Carotid Intima-Media Thickness and Liver Histology in Hemodialysis Patients with Nonalcoholic Fatty Liver Disease

Sergio Neri, M.D.,[1] S.S. Signorelli, M.D.,[1] R. Scuderi, M.D.,[1] M. Bruno, M.D.,[1] G. Bertino, M.D.,[1] A. Clementi, M.D.,[2] I. Torrisi, M.D.,[2] F. Fidone, M.D.,[1] A.B. Pagano, M.D.,[1] M. Malaguarnera, B.Sc.,[3] and R. Noto, M.D.[1]

ABSTRACT

The prevalence of atherosclerotic cardiovascular disease in chronic hemodialysis (HD) patients has been demonstrated to be higher than in healthy people. Severe liver fibrosis is strongly associated with early carotid atherosclerosis and it might reduce the survival of patients who undergo both renal replacement therapy and transplantation. We wanted to assess whether nonalcoholic fatty liver disease (NAFLD) was associated with altered intima-media thickness (IMT) in HD patients as an independent marker of subclinical atherosclerosis. We enrolled 42 patients undergoing HD and 48 patients with normal renal function, all of them with high levels of aminotransferases and an ultrasonographic diagnosis of liver steatosis. The control group consisted of 60 healthy subjects. Laboratory tests for inflammatory and oxidative markers, ultrasonographic liver evaluation, carotid IMT measurement, and liver biopsy were performed. Different degrees of fibrosis were detected in our study cohort. Worse liver histopathological scores and higher plasmatic levels of C-reactive protein, reactive oxygen species, and vascular cell adhesion molecule-1 were found in HD patients. Carotid IMT was significantly higher ($p < 0.005$) in patients with histological steatosis. HD patients may develop active and progressive chronic hepatitis faster than patients with normal renal function and the thickness of their carotid intima-media might be markedly increased. These two conditions seem to be independent on classical risk factors and on metabolic syndrome. They might be related to the high levels of oxidants and to the inflammatory state, which are typical of patients undergoing HD. Independently related with the traditional risk factors for cardiovascular disease, nonspecific inflammation and oxide-reductive imbalance may play an important role in the progression of NAFLD and atherosclerotic disease in HD patients.

KEYWORDS: Chronic hepatitis, nonalcoholic steatohepatitis, oxidative stress, fibrosis, intima-media thickness, hemodialysis

[1]Department of Internal Medicine and Systemic Diseases; [2]School of Nephrology; and [3]Research Center "The Extreme Senescence," University of Catania, Catania, Italy.

Address for correspondence and reprint requests: Sergio Neri, M.D., Department of Internal Medicine and Systemic Diseases, Policlinico di Catania, Via S. Sofia 87, 95123 Catania, Italy (e-mail: sergio.neri4@tin.it).

Int J Angiol 2011;20:149–156.
Published online: July 19, 2011.
DOI: http://dx.doi.org/10.1055/s-0031-1283218.
ISSN 1061-1711.

Chronic hemodialysis (HD) patients have a high risk to develop atherosclerotic cardiovascular disease. The available data, even though limited, suggest that some of the traditional cardiovascular risk factors do not play an important role in the HD population.[1]

Nonalcoholic fatty liver disease (NAFLD) is recognized as one of the leading causes of a wide spectrum of liver diseases, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and end-stage liver failure.[2,3]

Despite the lack of conclusive results, it has been shown that steatosis accelerates the progression of chronic liver damage in hepatitis C (HCV) patients with specific HCV genotypes and in patients with visceral obesity,[4] suggesting that severe liver fibrosis (caused by the progression of NAFLD) might reduce the survival in HD patients.

