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# Association Between Plasma Grem1 and Peritoneal Permeability Alterations in Dialysis Patients

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## ABSTRACT

**Introduction:** Alterations in the peritoneal membrane can cause trouble for adequate dialysis. We proposed to evaluate a possible relationship between peritoneal permeability and Grem1 protein in peritoneal dialysis (PD) patients.

**Methods:** Adult PD patients who undergoing dialysis for at least one year were included. Grem1 level was measured in plasma. The peritoneal equalization test (PET) was used to define peritoneal transporting properties. Dialysate to plasma ratio for creatinine (DPRC) value was used as the parameter of the permeability. The first and last DPRC values were compared to determine membrane alteration status.

**Results:** A total of 60 patients were enrolled. The mean age was 52.9±14.3 years. The average PD duration was 60.3 (24-86.5) months. The average Grem1 level was 164.9 (81.6-164.9) ng/mL. An overall 5.8% increase was determined in DPRC value. There was no statistically significant difference in Grem1 levels between increasing DPRC and non-increasing DPRC groups (p=0.783). According to PET classification: class elevation was observed in 31.7% (19) patients. The plasma Grem1 levels of these groups are as follows: 204.2 ng/mL in stable patients, 168.2 ng/mL in ascending patients, and 196.2 ng/mL in descending patients. There was no statistically significant difference between the groups in terms of Grem1 levels in One-Way ANOVA (p=0.709).

**Conclusion:** We did not identify any correlation between changes in peritoneal permeability and plasma Grem1 levels. However, we have emphasized the importance of novel biomarkers that could predict the changes in peritoneal permeability.

**Keywords:** Grem1, dialysis, peritoneal membrane, permeability

## Introduction

Grem1 (Gremlin1) is a member of the antagonists of bone morphogenetic proteins (BMP) pathway. The gene encodes a Grem1 is located in 15q13.3 and has three exons. Grem1 is a 184-amino acid glycoprotein and a cysteine knot-secreted protein (1). The antagonistic effect of the secreted protein encoded by this gene is presumably via direct binding to BMPs (2). Furthermore, Grem1 is a cell growth and differentiation factor that plays a key role in embryogenesis. It can regulate organogenesis, organ embodiment, and tissue differentiation. Regulation of BMPs by Grem1 is also essential for kidney and lung development (3).

The transforming growth factor-beta (TGF-β) pathway is implicated as a driver of fibrosis-related diseases. BMP-7 has an antagonistic effect on TGF-β signaling (4). In addition, experimental studies revealed that Grem1 promoted the proliferation and activation of fibroblast cells by enhancing fatty acid oxidation (5). The contribution of Grem1 to fibrosis has been shown in several organs (3).

The peritoneum is used as a semipermeable membrane in peritoneal dialysis (PD). Solute diffusion and ultrafiltration (UF) occur through this membrane. Alterations in the peritoneal membrane, such as inflammation, neoangiogenesis, and fibrosis, cause trouble for adequate dialysis (6). Increased peritoneal permeability can particularly result in UF failure. TGF-β1-induced epithelial-to-mesenchymal transition (EMT) plays a crucial role in alterations of the peritoneal membrane. Moreover, mesenchymal Grem1-mediated BMP antagonism is required for proper epithelial- mesenchymal signaling (7).

BMP antagonism through Grem1 may affect the transport features of the peritoneal membrane in clinical practice. In addition, this antagonism has not been investigated sufficiently in PD practice. Therefore, this study aimed to evaluate a possible relationship between Grem1 and the permeability of the peritoneal membrane in patients with PD.

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## Methods

This study was designed as a single-center study. Patients enrolled between 01.01.2020 and 01.01.2021. The study was approved by the Erciyes University Ethics Committee (approval number: 2019/678, date: 09.10.2019). Written informed consent was obtained from the patients by explaining the procedure of the study. This clinical investigation was conducted in accordance with the Helsinki Declaration.

Key inclusion criteria were age ≥18 and should have maintained PD for at least one year. The patients were not included in the study during the active peritonitis period. The minimum duration since the patient last experienced peritonitis was three months for inclusion. Patients with leakage of dialysate, dysfunction of the PD catheter, cirrhosis, ascites, or systemic infection findings were excluded. Furthermore, patients who underwent abdominal surgery during the follow-up period were excluded from the study.

