Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Changes in endothelial function before and after renal transplantation*

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Keywords
endothelial function, renal transplantation.

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*This study was supported by Turkish Kidney Foundation with grant number 2005-1.

Received: 18 November 2005
Revision requested: 23 December 2005
Accepted: 16 January 2006

doi:10.1111/j.1432-2277.2006.00288.x

Summary

Endothelial dysfunction is an early key event in the development of atherosclerotic cardiovascular disease observed in chronic renal failure patients. The role of renal transplantation (RTx) on endothelial dysfunction is still unclear. The aim of this study was to evaluate the endothelial function of chronic renal failure patients before RTx (while they were on hemodialysis, HD), and after RTx (at the 6th and 12th months) by a noninvasive method, brachial arterial ultrasound. A total of 22 (17 male, mean age: 33.9 ± 11.6 years) RTx recipients were enrolled in the study. Endothelium-dependent vasodilation (EDD) was assessed by establishing reactive hyperemia. EDD prior to transplantation was significantly lower when compared with EDD measured at the 6th and 12th months after RTx (EDD pretransplantation: 6 ± 3.7%, EDD at the 6th month of RTx: 8.3 ± 2.3% and EDD at the 12th month of RTx: 12.1 ± 3.6%, $P < 0.001$). When the EDD values measured at the 6th and 12th months of RTx were compared, measurements of the 12th month were found significantly higher than those of the 6th month ($P < 0.001$). Our results also showed that RTx has provided improvement in endothelial function by eliminating the uremic environment although not in the early post-RTx period.

Introduction

The mortality rate of patients on chronic hemodialysis (CHD) is high and cardiovascular disease is the leading cause of death. The predominant cardiac abnormalities in this population are left-ventricular hypertrophy (LVH), atherosclerotic heart disease and arrhythmias [1].

Endothelial dysfunction, an established early abnormality during the progression of atherosclerosis, has also been reported in CHD patients [2–4].

The maximum potential for prevention and reversibility of cardiovascular diseases would be expected with an intervention at an earlier stage of the atherosclerosis [5]. Recently endothelial dysfunction has been shown to precede the formation of atherosclerotic plaques. Endothelium is an active barrier between vascular wall and the blood. The main functions of the endothelium are the control of coagulation, fibrinolysis, vascular tone, and immune response. Potential causes of its dysfunction are inflammation, retention of L-arginine inhibitors, oxidative stress, hyperhomocysteinemia, dyslipidemia, hyperglycemia, and hypertension [5,6].

Renal transplantation (RTx) is the preferred treatment modality in patients with end-stage renal disease and most of the pathophysiological features related to uremia improve after transplantation; while arterial endothelial dysfunction may still persist. This is partly because of the effect of immunosuppressive drugs used, and partly because of uremic state that persists to some extent in the post-transplant period. It is known that some of the cardiovascular risk factors that have already been established in uremic milieu also still continue to exist in the post-transplant period [7,8].

High-resolution brachial ultrasonography is a noninvasive, simple and effective method developed in the last decade to assess endothelial function. This method correlates well with coronary artery endothelial function [9].

The aim of this study was to establish the degree of endothelial dysfunction in the CHD period and at the 6th and 12th months after RTx. We also aimed to evaluate the changes seen in cardiovascular risk factors affecting endothelial dysfunction.

Materials and methods

Study groups

The study population consisted of 22 patients (five females, 17 males). All were being followed-up in our transplant clinic. Patients with overt atherosclerotic disease, congestive heart failure, amyloidosis, and abnormal electrocardiography findings were excluded from the analysis.

Fifteen patients were dialyzed thrice a week and seven patients were dialyzed twice a week. The mean duration of dialysis was 4 h per session with 250–300 ml/min blood flow rate and with a dialysate flow of 500 ml/min. All patients were dialyzed with bicarbonate containing dialysate bath. Etiologies of renal diseases were primary glomerular diseases ($n = 2$), tubulo-interstitial nephritis ($n = 4$), diabetic nephropathy ($n = 2$), hypertensive nephrosclerosis ($n = 5$), other reasons ($n = 3$), and unknown etiology ($n = 4$). Patients were treated for more than 6 months with HD. Creatinin levels of patients were under 2 mg/dl in post-transplantation period.

