Cardiovascular Risk Assessment and Homocysteine and Leptin Levels in Peritoneal Dialysis and Hemodialysis Patients

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In the present study, we used high sensitivity C-reactive protein (hs-CRP) analysis in combination with lipid screening [which has been reported to be a more valuable risk marker than other novel markers such as homocysteine (Hcy) and lipoprotein a] to perform cardiovascular risk assessment in peritoneal dialysis (PD) and hemodialysis (HD) patients.

We selected 9 PD patients, 10 HD patients, and 9 control subjects for the study. In those patients, we determined levels of serum lipids, hs-CRP, Hcy, vitamin B_{12}, folic acid, and leptin.

Patients on PD had a significantly elevated hs-CRP concentration (3.14 ± 0.79 mg/L) and ratio of total cholesterol (TC) to high density lipoprotein (HDL) cholesterol (4.71 ± 0.40), and their cardiovascular risk was found to be three times that of control subjects. In HD patients, the elevation of hs-CRP was more profound (5.66 ± 1.30), but their TC:HDL ratio fell within the normal range (3.18 ± 0.13). However, a cardiovascular risk assessment of the HD group showed the same risk as in the PD group. Serum Hcy was also elevated in patients on PD (54.95 ± 18.08 µmol/L) and on HD (25.33 ± 3.70 µmol/L) as compared with healthy subjects (13.76 ± 0.94 µmol/L). Folic acid and vitamin B_{12} levels (needed to remethylate Hcy to methionine) were not compromised in the dialysis population. On the other hand, leptin secreted by adipose tissue was found to be mildly higher in PD patients (37.08 ± 12.59 ng/mL). The mean leptin level in control subjects was 14.14 ± 3.60 ng/mL. The proinflammatory and proangiogenic action of excess leptin may aggravate cardiovascular risk in PD patients.

Increased values of known risk factors were found in dialysis patients on PD and on HD. However, lower levels of HDL cholesterol, higher cardiovascular risk assessment and Hcy levels, and mildly increased leptin levels seem to increase the potential threat of vascular disease in PD patients more than in HD patients.

Key words
High-sensitivity C-reactive protein, homocysteine, leptin

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Introduction

Cardiovascular disease is a major cause of morbidity and mortality in patients with renal disease (1). However, known inherited and environmental risk factors—including hypertension, diabetes, smoking, and elevated lipids—cannot explain as many as one third of cardiovascular events in the general population (2).

Novel candidate risk factors for cardiovascular disease in patients with renal disease have recently attracted interest. High sensitivity C-reactive protein (hs-CRP), an acute phase reactant, has been reported to be a predictor of future coronary events among healthy men in the Physician's Health Study. Compared with men in the bottom quartile of hs-CRP measurements at baseline, men in the top quartile had twice the risk of future stroke, three times the risk of future myocardial infarction, and four times the risk of future peripheral vascular disease (3,4).

Current data suggest that the addition of lipid screening to hs-CRP can improve detection of absolute coronary risk (5). Risk estimates using hs-CRP in combination with lipid screening have been reported to be superior to assessments that evaluate just hs-CRP, homocysteine (Hcy), or lipoprotein a (6). However,

the cardiovascular risk assessment algorithm that uses hs-CRP and lipid screening has not been tested in dialysis patients. Moreover, the hormone leptin, which is secreted by adipose tissue and which regulates body fat mass through central satiety, has been suggested to exert proinflammatory, proangiogenic actions on human endothelial cells (7,8). Any disturbance of serum leptin values might therefore hypothetically provoke cardiovascular system disease.

In the present study, we measured levels of serum lipids, hs-CRP, Hcy, vitamin B_{12}, folic acid, and leptin to evaluate the risk of cardiovascular disease in peritoneal dialysis (PD) and hemodialysis (HD) patients.

Patients and methods

Study population

We selected 9 PD patients aged 39.14 ± 2.42 years and 10 HD patients aged 32.50 ± 2.37 years for the study. The primary renal diseases in these patients were chronic glomerulonephritis, pyelonephritis, and primary nephrosclerosis. The HD group consisted of long-term dialysis patients (mean dialytic course: 4 – 11 years) in whom dialysis anticoagulation was achieved using heparin. All patients received a daily oral vitamin supplement: folic acid 1.5 mg, vitamin B_{12} 30 μg, and vitamin B_6 10 mg. Erythropoietin was being administered to 71% of the HD patients and to 67% of the PD patients.

For comparative purposes, 9 healthy, age-matched subjects were also studied. Each subject gave informed consent to participate in the study.

