Quantitative evaluation and assessment of peritoneal morphologic changes in peritoneal dialysis patients

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Abstract

Background. Morphologic changes of the peritoneum such as peritoneal fibrosis and vasculopathy develop during peritoneal dialysis (PD). In 2002, Williams et al. reported microscopic characteristics of peritoneal changes in PD patients. These studies pointed out the importance of establishing a global standard for qualitative and quantitative histological evaluations. The objectives of the present study are (i) to verify the methods for assessing peritoneal thickness and classifying vasculopathy in peritoneal specimens using the assessment of Williams et al. and (ii) to propose a simple assessment that reflects clinical features such as PD duration and peritoneal function.

Methods. Parietal peritoneal samples were obtained from 35 patients that included 27 patients with PD and 8 uraemic patients without PD. In all samples, the maximum and average thicknesses of the submesothelial compact (SMC) zone were measured to assess peritoneal interstitial fibrosis using KS400 imaging analysis. Vasculopathy was also assessed by calculation of patency rates of the vascular lumens using the diameter and area, and by measurement of dimensions of vascular wall hyalinization in each vessel specimen.

Results. The median values of maximum and average thicknesses of the SMC zone exceeded 200 μm in uraemic patients without PD treatment. There was a significant relationship between the maximum and average thicknesses of the SMC zone (P < 0.0001). Four to 30 vessels were examined in each participant. Various grades of vasculopathy were observed in each specimen. According to the predominant vasculopathy found in each vessel, the prevalence of serious vasculopathy increased with increasing PD duration. Vascular patency calculated from wall thickness was significantly related to that calculated by

the area and to the thickness of hyalinization. Average vascular patency assessed from 5 to 10 vessels in each patient having diameters ranging from 10 to 40 μm was related to PD duration and to peritoneal function (D4/P).

Conclusions. A random-points measurement of average SMC thickness provides a descriptive evaluation of the severity of peritoneal fibrosis that minimizes artefacts during processing and avoids human error. In addition, the average patency in post-capillary venules appears to accurately reflect clinical features such as PD duration and peritoneal permeability.

Keywords: patency; peritoneal dialysis; peritoneal fibrosis; submesothelial compact (SMC) zone; vasculopathy

Introduction

Peritoneal dialysis (PD) is an attractive treatment for patients with end-stage kidney disease (ESKD), and the advantage of this therapy is that it prolongs residual renal function. However, long-term PD is associated with the development of functional and structural alterations in the peritoneal membrane [1–4]. Continuous exposure to biocompatible peritoneal dialysate components and repeated episodes of bacterial peritonitis may play major roles in producing alterations in peritoneal function and structure [5–9]. Peritoneal fibrosis is a characteristic finding in long-term PD, and is associated with mesothelial loss, severe thickening of the submesothelial compact (SMC) zone and vascular alterations [10–13]. Ultrafiltration failure, a common complication, is thought to cause morphological
alterations in the peritoneum. A more serious complication for long-term PD patients is encapsulating peritoneal sclerosis (EPS), which is sometimes life-threatening. EPS has been observed during PD but also after discontinuation of treatment [14–18].

A number of studies have described characteristic alterations of the peritoneum during long-term PD, and have reported a relationship between morphologic changes and specific clinical events [1,4,7,11,14]. For example, Williams et al. [1] performed a comprehensive analysis of morphologic changes, thickening of the SMC zone and vasculopathy in the peritoneal membrane of 212 patients. Fibrosis was defined as a maximal thickness of the SMC zone measuring >150 μm [1]. They reported that maximum SMC zone thickness was increased gradually with PD duration. However, thickness of the SMC zone in the peritoneum specimens was not homogenous, even when artefacts were removed during sampling. It is not known whether measurement of the maximal thickness of the SMC zone in specimens is a valid method for evaluating peritoneal fibrosis. We attempted to validate measurement methods of thickness using the maximal or average thickness of the SMC zone in peritoneal specimens.

