Possible role of IL-6 and TIE2 gene polymorphisms in predicting the initial high transport status in patients with peritoneal dialysis: an observational study

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ABSTRACT

Objectives: The aim of this study was to investigate the effect of interleukin (IL)-6 and TIE2 gene polymorphisms on baseline peritoneal transport property.

Design: An observational study.

Setting: Renji Hospital in Shanghai, China.

Participants: This study included 220 patients with continuous ambulatory peritoneal dialysis (PD).

Outcome measures: Patients were divided into 2 groups based on the results of an initial peritoneal equilibration test performed within 3 months of starting PD therapy: group 1 consisted of low/low average transporters (n=123), and group 2 consisted of high/high average transporters (n=97). We genotyped TIE2 and IL-6 polymorphisms and analysed their effects on baseline transport status.

Results: The genotype AT in IL-6 Rs13306435 and the genotype CC in TIE2 Rs639225 were both negatively associated with a higher initial peritoneal transport status (IL-6 Rs13306435: OR=0.408, 95% CI 0.227 to 0.736; TIE2 Rs639225: OR=0.188, 95% CI 0.044 to 0.806).

Conclusions: IL-6 and TIE2 polymorphisms are associated with baseline peritoneal transport property.

INTRODUCTION

Peritoneal dialysis (PD) is an effective renal replacement therapy for patients with end-stage renal disease (ESRD). Patients undergoing PD have significantly different small solute transport rates. The standard peritoneal equilibration test (PET) proposed by Twardowski et al in 1987 is the most widely used method to assess the peritoneal small solute transport rate. Patients can be divided into four types: high (H), high average (HA), low average (LA) and low (L) based on PET results. Studies have shown an association between high transport status and poor outcome. The results of a meta-analysis have indicated that for every 0.1 increase in the dialysate over plasma ratio for creatinine (D/P Cr), the relative risks for mortality and technique failure increase by 1.15 and 1.18, respectively. Compared with the mortality of the low transport group, that of the LA, HA and high transport groups increased by 21.9%, 45.7% and 77.3%, respectively. As technology advances, new peritoneal dialysate (icodextrin) and automated PD (APD) have been shown to improve the prognosis of high transporters. However, in developing countries, icodextrin and APD cannot be widely used for patients with PD. Initial high transport is still an important factor that influences the outcome of these patients without icodextrin.
or APD. Therefore, it is important to know the baseline peritoneal transport property before starting PD therapy. We can advise probable high transporter patients to choose haemodialysis (HD) or renal transplantation for renal replacement therapy. Researchers have attempted to find non-invasive biomarkers to predict the baseline peritoneal membrane function before starting dialysis. Previous studies found that age, gender and complications such as hypertension, diabetes and malnutrition might influence transport characteristics. However, they are not sufficient to predict high transport status.

In recent years, many studies have shown that genetic variants may play an important role in mechanisms contributing to the baseline variability in peritoneal transport. It has been suggested that chronic inflammation mediated by various inflammatory cytokines may have an effect on peritoneal transport. Studies have shown that the interleukin (IL)-6 level in peritoneal dialysates is associated with the peritoneal solute transport. Moreover, an increase in the effective solute exchange area caused by peritoneal vascular proliferation is also an important factor for high peritoneal transport status. The angiogenesis of the peritoneum induced by PD can be inhibited using sTie2/Fc in a uremic rat model.

The study aimed to determine whether TIE2 and IL-6 gene polymorphisms have an effect on the baseline peritoneal transport property and explore its possible role in predicting initial high transport status.

MATERIALS AND METHODS

Patient selection

All patients with PD having an initial PET performed within 3 months of starting PD therapy were included. Those who switched from failed renal allograft or maintenance HD were excluded. Two hundred and twenty patients with continuous ambulatory PD in the Peritoneal Dialysis Center, School of Medicine, in Shanghai Jiaotong University were enrolled in the study. Written informed consent was obtained from each patient.

