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Original Article

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Carotid atherosclerosis is associated with inflammation and endothelial cell adhesion molecules in chronic haemodialysis patients

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Abstract

Background. Recently emerging evidence suggests that endothelial adhesion molecules may participate in atherogenesis. The aim of the present report was to investigate the probable association of circulating ICAM-1, VCAM-1 and E-selectin with atherosclerotic disease in chronic haemodialysis (HD) patients.

Methods. One hundred and twelve HD patients and 50 age- and sex-matched healthy normotensive controls participated in the study. Atherosclerotic disease in both groups was assessed by measuring intima–media thickness (IMT) and plaque score of the common carotid arteries using an ultrasound scanner. In addition, in a follow-up study, the survival of 81 patients after a mean period of 26 months was analysed in relation to ICAM-1 and VCAM-1 levels.

Results. IMT and plaque score were significantly higher in HD patients compared with control subjects ($P<0.001$ and $P<0.0001$, respectively). The above ultrasonographic indices were correlated with age both in controls ($P=0.0001$ and $P=0.002$, respectively) and HD patients ($P=0.0001$ and $P=0.0001$, respectively). A significant relationship was observed between IMT and systolic blood pressure (BP) both in controls and in HD patients ($P=0.002$ and $P=0.01$, respectively). In HD patients, plaque score was also correlated with systolic BP ($P=0.02$). In HD patients, IMT and plaque score were correlated significantly with log CRP values ($P=0.01$ and $P=0.01$, respectively). Multivariate analysis showed that log CRP values were a strong independent contributor to plaque score ($P=0.01$). IMT was significantly correlated with ICAM-1 and VCAM-1 concentrations ($P=0.0001$ and $P=0.003$, respectively). Multivariate analysis showed that ICAM-1 concentrations were a strong independent correlate of IMT ($P=0.001$). E-selectin concentrations did not show any relation with IMT or plaque score. During the follow-up period, 13 of the 81 patients died. Survival analyses showed that patients with increased ICAM-1 had a shorter survival than patients with normal ICAM-1 values and that serum ICAM-1 levels were a strong predictor of death.

Conclusions. In HD patients, carotid atherosclerosis is associated with inflammation and circulating levels of soluble adhesion molecules ICAM-1 and VCAM-1. The correlations between serum ICAM-1 and IMT and ICAM-1 and survival may indicate that this molecule could be a marker of a process that contributes to the high mortality of HD patients.

Keywords: adhesion molecules; atherosclerosis; cardiovascular disease; haemodialysis; intima–media thickness; plaque score

Introduction

Atherosclerotic cardiovascular disease (CVD) is a significant cause of morbidity and mortality for patients with end-stage renal disease (ESRD). A marked increase in coronary heart disease incidence and death rates has been reported in haemodialysis (HD) patients when compared with an age-matched general population, as well as a significant increase when compared with non-uraemic population with hyperlipidaemia and hypertension [1]. Undoubtedly, ESRD is associated with a higher prevalence of several traditional and uraemia-related risk factors for atherogenesis, such as hypertension, hyperlipidaemia, diabetes mellitus, haemodynamic overload, anaemia and increased oxidative stress. However, the combination of the known risk factors accounts only partly for the particularly increased burden of atherosclerotic disease in HD patients, indicating

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that other factors yet to be defined are also probably triggered in this patient population [2].

Recently, emerging evidence suggests a central contribution of endothelial cell adhesion molecules in the pathogenesis and progression of atherosclerosis through their effects on leukocyte activation, cell migration and smooth-muscle cell proliferation [3]. E-selectin is a member of the selectin family that is expressed transitorily only on the surface of cytokine-activated endothelial cells, where it mediates leukocyte rolling, a step that immediately precedes adhesion. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are two members of the Ig-like supergene family of adhesion molecules that are normally expressed by endothelial cells. Cytokine activation produces a dramatic upregulation of their expression on the cell surface where they support the firm adhesion and subsequent transmigration of leukocytes [4]. We have previously demonstrated that the dialysis procedure itself induces a striking increase in soluble ICAM-1 and VCAM-1 concentrations independently of the type of dialysis membrane used. Moreover, a significant increase of serum ICAM-1 was observed in patients with established CVD such as a history of myocardial infarction, coronary artery bypass, angina pectoris, stroke or peripheral vascular disease [5]. Since no data were available concerning their levels in patients with subclinical atherosclerosis, we undertook an investigation of the probable association of circulating endothelial cell adhesion molecules with atherosclerotic disease as assessed by high-resolution ultrasonography of the common carotid arteries.

