Vascular Endothelial Cell Function and Cardiovascular Risk Factors in Patients With Chronic Renal Failure[1]

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ABSTRACT

Cardiovascular risk factors and markers of endothelial cell function were studied in nondiabetic patients with mild to moderate chronic renal failure. The transcapillary escape rate of albumin and the plasma concentrations of von Willebrand factor, fibrinogen, and plasma lipids were measured in 29 nondiabetic patients (GFR of 25 (11–44) mL/min × 1.73 m^2 (median and range)) and 14 normal subjects. The proportion of smokers was similar between the groups. In the patients, the plasma concentration of von Willebrand factor was elevated by 61% (1.27 ± 0.44 versus 0.79 ± 0.28 U/mL; $P < 0.01$) (mean ± SD) and that of fibrinogen was elevated by 72% (10.18 ± 4.14 versus 5.92 ± 2.01 μmol/L; $P < 0.01$). The plasma concentrations of lipoproteins showed an atherogenic pattern in the patients with increased levels of very low-density lipoprotein cholesterol (0.57 ± 0.31 versus 0.33 ± 0.13 mmol/L; $P < 0.01$) and triglycerides (1.26 ± 0.25 versus 0.71 ± 0.28 mmol/L; $P < 0.01$), but a decreased level of high-density lipoprotein cholesterol (1.23 ± 0.33 versus 1.46 ± 0.35 mmol/L; $P < 0.05$). Total cholesterol and low-density lipoprotein cholesterol were similar in the groups. The observed differences were further aggravated among smoking patients, particularly with respect to von Willebrand factor and triglycerides. The transcapillary escape rate of albumin was similar in the patients and the controls and was not correlated to the level of albuminuria. The combination of probable vascular injury and an elevated plasma concentration of fibrinogen may increase the risk of thrombotic episodes in the patients and, together with dyslipidemia and hypertension, may explain the increased cardiovascular morbidity and mortality in chronic nephropathy.

Key Words: *chronic renal failure, lipoproteins, fibrinogen, von Willebrand factor, transcapillary escape rate*

Cardiovascular disease is a frequent complication of chronic renal failure (CRF) (1), possibly related to the high frequency of dyslipidemia (2) and hypertension (3) in these patients. Other factors such as changes in the coagulation system, platelet hyperreactivity, and endothelial dysfunction or injury could be of importance as well. Thus, studies in insulin-dependent diabetic patients with clinical nephropathy have demonstrated probable vascular dysfunction as manifested by elevated plasma levels of von Willebrand factor (4) and an increased permeability of macromolecules through the vascular walls (5). These risk factors have not been well studied in patients with nondiabetic nephropathy. Therefore, the main purpose of this study was to examine vascular endothelial cell function as well as established cardiovascular risk factors in nondiabetic patients with mild to moderate CRF.

SUBJECTS AND METHODS

Patients

The study was carried out in 29 patients (12 women and 17 men), aged 22 to 69 years, with mild to moderate CRF, with plasma creatinine in the range of 163 to 476 μmol/L. The patients had one of the following clinical diagnoses: 5 patients had chronic glomerulonephritis, 6 had hypertensive nephrosclerosis, 2 had chronic pyelonephritis, 13 had polycystic kidney disease, and 3 had unclassified nephropathy. None had diabetes mellitus, and none received lipid-lowering drugs. All but one received antihypertensive medication. Twenty-four patients were treated with angiotensin-converting enzyme inhibitors alone or in combination with other drugs. Twelve patients received β-blockers and thiazides. Fourteen normal subjects (eight women and six men) in the age range of 32 to 73 years were studied as controls.

Ten patients (34%) and three normal subjects (21%) were smokers. All subjects gave their informed consent to participate in the study, which was approved by the research ethics committee of Copenhagen County, Denmark.

Laboratory Measurements

All patients and control subjects were studied after an overnight 8-h fast. A cannula was inserted in the antecubital vein in each arm. After a 30-min rest in the supine position, blood samples were collected. The plasma was separated immediately. Serum albumin was measured with an enzyme-linked immunosorbent (ELISA) assay (6), and serum creatinine was measured by a reaction rate kinetic principle eliminating pseudocreatinines (7). The plasma concentration of von Willebrand factor was measured with an ELISA (8), and plasma fibrinogen was determined as thrombine coagulable fibrinogen, as described by Jacobsen (9). Plasma cho-

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lesterol and triglyceride were determinated enzymatically (Boehringer Mannheim, Mannheim, Germany, CHOD-PAP method). Plasma high-density lipoprotein (HDL) cholesterol was determined after the precipitation of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) with phosphotungstic acid (Boehringer Mannheim). Plasma LDL cholesterol and VLDL cholesterol were calculated by Friedewald's formula: LDL cholesterol = total cholesterol − (HDL cholesterol + VLDL cholesterol), where VLDL cholesterol = 0.47 × triglyceride (10).