The peritoneal equalization test (PET) was used to define the transporting properties of the peritoneum. Standard PET was performed in this study as described by Twardowski (8). The patients were classified by the 4-h dialysate-to-plasma ratio (D/P) for creatinine. Four permeability groups were determined as follows: high, high average, low average, and low. Two PET values were compared for alterations in peritoneal membrane permeability. The initial PET was defined as performed when the patients started PD. The last PET was defined as performing concomitantly with Grem1 sampling. The D/P ratio for creatinine (DPRC) was used to calculate the alteration in peritoneal membrane permeability. 24-h urine and dialysate samples were used to calculate creatinine clearance. We used the International Society for Peritoneal Dialysis Guidelines for the diagnosis of peritonitis (9).

The Grem1 level was measured in plasma. Venous blood samples were centrifuged for 10 min at 5000 rpm (NF 400 centrifuges, Turkey). The samples were preserved at -80 °C until assessment. Grem1 levels were measured using commercially available ELISA kits (Elabscience Biotechnology Inc., Houston, TX, USA), according to the manufacturer's instructions and expressed as ng/mL. The detection range of the kits for Grem1 was 0.16-10 ng/mL. The inter-assay coefficient of variation for the kits was <10%.

### Statistical Analysis

The normality of the data was tested with the Shapiro-Wilk test; (q-q) and histogram plots. The Shapiro-Wilk test was employed to assess whether the numerical data followed a normal distribution. Numerical data showing a normal distribution are presented as mean ± standard deviation, whereas non-normally distributed numerical data are presented as median ($1^{st}$-$3^{rd}$ quartiles). Non-numerical (categorical) data are expressed as percentages. Statistical evaluations between the two periods were performed using the paired t-test for normally distributed parameters. Mann-Whitney U was used to compare the medians of non-normally distributed parameters. The relationships between categorical variables were tested using the chi-square test. ANOVA was used to determine differences between research results from three or more unrelated samples or groups. Data analysis was conducted using TURCOSA (Turcosa Analytics Ltd Co, Turkey, www.turcosa.com.tr) statistical software. The significance level was set as $p<0.05$.

## Results

In this study, 60 patients were enrolled and analyzed. The patients consisted of 31 females (51.7%) and 29 males (48.3%). The mean age of patients was 52.9±14.3 years. The median plasma Grem1 level of the patients was 164.9 (81.6-164.9) ng/mL. Demographic features and laboratory results of the patients are summarized in Table 1.

The distribution of patients according to the PD modality was as follows: 80% (n=48) continuous ambulatory peritoneal dialysis and 20% (n=12) automated peritoneal dialysis. The median PD duration of the patients was 60.3 (24-86.5) months. The median Kt/V urea was 2.02 (1.82-2.23). The median creatinine clearance was 62.1 (51.5-80.5) mL/min. The frequency of individuals who underwent at least one peritonitis attack during the follow-up period was 33.3% (n=18). The overall peritonitis rate was 0.1 episodes per year per individual.

The alteration of peritoneal membrane permeability was analyzed. The DPRC parameter was used to define the peritoneal membrane transport rate. The first and last PET results were compared to the calculation of changes in DPRC. High average was the most frequent subgroup, 35% (n=21) in the first PET and 46.7% (n=28) in the last PET. The results are summarized in Table 2.

First, peritoneal permeability alteration was analyzed according to DPRC values. The mean of the first DPRC value was 0.64±0.14, and the mean of the last DPRC value was 0.68±0.11 in all patients. An overall 5.8% increase was observed in the average DPRC value during the follow-up period. However, there was no statistically significant difference between the first and the last DPCR pairs (p=0.103). In addition, there

**Table 1. Demographic features and laboratory results of patients**

| Parameters | Descriptives |
|---|---|
| Gender | |
| Male | 29 (48.3%) |
| Female | 31 (51.7%) |
| Age (years) | 52.9±14.3 |
| BMI ($kg/m^2$) | 26.92±5.32 |
| Grem1 (ng/mL) | 164.9 (81.6-164.9) |
| BUN (mg/dL) | 53.04±12.66 |
| Creatinine (mg/dL) | 8.4±2.8 |
| Uric acid (mg/dL) | 5.5±1.2 |
| Calcium (mg/dL) | 9.0 (8.6-9.6) |
| Phosphorus (mg/dL) | 4.6±1.0 |
| PTH (pg/mL) | 419 (262-565) |
| Sodium (mEq/L) | 137.6±4.5 |
| Potassium (mEq/L) | 4.4 (4.0-4.8) |
| Albumin (g/dL) | 3.87±0.35 |
| Glucose (mg/dL) | 120.1 (92.2-129.2) |
| Hemoglobin (g/dL) | 11.5±1.7 |
| Leukocytes (cell/µL) | 7266±2529 |
| Platelet ($10^3$/µL) | 245.0±70.4 |

