Nephrol Dial Transplant (2006) 21: 203–207
doi:10.1093/ndt/gfi119
Advance Access publication 6 September 2005

Nephrology Dialysis Transplantation

*Original Article*

# Effect of renal transplantation on endothelial function in haemodialysis patients

Huseyin Kocak[1], Kaan Ceken[2], Asuman Yavuz[1], Selcuk Yucel[3], Alihan Gurkan[4], Okan Erdogan[4], Fevzi Ersoy[1], Gulsen Yakupoglu[1], Alper Demirbas[4] and Murat Tuncer[1]

[1]Department of Nephrology, [2]Department of Radiology, [3]Department of Urology and [4]Department of Surgery, Akdeniz University School of Medicine, Antalya, Turkey

Downloaded from https://academic.oup.com/ndt/article/21/1/203/1818964 by Akdeniz Universitesi user on 20 September 2021

## Abstract

**Background.** Haemodialysis patients (HD) have been characterized by a high incidence and prevalence of atherosclerotic cardiovascular disease. Based on the traditional cardiovascular risk factors in this population, we cannot explain this high incidence and prevalence. One of the mechanisms contributing to cardiovascular risk in HD patients may be to uraemic toxins. Cardiovascular risk factors and uraemic toxins themselves may cause endothelial dysfunction, which may play a pivotal role in the development and progression of atherosclerosis in this population. We hypothesized that elimination of uraemic toxins in response to renal transplantation (RTx) can improve endothelial function as assessed by flow-mediated dilatation of brachial artery in haemodialysis (HD) patients.

**Methods.** Endothelial function measured by flow-mediated dilatation of the brachial artery (FMD) and glyceryltrinitrate-induced dilatation of the brachial artery (NMD) were assessed twice, during haemodialysis treatment and after RTx in 30 chronic haemodialysis patients. All patients were characterized by absence of known atherosclerotic disease and traditional cardiovascular risk factors. We also studied age- and gender-matched 20 normotensive healthy controls.

**Results.** FMD values significantly improved after RTx ($6.69 \pm 3.1\%$ *vs* $10.50 \pm 3.0\%$, $P < 0.001$) in HD patients. FMD of patients both during haemodialysis and after RTx was lower than in healthy controls ($6.69 \pm 3.1\%$, $10.50 \pm 3.0\%$ *vs* $14.02 \pm 2.3\%$, $P < 0.001$ and $P < 0.01$, respectively). There was no change in NMD values after RTx in HD patients ($16.27 \pm 1.9\%$ *vs* $16.30 \pm 1.8\%$, $P > 0.05$). Also, NMD values in all patients were similar to healthy control values.

**Conclusions.** There is an improvement of endothelial function as assessed by FMD of the brachial artery after RTx in HD patients. This may be attributed to the elimination of uraemic toxins by successful RTx.

**Keywords:** endothelial dysfunction haemodialysis; renal transplantation

## Introduction

Haemodialysis (HD) patients have been characterized by a high incidence and prevalence of cardiovascular disease, which is the leading cause of death in these patients [1]. In HD patients, atherosclerosis is the most frequent cause of cardiovascular morbidity [2]. The endothelium plays a central role in the initiation of atherosclerosis. The endothelium senses and responds to changes in haemodynamic forces and blood-borne signals by releasing vasoactive substances such as nitric oxide (NO). Decreased production or availability of NO and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors cause endothelial dysfunction [3]. The term endothelial dysfunction is generally used to denote impairment of endothelium-dependent vasodilatation. It is well known that cardiovascular risk factors such as hypertension, diabetes mellitus and hyperlipidaemia may cause endothelial dysfunction. There are many modalities available to test endothelial function such as intracoronary studies, impedance plethysmographic studies, venous studies and measurement of flow-mediated vasodilatation in the brachial artery by ultrasonography [3,4]. Celermajer *et al.* [5] was the first to describe non-invasive assessment of flow-mediated vasodilatation in the brachial artery. In this technique, upper-arm occlusion for 5 min results in reactive hyperaemia after the cuff is released, and this causes increased shear stress resulting in flow-mediated

