Long-term peritoneal dialysis leads to encapsulating peritoneal sclerosis (EPS), which is a rare but often fatal complication. The pathogenesis of EPS is characterized by increased inflammation, neoangiogenesis, epithelial–mesenchymal transition (EMT), and fibrosis. Matrix metalloproteinase 2 (MMP-2), which degrades type IV collagen, plays an important role in pathogenesis. Clinical trials report that dialysate levels of MMP-2 can be used as an early marker of peritoneal sclerosis. We aimed to determine the association of MMP-2 with peritoneal function, histology, and effluent cytokine levels in an experimental EPS model in rats.

We evaluated data for 71 rats from our various studies using an experimental EPS model. Functional assessment was performed using a 1-hour peritoneal equilibration test with peritoneal dialysis fluid containing 3.86% glucose. Specimens of parietal peritoneum were examined with light microscopy for histologic evaluation. Parietal peritoneum thickness and submesothelial area were measured. Fibrosis, number of vessels, neovascularization, and cellular infiltration were evaluated by one pathologist. The relationships between MMP-2 and other parameters were analyzed using Pearson correlation analysis.

Dialysate levels of MMP-2 reflect both functional and histologic change in peritoneum. Levels of MMP-2 were negatively correlated with net ultrafiltration, effluent protein levels, and end (1-hour)–to–initial dialysate concentration ratio of glucose. Cytokines such as vascular endothelial growth factor, transforming growth factor beta, monocyte chemotactic protein 1, and osteopontin—which are known to play important roles in neovascularization, inflammation, and EMT leading to fibrosis—were correlated with MMP-2.

In peritoneal dialysis patients, MMP-2 levels may be an early marker of EPS and EMT.

Key words
Epithelial–mesenchymal transition, fibrosis, histology, inflammation, matrix metalloproteinase 2

Introduction
Peritoneal dialysis (PD) is one of the most widely used treatment choices for patients with end-stage renal failure. In long-term PD, peritoneal fibrosis, which leads to inadequate solute clearance and ultrafiltration (UF) failure, is a major complication. Encapsulating peritoneal sclerosis (EPS) is a rare but often fatal complication of PD (1). Inflammation, fibrosis, and angiogenesis are the main features of EPS pathophysiology. Not only is EPS associated with reduced peritoneal function, but also sometimes with clinical symptoms such as ileus. Although EPS is a rare complication (in the range 0.7% – 7.3%), it is an important one because of the mortality rate (2,3). The irreversible and progressive EPS process continues even if PD treatment ends. Even with a switch to hemodialysis or transplantation, the ongoing mortality risk is high (4,5). There are no definite diagnostic criteria, and diagnosis depends on suspicion. Treatment of EPS remains controversial.

The pathophysiology of EPS is characterized by increased profibrotic cytokines, inflammatory cell activity, and neovascularization. Accumulating evidence implicates epithelial–mesenchymal transition (EMT) as a potential mechanism for the development and progression of peritoneal fibrosis (5,6). In the process of fibrosis and sclerosis, tissue destruction and remodeling occur. Extracellular matrix components are arranged by a group of molecules called matrix metalloproteinases (MMPs). Among these enzymes, MMP-2 (also called gelatinase A) plays an important role by degrading basal membrane components such as type IV collagen and fibronectin (7). Expression of MMP-2 enhances various types of fibrosis and sclerosis.

In present study, we aimed to determine the association of MMP-2 levels with peritoneal

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function, histology, and dialysate cytokine levels in an experimental rat model of EPS.

**Methods**
We evaluated data for 71 nonuremic female Wistar albino rats [mean body weight: 175.7 ± 20.6 g (range: 114 – 220 g)] from our various experimental EPS model studies. All rats were housed in polycarbonate cages at 24°C room temperature, with a 12-hour light/dark cycle, and were fed a standard laboratory diet. The Animal Ethics Committee of Ege University Hospital approved the various study designs.

**Study protocol**
The rats from control (sham) groups received 2-mL intraperitoneal injections of isotonic saline daily for 3 weeks, without any other treatment. The rats from EPS groups (CG rats) received intraperitoneal injections (2 mL per 200 g body weight) of chlorhexidine gluconate (0.1%) and ethanol (15%) dissolved in saline, daily for 3 weeks and for an additional 3 weeks with or without an experimental treatment (sunitinib, aldactone, doxicycline, nebivolol).