Values are expressed as mean ± standard deviation, median ($1^{st}$-$3^{rd}$ quartiles). BUN: Blood urea nitrogen, PTH: Parathyroid hormone, DPRC: Dialysate to plasma ratio for creatinine, BMI: Body mass index

**Table 2. Features of patients according to PET results**

| Parameters | First PET | Last PET |
|---|---|---|
| DPRC | 0.64±0.14 | 0.68±0.11 |
| **Classification** | | |
| High | 10 (16.7) | 7 (11.7) |
| High average | 21 (35.0) | 28 (46.7) |
| Low average | 19 (31.6) | 23 (38.3) |
| Low | 10 (16.7) | 2 (3.3) |

Values are expressed as mean ± standard deviation, median (1st-3rd quartiles). DPRC: Dialysate to plasma ratio for creatinine, PET: Peritoneal equalization test

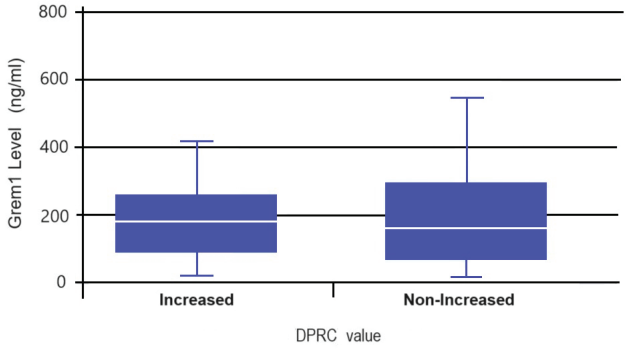


**Figure 1.** Comparison of Grem1 levels according to dialysate to plasma ratio for creatinine alteration status
DPRC: Dialysate to plasma ratio for creatinine

was no statistically significant difference between the increasing DPRC and non-increasing DPRC groups according to Grem1 levels (p=0.783). The results are shown in Figure 1.

Second, peritoneal permeability alteration was analyzed according to PET classification. Stable status was observed in 45% (27) patients, ascending in 31.7% (19) patients, and descending in 23.3% (14) patients. The median plasma Grem1 levels of these groups were as follows: 204.2 ng/mL in stable permeability class patients, 168.2 ng/mL in ascending permeability class patients, and 196.2 ng/mL in descending permeability class patients. In the One-Way ANOVA test, there was no statistically significant difference between the groups in terms of Grem1 levels (p=0.709). The results are shown in Figure 2.

There was no correlation between Grem1 and peritonitis attacks (p=0.256). A statistically significant weak correlation was determined between Grem1 levels and BMI in the Pearson analysis (r=-0.273, p=0.035). However, no statistically significant correlation was identified with important parameters such as the last DPRC (r=0.224, p=0.084), UF volume, Kt/V, and PD duration. The results are illustrated in Figure 3.

## Discussion

In this study, we focused on peritoneal permeability alterations, which are an important problem in PD practice. We investigated a possible relationship between Grem1 and peritoneal permeability; however, we have not identified any association. We chose Grem1 protein in this study because of its antagonistic effect against BMP-7. We considered that

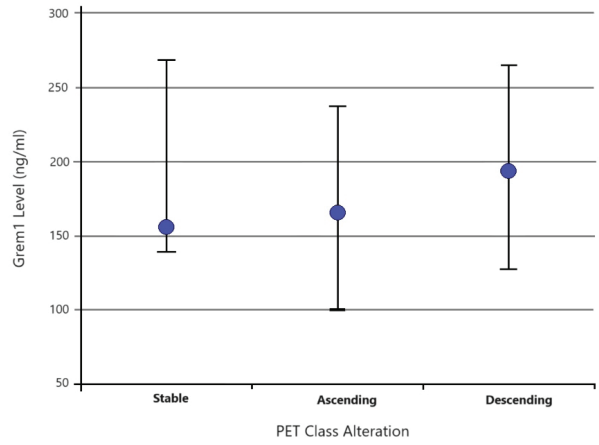


**Figure 2.** Comparison of Grem1 levels according to peritoneal equalization test class changing status
PET: Peritoneal equalization test