*Correspondence and offprint requests to*: Huseyin Kocak, MD, Department of Nephrology, Akdeniz University School of Medicine, Kampus 07070, Antalya, Turkey.
Email: hkocak@akdeniz.edu.tr

© The Author [2005]. Published by Oxford University Press on behalf of ERA-EDTA. All rights reserved.
For Permissions, please email: journals.permissions@oxfordjournals.org

vasodilatation of the brachial artery (FMD). Brachial artery FMD has been shown to correlate with measures of coronary endothelial function, which is the gold standard for measurement of endothelial function. The main advantages of brachial artery FMD measurement are the non-invasive nature and the ability to repeat multiple tests in the same patient or to construct a study in a large number of patients [3,4].

The well known atherosclerotic risk factors in HD patients are hypertension, diabetes mellitus, dyslipidaemia, inflammation and smoking [6]. Although traditional cardiovascular risk factors are highly prevalent in dialysis patients, it does not necessarily explain the extraordinary high risk of cardiovascular morbidity. One of the possible factors contributing to higher cardiovascular risk in HD patients may be the uraemic toxins [7]. It is described that HD patients have impaired FMD of conduit arteries [8,9]. Apart from hypertension, hyperlipidaemia and hyperglycaemia, which are highly prevalent in HD patients, the uraemic medium itself can cause endothelial dysfunction in these patients [10]. Short term elimination of uraemic toxins may result in increased FMD of the brachial artery in haemodialysis patients [11].

Herein, we hypothesize that the elimination of uraemic toxins in response to renal transplantation (RTx) can improve endothelial function. We therefore compared endothelial functional status in the same patients before and after renal transplantation by measurement of FMD of brachial artery non-invasively. The other aim of this study was to compare endothelial functional status of chronic renal failure patients with healthy control subjects.

## Subjects and methods

Thirty chronic HD patients and 20 normotensive healthy controls were included in this study. The aetiologies of chronic kidney disease disease were as follows: chronic glomerulonephritis ($n = 14$), chronic interstitial nephritis ($n = 10$) and unknown ($n = 6$). Informed consent was taken from all participants. This study was approved by the Ethics Committee on Human Research at Akdeniz University, Antalya, Turkey. Patients who were between 18 and 60 years of age, who were on haemodialysis for >6 months and were candidates for living-related kidney transplantation were included. All HD patients were haemodialysed with bicarbonate containing dialysate bath three times weekly and the duration of dialysis was 4 h per session. Patients with diseases that may interfere with endothelial function, such as diabetes mellitus and coronary artery disease, were excluded. Coronary artery disease was investigated with medical history, physical examination, ECG recordings and echocardiographic examination. No patient required antihypertensive medication or lipid-lowering drugs. All study subjects were non-smokers. The type of kidney transplantation performed in all patients was living-related. The maintenance immunosuppressive regimens after RTx were similar in all recipients (cyclosporine, mycophenolate mofetil and prednisolone).

Assessment of endothelial function in HD patients was performed twice: (i) 3 days before kidney transplantation, (ii) 14th day after kidney transplantation. Endothelial function was measured non-invasively as percentages of flow-mediated dilatation of the brachial artery in the non-dominant or non-fistula arm, as described previously [5]. Patients were investigated in a supine position after a 10 min rest in a temperature-controlled room, after 12 h overnight fasting. The arm was comfortably immobilized in the extended position in order to best visualize the brachial artery and the brachial artery was scanned in the longitudinal section 3–5 cm above the antecubital fossa using 10 MHz high resolution linear array transducer. After optimal transducer positioning, the skin was marked for reference for later measurements and the arm was kept in the same position throughout the study. All measurements of the brachial artery internal diameter were assessed at end-diastole (timed by QRS complex) and were calculated as the mean of three consecutive measurements. After baseline measurements of the brachial artery diameter were recorded, the cuff was inflated to 200 mmHg (or 50 mmHg higher than the systolic blood pressure) for 5 min to create forearm ischaemia. Subsequently, the cuff was deflated and the arterial diameter was measured at 45–60 s after deflation. Flow-mediated endothelium dependent dilatation of brachial artery was expressed as the percentage change in the brachial artery diameter from baseline to reactive hyperaemia. In addition, peak systolic velocity (PSV) was measured by Doppler in the centre of the artery at a 60° angle to the vessel, with a range gate of 1.5 mm. Baseline and the maximum PSV during the first 15 s after releasing the cuff was recorded and the change in PSV expressed as a percentage of the baseline PSV.