The urinary albumin was measured with an ELISA (6). The level of albuminuria was determined on the basis of the median urinary albumin excretion (UAE) in the last three 24-h urine collections performed at home during normal physical activity. This was done in order to take into account the high (50%) day-to-day variation of the 24-h UAE (6).

Transcapillary Escape Rate of Albumin

The transcapillary escape rate of albumin (TER_{alb}), which is the fraction of the intravascular mass of albumin leaving the vascular bed per hour, was measured as described by Feldt-Rasmussen (5). [^{125}I]human serum albumin (Code IFA-IT23S; Kjeller, Olso, Norway) was used. After an iv injection of the tracer (2 μCi) in one arm, seven blood samples were collected from the other arm during the first hour. Plasma radioactivity was measured in each sample with a well-type scintillation detector and expressed as counts per minute per gram of total plasma albumin. These ratios were plotted in a semilogarithmic scale, and the transcapillary escape rate was calculated as the disappearance rate constant (percent per hour). Plasma volume was determined by retropolation of the disappearance curve to zero time and from the injected volume of tracer (milliliters per 1.73 m^2). The intravascular mass of albumin was calculated as plasma volume times serum albumin (micromoles per 1.73 m^2), and the outflux of albumin from the intravascular compartments was calculated as IVM times Ter_{alb} (micromoles per hour × 1.73 m^2). Measurement of TER_{alb} has a coefficient of variation of around 8% (11).

The GFR was measured with a single injection of [^{51}Cr]EDTA, plotting the plasma disappearance of the tracer for 4 h. In the case of an expected GFR < 20 and > 15 mL/min, an additional blood sample was drawn 5 h after injection. When GFR was estimated below 15 mL/min, blood samples were drawn at 5 and 24 h after injection (12,13). The small underestimation (10%) of [^{51}Cr]EDTA clearance versus clearance of inulin was corrected for by multiplying the EDTA clearance by 1.10. This method has a coefficient of variation of around 4% (14).

Statistical Analysis

Results are given as mean and ± SD, median, and range. Mann-Whitney's test was used for comparison between groups. Spearman's correlations were calculated to evaluate the association between the measured variables.

RESULTS

The clinical data of the patients and normal controls are shown in Table 1. The patients and controls were not different in age and body mass index. The patients were moderately uremic with a GFR of 25 (11 to 44) mL/min × 1.73 m^2 and a plasma creatinine of 296 (163 to 476) μmol/L (median and range). Both their systolic blood pressure and their diastolic blood pressure were significantly increased when compared with those of the controls.

TABLE 1. Clinical data of patients and normal subjects[a]

Characteristic	Controls	Patients
Sex ratio (F/M)	8/6	12/17
Age (yr)	41 (28-73)	44 (22-69)
GFR (mL/min per 1.73 m^2)	94 (73-118)	25 (11-44)[b]
Creatinine (μmol/L)	86 (49-128)	296 (163-476)[b]
Systolic Blood Pressure (mm Hg)	115 (100-140)	140 (105-165)[b]
Diastolic Blood Pressure (mm Hg)	75 (50-95)	84 (70-100)[b]
Body Mass Index (kg/m^2)	23 (20-29)	25 (19-33)
Smoker/Nonsmoker	3/11 (21%)	10/19 (34%)

[a] Values are median (range).
[b] Denotes $P < 0.01$.

Plasma concentrations of both fibrinogen and von Willebrand factor were significantly elevated in the patients compared with the controls ($P < 0.01$) (Table 2). There was no correlation between GFR and plasma von Willebrand factor.

The TER_{alb} was identical in patients with CRF and normal controls, and Spearman's test revealed no positive correlation between the UAE and TER_{alb} in patients with CRF. The plasma volume, the intravascular mass of albumin, and the outflux of albumin were all similar in the patients and the normal subjects (Table 3).

