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Original Article

An allele of CRP promoter region -717 - a susceptible gene for peritoneal dialysis related peritonitis

Jian-Hua Wu*, An-Qi Nie*, Nai-Feng Guo, Xiao-Lan Chen

*Department of Nephrology, Affiliated Hospital of Nantong University, Jiangsu, China. *Equal contributors*

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Abstract: Peritoneal dialysis-related peritonitis is one of the most common complications and the leading cause of failure in peritoneal dialysis. In addition, peritonitis significantly increases hospitalization rates and mortality. To study the relationship between peritoneal dialysis related peritonitis and high-sensitivity C-reactive protein (hs-CRP) and its promoter region -717A > G gene polymorphism, A total of 68 peritoneal dialysis related peritonitis patients and 70 peritoneal dialysis patients without peritonitis and 60 healthy individuals in the control group were enrolled. Serum levels of hs-CRP were measured by turbidimetric immunoassay. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) was used to determine the frequency of CRP promoter -717A > G genotype and its allele frequencies. Compared with non-peritonitis patients, the levels of hs-CRP and serum phosphorus were higher in patients with peritonitis, and hemoglobin and serum albumin were lower ($P < 0.05$). Logistic multivariate regression analysis showed that baseline high hs-CRP levels were risk factors for peritoneal dialysis related peritonitis (OR = 2.228). There were no significant differences in the frequency distribution of AA, AG and GG and allele A and G in genotype -717A > G between the peritoneal dialysis group and the control group ($X^2 = 1.976$ and 1.893, $P > 0.05$). There was no significant difference in the frequency distribution of AA, AG and GG in -717A > G genotype in patients with peritonitis and non-peritonitis ($X^2 = 4.076$, $P > 0.05$), and the frequency distribution of allele A in peritonitis group was higher than that in non-peritonitis group ($X^2 = 4.639$, $P = 0.031$), suggesting that microinflammatory state is common in peritoneal dialysis patients. Elevated hs-CRP may be an independent risk factor for peritoneal dialysis-related peritonitis. The A allele of the CRP promoter region -717 may be a susceptible gene for peritonitis in peritoneal dialysis patients.

Keywords: Peritoneal dialysis, C reactive protein, gene polymorphism

Introduction

Peritoneal dialysis (PD) is one of the main renal replacement therapies for end-stage renal disease (ESRD) [1]. Peritoneal dialysis related peritonitis can lead to peritoneal failure and increase in hospitalization and mortality [2]. Some studies [3, 4] showed that there may be genetic and racial differences in the occurrence of peritoneal dialysis related peritonitis. Hs-CRP is a sensitive index for evaluating inflammation. Its level is related to genotype, and genotype can affect the progress of some diseases [5]. At present, few studies have explored whether hs-CRP is genetically related to peritonitis. Therefore, this study explored the relationship between hs-CRP and its gene polymorphism and peritoneal dialysis related peritonitis, in order to provide a new theoretical basis for revealing its pathogenesis and treatment.

Materials and methods

Research subjects

From January 2016 to December 2018, peritoneal dialysis patients admitted to the Department of Nephrology, Affiliated Hospital of Nantong University, were divided into peritonitis group and non-peritonitis group according to whether there was peritoneal dialysis related peritonitis in the past (within 3 months). Patients enrolled in the non-peritoneal group required dialysis for more than one year. In addition, healthy people were selected as the control group. All the candidates were Han Chinese and had no kinship with each other. Exclusion criteria: (1) non-infectious peritonitis; (2) other active inflammatory diseases occur within the past three months; (3) cardiovascular and cerebrovascular events occur within the

past three months; (4) malignant tumors; (5) mental diseases or an inability to cooperate. All patients were treated with Baxter dual system, Baxter PD4 low calcium (2.5%) lactate glucose (1.5%, 2.5%) dialysate, continuous ambulatory peritoneal dialysis (CAPD) or daytime ambulatory peritoneal dialysis (DAPD). According to the International Association for Peritoneal Dialysis (ISPD) guidelines [6]: (1) abdominal pain and/or turbidity of peritoneal effluent with or without fever; (2) peritoneal effluent white blood cell count > 100/ml, neutrophil ratio > 50%; and (3) pathogenic microorganisms grow in peritoneal effluent culture. With two or more of the above three items, the peritoneal-related peritonitis can be diagnosed.

Observation indicators and detection methods

The baseline clinical data at the time of admission were collected, including gender, age, body mass index (BMI), dialysis age and dialysis mode. Laboratory indicators include hemoglobin (Hb), platelet (Plt), serum albumin (Alb), renal function, whole parathyroid hormone (iPTH) and total cholesterol. Plasma hs-CRP levels were determined by immunoturbidimetric assay according to the kit (Beckman) instructions with a reference value < 3 mg/L.

