Systemic endotoxaemia in peritoneal dialysis patients

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Abstract

Previous reports have linked systemic endotoxaemia in dialysis patients to increased markers of inflammation, cardiovascular disease and mortality. Many peritoneal dialysis (PD) patients use acidic, hypertonic dialysates which could potentially increase gut permeability resulting in increased systemic endotoxaemia. However, the results from studies measuring endotoxin peritoneal dialysis (PD) patients have been discordant. As such we measured systemic endotoxin in a cohort of 55 PD outpatients attending for routine assessment of peritoneal membrane function; mean age 58.7±16.4 years, 32 (58.2%) male, 21 (38.2%) diabetic, median duration of PD treatment 19.5 (13-31) months, 32 (58.2%) using 22.7 g/L dextrose dialysates, and 47 (85.5%) icodextrin. The median systemic endotoxin concentration was 0.0485 (0.0043-0.103) EU/ml. We found no association between endotoxin levels and patient demographics, markers of inflammation, serum albumin, N-terminal pro-brain natriuretic peptide, extracellular volume measured by bioimpedance, blood pressure, peritoneal dialysis prescriptions or peritoneal membrane transporter status, or medications. The measurement of endotoxin can be affected by failure to effectively release protein bound endotoxin prior to analysis on the one hand, and on the other by contamination when taking blood samples, processing and storing the samples. Additionally, the presence of fungal β-glucan from fungal cell walls and the use of different assays to analyse endotoxin can also give differing results. These factors may help to explain the disparate results reported in different studies. Our study would suggest that

exposure to peritoneal dialysates does not affect systemic endotoxaemia, and that endotoxin is not a major cause of inflammation in adult PD outpatients.

Introduction

Patients with chronic kidney disease (CKD), especially dialysis patients are at increased risk of inflammation [1], which drives muscle wasting, malnutrition, and vascular calcification, cumulating in an increased risk of mortality [1,2]. There are many potential sources of inflammation, including direct inflammatory effects of uraemic toxins, to increased peri-odontal disease due to underlying kidney bone mineral disease, absorption of toxic products from the gastrointestinal biome, contamination of dialysis fluids and catheter related infections. As circulating i cytokines and other inflammatory mediators are normally cleared by the kidney, then patients with CKD would be expected to have elevated levels [3].

There has been recent interest in circulating endotoxin as a cause of inflammation in kidney dialysis patients [4,5]. Endotoxins are complex lipopolysaccharides, ranging in size from 10 to 1000 kDa (larger masses form due to hydrophobic aggregation), present in the cell wall of gram negative bacteria. Endotoxins trigger activation of the innate immune system, as well as activating monocytes and macrophages through their CD14/Toll like receptor 4 complex activation. As endotoxins are such potent activators of inflammation, there are

natural host defence mechanisms designed to rapidly bind and detoxify any circulating endotoxin.

Previous reports have linked circulating endotoxin levels with hypertension and extracellular volume overload [6,7] and systemic inflammation [5,6], whereas other reports have shown no association with volume status, or markers of systemic inflammation [4]. In view of the differing reports we set out to measure endotoxin and volume status in a cohort of peritoneal dialysis (PD) patients.

Methods

We measured plasma endotoxin in adult PD patients attending for peritoneal membrane assessment [8]. Patients who had peritonitis or PD catheter exit site infection or hospital admission in the preceding three months were excluded. In addition to standard laboratory biochemical measurements, we measure brain-natriuretic peptide (NT-proBNP) (Roche Integra, Roche diagnostics, Lewes, UK), and C reactive protein (CRP) with an assay with a detection limit ≤1.0 mg/L [9]. Blood samples for endotoxin were collected aseptically into sterile heparinised tubes, and plasma separated by centrifugation and stored at -80°C until assayed. All phlebotomy equipment, pipette tips and Eppendorf storage tubes were checked for endotoxin contamination, and all apparatus had no detectable endotoxin (<0.0005 EU/ml). Samples were assayed using endochrome-K lysate (Charles River Laboratories, France) with manufacturer supplied depyrogenated equipment, and the kinetic

chromogenic limulus amoebocyte lysate analysed using FLUOstar Omega microplate readers with MARS data analysis software (BMG Labtech, Offenburg, Germany) and read at 405 nm and compared to standard curves [10].

Extracellular water (ECW) and body composition were measured using multifrequency bioelectrical impedance (MFBIA) (InBody 720, Seoul, South Korea) [11], after patients had emptied their bladder and peritoneal dialysate drained out [12,13],

Patients provided informed consent for this observational study which was approved by London Camden and Islington research ethics committee (13/LO/0912) and registered (ISRCTN70556765). All patient data was anonymised.

