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Gastrointestinal-related Uremic Toxins in Peritoneal Dialysis: A Pilot Study with a 5-year Follow-up

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Background and Aims. P-cresyl sulfate (PCS) and indoxyl sulfate (IS) were not only novel but essential factors associated with cardiovascular disease and mortality in patients with chronic kidney disease and hemodialysis. However, little evidence exams the effect in peritoneal dialysis (PD) patients.

Methods. This pilot study recruited 46 stable PD patients in a single medical center. Serum levels of IS, PCS and biochemistry were measured concurrently. Clinical outcomes including cardiovascular, all-cause mortality and PD failure event were recorded during a 5-year follow-up.

Results. Serum levels of free and total PCS were lower in patients with residual renal function (11.67 ± 6.92, $p = 0.014$, 0.77 ± 0.48, $p = 0.046$, respectively). Multivariate Cox regression analysis showed age (HR: 1.07, $p = 0.01$), serum CO_2 (HR: 0.67, $p = 0.02$) and total PCS (HR: 1.05, $p < 0.01$) were independently associated with cardiovascular events; only free PCS (HR: 1.42, $p < 0.01$) reached significant correlation with all-cause mortality. Total IS (HR: 1.27, $p = 0.03$) significantly correlated with PD failure event after adjusting other confounding factors. Kaplan–Meier analysis revealed that patients with higher total and free PCS levels had higher cardiovascular events (log rank $p < 0.01$, log rank $p = 0.05$, respectively) and mortality event (log rank $p = 0.02$, log rank $p = 0.03$, respectively) than those with lower levels. In addition, total IS (log rank $p = 0.04$), total PCS (log rank $p = 0.01$) and free PCS (log rank $p < 0.01$) could independently predict PD failure event during the study period.

Conclusions. Our findings suggest PCS and IS may be a valuable surrogate in predicting poor clinical outcomes in PD patients. © 2013 IMSS. Published by Elsevier Inc.

Key Words: Protein-bound uremic toxin, p-Cresyl sulfate, Indoxyl sulfate, Peritoneal dialysis.

Introduction

Evidence has shown that cardiovascular disease (CVD) (1,2) is the main cause of death in patients with chronic kidney disease (CKD)—especially in those with end-stage renal disease (ESRD) (3). Atherosclerosis may be preceded by endothelial dysfunction and result in arterial stiffness in dialysis patients. Associated cardiovascular risk factors

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include traditional risk factors such as smoking, diabetes, hyperlipidemia and hypertension (4,5) as well as nontraditional risk factors such as hyperhomocysteinemia, calcium phosphate abnormality, intact parathyroid hormone excess and protein-bound uremic toxins (6–9).

Some investigators have recognized that indoxyl sulfate (IS) and P-cresyl sulfate (PCS), two protein-bound uremic toxins, had deleterious effects on endothelial function (10,11) and induced the production of reactive oxygen species in *in vitro* studies (12,13). IS and PCS were also regarded as a novel surrogate marker in predicting infection event, cardiovascular and all-cause mortality not only in CKD but also in hemodialysis (HD) patients (14–18). Moreover, our recent research showed serum levels of PCS and IS were associated with peripheral artery disease (PAD) and total PCS could be an important determinant of access viability other than traditional or nontraditional risk factors in HD patients (19). These results indicated that IS and PCS played a critical role on clinical outcomes in patients with uremia.

However, current studies exploring the effect of IS and PCS on clinical events were focused on a CKD or HD population. Whether this effect can be demonstrated in PD patients remains unclear. In this pilot study we aimed to elucidate the effect of IS and PCS on clinical outcomes in patients undergoing PD during a 5-year follow up.

Patients and Methods

Study Patients

Our study recruited 46 stable ESRD patients on PD from June–July 2007 in a single medical center. Patients with acute infection and cardiovascular events in the past 3 months, those with malignancy, or those <18 years were excluded from this study. The cause of ESRD included cGN, type 2 diabetic nephropathy, polycystic kidney disease and lupus nephritis. Patient characteristics and biochemical parameters were examined concurrently. Our study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of the Mackay Memorial Hospital. Informed consent was obtained from all patients.

Laboratory Assessment

Serum samples were obtained twice during the same week after signed permit for all patients. The following tests were performed: blood urea nitrogen (BUN, md/dL), creatinine (Cr, mg/dL), hemoglobin (Hb, g/dL), hematocrit (Hct, %), albumin (g/dL), bicarbonate (mmol/L), calcium (Ca, mg/dL), phosphate (P, mg/dL), intact-PTH (i-PTH, pg/mL), high-sensitive C-reactive protein (hs-CRP, mg/dL), IS (mg/L) and PCS (mg/L). Serum levels of hs-CRP were measured using a Behring Nephelometer II (Dade Behring, Tokyo, Japan). The bromocresol green method was used for determination of albumin. Residual renal function was calculated and expressed as renal Kt/V (rKt/V). Total Kt/V is equal to peritoneal Kt/V plus renal Kt/V. Patients with daily urine <100 mL were regarded as nonresidual renal function. The normalized protein catabolic rate (nPCR) (g/kg/day) was calculated as a measure of daily protein intake. Serum PCS, IS and hs-CRP were measured twice to obtain an average value. Other biochemical values were measured at the first sampling during after enrollment.

Serum IS and PCS were analyzed with LC-MS/MS (4000 QTRAP, Vernon Hills, IL). Briefly, serum samples were prepared and deproteinized by heat denaturation. HPLC was performed at room temperature using a dC18 column (3.0 $\times$ 50 mm, Atlantis, Waters, Milford, MA). The buffers used were (A) 0.1% formic acid and (B) 1 mmol NH_4OAc + 0.1% formic acid in 100% acetonitrile. The flow rate was 0.6 mL/min with a 3.5 min gradient cycling from 90% A/10% B–10% A/90% B. Under these conditions, both PCS and IS were eluted at 2.73 and 2.48 min, respectively. Standard curves for PCS and IS were set at 1, 5, 10, 50, 250, 500 and 1000 μg/L; both were processed in the same manner as the serum samples and correlated with the serum samples with average r^2 values of 0.996 $\pm$ 0.003. These samples were diluted if the IS or PCS concentration exceeded standard curve. Quantitative results were obtained and calculated in terms of their concentrations (mg/L). The sensitivity of this assay was 1 μg/L for PCS and 1 μg/L for IS.

Endpoint Evaluation

Patients were followed-up until July 31, 2012. During the study period, clinical events including PD failure event, cardiovascular events and all-cause mortality were reviewed by one independent physician. In order to control the data accuracy, the medical charts of study patients were reviewed for all dialysis and for surgeries. Only patients who experienced PD failure and were switched to HD were recorded as having PD failure events in this study. Cardiovascular event was defined as patients with any one of the following cardiovascular events including death from cardiac causes, myocardial ischemia, nonfatal myocardial infarction, ischemic stroke, or new onset of peripheral vascular disease, whichever developed first. Only one event of cardiovascular event per subject was included in the analysis. Deaths were accurately recorded and the cause of death was categorized as cardiovascular, infectious or other.

Statistical Analysis

Demographic data were expressed as mean $\pm$ standard deviation (SD). Paired and unpaired *t* tests were applied for comparison between groups of PD patients (residual vs. nonresidual function or with vs. without event). Cox regression model was used to analyze the relationship between