although a living kidney donation is generally considered safe in carefully selected living donors, concerns of possible adverse effects related to kidney donation remain, especially in younger and high-risk donors. In this study, we examined the changes in a panel of traditional and novel serum biomarkers linked with cardiovascular conditions in a cohort of 34 healthy living kidney donors with a mean age $\pm$ SD of 40 ± 10 years and estimated predonation glomerular filtration rate (GFR) of 86 ± 10 ml/min/1.73 m². At 6 months after donation, there were no significant changes in the clinical parameters including body mass index and blood pressure despite a significant decline in the mean estimated GFR to 60 ml/min/1.73 m². Among the panel of markers, the levels of symmetric dimethylarginine and fibroblast growth factor 23 increased significantly compared to baseline, suggesting that living kidney donation may result in changes in biomarkers that are associated with cardiovascular risk in other cohorts.

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Introduction

Kidney transplant remains a preferred treatment option for many patients with end-stage renal disease because of its superior survival compared to dialysis [1]. The waiting time for a deceased donor kidney transplant has increased significantly due to the growing number of patients awaiting transplant, while the supply of deceased donor kidneys has remained limited [2]. In addition to better graft survival compared to deceased donor kidney [3], living kidney donation increases the supply of donor kidneys and reduces the waiting time for kidney transplant recipient. Although living kidney donation is generally considered safe with low cardiovascular disease (CVD) risk in carefully selected donors [4, 5], concerns remain especially in younger and higher-risk donors, including ethnic minorities [6, 7]. The risk of CVD is greater when living kidney donors develop comorbidities like hypertension or diabetes after the donation. The current mandatory follow-up of living kidney donors after donation is only for 2 years with monitoring of blood pressure and urinalysis (http://optn.transplant.hrsa.gov/PoliciesandBylaws2/policies/pdfs/policy_172.pdf). Living kidney donors are known to have modest reduction in glomerular filtration rate (GFR) and some studies suggest an increase in microalbuminuria after

donation [8]. With long-term follow-up, living kidney donors have shown loss of circadian blood pressure rhythm on ambulatory blood pressure monitoring despite maintaining a normotensive status [9]. However, when this loss of circadian rhythm occurs has not been precisely established, and our recent data found no significant changes in blood pressure and the blood pressure circadian rhythm at 6 months after donation by ambulatory blood pressure monitoring [10].

It is well established that the CVD risk is linked to traditional serum biomarkers like glucose and cholesterol. Moreover, newer data suggest that novel biomarkers like fibroblast growth factor 23 (FGF-23) are also informative about kidney disease progression and death in patients with chronic kidney disease (CKD) [11, 12]. Other biomarkers such as symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA) are increased in CKD and associated with endothelial dysfunction and worse cardiovascular outcome [13]. However, little is known about changes in biomarkers after living kidney donation. Given that the postdonation decline of estimated GFR is on average about 30 ml/min/1.73 m², it is possible that biomarker concentrations in kidney donors increase to levels comparable to those found in patients with similar levels of kidney function from a parenchymal disease process and that they could contribute to the known increased risks of CVD associated with CKD. In this study, we examined a panel of traditional and novel serum biomarkers before and 6 months following living kidney donation. We specifically chose markers of endothelial function, inflammation, and mineral metabolism for this study.

Methods

Subjects

Living kidney donors were recruited from two academic transplant centers: the University of Pennsylvania in Philadelphia and Mount Sinai School of Medicine in New York. A total of 34 living kidney donors (14 from the University of Pennsylvania and 20 from Mount Sinai) had blood samples collected before and at 6 months after donation, and were included in these analyses. All subjects provided informed written consent and the study protocols were approved by the institutional review boards of the University of Pennsylvania and Mount Sinai School of Medicine.

Clinical and Hemodynamic Measures

For all subjects recruited from the Hospital of University of Pennsylvania, brachial blood pressures were recorded from the right arm in the seated position by the nursing staff in the transplant program using a Dinamap device (GE Healthcare, Milwaukee, Wisc., USA). Six-month follow-up blood pressures were per-

formed by the nursing staff in the Clinical and Translational Research Center using a Datascope device (Datascope, Mahwah, N.J., USA) located adjacent to the transplant program offices. Clinical and hemodynamic measurements were obtained in similar fashion using a Welch Allyn oscillometric device at Mount Sinai Medical Center.

