DIABETES INFLUENCES PERITONEAL MORPHOLOGY IN UREMIC PATIENTS AT THE INITIATION OF PERITONEAL DIALYSIS

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◆ **Background:** The peritoneum begins to undergo morphologic changes before the start of peritoneal dialysis (PD), particularly in diabetic patients. The present study was conducted to investigate the effects of diabetes on the peritoneum.

◆ **Methods:** This study involved 17 patients who began receiving PD and had diabetes as an underlying disease (DM group), and 30 patients without diabetes who served as a control group (nonDM group). At the start of PD, the parietal peritoneum was sampled to assess submesothelial connective tissue thickness, number of capillaries and postcapillary venules, and indications of vasculopathy (grades 0–3).

◆ **Results:** Submesothelial connective tissue thickness was significantly greater in the DM group than in the nonDM group ($p < 0.01$). The number of capillaries was significantly greater in the DM group ($p < 0.01$). Based on multivariate linear regression analysis, diabetes was identified as a significant independent variable of both submesothelial connective tissue thickness and number of capillaries ($p < 0.01$).

◆ **Conclusions:** In diabetic patients, morphologic changes of the peritoneum are marked at the start of PD.

replacement therapy aimed at improving not only quality of life and social activity for patients, but also prognosis (survival) (1). However, prolonged use of PD increases the risk of developing encapsulating peritoneal sclerosis in addition to ultrafiltration failure and insufficient dialysis (2,3). Thus, it is important to establish a detection method for encapsulating peritoneal sclerosis by evaluating morphologic change and function in the peritoneum. Prolongation of PD induces morphologic changes in the peritoneum, particularly denudation of mesothelial cells, thickening and appearance of sclerotic lesions in the submesothelial connective tissue, progression of peritoneal vasculopathy (that is, vascular lumen narrowing, vascular wall thickening), and hyperplasia of newly formed small vessels. These morphologic changes typically begin before the start of PD (4,5).

Factors that lead to deterioration of the peritoneum include uremia (6) and carbonyl stress attributable to glucose, glucose degradation products, advanced glycation end-products (7), acidic dialysate, plasticizers, and antiseptics, among others (2). Glucose, glucose degradation products, and advanced glycation end-products are considered to be the most important factors (7–10). Those findings suggest that peritoneal damage at the start of PD may be more severe in patients with diabetes than in those who are diabetes-free. However, few studies have evaluated morphologic changes in diabetic patients (4,5). Additionally, whether peritoneal permeability differs between diabetic patients and diabetes-free patients has not been established clinically (11–16). Thus, we examined morphologic changes of the peritoneum at the start of PD in diabetic patients with renal failure and compared the results with results in diabetes-free patients with renal failure.

Perit Dial Int 2013; 33(2):175–181 www.PDConnect.com
epub ahead of print: 01 Sep 2012 doi:10.3747/pdi.2011.00205

KEY WORDS: Morphologic change; diabetes; vascular density.

Peritoneal dialysis (PD) is used as renal replacement therapy for patients with end-stage renal failure and serves as an initial strategy for integrated renal

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Received 22 August 2011; accepted 8 February 2012

METHODS

SUBJECTS

The study involved 16 men and 1 woman (mean age: 55 years) who began receiving PD at the Fukuoka Red Cross Hospital and who provided written consent to participate in the study. The diabetes-free control group included 13 men and 17 women with underlying renal diseases including chronic glomerulonephritis ($n = 23$), renal sclerosis ($n = 1$), renal hypoplasia ($n = 1$), reflux nephropathy ($n = 1$), gouty kidney ($n = 1$), membranous nephropathy ($n = 2$), and malignant hypertension ($n = 1$).

