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Clinical Study

Effluent Markers Related to Epithelial Mesenchymal Transition with Adjusted Values for Effluent Cancer Antigen 125 in Peritoneal Dialysis Patients

Sonoo Mizuiri,[1] Hiromichi Hemmi,[2] Michitsune Arita,[2] Reibin Tai,[1] Yoshinari Hattori,[1] Atsuhiko Muto,[1] Yasunori Suzuki,[1] Yasushi Ohashi,[1] Ken Sakai,[1] and Atsushi Aikawa[1]

[1] *Department of Nephrology, Toho University School of Medicine, 6-11-1 Omori-Nishi, Ohta-ku, Tokyo 143-8541, Japan*
[2] *Department of Molecular Biology, Toho University School of Medicine, Tokyo 143-8541, Japan*

Correspondence should be addressed to Sonoo Mizuiri, sm210@med.toho-u.ac.jp

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Objectives. Epithelial mesenchymal transition (EMT) is important for peritoneal deterioration. We evaluated the association between peritoneal solute transport rate (PSTR) and effluent markers related to EMT with adjusted values for effluent cancer antigen 125 (CA125). *Methods*. One hundred five incident peritoneal dialysis (PD) patients on PD for 25 (12–68) months with biocompatible solutions were included in the study. Fast peritoneal equilibration test was used to evaluate PSTR. Effluent hepatocyte growth factor (HGF), bone morphogenic protein-7 (BMP-7), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), and CA125 at 4 h were measured. *Results*. Patients with dialysate/plasma creatinine $\geqq$0.82 showed significantly higher effluent HGF (240 versus 133 pg/mL, $P < .001$), VEGF, IL-6, and IL6/CA125 levels than the others but no significant differences in effluent HGF/CA125, BMP-7, and BMP7/CA125 were observed. *Conclusion*. Increase in the effluent HGF levels as a compensatory mechanism is a marker of peritoneal deterioration, but controversy remains regarding adjusted value for CA125.

1. Introduction

Resident fibroblasts and infiltrating inflammatory cells are considered to be the main entities responsible for structural and functional alterations in the peritoneum, but recent findings have demonstrated that new fibroblastic cells can arise from the local conversion of mesothelial cells by epithelial-to-mesenchymal transition (EMT) during the inflammatory and repair responses that are induced by peritoneal dialysis (PD) [1]. EMT of peritoneal mesothelial cells is associated with angiogenic stimuli and altered transport through common initiating growth factors and inflammatory cytokines [2, 3]. Hepatocyte growth factor (HGF) and bone morphogenic protein-7 (BMP-7) ameliorate high-glucose-induced EMT of the peritoneal mesothelium [4, 5].

As it is not possible to perform repeated peritoneal biopsies, the search for effluent markers of peritoneal damage and EMT is clinically important. However, the clinical significance of HGF and BMP-7 effluent levels with regard to these conditions remains unclear. It has also been reported that dialysate growth factor levels should be measured relative to the mesothelial cell mass, for example, relative to the level of cancer antigen 125 (CA125) [6, 7]. We evaluated the association between peritoneal membrane transport rate and the expression of effluent markers related to epithelial mesenchymal transition (HGF, BMP-7, vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6)) with adjusting the levels of these markers relative to the effluent CA125 concentration in patients on PD.

2. Subjects and Methods

2.1. Patient Selection. From December 2007 to December 2010, all incident PD patients, aged between 20 and 69, who were being treated at our unit were enrolled in the study ($n = 116$). The patients had been on continuous ambulatory peritoneal dialysis (CAPD) with dual-chamber bags, neutral-pH, and low-GDP glucose-based solutions for more than

6 months and had been clinically stable and peritonitis-free for at least 3 months before the study. Patients on automated peritoneal dialysis (APD) and patients who had received glucose polymer-based peritoneal dialysis solution were also included the study, but they were switched to CAPD with dual-chamber bags, neutral-pH, and low-GDP glucose-based solutions the day before the study. None of the subjects were on PD with conventional acidic PD solutions. The exclusion criteria included severe systemic disease, malignancy, and patients with elevated serum CRP levels. All eligible 105 incident PD subjects were included for this study, and 11 patients were excluded. The ethics committee of Toho University School of Medicine approved this study, and informed consent was obtained from all subjects.

