

Abnormalities of endothelial function in patients with predialysis renal failure

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Abstract

Background—Endothelial dysfunction plays an important role in the development of atherosclerotic vascular disease, which is the leading cause of mortality in patients with chronic renal failure.

Objective—To examine the relation between predialysis renal failure and endothelial function.

Design—Two groups were studied: 80 patients with non-diabetic chronic renal failure and 26 healthy controls, with similar age and sex distributions. Two indices of endothelial function were assessed: high resolution ultrasonography to measure flow mediated endothelium dependent dilatation of the brachial artery following reactive hyperaemia, and plasma concentration of von Willebrand factor. Endothelium independent dilatation was also assessed following sublingual glyceryl trinitrate. The patients were divided into those with and without overt atherosclerotic vascular disease.

Results—Although patients with chronic renal failure had significantly impaired endothelium dependent dilatation compared with controls (median (interquartile range), 2.6% (0.7% to 4.8%) *v* 6.5% (4.8% to 8.3%); $p < 0.001$) and increased von Willebrand factor (254 (207 to 294) *v* 106 (87 to 138) iu/dl; $p < 0.001$), there was no difference between renal failure patients with and without atherosclerotic vascular disease. Within the chronic renal failure group, endothelium dependent dilatation and von Willebrand factor were similar in patients in the upper and lower quartiles of glomerular filtration rate (2.7% (0.7% to 6.7%) *v* 2.8% (1.1% to 5.0%); and 255 (205 to 291) *v* 254 (209 to 292) iu/dl, respectively). Endothelium independent dilatation did not differ between the renal failure or control groups and was also similar in patients with renal failure irrespective of the degree of renal failure or the presence of atherosclerotic vascular disease.

Conclusions—Endothelial function is abnormal in chronic renal failure, even in patients with mild renal insufficiency and those without atherosclerotic vascular disease, suggesting that uraemia may directly promote the development of atherosclerosis early in the progression of chronic renal failure.

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Keywords: atherosclerosis; endothelial function; flow mediated dilatation; renal failure

Advances in the provision of renal replacement therapy have reduced deaths from uraemia but have revealed the clinical significance of atherosclerotic vascular disease in patients with chronic renal failure. Cardiovascular disease is the leading cause of mortality in these patients, with a 16-fold to 19-fold increased risk of myocardial ischaemia and infarction compared with control subjects.^{1–3} This accounts for much of the 70–80% reduction in life expectancy of patients with end stage renal disease.⁴

The response to injury hypothesis proposes that endothelial injury and dysfunction are the primary processes in the pathogenesis of atherosclerosis because they result in lipid accumulation, smooth muscle proliferation, and a tendency to vasospasm and thrombosis.⁵ Endothelial function can be assessed non-invasively using high resolution ultrasound to measure flow mediated endothelium dependent dilatation of the brachial artery, and also by determining plasma concentrations of circulating von Willebrand factor.⁶ Impaired endothelium dependent dilatation has been demonstrated in asymptomatic children and young adults with established risk factors for atherosclerosis, and there is a close correlation between brachial and coronary artery endothelial dysfunction.^{6–8} Von Willebrand factor is an

endothelium derived protein that is involved in the regulation of haemostasis.⁹ Raised plasma concentrations of circulating von Willebrand factor are associated with endothelial injury and predict the development and progression of cardiovascular disease.^{10,11} Reduced endothelium dependent dilatation and increased plasma von Willebrand factor levels have been demonstrated in patients with renal failure on maintenance haemodialysis.^{12–14} However, endothelial function may be altered not only by the process of dialysis itself but also by asymptomatic atherosclerotic disease which may be present in patients with end stage renal disease.¹⁵

This study was designed to assess endothelial function at an earlier stage of progressive renal failure in predialysis patients; to examine the relation between renal failure and endothelial function; and to determine whether abnormalities of endothelial function are related to the presence of clinically evident atherosclerotic vascular disease in patients with chronic renal failure.

