ORIGINAL RESEARCH

Protein Losses and Urea Nitrogen Underestimate Total Nitrogen Losses in Peritoneal Dialysis and Hemodialysis Patients

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Objective: Muscle wasting is associated with increased mortality and is commonly reported in dialysis patients. Hemodialysis (HD) and peritoneal dialysis (PD) treatments lead to protein losses in effluent dialysate. We wished to determine whether changes in current dialysis practice had increased therapy-associated nitrogen losses.

Design: Cross-sectional cohort study.

Methods: Measurement of total protein, urea and total nitrogen in effluent dialysate from 24-hour collections from PD patients, and during haemodiafiltration (HDF) and haemodialysis (HD) sessions.

Subjects: One hundred eight adult dialysis patients.

Intervention: Peritoneal dialysis, high-flux haemodialysis and haemodiafiltration.

Main Outcome Measure: Total nitrogen and protein losses.

Results: Dialysate protein losses were measured in 68 PD and 40 HD patients. Sessional losses of urea (13.9 [9.2-21.1] vs. 4.8 [2.8-7.8] g); protein (8.6 [7.2-11.1] vs. 6.7 [3.9-11.1] g); and nitrogen (11.5 [8.7-17.7] vs. 4.9 [2.6-9.5] g) were all greater for HD than PD, $P < .001$. Protein-derived nitrogen was 71.9 (54.4-110.4) g for HD and 30.8 (16.1-59.6) g for PD. Weekly protein losses were lower with HD 25.9 (21.5-33.4) versus 46.6 (27-77.6) g/week, but nitrogen losses were similar. We found no difference between high-flux HD and HDF: urea (13.5 [8.8-20.6] vs. 15.3 [10.5-25.5] g); protein (8.8 [7.3-12.2] vs. 7.6 [5.8-9.0] g); and total nitrogen (11.6 [8.3-17.3] vs. 10.8 [8.9-22.5] g). Urea nitrogen (UN) only accounted for 45.1 (38.3-51.0)% PD and 63.0 (55.3-62.4)% HD of total nitrogen losses.

Conclusion: Although sessional losses of protein and UN were greater with HD, weekly losses were similar between modalities. We found no differences between HD and HDF. However, total nitrogen losses were much greater than the combination of protein and UN, suggesting greater nutritional losses with dialysis than previously reported.

Introduction

MUSCLE WASTING IS associated with an increased risk of morbidity and mortality.[1] Patients with chronic kidney diseases are potentially at increased risk of muscle loss because of combinations of dietary restrictions, vitamin deficiencies, changes in anabolic hormones and insulin resistance, metabolic acidosis, and the effects of uremic toxins,[2] along with physical inactivity.[3] In addition, although dialysis treatments clear retained waste products of metabolism, dialysis is nonselective and can potentially clear useful nutrients, including glucose and proteins. Therefore, clinical guidelines have recommended greater dietary protein intakes for the general population to compensate for protein losses during dialysis.[4-6]

Older studies have reported removal of amino acids (varying between 6 and 12 g per hemodialysis [HD] session), some peptides, and small amounts of protein (≤1 to 3 g per dialysis session).[4-7] However, the practice of HD has changed over time, and previous reports of higher protein losses were associated with dialyzer reuse[7] and reprocessing the dialyzers with bleach.[8] Most centers now use dialyzers with larger pore sizes designed to deliver high-flux dialysis; and in Europe, the introduction of hemodiafiltration (HDF), adding convective transport which increases middle molecule clearances compared with diffusional clearance with HD, has the potential to increase nutritional losses.[5]

Similarly, older studies in peritoneal dialysis (PD) patients have suggested daily peritoneal dialysate losses of 5-15 g of protein and around 2-4 g of amino acids.[9] Again,

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PD has changed from intermittent PD with the introduction of continuous ambulatory peritoneal dialysis (CAPD), automated cycler peritoneal dialysis (APD), and changes in dialysates and connectology. The older studies have comprised small series, typically less than 30 patients, and with measurements of protein, peptides, or amino acids, but not total nitrogen losses. We therefore wished to measure total nitrogen losses in a contemporary cohort of dialysis patients, including patients treated by HDF, to determine whether nitrogen losses were different between modalities and were greater than in earlier studies.