Subjects and methods

Cross-sectional study

Subjects. Between January and April 2001, 112 adult patients on chronic maintenance HD (60 male, mean age 59 years, range 25–86 years) from the dialysis unit of the University Department of Nephrology at Hippokration General Hospital and from one affiliated outpatient dialysis centre consecutively entered the study. All patients had been stabilized on renal replacement therapy for >3 months (mean HD duration 74 months, range 5–372 months) and were clinically stable and free of active infection. Chronic renal failure was attributed to glomerulonephritis in 46 cases, tubulointerstitial nephritis in 35, polycystic kidney disease in 11, renovascular hypertension in four and was undetermined in 16 cases. Patients with diabetes mellitus, liver disease, autoimmune diseases or malignancies were excluded, in order to avoid the possible effects of these co-morbid conditions on cytokine production. None of the patients was receiving antibiotics, corticosteroids or cytotoxic drugs at the time of the study. All patients were receiving conventional 4-h HD, three times weekly, with bicarbonate dialysate and low-molecular-weight heparin as standard anticoagulation. Dialysis prescription was guided by a goal of achieving a value of $\geqslant 0.65$ for the urea reduction ratio and a value of Kt/V $\geqslant 1.2$. The above indices of adequacy of dialysis were calculated by the formula [(pre-dialysis urea)–(post-dialysis urea)/predialysis urea] and by the second-generation Daugirdas equation, respectively. Forty-eight of the 112 patients (42.9%) were routinely dialysed with modified cellulose membranes and 64 patients (57.1%) were routinely dialysed with polysulphone membranes. Seventy-five patients (67%) were receiving one or more antihypertensive drugs (calcium-channel blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, oral nitrates or beta-blockers) at the time of the study. Ninety-three patients (83.0%) were on recombinant human erythropoietin therapy and the mean dosage was 111 ± 51 IU/kg BW/week. Thirty-nine patients (34.8%) had clinical signs or a previous history of CVD. Of the 39 patients, 26 had a history of myocardial infarction, coronary artery bypass or clinical signs of angina pectoris, five had suffered a stroke and eight had peripheral vascular disease. The control group consisted of 50 age- and sex-matched apparently healthy subjects (27 male, mean age 56 years, range 25–85 years) who were recruited from hospital staff members and their relatives. These control subjects did not have any history of hypertension, diabetes mellitus and renal or vascular disease, and were receiving no drugs at the time of the study. Informed consent was obtained from each patient and control subject.

Blood pressure measurements. Pre-dialysis and post-dialysis blood pressure (BP) were calculated as the average value of all recordings (12 measurements, i.e. three measurements per week) obtained during the month preceding the study [6]. The mean value for pre-dialysis and post-dialysis BP was then obtained for each patient and was considered for statistical assessment. In control subjects, BP was measured after 15 min of recumbency and five measurements 2 min apart were averaged.

Laboratory methods. Blood samples from HD patients and control subjects were taken from a peripheral vein under fasting conditions. Samples from HD patients were collected in the morning of a midweek routine dialysis day. Serum samples were separated from clotted blood by immediate centrifugation (1500 *g* for 10 min), aliquoted and stored at -70°C until assay. Serum levels of the circulating adhesion molecules ICAM-1, VCAM-1 and E-selectin were measured by an enzyme-linked immunosorbent assay (ELISA) using commercially available standard kits (Quantikine human sICAM-1, sVCAM-1 and sE-selectin; Research & Diagnostic Systems Europe Ltd, Abington, UK). Sera were diluted 1/30, 1/75 and 1/25, respectively, for the quantitation of ICAM-1, VCAM-1 and E-selectin. The concentrations of these molecules were calculated by reference to standard curves performed with the corresponding recombinant molecule. All serum samples were tested in duplicate. The sensitivity of the ELISA system was 2 ng/ml, 2 ng/ml and 1 ng/ml for ICAM-1, VCAM-1 and E-selectin, respectively. Serum albumin, total cholesterol, triglycerides and HDL cholesterol were determined by routine techniques using an automated analyser (Olympus AU560, Hamburg, Germany). LDL cholesterol was calculated using the Friedewald formula. Serum CRP levels were measured by nephelometry. The detection limit was 3.75 mg/l and in the statistical evaluation all values <3.75 mg/l were treated as 3 mg/l.

Carotid ultrasonography. Ultrasonographic studies were performed with an Aloka Sonos SSD-1700 (Aloka, Tokyo, Japan) instrument using a 7.5 MHz high-resolution probe.