Unfortunately, these studies are often limited by the lack of information about the pretransplant histological severity of the chronic hepatitis. Moreover, the spuriously low serum aminotransferase levels in HD patients may not serve as a reliable predictor of liver disease progression. In addition, NAFLD is closely related to several features of metabolic syndrome which is associated to an increased risk of cardiovascular disease and premature death. Recent studies have shown that the severity of liver histopathology among NAFLD patients is strongly associated with early carotid atherosclerosis,[5] as assessed by intima-media thickness (IMT) ultrasound (US) examination. IMT is an independent predictor of cardiovascular mortality in the general population as well as in HD patients.[6,7]

To assess the possible relationship between NAFLD and increased IMT among patients undergoing HD, as a possible marker of subclinical atherosclerosis, independent on metabolic syndrome, insulin resistance, and classical cardiovascular risk factors, this prospective, cross-sectional study examined liver biochemical findings, liver histological features, and IMT measurements in a cohort of HD patients.

METHODS

From March 2004 to July 2009, we studied 90 subjects—42 undergoing HD (Group A) and 48 with normal renal function with increased aminotransferase levels and ultrasonographic diagnosis of liver steatosis (Group B). The control group consisted of 60 healthy subjects. The subjects were recruited from the outpatients of an internal medicine unit (University of Catania, Catania, Italy).

Age, sex, race, etiology, and duration of renal failure were recorded.

Hematological tests for liver function and other biochemical blood measurements were performed (Table 1) in the morning after a 12-hour overnight fast, using standard laboratory procedures.

Exclusion Criteria

- Age $\geq$65 years and waist circumference of 102 cm or less (men) or 88 cm or less (women)
- Smoke
- Obesity (with body mass index [BMI] $\geq$29 kg/m^2)

Table 1 Baseline Clinical and Hematological Characteristics of the Study Subjects, Grouped According to Their Status of Liver Steatosis ($n = 90$)

Variable Studied	Group A (42)	Group B (48)	p
Age (years)	47.1 ± 12.3	51.2 ± 8.4	NS
Gender	46/24	44/28	NS
BMI (kg/m^2)	24 ± 3	26 ± 2	NS
WHR	0.96 ± 0.04	0.98 ± 0.03	NS
Hemodialysis duration (months)	37.3 ± 26.6		
Systolic blood pressure (mm Hg)	129 ± 5	130 ± 4	NS
Diastolic blood pressure (mm Hg)	82 ± 3	83 ± 3	NS
HOMA-IR score	4.3 ± 0.2	3.5 ± 0.1	<0.05
Fasting glucose (mmol/L)	7.7 ± 0.2	7.4 ± 0.5	NS
Fasting Insulin (μU/L)	18.4 ± 3	12 ± 4	<0.05
Triglycerides (mmol/L)	2.09 ± 0.7	1.42 ± 0.9	NS
HDL cholesterol (mmol/L)	1.30 ± 0.3	1.24 ± 0.3	0.05
LDL cholesterol (mmol/L)	3.25 ± 0.2	3.22 ± 0.3	NS
AST (U/L)	55 ± 25	42 ± 28	NS
ALT (U/L)	58 ± 22	44 ± 25	NS
GGT (U/L)	35 ± 25	37 ± 30	NS

NS, not significant; BMI, body mass index; WHR, waist-to-hip ratio; HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase.

- Type 1 diabetes
- Microalbuminuria defined as an albumine–creatine ratio >3.5 with urinary protein concentration <200 mg/L (measured radioimmunologically from an early morning specimen)
- Altered uric acid levels
- Alcohol consumption ≥20 g/d (assessed through a separate interview with the subject, the referring physician, and family members) and history of alcohol addiction
- Recent history of acute illness or cardiovascular events
- Liver (other than NASH) or kidney diseases (creatinine >130 μmol/L and creatinine clearance <1.3 mL/s, assayed by autoanalyser)
- HCV detected by qualitative polymerase chain reaction (PCR) test (Amplicor PCR system, Roche Diagnostic System Inc., Brackburg, NJ); hepatitis A virus and hepatitis B virus infection (by serological markers using a kit, Abbott Laboratories, Chicago, IL); Epstein-Barr virus and cytomegalovirus infection (by immunofluorescence)
- Autoimmune diseases (presence of antinuclear, antimitochondrial, antismooth muscle, antiliver-kidney-microsome antibodies evaluated by indirect immunofluorescence)
- Wilson disease (by serum concentration of α_1 antitrypsin), hemochromatosis (by genetic assessment of HFE gene using reverse dot blot-real time PCR/FRET probes on blood leukocytes)

Subjects who usually smoked, dyslipidemic subjects (high-density lipoprotein [HDL] cholesterol <40 mg/dL and triglycerides >150 mg/dL by enzymatic method), individuals with cholelithiasis, and subjects who were chronically taking any medication, vitamins, and antioxidants were also excluded.

Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald's equation.

BMI was calculated by dividing the weight of patients (in kilograms) by the square of the height of patients (in meters).

Waist circumference (widest area between the lower rib margin and the iliac crest) and hip circumference (widest area over great trochanters) were measured and used to calculate the waist-to-hip ratio (WHR), as an index of regional fat distribution. Patients were considered to have visceral obesity if their waist circumference was higher than 102 cm in males or higher than 88 cm in females.

Blood pressure was measured with a standard mercury sphygmomanometer (the mean value between three consecutive readings in the sitting position on the right arm after 5-minute rest). Information on daily alcohol consumption and other lifestyle characteristics was obtained from all participants during the screening. Venous blood was taken after an overnight fast. Plasma liver function tests, plasma insulin, and other biochemical blood measurements were performed according to our central standard laboratory procedures. Glycosylated hemoglobin (HbA1c) was determined by ion-exchange chromatography.

Liver US scans were performed routinely before liver biopsy. The presence of hepatic steatosis was confirmed by ultrasonography using a high-resolution convex (7.8 MHz) linear electronic probe (Acuson Corporation, Mountain View, CA). The scans were performed according to conventional criteria by two trained operators who were blinded to all clinical and laboratory characteristics of the participants. Liver biopsies were performed in subjects with ultrasonographic evidence of steatosis and elevated transaminase levels to identify patients with suspected NASH. Liver biopsy samples were considered eligible for histological analysis if at least six portal spaces were observed. The histological diagnosis was made by a single blinded pathologist on sections stained with hematoxylin-eosin, Masson's trichrome stain, Perls' Prussian blue, and periodic acid-Schiff stain plus diastase.

NAFLD includes both simple fatty liver and NASH. A fatty liver was defined as the presence of steatosis with or without inflammation. A histological diagnosis of NASH required the presence of steatosis, lobular inflammation, hepatocytic ballooning, degeneration of hepatocytes (with or without perisinusoidal fibrosis), and Mallory hyaline bodies. Steatosis, flogistic activity, and fibrosis were assessed on the biopsy specimens according to standard criteria[8,9] by a single experienced pathologist.

Systolic and diastolic blood pressures were measured before and after each HD treatment using a standard mercury sphygmomanometer. The presence of hypertension was defined by the administration of antihypertensive agents and/or systolic blood pressure higher than 160 mm Hg and/or diastolic blood pressure higher than 95 mm Hg.

Diabetes was diagnosed according to the American Diabetes Association criteria.[10] Impaired glucose tolerance (IGT) was defined as a fasting glucose level higher than 100 mg/dL (5.6 mM) but less than 126 mg/dL (7 mM) or a 2-hour oral glucose tolerance test (OGTT) value higher than 140 mg/dL (7.8 mM) and lower than 200 mg/dL (11.1 mM). Diabetes was defined as fasting glucose level higher than 126 mg/dL (7 mM) in two measurements or random plasma glucose level less than 200 mg/dL or a 2-hour postmeal glucose level higher than 200 mg/dL (11.1 mM). Serum C-reactive protein (CRP) was measured (in ng/mL) using an ultrasensitive colorimetric competitive enzyme-linked immunosorbent assay (ELISA).[11]

