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Peritoneal sclerosis in peritoneal dialysis patients related to dialysis settings and peritoneal transport properties

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Peritoneal sclerosis in peritoneal dialysis patients related to dialysis settings and peritoneal transport properties.

Background. Peritoneal sclerosis is a histopathologic finding that is probably causative of long-term system failure in peritoneal dialysis (PD). It may be due to uremic toxicity and the influence of peritoneal dialysate components. We intended to clarify to which degree peritoneal fibrosis and vascular changes were associated with the modalities of PD and the peritoneal transport characteristics.

Methods. Peritoneal biopsies of 41 patients suffering from end-stage renal disease were examined. Sixteen patients were at the initiation of dialysis treatment, and 25 patients were studied during chronic PD treatment [9 patients on continuous ambulatory PD (CAPD) and 16 patients on automatic PD (APD)]. Twenty nonuremic patients undergoing abdominal surgery served as the controls. Samples were taken from the parietal peritoneum under standardized conditions and were examined by light microscopy using different staining techniques, semiquantitative grading, and computer-based histomorphometry.

Results. A marked loss of mesothelial cells during PD treatment was only observed in cases with two or more preceding episodes of peritonitis, but an increase of the submesothelial fibrous tissue was a common finding and was related to the cumulative glucose load on PD. Patients on PD also had a significantly increased density of small vessels and capillaries in the submesothelial peritoneal layer (12.8 ± 6.9 per field vs. 6.6 ± 2.9 in normal controls, $P < 0.01$). The wall/lumen index of the vessels was increased indicating vascular sclerosis. The degree of vascularization correlated with the amount of fibrous tissue. Patients characterized as high transporters according to the peritoneal equilibration test (PET) had an increased submesothelial fibrous layer. Patients on APD tended to have an increased membrane diameter submesothelial stroma and vascularization (P = NS), although they were treated for a shorter period of time than the CAPD group. Some of the morphologic changes described could already be observed in uremic patients before the onset of dialysis.

Conclusion. Further research focusing on the clinical and biochemical backgrounds leading to peritoneal membrane changes is of major importance for developing strategies to improve long-term survival on PD.

Key words: peritoneal sclerosis, uremic toxicity, dialysate, submesothelial fibrosis, end-stage renal disease, CAPD, APD, solute transport, vascularization.

One of the most important problems related to long-term peritoneal dialysis (PD) is the progressive change in peritoneal membrane function [1]. Clinically, the most relevant consequence is a progressive loss of ultrafiltration. The latter has been identified as the most frequent reason for system failure in PD [2]. Several studies support the assumption that peritoneal sclerosis is, at least in part, responsible for this feature [3–5]. However, the exact course of these alterations and the mechanisms is hardly understood. The morphological changes described for the peritoneum of patients on continuous ambulatory PD (CAPD) are mesothelial denudation, interstitial fibrosis, and the deposition of extracellular matrix (ECM) proteins resulting in a thickening of the peritoneal membrane. Electron microscopy revealed a characteristic replication of the basement membrane of the peritoneal capillaries [4], similar to diabetic nephropathy. Additionally, microvascular alterations were observed with severe fibrosis and hyalinization of the vascular media. These changes are usually described by the term "peritoneal sclerosis." The findings suggest that certain factors may exert a toxic and/or proliferative effect on fibroblasts and vascular smooth muscle cells of the peritoneum.

It is considered that the glucose and its degradation products in dialysis fluid are accelerating factors of peritoneal sclerosis [6]. On the other hand, the toxic effect of an acidic pH of the conventional PD fluids has been widely shown in cell culture experiments [7]. Although some typical morphological changes in PD have been deliberately described, it is still unclear to what extent these changes correspond to the clinical dialysis settings. Therefore, biopsy information on human peritoneal tissue is strongly needed as it may be a clue for a better understanding of the risk factors leading to peritoneal membrane failure. Normal controls are needed for

Table 1. Anthropometric and clinical data of the patients studied.