Laboratory methods

Venous blood was obtained from all subjects in the morning and, for HD patients, before the dialysis procedure. We measured serum total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides (TG), blood urea nitrogen (BUN), creatinine, and uric acid by standard automated clinical chemistry laboratory methods.

We used a highly sensitive immunoturbidimetric assay (Cobas Integra 400 chemistry analyzer: Roche Diagnostics, Basel, Switzerland) to analyze serum hs-CRP levels. Because values of hs-CRP greater than 15 mg/L likely indicate active inflammation or the presence of a chronic inflammatory condition, only the patients and controls whose hs-CRP values were lower than 15 mg/L were included in the study.

Concentration of Hcy was determined using a high-performance liquid chromatography–fluorometric method after samples had been treated with 7-fluorobenzo-2-oxa-1,3-diazol-4-sulfonamide to convert Hcy to a fluorescent compound. For the assay, DL-homocysteine was used as a standard and *N*-acetyl-L-cysteine as a internal assay standard (9).

Serum concentrations of vitamin B_{12} and folic acid were measured by radioimmunoassay (kit: Diagnostic Products Corp., Los Angeles, CA, U.S.A.). Serum leptin levels were determined by ELISA (kit: BioVendor Laboratory Medicine, Brno, Czech Republic). The lower detection limit was 0.5 ng/mL. Inter- and intra–assay variations were less than 8%.

Statistical analysis

Data are presented as mean ± standard error. All statistical analyses were carried out using the nonparametric Kruskal–Wallis test. A value of *p* less than 0.05 was considered statistically significant.

Results

Table I shows the serum concentrations of all measured parameters in the PD and HD patients and the control subjects. As expected, serum BUN, creatinine, and uric acid levels were high in both patient groups.

Values of total cholesterol and LDL cholesterol were found to be in the normal range, but TG levels were high in both patient groups. Values of HDL cholesterol were lower in the patients than in healthy subjects. The reduction in HDL cholesterol was more profound in the PD patients. The ratio of TC to HDL cholesterol (TC:HDL) was found to be significantly elevated only in PD patients. However, hs-CRP values were significantly higher in both patient groups than in the control subjects.

Using both hs-CRP and the TC:HDL ratio, a cardiovascular risk assessment detected a 3.3 times higher risk in PD patients and a 2.8 times higher risk in HD patients than the risk seen in the control subjects. But significantly elevated Hcy levels were found in the two patient groups as compared with the healthy subjects. The prevalence of hyperhomocysteinemia (>15 μmol/L) was about 100% in PD patients, 90% in HD patients, and 22% in healthy subjects.

When levels of folic acid and vitamin B_{12} were examined, the HD group was found to have significantly higher concentrations of vitamin B_{12} and folic acid as compared with the levels seen in control

TABLE I   Comparison of serum parameters (mean ± standard error) between peritoneal dialysis (PD) and hemodialysis (HD) patients and control subjects

	Controls	Samples (n)	PD	Samples (n)	HD	Samples (n)
Cholesterol (mg/dL)	200.0±19.6	7	174.7±14.7	8	146.5±6.6	10
LDL (mg/dL)	117.8±14.3	7	109.3±11.1	8	86.7±4.6	10
HDL (mg/dL)	60.8±.6	7	37.6±2.4[a]	8	42.5±5.5[a]	10
TC:HDL	3.18±0.13	8	4.71±0.40[a]	8	3.65±0.37	10
Triglycerides (mg/dL)	67.5±6.4	7	158.8±25.2[a]	8	112.9±11.5[a]	10
hs-CRP (mg/L)	0.71±0.17	8	3.14±0.79[a]	9	5.66±1.30[a]	9
Cardiovascular risk	1.20±0.06	8	4.01±0.70	8[a]	3.38±0.50[a]	10
Homocysteine (μmol/L)	13.76±0.94	9	54.95±18.08[a]	9	25.33±3.70[a]	10
Folic acid (nmol/L)	20.21±2.49	9	28.15±6.68	9	48.46±7.31[a,b]	10
Vitamin B_{12} (pmol/L)	156.12±26.46	9	301.27±39.08	9	639.63±54.66[a,b]	10
Leptin (ng/mL)	14.14±3.60	9	37.08±12.59	9	14.51±7.58	10
BUN (mg/dL)	13.50±1.62	8	54.22±9.10[a]	9	66.70±5.27[a]	10
Creatinine (mg/dL)	0.84±0.03	9	9.91±0.78[a]	9	6.05±0.31[a]	10
Uric acid (mg/dL)	3.25±0.29	6	6.22±0.63[a]	9	6.05±0.31[a]	10

[a] $p < 0.05$ as compared with control subjects.
[b] $p < 0.05$ as compared with PD patients
LDL = low-density lipoprotein; HDL = high-density lipoprotein; TC = total cholesterol; hs-CRP = high-sensitivity C-reactive protein; BUN = blood urea nitrogen.

subjects and in PD patients. Levels of those vitamins were also high in PD patients, but the levels were not found to be significantly high. These results were to be expected, because all patients were receiving folic acid, vitamin B_{12}, and vitamin B_6 supplementation.