Serum vascular cell adhesion molecule-1 (VCAM-1) levels were determined by ELISA (R&D System Europe, Abington, UK) after centrifugation of

whole blood diluted 1:10 in 0.13 M sodium citrate.[12] As a marker of systemic oxidative stress, we measured malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), plasma glutathione peroxidase (GSH-Px). Plasma MDA concentration was measured using a Beckmann high performance liquid chromatograph (HPLC) and a preconditioned C_{18} column washed with water and acetonitrile (85:15) after incubation with thiobarbituric acid.[13] A spectrocolorimetric assay (LPO-586 method Bioxytech) was used to measure plasma 4-HNE levels, as described by Esterbauer and Cheeseman.[13,14] Plasma GSH-Px was determined by a spectrophotometric assay as well (Hitachi model 200–20, Hitachi High-Technologies Corporation, Tokyo, Japan).[15]

Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR; index computed with the formula: fasting serum insulin in μIU/mL multiplied by fasting serum glucose in mM and divided by 22.5).[16] The top quartile of the control sample was 2.231; therefore, we considered it as the cut-off to define insulin resistance. Metabolic syndrome was defined by the Adult Treatment Panel III criteria.[17]

Information on daily alcohol consumption and other lifestyle characteristics were obtained from all participants during the screening to exclude patients with an alcohol intake greater than or equal to 20 g/d. Alcohol intake was assessed during separate interviews with the subjects and their family members by the referring physician using the World Health Organization Alcohol Use Disorders Identification Test.[18] Venous blood samples were taken after an overnight fast.

Carotid IMT[19] was measured by a computer-assisted method employing US B-mode imaging with a high-resolution (3.5 MHz) linear electronic probe (Apogèe CX 800m ATL-Philips, Arroyo Grande, CA). Carotid IMT was defined as the distance from the leading edge of the first echogenic line (corresponding to the lumen–intimal interface) to that of the second echogenic line (corresponding to the collagen-containing upper layer of the tunica adventitia). To exclude interobserver variability, this was performed by only one experienced ultrasonographer physician who was blinded to the patients' clinical and laboratory characteristics. An alteration of IMT was defined as a thickness higher than 0.85 to 1 mm as measured on the far wall of the common carotid artery at points 5, 10, and 15 mm proximal to the carotid bifurcation bilaterally at a site free of any discrete plaque. For each patient, three measurements on both the left and right common carotid arteries were taken on the anterior, lateral, and posterior projections of the near and far wall. An average of all the readings was used (coefficients of variation less than 7%).

The protocol was approved by the Ethical Committee of the Institutional Review Board of the University of Catania and a written informed consent was obtained from all participants. The Declaration of Helsinki on experimentation in humans has been observed in all aspects of this study.

Statistical Analysis

For each variable, the results are presented as mean ± standard deviation (SD). An unpaired two-tailed Student's *t*-test was used to calculate the difference between the means and one-way analysis of variance (ANOVA), and Bonferroni test was used for multiple group comparisons. The χ^2 test with Fisher's exact test correction was used to evaluate the differences between categorical variables. Multivariate analysis (MVA) was performed to assess the correlation between liver histology and IMT. Relative risk (RR) was measured using a 95% confidence interval (95% CI) using the approximation of Katz. *P* values less than 0.05 were considered to be statistically significant. Statistical analysis of the data was performed using SPSS 11.5 (SPSS Production, Chicago, IL).

RESULTS

Demographic and clinical features of the study participants are summarized in Table 1. The median age of HD patients included in the study was 37.3 ± 26.6 months. The mean age and gender were not significantly different between the groups. There was not a significant difference between hypertension and glycemia mean values in Groups A and B. The distribution of underlying nephropathies in Group A was as follows: chronic glomerulonephritis (44%), diabetic nephropathy (28%), nephroangiosclerosis (15%), polycystic kidney disease (13%), interstitial nephritis (11%), and idiopathic causes (17%).

Clinical Assessment (Groups A and B)

In Group A (containing 42 subjects on HD), 12 patients had diabetes, 18 had a HOMA-IR index higher than 2.231, and 12 had a HOMA index lower than 2.231.

In Group B (containing 48 subjects with normal renal function), 13 patients had diabetes, 19 had a HOMA index higher than 2.231, and 16 had a HOMA index lower than 2.231.

All patients had a BMI lower than 30. None of the patients had signs of cirrhosis or portal hypertension.