2.2. Study of Peritoneal Transport Kinetics and Effluent Markers. The study was performed cross-sectionally, and on the night before the study, all patients were asked to undergo PD using 2.5% glucose PD solution with a 10 h dwell time. After the dialysis fluid had drained completely, a standard fast peritoneal equilibration test (fast PET) was performed. The drainage volume and ultrafiltration volume were recorded at 4 h.Dialysate to plasma creatinine values (D/P creatinine) and effluent glucose were measured at 4 h, and effluent samples were taken at 4 h and immediately stored at −70°C until they were used to measure HGF, BMP-7,VEGF, IL-6, and CA125.

2.3. Measurement of Effluent Markers. The concentrations of CA125 and IL-6 in the effluent were measured using a chemiluminescent enzyme immunoassay with appropriate kits (Fujirebio, Tokyo, Japan) [8, 9]. The concentrations of VEGF, HGF, and BMP-7 were measured with commercially available immunoenzymometric assays according to the manufacturer's instructions (VEGF and HGF were measured with ELISA kits from Quantikine R & D Systems, Minneapolis, Minn, USA and BMP-7 was measured with an ELISA kit from RayBiotech Inc., Peterborough, UK).

2.4. Statistical Analysis. The data were not in the normal distribution, and nonparametric tests were performed in all analyses. The data are expressed as median values and 25% to 75% interquartile ranges (IQR). Differences between two groups were assessed by the Mann-Whitney U test. Differences considered to be associated with diabetes were assessed using the chi-square test. A P value less than .05 denoted the presence of significant difference.

3. Results

The clinical characteristics and the results of the fast PET in the subjects are shown in Table 1. The median (IQR) age was 55 (44–64) years old, and the median (IQR) PD duration was 25 (12–68) months for all patients. The patients were subdivided into two groups according to their peritoneal transport characteristics to allow statistical evaluations to be performed: the patients with high peritoneal transport rate (D/P creatinine $\geqq$ 0.82) and the "others" (D/P creatinine < 0.82).

Table 1: Clinical characteristics and the results of the fast peritoneal equilibration test.

Group	Patients with high transport rate	Others
n	14	91
Age (years old)	58 (44–68)	55 (46–64)
Diabetics	8/14	22/91
Duration of PD (months)	10 (7–11)**	32 (15–75)
Urine volume (mL/day)	400 (175–624)	100 (0–875)
Drainage volume (mL)	2120 (1963–2260)**	2370 (2265–2450)
D/P creatinine	0.90 (0.87–0.96)**	0.71 (0.60–0.75)
Dialysate glucose (mg/dL)	596 (469–668)**	834 (718–948)
Serum albumin (g/dL)	2.70 (2.69–2.98)**	3.40 (3.03–3.78)

Data are medians with 25 and 75% interquartile ranges in parentheses.
PD: peritoneal dialysis; D/P creatinine: dialysate to plasma creatinine level.
*P < .05 compared with others; **P < .001 compared with others.

There were significant differences between the two groups with regard to the duration of PD (P < .05) and serum albumin levels (P < .001). Furthermore, prevalence of diabetes was higher in the patients with high transport rate than the others, although the difference was not statistically significant (P = .08).

Effluent markers and effluent markers-to-effluent CA125 ratio in patients with high transport rate and others are shown in Table 2. Significantly higher effluent HGF, VEGF, and IL-6 levels were found in the patients with high transport rate compared to the others. No differences were observed in the effluent BMP-7 or CA125 levels between the two groups. With regard to effluent markers-to-effluent CA125 ratio, there was a significant difference only in effluent IL-6/CA125 levels between two groups. No significant differences were observed in effluent HGF/CA125, BMP-7/CA125, and VEGF/CA125 levels between two groups.