Each subject was examined in the supine position in a semi-dark room. In HD patients, the ultrasonographic study was performed during a mid-week non-dialysis day and within 2 weeks after blood sampling. The carotid artery was investigated bilaterally by the same expert radiologist (M.K.) who was unaware of clinical and laboratory data. Intima–media thickness (IMT) was defined as a low-level echo grey band that does not project into the arterial lumen and was measured at the diastolic phase as the distance between the leading edge of the first and second echogenic line. IMT was measured on the longitudinal views of the far wall of the distal segment of the common carotid artery, the carotid bifurcation and the initial tract of the internal carotid artery on both sides. Measurements were performed 0.5, 1 and 2 cm below and above the bifurcation (six measurements on each side) in a plaque-free arterial segment. When a plaque was observed in the region of carotid-artery measurements, the IMT was not measured. The average measurement of the obtained values was taken as IMT and it was considered abnormal when it exceeded 0.82 mm [7]. Carotid plaques were defined (and counted) either as faint grey echoes (soft plaques) or bright white echoes (calcified plaques) protruding into the arterial lumen. Plaque thickness was measured in a suitable longitudinal or transverse view. Plaque score was computed by summing maximum thickness in millimetres of plaques in each segment on both sides.

Follow-up study

In our previous study, serum ICAM-1 and VCAM-1 levels were measured in 81 patients, before and at the end of a routine midweek dialysis session and the results have been published recently [5]. These patients were enrolled between December 1999 and February 2000 and were followed up for 26.0 ± 3.8 months (range 11–28 months) after the assessment of pre- and post-dialysis serum adhesion molecule levels. During this follow-up period, 13 patients died. Each death was reviewed, all available medical information was collected including hospitalization records and an underlying cause was assigned. Survival was analysed in relation to the predialysis serum levels of ICAM-1 and VCAM-1 [5].

Statistical analysis

Data are expressed as mean $\pm$ SD and with range. The significance of differences in mean between the two groups was assessed by Student's *t*-test or Mann–Whitney test as appropriate. Differences in proportions were tested with the use of χ^2 statistic. Correlations were tested by regression analysis. Non-normally distributed variables were log-transformed before entering regression analysis. Multiple regression analysis with a forward elimination procedure was used to assess the combined influence of variables on IMT and plaque score values. The following variables were used: age, sex, smoking, HD duration, history of CVD, systolic and diastolic BP, serum cholesterol, triglycerides, HDL, LDL, log CRP, ICAM-1, VCAM-1 and E-selectin levels. Survival analysis was made by the Kaplan–Meier method. Probability of survival was also analysed by univariate Cox proportional regression analysis. Variables tested were age, sex, smoking, HD duration, hypertension, history of CVD, log CRP and serum ICAM-1 levels. The calculations were performed using Statview v. 4.5 statistical software (Abacus Concept Inc., Berkeley, CA, USA). A two-tailed *P*-value < 0.05 was considered statistically significant.

Results

The somatometric, haemodynamic and biochemical characteristics, as well as the risk factors for atherosclerosis of controls and HD patients are shown in Table 1. Triglycerides were significantly increased and HDL was significantly decreased in HD patients compared with control subjects. Thus, HD patients showed, as expected, a lipid profile different from that of healthy normotensive controls. In addition, systolic BP was elevated in HD patients whereas diastolic BP did not differ significantly between the two groups. BMI and smoking status were also similar in the two groups (Table 1).

Compared with control subjects, HD patients had significantly increased IMT and plaque score (0.65 ± 0.08 *vs* 0.89 ± 0.17 mm, $P < 0.001$ and 0.37 ± 0.73 *vs* 4.85 ± 4.56 mm, $P < 0.0001$, respectively). IMT was increased (> 0.82 mm) in 67 (59.0%) of HD patients. Atherosclerotic plaques were detected in 91 (81.2%) of HD patients and in 11 (22%) of controls. The difference in plaque occurrence between the two groups was significant ($P < 0.0001$). The number of plaques ranged from 1 to 3 in control subjects and from 1 to 9 in HD patients. In the latter group, all plaques were calcified.