Histopathological Features (Groups A and B)

Histological signs of steatosis were present in 82.5% of subjects of Group A and in 67.7% of subjects of Group B. Nineteen of the specimens (from Groups A and B) showed minimal Kupffer intracellular iron deposition. There was no statistical difference between the two groups ($p > 0.05$).

Table 2 Assessment of Fibrosis in Patients of Group A (42 HD Subjects) and Group B (48 with NAFLD and NASH)

	Fibrosis Score		
Metabolic Assessment	**Group A (42)**	**Group B (48)**	**Significance**
NAFLD			
Diabetes	1.91 ± 0.1	1.90 ± 0.1	<0.05
HOMA-IR >2.231	1.90 ± 0.3	1.90 ± 0.1	NS
HOMA-IR <2.231	1.65 ± 0.2	1.77 ± 0.2	<0.05
NASH			
Diabetes	1.95 ± 0.4	1.93 ± 0.2	<0.05
HOMA-IR >2.231	1.94 ± 0.3	1.92 ± 0.3	<0.05
HOMA-IR <2.231	1.91 ± 0.5	1.89 ± 0.2	<0.05

HD, hemodialysis; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; HOMA-IR, homeostasis model assessment-insulin resistance; NS, not significant.

According to the grading, a significant difference between the mean of specimen scores between Group A and Group B was not observed.

Fibrosis

Various degrees of fibrosis were present in 40% of subjects of Group A and in 16.2% of subjects of Group B. The results are summarized in Table 2. The histological features of cirrhosis (stage 4) were present in 8.6% of subjects of Group A and in 8.1% of subjects of Group B, without a statistically significant difference between the two groups.

Liver histopathological scores were strongly related ($r = 0.26$; 95% CI; $p < 0.001$) to the time since diagnosis of chronic renal failure was done. Age, sex, race, cause of renal failure, aminotransferase, and γGT levels were not associated with liver histology. In patients with NASH, a correlation between the grade of steatosis and serum level of ALT ($r = 0.40$; 95% CI; $p < 0.001$) and γGT levels ($r = 0.35$; 95% CI; $p < 0.001$) was observed, without a statistically significant difference between the two groups. Subjects with a larger WHR had significantly worse histological scores (6.8 in Group A and 7.6 in Group B) when compared with subjects with a normal WHR ($p < 0.005$). However, there was no statistically significant difference between Group A and Group B. Logistic regression analysis confirmed a significant relationship between NASH and higher waist circumference with RR of 0.368 (95% CI = 0.096–0.894; $p < 0.001$) and no statistical difference between the two groups.

The presence of diabetes, a HOMA score higher than 2.231, and NASH was associated ($p < 0.05$, $p < 0.05$, and $p < 0.001$, respectively) with a worse histological score. However, no statistically significant difference between the two groups was present.

Markers of Inflammation (Groups A, B, and C)

CRP levels were significantly higher in Group A (8.6 ± 21 ng/mL) than in Group B (5.2 ± 20 ng/mL, $p < 0.05$) and Group C (1.48 ± 0.6 ng/mL, $p < 0.001$). VCAM-1 levels were significantly higher ($p < 0.001$) in Group A (678 ± 58 ng/mL) than in Group B (452 ± 54 ng/mL; $p < 0.05$) and Group C (168 ± 25 ng/mL, $p < 0.001$; Table 3).

Oxidative Stress (Groups A, B, and C)

There was a significant difference between the groups in terms of oxidative stress. Higher MDA, 4-HNE, and lower erythrocyte GSH-Px activity were found in HD patients of Group A (2.1 ± 0.2, 2.2 ± 0.2, and 5.04 ± 0.01 μmol/dL, respectively) than in Group B (1.3 ± 0.1, 1.2 ± 0.2, and 5.9 ± 0.04 μmol/dL, respectively) and in