Before and after RTx, patients received similar antihypertensive medications (Table 1).

Patients with diabetes mellitus were using insulin therapy and these patients were the only ones using statins as lipid lowering regimen. During the hemodialysis period, 70% of subjects were taking erytropoetin therapy. Only five of our patients were smokers prior to transplantation.

The immunosuppressive regime of the RTx patients consisted of mycophenolate mofetil or azathiopurine, prednisone and Cyclosporine A (CsA) ($n = 11$) or tacrolimus ($n = 11$). Serum levels of FK506 and CsA levels were measured once monthly. Target trough values of CsA and FK506 levels were 100–200 and 5–15 ng/ml respectively. Cyclosporine A trough levels were measured by EMIT and FK506 by IMX (Abbott laboratories).

Table 1. Antihypertensive medications.

Medication	HD	RTx6	RTx12
Calcium channel blockers	10	12	13
Beta blockers	2	2	2
ARB or ACE inhibitors	2	2	1
Alpha adrenergic blockers	7	5	6

HD, hemodialysis; RTx, renal transplantation; ARB, angiotensin receptor blockers; ACE: angiotensin-converting enzyme.

Biochemical parameters of the study group such as serum glucose, creatinine, sodium, potassium, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and full blood count were taken 4–6 h after the hemodialysis session, and at the 6th and 12th months after RTx.

Systolic and diastolic blood pressures were measured on the right arm of the subject in an upright sitting position after at least 5 min rest using a mercury sphygmomanometer with an appropriate cuff size. Two readings were recorded for each individual. The average of two readings was defined as the subject's blood pressure.

High-resolution ultrasonography

Flow-mediated dilation (FMD) of the brachial artery following transient ischemia, which is a noninvasive method for assessing endothelial function was done according to method defined by Celermajer *et al.* [9] using high-resolution ultrasound machine (10 MHz transducer, attached to a standard Vingmed System Five, Norway). Prior to RTx, brachial artery assessments of patients were made 4–6 h after the last hemodialysis session. Post-transplantation brachial artery assessments were performed at 6th and 12th months and 12 h after the last dosage of the calcineurin inhibitors (CNI).

Patients were kept in supine position in the stable room temperature between 20 and 25 °C. All patients abstained from smoking and caffeine-containing drinks for at least 12 h. Brachial artery in the nonfistula arm in CHD patients was utilized. So as to best visualize the brachial artery, the arm was comfortably immobilized in the extended position and brachial artery was scanned in the longitudinal section 3–5 cm above the antecubital fossa using a 10 MHz high-resolution linear-array transducer. After optimal transducer positioning, the skin was marked for reference for later measurements and arm was kept in the same position throughout the study.

All measurements of the brachial artery internal diameter were taken at the end-diastole (timed by the QRS complex) and were calculated as the average of the measurements obtained during three consecutive cardiac cycles. After baseline measurements of the brachial artery were recorded, the cuff was inflated to 200 mmHg (or 50 mmHg higher than systolic blood pressure) for 5 min to create forearm ischemia. Subsequently, the cuff was deflated and the arterial diameter was measured at 60 s after deflation. All measurements were performed by single investigator blinded to clinical details and brachial artery measurements of the study groups and were recorded on VHS videotape (SVO-9500 MDP) for subsequent offline analysis.

Endothelium-dependent FMD was expressed as the percentage change in the brachial artery diameter from baseline to following reactive hyperemia.

Brachial artery FMD, CRP, and traditional cardiovascular risk factors were measured in the Firefighters and Their Endothelium (FATE) study, which recruited 1154 male participants with no known history of cardiovascular disease. FMD was 8.2% ± 3.5 (median 8.0) [10]. An acceptable reproducibility is reported as a mean difference of 2–3% in FMD over time (on a baseline vasodilation of about 10%) [11]. The intra-observer variability for measurements of brachial artery diameter was <5% in our study.

All study patients were examined with good medical and laboratory practice according to the recommendations set forth by the Declaration of Helsinki on Biomedical Research Involving Human Subjects [12]. A written informed consent was obtained from all of the patients.