Group	Age *years*	Body weight *kg*	Body height *cm*	Gender *m/f*	Serum creatinine *mg/dL*	Type of PD (CAPD/APD)	PET, D/P ratio creatinine %	Cumulative PD glucose load
Non-uremic patients ($N = 20$)	60.8 ± 13.6	71.7 ± 12.7	170 ± 6	12/8	0.9 ± 0.2	—	—	—
Uremic patient before the onset of PD ($N = 16$)	44.6 ± 16.5	73.1 ± 8.8	174 ± 11	9/7	8.0 ± 2.4	—	65.6 ± 8.4[a]	—
Uremic patient on chronic PD ($N = 25$)	49.2 ± 11.6	71.0 ± 8.7	174 ± 9	11/14	10.9 ± 3.6	9/16	74.1 ± 8.8	318.6 ± 227.6

Abbreviations are PET, peritoneal equilibration test; D/P, dialysate/plsama.
[a] Data measured 4 weeks after the initiation of PD.

the evaluation of PD biopsies, but the potential uremic changes of the peritoneum in the predialytic phase should also be monitored. Including the control groups mentioned, we intended to clarify to which degree peritoneal fibrosis and vascular changes were associated to PD modalities and peritoneal transport characteristics.

METHODS

Patients

Peritoneal biopsies of 41 patients suffering from end-stage renal disease were examined. Sixteen patients were at the initiation of dialysis treatment when elective catheter insertion was performed by a surgeon. Twenty-five patients were studied during chronic PD treatment when there was an indication for the interruption of PD treatment or catheter replacement (change to hemodialysis because of loss of ultrafiltration, $N = 2$; inadequate clearance targets, $N = 2$; kidney transplantation, $N = 11$; catheter replacement because of catheter obstruction, $N = 3$; catheter replacement because of relapsing exit-site and tunnel infections, $N = 7$; Table 1). All samples were collected in a noninfectious peritoneal state [that is, without signs of peritonitis: leukocytes in the dialysate <100/μL, C-reactive protein (CRP) <1.5 mg/dL]. As a control group, 20 nonuremic patients were examined who underwent unrelated abdominal surgery providing small parts of peritoneum from routine resection. No signs of peritoneal affection were present. All patients provided informed consent to participate in this study approved by the local ethic committee.

Clinical evaluation

Among the 25 PD patients, 16 had been on APD for at least three months. Cumulative dialysate volume and glucose load were evaluated from the start of PD. Dianeal® (Baxter Healthcare, McGaw Park, IL, USA) or CAPD® (Fresenius Medical Care, Deutschland GmbH, Bad Homburg, Germany) were used as standard solutions. None of the patients had more than 50% of the bags containing >2.5% glucose. A peritoneal equilibration test (PET) had been performed in all patients at least six months before biopsy examination. The dialysate-to-plasma creatinine concentration and the dialysate (D/Do) ratio of glucose were measured. Daily ultrafiltration, residual diuresis, and clearance data (Kt/V, weekly creatinine clearance) were also available in all patients. Peritonitis episodes were registered since the beginning of PD treatment.

Histologic methods

Parietal peritoneum specimens were obtained during surgery. Approximately 10 × 10 mm samples were fixed with neutral-buffered 3.7% formalin (pH 7.3) for 24 hours. Samples were embedded in paraffin and then cut into 5 to 7 μm sections. For light microscopic evaluation, three sections were stained with hematoxylin eosin, three sections with periodic acid-Schiff reagent to evaluate changes in the vascular wall, three sections with Sirius red to differentiate for collagen tissue, and three sections stained with Elastica van Gieson to detect elastic fibers.

Evaluation was performed with an Olympus light microscope (BX 50; Olympus Optical Co., Hamburg, Germany). On each of the three sections, three typical fields of view were evaluated at ×10 magnification. The results were then averaged. On a semiquantitative scale, the density of the mesothelial cell layer was evaluated (grade 3, normal cell density; grade 0, complete denudation). The thickness of the submesothelial connective tissue lamina was measured by a graded eyepiece. The amount of collagen and elastic fibrous tissue in the submesothelial layer was evaluated semiquantitatively. A computer-based histomorphometric analysis was made to examine microvascular changes. The software Image J (version 0.93e; National Institutes of Health, Bethesda, MD, USA) was used to quantitate the changes in a 16.4 billion color tif-file at a resolution of 300 dpi (×10 magnification). The number of vessels was counted, and the diameter of the vascular wall and the lumen was measured. The area of the vascular wall and the lumen was measured to establish a wall/lumen area ratio as a measure of vascular sclerosis.