At the end (6th week) of each study, a 1-hour peritoneal equilibration test with 25 mL 3.86% PD solution (Dianeal 3.36%; Eczacibasi–Baxter, Istanbul, Turkey) was performed. After 1 hour, ketamine HCl anesthesia (60 mL per kilogram body weight) was applied. Blood samples were then immediately collected by direct cardiac puncture, and dialysate samples were obtained without effluent leakage through a midline incision by insertion of a shortened dialysis catheter.

**Functional parameters**
Blood and dialysate urea were determined using an enzymatic kinetic method (Randox Laboratories, San Francisco, CA, USA) to calculate the dialysate-to-plasma ratio (D/P) of urea. The end (1-hour)–to–initial dialysate concentration ratio (Df/D0) of glucose was determined using the glucose levels from drained and uninfused dialysate. The net UF was calculated as the difference between the instilled and the drained volumes of dialysate.

**Structural parameters**
Tissue samples were obtained from the peritoneal cavity in areas other than the region of injection. The peritoneal membrane samples were fixed in 10% formalin and embedded in paraffin. Paraffin blocks were divided into 5-µm sections and stained with hematoxylin–eosin and Masson trichrome. All samples were examined by one pathologist who was unaware of the groups from which the samples originated. Peritoneal thickness, neovascularization, inflammation, and fibroblastic activity were evaluated. Thickness was measured using an ocular micrometer, and the other parameters were scored as counts of capillaries, mononuclear cells, and fibroblasts per high-power field at 400× magnification.

**Dialysate cytokine profile**
Dialysate cytokines, transforming growth factor β1 (TGFβ1), vascular endothelial growth factor (VEGF), and monocyte chemotactic protein 1 (MCp-1) were measured in drained dialysate using ELISA kits according to the manufacturer’s guidelines (Quantikine Rat kits: R&D Systems, Minneapolis, MN, USA).

**Statistics**
Study results are presented as mean ± standard deviation. The relationships between MMP-2 and other parameters were analyzed using Pearson correlation, and p < 0.05 was considered to be statistically significant.

**Results**
All rats had normal renal function tests and electrolyte, lipid, and protein levels (Table I). The peritoneum in the EPS groups showed severely disturbed functional and structural properties, characterized by UF failure, increased peritoneal thickness, and neovascularization. Functional changes such as UF and D/P protein were negatively correlated with levels of MMP-2 (Table II). Structural changes (dialysate cell count, number of vessels, and peritoneal thickness) were correlated with elevated MMP-2 levels (Table III). In CG rat (EPS) groups, VEGF, TGFβ1, MCP-1, and osteopontin were also found to be increased, and those cytokines were significantly correlated with MMP-2 (r = 0.59, 0.41, 0.62, and 0.74 respectively; Figure 1).

**Discussion**
Pathologic changes in the peritoneal membrane with long-term PD are characterized by a decrease or loss of mesothelial cells and enlargement of the submesothelial compact zone because of inflammation, interstitial fibrosis, and neovascularization. In the present study, CG injection resulted in EPS as previously described by Ishii et al. (8). In various EPS model studies by our
group, CG and alcohol injection resulted in significant change to the morphology and functional parameters of the peritoneum (9–12).

During the sclerotic or fibrotic process, destruction of the extracellular matrix, arrangement of the basal membrane, and neovascularization are regulated by MMPs (13). In an experimental EPS study, Hirahara et al. (14) showed that MMP-2 levels were associated with peritoneal injury, increased solute transport, and EPS progression. In the same study, those authors also reported that active or latent levels of MMP-2 in dialysate fluid and peritoneal tissue samples were correlated. We measured dialysate levels of latent MMP-2 (pro–MMP-2) and found the same increase.

In the present study, functional changes in peritoneum were assessed by dialysate protein levels, D/P urea, D/D₀ glucose, and UF loss. All those parameters were correlated with increased levels of MMP-2 as evidence of fibrosis. As a result of inflammation, the dialysate white blood cell count increased. Thickening of the peritoneum, accompanied by infiltration by inflammatory cells, was induced. In a recently published study, Yildirim et al. reported that levels of MMP-2 in PD fluid and tissue samples were correlated with an increase in the degree of fibrosis in an experimental EPS model using chlorhexidine (15). Our results seem to support those findings.

