Hemodiyaliz Hastalarında Karotis İntima Medya Kalınlığıyla Subklinik Aterosklerozun İlişkisi ve Klamidya ve Sitomegalovirüs İnfeksiyonunun Rolü

Inflammation as a Risk Factor for Carotid Intimal-Medial Thickening, a Measure of Subclinical Atherosclerosis in Hemodialysis Patients and the Role of Chlamydia and Cytomegalovirus Infection

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ÖZET

Giriş: Aterosklerotik vasküler hastalık (AVH) hemodiyaliz (HD) hastalarında en sık görülen komplikasyondur. Elde edilen veriler inflamasyonun aterosklerozun patogenezinde ve progresyonunda rol oynadığını düşündürmektedir. Bu çalışmadaki amacımız HD hastalarında aterosklerozun inflamasyon üzerindeki etkisini araştırmaktı.

Yöntem: 54 HD hastasında ve 52 kişilik kontrol grubunda intima medya kalınlığı (İMK) ölçüldü. Çalışmaya katılanların tümünde plazma lipid, glukoz, albümin, akut faz proteinleri ve klamidya ve CMV için IgG düzeyleri ölçüldü.

Bulgular: Ortalama İMK kontrol grubuna göre anlamlı olarak artmış bulundu (0.75 mm ve 0.56 mm, p<0.005). İki grupta da yaş ile İMK arasında ilişki saptandı. HD hastalarında CRP, SAA, Lp(a), fibrinojen ve ferritin seviyeleri artmış olarak bulunurken, albümin seviyeleri azalmıştı. Hastalarda İMK, CRP (R=0.29, p=0.019), SAA (R=0.69, p<0.001), Lp(a) (R=0.42, P=0.001), fibrinojen (R=0.57, p<0.001), ve klamidya pneumonia IgG seviyeleri (R=0.50, p<0.001) ile pozitif, albümin ile negatif korele idi. Karotis İMK ile; hipertansiyon, plazma lipid seviyeleri ve CMV arasında ilişki saptanmadı. Bununla beraber, CRP ve SAA; fibrinojen, Lp(a) ve klamidya ile pozitif albümin seviyeleri ile negatif korele bulundu.

Sonuç: HD hastalarında aterosklerotik değişikliklerin daha fazla olduğu görüldü ve inflamatuar sürecin ateroskleroz patogenezinde rol oynayabileceği sonucuna varıldı. Ayrıca bu çalışma geçirilmiş klamidyal infeksiyonların CMV infeksiyonlarından daha aterojenik olabileceğini düşündürmektedir.

Anahtar sözcükler: akut faz cevabı, ateroskleroz, inflamasyon; karotis intima media kalınlığı, klamidya pneumonia ve CMV infeksiyonu, hemodiyaliz

ABSTRACT

Objectives: Atherosclerotic vascular disease (AVD) is the most frequent complication seen in hemodialysis (HD) patients. Evidence suggests that inflammation may play a role in the pathogenesis and progression of atherosclerosis. Our aim was to evaluate the causative role of inflammation in atherosclerosis among HD patients.

Methods: Intima-media thickness (IMT) in carotid arteries was determined in 54 HD patients and 52 controls. Plasma levels of lipids, glucose, albumin and several acute phase proteins, and IgG titers against Chlamydia and CMV were measured in all subjects.

Results: Mean carotid IMT was significantly greater in HD patients than in controls (0.75 mm versus 0.56 mm, p<0.005). Increasing carotid IMT was associated with advancing age in both groups. While plasma levels of CRP, SAA, Lp(a), fibrinogen and ferritin were higher in HD patients, albumin levels were lower. In HD patients, carotid IMT was correlated positively with CRP (R=0.29, p=0.019), SAA (R=0.69, p<0.001), Lp(a) (R=0.42, P=0.001), fibrinogen (R=0.57, p<0.001), and with Chlamydia pneumonia IgG titers (R=0.50, p<0.001), and negatively with albumin levels (R=-0.33, p=0.02); there was no relationship between carotid IMT and hypertension, plasma lipid levels, and CMV. Moreover, CRP and SAA were positively correlated with fibrinogen, Lp(a) and Chlamydia, and inversely with albumin levels in HD patients.

Conclusions: We conclude that atherosclerotic changes are more common in HD patients than controls, and that inflammatory processes may play a role in the pathogenesis of atherosclerosis. Also, past Chlamydial infection might be more atherogenic than CMV.

