Progression of Endothelial Dysfunction, Atherosclerosis, and Arterial Stiffness in Stable Kidney Transplant Patients: A Pilot Study

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Abstract

Background Kidney transplant patients suffer from vascular abnormalities and high cardiovascular event rates, despite initial improvements post-transplantation. The nature of the progression of vascular abnormalities in the longer term is unknown. This pilot study investigated changes in vascular abnormalities over time in stable kidney transplant patients long after transplantation. Methods Brachial artery flow-mediated dilation (FMD), nitroglycerin-mediated dilation, carotid-femoral pulse wave velocity (cf-PWV), ankle-brachial pressure index, and common carotid artery intima-media thickness (CCA-IMT) were assessed in 18 kidney transplant patients and 17 controls at baseline and 3-6 months after. Results There was no difference in age (51±13 vs. 46±11; P=0.19), body mass index (26±5 vs. 25±3; P=0.49), serum cholesterol (4.54±0.96 vs. 5.14±1.13; P=0.10), systolic blood pressure (BP) (132±12 vs. 126±12; P=0.13), diastolic BP (82±9 vs. 77±8; P=0.10), or diabetes status (3 vs. 0; P=0.08) between transplant patients and controls. No difference existed in vascular markers between patients and controls at baseline. In transplant patients, FMD decreased (-1.52±2.74; P=0.03), cf-PWV increased (0.62±1.06; P=0.03), and CCA-IMT increased (0.35±0.53; P=0.02). No changes were observed in controls. Conclusions Markers of vascular structure and function worsen in the post-transplant period on long-term follow-up, which may explain the continued high cardiovascular event rates in this population.

Background

Patients with chronic kidney disease (CKD) have a higher burden of cardiovascular disease (CVD), including kidney transplant recipients [1]. Although cardiovascular risk factors improve in the immediate perioperative period, the long-term risk remains high [2, 3]. CVD is the commonest cause of death in transplant patients with a surviving graft, more
so than infection or malignancy [4].

Endothelial dysfunction, arterial stiffness, and accelerated atherosclerosis is common in stable kidney transplant patients and may contribute to the high cardiovascular event rate [3, 5, 6]. Endothelial dysfunction, a prerequisite to atherosclerosis, encompasses numerous maladaptive alterations adversely affecting vascular tone, haemostasis, and inflammatory processes within the arterial wall [7]. Both traditional and non-traditional risk factors in transplant patients can induce endothelial dysfunction [3, 8]. Calcification of the arterial wall is common in transplant patients and contributes to vascular stiffness [3, 9].

The nature of the changes in these vascular abnormalities in kidney transplant recipients is unknown. Previous studies mostly examined changes in the vascular properties of transplant patients pre-transplantation and immediately post-transplantation. They were not examined in stable transplant recipients long after transplantation. If these changes are adverse, they may be the result of novel risk factors post-transplantation and may be a cause of the high cardiovascular event rate. This pilot study investigated the changes in endothelial dysfunction, arterial stiffness, and atherosclerosis in stable kidney transplant recipients long into the post-transplant period.

Methods

Study population and design

Participants included patients recruited from the transplantation clinic, patient relatives, and staff volunteers. Patients were eligible if they were between 18 to 80 years of age with stable kidney function for ≥3 months (estimated glomerular filtration rate [eGFR]
change <5ml/min/1.73m²) and ≥6 months post-transplantation with or without previous dialysis. Exclusion criteria included a history of malignancy, heart failure, vasculitis, lupus, myocardial infarction or cerebrovascular event within 6 months or recent hospitalisation within 3 months prior to starting the study. The study was approved by the London South East Research Ethics Committee. All participants gave informed consent prior to their inclusion in the study. Vascular parameters were measured in a quiet vascular laboratory with a controlled temperature of 22-24 °C at recruitment (baseline visit) and 3-6 months after (second visit).

Clinical characteristics

A standardised questionnaire was used to obtain systematic information on the presence of cardiovascular risk factors. Weight, height, waist and hip circumference, and two blood pressure (BP) measurements were taken. Body mass index (BMI) and waist to hip ratio (WHR) were calculated.

Flow-mediated Dilation, Nitroglycerin-mediated Dilation, Common Carotid Artery Intima-Media Thickness, and Carotid-Femoral Pulse Wave Velocity

Brachial flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), and common carotid artery intima-media thickness (CCA-IMT) were performed as described in previous studies by our group [3]. Carotid-femoral pulse wave velocity (cf-PWV) was performed as described before [10].