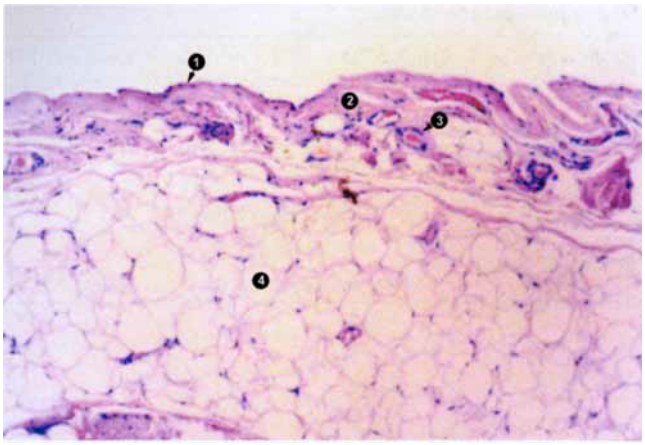

Fig. 1. Peritoneal biopsy from a 50-year-old patient with membranous glomerulonephritis who had been on ambulatory peritoneal dialysis (APD) for 31 months. The catheter was removed after a successful kidney transplant. The dialysate/plasma (D/P) ratio of creatinine was 68% by the peritoneal equilibration test (PET). The patient had experienced one, episode of peritonitis. The histological appearance of the parietal peritoneum is fairly normal. Key: (1) mesothelial cell layer; (2) submesothelial connective tissue; (3) stromal blood vessel; (4) adipose tissue. Hematoxylin-eosin stain ×10.

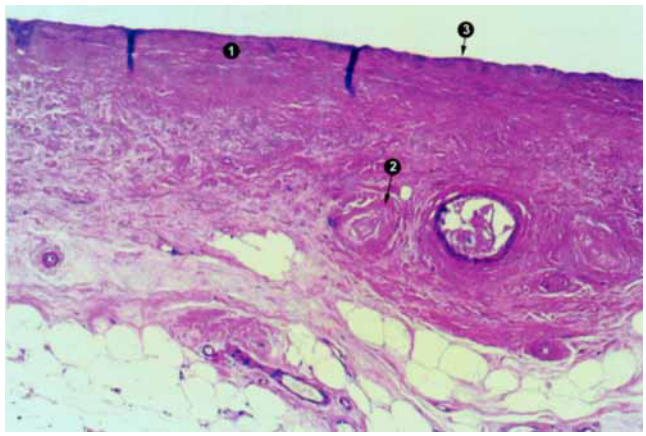

Fig. 2. Peritoneal biopsy from a 54-year-old patient with chronic glomerulonephritis who had been on continuous ambulatory peritoneal dialysis (CAPD) for 11 years, 8 months. The catheter was removed after a successful kidney transplantation. The D/P ratio of creatinine was 71% according to the PET. This patient had seven episodes of peritonitis in the past. The peritoneum demonstrates massive fibrosis by collagenous tissue (1) and vascular sclerosis (2). The mesothelial cells are rarified (3). PAS stain ×10.