Table 3 Markers of Inflammation and Oxidative Stress

	Group A (42)	**Group B (48)**	**Group C (60)**
CRP (ngr/mL)	$8.6 \pm 21^{*,\dagger}$	$5.2 \pm 20^{\dagger}$	1.48 ± 0.6
VCAM-1 (ngr/mL)	$678 \pm 58^{*,\dagger}$	$452 \pm 54^{\dagger}$	168 ± 25
MDA (μmol/dL)	$2.2 \pm 0.2^{*,\dagger}$	$1.3 \pm 0.1^{\dagger}$	0.89 ± 0.1
4-HNE (μmol/dL)	2.2 ± 0.2	1.2 ± 0.2	0.90 ± 0.2
GSH-Px (μmol/dL)	5.04 ± 0.01	5.9 ± 0.04	6.60 ± 0.02

*Significant difference with Group B.
†Significant difference with Group C.
CRP, C-reactive protein; VCAM-1, vascular cell adhesion molecule-1; MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal; GSH-Px, plasma glutathione peroxidase.

Table 4 Carotid Intima-Media Thickness in Patients with Steatosis, NASH, NAFLD Compared with Healthy Group C

	Group A (42)	Group B (48)	Group C (60)
NASH	$1.641 \pm 0.1^{*,\dagger}$	$1.332 \pm 0.1^{\dagger}$	0.864 ± 0.1
NAFLD	$1.430 \pm 0.3^{*,\dagger}$	$1.310 \pm 0.2^{\dagger}$	0.864 ± 0.1

*Significant difference ($p < 0.05$) with Group B.
†Significant difference ($p < 0.05$) with Controls.

the healthy patients of Group C (0.89 ± 0.1, 0.90 ± 0.2, and 6.6 ± 0.02 μmol/dL, respectively) (Table 3).

Intima-Media Thickness (Groups A, B, and C)

The carotid IMT (Table 4) was significantly higher ($p < 0.005$) in patients of Group A and Group B compared with the healthy subjects. The carotid IMT was significantly higher in patients of Group A (OR 0.326, 95% CI; $p < 0.001$) and Group B (OR 0.562, 95% CI; $p < 0.005$) with NASH/NAFLD than in healthy subjects. The carotid IMT was also higher in NAFLD/NASH patients in Group A than in patients in Group B with normal renal function (OR 0.284, 95% CI; $p < 0.0001$). The difference in carotid IMT values observed between the groups remained statistically significant when ANOVA was performed to adjust for potential confounders.

DISCUSSION

We assessed liver biochemical findings, liver histopathological features, and IMT US measurements in a cohort of HD patients with chronic hepatitis due to NAFLD. This analysis excluded patients with viral infections and conventional cardiovascular risk factors.

Our results have shown that patients with chronic end-stage renal disease (ESRD) undergoing HD can present with severe liver histological changes due to NAFLD/NASH and altered IMT.

In our study, the prevalence of various degrees of steatosis (NASH, NAFLD, and steatosis) trended to be higher among HD patients (66/80; 86.2%) compared with patients with normal renal function (54/80; 73.7%). In relation to the presence of NAFLD/NASH, HD subjects presented worse histological features with a higher incidence of fibrosis than the normal renal subjects. No association was found in our patients between fibrosis and aminotransferase levels, age or gender, metabolic syndrome, classical cardiovascular risk, and insulin resistance.

These findings seem to demonstrate a significantly increased correlation between NAFLD/NASH and increased IMT in the HD group when compared with patients with normal renal function and the healthy control group.

Previously published data have shown that steatosis accelerates the progression of liver damage in subjects with chronic HCV and that this steatosis correlates with a specific HCV genotype and visceral obesity.[4]

To date, there are not any clinical trials evaluating the prevalence and histopathological significance of liver steatosis in ESRD patients undergoing HD in relation to the presence and progression of both atherosclerosis and liver fibrosis.