Endothelium-independent dilatation was measured by further scans at rest and 4 min after sublingual administration of 0.4 mg glyceryltrinitrate (GTN), which is a direct NO donor. The time interval between the first and second measurements was 20 min for vessel recovery. The differences between resting diameter and diameter after GTN were expressed as glyceryltrinitrate-induced endothelium-independent dilatation (NMD). In addition, the same measurements of endothelial function were also performed in the control group.

In all subjects, blood was drawn at 8:00 am. Serum total cholesterol and triglyceride levels were measured by cholesterol oxidase and enzymatic colorimeric assay (Roche Modular Diagnostics, Basel, Switzerland). Serum glucose level was measured by the glucose oxidase method (Roche Modular Diagnostics). High density lipoprotein (HDL) cholesterol level was measured by direct HDL cholesterol assay and low-density lipoprotein (LDL) cholesterol level was calculated using the Friedwald formula. Serum creatinine level was measured by the Jaffé method. High sensitive C-reactive protein (hs-CRP) was measured by the nephelometric method.

### *Statistical analysis*

Statistical analysis was done by Graph Pad Prism Software (version 3.00). Numerical variables were given as mean ± standard deviation (SD). Numerical variables were compared with one-way analysis of variance (ANOVA) or unpaired Student's *t*-test when necessary. When distribution was abnormal, non-parametric tests were used. $\chi$-Square test was used for non-numerical data. Statistical significance was considered as assumed at $P < 0.05$.

Downloaded from https://academic.oup.com/ndt/article/21/1/203/1818964 by Akdeniz Universitesi user on 20 September 2021

**Table 1.** Baseline characteristics of the patients and control subjects

| Characteristic | HD patients ($n=30$) | Tx patients ($n=30$) | Control subjects ($n=20$) | $P$ |
|---|---|---|---|---|
| Age (years) | 38 ± 6 | 38 ± 6 | 37 ± 5 | ns |
| Male/female | 16/14 | 16/14 | 11/9 | ns |
| Time on dialysis (months) | 34 ± 20 | | | |
| Creatinine (mg/dl) | 8.4 ± 2.3 | 0.86 ± 0.22 | 0.78 ± 0.28 | <0.001[a] |

ns, nonsignificant; HD, haemodialysis; Tx, transplant.
[a]HD patients *vs* control and Tx patient.

**Table 2.** Haemodynamic, biochemical and endothelial function parameters

| | Patients on HD | Patients after RTx | Control | $P$ |
|---|---|---|---|---|
| SBP (mmHg) | 126 ± 9 | 128 ± 9 | 124 ± 10 | ns |
| DBP (mmHg) | 81 ± 5 | 82 ± 4 | 80 ± 5 | ns |
| Serum cholesterol (mg/dl) | 184.8 ± 7.8 | 185.4 ± 8.3 | 185.6 ± 7.3 | ns |
| HDL (mg/dl) | 25.5 ± 5.3 | 26.5 ± 4.9 | 27.1 ± 3.4 | ns |
| LDL (mg/dl) | 116.2 ± 10.8 | 116.3 ± 11.3 | 118.4 ± 4.8 | ns |
| TGL (mg/dl) | 214.4 ± 37.8 | 217.1 ± 39.9 | 201.8 ± 43.6 | ns |
| FBG (mg/dl) | 82.2 ± 9.4 | 84.1 ± 14.8 | 83.7 ± 9.2 | ns |
| FMD % | 6.6 ± 3.1 | 10.5 ± 3.0 | 14.0 ± 2.3 | <0.001[a] |
| NMD % | 16.2 ± 1.9 | 16.3 ± 1.8 | 17.1 ± 3.3 | ns |
| SCRP | 1.50 ± 0.21 | 1.49 ± 0.23 | 0.34 ± 0.16 | <0.001[b] |

[a]HD patients *vs* Tx patients and control.
[b]HD patients and Tx patients *vs* control.

