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ORIGINAL ARTICLE

Expression pattern of peritoneum IL-6 is associated with baseline peritoneal transport function in uremic patients before dialysis

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Abstract

OBJECTIVE: This study aimed to investigate the biological factors associated with baseline peritoneal transport in uremic patients before dialysis.
METHODS: Thirty patients with uremia were grouped according to their peritoneal dialysate creatinine/serum creatinine ratio (D/P) as high-transport (H, 16 cases) with D/P>0.65 and low-transport (L, 14 cases) with D/P≤0.65 one month after continuous ambulatory peritoneal dialysis treatment. Multi-inflammatory levels such as serum IL-6 and albumin, peritoneal IL-6 level, and microvessel density (MVD) of visceral peritoneal were compared and correlated between the two groups to determine the associated factors.
RESULTS: There were no significant differences in clinical parameters between the two groups (p>0.05). There were no significant differences in serum IL-6 and albumin between the two groups. However, peritoneal IL-6 and MVD in group H were significantly higher than group L (p=0.012, p=0.044), and they were positively correlated (r=0.368, p=0.045). Furthermore, baseline D/P was positively correlated with IL-6 expressions (r=0.640, p=0.000) and peritoneal MVD (r=0.476, p=0.008), and independently associated with peritoneal IL-6 expression (p=0.004).
CONCLUSIONS: The baseline peritoneal transport performance is associated with peritoneal IL-6 expression and MVD but not circulatory IL-6.

Abbreviations:

Alb	- albumin
BUN	- blood urine nitrogen
ELISA	- Enzyme-Linked Immunosorbent Assay
GFR	- glomerular filtration rate
Hb	- Hemoglobin
HE	- staining hematoxylin-eosin staining
HP	- high-power field
IL-6	- Interleukin- 6
MVD	- microvessel density
PET	- peritoneal equilibration test
PTH	- parathyroid hormone
RT-PCR	- Reverse Transcription-Polymerase Chain Reaction
Scr	- serum creatinine
TC	- Total Cholesterol
TP	- Total Protein
UA	- uric acid
VEGF	- Vascular endothelial growth factor

INTRODUCTION

Peritoneal dialysis is an alternative treatment for end-stage renal diseases (Li *et al.* 2017), and more than 15% of patients with kidney diseases worldwide have undergone peritoneal dialysis (Mehrotra *et al.* 2016). Peritoneal transport function is evaluated according to the permeability of micromolecule solutes (urea, glucose, creatinine, etc.), namely by the creatinine ratio in the peritoneal drainage fluid to the plasma (D/P, peritoneal equilibration test, PET) (Waniewski *et al.* 2013), which is also the main basis for the manner and prescription selection of peritoneal dialysis (Morgan *et al.* 1977). According to the D/P value, the peritoneal solute transport function is divided into the following types: D/P ranging 0.81-1.03 is defined as high transport, 0.65-0.8 as high average transport; 0.50-0.64 as low average transport; 0.34-0.49 as low transport (Moncrief 2017). High peritoneal solute transport is prone to causing water retention, hypertension, or heart failure, together with malnutrition, oxidative stress, or microinflammation, resulting in higher mortality (Sawai *et al.* 2011). Thus, high peritoneal transport status is considered an independent risk factor for high mortality in patients with peritoneal dialysis (Brimble *et al.* 2006), but its mechanism is not fully understood.

Baseline peritoneal transport function is mainly related to hereditary and uremic state (Davies 2014), and microinflammation is common in uremic patients. IL-6 and albumin are considered to be the markers of inflammation, and the expression of peritoneal IL-6 reflects local microinflammation in the abdominal cavity (Sikorska *et al.* 2016). The influence of systemic inflammation on peritoneal transport function is controversial, and it has been reported that serum IL-6 is associated with peritoneal transport function in patients with maintenance peritoneal dialysis (Pecoits-Filho *et al.* 2006). However, a study in Korea did not observe the correlation between serum IL-6 and peritoneal transport function (Pecoits-Filho *et al.* 2002a).