NAFLD is the second most common cause of chronic liver disease after HCV. NAFLD is characterized by fatty infiltration of liver cells. NAFLD is recognized as one of the leading causes of a wide spectrum of liver diseases, ranging from simple steatosis to end-stage liver failure. NAFLD is consistently associated with visceral obesity, dyslipidemia, insulin resistance, and Type 2 diabetes, suggesting that it can be considered as another feature of the metabolic syndrome.[20,21]

Growing evidence[20] suggests that the pathogenesis of NAFLD involves oxidative stress and inflammation. Alteration of metabolic glycemia control, malnutrition, inflammation, and oxidative stress are common conditions in HD subjects.[22–24] CRP is a member of the class of acute-phase reactants. The level of CRP rises dramatically during inflammatory processes occurring in the body. This increase is due to a rise in the plasma concentration of interleukin-6 (IL-6), which is produced predominantly by macrophages.[11] VCAM-1 is expressed on the surface of vascular endothelial cell in response to proinflammatory cytokines; several evidences[12] suggest that endothelial cell adhesion molecules may participate both in the initiation and progression of atherosclerotic vascular damage. MDA and 4-HNE are specifically stable compounds of free radical-induced lipid peroxidation formed during free radical-catalyzed peroxidation of arachidonic acid. MDA and 4-HNE are reliable markers of oxidative stress in vivo.[13–15] In addition, GSH-Px is an oxygen radical scavenging enzyme.[15] It is well known that the endothelium is a sensitive and elective target of free radicals cytotoxic action. Oxidative damage may promote atherosclerosis through cytotoxicity and endothelial damage with loss of its nonthrombogenetic properties.

Similar data have also been highlighted by other authors[25,26] who identified a correlation (through lipid peroxidation and oxidative DNA damage) between increase of serum and hepatic expression of oxidative markers and severity of necroinflammation and fibrosis in patients with nonalcoholic steatohepatitis. We have demonstrated an alteration in the oxide-reductive balance and an increase in both CRP and VCAM-1 levels in HD patients.

Several studies have suggested that the severity of liver histopathology among NAFLD patients is strongly associated with early carotid atherosclerosis;

this association is independent of classical risk factors, such as insulin resistance and the presence of metabolic syndrome.[21–24]

The histological features of NAFLD in this particular setting can be very heterogeneous, with unclear clinical significance and posttransplant outcomes.

IMT is a strong predictor of cardiovascular events in the general population. Moreover, it has been shown that carotid artery IMT could represent an independent predictor of cardiovascular mortality in dialysis patients.[27] Additionally, the prevalence of cardiovascular disease and traditional risk factors is high among renal transplant candidates.

In our study, an increased carotid IMT was significantly associated with the degree of liver steatosis and fibrosis in HD patients. Logistic regression showed that the severity of the histological features of NAFLD/NASH were independently associated with increased carotid IMT after adjustment for all potential confounders.

The present study has some limitations:

1. The cross-sectional design of our study precludes the establishment of causal or temporal relations between chronic liver diseases, carotid atherosclerosis, and the metabolic syndrome.
2. Exclusion of NAFLD was based on medical history, blood testing, and US imaging. However, the absence of NAFLD was not confirmed by liver biopsy due to ethical reasons.
3. We are aware that an assay of the oxide-reductive balance in vivo is very difficult. The method utilized in this work is not ideal, but nevertheless we believe that the simultaneous determination of various parameters and the homogeneity of the results obtained allow us to postulate the presence of a state of oxidative stress in HD patients.
4. Some patients can be misclassified as having steatosis and/or mild fibrosis because a liver biopsy is not a perfect "gold standard" and can underestimate the severity of fibrosis. This can occur even if all the biopsies are red by a single, experienced hepatopathologist in a blinded way choosing to include only the samples considered adequate.
5. We performed liver biopsy in all subjects presenting elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), and/or an ultrasonographic diagnosis of liver steatosis. However, 15 subjects with elevated AST/ALT did not have steatosis detected histologically.

