

Systemic and Intraperitoneal Proinflammatory Cytokine Profiles in Patients on Continuous Ambulatory Peritoneal Dialysis

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Our cross-sectional study included 44 patients (27 men, 17 women; mean age: 57.12 ± 16.66 years; mean dialysis treatment period: 3.59 ± 2.67 years) on continuous ambulatory peritoneal dialysis (CAPD). Of the 44 patients, 21 were using standard solutions (Stay-Safe, ANDY-disc: Fresenius Medical Care, Bad Homburg, Germany), and 23 were using biocompatible solutions (Gambrosol Trio: Gambro Lundia AB, Lund, Sweden; Stay-Safe Balance: Fresenius Medical Care). In all CAPD patients dialyzed longer than 6 months, we analyzed levels of interleukin 1 β (IL-1 β), tumor necrosis factor α (TNF α), and interleukin 6 (IL-6) in serum and dialysis effluent when patients were free of acute infection-related (CAPD peritonitis, exit-site infection, other acute infections) complications. In a control group of 20 patients with chronic renal failure [CRF (stages IV and V)], we also determined serum levels of the same cytokines.

Levels of the inflammatory cytokines were measured using specific commercial ELISA kits (BioSource, Camarillo, CA, U.S.A.). Statistical analysis of the results was performed using commercial statistics software for the PC (Statistica for Windows, rev. 4.5: StatSoft, Tulsa, OK, U.S.A.).

Serum levels of IL-1 β and IL-6 were not statistically significantly different between the patients on CAPD, regardless of the type of dialysis the used, and between the patients and the control group with CRF. Serum levels of TNF α , unlike those for IL-1 β and IL-6, were statistically significantly higher in patients on CAPD than in the control group with CRF (13.20 ± 3.23 pg/mL vs. 5.59 ± 4.54 pg/mL, $p < 0.001$,

Mann-Whitney test). Serum and effluent IL-1 β levels in patients on CAPD for less than 1 year and more than 1 year did not significantly differ, but effluent IL-6 levels were significantly higher than serum IL-6 levels in both groups of patients, and effluent IL-6 levels were significantly higher in CAPD patients dialyzed for more than 1 year than in patients dialyzed for less than 1 year. Serum and intraperitoneal (IP) levels of the examined cytokines did not significantly differ in patients on standard and biocompatible solutions, but a trend toward lower IP levels of IL-6 was seen in patients on biocompatible solutions. Residual renal function and number of episodes of CAPD peritonitis had no important effect on serum and IP levels of the examined cytokines.

Elevated serum levels of TNF α and significant local IL-6 production in our CAPD patients indirectly confirmed the importance of peritoneal dialysis (PD) in amplifying the chronic inflammation that substantially depends on duration of dialysis treatment.

Key words

Pro-inflammatory cytokines, CAPD, chronic inflammation

Introduction

Continuous ambulatory peritoneal dialysis (CAPD) has been a successful form of renal replacement therapy for more than 30 years. Long-term CAPD is associated with changes in the peritoneal membrane. Standard peritoneal dialysis solutions (PDSs) are bioincompatible because of their low pH, high glucose content, hyperosmolality, and increased concentration of glucose degradation products (GDPs).

Investigations conducted during recent years in animal models and in the clinic more and more suggest

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the importance of chronic inflammation, malnutrition, and atherosclerosis—"MIA syndrome"—as predictors of mortality in patients treated by chronic peritoneal dialysis (PD). Causes of inflammation in patients with chronic renal failure (CRF) are numerous, and the most important is reduced renal clearance of the inflammatory cytokines [interleukin 1 (IL-1), tumor necrosis factor α (TNF α), interleukin 6 (IL-6)], accumulation of advanced glycosylation end-products, heart failure, atherosclerosis *per se*, unrecognized chronic infections, and numerous genetic factors (1,2). As patients reach the terminal stage of CRF and after they are initiated onto PD, the foregoing factors are associated with infection-related (peritonitis, exit-site infection) complications of PD that are related to the bioincompatibility of standard PDSs. The unfavorable aspects of some PDSs not only affect peritoneal membrane structure (mesothelial cells, capillary endothelial cells, fibroblasts), but also the monocyte-macrophage (Mo-Mf) cells that constitute the dominant population of mobile cells that provide local host defense (3–5). The continuous nature of the CAPD process and the permanent removal of large numbers of cells with the dialysate effluent, together with the unfavorable effects of PDSs on Mo-Mf cells, result in a serious disturbance to phagocytic capacity and a reduced potential for differentiation into dendritic cells, which are the most potent peritoneal antigen-presenting cells.