Regarding the effect of local inflammation in the abdominal cavity on peritoneal transport function, it has been reported that the IL-6 concentration in peritoneal dialysis drainage fluid is associated with high transport status of baseline peritoneum (Jin *et al.* 2016). However, the impact of the expression of peritoneal IL-6 before dialysis on baseline peritoneal transport function has not been reported. This study investigated the relation between serum IL-6 and peritoneal tissue IL-6 on the baseline peritoneal transport function and possible mechanisms from the perspective of microinflammation.

MATERIALS AND METHODS

Patients and ethics approval

Thirty patients with uremia from Dalian Central Hospital were enrolled in this study. The study was conducted following the approval from the Ethics Committee of Dalian Central Hospital and performed according to the declaration of Helsinki. Before the study commenced, a written informed consent was obtained from each participant.

Experimental materials and detection of peritoneal specimens

Peritoneal tissue of each patient's purse-string suture site (1 cm^2) was sampled during peritoneal dialysis catheterization.

Serum specimen

Blood sample was obtained from each patient following a twelve hours fasting from food and water. A serum sample was collected and processed for measuring routine clinical tests and IL-6 by ELISA (DIACLONE company).

RT-PCR for IL-6

About 0.7 x 0.7 cm of peritoneal tissue was used for RT-PCR (TRANS). An upstream primer 5'-CTCTGGCTTGTTCCTCACTA- 3', and downstream primer 5'-ACAAATTCGGTACATCCTCG-3', with cycle conditions of 94 °C × 30 s plus 56 °C × 30 s and 72 °C × 1 min (a total of 39 cycles) were used.

Immunohistochemistry and HE staining

The remaining specimen (about 0.3 × 0.3 cm) of peritoneal tissue was fixed in 10% formalin overnight, followed by routine paraffin embedding, and sectioning. The slides were stained by HE and immunohistochemistry for CD34. Each slide was examined microscopically counting the CD34-specific vascular endothelial cells. The vessels with a diameter greater than 8 red blood cells or smooth muscles in the vessel wall were not counted. Under high-power microscopy (×400), the average value of all the selected fields of view were set as the microvessel density (MVD) value of this specimen.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 software, and each variable was tested for normality. All the measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). If the distribution is normal, t-test is used. If the distribution is not normal, Mann Whitney nonparametric test is used. The Pearson linear correlation analysis was used to analyze the association among parameters, with $p < 0.05$ being considered as statistical significance. The multivariate linear regression analysis was performed for the baseline peritoneal transport function, including age, gender, primary diseases, serum albumin, GFR, serum IL-6, peritoneal MVD, peritoneal IL-6, and baseline D/P among the different groups.

Tab. 1. Comparison of clinical parameters between group H and group L

	H	L	*p*
Age (years)	60.4±15.7	62.4±13.4	0.716
Gender (M:F)	8:8	8:6	0.708
Residual renal function (mL/min/1.73m^2)	3.03	3.45	0.280
Hb (g/L)	79	95	0.308
BUN (mmol/L)	33.33	34.28	0.934
Scr (umol/L)	590	968	0.480
UA (umol/L)	480	420	0.739
Alb (g/L)	35.9±3.9	34.9±3.3	0.456
TP (g/L)	59.4±7.5	59.1±7.7	0.916
TC (mmol/L)	3.9±1.3	4.3±1.1	0.324
TG (mmol/L)	1.8±1.6	1.4±0.7	0.967
PTH (pg/mL)	370	599	0.198
Ca (mmol/L)	2.00±0.26	2.01±0.13	0.895
P (mmol/L)	2.05±0.67	2.04±0.69	0.975
Serum IL-6 (ng/ml)	6.44	3.52	0.553

"±": mean ±SD

RESULTS

Grouping and clinical parameters

The 30 enrolled patients with uremia were firstly performed one-month continuous ambulatory peritoneal dialysis, and then performed PET and recorded the 4-hr creatinine ratio (D/P) of peritoneal drainage/serum. Because the peritoneal specimens of this study were obtained by initial peritoneal dialysis catheterization, the influence of other factors caused by peritoneal dialysis can be ruled out.

The patients were grouped according to their D/P values: group H, D/P> 0.65, group L: D/P≤ 0.65 (Waniewski *et al.* 2017). The patients in group H (16 cases) and in group L (14 cases) showed no statistical significance in the age, residual renal function, hemoglobin, blood urea nitrogen, serum creatinine, uric acid, albumin, total protein, total cholesterol, triglycerides, parathyroid hormone, Ca, or P (p>0.05). (Table 1).