Statistical analysis

Data is presented as mean ± standard deviation, median (interquartile range), or percentage. Data was analysed using D'Agostino & Pearson normality test, and standard statistical tests; t test and Mann Whitney U test, ANOVA, Kruskal Wallis and Chi square test, with appropriate post hoc corrections for multiple testing (Tukey or Dunn) and Spearman correlation. For multivariable models, nonparametric data was log transformed if required. Statistical analysis used Prism 7.0 (Graph Pad, San Diego, USA) and SPSS 24 (IBM SPSS Statistics, Armonk, New York, USA). Statistical significance was taken as $p<0.05$.

Results

We measured endotoxin in 55 patients (table 1). The median endotoxin concentration was 0.0485 (0.0043-0.103) EU/ml, with endotoxin undetectable (<0.005 Eu/ml) in 12 patients. There was no difference in endotoxin levels according to primary renal disease (table 1). There were no statistically significant correlations between endotoxin concentrations and any of the variables in table 1.

Neither multivariable models or binary logistic models (above and below median) showed any significant association between endotoxin concentrations and potential variables of interest (serum albumin, NT-proBNP, CRP, systolic blood pressure, pulse pressure, ECW, body composition, Davies Co-morbidity grade, primary renal disease, residual renal function, peritoneal membrane transporter status or peritoneal or total urea clearance).

Discussion

The results from previous studies reporting on systemic endotoxaemia in peritoneal dialysis patients have been discordant, both in terms of the circulating concentrations reported and association with systemic inflammation and outcomes. We report a median circulating endotoxin concentration of 0.05 Eu/ml, which compared to reports of as low as 0 Eu/ml [14], up to 15.9 Eu/ml [15]. Generally, PD patients from South East Asia have been reported to have greater endotoxin levels [16] than those from Western Europe [18].

Studies have used assays from different manufacturers, with varying detection limits from 0.005 to 0.01 to 1 Eu/ml [5,10,15]. As such this may

partially explain some of the differences reported between studies. These assays were originally developed to detect very low levels of endotoxin in water as part of sterility quality control procedures. As endotoxin is such a potent activator of inflammation, plasma endotoxin is highly regulated by binding to albumin and other proteins such as lipopolysaccharide binding protein, and intestinal alkaline phosphatase to minimise free plasma endotoxin. Measuring endotoxin therefore requires heat pre-treatment of samples to ensure that all endotoxin is freed from plasma protein and so becomes available for measurement. On the other hand, samples may be contaminated by addition of exogenous endotoxin from numerous sources including phlebotomy equipment, blood sampling tubes, storage tubes. More recently it has been recognised that the most common assay, the limulus Amoebocyte Lysate (LAL) assay is not endotoxin-specific and can be activated by (1→3)-β-glucan, a component of fungal cell walls leading to false positive signals [19]. Fungal peritonitis is more commonly reported from South East Asia than Europe [20,21], and differences in environmental exposure to fungi may account for the much higher endotoxin levels reported from Hong Kong and Taiwan [15,16].

Previous observational studies in dialysis patients have differed, with reports that patients with higher plasma endotoxin levels have better survival [18], whereas others described a greater incidence of cardiovascular disease and increased mortality [4]. We found no association between volume status and NT-proBNP, which is in keeping with previous European studies [4,17]. Studies in a highly selected small group of elderly patients with chronic kidney disease

185 suggested that endotoxin levels were positively associated with systemic blood
186 pressure and vascular stiffness [7], whereas we found no association with blood
187 pressure and endotoxin levels, and similarly others have shown no association
188 between cardiac magnetic resonance and pulse wave velocity findings with
189 endotoxin levels [4,17]. Similarly, there have been varying results reporting an
190 association between systemic endotoxin levels and markers of inflammation, with
191 studies reporting a positive association with CRP [5,18] and monocyte
192 chemoattractant protein-1 [15], whereas others have reported no association
193 with CRP [7], or the inflammatory cytkines interleukin-6 and tumour necrosis
194 factor alpha [14]. The largest study reporting a positive association between
195 endotoxin and CRP, also reported a negative association with albumin, and yet
196 patients with the greatest endotoxin levels had greater survival [18]. As assays
197 are designed to measure total endotoxin following protein denaturation, and as
198 any free endotoxin is rapidly bound in plasma by albumin, this may explain why
199 the majority of published studies (and our own) have failed to demonstrate any
200 association between endotoxin levels in healthy PD outpatients and inflammation.
201 This is supported by one study which measured circulating bacterial DNA, and
202 could only demonstrate that endotoxin levels could only account for
203 approximately 5% of the predicted levels from the observed bacterial DNA [16].
204 We found no association between the amount of peritoneal dialysis urea
205 clearance, peritoneal transporter status, use of hypertonic glucose dialysates or
206 icodextrin and systemic endotoxin levels, which is in keeping with previous
207 reports [18].