Ultrasonographic findings and risk factors for atherosclerosis

IMT and plaque score were significantly correlated with age both in control subjects ($r = 0.51$, $P = 0.0001$ and $r = 0.43$, $P = 0.0001$, respectively) and HD patients ($r = 0.46$, $P = 0.0001$ and $r = 0.41$, $P = 0.0001$, respectively) (Figure 1). In the latter group, the number of plaques in individual patients was also significantly correlated with age ($r = 0.42$, $P = 0.0001$). In addition, IMT was significantly correlated with systolic BP in both groups ($r = 0.43$, $P = 0.002$ and $r = 0.23$, $P = 0.01$,

Table 1. Somatometric, haemodynamic and biochemical characteristics and risk factors for atherosclerosis of 50 control subjects and 112 haemodialysis patients

	Controls	HD patients	*P*
Age (years)	56 ± 15	59 ± 14	NS
Sex (M/F)	27/23	60/52	NS
HD duration (months)		74 ± 77	
Kt/V		1.29 ± 0.17	
BMI	24.5 ± 2.5	23.3 ± 3.1	NS
Smoking (% of patients)	14 (28%)	29 (25.9%)	NS
Systolic BP (mmHg)	133 ± 12.6	141.3 ± 19.8	0.03
Diastolic BP (mmHg)	76.8 ± 8.3	78.6 ± 9.4	NS
Haemoglobin (g/dl)	14.6 ± 0.7	11.2 ± 1.4	0.0001
Albumin (g/dl)	4.8 ± 0.5	4.2 ± 0.4	NS
Total cholesterol (mg/dl)	201 ± 23	199 ± 46	NS
Triglycerides (mg/dl)	116 ± 39	179 ± 103	0.0001
HDL cholesterol (mg/dl)	48.0 ± 9.6	38.0 ± 11.2	0.0001
LDL cholesterol (mg/dl)	129 ± 19.5	125 ± 41	NS
CRP (mg/l)	3.9 ($<$3.75–6)	11.8 ($<$3.75–41.5)	

Values are expressed as mean $\pm$ SD and (%), as appropriate. CRP values are given as median values and ranges.

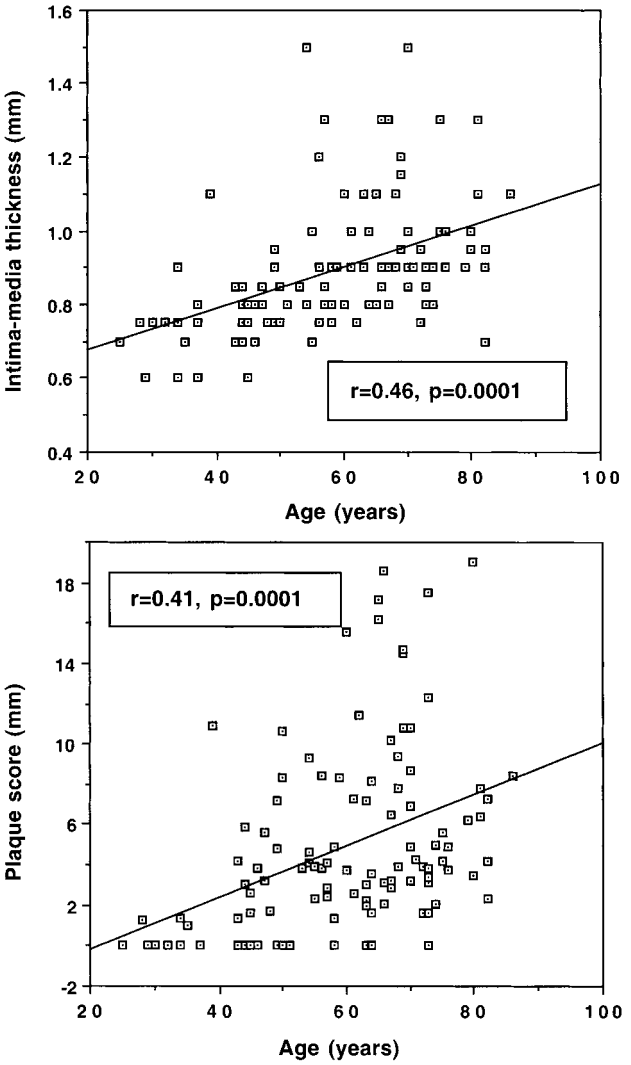

Fig. 1. Correlation between age and IMT (upper panel) and plaque score values (lower panel) in 112 haemodialysis patients.

respectively). In HD patients, plaque score was also correlated with systolic BP ($r=0.21$, $P=0.02$). Ultrasonographic parameters did not show any significant correlation with other classic cardiovascular risk factors in both groups, such as gender, smoking status, total cholesterol, triglycerides, HDL and LDL cholesterol or HD duration and Kt/V in HD patients. IMT, but not plaque score values, were significantly increased in patients with history of CVD compared with patients with negative history (0.95 ± 0.21 *vs* 0.86 ± 0.14 mm, $P=0.02$ and 5.3 ± 4.1 *vs* 4.6 ± 4.8 mm, $P=0.41$, respectively). In addition, history of CVD was significantly correlated with IMT in univariate regression analysis ($P=0.03$).