Peritoneal IL-6 and MVD correlated with high baseline D/P

There was no statistical significance in serum IL-6 and albumin between group H and group L (p>0.05, Table 1). However, the IL-6 level in peritoneal tissues of group H (2.3±1.2) was significantly higher than group L (1.3±0.7, p=0.005). Furthermore, the MVD value was significantly higher in group H compared to group L (3.9 ± 2.1 / HP vs. 2.5 ± 1.4 / HP, p = 0.044).

The baseline D/P was positively correlated with peritoneal IL-6 expression and peritoneal MVD (p=0.000; p=0.004), and peritoneal MVD was positively correlated with peritoneal IL-6 (p=0.045). Meanwhile serum IL-6 and albumin were not correlated with baseline D/P (Table 2).

The multivariate linear regression analysis showed that baseline D/P was independently associated with the expression of peritoneal IL-6 in peritoneal tissues (r=0.707, p=0.004).

DISCUSSION

The effect of microinflammation on baseline peritoneal transport characteristics in pre-dialysis uremic status is very important. This study is the first to report the association between pre-dialysis systemic and peritoneal microinflammation on peritoneal transport function in the Chinese population.

It has been reported that about half (47.4-66.5%) of patients with peritoneal dialysis have high or high average transport function at the beginning of peritoneal dialysis, and high /high average transport is the factor that leads to poor prognosis in patients (Rumpsfeld *et al.* 2006). Furthermore, IL-6 concentration in peritoneal dialysate was found to be associated with high solute transport in baseline peritoneum of dialysis patients (Cho *et al.* 2010).

The impact of inflammatory factor IL-6 on peritoneal transport function has been reported in different cases (Pecoits-Filho *et al.* 2002b; Rodrigues *et al.* 2007), but most of the previous reports focused on the effect of microinflammation on the peritoneal transport function in maintenance peritoneal patients, which may be obtained after dialysis. The present study, however, focused on the influence of micro-inflammation on

Tab. 2. Correlation analysis between baseline peritoneal transport function and peritoneal parameters/clinical parameters

		H	D/P	RT-PCR IL-6	Serum IL-6	MVD	Alb g/L	GFR	Primary diseases	Age	Gender
D/P	r	1	0.640**	0.098	0.506**	0.069	0.214	0.333	-0.137	0.093
	p		0.000	0.606	0.004	0.717	0.257	0.072	0.470	0.627
RT-PCR IL-6	r		1	0.216	0.368*	0.030	0.054	0.227	-0.039	-0.001
	p			0.252	0.045	0.874	0.776	0.227	0.839	0.997
Serum IL-6	r			1	0.049	0.033	-0.143	0.184	0.370*	-0.059
	p				0.798	0.862	0.452	0.332	0.044	0.757
MVD	r				1	0.143	0.063	0.212	-0.353	0.128
	p					0.451	0.743	0.261	0.055	0.501
Alb g/L	r					1	0.035	-0.109	0.167	0.076
	p						0.853	0.567	0.377	0.688
GFR	r						1	0.300	0.083	0.041
	p							0.107	0.663	0.830
Primary diseases	r							1	-0.357	-0.030
	p								0.053	0.876
Age	r								1	0.118
	p									0.536
Gender	r									1
	p									

**. Significantly correlated at the level of 0.01 (bilateral), *. Significantly correlated at the level of 0.05 (bilateral).

peritoneal transport function factors before dialysis. Macrophage, mesothelial cells, endothelial cells, and other inflammatory cells can produce IL-6 (Pecoits-Filho *et al.* 2002b; Sawai *et al.* 2011), suggesting that local inflammation presents in the peritoneum. In group H, peritoneal IL-6 was higher than group L and correlated with baseline D/P, implying that there were differences in the peritoneal microinflammation status between the two groups before dialysis.