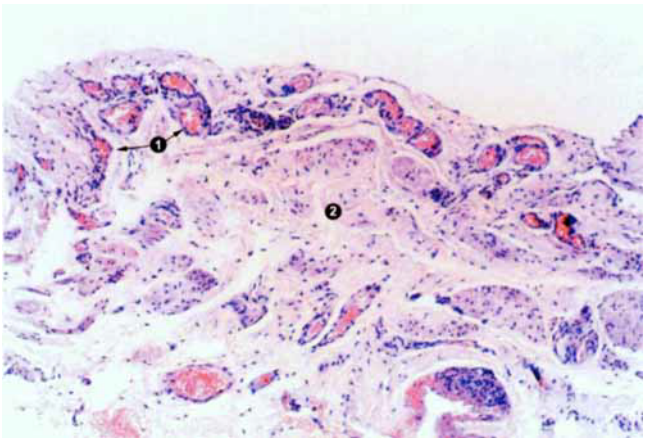

Fig. 3. Peritoneal biopsy from a 70-year-old patient with benign nephrosclerosis who had been on APD for 26 months. The catheter was removed after switching to hemodialysis due to a loss of ultrafiltration. The D/P ratio of creatinine was 84% according to the PET. This patient had one prior episode of peritonitis. There is marked hypervascularization in the peritoneal stroma (1). In addition to fibrous thickening, there is overt expansion of extracellular matrix (2). Hematoxylin-eosin stain, ×10.

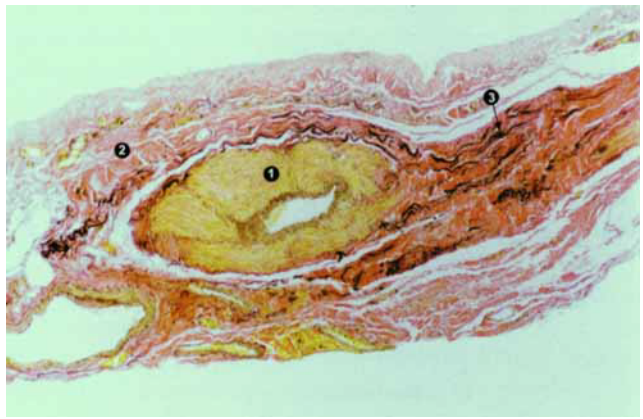

Fig. 4. Peritoneal biopsy from a 60-year-old patient with autosomal dominant polycystic kidney disease who had been on CAPD for 35 months. The catheter was removed due to relapsing tunnel infection. The PET showed a D/P ratio of 92% for creatinine. There was no prior episode of peritonitis. Severe perivascular fibrosis (1) and enhanced interstitial fibrosis (2) are prevalent (PAS stain, ×10). There is a distinct increase of elastic connective tissue (3) as shown by the Elastica van Gieson stain.

Statistics

Differences between the patient groups were tested by the *t* test for unpaired samples. Computation of correlation according to Pearson was performed for nominal variables, and the Spearman rank correlation was used to analyze correlations concerning the grading results of the histologic findings. All data are given as mean ± SD.

RESULTS

Mesothelial cell integrity

The mesothelial cell layer was rather well preserved in nonuremic patients (grade 2.0 ± 1.1). Uremic patients at the initiation of dialysis showed only slightly (P = NS) lower values (grade 1.7 ± 1.3; Fig. 1). Patients on PD had a mesothelial cell density comparable to the

Table 2. Histomorphometric data on the peritoneum in uremic patients before the onset of dialysis treatment and during peritoneal dialysis treatment

Group	Diameter of submesothelial fibrous tissue *μm*	Vessels per field of view *N*	Wall/lumen quotient of submesothelial vessels
Non-uremic patients	327 ± 163	6.6 ± 2.9	0.38 ± 0.45
Uremic patients before the onset of PD	429 ± 207[a]	9.7 ± 6.2[a]	1.04 ± 0.48
Uremic patients on chronic PD	492 ± 266[b]	12.8 ± 6.9[b]	1.35 ± 0.73[a]

Level of significance tested against non-uremic controls was [a] $P < 0.05$, [b] $P < 0.01$.

Table 3. Histomorphometric data on the peritoneum of patients on peritoneal dialysis grouped according to clinical parameters potentially related to the development of peritoneal sclerosis

Group	*N*	Time on PD *months*	Diameter of submesothelial fibrous tissue *μm*	Vessels per field of view *N*	Mesothelial cell density *score*
Type of PD:					
CAPD	9	63 ± 43	408 ± 241	11.3 ± 7.5	2.0 ± 1.3
APD	16	43 ± 24	537 ± 276	13.7 ± 6.8	1.9 ± 1.0
Cumulative peritoneal glucose load					
<250 kg	11	32 ± 20	379 ± 297	11.2 ± 5.0	2.0 ± 1.0
>250 kg	14	63 ± 34	579 ± 212[a]	14.2 ± 8.2	1.8 ± 1.0
D/P ratio of creatinine:					
<72%	9	62 ± 41	360 ± 257	12.2 ± 6.4	2.0 ± 1.0
>72%	16	43 ± 26	559 ± 246[a]	14.1 ± 6.8	1.9 ± 1.1

Level of significance tested against the corresponding gruop within each field.
[a] $P < 0.05$.

other groups (grade 1.9 ± 1.0). The subgroup of patients being long-term on PD (>48 months) did not differ from patients with a shorter time on PD; however, patients with two or more episodes of peritonitis in their history showed the lowest cell density (grade 1.2 ± 1.3).