Plasma concentrations of total cholesterol in the normal controls and patients with CRF were not significantly different. However, in the patients, the plasma concentrations of VLDL cholesterol and triglyceride were significantly higher ($P < 0.01$) and that of HDL cholesterol was significantly lower ($P < 0.05$). Twelve patients received β-blockers alone or in combination with thiazides. When these patients were compared with the 17 who received no such treatment, no significant difference was found in the plasma concentrations of cholesterol ($P = 0.5$), triglyceride ($P = 0.81$), LDL cholesterol ($P = 0.44$), or HDL cholesterol ($P = 0.71$). Thirty-four percent of the patients were smoking more than five cigarettes a day compared with 21% of the controls (not significant). All of the observed differences between patients and controls in Table 2 remained significant when including only nonsmoking patients versus all controls, smoking or nonsmoking. This was also the case with respect to von Willebrand factor (controls versus nonsmoking patients, 0.79 ± 0.28 versus 1.17 ± 0.29 U/ml [$P < 0.01$] and plasma triglyceride (0.71 ± 0.28 versus 1.49 ± 0.55 mmol/L [$P < 0.01$]). The risk factors in general were or tended to be aggravated among smoking patients.

DISCUSSION

The main new finding in this study of patients with mild to moderate uremia is the demonstration of an

TABLE 2. Plasma concentrations of von Willebrand factor, fibrinogen, and lipoproteins in smoking and nonsmoking patients with chronic renal failure and healthy controls[a]

Plasma Concentration	Controls	Patients	
		Nonsmokers	Smokers
N	14	19	10
von Willebrand Factor (U/mL)	0.79 ± 0.28	1.17 ± 0.29	1.64 ± 0.56[b]
Fibrinogen (μmol/L)	5.92 ± 2.01	11.06 ± 4.70[c]	11.54 ± 2.72[c]
VLDL Cholesterol (mmol/L)	0.33 ± 0.13	0.55 ± 0.21[c]	0.81 ± 0.33[c]
LDL Cholesterol (mmol/L)	2.99 ± 0.95	3.36 ± 0.81	3.03 ± 1.30
HDL Cholesterol (mmol/L)	1.46 ± 0.35	1.12 ± 0.39[d]	1.18 ± 0.25[d]
Triglyceride (mmol/L)	0.71 ± 0.28	1.49 ± 1.55	1.93 ± 0.70[b]
Total Cholesterol (mmol/L)	4.95 ± 0.59	5.07 ± 0.89	5.38 ± 1.00

[a] Results are mean ± SD.
[b] Denotes $P < 0.01$ from controls and nonsmokers.
[c] Denotes $P < 0.01$ from controls.
[d] Denotes $P < 0.05$ from controls.

TABLE 3. Intravascular mass, outflux, and escape of albumin in 29 patients with chronic renal failure and 14 normal controls[a]

Variable	Controls	Patients
Serum Albumin (μmol/L)	622 ± 33	603 ± 49
Plasma Volume (mL/1.73 m^2)	2,892 ± 330	3,040 ± 290
TER_{alb} (% per h)	5.8 ± 1.8	5.9 ± 2.8
Intravascular Mass of Albumin (μmol/1.73 m^2)	1,782 ± 176	1,805 ± 185
Outflux of albumin (μmol/h × 1.73 m^2)	103 ± 29	112 ± 46
UAE (mmol/24 h)		16.0 (0.0–71.6)

[a] Mean ± SD, except for UAE, which is given as median and range.

elevated level of circulating von Willebrand factor, which may suggest vascular endothelial cell dysfunction. The study is also the first to report an elevated concentration of plasma fibrinogen in this group of patients. Well-known cardiovascular risk factors, *i.e.*, an elevated blood pressure and atherogenic lipid abnormalities, were also present. Putative and established risk factors tended to be more strongly expressed in subgroups of patients who were smokers.

von Willebrand factor is a glycoprotein known to be synthesized in both vascular endothelial cells and megakaryocytes, the endothelial cells being the major source (15). von Willebrand factor is essential for platelet adhesion to the subendothelium (16). The stimulation of the endothelium with cytotoxic agents such as tumor necrosis factor and lymphotoxin induces the release of von Willebrand factor (17), and high plasma levels have, in prospective studies, been prognostic for myocardial infaction events (18). Thus, high plasma levels are generally regarded as an indicator of endothelial cell damage (19). Elevated von Willebrand factor has previously been demonstrated in patients undergoing regular hemodialysis treatment (20–22), but our study is the first to demonstrate an elevated plasma level of this factor also in patients with only mild to moderate uremia, suggesting endothelial cell dysfunction. Interestingly, no correlation was found between the level of GFR and the plasma concentration of von Willebrand factor. von Willebrand factor is a large-molecular-weight (0.5×10^6 to 20×10^6) multimer that will not normally be cleared by the kidneys, and hence, it is unlikely that the high plasma levels in uremia are caused by the decreased GFR *per se*.