Ultrasonographic findings and inflammation

Fifty-two of the 112 HD patients (46.4%) had abnormal CRP levels (normal range <3.75–5 mg/l). In HD patients, IMT and plaque score were significantly correlated with log-transformed CRP ($r=0.24$, $P=0.01$ and $r=0.23$, $P=0.01$, respectively). In addition, compared with patients with normal CRP, patients with elevated CRP (>5 mg/l) had significantly increased IMT (0.86 ± 0.16 *vs* 0.94 ± 0.18, $P=0.004$) and plaque score (4.04 ± 4.40 *vs* 5.77 ± 4.61 mm, $P=0.01$). Multivariate analysis showed that log-CRP values were a strong independent correlate of plaque score ($r=0.23$, $P=0.01$). Moreover, age and systolic BP retained also an independent effect on plaque score values (Table 2).

Ultrasonographic findings and endothelial adhesion molecules

Compared to control subjects, HD patients showed a significant increase in serum ICAM-1 (244 ± 56 *vs* 508 ± 202 ng/ml, $P<0.0001$) and VCAM-1 (1056 ± 122 *vs* 2375 ± 646 ng/ml, $P<0.0001$), but not in E-selectin (73 ± 41 *vs* 91 ± 56 ng/ml, $P=$NS) concentrations. In HD patients, adhesion molecule levels did not show any relation to HD duration or Kt/V. Serum levels of ICAM-1 and VCAM-1 were significantly correlated with IMT ($r=0.36$, $P=0.0001$ and $r=0.28$, $P=0.003$, respectively) (Figure 2). Moreover, compared to patients with normal IMT, patients with increased IMT values (>0.82 mm) had significantly elevated ICAM-1 (435 ± 166 *vs* 557 ± 211 ng/ml, $P=0.001$) and VCAM-1 (2163 ± 721 *vs* 2518 ± 551 ng/ml, $P=0.004$), but not E-selectin (86 ± 55 *vs* 99 ± 56 ng/ml, $P=0.23$).

Multivariate analysis showed that serum ICAM-1 concentrations were a strong independent correlate of IMT (Table 3). Because of the highly significant relationship between ICAM-1 and log CRP values ($r=0.36$, $P=0.0001$), the latter parameter was an independent correlate of IMT ($r=0.22$, $P=0.02$) only if serum ICAM-1 were excluded from the model. A history of CVD failed to be a significant correlate of IMT in multivariate analysis (Table 3). ICAM-1 and VCAM-1 concentrations failed to be a significant contributor to plaque score either on univariate or multivariate analyses. E-selectin concentrations

Table 2. Multivariate analysis of carotid plaque score

Independent variables	*r*	*P*
Age	0.36	0.0001
Systolic BP	0.22	0.03
log CRP	0.23	0.01
Out of model		
Sex	−0.17	0.80
Smoking	−0.03	0.73
CVD	−0.11	0.25
HD duration	0.15	0.13
Diastolic BP	0.10	0.31
Cholesterol	0.09	0.36
Triglycerides	0.11	0.26
HDL	0.05	0.60
LDL	0.18	0.06

Data are partial correlation coefficients and *P*. Carotid plaque score multiple $r=0.52$, $P=0.0001$.