4. Discussion

It was reported that solute transfer increases and ultrafiltration declines with time during peritoneal dialysis treatment [10] and that a high transport status is observed after 6 years dialysis treatment and subsequently develops into encapsulating peritoneal sclerosis [11–13]. In contrast with previous reports [10–13], the patients with high transport rate in our study had not undergone PD treatment for a longer period than the other group. Differences between the PD solutions might partly explain the different results since all our patients were treated with new biocompatible solutions whereas the patients in previous reports were treated with conventional nonbiocompatible solutions. However, the patients with high peritoneal transport rate in our study showed a higher prevalence of diabetes and hypoalbuminemia, as reported previously [14–16].

Table 2: Effluent markers and effluent markers-to-effluent cancer antigen 125 ratio in patients with high transport rate and others.

Group	Patients with high transport rate	Others
n	14	91
Effluent HGF (pg/mL)	240.0 (197.5–319.3)***	133.0 (107.0–216.0)
Effluent HGF/CA125 (pg/U)	19.0 (10.8–33.2)	13.7 (8.0–25.9)
Effluent BMP-7 (pg/mL)	8.3 (7.4–9.4)	7.5 (4.8–9.1)
Effluent BMP-7/CA125 (pg/U)	0.6 (0.5–0.8)	0.6 (0.3–1.2)
Effluent VEGF (pg/mL)	33.0 (29.0–34.0)**	25.0 (20.0–31.0)
Effluent VEGF/CA125 (pg/U)	2.5 (1.7–3.7)	2.3 (1.4–3.7)
Effluent IL-6 (pg/mL)	23.8 (13.9–35.1)*	11.6 (6.7–24.0)
Effluent IL-6/CA125 (pg/U)	1.9 (1.3–2.2)*	1.1 (0.6–1.8)
Effluent CA125 (U/mL)	13.3 (7.6–18.9)	11.1 (6.3–20.0)

Data are medians with 25 and 75% interquartile ranges in parentheses.
HGF: hepatocyte growth factor, BMP-7: bone morphogenic protein-7, VEGF: vascular endothelial growth factor, IL-6: interleukin-6, CA125: cancer antigen 125.
*$P < .05$ compared with others, **$P < .01$ compared with others, ***$P < .001$ compared with others.

EMT of mesothelial cells is associated with high peritoneal transport [17]. There is emerging evidence that the mesenchymal conversion of mesothelial cells is an important mechanism for the failure of peritoneal membrane function [18–20]. High levels of glucose, glucose degradation products, a low-PD solution pH, inflammation, and angiotensin II are responsible for the production of transforming growth factor β (TGF-β) and VEGF, which induce EMT, by mesothelial cells [1]. TGF-β is a key regulator of EMT [1, 20]; however, the measurement of TGF-β is not easy because of its low concentration in dialysis effluent fluids [6]. In addition, it is not clear whether measuring the amount of TGF-β protein in peritoneal fluid, in which it is mostly found in an inactive state, that is, bound to a latency-associated protein, is reflective of the tissue levels of active TGF-β [6, 21] and a previous study found no differences in TGF-β at any time in a comparison of patients treated with low-GDP solution and patients treated with high-GDP solutions [6]. VEGF was found to be locally produced in the peritoneal tissue of patients undergoing peritoneal dialysis, and effluent VEGF was found to be correlated with solute transport but not the TGF-β1 level [22, 23].

IL−6 is a cytokine involved in the acute-phase inflammatory reaction, and dialysate IL-6 levels and VEGF concentrations are associated with a high peritoneal solute transport rate [24]. It has also been reported that HGF and BMP-7 ameliorate high-glucose-induced EMT in the peritoneal mesothelium [4]. Furthermore, it was reported that measuring the dialysate VEGF, level relative to the effluent CA125 level revealed a significant association with EMT, whereas unadjusted levels of the growth factor did not [6]. Thus, we studied the relationship between peritoneal transport characteristics and effluent HGF, BMP-7, VEGF and IL-6 levels and their values relative to the effluent CA125 concentration, focusing on EMT in patients being treated with PD using new, biocompatible PD solutions.