A PD catheter was inserted under epidural anesthesia in each patient. At the start of PD, 2 L of dialysate containing 1.5% glucose was used, and the bag was exchanged 3 times daily. Table 1 shows the characteristics of the patients with diabetes. All diabetic patients received insulin therapy before beginning PD and had a

TABLE 1
Clinical Characteristics^a of the Study Patients

Variable	Patient group	
	With diabetes	Without diabetes
Patients (n)	17	30
Sex (n men:women)	16:1 ^b	13:17
Age (years)	55±2	49±3
Total protein (g/dL)	6.4±0.2	6.5±0.1
Albumin (g/dL)	3.5±0.1	3.8±0.1
Serum glucose (mg/dL)	134±15 ^b	105±6
BUN (mg/dL)	74.5±4.2	93.5±6.8
Serum creatinine (mg/dL)	8.12±0.34	9.34±0.71
Hemoglobin (g/dL)	9.4±0.3	8.6±0.2
Degree of proteinuria (g/gCr)	5.1±0.6 ^c	3.4±0.5
Total cholesterol (mg/dL)	181±9	185±9
Triglycerides (mg/dL)	152±24	140±19
CRP (mg/dL)	0.23±0.07	0.17±0.06
Systolic BP (mmHg)	158±5	151±4
Diastolic BP (mmHg)	86±3	86±3
Patients [n (%)] with		
Use of RAS inhibitors	13 (76)	22 (73)
Use of beta-blockers	11 (65) ^b	9 (30)
History of abdominal surgery	7 (41)	13 (43)

BUN = blood urea nitrogen; gCr = grams of creatinine; CRP = C-reactive protein; BP = blood pressure; RAS = renin-angiotensin system.

^a Unless otherwise specified, values are given as mean ± standard error of the mean.

^b $p < 0.05$ vs patients without diabetes.

^c $p < 0.01$ vs patients without diabetes.

mean HbA1c of 5.9% (range: 4.5% – 8.2%) at the start of PD. No patients had a history of peritonitis or developed peritonitis within 1 month after PD start.

PERITONEAL BIOPSY

At the time of PD catheter insertion, the parietal peritoneum was sampled. All operative manipulations were performed by the same surgeon, who was experienced in PD catheter insertion and biopsy. After making a paramedian incision at a point twice the width of a finger lateral to the umbilicus, the rectus abdominis muscle anterior sheath was incised and separated to expose the posterior sheath and peritoneum. Parietal peritoneum (1.5×1.5 cm) and rectus abdominal muscle posterior sheath were collected at a point 3 cm below the PD catheter insertion site. The sample was developed on a 2×2-cm section of cellophane such that the posterior sheath faced the cellophane's surface, with mesothelial cells on the top side. Because long-term exposure of the peritoneum to air causes degeneration of the mesothelial cells (17), we minimized the length of time from sampling to fixation. We applied the following experimental parameters:

- The peritoneum was sampled before PD catheter insertion.
- No electrical stimulation (electrocautery) was applied.
- The peritoneum was sampled without direct contact to avoid mechanical injury.
- The sampled peritoneum was developed on cellophane without excessive tensioning and was immediately placed into fixative.

The sample was fixed at room temperature for 24 hours in 10% phosphate-buffered formalin, embedded with paraffin, cut into 3-μm sections, and fixed on a glass slide. Hematoxylin and eosin staining, azan staining, and elastica-van Gieson staining were performed. Samples were evaluated using an Olympus light microscope (Bx41; Olympus, Tokyo, Japan).

HISTOLOGIC ANALYSES

Two examiners, a nephrologist and a pathologist, evaluated the samples. The peritoneum was evaluated to determine the presence or absence of mesothelial cell denudation, acellular sclerotic changes in submesothelial connective tissue, submesothelial connective tissue thickness, vasculopathy, and microvascular density.

Submesothelial connective tissue thickness was evaluated based on the submesothelial compact zone (SCZ) thickness from immediately before the mesothelial cell

layer to the fat tissue layer. The mean of this parameter at 10 randomly selected sites was used in the analysis.