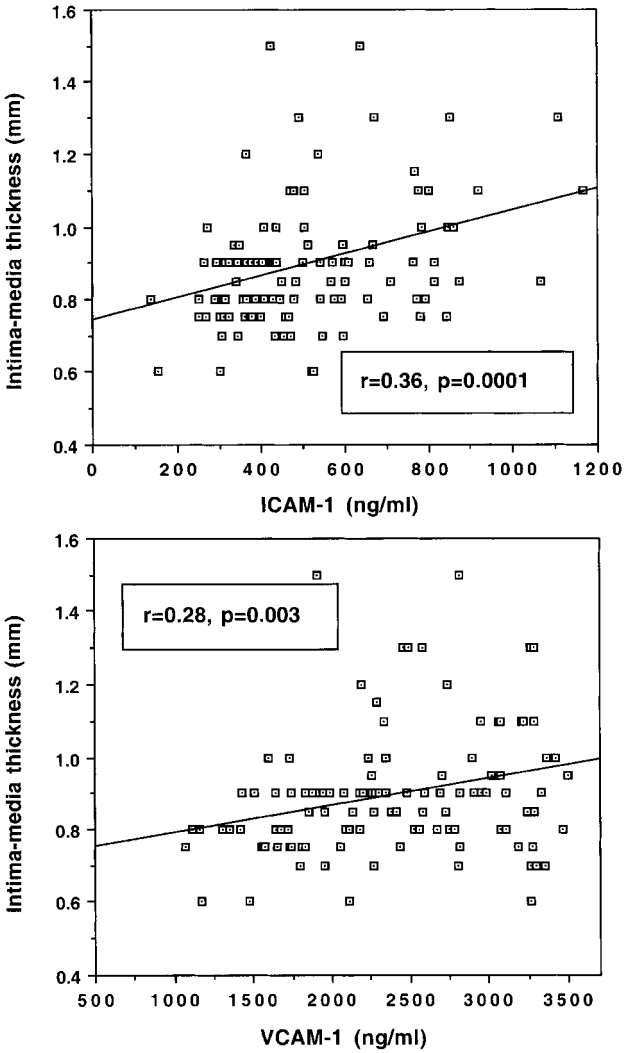

Fig. 2. Correlation between IMT and serum ICAM-1 (upper panel) and VCAM-1 levels (lower panel) in 112 haemodialysis patients.

Table 3. Multivariate analysis of carotid IMT

Independent variables	*r*	*P*
Age	0.42	0.0001
Systolic BP	0.24	0.01
ICAM-1	0.33	0.001
Out of model		
Sex	0.17	0.08
Smoking	0.02	0.78
CVD	0.03	0.75
HD duration	−0.04	0.60
Diastolic BP	−0.03	0.69
Cholesterol	−0.03	0.71
Triglycerides	−0.04	0.74
HDL	−0.05	0.63
LDL	0.03	0.78
log CRP	0.13	0.20
VCAM-1	0.04	0.72

Data are partial correlation coefficients and *P*. Carotid IMT multiple $r=0.62$, $P=0.0001$.

showed no correlation with either IMT or plaque score values.

Endothelial adhesion molecules and survival

After a mean follow-up period of 26 months, 13 of the 81 patients (16%) had died, 8 (61.5%) from cardiovascular causes. Serum ICAM-1 levels were higher in patients who died compared to those who survived (649 ± 172 *vs* 490 ± 167 ng/ml, $P=0.003$). Similarly, ICAM-1 levels were higher in patients who died from cardiovascular causes compared to those who survived (698 ± 183 *vs* 490 ± 167 ng/ml, $P=0.001$). Serum VCAM-1 levels tended also to be greater in non-survivors compared to survivors but this difference failed to reach statistical significance (2357 ± 578 *vs* 2060 ± 654 ng/ml, $P=0.12$). Kaplan–Meier survival analysis showed that patients with increased serum ICAM-1 had a shorter survival compared to patients with normal ICAM-1 concentrations (normal range 126–425 ng/ml) (Figure 3). The relationship between serum ICAM-1 and survival was also analysed by univariate Cox regression analysis and this showed that ICAM-1 levels were a strong predictor of death. Age, sex, smoking, HD duration, hypertension, history of CVD and log CRP failed to be statistically significant predictors of mortality (Table 4).

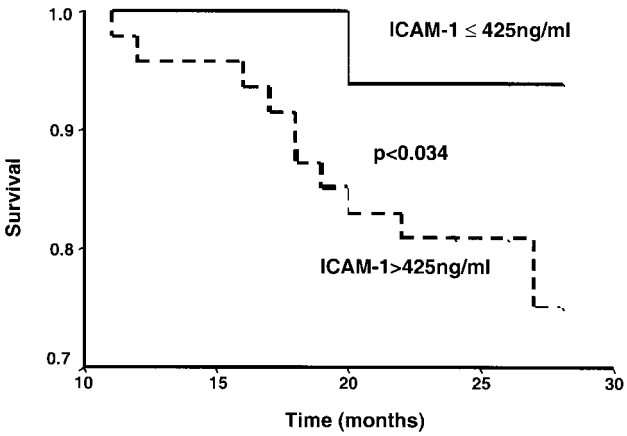

Fig. 3. Kaplan–Meier survival curves in 81 haemodialysis patients with serum ICAM-1 levels greater than ($n=47$) and less than ($n=34$) the upper limit of normal range (126–425 ng/ml).