Kidney function was determined using the estimated GFR, calculated from the simplified Modification of Diet in Renal Disease (MDRD) equation [14] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [15] in blood drawn before donation and 6 months after donation.

Assays for Biomarkers

Fasting blood samples were obtained before and 6 months after donation. The blood samples were immediately placed on ice and centrifuged at 3,000 r.p.m. for 10 min at 4°C. The serum was separated and immediately stored at -70°C before analysis.

Arginine, homoarginine ADMA, and SDMA were measured simultaneously by high-performance liquid chromatography with fluorescence detection [16]. Plasma, 0.2 ml, was mixed with 0.1 ml of a 40-μM solution of the internal standard L-NMMA and 0.8 ml of phosphate-buffered saline. This mixture was applied to Oasis MCX solid-phase extraction columns (Waters, Milford, Mass., USA) for the extraction of basic amino acids. The columns were consecutively washed with 1.0 ml of 100 mM HCl and 1.0 ml of methanol. Analytes were eluted with 1.0 ml of concentrated ammonia/water/methanol (10/40/50). After evaporation of the solvent under nitrogen, the amino acids were derivatized with an *o*-phthalaldehyde reagent containing 3-mercaptopropionic acid. The derivatives were separated by isocratic reversed-phase chromatography on a Symmetry C18 column (3.9 × 150 mm, 5-μm particle size; Waters). Potassium phosphate buffer (50 mM; pH 6.5) containing 8.7% acetonitrile was used as the mobile phase at a flow rate of 1.2 ml/min and a column temperature of 30°C. Fluorescence detection was performed at excitation and emission wavelengths of 340 and 455 nm, respectively. After elution of the last analyte, strongly retained compounds were quickly eluted by a strong solvent flush with 50% acetonitrile, resulting in a total analysis time of 25 min. The intra-assay coefficients of variations (CVs) for arginine, ADMA, and SDMA were 3.5, 4.6, and 3.8%, respectively. The interassay CVs for arginine, ADMA, and SDMA were 4.1, 5.7, and 6.4%, respectively.

FGF-23 was measured by ELISA (second-generation C terminal) using kits from Immotopics (San Clemente, Calif., USA). Intra- and interassay CVs were less than 4.5 and 6.2%, respectively. Inflammatory cytokines, TNF-1α and IL-6, were measured by ELISA kits from R&D Systems (Minneapolis, Minn., USA). Intra- and interassay CVs were less than 5.9 and 6.8%, respectively. hsCRP was measured using a Roche Analyzer with intra- and interassay CVs that were less than 6.3 and 7.5%, respectively.

Statistics

Data are presented as means ± SD. Descriptive statistics were used to characterize subject demographics and clinical parameters. A paired *t* test was used to evaluate change in hemodynamic and laboratory parameters within subjects over the study time points. Analyses were performed using STATA v11.0 (SAS Institute, Cary, N.C., USA). Pearson's correlation coefficient was used to evaluate relationships between estimated GFR and biomarkers.

Table 1. Characteristics of study participants

Parameter	Baseline	6 months after donation	p
Age, years	40.2±10.08	–	
Male	17 (50)	–	
BMI, kg/m ²	26.3±4.1	26.0±4.2	NS
SBP, mm Hg	125±10	115±8	<0.001
DBP, mm Hg	76±7	74±6	NS
Serum creatinine, mg/dl	0.9±0.2	1.3±0.2	<0.001
MDRD eGFR, ml/min/1.73 m ²	86±10.3	60±11.7	<0.001
CKD-EPI eGFR, ml/min/1.73 m ²	96±17.3	64±14.2	<0.001

Values are means ± SD or n (%). SBP = Systolic blood pressure; DBP = diastolic blood pressure.