## Results

Baseline characteristics of patients and healthy controls are given in Table 1. Age and gender were similar in the two groups. The mean serum creatinine in HD patients was higher than in the control group (8.4 ± 2.3 *vs* 0.7 ± 0.2 mg/dl, $P<0.001$). After RTx, mean serum creatinine levels in both groups were similar (0.8 ± 0.2 *vs* 0.7 ± 0.2 mg/dl, $P>0.05$). Biochemical, haemodynamic and endothelial function parameters are shown in Table 2. Systolic blood pressure (SBP), diastolic blood pressure (DBP), serum cholesterol, HDL, LDL, triglyceride (TGL) and fasting blood glucose (FBG) levels and NMD were similar in all groups ($P>0.05$). Sensitive CRP levels were found to be similar during dialysis and after transplantation in some patients (Table 2). The patients' sensitive CRP levels were higher than in healthy controls ($P<0.001$).

Endothelial function as the percentage of FMD was significantly lower in patients either during HD or after RTx, compared to healthy controls (6.69 ± 3.15%, 10.50 ± 3.03% *vs* 14.02 ± 2.36%, respectively, $P<0.001$ and $P<0.01$), but NMD were similar in all groups (16.27 ± 1.93%, 16.30 ± 1.86% *vs* 17.16 ± 3.38%, respectively, $P>0.05$). In haemodialysis patients, although there was an improvement in FMD with transplantation (6.69 ± 3.15% *vs* 10.50 ± 3.03%, $P<0.001$; Figure 1), there was no significant change in NMD (16.27 ± 1.93 *vs* 16.30 ± 1.86%, $P>0.05$). In addition, as a % increase in PSV was similar in patients during haemodialysis or after RTx (243 ± 45 *vs* 260 ± 60, $P>0.05$), % increase in PSV was also similar between patients either during haemodialysis or after RTx and control (243 ± 45, 260 ± 60 *vs* 267 ± 46, $P>0.05$).

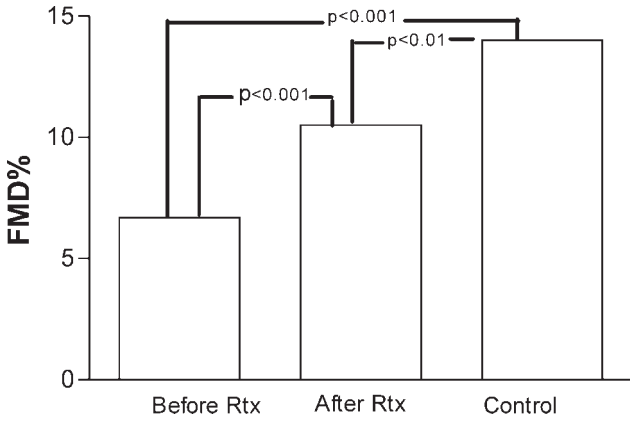



**Fig. 1.** Comparision of flow mediated dilatation (FMD) of brachial artery as FMD% in patients before RTx and after RTx with control.

## Discussion

Endothelium-derived NO, synthesized by the endothelial NO synthase from the precursor L-arginine, is not only a major mediator of endothelium-dependent vasodilation but is also critically involved in the regulation of other protective properties of the healthy endothelium. NO has anti-inflammatory, antithrombotic, anticoagulant and profibrinolytic properties.

Downloaded from https://academic.oup.com/ndt/article/21/1/203/1818964 by Akdeniz Universitesi user on 20 September 2021

Decreased bioavailability of NO in vascular endothelium can initiate the atherosclerotic process. Hyperlipidaemia, diabetes mellitus, hypertension and smoking may cause endothelial dysfunction by causing decreased NO bioavailability [4,5]. Patients with hypertension, diabetes, hyperlipidaemia and smoking habitus were excluded in our study.