Patients and Methods

Aliquots of PD dialysate effluents were obtained from adult PD patients attending for peritoneal membrane testing.[10] Pooled samples from 24-hour PD effluents were obtained from patients treated by automated peritoneal dialysis (APD) with and without a daytime exchange, and from individual dialysate effluents for those patients treated by CAPD. Aliquots of HD effluents were sampled at 5, 30, and 60 minutes, and then at the end of the dialysis session in patients attending out-patient dialysis sessions, and losses were calculated by area under the curve. Dialysate effluent measurements were then adjusted for dialysate volumes to determine total sessional/daily losses.

Serum total protein and HD dialysate effluent protein were measured by colorimetric assays. PD dialysate protein was measured using pyrogallol red-molybdate (PRM) (Hitachi 726 auto analyser, Maidenhead, UK). This method is linear up to 2.14 g/L, and higher concentration samples were diluted to bring them into range.[9] Protein was also measured using a modified Lowry method (BioRad DC protein assay, BioRad, Hemel Hempstead, UK). We also tested to ensure that urea did not interfere with the BioRad protein assay. The commonly used conversion factor of 6.25 was used to determine the protein equivalent of nitrogen (i.e., 6.25 g protein is equivalent to 1 g nitrogen).

Effluent dialysate urea concentration was determined by the diacetyl monoxime colorimetric assay, using appropriate standards.[11] This test was specifically chosen because most "urea" assays (glutamate dehydrogenase based) used by chemical pathology laboratories actually assay urea plus ammonia. Testing confirmed that exogenous ammonia did not interfere with the assay. Dialysate urea was also measured using the Cobas UREAL assay (Roche Diagnostics, West Sussex, UK), which assays urea plus ammonia. Dialysate urea nitrogen (UN) was determined by adjusting for the nitrogen content of urea; 1 mole urea is equivalent to 28 g nitrogen.

Total nitrogen concentration was determined using an Antek MultiTek® nitrogen analyser (MultiTek, Houston, TX) and Antek MultiTek Software (version 2.0.0.0) by PAC (Houston, TX). The mean of 3 measurements was recorded, and if the relative standard deviation of the 3 measurements was >5%, then sample measurements were repeated. Coefficient of variation of the assay ranged from 0.7 to 1.0%.

All HD patients were dialyzed using high-flux polysulfone hemodialyzers (Elisio-H™, Nipro Europe, Zaventem, Belgium),[12,13] dialyzer surface area 1.5-2.1 m^2, and BBraun Dialog + machines (B.Braun, Melsungen, Germany). Patients were either treated by HD or on-line HDF. Low-molecular-weight heparin was used for dialysis circuit anticoagulation.[14] Dialysate water quality met current national bacteriological and chemical standards for ultrapure water. All PD patients used lactate-based PD fluids, and 7.5% icodextrin for overnight exchange with CAPD and daytime exchange with APD (Baxter Health Care, Deerfield, IL).

Statistical Analysis

Results are expressed as mean ± standard deviation, or median and interquartile range, or percentage. Student's *t* test was used for parametric and the Mann–Whitney *U* test for nonparametric data, with appropriate correction for multiple analyses where appropriate, and Spearman correlation test was used for nonparametric data. Statistical analysis was performed using Graph Pad Prism (version 7.0, Graph Pad; San Diego, CA), Statistical Package for Social Science, version 24.0 (IBM Corporation, Armonk, NY), and Analyse-It (Analyse IT 4.0, Leeds, UK). Statistical significance was taken at or below the 5% level.

This project was registered at Integrated Research Application System reference number 191812/893749/14/564 and approved by the National Research Ethics (Manchester) and the Hospital Research and Development Service, and complied with National Health Service (NHS) guidelines (UK NHS guidelines for clinical audit and service development). Individual consent was waived as we only analyzed waste samples. In keeping with the Hospital Trust policy, no patient identifiable data were used.

Results

We measured dialysate protein losses in 68 PD and 40 HD patients (Table 1). The majority of patients were treated by APD and HDF. Predialysis serum urea and hemoglobin were significantly greater in the HDF cohort (Table 2), and urea was greater in patients treated by CAPD compared with APD, who used more dialysate per day (Table 3). Using the PRM method, the amount of protein in effluent dialysate was below the limit of detection (0.4 mg/L) for HD patients, compared with a mean of 0.09 mg/L (range 0.005-1.5 mg/L) with the BioRad DC assay. Daily protein dialysate losses in the PD patients were found to be 4.86 ± 4.09 g/day using the PRM method and 7.79 ± 4.96 with the BioRad DC assay. Correlation between assays, $r^2 = 0.35$, $P < .001$, although the mean Bland–Altman bias was 3.3 (95% limits of agreement −4.2 to 10.8 g), there was a systematic bias with the PRM method giving higher results when protein concentrations