Chronic inflammation in patients treated by chronic PD results in the development of malnutrition, with inflammatory cytokines having the most important role in that process. Association of chronic inflammation and cardiovascular disease in patients on chronic PD has been well documented, but whether the markers of inflammation [C-reactive protein (CRP), fibrinogen, ferritin, IL-6, lipoprotein a] are epiphenomena of the existing process of atherosclerosis or are included in its onset and progression remains unknown (1,3,5,6).

In the present study, we examined the effects of CAPD solutions (standard vs. biocompatible) and duration of PD on the local and systemic profiles of proinflammatory cytokines (IL-1 β , TNF α , and IL-6) in patients on CAPD.

Patients and methods

Our cross-sectional study included 44 stable prevalent CAPD patients (27 men, 17 woman; mean age: 57.12 ± 16.66 years) of whom 21 were using standard solutions (Stay-Safe, ANDY-disc: Fresenius Medical

Care, Bad Homburg, Germany), and 23 were using biocompatible solutions (Gambrosol Trio: Gambro Lundia AB, Lund, Sweden; Stay-Safe Balance: Fresenius Medical Care). Mean dialysis duration was 3.59 ± 2.67 years. In all CAPD patients dialyzed for longer than 6 months, we analyzed levels of IL-1 β , TNF α , and IL-6 in serum and dialysis effluent during a period when patients were free of acute infection-related (CAPD peritonitis, exit-site infection, other acute infections) complications. A control group included 20 patients with CRF (stages IV and V), whose serum levels of the examined cytokines were also determined.

Levels of the proinflammatory cytokines were determined using specific commercial ELISA kits (BioSource, Camarillo, CA, U.S.A.). The lowest threshold of detectability for IL-1 β was 1 pg/mL; for IL-6, 2 pg/mL; and for TNF α , 1.7 pg/mL.

Blood samples were taken in the morning, when the overnight exchange was being drained and the peritoneal cavity was being filled with fresh PDS. Samples for determination of cytokine levels were taken from the dialysis effluent in the drainage bag from the overnight exchange. A peritoneal equilibration test was also performed in all patients to evaluate the transport characteristics of the peritoneal membrane.

Statistical analyses of the results use both descriptive and analytic statistics. The statistical significance between particular groups was checked using the Student *t*-test and the *s*-test, and correlations were examined using Pearson correlation analysis. The significance of differences in frequencies between the various groups was checked using the χ^2 test. Data were processed using commercial statistical software for the PC (Statistica for Windows, rev. 4.5: StatSoft, Tulsa, OK, U.S.A.).

Results

Serum levels of IL-1 β and IL-6 were not statistically significantly different in patients on CAPD and in control subjects with CRF, regardless of the PDS used. Serum levels of TNF α , unlike of those of IL-1 β and IL-6, were statistically significantly higher in patients on CAPD than in control subjects (13.20 ± 3.23 pg/mL vs. 5.59 ± 4.54 pg/mL, $p < 0.001$ by Mann-Whitney test; Figure 1).

Tables I and II present intraperitoneal levels of the examined proinflammatory cytokines in patients on CAPD by treatment duration (up to 12 months, and

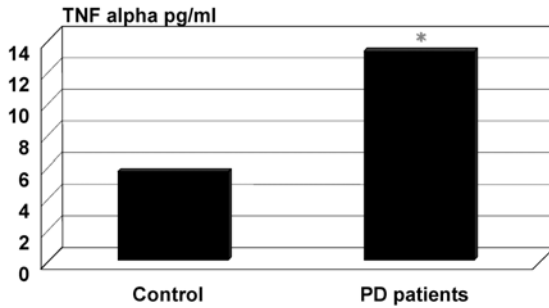


FIGURE 1 Serum levels of tumor necrosis factor (TNF) α in patients on continuous ambulatory peritoneal dialysis (PD). * $p < 0.001$ by Mann–Whitney test.

more than 1 year). The tables make it clear that the serum and effluent IL-1 β levels in patients on CAPD do not significantly differ by dialysis duration. In both groups of patients, effluent IL-6 levels were significantly higher than serum levels were, but effluent IL-6 levels in CAPD patients dialyzed for more than 1 year were significantly higher than they were in patients treated for up to 12 months.

Table III presents the effect of PDS biocompatibility on intraperitoneal levels of IL-1 β , IL-6, and TNF α . As can be seen, serum and intraperitoneal levels of the examined cytokines are not significantly different in patients on standard and on biocompatible PDSs, regardless of the trend toward a decline in intraperitoneal IL-6 levels in patients on biocompatible PDSs.