Our study suggests that HD patients may have both a more active and a more progressive chronic hepatitis than patients with normal renal function. Additionally, these patients have an increased IMT when compared with patients with normal renal function. Several cross-sectional and longitudinal studies have shown that the metabolic syndrome may play a key role in the pathophysiology of (carotid) atherosclerosis and NAFLD. In addition, insulin resistance strongly correlates with NASH and subclinical and clinical cardiovascular disease.[28] However, the significant differences in carotid IMT values observed in patients with NAFLD/NASH and normal HOMA scores showed that NAFLD/NASH might influence or accelerate the progression of atherosclerosis independently on the adverse effects of insulin resistance. This association can be independent on classical cardiovascular, insulin resistance, and other features of metabolic syndrome. Altered oxide-reductive balance and an elevated inflammatory state associated with a higher cytokine release are particularly common in ESRD treated with HD and are involved both in the pathophysiology of atherosclerosis and NAFLD. This suggests that nonspecific inflammation and oxide-reductive imbalance (nontraditional risk factors for cardiovascular disease) may have a possible pathogenetic role in the progression of NAFLD and atherosclerosis in HD population

In addition, our data support the possibility that US and histological liver assessment can help physicians identify patients with a higher risk of atherosclerotic progression. All dialysis subjects with severe liver steatosis and elevated transaminase plasma levels could benefit from further liver screening including incidental liver histology.

REFERENCES

1. Cheung AK, Sarnak MJ, Yan G, et al. Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. Kidney Int 2000;58(1):353–362
2. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346(16):1221–1231
3. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. Gastroenterology 2002;122(6):1649–1657
4. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 2001; 33(6):1358–1364
5. Targher G, Bertolini L, Padovani R, et al. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. Diabetes Care 2006;29(6): 1325–1330
6. 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(11): 2458–2464
7. Nishizawa Y, Shoji T, Maekawa K, et al. Intima-media thickness of carotid artery predicts cardiovascular mortality in hemodialysis patients. Am J Kidney Dis 2003;41(3, Suppl 1): S76–S79
8. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for non alcoholic fatty liver disease. Hepatology 2005;4:1313–1321

9. Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis 2004;24(1):3–20
10. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2004;27(Suppl 1):S5–S10
11. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997;43(1):52–58
12. Fiotti N, Giansante C, Ponte E, et al. Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease. Atherosclerosis 1999;145(1):51–60
13. Yagi K. Assay for serum lipid peroxide level and its chemical significance. In: Yagi K ed. Lipid Peroxides in Biology and Medicine. New York: Academic Press; 1982:223–242
14. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 1990;186:407–421
15. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70(1):158–169
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7): 412–419
17. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. Diabetes Care 1997;20(7):1087–1092
18. World Health Organization. AUDIT. The Alcohol Use Disorders Identification Test: Guidelines for Use in Primary Health Care. Genève, Switzerland: WHO; 1992.
19. O'Leary DH, Polak JF. Intima-media thickness: a tool for atherosclerosis imaging and event prediction. Am J Cardiol 2002;90(10C):18L–21L
20. Cortez-Pinto H, de Moura MC, Day CP. Non-alcoholic steatohepatitis: from cell biology to clinical practice. J Hepatol 2006;44(1):197–208
21. Targher G, Bertolini L, Padovani R, et al. Increased prevalence of cardiovascular disease in Type 2 diabetic patients with non-alcoholic fatty liver disease. Diabet Med 2006;23(4):403–409
22. Targher G, Bertolini L, Padovani R, Rodella S, Arcaro G, Day C. Differences and similarities in early atherosclerosis between patients with non-alcoholic steatohepatitis and chronic hepatitis B and C. J Hepatol 2007;46(6): 1126–1132
23. Stenvinkel P, Heimbürger O, Paultre F, et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney Int 1999;55(5):1899–1911
24. Chen J, Muntner P, Hamm LL, et al. The metabolic syndrome and chronic kidney disease in U.S. adults. Ann Intern Med 2004;140(3):167–174
25. Seki S, Kitada T, Sakagushi H, Katani K, Wakasa K. In situ detection of lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver disease. J Hepatol 2002;37(1): 56–62
26. Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2004;99(8):1497–1502
27. Volzke H, Robinson DM, Kleine V, et al. Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. World J Gastroenterol 2005;11(12):1848–1853
28. Bressler P, Bailey SR, Matsuda M, DeFronzo RA. Insulin resistance and coronary artery disease. Diabetologia 1996; 39(11):1345–1350