In diabetic patients with microalbuminuria, the presence of high levels of von Willebrand factor (4), together with an increased endothelial permeability for albumin, has supported the hypothesis that microalbuminuria is an early marker of generalized vasculopathy in Type I (23) as well as in Type II (24) diabetic patients. We did not find any correlation between the plasma level of von Willebrand factor and albuminuria in this study of nondiabetic uremic patients, and the TER_{alb} was similar in the patients and normal controls. Further, there was no association between TER_{alb} and albuminuria. Thus, the failure to demonstrate any generalized vascular leakage of albumin, taken together with the elevated plasma concentration of von Willebrand factor in patients with moderate uremia, suggests that these patients have a dysfunction of endothelial cells qualitatively different from that of albuminuric diabetic patients. Although it is likely that albuminuria and endothelial dysfunction have a common pathogenetic mechanism in diabetic patients (22), it would seem reasonable to propose that endothelial dysfunction is directly caused by uremia in nondiabetic patients with CRF. The fact that elevated plasma levels of von Willebrand factor can reflect different types of injury may be suggested because it is also seen in atherosclerosis and inflammatory vascular disease (25).

The role of an elevated plasma concentration of fibrinogen as an independent risk factor for myocardial infarction and stroke (26–28) has been clearly demonstrated in the general population and also in patients with nephrotic syndrome and end-stage renal

failure (29). It has not previously been studied in patients with only mild to moderate uremia. The combination of endothelial dysfunction and hyperfibrinogenia might increase the risk of thrombotic episodes in the patients.

The presence of hypertension and atherogenic lipoprotein abnormalities with elevated plasma concentrations of triglyceride and VLDL cholesterol and reduced HDL cholesterol concentration is in accordance with previous studies (2,30). All patients but one received antihypertensive medications. Twelve patients were treated with β-blockers alone or in combination with thiazides. These drugs may induce changes in the plasma concentrations of lipoproteins. Compared with the 17 patients who did not receive such treatment, no significant differences between any of the measured cardiovascular risk factors were demonstrated.

Smoking, a well-established cardiovascular risk factor, was also accounted for in this study. About one-third of the patients were smoking more than five cigarettes a day. The smoking patients had significantly elevated plasma concentrations of triglyceride and von Willebrand factor compared with the nonsmoking patients. The nonsmoking patients, however, still showed higher plasma concentrations of these parameters as compared with the healthy controls. This suggests that smoking may add to the increased cardiovascular mortality in patients with CRF. However, of the observed differences in the risk profiles with respect to hyperfibrinogenemia, endothelial dysfunction, and lipoprotein, patterns also remained significant when comparing only nonsmoking patients with the control group. In conclusion, this study has added hyperfibrinogenemia and endothelial dysfunction to the array of cardiovascular risk factors present in mild to moderate uremia, hopefully paving the way for new therapeutic and preventive measure in these patients.