Williams et al. [1] evaluated vasculopathy semi-quantitatively and classified the observed vascular features into five grades using histologic semi-quantitative assessment as follows: Grade 0 as normal, Grade 1 with thickness of the subendothelial hyaline material of <7 μm, Grade 2 with thickness of subendothelial hyaline material of >7 μm without luminal distortion or narrowing, Grade 3 with luminal distortion or narrowing, and Grade 4 with luminal obliteration. Data for the worst lesion in each sample were also recorded. Honda et al. [4] also evaluated the relationship between morphologic and functional peritoneal alterations in a quantitative study. They pointed out that the average peritoneal thickness and lumen/vessel diameter ratio were useful as morphologic parameters to qualify the severity of peritoneal alterations in uraemic and PD patients. They also noted that the average peritoneal thickness was related to PD duration [4]. Vasculopathy that included occlusion and thickening of the vascular walls in the post-capillary venules and capillaries also progressed in relation to PD duration [1].

The prevalence of ultrafiltration failure (UFF) and high peritoneal permeability were increased with PD duration in previous reports [7]. Furthermore, long-term PD treatment is a major factor for the development of EPS [19]. Some EPS patients showed UFF or high peritoneal permeability before discontinuation of PD [17]. As mentioned above, it is important to assess whether patients should switch from PD to haemodialysis in order to prevent EPS, and to establish universal guidelines for this purpose. It is imperative to determine the characteristics of peritoneal alterations in PD patients and to establish a global standard of morphological assessment that involves clinical features and prognosis. We believe that it is important to establish an objective assessment for vasculopathy that is easy for nephrologists and does not require expert pathological knowledge or experience. This assessment should minimize artefacts and human errors, and should reflect clinical features.

The purposes of the present study are (i) to verify the methods for assessing peritoneal thickness and for classifying vasculopathy in peritoneal specimens using the assessment of Williams et al. and (ii) to propose a simple assessment that reflects clinical features.

Materials and methods

Patients and sample analysis method

Specimens were collected at the Juntendo University Hospital (Tokyo, Japan), from 1989 to 2006, after obtaining informed consent and approval from the Ethics Committee. Histologic assessments of the specimens were performed by two examiners: one pathologist (T. Kumasaka) and one nephrologist (T. Shimaoka). All specimens were assessed by these two examiners who were unaware of patient characteristics and clinical details.

Peritoneal biopsy

Anterior partial peritoneum specimens at least 5 cm from the site of original catheter insertion were obtained. The peritoneal samples were immediately fixed in formalin solution and then stained with haematoxylin and eosin (HE), periodic acid–Schiff (PAS), Masson trichrome (MT), or elastic van Gieson (EVG). Histological analysis was performed using Imaging System KS400 (Kortron Elektronik GmbH, Germany).

Histological analysis

SMC zone

The thickness of the SMC zone, which is a submesothelial interstitial layer between the mesothelial surface and upper border of the peritoneal adipose tissue, was measured as an indicator of peritoneal fibrosis. According to the report of Williams et al. [1], the maximal thickness of the SMC zone was measured in each section oriented perpendicular to the serosal surface in micrometres. Average thickness of the SMC zone was calculated from the area measured in each specimen. For measurement of the average thickness, calculating from the area element, an outline of the SMC zone was traced in each specimen while avoiding artefacts such as compression or rupture. Using the assumption that the area approximated a square, each area was divided by its width using Imaging System KS400 (Figure 1A).

Evaluation and assessment of vasculopathy

All capillaries, post-capillary venules, and venules within the SMC zone in each specimen were identified by light microscopy to evaluate vascular findings. Vasculopathy was examined semi-quantitatively by measuring the thickness of subendothelial hyaline material in the vascular wall, and vascular luminal patency was measured as distortion, narrowing and obliteration in each vessel as reported by Williams et al. [1]. Thickness of the subendothelial hyaline material of the obliterated vessel was calculated as half of its outer diameter. We evaluated vasculopathy using patency of the vascular lumen calculated by the outer diameter (Figure 1B). We also evaluated vasculopathy using patency of the vascular lumen which was calculated from the vessel area using the KS400 Imaging System.