Submesothelial fibrosis

Submesothelial thickening of connective tissue was a common finding in PD patients. It was predominantly characterized by an increase of collagen tissue. The amount of elastic fibers usually increased in parallel. The diameter of the submesothelial fibrous layer was 492 ± 266 μm in PD patients as compared with nonuremic controls (327 ± 163 μm, $P < 0.01$; Table 2). The cumulative glucose load was positively correlated to submesothelial fibrosis ($r = 0.306$, $P < 0.05$; Table 3). Even in the nondialyzed uremic patients, we found some significant increase of submesothelial fibrosis as compared with controls ($P < 0.05$; Table 2).

Mirovascular changes

Patients on PD had a significantly increased density of small vessels and capillaries in the submesothelial peritoneal layer (12.8 ± 6.9 per field vs. 6.6 ± 2.9 in normal controls, $P < 0.01$; Table 2). Again, in uremic, nondialyzed patients, the density of vessels was also somewhat increased (9.7 ± 6.2, $P < 0.05$). The diameter of the vascular wall as related to the free vessel lumen increased during PD by the development of perivascular fibrosis (Fig. 4). To correct for different vessel sizes, the quotient of the area of wall to lumen was calculated. This ratio was significantly increased in PD patients (1.35 ± 0.73 vs. 0.98 ± 0.49 in controls, $P < 0.05$). It was still close to normal in nondialyzed uremic patients (1.04 ± 0.48).

Histomorphometric changes as related to peritoneal transport and the type of PD

We compared the histomorphometric findings of 9 patients on CAPD for 63 ± 43 months and 16 patients on APD for 43 ± 24 months. Although having a shorter amount of time on PD, we found the submesothelial connective tissue layer thicker in APD patients (537 ± 276 μm) as compared with CAPD patients (408 ± 241 μm, P = NS). In parallel, the number of vessels per field also tended to be increased in APD (14.6 ± 5.9 vs. 11.3 ± 7.5 in CAPD, P = NS). The grade of peritoneal mesothelialization was not different between both groups (Table 3).

Patients on PD were divided according to their PET test into a group representing a high peritoneal transport type with a four-hour dialysate/plasma (D/P) ratio of creatinine >72% and a group having a lower transport of <72%. High transporters had a significantly increased submesothelial fibrous layer (559 ± 246 μm) compared with the low transporters (360 ± 257 μm, $P < 0.05$). The density of vascularization also tended to be increased in

high transporters (14.4 ± 6.8 vs. 12.1 ± 6.4, P = NS; Table 3). The diameter of the submesothelial fibrous layer and the degree of vascularization were positively correlated ($r = 0.459$, $P < 0.05$).