Keywords: acute phase response, atherosclerosis, inflammation, carotid intima media thickness, Chlamydia pneumonia and CMV infection, hemodialysis

2005;15 (1) 84-92

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Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in patients with end-stage renal disease (ESRD) on hemodialysis (HD) (1,2). This frequently is attributed to accelerated atherosclerosis (3). Despite significant progress in dialysis technology, the prevalence of atherosclerotic CVD has not decreased during the last decade. The increased risk of these complications is related, in part, to a higher prevalence of conditions that are recognized to be risk factors for CVD in the general population, such as older age, hypertension, hyperlipidemia, diabetes and smoking (4). The excess risk also may be due to hemodynamic and metabolic factors characteristic of uremia, including hyperparathyroidism, higher levels of thrombogenic factors and hyperhomocysteinemia (1,3).

Recently, it was suggested that inflammation may be important in the pathogenesis of atherosclerotic CVD, and atherosclerosis has been characterized as a pro-inflammatory condition (5). Inflammation is associated with activation and proliferation of macrophages, endothelial cells and smooth-muscle cells, and the presence of pro-inflammatory mediators including cytokines, growth factors, complement and oxidized low-density lipoproteins. Previous studies have also reported on associations between atherosclerosis and certain persistent bacterial and viral infectious agents, such as Chlamydia pneumonia and cytomegalovirus (CMV) (6,7). During inflammation and infection, the plasma concentrations of various proteins, alternatively known as acute phase reactants or acute phase proteins, can rise (8). C-reactive protein (CRP) and serum amyloid A (SAA) are prominent acute phase reactants, and plasma concentrations of these proteins are directly correlated with the presence and severity of atheresclerotic CVD (9). Fibrinogen and lipoprotein (a) (Lp(a)) are other well-known pro-inflammatory markers which might also be involved in events leading to cardiovascular complications (10,11). On the other hand, it has been reported that the plasma concentrations of CRP and other acute phase proteins are elevated in dialysis patients (12). Although an association between elevated serum CRP levels and mortality rate has been reported in dialysis patients (13), the exact mechanisms behind this and other associations remain unknown. It may be related to acceleration of the atherosclerotic CVD.

To assess for possible interactions between inflammation, chronic infection and asymtomatic atherosclerosis in HD patients, in this study we determined the intima-media thickness (IMT) of carotid arteries, using high-resolution B-mode ultrasound, as an indicator of preclinical atherosclerosis. We also measured plasma levels of pro-inflammatory markers (CRP, SAA, albumin, Lp(a), fibrinogen, ferritin) and IgG antibody titers for Chlamydia pneumonia and CMV. We had two main objectives: 1) to evaluate whether there is any correlation between carotid artery IMT and plasma levels of any of the inflammatory markers; and 2) to evaluate whether there is any correlation between carotid artery IMT and immunoglobulin G (IgG) titers against Chlamydia or CMV.

Materials and Methods

Patients

Fifty-four patients (29 male, 25 female; mean age ± SD = 48 ± 16 years, range 23-64) were recruited from the list of adult patients currently undergoing hemodialysis at our institution. All were receiving maintenance HD three times weekly utilizing a polysulphane membrane. The mean duration of dialysis was 89 ± 58 months (range: 9-252 months). The causes of ESRD were chronic glomerulonephritis in 16 patients, malignant nephrosclerosis in 10 patients, chronic pyelonephritis in 7 patients, nephrolithiasis in 5 patients, polycystic kidney disease in 3 patients, and idiopathic renal disease in 13 patients. Patients who were diabetic or more than 65 years of age were excluded, as were patients having clinical signs of overt infection and/or inflammation, or overt atherosclerotic CVD at time of the examination. All HD patients were dialyzed using conventional bicarbonate-buffered dialysate. All HD patients were receiving 3 g/d of calcium carbonate as a phosphate binder, vitamin supplementation (B, C and D), and an oral preparation of essential amino acids. Medications taken by patients included antihypertensive drugs (32/54, 60%), erythropoietin (38/54, 70%) and oral iron sulfate (47/54, 85%). HD patients were matched with 52 control subjects (28 male, 24 female; 46 ± 24 years, 24-62) by age, sex and body mass index (BMI). None of the control subjects had uremia, hyperlipidemia, hypertension, diabetes, cardiovascular disease, cerebrovascular disease or peripheral vascular disease. All subjects provided informed consent to participate, in accor-