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REFERENCES

1. **Ordonez JD, Hiatt R, Killebrew E, Fireman B:** The risk of coronary artery disease among patients with the nephrotic syndrome. Kidney Int 1990;37:243A.
2. **Appel G:** Lipid abnormalities in renal disease. Kidney Int 1991;39:169–183.
3. **MA KW, Greene EL, Raij L:** Cardiovascular risk factors in chronic renal failure and hemodialysis populations. Am J Kidney Dis 1992;6:505–513.
4. **Jensen T:** Increased plasma concentration of von Willebrand factor in insulin-dependent diabetic patients with incipient nephropathy. BMJ 1989;298:27–28.
5. **Feldt-Rasmussen B:** Increased transcapillary escape rate of albumin in type 1 (insulin-dependent) diabetic patients with microalbuminuria. Diabetologia 1986;29: 282–286.
6. **Feldt-Rasmussen B, Dinesen B, Deckert M:** Enzyme immuno assay - an improved determination of urinary albumin in diabetics with incipient nephropathy. Scand J Clin Lab Invest 1985;45:539–544.
7. **Larsen K:** Creatinine assay by a reaction-kinetic principle. Clin Chim Acta 1972;45:209–217.
8. **Ingerslev JA:** Sensitive ELISA for von Willebrand factor (vWf:Ag). Scand J Clin Lab Invest 1987;47:143–149.
9. **Jacobsen K:** Method for the determination of fibrinogen in plasma. Scand J Clin Lab Invest 1955;14 [Suppl]:14–16.
10. **Friedewald WT, Levy RI, Frederickson DS:** Estimation of the concentration of low-density cholesterol in plasma without use of preparative ultracentrifuge. Clin Chem 1972;18:499–502.
11. **Parving H-H, Nielsen SL, Lassen NA:** Increased transcapillary escape rate of albumin, IgG and IgM during angiotensin II induced hypertension in man. Scand J Clin Lab Invest 1974;34:111–118.
12. **Brøchner-Mortensen J:** A simple method for the determination of glomerular filtration rate. Scand J Clin Lab Invest 1972;30:271–274.
13. **Brøchner-Mortensen J:** Reliability of rutine clearence methods of assessment of glomerular filtration rate in advanced renal insufficiency. Scand J Clin Lab Invest 1981;41:91–97.
14. **Brøchner-Mortensen J, Rødbro P:** Selection of routine method for determination of glomular filtration rate in adult patients. Scand J Clin Lab Invest 1976;36:35–45.
15. **Jaffe EA, Hoyer LW, Nachman RL:** Synthesis of von Willebrand factor by cultured human endothelial cells. Proc Natl Acad Sci 1987;79:117–123.
16. **Sakariassen KS, Bolhuis PA, Sixma JJ:** Human blood platelet adhesion to artery subendothelium is mediated by factor VIII-von Willebrand factor bound to the subendothelium. Nature 1979;279:636–638.
17. **Kahaleh MB, Smith EA, Soma Y, LeRoy EC:** Effect of lymphotoxin and tumor necrosis factor on endothelial and connective tissue cell growth and function. Clin Immunol Immunopathol 1988;49:261–272.
18. **Nilsson TK, Norberg B, Wallberg-Johnson S, Dahlqvist SR:** Von Willebrand factor in plasma as risk indicator for cardiovascular events. J Intern Med 1991;229:557–558.
19. **Boneu B, Abbal M, Plante J, Bierme R:** Factor VIII complex and endothelial damage. Lancet 1975;1:1430.
20. **Tomura S, Nakamura Y, Degughi F, *et al.*:** Plasma von Willebrand factor and thrombomodulin as markers of vascular disorders in patients undergoing regolar hemodialysis therapy. Thromb Res 1990;58:413–419.
21. **Turney JH, Woods HF, Fewell MR, Weston MJ:** Factor VIII complex in uraemia and effects of haemodialysis. BMJ 1981;282:1653–1656.
22. **Warrel PR, Hultin MB, Coller B:** Increased factor VIII/von Willebrand factor antigen and von Willebrand activity in renal failure. Am J Med 1979;66:226–228.
23. **Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A:** Albuminuria reflects widespread vascular damage. The steno hypothesis. Diabetologia 1989;32:219–226.
24. **Schmitz A:** The kidney in non-insulin-dependent diabetes. Acta Diabetol 1992;29:47–69.
25. **Blann AD:** von Willebrand factor as a marker of injury to thr endothelium in inflammatory vascular disease. J Rheumatol 1993;20:1469–1471.
26. **Kannel WB, Wolf PA, Castelli WP, D'Agostina RB:** Fibrinogen and risk of cardiovascular disease. The Framingham study. JAMA 1987;258:1183–1186.
27. **Meade TW, Mellows S, Brozovic M, *et al.*:** Haemostatic function and ischaemic heart disease. Principal results of the Northwick Park Heart Study. Lancet 1986;2:533–537.
28. **Qizilbash N, Jones L, Warlow C, Mann J:** Fibrinogen and lipid concentrations as risk factors for transient ischaemic attacks and minor ischaemic strokes. BMJ 1991; 303:605–609.
29. **Vaziri ND, Gonzales E, Barton CH, Chen HT, Nguyen Q, Arquilla M:** Factor XIII and its substrates, fibronectin, fibrinogen, and α_2-antiplasmin, in plasma and urine of patients with nephrosis. J Lab Clin Med 1991;117:152–156.
30. **Mendez A, Perez GO, Goldberg RB, Hsia SL:** Lipid and lipoprotein levels in undialyzed patients with chronic renal failure. Am J Med Sci 1987;293:164–170.