Serum leptin levels tended to be higher in PD patients than in control subjects and HD patients, but the difference did not reach statistical significance. The prevalence of elevated leptin levels (>7.8 ng/mL) was about 89% in PD patients, 40% in HD patients, and 55% in healthy control subjects.

Discussion

In the present study, we evaluated cardiovascular disease risk in PD and HD patients. To that end, we measured serum lipid profiles and levels of hs-CRP, Hcy, folic acid, vitamin B_{12}, and leptin.

End-stage renal disease managed by PD and HD was not associated with increased serum total cholesterol or LDL cholesterol. However, reduced levels of HDL cholesterol were observed in serum from both patient groups, in accord with previous reports (10).

In past studies, CRP, a major systemic marker of inflammation, was reported to induce adhesion molecule expression in human endothelial cells, supporting involvement of CRP in the atherosclerotic process (11). Earlier laboratory assay methods were not sufficiently sensitive to measure blood levels of CRP within

the normal range (<10 mg/L); however, the recent development of high-sensitivity assays for CRP has permitted mild elevation of CRP to be detected even within the normal range. Reliable and fully automated high-sensitivity assays for CRP are now widely available. Because a direct positive association between hs-CRP and future coronary events was previously reported (3,4), the elevated hs-CRP observed in our patient groups might alone be a predictor of vascular risk.

The combination of TC:HDL ratio with hs-CRP value has been suggested to be a significantly strong predictor of cardiovascular events (5). Evaluating PD patients and HD patients with the cardiovascular risk assessment algorithm that uses hs-CRP and TC:HDL ratio, the patients were found to have an approximately three times greater risk than control subjects had.

We also measured Hcy levels—another risk factor for cardiovascular disease—in dialysis patients and found the levels in both patient groups to be higher than the levels in control subjects.

The many studies comparing Hcy levels in PD patients with those in HD patients have produced conflicting results (12). One study documented a higher prevalence of hyperhomocysteinemia in HD patients. On the other hand, Kim *et al.* (13) and Suliman *et al.* (14) reported higher Hcy levels in PD patients, results that are consistent with our findings.

Possible causes of high serum Hcy levels in chronic renal failure may include reduced renal clearance, impaired degradation by the kidney, and deficiency of, or abnormally high requirement for, folic acid, vitamin B_{12}, and vitamin B_6. Because urinary Hcy excretion is only minimal in humans (15), loss of urinary excretion does not explain the hyperhomocysteinemia found in renal failure. The loss of active catabolism of Hcy in renal tubular cells is therefore the more attractive hypothesis, in view of the large quantities of Hcy found in renal patients.

On the other hand, vitamin B_{12} and folic acid are needed in the remethylation pathway of Hcy to methionine. Lack of folic acid and vitamin B_{12} may be a possible cause of high Hcy serum concentrations. Although serum levels of folic acid and vitamin B_{12} were adequate in our patient groups, especially in the HD patients, those levels appear insufficient to prevent hyperhomocysteinemia, a finding that is consistent with previous studies (16).

Accordingly, we suggest that an intra-renal mechanism, such as a reduced ability of the kidney to metabolize Hcy, rather than an insufficiency of vitamin B_{12} and folic acid, may be the primary cause of hyperhomocysteinemia in end-stage renal failure. We observed elevated Hcy levels in our patient groups, but the mechanism of the relationship between elevated Hcy and cardiovascular disease remains to be established.

In our study, serum leptin levels tended to be higher in PD patients than in control subjects and HD patients, although the differences did not reach statistical significance. The mild increase of serum leptin levels in PD patients might be caused by the continuous glucose load that these patients receive, which increases body fat mass and, consequently, serum leptin levels. Obesity and hyperinsulinemia are major stimulators of leptin production and are usually associated with insulin resistance, dyslipidemia, cardiovascular disease, hypertension, and diabetes mellitus type 2 (17). For these reasons, the elevated leptin values in our PD patients might give rise to potentially dangerous side effects within the cardiovascular system as previously described (18).

Conclusions

Increased values of known risk factors were found in PD and HD patients alike. Lower HDL cholesterol levels, higher cardiovascular risk assessments, higher Hcy levels, and mildly increased leptin levels seem to produce a greater potential threat for vascular disease in PD patients than in HD patients.

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