Ankle-Brachial Pressure Index
Ankle-brachial pressure index (ABPI) was assessed with a Doppler instrument. The ABPI of each leg was calculated by dividing the highest systolic BP in the desired leg between the measurement of the dorsalis pedis and posterior tibial artery divided by the highest systolic BP between the two arms. The mean ABPI value for each participant was derived from the average between the ABPI of the right and left leg.

*Laboratory measurements*

Blood and urine samples were collected for biochemical analysis at a standard clinical reference laboratory at St. George’s University Hospitals NHS Foundation Trust. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 equation [11].

*Statistical analyses*

Continuous variables were analysed with an independent samples t-test, categorical variables using a chi-squared test, and within-group comparisons using a paired t-test. Bivariate correlations between continuous variables were calculated using Pearson’s correlation coefficient (r). Point-Biserial correlation ($r_{pb}$) was used to determine correlations between binary and continuous variables. A two-sided $P$-value of <0.05 was used to determine statistical significance. Analyses were performed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

*Results*

18 kidney transplant patients and 17 healthy volunteers were enrolled in the study. The
mean number of days between the first and second visit for transplant patients was 161 ± 36 days and 185 ± 52 days for controls. The mean duration on dialysis (haemodialysis and/or peritoneal dialysis) before transplantation was 29 ± 14 months (median 29, interquartile range [IQR] 28). The median time since transplantation at recruitment was 86 months with an IQR of 123 months. Transplant patients were taking up to 3 of 6 different immunosuppressants at recruitment. The immunosuppressants used were Tacrolimus (89%), Mycophenolate Mofetil (39%), Prednisolone (44%), Azathioprine (22%) and Sirolimus (6%). The aetiology of CKD in our transplant patients were the following: autosomal dominant polycystic kidney disease (4), IgA nephropathy (4), hypertension (2), pyelonephritis (1), focal segmental glomerulosclerosis (1), gout nephropathy (1), and type 2 diabetes mellitus (1). Four transplant patients had an unclear aetiology to their CKD.

Baseline parameters in study participants

Table 1 shows the baseline clinical characteristics and circulating biomarkers in study participants. The kidney transplant patient group had reduced eGFR, more dyslipidaemics, and elevated parathyroid hormone and N-terminal-pro-brain natriuretic peptide levels. Table 2 shows the baseline vascular structure and function of participants. No difference existed between transplant patients and controls.

Changes in clinical, circulating biomarkers, and in cardiovascular structure and function in study participants

Table 1 shows changes in clinical characteristics and circulating biomarkers in participants. In controls, systolic BP (SBP) decreased, while vitamin D and corrected
calcium levels increased. In transplant patients, vitamin D levels also increased while eGFR declined. Vascular structure and function did not change in controls (Table 2). In transplant patients, brachial FMD decreased (-1.52 ± 2.74; \(P=0.03\)), while cf-PWV (0.62 ± 1.06; \(P=0.03\)) and CCA-IMT increased (0.35 ± 0.53; \(P=0.02\)) (Table 2).

**Association of changes in FMD, cf-PWV, and CIMT in kidney transplant patients**

No significant correlation existed between the decline in eGFR and changes in FMD (\(r = 0.21; P=0.42\)), cf-PWV (\(r = 0.30; P=0.23\)), or CIMT (\(r = 0.37; P=0.15\)). Changes in FMD were not associated with age (\(r = 0.43; P=0.08\)), BMI (\(r = 0.45; P=0.05\)), WHR (\(r = 0.31; P=0.21\), gender (\(r_p = 0.18; P=0.48\)), DM (\(r_p = 0.18; P=0.47\)), SBP (\(r = -0.32; P=0.07\)), or diastolic BP (DBP) (\(r = -0.03; P=0.89\)). Changes in PWV were not associated with age (\(r = 0.18; P=0.49\)), BMI (\(r = 0.27; P=0.29\)), WHR (\(r = 0.14; P=0.57\)), gender (\(r_p = 0.16; P=0.54\), DM (\(r_p = -0.14; P=0.59\)), SBP (\(r = 0.30; P=0.08\)), or DBP (\(r = 0.08; P=0.64\)). Changes in CCA-IMT were not associated with age (\(r = -0.10; P=0.71\)), BMI (\(r = -0.08; P=0.78\), WHR (\(r = -0.29; P=0.26\)), gender (\(r_p = 0.14; P=0.59\)), DM (\(r_p = -0.25; P=0.34\)), SBP (\(r = 0.23; P=0.19\)), or DBP (\(r = 0.04; P=0.81\)).