Residual renal function and number of CAPD peritonitis episodes did not have any significant effect on serum and intraperitoneal levels of the examined cytokines (Tables IV and V). At the time of the analysis, serum and intraperitoneal levels of cytokines also were not significantly different in CAPD patients with low, moderately low, moderately high, or high peritoneal transport characteristics, although a trend toward higher levels of all examined cytokines in effluent was noted with an increased dialysate-to-plasma (D/P) creatinine of 0.34 – 1.03.

Discussion

The uremic syndrome is a complex condition that results from retention of waste compounds that would normally be excreted into the urine or catabolized by the kidneys. In addition, inflammation has been implicated in symptoms associated with uremia, including

TABLE I Serum and effluent levels of cytokines in patients on peritoneal dialysis for up to 12 months

Cytokine		Patients (n)	Mean	SD
IL-1 β	Serum	10	1.14	1.94
	Effluent	7	0.72	1.38
IL-6	Serum	10	5.19	8.99
	Effluent	7	69.98 ^a	17.38
TNF α	Serum	10	12.26 ^a	3.23
	Effluent	7	2.83	1.42

^a Statistically significant.

IL-1 β = interleukin 1 β ; IL-6 = interleukin 6; TNF α = tumor necrosis factor α ; SD = standard deviation.

TABLE II Serum and effluent levels of cytokines in patients on peritoneal dialysis (PD) for more than 12 months

Cytokine		Patients (n)	Mean	SD
IL-1 β	Serum	33	0.99	3.86
	Effluent	32	1.18	1.86
IL-6	Serum	33	3.60	4.12
	Effluent	33	145.02 ^a	99.83
TNF α	Serum	33	13.21 ^a	4.54
	Effluent	33	3.14	2.03

^a Statistically significant.

IL-1 β = interleukin 1 β ; IL-6 = interleukin 6; TNF α = tumor necrosis factor α ; SD = standard deviation.

TABLE III Serum and effluent cytokine levels in patients on standard (Std) and biocompatible (Bio) solutions

Cytokine	Solution type	Serum		Effluent	
		Mean	SD	Mean	SD
IL-1 β	Std	1.31	4.62	0.90	1.67
	Bio	0.66	1.39	1.27	1.85
IL-6	Std	4.44	6.99	135.55 ^a	114.08
	Bio	3.33	3.21	123.60 ^a	72.27
TNF α	Std	13.19 ^a	5.56	3.51	2.90
	Bio	13.22 ^a	2.84	3.07	1.59

^a $p < 0.001$ (Mann–Whitney test).

SD = standard deviation; IL-1 β = interleukin 1 β ; IL-6 = interleukin 6; TNF α = tumor necrosis factor α .

its role in MIA syndrome. About 30% – 50% of pre-dialysis and PD patients are recognized to have serologic evidence of an activated inflammatory response, with elevated serum levels of CRP and IL-6 (1,4,6–11). Acute and chronic-phase response may be influenced

TABLE IV Effect of residual renal function on cytokine levels

Cytokine (mL/24 h)	Diuresis	Serum		Effluent	
		Mean	SD	Mean	SD
IL-1 β	0–200	1.89	5.49	1.66	2.53
	>200	0.49	1.22	0.77	1.08
IL-6	0–200	4.62	4.67	137.14	83.37
	>200	3.51	5.95	125.44	102.44
TNF α	0–200	14.61	4.65	3.38	2.29
	>200	12.39	4.16	3.25	2.40

SD = standard deviation; IL-1 β = interleukin 1 β ; IL-6 = interleukin 6; TNF α = tumor necrosis factor α .

TABLE V Serum and effluent cytokine levels in patients with and without peritonitis episodes

Cytokine	Peritonitis episodes	Serum		Effluent	
		Mean	SD	Mean	SD
IL-1 β	0	0.82	1.46	0.62	0.94
	1–2	1.25	4.01	0.82	1.48
	>2	0.32	0.72	1.69	2.23
IL-6	0	2.66	3.18	123.20	111.43
	1–2	3.88	6.06	117.53	98.96
	>2	4.01	3.79	159.18	80.94
TNF α	0	12.80	3.48	2.58	1.67
	1–2	13.35	4.50	3.19	2.37
	>2	12.79	4.37	3.54	2.34

SD = standard deviation; IL-1 β = interleukin 1 β ; IL-6 = interleukin 6; TNF α = tumor necrosis factor α .

by a number of non-dialysis-related and dialysis-related factors such as age, race, residual renal function, sex, and bioincompatibility of PD fluids (because of high concentrations of glucose, GDPs, lactate). Residual renal function has an important role in the inflammatory process. Deterioration of renal function has been associated with significant increases in serum cytokine levels (3,6,8,10).