Methods

SUBJECTS

Eighty patients with chronic renal failure (serum creatinine > 130 $\mu\text{mol/l}$) were recruited

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Table 1 Definitions of clinical criteria for the presence of atherosclerotic vascular disease and risk factors

Clinical criteria	Definitions
Coronary artery disease	Any one of the following: documented history of myocardial infarction confirmed by Q waves on a 12 lead ECG; typical anginal chest pain confirmed by evidence of inducible ischaemia (exercise ECG, or myocardial perfusion scan); angiographic evidence; coronary revascularisation procedure
Cerebrovascular disease	Documented episode of sudden onset neurological deficit
Peripheral vascular disease	Any of the following: documented history of intermittent claudication or abdominal aortic aneurysm; angiographic evidence; peripheral artery revascularisation procedure; amputation for vascular disease
Renovascular disease	Angiographic evidence
Smoking history	Subjects who had smoked in the last year
Hypertension	Subjects on antihypertensive treatment, or with a systolic blood pressure ≥ 160 mm Hg or a diastolic blood pressure ≥ 90 mm Hg
Hypercholesterolaemia	Subjects receiving lipid lowering treatment or with fasting plasma total cholesterol > 6.5 mmol/l
Family history	Presence of vascular disease in a first degree relative < 65 years

from the predialysis clinic at the Queen Elizabeth Hospital, Birmingham. There were 58 men and 22 women, with a median (interquartile range) age of 65 (56 to 71) years. Twenty six healthy controls with no history of vascular disease (61 (51 to 72) years; 17 men, nine women) were recruited from a nearby general practice. Subjects with diabetes mellitus were excluded.

STUDY PROTOCOL

The study was approved by the South Birmingham local research ethics committee, and written informed consent was obtained from all participants. Following the discontinuation of vasoactive drugs for 18 hours and a 12 hour fast, all subjects underwent a brachial artery ultrasound scan. Subjects were screened by a standardised doctor administered questionnaire, using clinical criteria defined prospectively (table 1), for the presence of atherosclerotic vascular disease and risk factors. The answers were confirmed by a review of the hospital case notes. After a 10 minute period of rest, blood pressure was recorded twice in the sitting position using a standard sphygmomanometer, and fasting blood samples were taken for estimation of serum creatinine and lipid profile. The glomerular filtration rate was calculated from the formula of Cockcroft and Gault.¹⁶ Circulating plasma concentrations of von Willebrand factor were measured using enzyme linked immunosorbent assay kits (Department of Rheumatology, University of Birmingham, Birmingham, UK).

HIGH RESOLUTION ULTRASONOGRAPHY OF THE BRACHIAL ARTERY

The ultrasound procedure was performed according to the method of Celermajer *et al.*⁶ In healthy arteries, reactive hyperaemia following transient occlusion increases shear stress, which results in vasodilatation mediated by endothelium derived nitric oxide.^{17, 18} Endothelium independent dilatation, a reflection of vascular smooth muscle function, can be assessed by measuring changes in the brachial artery diameter following sublingual administration of the nitric oxide donor glyceryl trinitrate.

Subjects were kept supine in a stable room temperature between 20–25°C, with their right arm comfortably immobile in the extended position to allow access to the brachial artery. None of the subjects studied had an arterio-venous fistula. A single investigator performed all imaging and analysis. A B mode scan was obtained of the right brachial artery in cross section between 5 cm to 12 cm proximal to the antecubital fossa, using a 7.5 MHz phased array transducer attached to a Sigma 44 HVD system (Kontron Instruments, Montigny le Bretonneux, France). After optimal transducer positioning, a resolution box function was used to magnify the images. Depth and gain settings were adjusted to maximise the definition of anterior and posterior media to intima interfaces, which were used to demarcate the brachial artery diameter. This diameter was calculated as the average of measurements made during four cardiac cycles, incident with the R wave of the electrocardiograph trace. All measurements were recorded on super VHS videotape for subsequent off line analysis.