Results

Table 1 lists the demographic and clinical information of the cohort. At the time of donation, the mean age was 40 ± 10 years. The mean BMI of this cohort was 26.3 ± 4.1 before donation and remained unchanged 6 months after donation. Both the mean systolic and diastolic blood pressures were normal in this group before donation and remained normal at 6 months after donation. The mean creatinine level increased from 0.9 ± 0.2 mg/dl before donation to 1.3 ± 0.2 at 6 months after donation, with an estimated GFR decreasing from 86 ± 10 ml/min/1.73 m² before donation to 60 ± 12 at 6 months after donation using the MDRD equation. A similar magnitude of reduction in eGFR occurred using the CKD-EPI equation. The demographics of the cohort in table 1 are similar to the 88 subjects screened in the transplant program at the University of Pennsylvania during the time period of the study.

Table 2 summarizes the changes in the panel of biomarkers from baseline to 6 months after donation. Among the markers of endothelial function, SDMA showed a significant increase in its level at 6 months after donation, while ADMA and arginine showed a trend towards increases but failed to reach statistical significance. FGF-23 demonstrated a marginally significant increase at 6 months after donation. The inflammatory markers failed to show significant changes at 6 months after donation.

There was a modest correlation between eGFR and FGF-23 ($r = -0.34$; $p = 0.069$) and a weaker correlation of eGFR with SDMA ($r = -0.24$; $p = 0.183$) before donation. The relationship between eGFR and FGF-23 was similar after donation ($r = -0.37$; $p = 0.055$), but the correlation between eGFR and SDMA was weaker ($r = 0.12$; $p =$

Table 2. Levels of biomarkers before the donation and 6 months after the donation

Marker	Before donation	6 months after donation	p
ARG, μM	66.7±18.8	74.9±17.5	0.078
hARG, μM	2.07±0.7	2.02±0.6	0.761
ADMA, μM	0.33±0.07	0.36±0.08	0.064
SDMA, μM	0.47±0.11	0.62±0.15	<0.001
FGF-23, RU/ml	54.0±27.9	70.0±32.9	0.041
TNF-1α, pg/ml	2.03±1.53	2.60±1.48	0.143
IL-6, pg/ml	1.59±0.97	1.79±1.30	0.501
hsCRP, mg/l	3.89±6.38	2.14±2.60	0.367

Values are means ± SD. ARG = Arginine; hARG = homoarginine.

0.510). The correlation of other biomarkers with eGFR before or after donation was not significant (p value generally >0.2 for all pairwise correlations).

Discussion

Our cohort of 34 relatively young living kidney donors with a mean age of 40 years and a BMI in the overweight range had an expected decline in estimated GFR (26 ml/min/1.73 m² on average) at 6 months after donation. Markers of endothelial function and bone metabolism demonstrated significant changes related to the decline in GFR, but the inflammatory markers showed no significant changes. While the mean diastolic blood pressure remained unchanged, the mean systolic blood pressure was significantly lower at 6 months after donation as we reported previously [10]. The changes in estimated GFR we observed are similar to those recently reported by Kasiske et al. [17] 6 months after donation.

ADMA is an endogenous inhibitor of endothelial nitric oxide synthase and has been implicated in the pathogenesis of endothelial dysfunction and atherosclerosis due to reduced production and availability of endothelium-derived nitric oxide. Elevated ADMA levels predict worse CVD outcomes in patients with hypertension, diabetes [18], proteinuria [19, 20], CKD [20, 21], stroke [22], peripheral vascular disease [23], ischemic heart disease [18, 20, 24], and congestive heart failure [25–27]. In a cohort of 24 healthy living kidney donors, Kielstein et al. [28] showed that ADMA levels decreased transiently after donation followed by significant elevation at 7 days after donation. We observed a trend towards higher ADMA

levels at 6 months after donation compared to baseline, which suggests a predisposition to endothelial dysfunction after donation.

SDMA, a structural isomer of ADMA eliminated by renal excretion, inhibits endothelial nitric oxide production by limiting the availability of L-arginine, the main substrate for nitric oxide synthase. In longitudinal studies, SDMA levels correlate with poorer CVD outcomes including myocardial infarction, stroke, and death [29–31]. Recent data also suggests that SDMA is involved in inflammation by inducing the production of mediators including IL-6, TNF- α , and NF- κ B [32]. In a recent study of 24 healthy living kidney donors, Kielstein et al. [33] showed that SDMA increased significantly within 6 h after unilateral nephrectomy and remained persistently elevated up to 7 days afterwards. We observed that SDMA was significantly elevated 6 months after donation, suggesting that the effects of living kidney donation may persist chronically, though we did not see an increase in systemic markers of inflammation. In our subjects, although both IL-6 and TNF- α were numerically higher after donation, they were not statistically significantly higher, which is likely because of the small numbers and variability in their measurement.

