Original Paper

The Association Between Peritoneal Charge Barrier Dysfunction and Protein Lost During Continuous Ambulatory Peritoneal Dialysis

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Key Words

Amylase • Charge barrier • Continuous ambulatory peritoneal dialysis • Hypoalbuminemia • Protein Malnutrition

Abstract

Background/Aims: The main purpose of the present study was to determine the effect of peritoneal charge barrier dysfunction on hypoalbuminemia during CAPD. **Methods:** We measured the association of dialysis dose, peritoneal equilibration test (PET) results (ratio of dialysate and plasma creatinine), and peritoneal charge barrier index (ratio of pancreatic and salivary α -amylase clearance) on protein loss in 33 patients on maintenance CAPD. All patients were from a single institution and were diagnosed with chronic nephritis (n = 18 cases), diabetic nephropathy (n = 8), hypertension (n = 5), and hepatitis B virus-associated glomerulonephritis (n = 2). **Results:** The mean ($\pm$ SD) dialysate protein loss was 4.04 g ($\pm$ 1.97) per day. Protein loss was positively correlated with dialysis dose (r = 0.438, p = 0.01) but was not significantly correlated with PET results. The mean ($\pm$ SD) peritoneal charge barrier index was 6.12 ($\pm$ 21.20) and was inversely correlated with protein loss into the peritoneal dialysate (r = -0.532, p < 0.01). **Conclusions:** Taken together, our study of CAPD patients indicates that protein loss into the peritoneal dialysate increases with peritoneal dialysis dose and with disruption of the peritoneal charge barrier.

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Introduction

Hypoalbuminemia is a major complication in patients with peritoneal dialysis and is significantly associated with increased incidence of cardiovascular events and mortality in these patients [1-3]. Hypoalbuminemia is an important consideration for improving

technique survival of patients being treated with hemodialysis (HD) and peritoneal dialysis (PD) [4]. PD patients may experience hypoalbuminemia due to inflammation, inadequate dialysis, impaired appetite, or protein loss during PD [5, 6]. The daily dialysate protein loss in PD patients can be up to 10.5 g, most of which is albumin [7]. Thus, protein loss during CAPD can ultimately lead to hypoalbuminemia and generalized edema due to osmotic effects. It is clearly important to understand the mechanism of protein loss during CAPD in order to develop protocols that can prevent and treat this problem. Many studies have reported that patients with high peritoneal transport have poor prognosis [8, 9].

The peritoneum functions as a molecular and charge barrier, similar to the glomerular basement membrane [10]. Thus, it is generally believed that disruption of the peritoneal barrier will cause negatively charged molecules (such as albumin) to pass through more readily, leading to significant protein loss [11], although there is some question about the relative importance of the peritoneum as a molecular barrier vs. a charge barrier [12]. However, the effect of disruption of the peritoneal charge barrier on protein loss in PD patients has not yet been established.

The methods currently used to assess charge barrier disruption include traditional histological staining, immunohistochemical staining, biochemical assays, isotopic methods, and measurement of peritoneal clearance. These methods can be complicated, are generally not applicable in clinical practice, and are unable to accurately quantify *in vivo* changes of the peritoneal charge barrier [13]. Endogenous proteins have been used to measure the glomerular charge barrier [14] and may also be useful for measurement of the peritoneal charge barrier. For example, pancreatic α -amylase (PAM) and salivary α -amylase (SAM) have the same molecular weight (56 kDa) and diameter (2.9 nm), but PAM has a neutral isoelectric point (pI = 7.0) whereas SAM has an acidic isoelectric point (pI = 5.9~6.4) [13]. Thus, the more negatively charged form (SAM) will have a lower clearance rate than the neutral form (PAM) due to the presence of the peritoneal charge barrier, and the ratio of the clearance rates of these two α -amylases will change upon disruption of the peritoneal charge barrier [15, 16].

The main purpose of the present study was to determine the effect of peritoneal charge barrier dysfunction on protein loss during CAPD.

Materials and Methods

Subjects and CAPD protocol

This study was conducted from August 2005 to August 2009 and was approved by the Institutional Review Board of Fuzhou General Hospital (Fuzhou City, China). All patients provided informed consent. The inclusion criteria were patients with primary diseases including chronic nephritis (n = 18 cases), diabetic nephropathy (n = 8), hypertension (n = 5), and hepatitis B virus-associated glomerulonephritis (n = 2). The exclusion criteria were patients with peritonitis, heart or liver dysfunction, infection, inflammation, or tumors. All patients underwent CAPD 3 or 4 times per day (dwell time of 4 h per session) using a 1.5% glucose sodium lactate peritoneal dialysis solution, Dianeal (Baxter, Guangzhou, China), although the time was shortened for high transporters based on PET results (see below). All patients were enrolled after they were on CAPD for two months, and all measurements were performed immediately after sample collection.