Statistical analysis

Results were given as percentage change from baseline values. Descriptive statistical results are presented in tables as mean ± standard deviations. Mann–Whitney *U*-test and independent sample *t*-test were used for statistical analysis. The difference was considered statistically significant when $P < 0.05$. Paired samples *t*-test was used to compare FMD values measured before and after RTx. Comparisons between the 6th and 12th months' measurements were also evaluated with paired samples tests.

Results

The mean age of the patients was 33.9 ± 11 (range: 18–56) years. The mean body mass index of the patients was 23.1 ± 4.2 kg/m^2. The mean hemodialysis duration prior to RTx was 30 ± 25 (range: 7–125) months. No significant difference was found when the hemodynamic parameters and lipid profiles were compared prior to transplantation and 6 and 12 months after the transplantation. Serum creatinine values were significantly higher in CHD patients compared with those received RTx therapy. But there were not significant differences at the 6th or 12th months in creatinine levels post-transplantation. The hemoglobin levels after RTx recipients were significantly higher when compared with CHD period, but hemoglobin levels did not differ with the duration of the transplantation. The biochemical and hemodynamic parameters are summarized in Table 2.

When the brachial artery basal diameters were compared between post-transplantation and CHD period, no difference was found at any time; on the other hand, FMD (Endothelium dependent) prior to transplantation was significantly lower when compared with FMD values measured at the 6th and 12th months of RTx therapy (FMD pretransplantation: 6 ± 3.7%, FMD at the 6th month of RTx: 8.3 ± 2.3% and FMD at the 12th month of RTx: 12.1 ± 3.6%, $P < 0.001$). When FMD values measured at the 6th and 12th months of RTx were compared, the values found at the 12th month were significantly higher than that in the 6th month ($P < 0.001$). Brachial artery measurements are summarized in Table 3.

Flow-mediated dilation values of the five patients who were smokers prior to transplantation were compared with the FMD values of nonsmokers. There was no statistically significant difference between them. After the RTx, all of these smokers quit smoking.

Table 2. Hemodynamic and laboratory parameters.

	HD	6th month post-RTx	12th month post-RTx
Systolic BP (mmHg)	135 ± 31	129 ± 17	130 ± 19
Diastolic BP (mmHg)	86 ± 18	83 ± 16	84 ± 20
Glucose (mg/dl)	79 ± 19	81 ± 22	80 ± 18
Creatinin (mg/dl)	9.4 ± 2.4*	1.4 ± 0.2	1.5 ± 0.3
Total cholesterol (mg/dl)	185 ± 30	180 ± 23	177 ± 22
HDL-Chol. (mg/dl)	43 ± 7	42 ± 7	44 ± 5
LDL-Chol. (mg/dl)	106 ± 26	107 ± 25	109 ± 21
Triglycerides (mg/dl)	156 ± 51	151 ± 44	160 ± 40
Hgb (%gr)	9.6**	13.2	13.7
CNI-Level (ng/ml)			
CsA	–	203 ± 42	192 ± 38
FK 506	–	10.5 ± 3.1	11.1 ± 2.9

RTx, renal transplantation; BP, blood pressure; NS, nonsignificant; Chol, cholesterol; Hgb, hemoglobin; CNI, calcineurin inhibitor; CsA, Cyclosporine A. *$P < 0.001$ versus 6th and 12th months post-RTx group; **$P < 0.01$ versus 6th and 12th months post-RTx group.

Table 3. Brachial artery measurements.

	HD	6th month post-RTx	12th month post-RTx
Basal diameter (mm)	3.9 + 0.6	3.8 + 0.4	3.7 + 0.4
FMD (%)	5.6 + 3.7*	8.3 + 2.3**	12.1 + 3.6

RTx, renal transplantation; FMD, flow-mediated dilation. *$P < 0.001$ versus 6th and 12th month post-RTx group; **$P < 0.01$ versus 12th month post-RTx group.

Discussion

Endothelial dysfunction is a well-known complication of chronic uremia, while etiology is multi-factorial.