Each study was composed of artery diameter measurements as follows: (1) first baseline after 10 minute period of rest to allow acclimatisation; (2) endothelium dependent dilatation 60 to 90 seconds after the response to reactive hyperaemia, induced by the sudden deflation of a pneumatic cuff placed on the ipsilateral forearm and inflated to a pressure 100 mm Hg above systolic for five minutes; (3) second baseline after further 10 minute rest period to allow vessel recovery; (4) endothelium independent dilatation four minutes after sublingual administration of 800 μ g glyceryl trinitrate spray.

At the end of each rest period the baseline velocity was assessed by Doppler ultrasonography. The average baseline diameter and blood flow velocity was calculated from the first and second baseline recordings. The peak increase in blood flow velocity in response to reactive hyperaemia was recorded as the maximum velocity in a single cardiac cycle within the first 15 seconds after cuff deflation and was expressed as a percentage of the average baseline velocity. This was used as a quantitative estimate of reactive hyperaemia. Endothelium dependent and independent dilatation were expressed as the percentage change in the brachial artery diameter from baseline following reactive hyperaemia and sublingual glyceryl trinitrate, respectively.

REPEATABILITY

Intraobserver variability was calculated, based on 17 subjects. The coefficients of variation for baseline diameter, endothelium dependent dilatation, and endothelium independent dilatation were 2.1%, 3.7%, and 2.9%, respectively.

STATISTICAL ANALYSIS

Data were analysed using SPSS for Windows 9.0. Medians and interquartile ranges were used to describe continuous variables. The distribution of discrete and continuous variables between groups was compared using χ^2 and

Table 2 Baseline characteristics of the renal failure and control groups

	Control	Renal failure
Number	26	80
Age (years)	61 (51 to 72)	65 (56 to 71)
Sex (M:F)	17:9	58:22
Smoking history	13	49
Family history	8	30
Hypertension	12	72***
Hypercholesterolaemia	2	26**
BMI (kg/m ²)	25.8 (24.6 to 29.1)	27.2 (24.3 to 29.9)
Creatinine (μmol/l)	96 (90 to 107)	245 (188 to 325)***
GFR (ml/min)	78.3 (62.3 to 93.1)	28.9 (20.3 to 37.6)***
Baseline diameter (cm)	0.48 (0.39 to 0.52)	0.51 (0.45 to 0.56)*
Increase in velocity (%)	208 (176 to 229)	196 (169 to 218)

Continuous variables are expressed as median (interquartile range). Significance was assessed using χ^2 and Mann-Whitney tests as appropriate.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

BMI, body mass index; GFR, glomerular filtration rate.

Mann-Whitney tests. The Kruskal-Wallis test was used to compare continuous variables by quartiles of calculated glomerular filtration rate. Multiple regression analyses were used to correct for differences in baseline diameter and known cardiovascular risk factors. The test results are presented as two tailed values and significance was inferred at $p < 0.05$.

Results

COMPARISON OF RENAL FAILURE AND CONTROL GROUPS

The baseline characteristics for the renal failure and control groups are shown in table 2. The significant differences, other than renal function, were the higher prevalence of hypertension and hypercholesterolaemia and the larger median baseline brachial artery diameter in the renal failure group.

Differences in endothelial function between patients and control are illustrated in fig 1. Endothelium dependent dilatation was significantly impaired in patients with renal failure compared with controls (2.6% (0.7% to 4.8%) *v* 6.5% (4.8% to 8.3%); $p < 0.001$). This difference remained significant after correction for the presence of vascular risk factors and for baseline diameter. Analysis of the crude data also suggested a significant impairment of endothelium independent dilatation in patients with renal failure (9.9% (7.8% to 13.8%) *v* 14.5% (10.6% to 19.0%)) but this was not significant after correction for differences in baseline diameter. Plasma von Willebrand factor concentration was significantly raised in the patients with renal failure compared with the controls (254 (207 to 294) *v* 106 (87 to 138) iu/dl; $p < 0.001$), a difference that remained after correction for vascular risk factors.