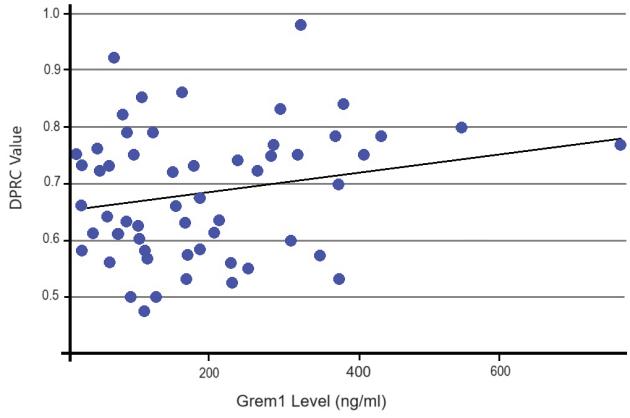


**Figure 3.** Correlation analysis of the last dialysate to plasma ratio for creatinine value and Grem1 levels
DPRC: Dialysate to plasma ratio for creatinine

determining a possible relationship would elucidate the pathogenesis of peritoneal membrane permeability change and may also contribute to treatment options.

In our cohort, we observed an increased average DPRC value (5.8%) during the follow-up period. In addition, increasing peritoneal permeability according to PET classification was observed in 37.1% of the patients at the end of the follow-up. Peritoneal membrane permeability is essential in PD practice. It is among the highest ranks in the factors affecting the performance of dialysis. Membrane permeability should be considered when determining the appropriate solutions and dwell durations for the patients. In addition, adequate clearance of uremic toxins and UF volume are directly affected by membrane permeability (10).

Extracellular signaling molecules such as TGF-β are implicated as drivers of fibrosis-related diseases. In particular, their role in diabetic nephropathy has been well demonstrated. Within these intricate mechanisms, Grem1 is involved as a BMP7 antagonist. In addition,

BMP7 has antagonistic effects against TGF-β and fibrosis (11). As a result, these cytokines and advanced glycation end products play a crucial role in peritoneal membrane fibrosis. Furthermore, long-term PD can lead to peritoneal membrane deterioration, such as increased membrane transport rate (12).

The association between BMP-7 levels in PD effluent fluid and peritoneal transport characteristics has been demonstrated in several studies. The reduction of peritoneal thickness by BMP-7 was shown in animal PD models. Yu et al. (13) also observed that high glucose exposure by dialysate decreased the expression of BMP-7 protein. In a mouse model, Grem1 overexpression in the peritoneum-induced submesothelial thickening, fibrosis, and angiogenesis. In addition, Grem1 was associated with decreased expression of BMP-4 and BMP-7. These effects of Grem1 resulted in EMT in mice (14).

Grem1 levels are increased in renal fibrotic conditions, including acute kidney injury, diabetic nephropathy, chronic allograft nephropathy, and immune glomerulonephritis (3). In particular, its crucial role is well demonstrated in diabetic kidney disease via many studies. A *Grem1* gene variant associated with diabetic nephropathy. Grem1 mRNA levels correlate with serum creatinine levels and tubulointerstitial fibrosis in diabetic nephropathy (15). In the literature review, it was seen that Grem1 has been less investigated on peritoneal permeability. Moreover, we noticed that these were almost all experimental studies and the clinical-based study was not much. Ruiz-Carpio et al. (16) did not determine any association between a high peritoneal transport status and PD effluent Grem1 levels in PD patients. In the aforementioned study, Grem1 was used as a biomarker of the genetic reprograming of EMT. Similarly, a hypothetically expected relationship could not be determined in our study either.

### Study Limitations

Several limitations could have influenced the results of this study. First, we measured Grem1 in PD effluent fluid. This assessment can evaluate the possible relationship. In addition, we did not assess UF failure status in this cohort. Subgroup analysis could be conducted in patients with UF failure. Furthermore, if the initial Grem1 levels of the patients had been measured, the relationship between changes in Grem1 levels relative to the baseline and alterations in peritoneal permeability could have been examined.

## Conclusion

We did not identify any correlation between changes in peritoneal permeability and plasma Grem1 levels. However, we have emphasized the importance of novel biomarkers that could predict changes in peritoneal permeability in this study. Finally, PD clinical practice requires more research on membrane pathophysiology to attain the best clinical outcomes.

**Ethics Committee Approval:** This study was designed as a single-center study. Patients enrolled between 01.01.2020 and 01.01.2021. The study was approved by the Erciyes University Ethics Committee (approval number: 2019/678, date: 09.10.2019).

**Informed Consent:** Written informed consent was obtained from the patients by explaining the procedure of the study.

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