Consistent with previous reports, VEGF and IL-6 levels were significantly different between patients with high transport rate and others [24]; however, effluent HGF levels showed bigger difference in these two groups in our study. We considered that using a low-GDP, neutral-pH, dual-chamber bag PD solution also causes EMT since high glucose itself induces EMT in cultured human peritoneal mesothelial cells [4]. According to previous studies, it is conceivable that the mesothelial cells of patients with high transport rate undergo EMT and display decreased production of HGF and BMP-7. However, in our study, the high transport rate group showed increased effluent HGF concentrations. HGF is a heterodimeric molecule composed of a 69 kDa alpha subunit and a 34 kDa beta subunit (Entrez Gene ID: 3082). Its peritoneal permeability is expected to be poor, and so the HGF protein detected in the effluent may be produced locally. Yu et al. demonstrated that human peritoneal mesothelial cells constitutively synthesized HGF [4]. In a previous study, high-glucose-induced EMT in the peritoneal mesothelium was reversed by HGF treatment, suggesting a link between decreased HGF expression and EMT in human peritoneal mesothelial cells [4]. HGF also prevented peritoneal fibrosis in a rat model of EPS [25]. However, Rampino et al. showed that treatment with high-dosage HGF (50 pg/mL) and the HGF released during peritonitis in humans may facilitate repair through mesothelial cell growth, but may also contribute to peritoneal fibrosis including cell detachment, fibroblast-like phenotype changes, and collagen synthesis [26]. These findings suggest that an antifibrotic effect of HGF may be dosage dependent with variable therapeutic dosages that depend on experimental conditions and types of animal model. We considered that unexpected increase in the HGF levels has been proposed as a compensatory mechanism in patients with high peritoneal transport rate. High effluent HGF may be a marker of peritoneal deterioration since high HGF levels coexist with high peritoneal transport rate.

In contrast with Szeto et al.'s report [27], no difference in BMP-7 was demonstrated by the difference in D/P creatinine in our study. Their results showed that the PD effluent BMP-7 level displayed a significant correlation with the change in the D/P creatinine level but was not significantly correlated with the D/P creatinine level at 4 or 52 weeks in new PD patients. However, we only studied the D/P creatinine level in incident CAPD patients at one time point, which may account for our different results. We consider that it is difficult to interpret EMT using measurements of effluent BMP-7 concentrations taken at one time point alone.

The number or mass of mesothelial cells could affect the levels of intraperitoneal growth factors in CAPD patients.

It was reported that the CA125 levels in peritoneal effluent were higher in patients treated with low-GDP solution than in those treated with conventional solution [28]. Do et al. observed differences in dialysate-VEGF/CA125 levels between the low- and high-GDP groups during the initial 12 months, but did not observe any difference in the unadjusted VEGF concentration [6]. From our data, patients with high transport rate displayed higher HGF, VEGF, and IL-6 levels. While, effluent HGF/CA125, and VEGF/CA125 levels were not significantly different between patients with high transport rate and others. Furthermore, IL-6/CA125 effluent level did not show a stronger relation with D/P creatinine than unadjusted IL-6. Breborowicz reported that CA 125 does not a good index of the number of mesothelial cells or their functional properties, because the amount of CA125 released from mesothelial cells is not only depend on the number of cells, but also on their properties, age of cell donor, and environmental factors [29]. We consider that the effluent concentrations of growth factors should be measured relative to mesothelial mass integrity and that the CA125 effluent level may not be a suitable surrogate marker for this purpose.

Our study has certain limitation. The small number of the patients in the high transport group and shorter duration of the PD in this group may affect the results. In conclusion, increase in the effluent HGF levels as a compensatory mechanism is a marker of peritoneal deterioration, but controversy remains regarding the adjustment of markers for CA125.

Conflict of Interests

The authors declare that there is no conflict of interests.

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