Biochemical measurements

Fasting serum albumin, urea, creatinine, total cholesterol (TC), and triglycerides (TG) were measured in the morning using an automatic biochemical analyzer (AU2700, Olympus, Japan). Peritoneal urea, Kt/V, and creatinine clearance (CCr) were then calculated. A dialysate sample (10 mL) was used for measurement of protein concentration and calculation of total protein loss. Peritoneal dialysate was collected at 24 h intervals for measurement of volume (PV) and protein (PP). Daily PV is the total amount of abdominal drainage (mL) in 24 h. PP was simply calculated as the product of the concentration of dialysate protein and the PV. Pancreatic α -amylase (PAM) and total α -amylase (TAM) were measured with kits from Randox Laboratories (Antrim, UK).

Charge barrier calculation

Salivary α -amylase (SAM) was calculated as the difference of PAM and TAM. The following variables were then calculated: peritoneal dialysate salivary α -amylase (PSAM); peritoneal dialysate pancreatic α -amylase (PPAM); serum salivary α -amylase (SSAM); and serum pancreatic α -amylase (SPAM). Finally, the charge barrier index was calculated as the ratio of the clearance of PAM and SAM (CPAM/CSAM), where $CPAM = PPAM \times \text{peritoneal dialysate volume} / SPAM$ and $CSAM = PSAM \times \text{peritoneal dialysate volume} / SSAM$.

Peritoneal equilibration test

Patients were divided into two groups based on peritoneal equilibration test (PET) results [17], which measured the dialysate/plasma ratio (D/P) of Cr. Dialysate samples for this test were collected after 4 h of dialysis. The high transport group ($n = 16$) consisted of patients who had high or high-average transport (range: 0.65-1.04), and the low transport group ($n = 17$) consisted of patients with low or low-average transport (range: 0.34-0.65).

Statistical analysis

Data are summarized as means $\pm$ standard deviations (SDs) and median with ranges (min to max), except that sex was expressed as n (%) of males. Protein measurements are summarized as means $\pm$ SDs before and after PD and compared with a paired t -test. Spearman correlation analysis was performed to assess the relationship of peritoneal dialysate protein loss with peritoneal dialysate time, peritoneal dialysate dosage, D/P of Cr, peritoneal dialysate, and serum amylase because of non-normal distribution. The relationship of charge barrier and peritoneal dialysate protein loss was analyzed by Pearson correlation analysis. All statistical assessments were two-tailed and a p -value less than 0.05 was considered significant. Statistical analyses were performed using PASW 18.0 statistics software (SPSS Inc, Chicago, IL, USA).

Results

A total of 33 consecutive CAPD patients were enrolled (Table 1). There were 28 males and 5 females, the average ($\pm$ SD) age was 50.85 ($\pm$ 15.17) years, the average duration of dialysis was 238.03 ($\pm$ 220.12 days) and the average dialysis dosage was 5.82 ± 1.16 L per day. Table 1 also summarizes the baseline demographic and clinical characteristics, urine analysis results, peritoneal dialysis variables, peritoneal dialysate and serum amylase activities, and charge barrier index values of all enrolled patients.

In this study, we used the equilibration between dialysate and plasma creatinine after 4 h (D/P of Cr) to characterize transport status. Thus, we classified patients as having low transport ($n = 17$, range: 0.34-0.65, mean $\pm$ SD: 0.61 ± 0.11) or high transport ($n = 16$, range: 0.65-1.04, mean $\pm$ SD: 0.80 ± 0.18). The mean protein loss was not significantly different in these two groups (low transport: 3.72 ± 2.03 , high transport: 4.36 ± 1.91 ; $p = 0.367$). Correlation analysis of protein loss and peritoneal dialysis variables indicated that protein loss was positively and significantly correlated with peritoneal dialysate dosage ($r = 0.438$, $p = 0.011$, Figure 1). However, there was no correlation of peritoneal protein loss with albumin ($r = -0.134$, $p = 0.457$).