Table I. Clinical and biochemical characteristics of hemodialysis patients and control subjects

	Hemodialysis patients (N=54)	Controls (N=52)	P
Age, years	48±16	46±24	NS
Gender, M/F	29/25	28/24	NS
BMI, kg/m^2	28.95±1.96	29,26±1.18	NS
Number of smokers (%)	7/54 (13%)	6/52 (12%)	NS
Duration of dialysis, months	89±58		
SBP, mmHg	*136±64	118±56	<0.001
DSP, mmHg	*87±24	72±32	<0.001
Serum glucose, mg/dL	93.38±31.97	90.89±24.12	NS
Lipid Profiles			
Triglyserides, mg/dL	154.19±60.96	114.3±66.30	NS
Cholesterol, mg/dL	150.49±31.92	182.64±46.76	<0.05
HDL-cholesterol, mg/dL	32.31±5.87	36.67±14.03	<0.05
LDL-cholesterol, mg/dL	98.26±24.52	116.13±126.78	<0.05
VLDL-cholesterol, mg/dL	33.41±12.32	31.30±13.01	NS

Data are mean ± SEM
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein
*on treatment with antihypertensive medication (32 patients)

Table II. Carotid intima-media thickness and inflammatory status of hemodialysis patients and control subjects

Hemodialysis patients	Controls (N=54)	P (N=52)	
Left IMT, mm	0.76±0.25	0.57±0.11	<0.005
Right IMT, mm	0.73±0.25	0.55±0.10	<0.005
Mean IMT, mm	0.75±0.25	0.56±0.10	<0.005
Inflammatory Markers			
CRP, mg/dL	1.16±0.98	0.68±0.59	<0.04
SAA, ng/dL	135.78±123.70	30.28±27.46	<0.001
Lp(a), mg/dL	34.87±3.8	27.47±6.5	<0.001
Ferritin, ng/mL	371.19±395.12	146.34±126.23	<0.005
Fibrinogen, mg/dL	346.30±32.94	269.95±57.88	<0.005
Albumin, g/dL	3.86±0.21	4.27±0.19	<0.001
Seropositivity, IgG titers			
Chlamidya pneumonia, >100	46/64	28/52	NS
CMV, >250	39/64	25/52	NS

Data are mean ± SEM
Abbreviations: IMT, intima-media thickness; CRP, C-reactive protein; SAA, serum amyloid A; Lp(a), lipoprotein (a); CMV, cytomegalovirus

dance with the ethical principles for human investigations, as outlined in the 2nd Helsinki Declaration.

Weight and height were determined and BMI was calculated for all patients and controls. A medical history and physical examination were performed and recorded for each subject, and each underwent tests for routine biochemical and hematological monitoring. Blood pressure (BP) was measured using a mercury sphygmomanometer, and cuffs were adapted to arm circumference after 15 minutes in a recumbent position. Hypertension was defined as systolic BP >140 mmHg and/or diastolic BP >90 mmHg and/or a history of current antihypertensive drug treatment. Information regarding smoking history was obtained by interviews.

Blood Samples

All samples were taken from patients and controls in the fasting state after a 30-min rest in the sitting position. Blood samples were drawn from the large antecubital veins. All venipunctures were carried out without interruption of venous flow and using a 19-gauge butterfly needle connected to a plastic syringe. None of the patients had received anticoagulants, except for heparin given during HD. Fifteen milliliters of blood were drawn and transferred to polypropylene tubes containing 1 mL trisodium citrate (0.109 mol/L) to determine the levels of analytes. The tubes then were centrifuged at 3000 rpm for 15 minutes at +10°C to +18°C. The supernatant plasma samples were stored in plastic tubes at -30°C, until assayed.

Assays

Triglycerides and cholesterol were measured by commercial colorimetric assay (GPOPAP and CHOP-PAP kits, respectively; Boehringer-Mannheim, Germany). High density lipoprotein (HDL)-cholesterol in plasma was determined by means of a precipitation-based method using phosphotungustic acid. Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Plasma glucose determinations were obtained from venous sampling after 12 hours of overnight fasting, using the glucose-oxidase method (Boehringer-Manheim, Germany). Plasma fibrinogen determination was made by means of the clotting method described by Clauss (STA compact analyzer). Lp(a) concentrations were measured by ELISA (Boehringer-Mannheim, Germany); the detection limit of this assay was 0.5 mg/dL. The intra- and inter-assay coefficients of variation were 5-12%and 2-6%, respectively. Serum albumin levels were measured via the bromocresol green method. CRP was measured by nephelometric analysis (NA Latex CRP Reagent: Behring Institute, Marburg, Germany); the normal range for CRP is less than 0.8 mg/dL. SAA was analyzed by enzyme-linked immunosorbent assay (Hemagen Kit: Hemagen Diagnostics Inc., Waltham, MA, USA). Chlamydia pneumonia IgG was detected by immunofluorescence; IgG titers of 100 or greater were considered positive. CMV IgG also was determined by immunofluorescence, and titers of 250 or greater were considered positive.