No method has been established for the evaluation of peritoneal vasculopathy (4,5,7,18–22). In several previous reports, evaluation was made at the capillary level (vascular diameter, up to 25 μm) to the postcapillary venule (PCV) level (vascular diameter, 25 – 50 μm). In the present study, vasculopathy was evaluated semi-quantitatively using a grading system based on vascular wall thickening and vascular lumen stenosis at the PCV level (7). Blood vessels with the largest changes were evaluated on a 4-grade scale (Figure 1): grade 0 (no change), grade 1 (wall thickening and less than 50% luminal stenosis), grade 2 (wall thickening and more than 50% luminal stenosis), and grade 3 (complete obstruction). Methods of microvascular density measurement reported to date include a method for counting the number of vessels per visual field (18–20) and a method

for counting vessels per millimeter of peritoneal tissue, among others (4,21,22). Because microvascular density appears to be affected by peritoneal thickness, we measured the number of capillaries and PCVs per millimeter of the peritoneal section. The number of vessels within the SCZ closer to the peritoneal cavity from the fat tissue layer was measured at 5 randomly selected sites, and the mean value was used in the analysis.

BLOOD AND URINE SAMPLING

Blood sampling was conducted with the patient fasting and before PD catheter insertion. Blood urea nitrogen (urease ultraviolet method), serum and urinary creatinine (creatinase–sarcosine oxidase–POD method), C-reactive protein (latex enhanced immunoturbidimetric method), total cholesterol (enzymatic method), triglycerides (enzymatic method), glucose (glucose

(A)

(B)

(C)

(D)

Figure 1 — Grades of vasculopathy. (A) Grade 0, no pathologic changes. (B) Grade 1, vascular wall thickening and less than 50% luminal narrowing. (C) Grade 2, vascular wall thickening and 50% or greater luminal narrowing. (D) Grade 3, complete luminal occlusion. Arrows, postcapillary venules (defined as blood vessels with a diameter in the range 25 – 50 μm).

oxidase method), total serum protein (biuret method), and albumin (bromocresol green method) were measured using a Technicon AutoAnalyzer (Hitachi 7700: Hitachi, Tokyo, Japan). Hemoglobin concentration was measured using the sodium lauryl sulfate-hemoglobin method (XE-2100: Sysmex, Kobe, Japan). Urinary protein was measured using the pyrogallol red method (Hitachi 7180: Hitachi).

STATISTICAL ANALYSES

Results are presented as means $\pm$ standard error of the mean. The statistical analyses were performed using the StatView software program (Abacus Concepts, Berkeley, CA, USA). The Mann-Whitney U-test was used to compare unpaired data. Potential associations between categorical data were analyzed using the chi-square test. Multivariate linear regression analysis was used to test the independent predictive value of variable risk factors with respect to SCZ thickness and microvascular density. The analysis was carried out numerically using dummy variables, assigning 0 to the "absence" of diabetes, use of renin-angiotensin system inhibitors or beta-blockers, and history of abdominal surgery; 1 to the "presence" of the foregoing factors; and 0 to "male" and 1 to "female." A p value less than 0.05 was considered statistically significant.

RESULTS

PATIENT CHARACTERISTICS

The DM and nonDM groups showed no significant difference in age, but men constituted a larger percentage of the DM group ($p < 0.05$, Table 1). There were no significant intergroup differences in serum protein, albumin, creatinine, total cholesterol, triglycerides, hemoglobin, or C-reactive protein. Fasting blood glucose and urinary protein were significantly higher in the DM group than in the nonDM group ($p < 0.01$). Patients treated with renin-angiotensin system inhibitors or those with a history of abdominal surgery showed no significant intergroup differences, but the percentage of patients concomitantly treated with beta-blockers was significantly higher in the DM group than in the nonDM group (65% vs 30%, $p < 0.05$).

HISTOLOGIC EXAMINATION OF THE PERITONEUM

Mesothelium and Submesothelial Connective Tissue: Mesothelial cells were retained in all cases. Submesothelial connective tissue thickness was

significantly greater in the DM group ($209 \pm 24 \mu\text{m}$) than in the nonDM group ($95 \pm 9 \mu\text{m}$, $p < 0.01$, Figure 2). All patients were free of acellular sclerotic changes of the peritoneum.

Vasculopathy and Microvascular Density: In the DM group, vasculopathy was rated as grade 0 in 2 cases, grade 1 in 13 cases, grade 2 in 2 cases, and grade 3 in no cases. In the nonDM group, vasculopathy was rated as grade 0 in 13 cases, grade 1 in 15 cases, grade 2 in 2 cases, and grade 3 in no cases. Thus, the percentage of severe vasculopathy cases was significantly higher in the DM group than in the nonDM group (Figure 3).