Previous studies have differed widely in reporting endotoxin levels in kidney dialysis patients, with some reporting similar levels for PD and hemodialysis patients [4] and others that PD patients have much lower values [14]. Small, but highly detailed studies have failed to demonstrate an effect of endotoxin levels on blood flow in the abdomen in PD patients, or vascular stiffness or vascular permeability with increased extracellular fluid [17]. Our study reports much lower endotoxin levels than previously reported by earlier observational studies [5,8,16]. We were unable to demonstrate any association between systemic endotoxin levels and markers of inflammation of extracellular volume excess, in keeping with more recent reports [17]. Whether these differences in reports relate to the methods used to take blood samples, sample processing, contamination with fungal β- glucan and different assays remains to be determined. However, our study would suggest that systemic endotoxaemia is not the major cause of inflammation in PD patients.

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328 Table 1. Patient demographics, peritoneal dialysis prescriptions, body
329 composition and laboratory investigations Results expressed as integers, mean
330 ±standard deviation, median (interquartile range) or percentage.
331

variable	
Male gender	32 (58.2%)
Age years	58.7 ±16.4
Diabetic	21 (38.2)
Ethnicity White/Asian/Black	20(36.4%);12(21.8%);23 (41.8%)
Months of peritoneal dialysis treatment	19.5 (13 – 31)
Endotoxin levels : primary renal disease	
Diabetic nephropathy	0.036 (<0.005-0.158) Eu/mL
Hypertensive renal disease	0.049 (0.013-0.075) Eu/mL
glomerulonephritis	0.078 (0.017-0.107) Eu/mL
Interstitial nephritis	0.042 (0.019-0.114) Eu/mL
Vasculitis	0.045 (0.022-0.076) Eu/mL
PD mode CAPD/APD/CCPD	16(29.1%);8(14.5%);31(56.4%)
Icodextrin L/day	2.0 (1.15-2.0)
Icodextrin usage	47 (85.5%)
22.7 g/L dextrose L/day	4.5 (0 -8.4)
22.7 g/L dextrose usage	32 (58.2%)
Weekly urinary Kt/Vurea	0.68 (0.09 – 1.98)
Weekly peritoneal Kt/Vurea	1.31 (0.88 -1.83)
4 hour dialysate creatinine/serum creatinine	0.74 ±0.12
Combined urinary urea and creatinine clearance ml/min	3.0 (0.3 – 7.9)
Systolic blood pressure mmHg	143 ± 27.1
Pulse pressure mmHg	35.7 ±16.2
Intracellular water L	22.9 ±5.2
Extracellular water L	14.8 ±3.4
Weight kg	74.5 ± 16.5
Skeletal muscle mass kg	27.6 ± 6.8
Fat mass kg	23.4 ± 9.9
Body mass index kg/m^2	26.6 ± 5.0
Protein nitrogen accumulation g/kg/day	0.97 ± 0.27
Glycated haemoglobin mmol/mol	34.4 (32.2 – 46.4)
Haemoglobin g/L	108 ±20.4
Serum albumin g/L	38.1 ±3.5
Serum corrected calcium mmol/L	2.34 ±0.14
Serum phosphate mmol/L	1.58 ±0.42
C reactive protein g/L	2.0 (1.0-8.0)
Blood glucose mmol/L	5.8 (4.8 – 8.2)
Serum sodium mmol/L	136 ±4.3
Serum potassium mmol/L	4.3 ±0.5

Serum urea mmol/L	19.9 ±5.5
Serum creatinine umol/L	739 (523 – 1075)
N terminal probrain natriuretic peptide pg/mL	2233 (894 – 6317)
Number of anti-hypertensive medications	1 (0.25 – 2.0)
Patients prescribed anti-hypertensives	40 (72.7%)
prescription calcium binders tablets/day	0 (0-3)
prescription non-calcium binders tablets/day	0 (0-2.7)
Davies co-morbidity grade	1 (0-1)

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