Ultrasonography

Subjects were examined in the supine position with their head turned 45° away from the side being scanned. The ultrasound system used was a *Toshiba Sonolayer SSA 270 A* (Toshiba Medical Systems, Japan), equipped with a 7.5 Mhz linear array transducer. The left and right common carotid arteries were examined in the anterolateral, posterolateral, and mediolateral planes. Measurement of the IMT was performed from the near wall of the common carotid artery at its segment, 1 cm proximal to the bifurcation, in each plane, so that a total of six measurements (three on each side) were obtained on each patient. The IMT, as defined by Pignoli et al, was measured as the distance from the inner echogenic line (which represents the lumen-intimal interface) to the outer echogenic line (which represents the collagen-containing layer of the tunica adventitia or the media-adventitia interface) (14). All scanning was conducted by one radiologist who was experienced in carotid Doppler imaging; the radiologist was blinded to subject group and laboratory values. Variability in arterial wall thickness measurements was examined by means of repeated scans and readings on volunteer participants with observed reliability coefficients of 0.76.

Statistical Analysis

All group means were presented as mean +/- standard deviation. Inter-group comparisons of continuous variables were performed using nonparametric Mann-Whitney U-Wilcoxon rank sum tests. Bivariate correlations were performed by Pearson correlation test; an a priori designation system was adopted of $R < 0.40$, weakly correlated; $R = 0.40$-

0.69, moderately correlated; and R ≥ 0.70, strongly correlated. Linear regression analysis was performed for multivariate analysis. All data were analyzed using SPSS (Version 6.0) for Windows (SPSS Inc.) with differences at $p < 0.05$ interpreted as statistically significant.

Results

The baseline clinical and biochemical characteristics of HD patients and control subjects are presented in Table I. There were no differences between the HD and control groups with respect to mean age, sex, BMI, and smoking history. HD patients had higher mean systolic BP (136 ± 64 mmHg vs 118 ± 56 mmHg) and mean diastolic BP (87 ± 24 mmHg vs 72 ± 32 mmHg) versus controls ($p<0.001$). The plasma levels of total cholesterol, LDL-cholesterol, and HDL-cholesterol were significantly lower in the HD group ($p<0.05$). Although HD patients tended towards slightly higher plasma triglyceride levels than controls, this difference was not statistically significant; neither were the mean plasma glucose and VLDL levels different.

The IMT values of the left and right carotid arteries in the HD patients (0.76 ± 0.25 mm and 0.74 ± 0.25 mm, respectively) were significantly greater than in age-matched control subjects (0.57 ± 0.11 mm and 0.55 ± 0.10 mm, respectively) ($p<0.005$) (Table II). Mean carotid IMT also was greater in the HD patients (0.75 ± 0.25 mm vs 0.56 ± 0.10 mm) ($p<0.005$) (Figure 1). Although the IMT tended towards being higher on the left side than the right side in HD patients and also in controls, the differences fell just short of statistical significance ($p=0.055$). The mean carotid IMT was significantly correlated with age, both among HD patients ($R=0.47$, $p<0.001$) and controls ($R=0.69$, $p=0.001$). Univariate analysis among HD patients showed that mean carotid IMT was weakly correlated with plasma levels of CRP ($R=0.29$, $p=0.019$) (Figure 2A), and moderately correlated with SAA ($R=0.69$, $p<0.001$) (Figure 2B), Lp(a) ($R=0.42$, $P=0.001$) (Figure 3A) and fibrinogen ($R=0.57$, $p<0.001$) (Figure 3B). IMT also was moderately correlated with Chlamydia pneumonia IgG titers ($R=0.50$, $p<0.001$). Mean carotid IMT was negatively correlated with serum albumin levels, albeit only weakly ($R=-0.33$, $p=0.02$) (Figure 4). There were no statistically-significant correlations between carotid IMT and BMI, mean systolic or diastolic BP, use of erythropoietin, duration of hemodialysis, duration of smoking, plasma levels of glucose, lipids, ferritin, or CMV IgG titers in HD patients.