The number of capillaries per millimeter of peritoneal tissue was significantly greater in the DM group (13.1 ± 0.9) than in the nonDM group (7.8 ± 0.4 , $p < 0.01$, Figure 4). The number of PCVs did not differ significantly between the DM (1.2 ± 0.2) and nonDM groups (0.9 ± 0.1).

Figure 2 — The thickness of submesothelial compact zone was significantly higher in diabetic patients than in patients without diabetes. DM = patients with diabetes; nonDM = patients without diabetes. * $p < 0.01$.

Figure 3 — Grades of vasculopathy were significantly more severe in diabetic patients than in patients without diabetes. DM = patients with diabetes; nonDM = patients without diabetes. The numbers in the bars indicate the number of patients at each grade. * $p < 0.05$.

Figure 4 — The number of capillaries was higher in diabetic patients than in patients without diabetes. DM = patients with diabetes; nonDM = patients without diabetes. * $p < 0.01$.

Multivariate Analysis of Factors Determining Submesothelial Connective Tissue Thickness and Capillary Numbers:

Factors determining the degree of submesothelial connective tissue thickness were explored using a multivariate linear regression analysis with these independent variables: albumin, hemoglobin, blood glucose, age, sex, presence or absence of diabetes, renin-angiotensin system inhibitor use, beta-blocker use, and history of abdominal surgery. The presence or absence of diabetes was found to be a significant independent factor of submesothelial connective tissue thickness (Table 2).

Factors determining the number of capillaries were explored in a similar manner using multivariate linear regression analysis. The presence or absence of diabetes was found to be a significant independent factor for the number of capillaries (Table 3).

DISCUSSION

The present work shows that, compared with diabetes-free patients, diabetic patients have more submesothelial connective tissue thickening and higher capillary density in the peritoneum at the start of PD. Additionally, results of a multivariate analysis indicate that the peritoneum is not significantly affected by nutrition status, anemia severity, previous abdominal surgery, or use of renin-angiotensin system inhibitors (23) or beta-blockers. Diabetes was identified as an independent risk factor causing submesothelial connective tissue thickening and elevation in capillary density. Given that no earlier studies have evaluated such variables in detail, the present

TABLE 2
Multivariate Linear Regression Examining Factors Predictive of the Thickness of the Submesothelial Compact Zone ($r^2 = 0.48$)

Variable	Standardized regression coefficient	p Value
Age	-0.097	NS
Sex (female, 1; male, 0)	-0.103	NS
Albumin	-0.220	NS
Hemoglobin	-0.003	NS
Serum glucose	0.188	NS
Diabetes mellitus (with, 1; without, 0)	0.480	0.0042
Taking RAS inhibitors (yes, 1; no, 0)	-0.122	NS
Taking beta-blocker (yes, 1; no, 0)	0.015	NS
History of abdominal surgery (yes, 1; no, 0)	0.050	NS

NS = nonsignificant; RAS = renin-angiotensin system.

TABLE 3
Multivariate Linear Regression Examining Factors Predictive of the Number of Capillaries ($r^2 = 0.57$)

Variable	Standardized regression coefficient	p Value
Age	0.143	NS
Sex (female, 1; male, 0)	-0.293	NS
Albumin	-0.028	NS
Hemoglobin	-0.092	NS
Serum glucose	0.072	NS
Diabetes mellitus (with, 1; without, 0)	0.457	0.0029
Taking RAS inhibitors (yes, 1; no, 0)	0.249	NS
Taking beta-blocker (yes, 1; no, 0)	0.048	NS
History of abdominal surgery (yes, 1; no, 0)	0.040	NS

NS = nonsignificant; RAS = renin-angiotensin system.

results may be important for elucidating the mechanism of morphologic changes in the peritoneum caused by glucose toxicity.

When the peritoneum is examined histologically, adequate care during peritoneal sampling is required to avoid mechanical injury to the peritoneal surface. If the peritoneal surface is damaged, subsequent evaluation is difficult. von Ruhland *et al.* (17) conducted simulations on various types of surgical trauma to the rat peritoneum and evaluated the severity of mesothelial cell injury, reporting that peritoneal mesothelial cells can undergo

degeneration simply after exposure to ambient air or massage of the peritoneum. In our study, all peritoneal samples were collected by the same surgeon. Thus, evaluating the peritoneum was possible given the identical conditions used for all study samples.

