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Removal of uremic retention products by hemodialysis is coupled with indiscriminate loss of vital metabolites

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ABSTRACT

Background: Although dialysis ameliorates uremia and fluid and electrolytes disorders, annual mortality rate remains high in dialysis population reflecting its shortcoming in replacing renal function. Unlike the normal kidney, dialysis causes dramatic shifts in volume and composition of body fluids and indiscriminate removal of vital solutes. Present study was undertaken to determine the impact of hemodialysis on plasma metabolites in end-stage renal disease (ESRD) patients.

Methods: 80 hemodialysis patients and 80 age/gender-matched healthy controls were enrolled in the study. Using ultra performance liquid chromatography-high-definition mass spectrometry, we measured plasma metabolites before, during, and after hemodialysis procedure and in blood entering and leaving the dialysis filter.

Results: Principal component analysis revealed significant difference in concentration of 214 metabolites between healthy control and ESRD patients' pre-dialysis plasma (126 increased and 88 reduced in ESRD group). Comparison of post-dialysis with pre-dialysis data revealed significant changes in the 362 metabolites. Among ESI^+ metabolites 195 decreased and 55 increased and among ESI^- metabolites 82 decreased and 30 increased following hemodialysis. Single blood passage through the dialyzer caused significant changes in 323 metabolites. Comparison of ESRD patients' post-hemodialysis with healthy subjects' data revealed marked differences in metabolic profiles. We identified 55 of the 362 differential metabolites including well known uremic toxins, waste products and vital biological compounds.

Conclusion: In addition to uremic toxins and waste products hemodialysis removes large number of identified and as-yet un-identified metabolites. Depletion of vital biological compounds by dialysis may contribute to the high morbidity and annual mortality rate in this population.

1. Introduction

Pandemics of type-2 diabetes and hypertension over the past 2 decades have resulted in a dramatic rise in prevalence of chronic kidney disease (CKD) and expanded use of dialysis modalities for renal replacement therapy worldwide. Although chronic dialysis modalities are highly effective in ameliorating uremia and fluid and electrolytes disorders, overall annual mortality rate remains high in dialysis-dependent population. This reflects the shortcomings of dialysis modalities in replacing normal kidney functions. Under normal conditions via continuous excretion of metabolic waste products and excess fluid and electrolytes, kidney plays a central part in maintaining homeostasis of the volume and composition of body fluids and blood pressure. In contrast, volume and composition of body fluids and arterial pressure undergo dramatic shifts during the inter- and intra-dialytic periods. These events can lead to adverse consequences. For instance, intradialytic and post-dialytic hypotension which is a common complication of hemodialysis is associated with numerous adverse consequences

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including cerebral atrophy [1] and increased mortality rate [2]. In addition, rapid removal of urea and other osmotically active substances together with delayed osmotic equilibrium of the intracellular fluid with extracellular fluid compartment contributes to intradialytic hypotension and neurological disorders [3]. In an attempt to achieve a greater uremia control, fluid and electrolyte homeostasis while simultaneously minimizing the magnitude of the inter- and intra-dialytic changes in hemodynamics and body fluid composition, several clinical trials have explored the effect of longer and more frequent dialysis regimens on clinical outcomes in end-stage renal disease (ESRD) patients. The ADEMEX trial which had enrolled 965 peritoneal dialysis patients, compared a 2-year, high intensity dialysis protocol (Kt/V = 2.0, creatinine clearance = 60 L/week) with a standard dialysis protocol. The study revealed no difference in patient survival, suggesting that more intensive elimination of urea and other water-soluble small molecular weight substances is not beneficial [4]. Similarly, no difference in annual mortality rate was observed in the HEMO trial which had enrolled 1846 chronic hemodialysis patients randomized to either high-intensity (Kt/V = 1.45 and urea reduction ratio = 75%) or standard-intensity (Kt/V = 1.05 or urea reduction ratio = 65%) hemodialysis treatment [5]. Likewise the FHN trial [6] which compared the outcomes in a group of ESRD patients randomized to high hemodialysis intensity (6 dialysis sessions/week), with those in a group of patients undergoing standard hemodialysis therapy (3 dialysis sessions/week) showed no significant difference in cognition, serum albumin or requirement for erythropoiesis-stimulating agents. Moreover, despite significant increase in Kt/V between the two groups (2.57 in conventional vs. 3.6 in intensive group), patients randomized to high intensity hemodialysis had a higher annual mortality rate than those assigned to conventional dialysis. Finally the ACTIVE (A Clinical Trial of Intensive Dialysis) trial in which participants were randomly assigned to 24 or more hours versus 12 to 15 h of hemodialysis/week and followed for up for 12 months revealed no significant reduction in mortality among patients enrolled in the extended dialysis program [7]. Taken together, these observations suggest that the high morbidity and annual mortality rate in ESRD populations maintained on dialysis modalities are not exclusively due to inadequate control of uremia, fluid and electrolytes disorders or the magnitude of dialysis-induced hemodynamic stress, and disequilibrium in body fluid compartments. Thus other factors must also contribute to high morbidity and annual mortality rate in this population.

