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Association between Markers of Inflammation, Fibrosis and Hypervolemia in Peritoneal Dialysis Patients

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Key Words
Bioelectrical impedance · Hypervolemia · Interleukin-6 · Transforming growth factor-β · Fibrosis

Abstract
Background/Aim: Volume expansion in peritoneal dialysis (PD) patients is associated with left ventricular hypertrophy. The link between inflammation and hypervolemia has not been extensively studied. The aim of this study was to determine if an association exists between hypervolemia and markers of inflammation in PD patients. ***Methods:*** In this cross-sectional study of 22 prevalent PD patients, volume was determined by bioelectrical impedance analysis. Serum and peritoneal effluent interleukin-6 (IL-6) and peritoneal transforming growth factor (TGF)-β_1 were measured. A fast peritoneal equilibration test determined peritoneal transport status. ***Results:*** Bioimpedance-derived measures of hypervolemia correlated with peritoneal effluent IL-6 and TGF-β_1. Peritoneal IL-6 was also associated with high peritoneal transport status. ***Conclusions:*** Markers of inflammation and fibrosis (peritoneal IL-6 and TGF-β_1) are associated with markers of hypervolemia.

Introduction

Cardiovascular (CV) disease is the leading cause of death in peritoneal dialysis (PD) patients [1]. Two recent randomized controlled trials have shown that a higher clearance on PD is not associated with a reduced CV burden and mortality [2, 3]. However, volume expansion in PD patients is associated with left ventricular hypertrophy and dilatation, hypertension and progressive CV disease [4]. Control of volume has led to a reversal of left ventricular hypertrophy and hypertension [5]. Due to this, the focus for reducing mortality in patients with end-stage renal disease has shifted from optimizing small solute clearance to controlling volume expansion. Accurate volume assessment in PD patients is difficult due to unreliable or impractical measurement techniques [6]. Recently, bioelectrical impedance analysis (BIA) has been proposed as an adjunct in determining volume status [7].

BIA is a technique that uses electrical properties of different tissues to distinguish and quantify extracellular water (ECW) and total body water (TBW) volumes. Phase angle is a derived measure from BIA and has been shown to be a predictor of death in PD patients [8]. BIA has been used in PD patients to determine volume status, and this

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device correlates well with gold standard isotope dilution methods [9]. The ratio of ECW/TBW has also been shown to correlate with clinical evidence of hypervolemia [10]. The impedance ratio is a derived measure from raw data at 5:200 kHz, and this correlates inversely with increased extracellular fluid and may also be a marker of poor prognosis [11].

Inflammation, as measured by C-reactive protein (CRP) is a recognized risk factor for CV death in the general population and in patients with end-stage renal disease [12]. Postulated mechanisms have included increased oxidative stress [13], occult/chronic infections [14] and endotoxemia in hypervolemic states [15]. In addition, the myocardium is capable of releasing cytokines which are augmented by adrenergic stimulation, and these cytokines have been implicated in the development of left ventricular dysfunction and cardiac myocyte apoptosis [15].

In PD patients, an association between hypervolemia and inflammation has been suggested [16]. For example, Chung et al. [17] found an association between inflammation, increased peritoneal membrane solute transport and loss of residual renal function.

The aim of this study was to determine if an association exists between hypervolemia, as measured by BIA and serum, and markers of peritoneal inflammation and fibrosis.

Subjects and Methods

Study Design and Population

This is a cross-sectional study consisting of 22 stable, consenting PD patients who were recruited from one of the affiliated teaching hospitals associated with the McMaster University (St. Joseph's Healthcare), Hamilton, Ont., Canada. Study protocol was approved by the St. Joseph's Healthcare Research Ethics Board. Patients were included in the study if they were 18 years of age or older and on PD for at least 3 months. Exclusion criteria included a history of peritonitis in the previous 3 months, history of limb amputation, presence of a pacemaker or defibrillator and inability to provide informed consent.

Data Collection from Chart Review

Patient charts were reviewed to collect demographic information, a list of comorbidities and medications (dichotomous variables). Data to complete the Davies comorbidity score was extracted [18].

Inflammation and Fibrosis Markers

On the day of testing, 2 h into the dwell for the peritoneal equilibration test (PET), a serum interleukin (IL)-6 concentration and CRP (Cardiophase® hs-CRP, Dade Behring, Inc., Newark, Del., USA) were drawn. Peritoneal effluent from the overnight dwell was assayed for IL-6 and transforming growth factor (TGF)-β_1 by ELISA (R&D Systems, Minneapolis, Minn., USA). Total TGF-β_1 was assayed by activating the samples according to standard protocol.