Study of peritoneal transport

A standard PET was performed for each of the enrolled patients. Dialysate as well as plasma creatinine and glucose levels were measured at 4 hours using 2 L of 2.5% glucose dialysis fluid. Creatinine dialysate to plasma ratios at 4 hours (D/P Cr) were calculated. Patients were classified into four types based on the D/P Cr value: H (D/P Cr>0.8), HA (D/P Cr 0.66–0.8), LA (D/P Cr 0.5–0.65) and low transporters (D/P Cr<0.5). Then they were divided into two groups: group 1 consisted of L/LA transporters, and group 2 consisted of H/HA transporters. The residual urine volume was assessed after 24 hours of urine collection. Weekly peritoneal Kt/V (peritoneal Kt/V) and residual urine Kt/V (urine Kt/V) were calculated and presented as total weekly Kt/V (total Kt/V).

DNA extraction and genotyping

DNA was extracted from whole blood using a DNA purification kit (Promega, USA). The single nucleotide polymorphisms (SNPs) of IL-6 and TIE2 were genotyped by a single base primer extension assay. The genomic DNA flanking the SNP was amplified by PCR using forward and reverse primer pairs (tables 1 and 2), and standard PCR regents in a 10 μL reaction volume containing a 20 ng DNA sample, 0.4 μmol of each primer, a 10× PCR buffer, 0.4 μmol dNTPs (Generay Biotech, China), 10 mmol MgCl₂ and 0.25 units HotStarTaq DNA Polymerase (QIAGEN, Germany). After 40 cycles of PCR (MJ Research PT-100), the products were purified by 2 U shrimp alkaline phosphatase (SAP) and 2 U exonuclease I (Epicentre). The purified amplification products (2 μL) were added into a SNaPShot Multiplex Ready reaction mixture (Applied Biosystems) containing 1 μL of genotyping primer for the primer extension reaction (tables 1 and 2). The primer extension reaction was carried out with 25 cycles at 96°C for 10 s, 50°C for 5 s and 60°C for 30 s. The reaction products were purified by 0.5 U SAP. The final reaction samples (0.5 μL) were added into 9.25 μL Hi-Di formamide (Applied Biosystems) and 0.25 μL GS-120 LIZ (Applied Biosystems). The mixture was incubated at 95°C for 5 min and then analysed by electrophoresis using the ABI Prism 3730xl DNA analyser (Applied Biosystems). Results were analysed using GeneScan analysis software (Applied Biosystems).

| SNP          | Primer sequences of IL-6 for genotyping using SNapShot assay | Target    |
|--------------|-------------------------------------------------------------|-----------|
| rs1800795    | AACCTCCTCTAAGTGGGCTG GGTGGGGCTGATTGGAAAC TCCCCCTAGTTGTGTCTTGC | TCCCCCTAGTTGTGTCTTGC CCAAGCCAGTTCTACAAACAGCC |
| rs1800796    | TCCCCCTAGTTGTGTCTTGC CCAAGCCAGTTCTACAAACAGCC                |           |
| rs13306435   | GAAGGGTCTCTACTCAGAGCA GTTGGGTCAGGGGTTGTTAT TCCCCCTAGGGCAAGAATCTAGA |           |

IL, interleukin; SNP, single nucleotide polymorphism.
Statistical analysis

Statistical analysis was conducted using SPSS V.17.0. All categorical data were presented as absolute counts or percentages, and mean and SD were provided for continuous data. To compare the differences between two baseline transport groups, categorical data were analysed by Fisher’s exact test and continuous variables were analysed by an unpaired t-test. Logistic regression analysis was applied to determine whether polymorphism of IL-6 and TIE2 affected the baseline peritoneal transport status. p Values of <0.05 were considered statistically significant.

RESULTS

Clinical parameters between different transport groups

In total, 220 patients were enrolled in this study. The average age of the patients was 52.54±14.56 years; the male-to-female ratio was 118:102 and the average body mass index was 21.83±3.47 kg/m². Residual renal function was 3.98±3.47 mL/min. The causes of ESRD were as follows: chronic glomerulonephritis (n=71; 32.3%), diabetic nephropathy (n=32; 14.5%), hypertensive nephropathy (n=9; 4.1%) and other/unknown (n=107; 48.6%). Based on the first PET results, there were 97 patients (44.1%) in the H/HA group, and 123 patients (55.9%) in the L/LA group. Comparisons of clinical characteristics between the two groups are shown in table 3.