Statistical analysis

Correlations between the different parameters were analysed by two-tailed Student’s t-tests or by regression analysis. ANOVA was used to determine the differences among multiple groups. Non-parametric methods, such as Spearman’s rank correlations and Wilcoxon’s rank-sum tests, were used for correlation and two-sample comparisons, respectively. Data were expressed as means ± SD or median values. P < 0.05 was considered significant. These statistical analyses were performed with StatView 5.0 software (Abacus Concepts).

Results

Patients and samples

The specimens were of sufficient size for analysis and contained several layers of peritoneum. Thirty-five specimens obtained from 35 patients were adequate for histolog-
Assessment of peritoneal morphologic evaluation in PD patients

The patients consisted of 28 males and 7 females. The mean age at the time of peritoneal biopsy was 51.6 ± 12.1 years. The causes of ESKD in these patients included chronic glomerulonephritis in 23 patients, diabetic nephropathy in 4 patients, hypertensive nephrosclerosis in 6 patients, polycystic kidney disease in 1 patient and other pathologies. Eight patients had a uraemic condition without dialysis. Twenty-seven patients had undergone PD from 20 to 166 months (94.7 ± 45.4 months) using a conventional glucose-based PD fluid (Dianeal®; Baxter, Tokyo, Japan) with a regimen of 4–5 exchanges daily. The reasons for PD withdrawal were UFF in 10 patients, under-dialysis in 5 patients, bacterial peritonitis in 4 patients, abdominal surgery, which was not related to PD, in 2 patients, long PD duration in 2 patients, suspected EPS in 1 patient, catheter-related infection that did not cause peritonitis in 1 patient, drainage failure in 1 patient, and an alternate reason in 1 patient. Four patients had recent bacterial peritonitis before peritoneal biopsy, as indicated by clinical findings and laboratory data that included cell counts. Recovery from peritonitis was diagnosed as levels of C-reactive protein (CRP) and white blood cell counts within the normal range and cell numbers in peritoneal effluent that were <100 per 3 mL. Peritoneal biopsy was performed at least 3 weeks after complete recovery from peritonitis.

**Fig. 1.** Quantitative evaluation of SMC zone thickness and vasculopathy. (A) Maximum thickness and average thickness of the SMC zone (a) in the peritoneum were measured using Imaging Analysis System KS400. To calculate the average SMC thickness, SMC area with the edge coloured yellow was divided by the SMC width (b). a: Maximum thickness of the SMC zone, b: SMC width. (B) Vasculopathy was determined from the thickness of the subendothelial hyalinized vascular walls. The severity of luminal narrowing and the ratio of luminal diameter to external vessel diameter or of luminal area to whole vessel area were measured using Imaging Analysis System KS400. a: External diameter of vessel, b: Luminal diameter of vessel, c: Subendothelial hyalinized vascular wall. Patency calculated by the diameter was shown as a diameter ratio, b/a (%). The grey area and blank area indicate the whole vascular wall area and luminal area, respectively. Patency calculated by the area was shown as the ratio of the whole vascular area (A + B) to luminal area (B).

**Measurement of SMC zone thickness**

The relationship between PD duration and maximal SMC zone thickness as well as average SMC zone thickness is shown in Figure 2. Both the maximum SMC zone thickness and the average SMC zone thickness were significantly related to PD duration (maximum; P < 0.0001, average; P < 0.0001, respectively). Both the maximum and average SMC zone thicknesses exceeded 200 μm in uraemic patients before initiation of PD (285.64 ± 115.35 μm maxi-
Assessment of vasculopathy

Selection of vascular findings. Five hundred and seventy-four vessels were evaluated from peritoneal specimens having various grades of vasculopathy in 35 patients. Among the vessels, 492 (85.7%) were quantitatively evaluated for morphology and were classified into Grade 0–4 according to the vasculopathy grading system of Williams et al. [1]. From 4 to 30 vessels per patient (14.1 ± 7.7 vessels analysed in each participant) were used for vasculopathy grading. When the vessels were graded as the most severe vasculopathy found in each vessel, 17 out of 35 patients (48.6%) were Grade 4, and only 20% were Grade 0. However, when the vessels were graded as the predominant vasculopathy found in each vessel, 22.9% of patients were Grade 4, and 37.1% were Grade 0. When assessed as the predominant vasculopathy, 18 out of 35 patients (51.4%) had a lower grade than when assessed as the severest grade. Eight out of 17 patients in Grade 4, assessed by the severest finding, shifted to Grade 1 or 0 when the specimens were assessed by the predominant grade.