Peritoneal Equilibration Test

The transport property of the peritoneal membrane was determined by the fast PET [19], and transport status was established based on the 4-hour dialysate/plasma (D/P) creatinine and recorded as a continuous variable. Drain volume over the 4-hour fast PET was recorded as the ultrafiltration volume.

BIA Measurement

A multifrequency BIA device (QuadScan 4000®, Bodystat, Inc., Douglas, UK) was used to determine volume status. BIA measurements were performed after drainage of overnight dwell and prior to performing the PET. Four skin electrodes were placed on the dorsum of the hand and foot on the side free from vascular access. Three measurements at 4 frequencies (5, 50, 100 and 200 kHz) were recorded, after the patient had been supine for 15 min as described by the manufacturer [20]. The mean of the 3 measurement values was used for analyses. Patients' resistance, reactance, impedance and phase angle values were measured at 50 kHz and body composition was determined by Bodystat® software. The impedance ratio of 5:200 kHz was calculated. ECW and TBW volumes were calculated from regression-based equations as per manufacturer's software, and a ratio of ECW:TBW was determined.

Statistics

All analyses were performed with the statistical package SPSS version 13 (SPSS, Inc., Chicago, Ill., USA). Descriptive statistics outlining baseline characteristics included the reporting of means and SD values for continuous variables and proportion and percentage for discrete variables. Logarithmic transformation was undertaken for variables that were not normally distributed in our population. Correlations are reported as the Spearman rank correlation coefficients. Two-sided p values were calculated with statistical significance set at alpha ≤ 0.05.

Results

The average patient age was 61 years and the average time on PD was 2.1 years. One third of the patients had diabetes; the cohort was equally divided between low and moderate Davies comorbidity index categories with 1 patient in the high comorbidity category. The glucose polymer solution icodextrin was being used by 68% of the PD patients.

The average IL-6 concentration in the PD effluent in the entire cohort was 270 $\pm$ 3 pg/ml while the corresponding serum IL-6 concentration was 19 $\pm$ 1 pg/ml.

The BIA measurement was repeated 3 times in each patient. The correlation between these 3 repeated measures for both reactance and resistance was >0.99. Asso-

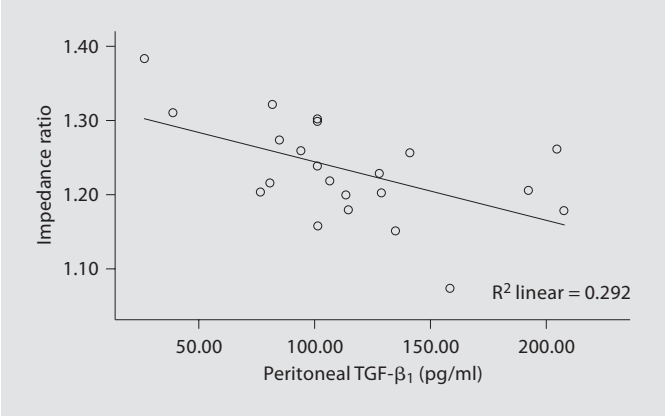

Fig. 1. Correlation between impedance ratio and peritoneal TGF-β_1 levels.

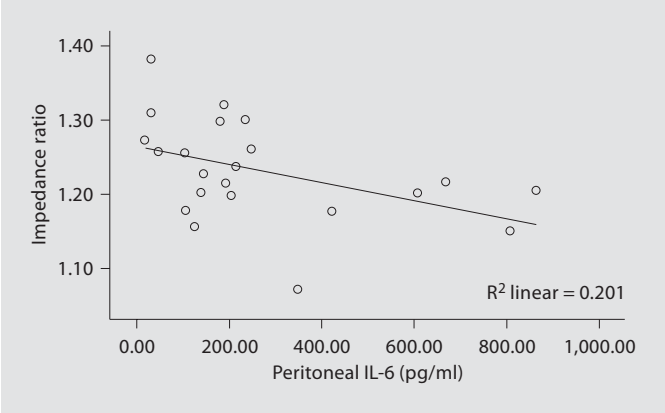

Fig. 2. Correlation between impedance ratio and peritoneal IL-6 levels.