Distribution of IL-6 and TIE2 polymorphisms in different transport groups

The distributions of IL-6 and TIE2 genotypes in the peritoneal transport groups are summarised in tables 4 and 5. Distributions of the 24 alleles (12 polymorphisms) were within the Hardy-Weinberg equilibrium. For the IL-6 polymorphism, there was a statistically significant correlation between the AT genotype of rs13306435 and the peritoneal transport group (p=0.023). For the TIE2 polymorphism, the distribution of rs10967789 and...

| SNP         | PCR-L                  | PCR-R                  | Target                        |
|-------------|------------------------|------------------------|-------------------------------|
| rs10967717  | TTTCTCCTGGTGGTAGGAA    | GGGCTACTGGGATCTGAC     | GAGGATATTAGATTCTCTCAGGC       |
| rs657867    | AGGCTGTTGCCTAAGGGA     | CCACTATGAGTTTGAATGGGA  | CAAATTGTGCAAGAATCACACG        |
| rs639225    | TTCTTCTCCTCAACCAGAA    | TCACATCAACGTGCTGTTCT   | GTTCCTCTTTGACATTTCGCC         |
| rs542913    | AGGGTTGGGTCTTCCCTCTC   | GAGCTTGCTCTAAGGGGA     | CATTCTCCTTTGACATTTCGCC        |
| rs373718    | GATGGTTCCCGAGGGCAAGAGA | TTTCCAGGGGACACAGTAT    | TTTCGTCAGGGTCAAGAGGGTA        |
| rs2273719   | TGCCACTGTTTGTCTTCCAG    | ACCGGCTGACTTTTGCTAGAG  | AGGCAACCCCTACTTGGCG           |
| rs2273718   | ATGGGCTGAAATCGAATGC    | AACCTGTAATACAGGGTCATTG | AGTCTTACATTTGCTTGT            |
| rs10967789  | ATGGGCTGAAATCGAATGC    | AACCTGTAATACAGGGTCATTG | AGTCTTACATTTGCTTGT            |
| rs9987817   | CTGGTGTATTCTTGAGGAGAC  | CACCTTCTGGACAGTCAAGTG  | GATAGACAGTGAAGAGCAAAAGA       |

SNP, single nucleotide polymorphism.

Table 3 Comparison of clinical characteristics and peritoneal parameters between the peritoneal transport groups of 220 patients with peritoneal dialysis

|                  | L/LA (n=123) | H/HA (n=97) | p Value |
|------------------|-------------|-------------|---------|
| Age (year)       | 52.85±14.94 | 52.27±14.42 | 0.761   |
| Male (%)         | 68 (55.3)   | 59 (61.8)   | 0.281   |
| BMI (kg/m²)      | 21.82±3.58  | 21.85±3.03  | 0.939   |
| Hypertension (%) | 94 (68.1)   | 75 (70.1)   | 0.740   |
| Periods (d)      | 48 (39, 69) | 47 (35, 68) | 0.924   |
| Serum albumin (g/L) | 104.6±22.67 | 99.13±21.29 | 0.056   |
| hs-CRP (mg/L)    | 2.65 (0.71, 3.83) | 2.58 (1.38, 3.86) | 0.657   |
| ACEI/ARB (%)     | 64 (52)     | 53 (54.6)   | 0.624   |
| D4/D0 (glucose)  | 0.44±0.08   | 0.34±0.08   | <0.01   |
| D/P Cr           | 0.55±0.08   | 0.74±0.07   | <0.01   |
| RRF (mL/min)     | 4±2.92      | 3±2.63      | 0.901   |
| NPCR (g/(kg.d))  | 1.05±0.6    | 0.96±0.26   | <0.01   |
| UF (mL)          | 160 (200, 520) | 80 (45, 500) | 0.004   |
| Urine volume (mL) | 1000 (560, 1400) | 1000 (500, 1500) | 0.217   |
| Kt/V             | 2.23±0.58   | 2.20±0.56   | 0.647   |

ACEI: ACE inhibitors; ARB, angiotensin-receptor blockers; BMI, body mass index; DM, diabetes mellitus; D/P Cr, dialysate over plasma ratio for creatinine; H, high; HA, high average; hs-CRP, high-sensitive C reactive protein; L, low; LA, low average; PET, peritoneal equilibration test; RRF, residual renal function; UF, ultrafiltration.

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rs639225 genotypes differed significantly between the two groups (p=0.039 and 0.047, respectively).