Improvement of endothelial function by dialysis reveals that endothelial dysfunction in renal failure could be a consequence of accumulation of uraemic toxins in the body [11]. Of the many different molecules that have been proposed as possible uraemic toxins, some have the potential to inhibit endothelial function, including $N^{G}N^{G}$-dimethyl-L-arginine (asymetrical dimethylarginine, ADMA, an inhibitor of endothelial NO production) and homocysteine [12,13]. We do not know yet exactly which uraemic toxin is responsible for the deterioration of FMD in haemodialysis patients.

In our study, we found that HD patients have endothelial dysfunction. This result confirms previous studies concerning endothelial functional assessment in haemodialysis patients [8,9]. Impairment of FMD in our HD patients may result from decreased bioavailability of NO in the vascular endothelium, since we know that NO is responsible for the FMD flow mediated dilatation of the brachial artery [14]. Similar NMD values in HD patients and healthy control supports decreased NO bioavailability in these patients' vascular endothelium, because nitrate was given as a direct NO source in non-invasive assessment of brachial artery endothelial function.

The main finding in this study was the improvement of endothelial function in HD patients after RTx. Recently, Passaur *et al.* [15] also showed improvement of endothelial function in ESRD patients after RTx. This study was performed in a small group by an invasive technique (forearm blood flow by venous occlusion plethysmography). In our study, we used brachial artery FMD measurement for the assessment of endothelial function. The main advantage of brachial artery FMD measurement is that it is a non-invasive test, can be repeated in the same patient and can be used to study large numbers of patients in a relatively short period of time [4]. As we do not know which uraemic toxin(s) are responsible for the deterioration of FMD in our HD patients, we could not determine whether specific uraemic toxin(s) eliminated by the transplanted kidney have improved the endothelial function.

CRP is an acute phase protein that increases in many conditions such as infection, inflammation and trauma. In dialysis patients, CRP levels were found to be higher than in the general population. Although factors responsible for increased CRP in dialysis patients are not completely understood, dialysate contamination, reduced clearance and Chlamydia infection are suggested as contributing factors [12,16]. We found that hs-CRP levels in our patients either during HD or after RTx were higher than in healthy controls. Any factor mentioned above might be responsible for increased CRP level in our patients. If we consider CRP level as an inflammatory marker, our results may suggest that dialysis and also transplantation might be a more inflammatory state than normal.

Inflammation and endothelial dysfunction are associated [16]. Inflammation status during dialysis and after transplantation was similar in our patients. In this study, hs-CRP levels in patients were higher than in healthy controls. This suggested that dialysis and also transplantation has a more inflammatory state than in the normal population.

Another major finding of this study was that FMD levels in RTx patients were also significantly lower than in normal subjects. One of the explanations for the decreased FMD level in RTx compared to healthy controls might be that the time elapsed between the transplantation operation and endothelial function measurement is too short for full recovery of the endothelium. According to current knowledge, we do not know yet how much time should elapse for full recovery of endothelium after any operation. So if we consider other conditions that may effect the endothelium, immunosuppressive drugs received in RTx were the only different parameter from healthy controls, other factors that may interfere with FMD levels such as fasting mean blood glucose level, mean systolic and diastolic blood pressures and mean serum cholesterol levels were similar between RTx patients and healthy control subjects. It was recently, shown that cyclosporine can lead to decreased stimulated and basal NO production, which may cause endothelial dysfunction, in RTx patients [17]. Tacrolimus also causes impaired vascular endothelial function [18]. Cyclosporine as an immunosuppressive drug in our patients may be responsible for the decreased FMD levels by causing decreased NO bioavailability.

There are some limitations of this study. One of the limitations is the technique we have used in measuring endothelial function itself. Although brachial artery FMD measurement by ultrasonography is a non-invasive and highly reproducible technique, it is also an operator-dependent one. However, despite that, brachial artery FMD measurement by ultrasonography is preferred as a testing modality by other authors [4]. Another point is that there might be bias for measurement of endothelial function because this study was open-ended.

Although endothelial dysfunction precedes coronary artery atherosclerotic disease in cardiac transplant patients, as has been shown in a study by Fish *et al.* [19], we do not know whether endothelial dysfunction observed in renal transplant patients can predict future cardiovascular events. Renal transplantation may improve endothelial function in HD patients by eliminating uraemic toxins and may decrease cardiovascular risk.