Table 2 shows the results of correlation analysis of peritoneal dialysate protein loss with peritoneal and serum dialysate α -amylases. The results indicate that protein loss was positively correlated with peritoneal dialysate salivary α -amylase (PSAM, $r = 0.426$, $p = 0.013$), but negatively correlated with peritoneal dialysate pancreatic α -amylase (PPAM, $r = -0.384$, $p = 0.027$) and serum salivary α -amylase (SSAM, $r = -0.613$, $p < 0.001$). There was no significant relationship of peritoneal dialysate protein loss with serum pancreatic α -amylase (SPAM, $r = -0.183$, $p = 0.308$). There was a negative correlation of peritoneal electric charge and protein loss in all 33 patients ($r = -0.584$, $p < 0.01$), but no significant difference of high transporters and low transporters.

We calculated the charge barrier index as the ratio of the clearance of PAM and SAM (CPAM/CSAM). Among all 33 patients, the mean charge ratio ($\pm$ SD) was $6.12 (\pm 21.20)$.

Table 1. Demographic and clinical characteristics, peritoneal dialysis variables, and peritoneal dialysate and serum α -amylase activities of enrolled patients (n = 33)

	Mean $\pm$ SD	Median (Range)
Demographic/clinical variable		
Age, yrs	50.85 $\pm$ 15.17	49 (20 to 80)
Sex, males (%)	28 (84.8)	
Weight, kg	61.70 $\pm$ 8.49	63 (45 to 78)
SBP, mmHg	142.18 $\pm$ 14.58	140 (120 to 186)
DBP, mmHg	84.06 $\pm$ 10.81	85 (51 to 102)
Urine volume, mL	1281.82 $\pm$ 680.63	1450 (50 to 3100)
CCr, mL/min	7.95 $\pm$ 2.54	7.96 (4.89 to 16.21)
Peritoneal dialysis variable		
Peritoneal dialysate time, days	238.03 $\pm$ 220.115	150 (60 to 1215)
Peritoneal dialysate dosage, L	5.82 $\pm$ 1.16	6 (4 to 8)
D/P of Cr	0.70 $\pm$ 0.17	0.68 (0.46 to 1.36)
Peritoneal dialysate protein loss, g/24 h	4.04 $\pm$ 1.97	4.23 (0.70 to 7.93)
Peritoneal dialysate and serum α -amylase activity*		
PSAM, U/L	7.2 $\pm$ 10.15	5 (1.8 to 60.8)
PPAM, U/L	5.14 $\pm$ 6.4	2.6 (0.2 to 30.2)
SSAM, U/L	57.2 $\pm$ 31.83	50.2 (2.4 to 140)
SPAM, U/L	29.95 $\pm$ 22.65	24.6 (3.69 to 91.3)
Charge barrier index**, CPAM/CSAM	6.12 $\pm$ 21.20	0.82 (0.1 to 122.5)

Data are expressed as means $\pm$ SDs and median with range (min to max) except for sex, which is given as n (%). Abbreviations: yrs, years old; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCr, clearance creatinine; D/P of Cr, dialysate/plasma ratio of creatinine; PSAM, peritoneal dialysate saliva α -amylase; PPAM, peritoneal dialysate pancreatic α -amylase; SSAM, serum saliva α -amylase; SPAM, serum dialysate pancreatic α -amylase. *Transperitoneal transport of amylases was measured three times and averaged for each patient.

**Charge barrier index was calculated as the ratio of clearance of PAM and SAM (CPAM/CSAM), where CPAM = PPAM $\times$ peritoneal dialysate volume/SPAM and CSAM = PSAM $\times$ peritoneal dialysate volume/SSAM.

Fig. 1. Scatter plot of peritoneal dialysate protein loss with peritoneal dialysate dosage. The coefficient of Spearman correlation analysis was observed as 0.438 (P=0.011).

and the range was 0.1 to 122.5 (Table 1). Finally, we analyzed the correlation of peritoneal dialysate protein loss with $\text{Ln}(\text{CPAM/CSAM})$ (Figure 2). In this analysis, the CPAM/CSAM was log-transformed to assure a normal distribution. The results indicate a significant negative correlation of peritoneal dialysate protein loss with $\text{Ln}(\text{CPAM/CSAM})$ ($r = -0.532$, $p = 0.001$).