Although, some studies indicated chronic endothelial injury in renal transplant patients as well, the effect of RTx on endothelial dysfunction has not been well assessed so far [7,13,14].

The present study showed that EDD was significantly lower in CHD patients when compared with RTx patients. Furthermore, impaired EDD was less prominent among RTx having longer post-transplant period.

In recent years, the vascular endothelium has been recognized as an endocrine organ with important physiological functions including modulation of vascular tone, vascular structure, and interaction of blood components with the vessel wall [5]. Reduced availability of nitric oxide (NO) to the endothelium commonly shows endothelial dysfunction.

In addition to the traditional risk factors identified in the general population such as hyperlipidemia, hypertension, diabetes mellitus, tobacco use, menopause, and physical inactivity, risk factors secondary to uremia such as dyslipidemia, prothrombotic factors, hyperhomocysteinemia, hemodynamic overload, anemia, increased oxidative stress, hypoalbuminemia, and electrolyte abnormalities all contribute to the endothelial dysfunction in this population, thus to the increased cardiovascular morbidity and mortality in chronic uremic patients [2,4,15]. Previous studies have shown that the reduction of circulating inhibitors of endothelial function by hemodialysis is associated with impaired endothelial function, which can lead to a speculation that endothelial toxins should be cleared efficiently after successful RTx [14,16].

Renal insufficiency influences NO activity via endothelial dysfunction, decreased arginine synthesis by the kidney, responses to arginine analogs that act as NO synthase inhibitors, increased cytokine activity, and altered oxidation of cells [17,18].

The results of our study confirmed the regression of endothelial dysfunction with RTx. The uremic state, hemodynamic overload, anemia, electrolyte imbalance, oxidative state all regress after transplantation, while endothelial dysfunction may still persist to some extent. Cyclosporine A increases superoxide production, which metabolizes NO. On the other hand, NO synthase is a calcium-calmodulin-dependent enzyme; CNIs may directly inhibit the production of NO by inhibition calmodulin. Hypertension, hyperlipidemia, and hyperglycemia are all common side effects with the use of CNIs. As a result, while patients are rescued from negative variables like uremia after RTX, they become exposed to factors which have negative influence on the endothelium like CNI. However, considering the results of our study, when we take into account the profit–risk ratio between these two factors, we might conclude that there will be recovery from uremia and as a result improvement or at least stabilization of endothelial functions. Some studies, which have examined endothelial function in only the post-RTx period, and associations between these factors and endothelial dysfunction, and were focused largely in the last decade, explain why endothelial dysfunction still persists to a lesser extent in the post-transplant period as even most of the risk factors of the chronic uremic period diminish [7,13,16,19,20].

Similarly, previous studies have also showed that apart from the traditional risk factors, renal impairment in itself impairs endothelial dysfunction when multiple regression analysis has been performed to evaluate solely the effect of chronic uremia [8,15].

Drugs, which may influence endothelial functions such as antihypertensives, antihyperlipidemics and CNIs were used in similar ratios by patients during the hemodialysis period and after RTx.

Apart from the above results, findings of significantly increased EDD values 12 months after transplantation, when compared with 6 months after RTx can be explained by two possible mechanisms. One of them is the possibility of that higher CNI dosages and trough levels in the early post-transplant period is more detrimental to endothelial function than the cumulative doses of CNIs (specially Cyclosporin A) [13,21]. The other explanation is related to ongoing chronic uremic state in early post-transplant months.

Despite its widespread use, high-frequency ultrasonographic imaging of the brachial artery has some technical and interpretive limitations. Flow-mediated vascular reactivity may be affected by temperature, food, drugs, sympathetic stimuli, environment, patient's position, use of the upper or forearm. The timing of measurements is also important. Several studies have suggested that the maximal increase in diameter occurs approximately 60 s after the release of the occlusive cuff, or 45–60 s after peak reactive hyperemic blood flow [11].

In conclusion, we determined that CHD patients have significantly impaired endothelial function when compared with RTx recipients. The degree of endothelial function increases inversely with shorter post-transplantation period. Further efforts, therefore, are needed to apply the knowledge we have gained so far with endothelial dysfunction, to determine markers of subclinical atherosclerotic cardiovascular disease.

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