Table 4. Univariate Cox regression analysis for overall mortality

Variable	Hazard ratio	95% Confidence interval	*P*
ICAM-1	1.004	1.001–1.007	0.004
Age	0.998	0.957–1.041	0.936
Sex	0.801	0.262–2.450	0.697
Smoking	2.029	0.450–9.158	0.357
HD duration	0.998	0.991–1.006	0.628
Hypertension	2.312	0.755–7.076	0.142
CVD	1.647	0.453–5.985	0.449
log CRP	0.522	0.115–2.361	0.399

Discussion

The introduction of high-resolution ultrasonography has provided a reliable, reproducible and non-invasive method for detecting and monitoring the progression of subclinical atherosclerosis. The present study demonstrated that IMT and plaque score, as well as the incidence of plaque occurrence, were significantly increased in HD patients compared with age and sex-matched controls. The above findings concur with previous reports and further support the hypothesis of an 'accelerated atherogenesis' in this patient population [8,9].

In recent years, systemic inflammation has been regarded as a cardiovascular risk factor both in the general population and in ESRD patients [10,11]. In accordance with previous reports, the present study demonstrated a significant association between IMT and CRP values [12,13]. In addition, CRP was found to be a significant contributor to plaque score on both univariate and multivariate analysis, indicating that CRP is a valuable surrogate marker for atherosclerotic vascular damage.

One key event in the initiation and progression of atherosclerosis is the adhesion of circulating leukocytes to the vascular wall and subsequent transendothelial migration mediated by interaction of leukocyte surface receptors with their ligands on endothelial cells. It is of interest that adhesion molecules ICAM-1, VCAM-1 and E-selectin, expressed on the surface of vascular endothelial cells in response to pro-inflammatory cytokines, have been detected by immunohistochemistry in human atherosclerotic lesions, and their expression was found to be correlated with intimal infiltration by T-lymphocytes and monocytes/macrophages [14]. However, studies examining the potential value of circulating levels of adhesion molecules as molecular markers for atherosclerosis in different clinical settings in non-renal populations have yielded contradictory results [15–17]. Moreover, the probable significance of these molecules as markers of atherosclerotic vascular damage in stable HD patients has not been investigated.

An interesting finding in the present study was the highly significant association observed between carotid IMT and serum ICAM-1 and VCAM-1 levels. Multivariate analysis showed that ICAM-1 levels were a strong independent correlate of IMT, whereas CRP became a significant correlate of IMT only when ICAM-1 levels were excluded from the analysis. Furthermore, this association appeared to be minimally influenced by a number of traditional or uraemia-related risk factors for atherosclerosis, including previous cardiovascular complications. The above data support the concept that measurements of these molecules could probably help to construct a better model for predicting atherosclerotic disease than models based only on classic risk factors and other markers of inflammation.

ICAM-1 and VCAM-1 concentrations were not correlated with plaque score either on univariate or multivariate analyses. The relative contribution of these molecules to the initiation and progression of atherosclerotic vascular damage has yet to be defined. Nevertheless, the natural history of atherosclerotic vascular changes is well documented and known to progress through the stage of intimal thickening to fully developed plaques and obstructive lesions. Consequently, the above results may indicate that these molecules could have a more important role on the early pathophysiological events than on the more advanced stages of atherosclerosis.

E-selectin concentrations failed to be a significant correlate of carotid atherosclerosis either on univariate or on multivariate analyses. However, these findings do not exclude a role for this protein in atherogenesis since E-selectin is only transitorily expressed on the surface of activated endothelial cells, and its expression returns rapidly to basal levels, even in the presence of continued cytokine exposure [4]. In addition, the probable effect of haemodialysis has been poorly investigated. To our knowledge, there is only one study that reported a significant post-dialysis increase in E-selectin levels using cuprophane and polysulphone, and not cellulose membranes, but the concentrations were still within the normal range [18]. We have also measured pre- and post-dialysis serum E-selectin in 41 patients and were unable to find any significant alterations using either polysulphone or modified cellulose membranes (unpublished data).