It should be noted that in addition to removing excess fluids, electrolytes and metabolic waste products dialysis modalities can indiscriminately remove biologically important circulating small molecular weight compounds. This is unlike the normal kidney in which the vital molecules present in the glomerular filtrate are reabsorbed by proximal tubules. Therefore, inevitable losses of biologically important small molecules may, in part, contribute to high morbidity and annual mortality rate in dialysis-dependent patients and account for the reported lack of benefit or worsened outcomes with increased dialysis intensity [4–6]. While much is known about the efficacy of dialysis modalities in the management of CKD associated fluid, electrolyte disorders and clearance of the uremic retention metabolites, little attention has been paid to the indiscriminate loss of biologically important compounds and its potential contribution to the high morbidity and annual mortality rate in this population. Metabolomics has been widely applied to disease diagnosis [8–10], drug discovery [11–15], toxicity evaluation [16–18] and disease action mechanism [19–21]. Ultra performance liquid chromatography-high-definition mass spectrometry (UPLC-HDMS) has been widely used for metabolomics due to its enhanced analytic speed and sensitivity [22–25]. Using UPLC-HDMS in ESI^+ and ESI^- modes to measure plasma metabolites before, during, and after hemodialysis procedure, the present study was undertaken to determine the effect of hemodialysis on blood metabolome in patients with ESRD.

Table 1
Summary of clinical and demographic baseline characteristics of patients with CKD and health control subjects in this study.

	Healthy controls	ESRD patients
Number	80	80
Age (years)	49.1 ± 14.8	53.7 ± 15.3
Women (%)	65	44
Pre-dialysis body weight (kg)	NA	61.0 ± 12.5
Post-dialysis body weight (kg)	NA	58.1 ± 12.2
Body weight change (kg)	NA	2.95 ± 1.21
Pre-dialysis SBP (mm Hg)	NA	146.6 ± 18.9
Pre-dialysis DBP (mm Hg)	NA	85.6 ± 12.8
Post-dialysis SBP (mm Hg)	NA	139.3 ± 16.0##
Post-dialysis DBP (mm Hg)	NA	81.1 ± 7.5##
eGFR (mL/min/1.73m^2)	108.72 ± 37.77	0.00**
BUN (mg/dL)	12.16 ± 3.31	68.82 ± 25.88**
Plasma creatinine (mg/dL)	0.68 ± 0.14	12.03 ± 3.53**
Uric acid (mg/dL)	5.52 ± 1.59	7.45 ± 1.77**
Triglycerides (mg/dL)	4.39 ± 1.42	5.26 ± 1.84*
Total cholesterol (mg/dL)	12.63 ± 3.66	11.79 ± 2.82
LDL-C (mg/dL)	6.49 ± 2.18	7.14 ± 2.68
HDL-C (mg/dL)	5.35 ± 1.70	4.03 ± 1.09*
Total protein (g/dL)	7.27 ± 0.33	6.6 ± 0.73**
Albumin (g/dL)	4.73 ± 0.42	3.9 ± 0.39**
White blood cell ($\times 10^9$/L)	5.62 ± 1.16	5.28 ± 1.69
Hemoglobin (g/dL)	13.8 ± 1.7	9.5 ± 1.8**
Blood platelet ($\times 10^9$/L)	214.25 ± 53.18	172.35 ± 62.51**

Data are expressed as the means ± standard deviation.
* P < 0.01.
** P < 0.001 compared with health control group.
P < 0.01 compared with pre-dialysis.

2. Methods

2.1. Patients and controls

80 stable patients (45 men and 35 women, age: 53.7 ± 15.3 years) with ESRD and 80 age- and gender-matched healthy control persons were enrolled in the study. The ESRD patients were maintained on 3.5-h hemodialysis sessions 3 times per week for an average of 4 years at the Affiliated Hospital of Shanxi Institute of Traditional Chinese Medicine and Xi'an No. 4 Hospital, Xi'an, China. Healthy controls were excluded if they had any of the following conditions: cardiovascular disease, diabetes, hypertension, kidney dysfunction or use of regular medications. The clinical and demographic features of the study population are provided in Table 1.

Polysulfone dialyzers and the dialysis solution containing Na 140 mmol/L, K 2.0 mmol/L, Ca 1.50 mmol/L, Mg 0.5 mmol/L, Cl 107.0 mmol/L, $HCO3^-$ 34.0 mmol/L, $CH3\text{-}COO^-$ 5.0 mmol/L were used in all dialysis sessions. Dialysate flow rate and blood flow rate were 500 mL/min and 260 mL/min respectively. Heparin was used for systemic anticoagulation during hemodialysis procedure. Blood was drawn from the arterial line immediately before hemodialysis, 30 min after the onset of dialysis and at the end of the hemodialysis procedure. In addition, samples were taken from blood entering and leaving the dialyzer at 10 min. Dialysate effluent was collected at 10 min, 30 min and immediately before the end of hemodialysis session. Blood samples were immediately centrifuged at 3000 r/min for 10 min at 4 °C and plasma was separated and stored at − 80 °C. The study was conducted in accordance with the Helsinki declaration and approved by the institutions' ethics committees. Signed informed consent was obtained from all patients prior to their inclusion in the study.

2.2. Biochemical assays

Plasma creatinine (PCr), blood urea nitrogen (BUN), total cholesterol, LDL-C, HDL-C, albumin, total protein, uric acid and triglyceride were analyzed by Olympus AU640 automatic analyzer. White blood