We evaluated vasculopathy based on the grade of wall thickening and lumen stenosis (7) and microvascular density on the basis of the number of vessels per millimeter of peritoneal tissue (4,21,22). The evaluation revealed that, compared with diabetes-free patients, patients with diabetes often had advanced vasculopathy and higher capillary density. However, no markedly advanced vasculopathy cases were detected in either group. Importantly, in the DM group, a high percentage of the observed vasculopathy was rated grade 1. In a rat model of diabetes, De Vriese *et al.* (8) reported an elevated density of small vessels, with a premature vascular network, and an increase in peritoneal permeability. Thus, hyperplasia of newly formed vessels may precede progression of vasculopathy of existing small vessels.

Few published papers have performed a morphologic evaluation of the peritoneum at the start of PD. Only two such papers addressing the influence of diabetes are available. Williams *et al.* (4) evaluated SCZ thickness, vascular density, and vasculopathy based on hyalinosis in the peritoneal microvascular wall and severity of peritoneal microvascular stenosis. Vasculopathy was noted in 4 of 10 diabetic patients, but in only 14 of 63 diabetes-free patients. Honda *et al.* (5) evaluated morphologic changes of the peritoneum by SCZ thickness and by lumen/vessel diameter ratio at the PCV level. They reported that, compared with diabetes-free patients at the start of PD, diabetic patients had significantly thicker submesothelial connective tissue and a lower lumen/vessel diameter ratio. The present findings regarding SCZ thickness and vasculopathy are identical to those reported in the earlier major studies. However, in those earlier studies, microvascular density was not evaluated according to the presence or absence of diabetes. It is therefore not possible to compare the findings of the present study (in which such an evaluation was conducted for the first time) with the findings in previous studies. Moreover, no previous study has reported a multivariate analysis evaluating in detail the factors affecting morphologic changes in the peritoneum.

Morphologic changes in the peritoneum have been shown to be present before the start of PD, regardless of the presence or absence of diabetes (4,5). The present study did not evaluate peritoneal samples from healthy individuals; however, compared with the approximately 50 – 60 μ m submesothelial connective tissue reported for healthy individuals by the previously mentioned studies,

the submesothelial connective tissue from the patients in the present study showed thickening.

The mechanisms resulting in morphologic and functional changes in the peritoneum related to diabetes have been studied primarily using animal models. Carbonyl stress attributable to high blood glucose levels, uremic toxins (10,24), and oxidative stress (25) can increase the expression of endothelial nitric oxide synthase (10,19) and vascular endothelial growth factor (8) in the peritoneal small vessels. Those increases in expression lead to increased peritoneal permeability and new blood vessel formation, stimulating the deposition of plasma containing fibrin in the submesothelial connective tissue (26). When that change is combined with profibrotic factors such as transforming growth factor β (9), fibrosis of the submesothelial connective tissue is stimulated, resulting in thickening. In the present study, the validity of those hypotheses was confirmed in diabetic patients.

Morphologic changes in the peritoneum associated with glucose toxicity may be affected by the degree of blood glucose control. In rats, capillary hyperplasia occurs in the presence of diabetes, resulting in increased micromolecular permeability across the peritoneum; however, blood glucose control with insulin therapy suppresses those changes (10). Thus, peritoneal changes may be severe in patients with persistent elevated blood glucose levels. Further studies should include an analysis of diabetes duration, insulin treatment period, and long-term status of blood glucose control.

Limitations of the present study include

- lack of a comparison with healthy individuals,
- lack of an analysis of capillary structural changes using electron microscopy, and
- an unknown duration of diabetes and time course of blood glucose in the patients of the DM group.

CONCLUSIONS

The present study was designed to evaluate the influence of diabetes on the peritoneum at the start of PD. Compared with diabetes-free patients, diabetic patients showed significant morphologic changes.

DISCLOSURES

The authors have no financial conflicts of interest to declare.

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