To validate our grading of vasculopathy, we examined the relationship between PD duration and vasculopathy. Thirty-five subjects were divided into four groups as follows: 8 subjects had uraemia before PD treatment, 6 had PD for <5 years, 8 had PD for 5–10 years and 13 had PD for >10 years. (A) Distribution of vasculopathy according to the severest vascular finding. Fifty percent (3/6) of patients on PD for <5 years, 67% (6/8) of patients on PD for 5–10 years, and 62% (8/13) of patients on PD for >10 years were Grade 4. No patient was Grade 0 after initiation of PD. (B) Distribution of vasculopathy according to predominant vascular findings in all vessels with the predominant finding shown. Grade 4 patients were found only at 5 years after initiation of PD. The prevalence of severe cases increased with PD duration.

Fig. 2. Correlation between PD duration and maximum SMC zone thickness or average SMC zone thickness. Both maximum SMC zone thickness and average SMC zone thickness gradually increased with PD duration. These SMC zone thicknesses were significantly related to PD duration (maximum; \( P < 0.0001 \), average; \( P < 0.0001 \)). Median values of maximum and average SMC zone thickness exceeded 200 μm in uraemic patients before PD treatment.

Fig. 3. Comparison of vasculopathy in uraemic and PD peritoneum. Thirty-five subjects were divided into four groups: 8 subjects had uraemia before PD treatment, 6 had PD for <5 years, 8 had PD for 5–10 years and 13 had PD for >10 years. (A) Distribution of vasculopathy according to the severest vascular finding. Fifty percent (3/6) of patients on PD for <5 years, 67% (6/8) of patients on PD for 5–10 years, and 62% (8/13) of patients on PD for >10 years were Grade 4. No patient was Grade 0 after initiation of PD. (B) Distribution of vasculopathy according to predominant vascular findings in all vessels with the predominant finding shown. Grade 4 patients were found only at 5 years after initiation of PD. The prevalence of severe cases increased with PD duration.

Fig. 4. Distribution of assessable vessel's size during PD. Diameters of the analysed vessel ranged from 3.9 to 64.94 μm. Fourteen percent of vessel diameters were <10 μm, 55% were from 10 to 20 μm, and 31% were >20 μm.
67% (6/8) of patients on PD for 5–10 years, and 62% (8/13) of patients on PD for >10 years. After PD initiation, no patient was Grade 0. A majority of vasculopathy was Grade 4 in each of the PD duration groups (Figure 3A). The distribution of vasculopathy according to the predominant vascular findings for all vessels is shown in Figure 3B. No patients showed Grade 4 until 5 years after initiation of PD (Figure 3B). The prevalence of severe grades increased with PD duration, whereas unexpectedly, the prevalence of the mild vasculopathy decreased (Figure 3B). However, there were only a few patients with Grade 2 or 3 that were found in the study (Figure 3A and B).

Parameters of vasculopathy. We examined the relationship between patency of the vascular lumen, calculated by the diameter and the area (Figure 1B). The patency calculated by diameter (Patency dia) was significantly related to the area (Patency area) (Patency area = 2.608 + 0.82 × Patency dia, $R^2 = 0.890, P < 0.0001$). A significant relationship between Patency dia and thickness of the subendothelial hyalinized material (Thickness) was also observed (Patency dia = 61.186 − 3.611 × Thickness, $R^2 = 0.317, P < 0.0001$). Patency area was also correlated with Thickness (Patency area = 50.219 − 3.508 × Thickness, $R^2 = 0.396, P < 0.0001$). In the intact vessels without subendothelial hyalinized material, the patency rate of vessel lumens ranged from 30% to 90%. Therefore, vessel size may affect patency. The vessels were of various sizes, and included capillaries and post-capillary venules (Figure 4). Fifty percent of assessable vessels in uremic patients were <10 μm in diameter. Since 34.7% of assessable vessels with diameters of <10 μm were in uremic patients without dialysis, assessment of vasculopathy in capillaries was performed primarily on uremic patients. The prevalence of vessels having <10 μm diameter decreased with PD duration (43.1% in the uremic condition, 14.9% by 5 years, 11.9% from 5 to 10 years and 8.8% for >10 years). To assess the validity of vascular size for vasculopathy grading, all vessels were divided into three groups by diameter as follows: <10 μm ($n = 72$), 10–20 μm ($n = 275$) and >20 μm ($n = 145$). The relationship between patency and thickness of the subendothelial hyalinized material was evaluated in each group. Vessels without hyalinized thickened walls accounted for 45.8% in the vessel group having an external diameter <10 μm, and 10 (30.3%) of the vessels showed