ICAM-1 levels were found significantly increased in patients who died during a follow-up period of 26 months compared with those who survived. Moreover, in accordance with a previous report in predialysis patients [19], survival analyses showed that patients with increased ICAM-1 levels had a shorter survival than patients with normal ICAM-1 levels, and that serum ICAM-1 levels were a strong predictor of death. It has been demonstrated that common carotid artery IMT is a strong predictor of cardiovascular events and mortality [20]. The correlations that emerged between ICAM-1 and IMT and ICAM-1 and survival may indicate that this molecule could be a marker of a process that contributes to the high morbidity and mortality of ESRD population. Unfortunately, no IMT measurements were performed in our previous study, to enable us to examine the relative influence of both parameters on survival. In addition, as the present patient numbers were small, this association must be interpreted extremely cautiously and further prospective studies in larger patient numbers are needed to confirm this interesting finding.

In conclusion, in HD patients carotid atherosclerosis is associated with inflammation and circulating levels of ICAM-1 and VCAM-1. Serum ICAM-1 appeared a strong predictor of death in survival analyses. Larger additional studies are needed to determine the potential value of these proteins as surrogate markers for early atherosclerosis and for global cardiovascular risk assessment in this high-risk population.

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References

1. Ma KW, Greene EL, Raij L. Cardiovascular risk factors in chronic renal failure and hemodialysis populations. *Am J Kidney Dis* 1992; 19: 505–513
2. Cheung AK, Sarnak MJ, Yan G *et al*. Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. *Kidney Int* 2000; 58: 353–362
3. Persson J, Blann AD, McCollum CN. Circulating endothelial cell/leukocyte adhesion molecules in atherosclerosis. *Thromb Haemost* 1994; 72: 151–154
4. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood* 1994; 84: 2068–2101
5. Papagianni A, Alexopoulos E, Giamalis P *et al*. Circulating levels of ICAM-1, VCAM-1 and MCP-1 are increased in haemodialysis patients. Association with inflammation, dyslipidaemia and vascular events. *Nephrol Dial Transplant* 2002; 17: 435–441
6. Zoccali C, Mallamaci F, Tripepi G *et al*. Prediction of left ventricular geometry by clinic, pre-dialysis and 24-h ambulatory BP monitoring in hemodialysis patients. *J Hypertens* 1999; 17: 1751–1758
7. Aminbakhsh A, Mancini GB. Carotid intima media thickness measurements. What defines an abnormality? A systematic review. *Clin Invest Med* 1999; 22: 149–157
8. Kawagishi T, Nishizawa Y, Konishi T *et al*. High-resolution B-mode ultrasonography in evaluation of atherosclerosis in uremia. *Kidney Int* 1995; 48: 820–826
9. Savage T, Clarke AL, Giles M, Tomson CRV, Raine AEG. Calcified plaque is common in the carotid and femoral arteries of dialysis patients without clinical vascular disease. *Nephrol Dial Transplant* 1998; 13: 2004–2012
10. Danesh J, Whincup P, Walker M *et al*. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *Br Med J* 2000; 321: 199–204
11. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 1999; 55: 648–658
12. Stenvinkel P, Heimbürger O, Paultre F *et al*. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int* 1999; 55: 1899–1911
13. Zoccali C, Benedetto FA, Mallamaci F *et al*. Inflammation is associated with carotid atherosclerosis in dialysis patients. *J Hypertens* 2000; 18: 1207–1213
14. O'Brien KD, McDonald TO, Chait A, Allen MD, Alpers CE. Neovascular expression of E-selectin, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in human atherosclerosis and their relation to intimal leukocyte content. *Circulation* 1996; 93: 672–682
15. De Catarina R, Basta G, Lazzerini G *et al*. Soluble vascular cell adhesion molecule is a biohumoral correlate of atherosclerosis. *Arterioscler Thromb Vasc Biol* 1997; 17: 2646–2654
16. Hwang SJ, Ballantyne CM, Sharrett AR *et al*. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart cases: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 1997; 96: 4219–4225
17. Rohde LE, Lee RT, Rivero J *et al*. Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* 1998; 18: 1765–1770
18. Mrowka C, Heintz B, Sieberth H-G. Is dialysis membrane type responsible for increased circulating adhesion molecules during chronic hemodialysis? *Clin Nephrol* 1999; 52: 312–321
19. Stenvinkel P, Lindholm B, Heimbürger M, Heimbürger O. Elevated serum levels of soluble adhesion molecules predict death in predialysis patients: association with malnutrition, inflammation and cardiovascular disease. *Nephrol Dial Transplant* 2000; 15: 1624–1630
20. Benedetto FA, Mallamaci F, Tripepi G, Zoccali C. Prognostic value of ultrasonographic measurement of carotid intima media thickness in dialysis patients. *J Am Soc Nephrol* 2001; 12: 2458–2464

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