Serum levels of IL-1 and IL-6 in our patients with CRF and on CAPD did not significantly differ, which may suggest that PD *per se* does not lead to the additional stimulation of systemic inflammation. An explanation for these results is extremely complex and requires additional investigations concerning the half-life of these cytokines in patients with CRF and on CAPD, the methods of their inactivation, and the relative contribution of renal function and of PD in their clearance (1,3,12–15).

The kidney usually excretes TNF α ; in dialysis patients, TNF α accumulates. Serum levels of TNF α in

patients on CAPD were significantly higher than they were in patients with CRF ($p < 0.001$), which may suggest specificity of TNF α as a marker of chronic inflammation in these patients, but also of impaired renal and peritoneal clearance. The statistically significantly higher serum levels of TNF α , as compared with overnight exchange effluent levels of this cytokine, in patients on CAPD provide confirmation of the marker hypothesis ($p < 0.001$). Low effluent TNF α levels in CAPD patients without signs of peritonitis confirm results from other authors as well. On the first day of CAPD peritonitis onset, effluent levels of TNF α can rise to up to 16 times the normal level (16–20). Residual renal function, transport characteristics of the peritoneal membrane, number of peritonitis episodes, PDS biocompatibility, and duration of dialysis did not significantly affect serum and overnight exchange effluent levels of TNF α in our CAPD patients.

Although it is evident that the number of proinflammatory and anti-inflammatory cytokines and soluble cytokine receptors orchestrate the inflammatory response, available data suggest that IL-6 plays a key role in these events (6,18,21). Unlike TNF α , IL-6—which is part of a family of 20 kD polypeptide cytokines that are secreted from various cells, including mesothelial cells, fibroblasts, macrophages, adipocytes, monocytes, and endothelial cells (18,19,21–23)—is locally secreted in considerable levels in the peritoneum of stable CAPD patients. Serum and effluent levels of IL-6 in our CAPD patients on standard and on biocompatible PDSs undoubtedly confirm this fact.

Activation of the local inflammatory response in stable CAPD patients is influenced mostly by bioincompatibility of the PDS (high levels of glucose and GDPs, low pH, lactate as a unphysiologic buffer) and uremia *per se*. In time, the peritoneum assumes the characteristics of a chronically inflamed organ with typical repercussions on the processes of ultrafiltration, arterial hypertension control, nutrition status in dialyzed patients, and cardiovascular comorbidity and mortality (8–13).

The importance of PDS bioincompatibility in the onset and development of local chronic inflammation is confirmed by the significantly lower effluent IL-6 levels in CAPD patients dialyzed for up to 12 months as compared with patients dialyzed for longer than 1 year ($p < 0.05$ by Mann–Whitney test). Importantly, this result confirms the absence of a significant difference in effluent levels of IL-6 in our patients

without, with 1 – 2, or with more than 2 CAPD peritonitis episodes.

Keeping in mind all the local and systemic consequences stemming from bioincompatibility of standard PDS, we are currently witnessing great efforts by the pharmaceutical industry and clinical physicians and researchers in the field of PD to formulate the most biocompatible solutions possible for CAPD and automated PD.

Experimental and clinical results with neutral PDSs (bicarbonate or bicarbonate/lactate as buffer, significantly lower levels of GDPs, in two- or three-chamber bags) undoubtedly confirm that use of such solutions lowers levels of IL-6 and vascular endothelial growth factor in effluent and the subsequent intraperitoneal inflammation and angiogenesis development (22,23).

Effluent levels of IL-6 in our patients on standard and available biocompatible PDSs also show a tendency to decline in patients on biocompatible solutions even when those solutions did not contain bicarbonate (that is, bicarbonate/lactate as the physiologic buffer of the human body). Results from other authors show that use of bicarbonate/lactate PDSs may help to restore peritoneal homeostasis and therefore membrane function in PD. Furthermore, use of the more biocompatible PDSs improved inflow pain, enhanced the phagocytic activity of peritoneal macrophages, and reduced accumulation of advanced glycation end-products in the peritoneal cavity (18,19,21,23).

All these advantages of the biocompatible PDSs show their positive effects in clinical applications. These solutions should result in further-reduced mortality rates in PD patients and should also increase technique survival (21,23).

Conclusions

Our results convincingly demonstrate the importance of proinflammatory cytokines as crucial molecules of the local and systemic inflammatory response. Elevated serum TNF α levels and the significant intraperitoneal IL-6 production in our CAPD patients indirectly confirm the importance of PD itself in the amplification of the chronic inflammation that is considerably influenced by dialysis treatment duration. The positive effects of biocompatible PDSs on peritoneal homeostasis and preservation of peritoneal membrane function require additional clinical confirmation in future clinical studies with much larger numbers of PD patients.

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