Plasma levels of CRP, SAA, Lp(a), fibrinogen and ferritin were significantly higher in HD patients than controls, and HD patients also had significantly reduced serum albumin levels (Table II). There were no inter-group differences with respect to Chlamydia pneumonia IgG and CMV IgG titers (Table II). CRP levels were moderately correlated with age ($R=0.58$, $p<0.001$), Lp(a) ($R=0.48$, $p<0.001$) and fibrinogen ($R=0.54$, $p<0.001$); weakly correlated with SAA ($R=0.34$, $p=0.019$); and inversely correlated with albumin ($R= -0.55$, $p<0.001$). There also were moderate positive correlations between SAA levels and age ($R=0.43$, $p=0.002$), Lp(a) ($R=0.58$, $p<0.001$) and fibrinogen ($R=0.57$, $p<0.001$) and an inverse correlation with albumin levels ($R=-0.37$, $p=0.009$), similar to CRP. In addition to being correlated with CRP and SAA, Lp(a) levels also correlated moderately with fibrinogen ($R=0.66$, $p<0.001$). In the hemodialysis group, titers of Chlamydia pneumonia IgG were weakly correlated with plasma levels of CRP ($R=0.35$, $p=0.03$), and moderately correlated with SAA ($R=0.45$, $p=0.001$) and fibrinogen ($R=0.45$, $p=0.001$). There was no correlation between CMV IgG titers and plasma levels of any of the inflammatory markers.

Discussion

It is now well-recognized that the prevalence of atherosclerotic CVD is significantly higher in dialysis patients than in non-uremic controls (1-3,15). Ultrasound measurements of IMT in the carotid artery are increasingly used to assess preclinical, generalized atherosclerosis as a non-invasive study in the general population, and also in dialysis patients. Most (16,17), but not all, studies have identified increased carotid IMT in HD patients versus healthy controls. Savage et al reported that carotid IMT among uremic patients did not differ from that of controls (18). However, these investigators observed, in the same study, that the prevalence of calcified plaques was increased in the carotid and femoral arteries of HD patients (18). Consistent with most previous studies (16,17), we showed that mean carotid IMT is greater in HD patients than in age- and sex-matched healthy controls.

Age, hypertension, hyperlipidemia and/or abnormalities in lipid metabolism, and smoking are well-known risk factors for atherosclerotic CVD in

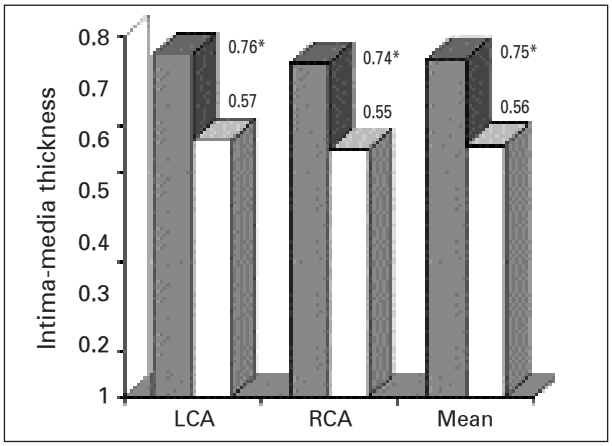

Figure 1. Mean (±SEM) values of intima-media thickness (mm) in the left carotid artery (LCA) and right carotid artery (RCA) in hemodialysis patients and controls (*p<0.005) (gray column, hemodialysis patients; white, controls).