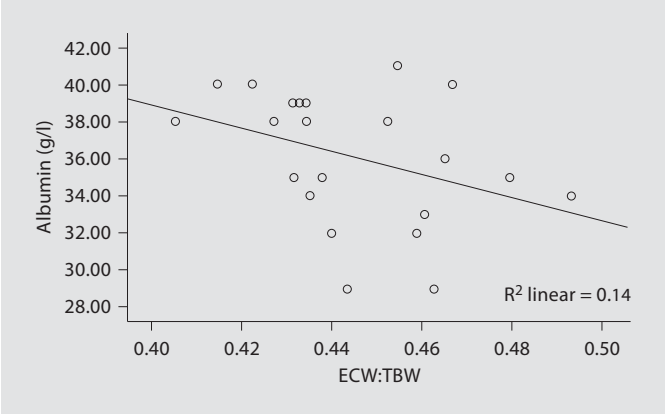

Fig. 3. Correlation between ECW:TBW and serum albumin.

Table 1. Significant correlations between measures of hypervolemia and inflammation/fibrosis

Measure of hypervolemia	Markers of inflammation and fibrosis	r	p
Impedance ratio	IL-6 PD	−0.47	0.03
	TGF-β_1 PD	−0.55	0.008
	albumin (serum)	0.74	<0.0001
Phase angle (50 kHz)	IL-6 PD	−0.42	0.05
	TGF-β_1 PD	−0.51	0.015
	albumin (serum)	0.74	<0.0001
ECW:TBW	IL-6 PD	0.36	0.10
	albumin	−0.42	0.05
D/P creatinine	IL-6 PD	0.52	0.01
Ultrafiltration volume	IL-6 PD	−0.47	0.03

ciations between different measures of volume status as determined by BIA and markers of inflammation and peritoneal injury are presented in table 1. Figures 1 and 2 show the correlation between peritoneal TGF-β_1 and IL-6 levels with the impedance ratio. Serum albumin correlated inversely with volume expansion as determined by ECW:TBW ($r = -0.42$, $p = 0.05$) (fig. 3) as well as other BIA measurements (impedance ratio: $r = 0.74$, $p < 0.0001$; phase angle (50 kHz): $r = 0.74$, $p < 0.0001$). Body mass index correlated inversely with ECW:TBW ($r = -0.50$, $p = 0.03$). In our study, D/P creatinine was associated with the impedance ratio ($r = -0.41$, $p = 0.05$), phase angle ($r = -0.40$, $p = 0.06$) and peritoneal IL-6 (table 1). Ultrafiltration volume correlated negatively with D/P creatinine ($r = -0.62$, $p = 0.003$) and was also associated with peritoneal IL-6 (table 1). Neither ultrafiltration volume ($r = -0.37$, $p = 0.10$) nor D/P creatinine ($r = 0.34$, $p = 0.12$) correlated significantly with peritoneal TGF-β_1 concentration. However, peritoneal TGF-β_1 concentration was associated with impedance ratio ($r = -0.55$, $p = 0.008$), phase angle ($r = -0.51$, $p = 0.015$) and the use of icodextrin ($r = 0.63$, $p = 0.002$). There was no correlation between peritoneal TGF-β_1 concentration and years on PD.

log CRP correlated with peritoneal IL-6 ($r = 0.46$, $p = 0.06$). The Davies comorbidity score approached a statistically significant correlation with peritoneal IL-6 ($r = 0.38$, $p = 0.08$). Peritoneal IL-6 was also associated with peritoneal TGF-β_1 ($r = 0.65$, $p = 0.001$) and the presence of diabetes ($r = 0.52$, $p = 0.01$).

Discussion

The main findings of this study are that the peritoneal effluent concentration of the inflammatory marker IL-6 is associated with markers of hypervolemia and higher peritoneal transport status. Increased PD effluent IL-6 has been observed previously [1] and is indicative of local intraperitoneal production of IL-6.

Of interest, we found that the peritoneal effluent TGF-β_1 concentration was associated inversely with the BIA measurements of phase angle and impedance ratio. TGF-β_1 is intimately linked with peritoneal injury, fibrosis, and impaired membrane function [21]. The association between inflammatory markers and hypervolemia has been previously described [16]. However, the association between the fibrogenic cytokine TGF-β_1 and hypervolemia is intriguing. Zweers et al. [22] have demonstrated that peritoneal effluent vascular endothelial growth factor correlated with solute transport, but TGF-β_1 did not. Our findings are in agreement with this, in that effluent TGF-β_1 did not correlate with solute transport. However, TGF-β_1 may be a marker for peritoneal fibrosis, and this may be associated with ultrafiltration dysfunction and subsequent hypervolemia. The peritoneal effluent TGF-β_1 concentration also correlated with the use of icodextrin. In this cross-sectional study, we cannot deduce whether icodextrin directly upregulates TGF-β_1 or, more likely, if patients with peritoneal injury and fibrosis are preferentially treated with icodextrin to maintain ultrafiltration. Currently, there is conflicting evidence as to the direct effect of icodextrin on peritoneal mesothelial cells in culture [23, 24].