Table 3 Endothelium dependent and independent dilatation of the brachial artery and von Willebrand factor concentration according to degree of renal impairment

	Controls	First quartile	Second quartile	Third quartile	Fourth quartile
GFR (ml/min)	78 (62 to 93)	49 (46 to 54)	32 (31 to 36)	24 (22 to 27)	16 (14 to 18)
Baseline diameter (cm)	0.48 (0.39 to 0.52)	0.51 (0.45 to 0.55)	0.53 (0.47 to 0.57)	0.50 (0.45 to 0.52)	0.51 (0.46 to 0.54)
Increase in velocity (%)	208 (176 to 229)	206 (190 to 225)	202 (180 to 218)	167 (151 to 215)	178 (169 to 213)
EDD (%)	6.5 (4.8 to 8.3)	2.7 (0.7 to 6.7)	2.3 (0.1 to 4.8)	1.9 (1.5 to 4.3)	2.8 (1.1 to 5.0)
EID (%)	14.5 (10.6 to 19.0)	11.0 (8.1 to 13.4)	9.1 (6.2 to 11.0)	9.8 (8.8 to 16.8)	9.8 (7.6 to 14.6)
vWF (iu/dl)	106 (87 to 138)	255 (205 to 291)	223 (185 to 276)	273 (223 to 298)	254 (209 to 292)

Results are expressed as median (interquartile range). Significance was assessed with the Kruskal-Wallis test.

EDD, endothelium dependent dilatation; EID, endothelium independent dilatation; GFR, glomerular filtration rate; vWF, von Willebrand factor.

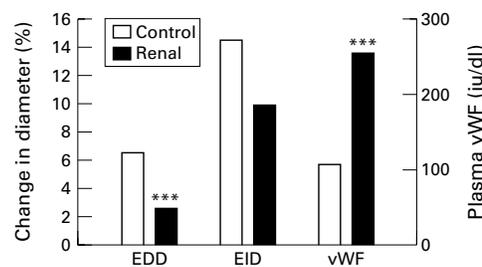


Figure 1 Endothelium dependent and independent dilatation of the brachial artery and von Willebrand factor concentration in the renal failure and control groups. EDD, endothelium dependent dilatation; EID, endothelium independent dilatation; vWF, von Willebrand factor; *** $p < 0.001$ after controlling for differences in the baseline characteristics.

ENDOTHELIAL FUNCTION ACCORDING TO DEGREE OF RENAL IMPAIRMENT

The renal failure group was divided by the calculated glomerular filtration rate into quartiles with declining renal function from the first to the fourth quartiles. There were no significant differences in age, sex distribution, body mass index, smoking and family history, prevalence of hypertension and hypercholesterolaemia, and the presence of vascular disease between the four quartiles. The results for endothelium dependent dilatation and endothelium independent dilatation of the brachial artery and plasma von Willebrand factor concentration are shown in table 3. The baseline diameter and increase in velocity were similar across the quartiles. There was no significant difference in endothelium dependent dilatation, endothelium independent dilatation, or plasma von Willebrand factor concentrations between the quartiles of calculated glomerular filtration rate in patients with renal failure.

COMPARISON OF THE RENAL FAILURE PATIENTS WITH AND WITHOUT CLINICALLY EVIDENT ATHEROSCLEROTIC VASCULAR DISEASE

Clinically evident atherosclerotic vascular disease was diagnosed in 29 of the renal failure patients: coronary artery disease ($n = 22$), peripheral vascular disease ($n = 3$), cerebrovascular disease ($n = 3$), and renovascular disease ($n = 1$). This group was significantly older than those without atherosclerotic vascular disease (66 (62 to 72) *v* 61 (47 to 71) years; $p < 0.05$) but there were no significant differences in other baseline characteristics between these two groups. After correction for age, there was no difference in endothelium dependent dilatation between renal failure patients with and without atherosclerotic vascular disease (1.6% (0.3% to 3.4%) *v* 3.8% (1.4% to 5.2%)). Similarly, there was no

significant difference in either endothelium independent dilatation or plasma von Willebrand factor concentration between renal failure patients with and without atherosclerotic vascular disease (9.9% (7.1% to 13.0%) *v* 10.0% (8.4% to 14.7%), and 254 (227 to 294) *v* 246 (201 to 293) iu/dl, respectively).