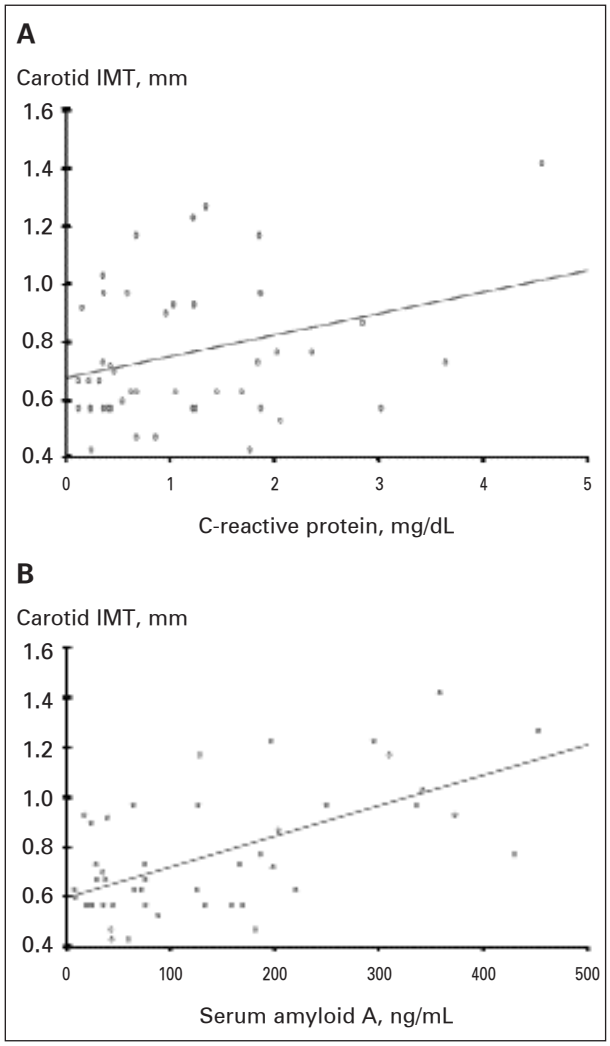

Figure 2. Correlation between mean carotid intima-media thickness (IMT) with C-reactive protein levels (R=0.29, p=0.019) (A), and serum amyloid A levels (R=0.69, p<0.001) (B) in hemodialysis patients.

the general population (4). These risk factors also commonly are observed among ESRD patients, and evidence suggests, albeit inconclusively, that these factors have some predictive value for cardiovascular complications in these patients (1). Our finding that advancing age was correlated with increasing carotid IMT, both in HD patients and controls, is entirely consistent with these results from prior studies (1-4).

Hypertension is a frequent complication of ESRD. An association between high BP and occlusive vessel wall changes has been identified in HD patients, and rigorous control of BP has led to a significant decrease in the incidence of myocardial ischemia (19). However, some studies have failed to demonstrate any correlation between BP and carotid artery lesions (16,20). We found no link between BP and carotid IMT in our HD patients, consistent with these latter studies. It may be that antihypertensive therapy causes regression of IMT or variability of blood pressure in HD patients.

Abnormalities in lipid metabolism have been described in patients with ESRD (21). The resultant alterations in serum lipid levels should, at least theoretically, contribute to the pathogenesis of uremic atherosclerosis. However, no large prospective study to date has examined whether disturbances in lipoprotein metabolism constitute a significant cardiovascular risk factor in the uremic population. Previously-reported studies, which have included small numbers of patients or have been retrospective in nature, have yielded conflicting results (19,22,23). We did not observe any correlation between serum lipid levels and carotid IMT in our HD patients. This somewhat unexpected finding may be related to the relatively small numbers of subjects in our study. In addition, abnormalities in lipoprotein composition and/or in LDL oxidation may be the factor(s) responsible for atherogenesis, rather than the actual elevations in lipoprotein levels we generally observe in uremic patients.

Several recent studies have demonstrated that inflammation may play a pivotal role in the develop-