DISCUSSION

Microscopic examination of biopsies from patients exposed to varying periods of PD treatment showed significant morphological deviations from normal, particularly in the submesothelial stroma. However, the number or density of the mesothelial cells did not vary significantly in our study, except in those patients who experienced repetitive episodes of peritonitis. However, by electron microscopy changes in the mesothelial ultrastructure associated with a loss of microvilli and hyperplasia of the rough endoplasmic reticulum have been shown [4]. Reduplication and thickening of the mesothelial basement membrane is a condition that was only found in patients exposed to PD and resembled the changes seen in diabetic patients. A layer of areolar tissue underlies both parietal and visceral mesothelium. It is composed of oriented bundles of collagen fibers and retiform elastic laminae in a ground matrix substance. Normally, the thin submesothelial tissue lamina is relatively acellular, with fibroblasts and mast cells being the predominant cell types. To compare the thickness of the submesothelial connective tissue layer between different subjects, samples of the parietal peritoneum should be taken from the same site. Increased fibrosis of the submesothelial layer was a typical finding we found associated with PD treatment. Collagenous and elastic fibers increased in parallel. The density of cells in the stroma, however, was not increased, and in some cases, there was even the "cellular desert" as described by Dobbie in the so-called tanned peritoneum [8]. We found this typical constellation in two patients being more than eight years on PD (Fig. 2). The finding that peritoneal sclerosis is mainly a complication of long-term PD makes it likely that the continuous exposure of the peritoneal membrane to dialysis fluids is a pathogenetic factor. Cultured mesothelial cells are damaged by a high-glucose concentration and by the combination of lactate and low pH [9]. The exposure of the peritoneal cells to glucose may induce advanced glycosylation end products (AGEs), which have been detected in the peritoneal tissue of nondiabetic CAPD patients [10]. The loss of the mesothelial cell integrity may additionally facilitate the diffusion of glucose and its degradation products into deeper peritoneal layers, and in this manner enhance the formation of AGEs. It has also been shown that the concentration of certain growth factors like transforming growth factor-β (TGF-β) and vascular endothelial growth factor is increased in the peritoneal fluid [11] and that AGEs are capable of stimulating the release of TGF-β and other growth factors and cytokines [12]. TGF-β has been reported to be the main mediator involved in the expansion of the ECM with deposition of collagen IV in diabetic nephropathy. We could demonstrate that peritoneal fibrosis was linked to cumulative peritoneal glucose load. However, thus far the question of whether the type of dialysis may also influence the peritoneal structure has not been investigated. We found that the biopsies from APD patients showed some increased submesothelial fibrosis as compared with CAPD, although the latter group was even longer on PD treatment. However, probably because of the limited sample number, this finding did not reach significance. It may be speculated that the more frequent exposure of fresh "nonequilibrated" dialysate fluids in APD may promote peritoneal sclerosis. As APD represents an important tool to improve clearance targets and to preserve patients from being switched to hemodialysis [13], this problem requires further research. New solutions with higher biocompatibility (for example, with bicarbonate as a buffer or new osmotic agents) may be a clue to solve this problem.

The evaluation of peritoneal transport capacity is usually performed by the PET test, as suggested by Twardowski [14]. We found that patients having a high transport rate (D/P ratio of creatinine >72%) also showed a significantly higher degree of peritoneal sclerosis.

It is not fully understood why an increased interstitial fibrosis and thickening of the microvascular walls as found in our study are accompanied by an increased permeability of small solutes. However, we could demonstrate that in PD patients, there is a significant increase of submesothelial vascularization. Interestingly, vasularization tended to be increased in APD over CAPD and in patients who were characterized as high transporters. An enhanced neovascularization of the peritoneum may explain that diffusive peritoneal transport is steady or even increases with time on PD despite a thickening of the peritoneal wall. Neoangiogenesis may be promoted by vascular endothelial growth factor that was found in the PD fluid in a higher concentration as expected by mere diffusion from the blood [11]. Even the enhanced perivascular fibrosis we found in long-term PD patients, which should rather decrease diffusive transport, seemed to be overcompensated by the number of vessels found. So far, detailed knowledge about the change of extracellular matrix (ECM) proteins under PD treatment is not available. However, it is imaginable that the intrinsic permeability of the ECM in the interstitium of the peritoneum can also increase, thereby facilitating small solute transport.

Another interesting finding was the fact that some of the peritoneal membrane alterations could also be detected in the group of the uremic patients before the onset of PD. Particularly, some degree of submesothelial fibrosis was prevalent. Biochemical alterations inherent

to uremia may promote this process independently from PD. Dobbie, Lloyd, and Gall described abnormalities of the mesothelium in one third of their patients just before the onset of PD [4]. A process of enhanced fibrosis was also described by Rambausek et al, who found an increase of intermyocardiocytic fibrous tissue in the heart of uremic patients [15].

Further research focusing on the complex mechanisms that lead to peritoneal membrane remodeling is of major importance for developing strategies to improve long-term survival of patients on PD.

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