Homoarginine is a cationic amino acid derived from lysine. It may increase the nitric oxide availability and enhance endothelial function by serving as a precursor of nitric oxide, increasing the intracellular concentration of L-arginine. It inhibits the enzyme arginase which competes with nitric oxide synthase for the key substrate L-arginine. Data from the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study and Diet Deutsche Diabetes Dialysis (4D) Study indicate that homoarginine levels are independently associated with cardiovascular and all-cause mortality in patients referred for coronary angiography and in patients undergoing hemodialysis [34]. Although we observed a numerical decrease in homoarginine levels at 6 months after donation, the changes were not statistically significant.

FGF-23 regulates phosphorous metabolism by increasing phosphate excretion. Serum levels of FGF-23 increase progressively as kidney function deteriorates. Elevated FGF-23 is an independent risk factor for mortality in both incident and prevalent dialysis patients [35, 36], and a predictor of more rapid progression of renal function in early stages of both nondiabetic and diabetic CKD [37, 38]. Recently published data from the large multicenter prospective observational Chronic Renal Insufficiency Cohort (CRIC) study observed that FGF-23 elevations predicted mortality across the entire spectrum of

CKD, and predicted end-stage renal disease in patients with early-stage CKD with estimated GFR above 30 ml/min/1.73 m² [39]. Data from both animal and clinic studies demonstrated that FGF-23 elevation plays a causal role in the pathogenesis of left ventricular hypertrophy [40]. In our study, we observed increases in FGF-23 levels at 6 months after donation, consistent with the modest reduction in GFR. In a recent report comparing kidney donors 5 years after donation with age-matched controls, Young et al. [41] observed FGF-23 levels that were 30% higher, which is similar to the change we observed in our donors at 6 months. We did not measure parathyroid hormone concentrations in our study, but parathyroid hormone was higher in donors compared with either pre-donation values in the donors or control subjects in the reports by Kasiske et al. [17] and Young et al. [41].

In a cohort of 24 healthy living kidney donors, Kielstein et al. [28] found that inflammatory mediators including CRP and IL-6 increased acutely after donation followed by a progressive decline, and the levels remained significantly elevated at 7 days after donation. In our cohort, however, we did not observe significant changes in inflammatory markers 6 months after donation. In the study by Kasiske et al. [17], CRP was slightly increased at 6 months after donation in donors, with a similar change in CRP also noted in the controls.

Our study has several limitations. The sample size is modest, follow-up was limited to a single visit at 6 months, and the study was not designed to determine whether there are clinical consequences to the changes in biomarkers we observed in our cohort. A larger study and a CKD control group with which to compare both the biomarker concentrations and the course of kidney function over time would be the ideal next step in this area.

In summary, we observed that living kidney donation, commonly accepted as a relatively safe procedure has virtually no short-term clinical adverse consequences on common clinical factors such as blood pressure and BMI. The observed reduction in estimated GFR in our cohort is comparable to other studies of similar living kidney donor populations. We observed significant changes in several biomarkers which are associated with adverse effects in endothelial function and bone metabolism at 6 months after living kidney donation, though we lack longitudinal data with which to frame the clinical consequence of these changes. Given the pressure to expand living kidney donation, our findings could be important in clarifying the role of representative biomarkers like SDMA and FGF-23 in patients with parenchymal kidney disease concerning their effects in patients with a surgical reduction in kidney

function who are otherwise relatively healthy, and could also argue for considering longer follow-up of the living kidney donor population.

NCRR grant (No. UL1RR024134). The content is the responsibility of the authors alone and does not necessarily reflect the views or policies of the AHA, Macy Foundation, or National Institutes of Health.

Acknowledgments

This work was supported in part by a Clinical Research Program award from the American Heart Association, Macy Foundation grant, NIH/NIDDK grant (No. 5K23DK076619), and NIH/

Disclosure Statement

None to declare.

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