Neovascularization was also induced and was correlated with levels of MMP-2. Basement membrane degradation by MMP-2 may be an important stage in the formation of new blood vessels. Dialysate levels of TGFβ1, MCP-1, VEGF, and osteopontin, which play roles in inflammation, neovascularization, and the process of fibrosis, were increased. Previous studies on EMT have reported that osteopontin is a cytokine important to the regulation of EMT. In the present study, osteopontin levels were correlated with MMP-2. That finding can be interpreted to suggest that MMP-2 is an indicator of EMT.

### Table I: Serum biochemical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>173.4±78.6</td>
<td>80.0–602.0</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>52.7±16.5</td>
<td>29.0–99.0</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.57±0.09</td>
<td>0.36–0.80</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>135.3±6.8</td>
<td>105.0–147.0</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>5.56±0.97</td>
<td>3.70–8.50</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.45±1.42</td>
<td>3.80–11.40</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.13±1.32</td>
<td>1.40–7.70</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>56.3±17.0</td>
<td>33.0–90.0</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>52.5±24.7</td>
<td>24.0–99.0</td>
</tr>
<tr>
<td>Protein (g/dL)</td>
<td>6.26±1.47</td>
<td>2.80–9.40</td>
</tr>
</tbody>
</table>

### Table II: Relationship between functional parameters of peritoneum and matrix metalloproteinase 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Range</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net UF (mL)</td>
<td>4.76±3.70</td>
<td>–5.00 to 10.30</td>
<td>–0.37</td>
</tr>
<tr>
<td>D/P urea</td>
<td>0.65±0.17</td>
<td>0.36 to 1.00</td>
<td>0.58</td>
</tr>
<tr>
<td>D/D₀ glucose</td>
<td>0.30±0.14</td>
<td>0.11 to 0.72</td>
<td>–0.49</td>
</tr>
<tr>
<td>D/P Protein</td>
<td>26.55±16.97</td>
<td>10.64 to 90.9</td>
<td>0.38</td>
</tr>
</tbody>
</table>

UF = ultrafiltration; D/P = dialysate-to-plasma ratio; D/D₀ = end (1-hour)–to–initial dialysate concentration ratio.

### Table III: Relationship between structural parameters of peritoneum and matrix metalloproteinase 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Range</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritoneal thickness (µm)</td>
<td>84.8±60.3</td>
<td>4.2–185.2</td>
<td>0.59</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.78±0.76</td>
<td>0.00–2.00</td>
<td>0.27</td>
</tr>
<tr>
<td>Vascularity (n vessels)</td>
<td>6.04±6.28</td>
<td>0.00–28.00</td>
<td>0.57</td>
</tr>
<tr>
<td>Vessel/submesothelial area</td>
<td>81.2±71.0</td>
<td>0.0–258.9</td>
<td>0.41</td>
</tr>
<tr>
<td>White blood cells (n)</td>
<td>582±425</td>
<td>30–1920</td>
<td>0.40</td>
</tr>
</tbody>
</table>
In previous studies in rats, MMP-2 was shown to be produced by peritoneal cells (14,16,17). Selgas et al. demonstrated that many biologic mechanisms for fibrotic and sclerotic process are connected with EMT (18). Yáñez–Mó et al. noticed that EMT of peritoneal cells begins with the first dialysis session (5). During PD, an ongoing process of EMT is regulated by several cytokines, including osteopontin and MMP-2 (19).

**Conclusions**

Our results suggest that MMP-2 may be useful as a marker of peritoneal injury, increased solute transport, EMT, and progression to EPS. We believe that the level of MMP-2 is a useful tool for demonstrating membrane viability and survival of patients with EPS. Matrix metalloproteinase 2 may be used as an early diagnostic marker for EMT and EPS.

**Acknowledgment**

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**Disclosures**

The authors have no financial conflicts of interest to declare.

**References**


6 Lee HB, Ha H. Mechanisms of epithelial–mesenchymal transition of peritoneal mesothelial

![Figure 1](image-url) Relationship between dialysate levels of matrix metalloproteinase 2 (MMP-2) and of (A) osteopontin, (B) vascular endothelial growth factor (VEGF), and (C) monocyte chemoattractant protein 1 (MCP-1).


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