Gillerot *et al.* (2005) observed that the IL-6 concentrations in serum and dialysate, as well as the solute transport parameters, of patients with genotypes of GC and CC were significantly higher than those with GG phenotype. This may be related to the IL-6 gene polymorphism. Retrospective analysis has showed that peritoneal IL-6 expression is an independent determinant of baseline peritoneal transport function (Oh *et al.* 2010). The ability of IL-6 to independently affect peritoneal transport properties may be related to its biological activity, which binds to soluble IL-6 receptor (sIL-6R) to form a complex that induces inflammatory cells to synthesize and secret CC chemokine, and monocyte chemoattractant protein-1. The latter processes attracts neutrophils and lymphocytes and induces the synthesis and secretion of other molecules, such as MCP-3/IL-8, adhesion molecules (ICAM-1, VCAM-1), or angiogenic molecules (VEGF) (Ishii *et al.* 2018).

Vascular endothelial growth factor (VEGF) is an important factor regulating blood vessels. It is positively expressed in peritoneal microvascular endothelial cells and peritoneal mesothelial cells and can induce the angiogenesis by stimulating endothelial cell proliferation, migration and tubular structure formation, thus leading to vascular proliferation, and blood vessel dilation, leading to an increase in vascular permeability and solute transport. Therefore, we conclude that IL-6 may affect peritoneal transport function indirectly by affecting microvascular proliferation. Herein, MVD in group H was significantly higher than group L, and MVD was significantly positively correlated with baseline peritoneal transport function. In addition, MVD and peritoneal tissue IL-6 expression were significantly positively correlated. Thus, IL-6 promotes peritoneal vascular proliferation and increases capillaries' permeability by regulating the release of downstream vasoactive VEGF, which then indirectly affects baseline peritoneal transport function.

Albumin is a widely recognized marker of inflammation and nutrition, as well as a predictor of prognosis in patients with peritoneal dialysis or chronic kidney disease Amdur *et al.* 2016). In this study, there was no difference in serum albumin between group H and group L. Serum IL-6 level is significantly elevated in most patients with end-stage renal

diseases (Samouilidou *et al.* 2014). Epidemiological analysis has suggested that serum IL-6 level is associated with prevalence and mortality of patients with hemodialysis and peritoneal dialysis (Pecoits-Filho *et al.* 2002a). There was no difference in IL-6 between group H and group L in this study. Correlation analysis showed no correlation with peritoneal transport function ($p<0.05$). In summary, this study shows that there is no correlation between systemic microinflammation and baseline peritoneal transport function before dialysis, suggesting that systemic microinflammation and peritoneal local inflammation before dialysis can't be equated, which is consistent with many studies (Pecoits-Filho *et al.* 2002b; Rodrigues *et al.* 2007), but the specific mechanism is still uncertain.

Previous studies have shown that baseline peritoneal transport characteristics are associated with age, gender, diabetes, or hypoproteinemia (Churchill *et al.* 1998), and associated with gender and more residual urine volume (Rumpsfeld *et al.* 2004). The results obtained in different clinical studies are not the same. Many studies have shown that the peritoneal transport function of peritoneal dialysis patients is different between different genders (Rumpsfeld *et al.* 2004, Mojahedi *et al.* 2007, Fang *et al.* 2019). Male patients show more high transport, which may be related to the larger body surface area in males than females. For adults, the peritoneal transport three-hole model shows a positive linear correlation between membrane pore area and body surface area, in which the membrane pore area is positively correlated with the volume of solute and water transport, so it may explain that peritoneal solutes in male patients with peritoneal dialysis are more in high transport status. However, no impact of gender, age, or various clinical factors on baseline D/P was observed in this study, indicating that clinical factors may not be the determinants of baseline peritoneal transport function. Also, in the present study, the patient number was very small which may limited observing a gender difference.

CONCLUSIONS

Although the patients exhibited different peritoneal transport characteristics, there was no statistical difference in the corresponding clinical indicators before dialysis. However, there was statistical significance in the expression of local IL-6, which is the pathophysiological basis of the difference in the baseline peritoneal transport among different patients. Baseline peritoneal transport characteristics is positively correlated with the expression of IL-6 in the peritoneal tissue. MVD is also correlated with the expression of IL-6 in the peritoneum, suggesting that local inflammation of the peritoneal cavity may indirectly affect the peritoneal microvascular volume and the peritoneal transport characteristics.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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