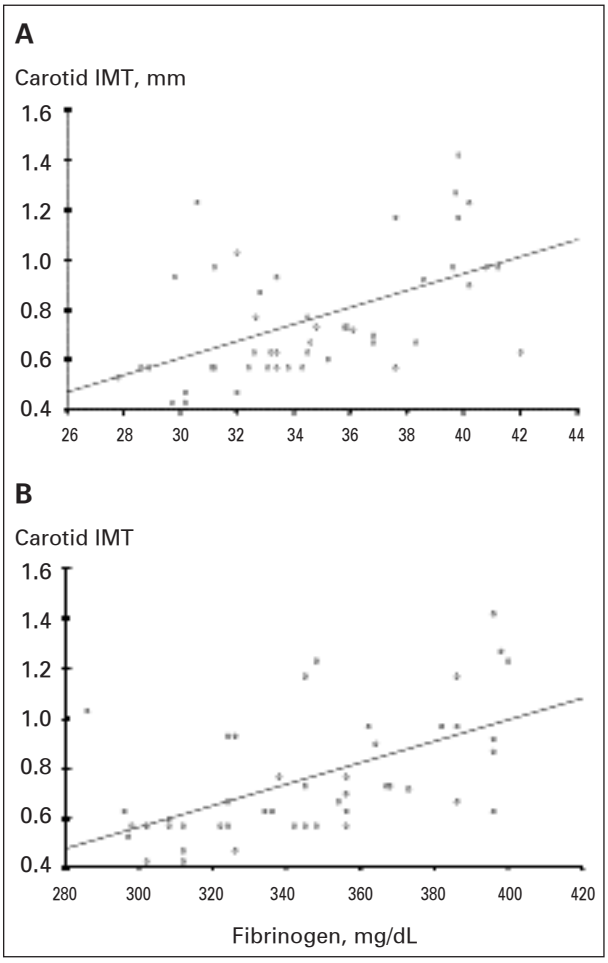

Figure 3. Correlation between mean carotid intima-media thickness (IMT) and lipoprotein (a) levels (R=0.29, p=0.019) (A), and fibrinogen levels (R=0.69, p<0.001) (B) in hemodialysis patients.

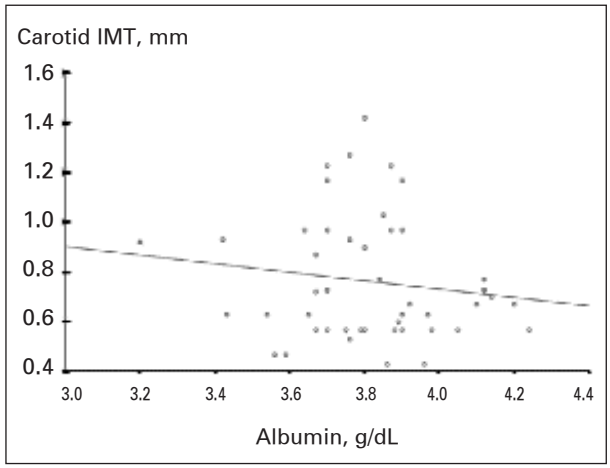

Figure 4. Correlation between mean carotid intima-media thickness (IMT) and serum albumin levels in hemodialysis patients (R=-0.33, p=0.02).

ment of atherosclerosis and death from CVD (9,10, 24). Some investigators have reported on patients with unstable angina, a poorly understood condition, and have found that the circulating acute phase reactants, CRP and SAA protein, both sensitive indicators of inflammation, predict clinical outcome; i.e., that patients with elevated levels have more ischemic episodes and higher rates of mortality (9,10). Interestingly, CRP and other acute phase proteins also have been reported to be elevated in ESRD patients (12,25). It is not clear what activates the acute phase response in HD patients. This may be related to the HD procedure, biocompatibility of the dialysis membranes, or the inevitability of multiple hospitalizations because of infections and/or other causes. Recently, McIntyre et al suggested that serum CRP is a marker of infection and inflammation in regular dialysis patients (26). It remains completely unknown whether there is any link between the increased levels of acute phase proteins and morbidity and mortality in dialysis patients.

Bergström et al reported that an elevated concentration of CRP was the most powerful predictor of death in ESRD patients (13). Other investigators have suggested that an activated acute phase response is closely related to high levels of atherogenic vascular risk factors and cardiovascular death (27-29). Ikizler et al have demonstrated that CRP was a powerful predictor of hospitalization in HD patients (30). Consistent with previous studies (25-30), we found that plasma levels, both of CRP and SAA, were elevated in HD patients compared with healthy controls. Furthermore, our study showed that there was a significant positive correlation between carotid IMT and plasma levels of CRP and SAA.

We also found that plasma levels of CRP and SAA were positively correlated with Lp(a) and fibrinogen levels in HD patients. Lp(a) is a genetically-determined independent risk factor for atherosclerosis in the general population (31). Lp(a) also has been shown to have the characteristics of an acute phase reactant (32). Recent studies have suggested that there is an association between high plasma levels of Lp(a) and the other acute phase reactants, such as CRP, SAA and fibrinogen in HD patients, ab-

solutely consistent with our findings (32,33). In addition, Lp(a) has been found to be elevated in patients with ESRD and further evidence suggests that elevated Lp(a) may predict cardiovascular risk and mortality in this population group as well (34,35). However, its predictive value remains controversial. Some investigators argue that the risk increases only because other lipid risk factors are present (36). In the present study, plasma levels of Lp(a) were higher in HD patients than in controls. Although elevated plasma levels of Lp(a) correlated significantly with carotid IMT, there was no relationship between carotid IMT and other lipid risk factors. Taken together, these findings suggest: 1) that Lp(a) may be one of the more important risk factors for uremic atherosclerosis; 2) that this increased risk may be related to its being a marker of inflammation, rather than to its association with lipid disorders; and 3) that inflammation is the mechanism by which this increased atherogenesis occurs.