### Additional Information

- **Fig. 5.** Correlation between thickness of subendothelial hyalinized material and patency calculated from diameter among different vessel sizes. (A) Relationship between thickness of subendothelial hyalinized material and patency in vessels with an external diameter of <10 μm. There was a negative correlation between the thickness of subendothelial hyalinized material and patency calculated from diameter ($P < 0.0001$). The thickness of subendothelial hyalinized material in all obstructed vessels was <5 μm. The patency of vessels without hyalinized thickening ranged from 50% to 80%. (B) Relationship between the thickness of subendothelial hyalinized material and patency in vessels with an external diameter ranging from 10 to 20 μm. There was a negative correlation between the thickness of subendothelial hyalinized material and patency calculated by diameter ($P < 0.0001$). The thickness of subendothelial hyalinized material in all obstructed vessels ranged from 5 to 10 μm. The patency of vessels without hyalinized thickening ranged from 40% to 85%. (C) Relationship between the thickness of subendothelial hyalinized material and patency of vessels with an external diameter of >20 μm. There was a negative correlation between the thickness of subendothelial hyalinized material and patency calculated from the diameter ($P < 0.0001$). The thickness of subendothelial hyalinized material in all obstructed vessels was >10 μm. The patency of vessels without hyalinized thickening was >60%.
<50% patency (Figure 5A). Obliteration was observed in all vessels having a wall thickness of >3 μm (Figure 5A). A patency prevalence of <50% in vessels without hyalinized thickened walls was found in 6.7% of vessels having diameters ranging from 10 to 20 μm (Figure 5B). Minimal vascular alteration tended to be exaggerated in small vessels having diameters <20 μm (Figures 5A and B). No vessel having <50% patency without hyalinized thickened walls was found in vessels with an external diameter of >20 μm (Figure 5C). The use of vascular patency as an assessment of vasculopathy was enhanced by the minute changes in capillary wall thickness.

To examine the relationship between vascular patency and PD duration, 35 patients were divided into four groups according to PD duration. Patency in all observed vessels, including capillaries and post-capillary venules, was significantly decreased with increasing PD duration (63.9 ± 13.0% in patients before PD, 54.8 ± 18.5% in PD <5 years, 44.7 ± 26.1% in PD for 5–10 years, and 34.1 ± 28.4% in PD >10 years; Figure 6). Patency in patients before PD (n = 8), on PD <5 years (n = 5), on PD for 5–10 years (n = 8) and on PD for >10 years (n = 14) was 67.3 ± 8.2%, 55.5 ± 9.6%, 46.1 ± 19.0% and 36.7 ± 17.4%, respectively (Figure 6). Patency significantly decreased with increasing PD duration.

Twenty-six patients were divided into two groups by the peritoneal equilibrium test (PET). PET was performed within 6 months before or after peritoneal sampling. Patency in 13 patients with high transport was remarkably lower than that in patients with low transport (P < 0.01, Figure 7).

Discussion

The purpose of the present study was to validate the previous morphologic evaluation reported by Williams et al. [1] and to propose a simple method having fewer variations caused by human error and less bias for the evaluation of peritoneal alterations in PD patients.

Results from the present study indicated the following: (i) for the assessment of peritoneal fibrosis development, measurement of SMC zone maximal thickness can be substituted for measurement of the average thickness; (ii) vascular patency, calculated by the diameter, showed a close positive relationship with the hyalinized subendothelial vascular wall thickness; and (iii) for the selection of vascular lesions for classification in each sample, it is preferable to use the predominant finding. In addition, when we assessed vasculopathy, it was preferable to evaluate the post-capillary venules having diameters ranging from 10 to 40 μm.