We used different measures of volume status since there is debate in the literature as to the ideal bioimpedance-derived measure of volume [25]. BIA measures can be derived from assumption-based regression equations (ECW:TBW) or through the use of measured electrical properties (impedance ratio, phase angle) which do not require any physiological assumptions. A low phase angle is recognized as a surrogate marker predicting hypervolemia and death in PD patients [8]. Phase angle is a complex number and may reflect a state of volume overload and/or reduced body cell mass [26]. Inflammation has been found to be associated with fluid retention [16], malnutrition [27] and hyperglycemia [28]. In our study, phase angle (50 kHz) was associated with markers of inflammation, peritoneal fibrosis, the presence of diabetes, and higher peritoneal transport status. Chung et al. [17] also found that inflammation predicted death in their cohort of PD patients. The novel association between phase angle and peritoneal TGF-β_1 again reinforces the possibility that peritoneal injury and fibrosis predispose to ultrafiltration dysfunction and volume expansion.

An association between higher peritoneal transport status and hypervolemia was also identified, and this is in agreement with previous studies [17]. Tzamaloukas [29] has identified clinical correlates of hypervolemia including a high transport status and reduced ultrafiltration volume. Similar to Chung et al. [17], we noted that a reduction in ultrafiltration volume was associated with increased inflammation.

Proposed mechanisms to explain the association between hypervolemia and inflammation include: (1) bowel wall edema that may lead to enhanced gut permeability, and bacterial endotoxin translocation which has been described in patients with chronic congestive heart failure [15], (2) chronic/occult infections [14], (3) suppression of aquaporin-1 channels due to inflammation [30], and (4) use of hypertonic bioincompatible solutions in an attempt to achieve dry weight. Exposure of peritoneal tissues to high glucose levels may lead to inflammation and fibrosis through the action of advanced glycation end products [31, 32].

Limitations of this study include the small sample size and the cross-sectional observational design. Due to our limited sample size, a multivariable analysis was not performed as such a model would be too unstable. Despite this, interesting associations between peritoneal inflammation, fibrogenesis and hypervolemia are observed, and further longitudinal studies are needed to determine if inflammation predisposes to peritoneal fibrosis and volume expansion, and if this is predictive of adverse outcomes.

In conclusion, we have found that inflammatory and fibrogenesis markers are associated with a hypervolemic state. The potential to modify volume status renders this an interesting area for any longitudinal study evaluation to ascertain if a causal pathway between inflammation, fibrosis and fluid status exists.