*Conflict of interest statement*. None declared.

Downloaded from https://academic.oup.com/ndt/article/21/1/203/1818964 by Akdeniz Universitesi user on 20 September 2021

## References


1. Locatelli F, Marcelli D, Conte F *et al*. Cardiovascular disease in chronic renal failure: the challenge continues. Registro Lombardo Dialisi e Trapianto. *Nephrol Dial Transplant* 2000; 15 [Suppl 5]: 69–80
2. Lindner A, Charra B, Sherrard D, Scribner BM. Accelerated atherosclerosis in prolonged maintenance haemodialysis. *N Engl J Med* 1974; 290: 697–702
3. Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. *Circulation* 2002; 105: 546–549
4. Anderson TJ. Assessment and treatment of endothelial dysfunction. *J Am Coll Cardiol* 1999; 34: 631–638
5. Celermajer DS, Sorensen KE, Gooch VM *et al*. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992; 340: 1111–1116
6. Locatelli F, Pozzoni P, Tentori F, Del Vecchio L. Epidemiology of cardiovascular risk in patients with chronic kidney disease. *Nephrol Dial Transplant* 2003; 18 [Suppl 7]: vii2–vii9
7. Cheung AK, Sarnak MJ, Yan G *et al*. Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. *Kidney Int* 2000; 58: 353–362
8. van Guldener C, Lambert J, Janssen MJ, Donker AJ, Stehouwer CD. Endothelium-dependent vasodilatation and distensibility of large arteries in chronic haemodialysis patients. *Nephrol Dial Transplant* 1997; 12 [Suppl 2]: 14–18
9. Joannides R, Bakkali EH, Le Roy F *et al*. Altered flow-dependent vasodilatation of conduit arteries in maintenance haemodialysis. *Nephrol Dial Transplant* 1997; 12: 2623–2628
10. Passauer J, Bussemaker E, Range U, Plug M, Gross P. Evidence *in vivo* showing increase of baseline nitric oxide generation and impairment of endothelium-dependent vasodilatation in normotensive patients on chronic hemodialysis. *J Am Soc Nephrol* 2000; 11: 1726–1734
11. Cross JM, Donald A, Patrick J *et al*. Dialysis improves endothelial function in humans. *Nephrol Dial Transplant* 2001; 16: 1823–1829
12. Zoccali C, Mallamaci F, Tripepi G. Traditional and emerging cardiovascular risk factors in end-stage renal disease. *Kidney Int Suppl* 2003; 85: S105–S110
13. Goligorsky MS. Endothelial cell dysfunction and nitric oxide synthase. *Kidney Int* 2000; 58: 1360–1376
14. Joannides R, Haefeli WE, Linder L, Richard V *et al*. Nitric Oxide is responsible for flow-dependent dilation of human peripheral conduit arteries in vivo. *Circulation* 1995; 91: 1314–1319
15. Passauer J, Bussemaker E, Lassig G, Gross P. Kidney transplantation improves endothelium-dependent vasodilation in patients with end-stage renal disease. *Transplantation* 2003; 75: 1907–1910
16. Bolton CH, Downs LG, Victory JG *et al*. Endothelial dysfunction in chronic renal failure: roles of lipoprotein oxidation and pro-inflammatory cytokines. *Nephrol Dial Transplant* 2001; 16: 1189–1197
17. Morris ST, Mc Murray JJ, Rodger RS Farmer R, Jardine AG. Endothelial dysfunction in renal transplant recipients maintained on cyclosporine. *Kidney Int* 2001; 57: 1100–1106
18. Ovuworie CA, Fox ER, Chow CM *et al*. Vascular endothelial function in cyclosporine and tacrolimus treated renal transplant recipients. *Transplantation* 2001; 72: 1385–1388
19. Fish RD, Nabel EG, Selwyn AP *et al*. Responses of coronary artery in cardiac transplant patients to acetylcholine. *J Clin Invest* 1988; 81: 21–31


*Received for publication: 22.3.05*
*Accepted in revised form: 11.8.05*

Downloaded from https://academic.oup.com/ndt/article/21/1/203/1818964 by Akdeniz Universitesi user on 20 September 2021