**Parameters for peritoneal solute transport rate for different genotypes**

We further compared the peritoneal solute transport rate among groups with the three SNPs (rs13306435, rs10967789 and rs639225). We found no statistically significant difference in the data of D/P Cr and D/D0 glucose (the ratio of dialysate glucose concentrations at 0 and 4 hours) among different genotypes (table 6).

**Roles of IL-6 and TIE2 gene polymorphisms in predicting initial peritoneal high transport status**

With possible clinical factors controlled in a multiple logistic regression model, the genotype AT in IL-6 Rs13306435 and CC in TIE2 Rs639225 were both negatively associated with a higher initial peritoneal transport status (IL-6 Rs13306435: OR=0.408, 95% CI 0.227 to 0.736; TIE2 Rs639225: OR=0.188, 95% CI 0.044 to 0.806; table 7).

**DISCUSSION**

The relationship between gene polymorphisms and disease has been receiving greater attention from researchers. It has been shown that SNPs of IL-6 may influence the development of cardiovascular disease,28 cancer,29 30 fractures31 and autoimmune diseases.32–34 In contrast, there is less available research regarding TIE2 gene polymorphisms, except for a study on the relationship between rs638203/rs639225 and vascular malformations.35

In this study, we investigated the effect of genetic polymorphisms of IL-6 and TIE2 on the baseline peritoneal transport property. Results showed that IL-6 and TIE2 gene polymorphisms were both negatively associated with initial high transport status. The genotypes of rs13306435 and rs639225 were shown to be independent predictors of initial high transport status in patients with PD.

Initial transport status can determine the patients’ dialysis prescription, which may influence the outcome for patients with PD. Previous studies have shown an association between initial high transport status and poor outcome. Although icodextrin and APD have been shown to improve the prognosis of high transporters, most of the patients with PD in developing countries are unable to use them. In China, for instance, icodextrin has not been approved for sale yet, and few patients can afford the cost of APD therapy. Therefore, predicting
the baseline transport status is important to select a better treatment strategy. Our study provided a potential solution to predict initial high transport status before beginning PD.

There have been several genetic studies of peritoneal solute transport rate in patients with PD. Polymorphisms of endothelial nitric oxide synthase, receptor of advanced glycation end products and transforming growth factor were not associated with initial peritoneal transport property. A functional study of the polymorphisms is required in the future.

Angiogenesis in the peritoneum could directly increase the effective solute exchange area and influence the transport characteristics. Previous studies have found that polymorphisms of vascular endothelial growth factor were not associated with initial peritoneal transport type. Ang/Tie2 has recently been confirmed to play an important role in angiogenesis in the peritoneum. Whether the polymorphisms of TIE2 are involved in initial peritoneal transport status remains unknown. In this study, we carefully selected nine SNPs with MAF>0.05 in the Chinese Han population to investigate their possible role in predicting high transport status. The results showed that TIE2 could be a new factor in predicting the baseline transport type. This is the first study to explore the possible association between TIE2 gene polymorphisms and characteristics of peritoneal transport. Rs639225 is located in exon 13 of TIE2. A study reported its association with venous malformation and presumed that this polymorphism might cause abnormal splicing of TIE2 into a defective protein.

Table 7

| OR   | 95% CI      | p Value |
|------|-------------|---------|
| 0.188| 0.044 to 0.806 | 0.024   |
| 0.408| 0.227 to 0.736 | 0.043   |
| 0.966| 0.930 to 1.004 | 0.081   |
| 1.401| 0.519 to 3.788 | 0.506   |
| 3.28 | 0.952 to 11.360| 0.060   |
| 0.996| 0.987 to 1.005 | 0.401   |
| 1.081| 0.964 to 1.212 | 0.182   |
| 0.898| 0.796 to 1.014 | 0.083   |
| 1.061| 0.371 to 1.632 | 0.197   |
| 0.984| 0.796 to 1.014 | 0.192   |

DM, diabetes mellitus; hs-CRP, high-sensitive C reactive protein; IL, interleukin; PET, peritoneal equilibration test.

In conclusion, IL-6 and TIE2 polymorphisms are associated with baseline peritoneal transport property. A functional study of the polymorphisms is required in the future.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethical committee of Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China.

Data sharing statement The technical appendix, statistical code and data set are available from the corresponding author.

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