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References

1 Collins AJ, Hao W, Xia H, et al: Mortality risks of peritoneal dialysis and hemodialysis. Am J Kidney Dis 1999;34:1065–1074.
2 Paniagua R, Amato D, Vonesh E, et al: Effects of increased peritoneal clearances on mortality rates in peritoneal dialysis: ADEMEX, a prospective, randomized, controlled trial. J Am Soc Nephrol 2002;13:1307–1320.
3 Lo WK, Ho YW, Li CS, et al: Effect of Kt/V on survival and clinical outcome in CAPD patients in a randomized prospective study. Kidney Int 2003;64:649–656.
4 Enia G, Mallamaci F, Benedetto FA, et al: Long-term CAPD patients are volume expanded and display more severe left ventricular hypertrophy than haemodialysis patients. Nephrol Dial Transplant 2001;16:1459–1464.
5 Fagugli RM, Reboldi G, Quintaliani G, et al: Short daily hemodialysis: blood pressure control and left ventricular mass reduction in hypertensive hemodialysis patients. Am J Kidney Dis 2001;38:371–376.
6 Jaeger JQ, Mehta RL: Assessment of dry weight in hemodialysis: an overview. J Am Soc Nephrol 1999;10:392–403.
7 Piccoli A: Identification of operational clues to dry weight prescription in hemodialysis using bioimpedance vector analysis. The Italian Hemodialysis-Bioelectrical Impedance Analysis (HD-BIA) Study Group. Kidney Int 1998;53:1036–1043.
8 Mushnick R, Fein PA, Mittman N, Goel N, Chattopadhyay J, Avram MM: Relationship of bioelectrical impedance parameters to nutrition and survival in peritoneal dialysis patients. Kidney Int Suppl 2003;87:S53–S56.
9 Konings CJ, Kooman JP, Schonck M, et al: Assessment of fluid status in peritoneal dialysis patients. Perit Dial Int 2002;22:683–692.
10 Plum J, Schoenicke G, Kleophas W, et al: Comparison of body fluid distribution between chronic haemodialysis and peritoneal dialysis patients as assessed by biophysical and biochemical methods. Nephrol Dial Transplant 2001;16:2378–2385.
11 Itobi E, Stroud M, Elia M: Impact of oedema on recovery after major abdominal surgery and potential value of multifrequency bioimpedance measurements. Br J Surg 2006;93:354–361.
12 Racki S, Zaputovic L, Mavric Z, Vujicic B, Dvornik S: C-reactive protein is a strong predictor of mortality in hemodialysis patients. Ren Fail 2006;28:427–433.
13 Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM: The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. Kidney Int 2002;62:1524–1538.
14 Haubitz M, Brunkhorst R: C-reactive protein and chronic *Chlamydia pneumoniae* infection – long-term predictors for cardiovascular disease and survival in patients on peritoneal dialysis. Nephrol Dial Transplant 2001;16:809–815.
15 Niebauer J, Volk HD, Kemp M, et al: Endotoxin and immune activation in chronic heart failure: a prospective cohort study. Lancet 1999;353:1838–1842.
16 Avila-Díaz M, Ventura MD, Valle D, et al: Inflammation and extracellular volume expansion are related to sodium and water removal in patients on peritoneal dialysis. Perit Dial Int 2006;26:574–580.
17 Chung SH, Heimburger O, Stenvinkel P, Bergstrom J, Lindholm B: Association between inflammation and changes in residual renal function and peritoneal transport rate during the first year of dialysis. Nephrol Dial Transplant 2001;16:2240–2245.
18 Davies SJ, Phillips L, Naish PF, Russell GI: Quantifying comorbidity in peritoneal dialysis patients and its relationship to other predictors of survival. Nephrol Dial Transplant 2002;17:1085–1092.
19 Twardowski ZJ, Nolph KD, Khanna R: Peritoneal equilibration test. Peritoneal Dialysis Bulletin 1987;7:138–147.
20 Bodystat® QuadScan 4000 Reference Manual, Bodystat, Inc., 2001.
21 Margetts PJ, Oh KH, Kolb M: Transforming growth factor-beta: importance in long-term peritoneal membrane changes. Perit Dial Int 2005;25(suppl 3):S15–S17.
22 Zweers MM, Struijk DG, Smit W, Krediet RT: Vascular endothelial growth factor in peritoneal dialysis: a longitudinal follow-up. J Lab Clin Med 2001;137:125–132.
23 Ha H, Cha MK, Choi HN, Lee HB: Effects of peritoneal dialysis solutions on the secretion of growth factors and extracellular matrix proteins by human peritoneal mesothelial cells. Perit Dial Int 2002;22:171–177.
24 Conti G, Amore A, Cirina P, Peruzzi L, Balegno S, Coppo R: Glycated adducts induce mesothelial cell transdifferentiation: role of glucose and icodextrin dialysis solutions. J Nephrol 2008;21:426–437.
25 Kuhlmann MK, Zhu F, Seibert E, Levin NW: Bioimpedance, dry weight and blood pressure control: new methods and consequences. Curr Opin Nephrol Hypertens 2005;14:543–549.
26 Pillon L, Piccoli A, Lowrie EG, Lazarus JM, Chertow GM: Vector length as a proxy for the adequacy of ultrafiltration in hemodialysis. Kidney Int 2004;66:1266–1271.
27 Kalantar-Zadeh K, Ikizler TA, Block G, Avram MM, Kopple JD: Malnutrition-inflammation complex syndrome in dialysis patients: causes and consequences. Am J Kidney Dis 2003;42:864–881.
28 Dandona P, Chaudhuri A, Ghanim H, Mohanty P: Insulin as an anti-inflammatory and antiatherogenic modulator. J Am Coll Cardiol 2009;53:S14–S20.
29 Tzamaloukas AH: Risk of extracellular volume expansion in long-term peritoneal dialysis. Adv Perit Dial 2005;21:106–111.
30 Stoenoiu MS, Ni J, Verkaeren C, et al: Corticosteroids induce expression of aquaporin-1 and increase transcellular water transport in rat peritoneum. J Am Soc Nephrol 2003;14:555–565.
31 De Vriese AS, Flyvbjerg A, Mortier S, Tilton RG, Lameire NH: Inhibition of the interaction of AGE-RAGE prevents hyperglycemia-induced fibrosis of the peritoneal membrane. J Am Soc Nephrol 2003;14:2109–2118.
32 Mortier S, De Vriese AS, Lameire N: Recent concepts in the molecular biology of the peritoneal membrane – implications for more biocompatible dialysis solutions. Blood Purif 2003;21:14–23.