**Discussion**

This study shows the worsening of vascular structure and function in stable kidney transplant patients, where FMD decreased while cf-PWV and CCA-IMT increased. Change in eGFR was not associated with changes in FMD, CCA-IMT, or cf-PWV. Traditional risk factors including age, BMI, WHR, gender, DM, SBP, and DBP did not correlate with the changes seen.

CKD patients exhibit endothelial dysfunction as measured by FMD [3, 5]. In previous
studies, transplantation has been shown to improve FMD, acutely and at 12 months [12, 13]. This recovery may be due to improvements in traditional and uraemia-related non-traditional risk factors [6]. Despite this, FMD values were often still lower compared to controls [5, 6].

CKD patients demonstrate accelerated atherosclerosis as evidenced by high CCA-IMT [3, 6]. The impact of renal transplantation on CCA-IMT is conflicting. One study demonstrated CCA-IMT to progressively increase after 2, 4, and 6 months post-transplantation [14]. Another reported improvements 6 months after transplant [15]. Despite this, values are often still higher compared to the general population [3, 15].

Cf-PWV is a marker of arterial stiffness and predicts the appearance of CVD in CKD, including in transplant patients [10, 16, 17]. Studies evaluating the progression of arterial stiffness over time is conflicting in transplant patients. One study reported no significant change at 12 months after transplantation, while another reported an improvement [17, 18]. Bachelet-Rousseau’s group compared cf-PWV progression in transplant waitlisted patients who were eventually transplanted or were still transplant-pending. No difference in cf-PWV was observed at baseline and upon 1-year follow-up with a short median time of 6.3 (3.8-10.1) months post-transplantation [19]. In contrast, Strozecki’s group showed cf-PWV to progress in transplant patients who were enrolled much later at 36 ± 27 months post-transplantation [20].

Most studies evaluating changes in FMD, CCA-IMT, and cf-PWV in transplant patients do so in immediately post-transplanted subjects. Improvements or non-progression in these parameters shortly after transplantation does not exclude a reversal in recovery. Unlike these studies, our patients were recruited long into the post-transplant period at a median of 86 months post-transplantation. Transplantation improves several traditional and non-traditional cardiovascular risk
factors in the immediate post-transplant period. However, during long-term follow-up, other factors related to immunosuppression and weight gain may prevent further improvement and eventual deterioration.

Limitations
Our study had a small sample size, hence it was difficult to reveal correlations between traditional and non-traditional risk factors with the changes observed.

Conclusions
Our study demonstrates that progressive worsening in surrogate markers of vascular structure and function occur in the post-transplant period upon long-term follow-up. Although transplantation initially alleviates the cardiovascular burden, the vascular disease progress in the long-term beyond the initial ‘honeymoon’ period.

Abbreviations
Brachial artery flow-mediated dilation (FMD)
Carotid-femoral pulse wave velocity (cf-PWV)
Common carotid artery intima-media thickness (CCA-IMT)
Blood pressure (BP)
Chronic kidney disease (CKD)
Cardiovascular disease (CVD)
Estimated glomerular filtration rate (eGFR)
Body mass index (BMI)
Waist to hip ratio (WHR)
Ankle-brachial pressure index (ABPI)
Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)
Interquartile range (IQR)
Declarations

**Ethics approval and consent to participate:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was submitted to and approved by the London South East Research Ethics Committee. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

**Consent for publication:** Not applicable.

**Availability of data and material:** Anonymised data is available for review with the lead author for audit and review.

**Competing Interests:** DB has received Grants from British Heart Foundation PG 10/71/28462; DB has received honorarium from AstraZeneca, Vifor Pharma, and Pfizer. JJ, NH, RR, RL-J, and JK have no conflict of interest.