Fibrinogen is another acute phase protein that serves as an independent risk factor for atherosclerosis (37). This factor also enhances blood coagulation, and elevated levels are associated with thrombosis. Recent studies have demonstrated that serum levels of fibrinogen are elevated in ESRD patients, and hyperfibrinogemia represents an important risk factor for atherosclerosis among HD patients (38, 39). Plasma fibrinogen levels also were elevated in our HD patients, and these elevated levels were correlated with carotid IMT. This is not surprising, because thrombosis long has been known to be involved at two critical points in the development of atherosclerotic CVD: first in atherogenesis, and second in the conversion of stabile ischemic heart disease to acute coronary syndrome. We conclude that similar changes in plasma Lp(a) and fibrinogen can be found in patients exhibiting acute phase responses, as reflected by elevated levels of CRP and SAA, indicating that an inflammatory condition might be responsible, at least in part, for the alterations in lipoprotein metabolism and fibrinolysis seen in ESRD patients.

Recently, an active area of research has been investigating the possibility that infectious processes may contribute to atherosclerosis. Several surveys of the general population have uncovered correlations between the prevalence of atherosclerosis and the presence of at least two infectious agents, Chlamydia pneumonia and CMV (6,7). Increased titers of antibodies to these organisms have been used as predictors of further adverse events in patients who already have had one atherosclerotic cardiovascular event. Nonetheless, there is no direct evidence that these organisms play any causative role in generating atherosclerotic lesions. Various potential causative mechanisms have been proposed, including local inflammation of arterial walls due to direct infection with these agents; possible effects on blood coagulation and fibrinolysis; effects on lipid metabolism; and alterations in the metabolism of cytokines and other inflammatory mediators (40). There are not enough data available to draw any firm conclusions about the relationship between these agents and atherosclerosis in ESRD patients. Recently, Zoccali et al reported that anti-chlamydia IgG titers are correlated with the number of carotid plaques and also with CRP levels (41). In the present study, while high titers of Chlamydia pneumonia IgG antibodies correlated significantly with carotid IMT, no significant correlation was found between CMV IgG antibody titers and carotid IMT. Moreover, Chlamydia pneumonia IgG titers correlated with CPR, SAA, and fibrinogen. In contrast to Nieto et al (42), we did not find any link between CMV infection and Lp(a), fibrinogen and any of the inflammatory markers. These findings suggest that Chlamydia pneumonia infection might be more atherogenic than CMV infection. We further concluded that there may be an atherogenic link between Chlamydia infection, inflammation and hypercoagulability.

Other major findings derived from this study are that serum albumin levels were lower in the HD group than in controls and that this reduced level correlates inversely with carotid IMT. Malnutrition is the most powerful predictor of death in ESRD patients (43). It has been shown that low serum albumin levels, usually used as an index of malnutrition, also are associated with high cardiovascular mortality in patients who have undergone renal transplantation (44). Although malnutrition has been targeted as the primary explanation for the low levels of albumin, several other factors, including inflammation, hormone deficiency states or a combination of these processes, also may contribute (45). It generally is accepted that albumin is a *negative acute phase protein*; that is, that while plasma concentrations of CRP and other acute phase reactants increase during infammation, albumin concentrations fall, even in the absence of malnutrition. Supportive evi-

dence is that a negative correlation exists between serum albumin and several acute phase proteins, such as CPR, SAA and fibrinogen (28-30). Consistent with this prior work, serum albumin levels were inversely correlated with CRP, SAA, Lp(a), and fibrinogen in our subjects. Taken together, all these findings suggest that there are links between malnutrition, hypoalbuminemia, inflammation and atherosclerosis.

In conclusion, our results indicate that atherosclerotic changes, reflected by increasing carotid IMT, are more common in HD patients than in controls, and suggest that the role of inflammatory processes in the pathogenesis of atherosclerosis may be more important than those of other established cardiovascular risk factors in this population. Further clinical studies are warranted to clarify the causative mechanisms by which inflammation induces atherosclerosis in patients receiving hemodialysis.

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