In previous studies, morphological alterations were assessed through loss or degeneration of the mesothelium, submesothelial thickening (variably described as fibrosis or sclerosis), changes in the structure and number of blood vessels, and vascular basement membrane reduplication [5–8,12–16]. Williams et al. [1] noted that the maximum thickness of the SMC zone, used for diagnosis of peritoneal fibrosis, gradually increased with increasing PD duration [1]. In their assessment, peritoneal fibrosis was defined as SMC zone thickness of >150 μm, measured as the maximum thickness in a specimen [1]. Honda et al. also reported that the average peritoneal thickness was 62.4 μm in normal subjects and 120 μm in uraemic patients [4]. In the present study, the mean of both the maximum and average SMC zone thickness exceeded 200 μm in uraemic patients before initiation of PD. Therefore, peritoneal fibrosis may represent a ubiquitous response to non-specific stimuli such as uraemia, bioincompatible dialysates, recurrent infection and inflammation.

To validate the measurements of peritoneal thickness using the maximum or average SMC zone in specimens, we used several measurement methods described in previous reports [1,19]. The average peritoneal thickness calculated by the measured SMC area was significantly related to the maximum thickness in our specimens. Measurement of the maximum thickness is a simple method for evaluating peritoneal fibrosis. To minimize artefacts in processing and to avoid human errors or bias, SMC thickness as a descriptive evaluation for the severity of peritoneal fibrosis should be measured using the average SMC thickness calculated from random-points measurements.
In the current study, we propose a grading system of vasculopathy that can be performed in an objective, straightforward manner according to predominant findings in each specimen, and that can be used by nephrologists without expert pathological knowledge or experience. Adequate vessel size and numbers may minimize artefacts and human errors to aid in objective measurements. As a morphologic parameter, patency is an objective and simple measurement. Patency should be related to clinical parameters such as PD duration or peritoneal permeability. Williams et al. [1] demonstrated the importance of vasculopathy and classified vasculopathy semi-quantitatively based on the severest finding. Vasculopathy was divided into five grades (0, 1, 2, 3, and 4), based on thickness of the subendothelial hyaline material and vascular luminal patency measured as distortion, narrowing and obliteration [1]. Several studies have reported that prevalence of advanced vasculopathy in the peritoneum increases with PD duration [1,17]. In the present study, vasculopathy was classified using the severest grade of vasculopathy in a specimen, which were obliterated vessels (Grade 4 vasculopathy) that were already present within 5 years after discontinuation of PD. To avoid bias, assessments using predominant findings are preferable. The average number of assessable vessels was 14.1 ± 7.7 (range, 4–30 vessels) in each patient in this study. Since it is difficult to evaluate all vessels in a specimen, findings from 5 to 10 vessels in each patient should be selected as predominant findings in vasculopathy assessment.

Calculation of patency by diameter or area of vessels is a simple method for classifying vasculopathy. Honda et al. [4] measured wall thicknesses of the affected post-capillary venules and calculated patency from the short axis of the vessel cut surface as an index of luminal patency. They found that patency gradually decreased with increasing PD duration. Therefore, patency based on diameter is a simple method that can reduce the bias caused by conventional semi-quantitative methods.

Honda et al. [4] selected post-capillary venules with diameters ranging from 25 to 50 μm for morphologic measurements, because patency was influenced by the size of the blood vessels examined. Although capillary patency was related to the thickness of the subendothelial hyalinated wall, the number of assessable capillaries decreased with PD duration in this study, and patency varied in each specimen. This may be caused by a resemblance between obliterator capillaries and nerve bundles and because of angiogenesis during prolonged PD. An objective assessment of vasculopathy reflecting PD duration and peritoneal function can be obtained from the average vascular patency among 5–10 vessels with diameters ranging from 10 to 40 μm.

In conclusion, we propose a more quantitative and descriptive assessment for clinical use that uses the average patency in post-capillary venules with diameters of >10 μm to provide a simple assessment that reflects clinical features in vasculopathy.

Conflict of interest statement. None declared.