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Tables

Table 1. Clinical characteristics and circulating biomarkers in study participants
| Parameter                                      | Healthy controls (n=17) |       |       |
|-----------------------------------------------|-------------------------|-------|-------|
|                                               | Baseline                | Second visit | P-value |
| Age (years)                                   | 45.82 ± 10.85           | NA    | NA    |
| Gender M/F                                    | 5/12                    | NA    | NA    |
| Body mass index (kg/m²)                       | 24.59 ± 2.59            | 25.00 ± 2.66 | 0.09   |
| Waist/Hip                                     | 0.82 ± 0.07             | 0.80 ± 0.06 | 0.10   |
| Systolic blood pressure (mmHg)                | 125.53 ± 12.39          | 119.76 ± 10.62 | 0.02   |
| Diastolic blood pressure (mmHg)               | 77.24 ± 7.61            | 76.24 ± 8.70 | 0.64   |
| Smoking status past/present/never             | 0/6/11                  | NA    | NA    |
| Diabetes mellitus                             | 0                       | NA    | NA    |
| History of IHD                                | 0                       | NA    | NA    |
| Dyslipidaemia                                 | 3                       | NA    | NA    |
| Haemoglobin (g/L)                             | 134.29 ± 11.86          | 138.71 ± 9.31 | 0.02   |
| Albumin (g/L)                                 | 39.35 ± 2.47            | 40.12 ± 2.32 | 0.19   |
| Urea (mmol/L)                                 | 4.26 ± 0.95             | 4.60 ± 1.11 | 0.16   |
| Creatinine (mcmol/L)                          | 69.65 ± 16.63           | 69.65 ± 15.66 | 1.00   |
| eGFR (mL/min/1.73m²)                          | 97.59 ± 15.59           | 97.29 ± 15.55 | 0.83   |
| Total cholesterol (mmol/L)                    | 5.14 ± 1.13             | 5.34 ± 1.21 | 0.16   |
| HDL (mmol/L)                                  | 1.71 ± 0.56             | 1.75 ± 0.62 | 0.51   |
| LDL (mmol/L)                                  | 3.24 ± 1.71             | 3.07 ± 0.89 | 0.63   |
| Total cholesterol/HDL                         | 3.29 ± 1.27             | 3.32 ± 1.26 | 0.78   |
| Non-HDL (mmol/L)                              | 3.44 ± 1.04             | 3.60 ± 1.04 | 0.09   |
| Triglyceride (mmol/L)                         | 1.05 ± 0.58             | 1.05 ± 0.70 | 0.97   |
| Glucose (mmol/L)                              | 4.71 ± 0.39             | 4.82 ± 0.40 | 0.35   |
| Corrected calcium (mmol/L)                    | 2.35 ± 0.05             | 2.39 ± 0.07 | 0.03   |
| Inorganic phosphate (mmol/L)                  | 1.11 ± 0.13             | 1.17 ± 0.14 | 0.05   |
| PTH (pmol/L)                                  | 4.98 ± 1.57             | 4.53 ± 1.09 | 0.19   |
| Vitamin D (nmol/L)                            | 43.65 ± 22.03           | 67.41 ± 30.41 | <0.01 |
| Troponin T (ng/L)                             | 2.35 ± 1.46             | 2.76 ± 1.72 | 0.13   |
| NT-pro-BNP (ng/L)                             | 43.65 ± 25.57           | 40.41 ± 40.53 | 0.62 |
| hsCRP (mg/L)                                  | 1.90 ± 3.51             | 1.19 ± 1.64 | 0.45   |
| Iron (mcmol/L)                                | 18.75 ± 6.40            | 21.56 ± 7.72 | 0.06   |
| Transferrin (g/L)                             | 2.82 ± 0.32             | 2.83 ± 0.36 | 0.78   |
| Ferritin (mcmol/L)                            | 82.69 ± 78.98           | 87.69 ± 73.04 | 0.55   |
| Transferrin saturation (%)                    | 27.50 ± 11.27           | 31.19 ± 12.58 | 0.08 |
| ACR (mg/mmol)                                 | 0.49 ± 1.35             | 0.34 ± 0.78 | 0.76   |
| Parameter            | Healthy controls (n=17) |       |       |       |
|----------------------|-------------------------|-------|-------|-------|
|                      | Baseline                | Second visit |        |       |
| Brachial FMD (%)     | 4.63 ± 3.02             | 3.51 ± 2.73 | 0.33   | 4.3   |
| Brachial NMD (%)     | 16.00 ± 5.47            | 17.17 ± 5.18 | 0.31   | 15.1  |
| Cf-PWV (m/s)         | 6.96 ± 1.26             | 7.17 ± 1.50 | 0.51   | 7.8   |
| Mean ABPI            | 1.18 ± 0.08             | 1.21 ± 0.11 | 0.43   | 1.2   |
| Mean CCA-IMT (mm)    | 5.54 ± 1.08             | 5.73 ± 1.34 | 0.22   | 5.7   |

Legend: n number of participants, FMD flow-mediated dilation, NMD nitroglycerin-mediated dilation, Cf-PWV carotid-femoral pulse wave velocity, ABPI ankle-brachial pressure index, CCA-IMT common carotid intima-media thickness

Data presented as mean ± SD