Table 2. Correlation of peritoneal dialysate protein loss with peritoneal dialysate and serum amylase (n = 33)

Amylase	Coefficient of correlation with peritoneal dialysate protein loss	P-value
PSAM, U/L	0.426	0.013*
PPAM, U/L	-0.384	0.027*
SSAM, U/L	-0.613	<0.001*
SPAM, U/L	-0.183	0.308

Abbreviations: PSAM, peritoneal dialysate saliva α -amylase; PPAM, peritoneal dialysate pancreatic α -amylase; SSAM, serum saliva α -amylase; SPAM, serum dialysate pancreatic α -amylase. Coefficients of correlation and *p*-values were calculated as *via* Spearman's rank correlation analysis due to the non-normal distribution of peritoneal dialysate and serum α -amylase data. **p* < 0.05 indicates significant correlation.

Fig. 2. Relationship of peritoneal charge barrier ($\ln[\text{CPAM}/\text{CSAM}]$) and peritoneal dialysate protein loss (g/24 h). The Pearson *r*-value was -0.532 (*p* = 0.001). The charge barrier was calculated as the ratio of clearance between PAM and SAM (CPAM/CSAM), where $\text{CPAM} = \text{PPAM} \times \text{peritoneal dialysate volume}/\text{SPAM}$ and $\text{CSAM} = \text{PSAM} \times \text{peritoneal dialysate volume}/\text{SSAM}$.

Discussion

Hypoalbuminemia is a common complication in PD patients, and protein loss during PD is a major cause of this problem. In this study, 20 of our 33 CAPD patients (61%) had hypoalbuminemia (serum albumin < 3.5 g/dL), somewhat higher than previous research which indicated the incidence of hypoalbuminemia in PD patients was 20–40%, the incidence of severe hypoalbuminemia was 8%, and that hypoalbuminemia increased with the duration of PD [7].

The mean serum albumin level in our PD patients was 3.16 ± 0.53 g/dL after PD, below the normal lower limit of 3.5 g/dL. This could be due to urinary protein loss, consumption of a low-protein diet, anorexia, and/or increased catabolism. After PD, our patients experienced a significant decline in serum albumin but a significant increase in triglycerides. These changes may be related to loss of residual renal function, inadequate dialysis, and/or changes in patient appetite, stamina, energy level, inflammation status, or cardiovascular function [18]. The peritoneal clearance of solute may lead to loss of some nutrients, such as amino acids and proteins, which can negatively impact nutritional status [6]. Our results showed that the mean dialysate protein loss was 4.04 ± 1.97 g/day. We also assessed the relationship of protein loss with nutritional status and found that protein loss was not significantly associated with serum albumin, cholesterol, or triglycerides (data not shown).

Thus, it is possible that compensatory liver protein synthesis was simply inadequate, similar to what occurs in the pathophysiology of nephrotic syndrome.

Our study of the mechanism of protein loss during PD indicated that PD dose was positively associated with protein loss. The reason for this result is not entirely clear, but it may be due to increased albumin convection subsequent to the increased solute exchange and ultrafiltration upon dose increase. An increased duration of PD may increase the permeability of the peritoneum and lead to peritoneal fibrosis, ultrafiltration failure [19], and possibly protein loss. However, our results indicated no significant association between duration of PD and protein loss (data not shown).

Albumin may be lost by convection, raising the question of whether the transport characteristics of the peritoneum itself may affect protein loss. We found no such relationship in the present study. A previous report suggested that the transport and clearance of macromolecular solutes is mainly molecular-selective in CAPD patients, and the charge barrier appears to play no role in this process [20]. Instead, these researchers concluded that the higher peritoneal clearance of neutral pancreatic amylase relative to anionic salivary amylase could be due to release of the former from the pancreas. However, we observed significant association between the charge barrier and peritoneal dialysate protein in our 33 CAPD patients. This difference may be explained by the inclusion of peritonitis patients in this previous study, but the exclusion of peritonitis patients in the present study. Albumin is negatively charged and is the major protein in the dialysate, so our data suggest that the disruption of the peritoneal charge barrier results in increased albumin leakage.

The present study had several limitations that should be noted. The sample size was relatively small and all patients were from a single medical center. Second, this was a single-arm study, and there was no control or experimental group. A large multi-center and multi-arm trial would clearly provide more reliable results. Finally, we cannot exclude the possibility that recorded peritoneal amylase levels are exclusively driven by transport across the peritoneal membrane.

Conclusion

Our results suggest that disruption of the peritoneal charge barrier plays an important role in dialysate protein loss in CAPD patients and confirmed that dialysate protein loss was positively associated with dialysis dose. In addition, the level of dialysate protein loss was negatively associated with the charge barrier index. These results suggest that protein loss in PD patients may be reduced if the dialysate is supplemented with negatively charged additives.

Conflict of Interests

The authors declare that they have no conflict of interest.

Acknowledgements

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