Discussion

Endothelial dysfunction precedes the development of plaques in animal models and develops initially in humans at coronary branch points, the same sites at which advanced plaques are later formed.^{19,20} Thus endothelial dysfunction is thought to be the initial event in a process that culminates in atherosclerosis.⁵ This study showed that two indices of endothelial function, endothelium dependent dilatation and plasma von Willebrand factor concentration, were abnormal in patients with chronic renal failure when compared with healthy subjects. These abnormalities were independent of differences in known vascular risk factors and vessel size between the two groups and suggest that chronic renal failure may directly induce endothelial dysfunction, thereby promoting the development of atherosclerosis.

The finding that endothelial function is equally impaired in renal failure patients with and without clinically evident atherosclerotic vascular disease lends support to the suggestion that endothelial dysfunction precedes the development of atheroma in renal failure. It is also possible that clinically silent atheroma is present in most patients with renal failure by the time of presentation.

Previous studies examining endothelial function in chronic renal failure have shown a significant reduction in endothelium dependent dilatation and raised concentrations of several endothelium derived proteins including endothelin-1, thrombomodulin, and von Willebrand factor.^{12-14,21-23} However, the subjects in these trials were either patients with advanced renal impairment (median glomerular filtration rates of < 25 ml/min) or those receiving renal replacement therapy. This present study shows that endothelial function is equally impaired in patients with biochemically mild renal failure and in those with more advanced renal disease, suggesting that endothelial dysfunction is already present early in progressive renal failure. Further studies are required to assess endothelial function in a larger number of individuals with glomerular filtration rates of between 50 and 80 ml/min to determine the level at which endothelial dysfunction is induced. However, the Cockcroft formula is a poor measure of glomerular filtration rate in patients with preserved renal function, and investigation of endothelial function in the mildest or subclinical forms of renal failure would require a more accurate estimate of the degree of renal impairment.

Nitric oxide regulates vessel tone, inhibits platelet activation, adhesion, and aggregation, inhibits smooth muscle proliferation, and modulates endothelial cell-leucocyte interactions.²⁴⁻²⁶ Impaired bioavailability of nitric oxide, as suggested by the reduction in

endothelium dependent dilatation in patients with chronic renal failure, may contribute to the pathogenesis of atherosclerosis in uraemia. Although the precise mechanisms are unknown, uraemic serum contains various substances that may either reduce endothelial production of nitric oxide or promote nitric oxide breakdown, including asymmetrical dimethylarginine,²⁷ homocysteine,^{24,28} and oxidatively modified low density lipoproteins.²⁹

Prevention and treatment of vascular disease is currently targeted at renal patients with advanced disease; however, our study would suggest that these strategies should be directed at patients at earlier stages of progressive renal failure before irreversible endothelial damage occurs. This is particularly important, as moderate renal dysfunction is surprisingly common—8% of 6000 middle aged individuals from the Framingham population were found to have mild renal insufficiency (serum creatinine 120 to 265 μ mol/l).³⁰ In up to half of all patients, the incidence of coronary artery disease cannot be explained by conventional risk factors. Therefore the study of the aetiology of coronary artery disease in patients with renal failure may provide valuable insights into mechanisms in the general population.

In conclusion, we have shown in this study that endothelial function is abnormal in chronic renal failure, even in patients with biochemically mild renal insufficiency and in those without clinically evident atherosclerotic vascular disease. This suggests that uraemia may directly promote the development of atherosclerosis early in the progression of chronic renal failure.

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