CRP gene polymorphism determination

(1) Peripheral venous blood EDTA anticoagulation, centrifuged blood cells were stored at -70°C frozen storage. DNA was extracted from the UNIQ-10 column DNA extraction kit (Shanghai Shenggong). (2) Design the hs-CRP promoter region -717A/G gene primer [7], upstream primer: 5'-GAC TCC TGC CTG AAG CTT TAC ATA-3'; downstream primer: 5'-ATA CAT GTG CCA TGC TGG TGT-3'. (3) Amplification was carried out using a PE 9600 PCR instrument (reagents were purchased from Shanghai Biotech). The 50 ul PCR reaction system comprises primers each of 0.5 umol/L, 10×PCR Buffer 5 ul, dNTP 0.2 mmol/L, Taq enzyme 2.0 U and genomic DNA 100 ng. Cyclic parameters: pre-denaturation at 95°C for 5 min, denaturation at 94°C for 1 min, annealing at 62°C for 32 Sec, 72°C for 8 min, a total of 32 cycles. (4) The PCR product was digested with restriction endonuclease Bsh1236 I (FERNMENT), the reaction system was 30 ul, and the PCR product was 10 ul, Bsh1236 I 2 U, and digested at 37°C for 16 h. The digested product was identified by 2% agarose gel electrophoresis containing olfactory ethidium.

Statistical analysis

Normal distribution measurements are represented by mean ± standard deviation ($\bar{X}$±SD), while non-positive distribution measurements are represented by median (quartile spacing). One-way ANOVA was used for comparison among groups, and SNK-q method was used for comparison. Hardy-Weinberg equilibrium test was used to examine the population distribution of polymorphic gene frequencies, and chi-square test was used to compare genotype and allele frequencies among groups. Logistic regression analysis was used for multivariate analysis. The statistical significance was set at $P < 0.05$. SPSS 20.0 statistical software was used for statistical analysis.

Results

General information of patients

A total of 138 patients with peritoneal dialysis were enrolled in this study, of whom 67 (48.5%) were women, with an average age of 48.7±14.2 years. In the control group, there were 60 cases, including 31 females (51.67%), with an average age of 46.5±16.7 years. Among the patients undergoing peritoneal dialysis, 89 cases (64.5%) were treated with CAPD, 49 cases (35.5%) were treated with DAPD, and the median dialysis age was 34.5 months. Compared with the control group, serum creatinine (Scr), hs-CRP, iPTH and serum phosphorus in peritonitis group and non-peritonitis group were higher, while Hb and Alb were lower (all $P < 0.05$). Compared with non-peritonitis group, hs-CRP and serum phosphorus in peritonitis group were higher, Hb and Alb were lower, and the difference was statistically significant. (All $P < 0.05$), as shown in **Table 1**.

Influencing factors of peritonitis

Regarding peritonitis as dependent variable and hs-CRP, serum phosphorus, Hb and Alb as independent variables in univariate analysis, a binary logistic multiple regression analysis was performed. The results showed that hs-CRP entered the regression model ($P = 0.036$), and its OR value was 2.228, 95% CI (0.839, 3.426), as shown in **Table 2**, suggesting that elevated

Table 1. General comparison of 3 groups

Group	Control group (n = 60)	Non-peritoneitis group (n = 70)	Peritonitis group (n = 68)
age (year)	48.5±16.7	47.40±15.00	49.91±13.20
Female (number, %)	31 (51.67%)	35 (50.00)	32 (47.06)
Dialysis age	-	33.8 (18.5, 60.6)	35.1 (20.2, 59.4)
BMI (kg/m^2)	20.96±4.27	21.18±4.80	20.31±5.07
Serum creatinine (umol/L)	73.7±15.3	901.71±392.0*	886.7±385.7*
Hemoglobin (g/L)	138.4±14.2	110.6±14.0**	101.4±12.5*
Platelets (10^9/L)	204.59±48.4	196.0±40.5	200.53±54.6
Serum albumin (mg/L)	46.019±5.64	40.04±4.82*	34.74±3.63$^{*,\#}$
Total cholesterol (mmol/L)	4.55±0.88	4.82±0.92	4.74±1.03
Serum phosphorus (mmol/L)	1.36±0.33	1.64±0.47*	1.78±0.55$^{*,\#}$
hs-CRP (mg/L)	1.55±0.60	3.36±1.37*	4.85±1.80$^{*,\#}$
iPTH (pg/mL)	48 (15, 85)	298.4 (173.5, 468.5)*	316.7 (187.0, 519.9)*

Note: $^*P < 0.05$ compared with the control group, $^{**}P < 0.01$ compared with the control group, $^\#P < 0.05$ compared with the peritonitis group; BMI: body mass index; hs-CRP: hypersensitive C-reactive protein; iPTH: whole parathyroid hormone.

Table 2. Multivariate Logistic regression analysis of peritoneal-related peritonitis

	β	*SE*	*OR*	*Wald*	*95% CI*	*P*
Hemoglobin	2.335	0.667	2.112	6.232	0.543-2.104	0.076
Serum albumin	4.213	0.834	0.783	4.001	0.321-1.255	0.064
Serum phosphorus	0.865	0.357	1.210	3.228	0.564-2.026	0.122
hs-CRP	1.654	0.752	1.765	9.442	0.839-3.426	0.036

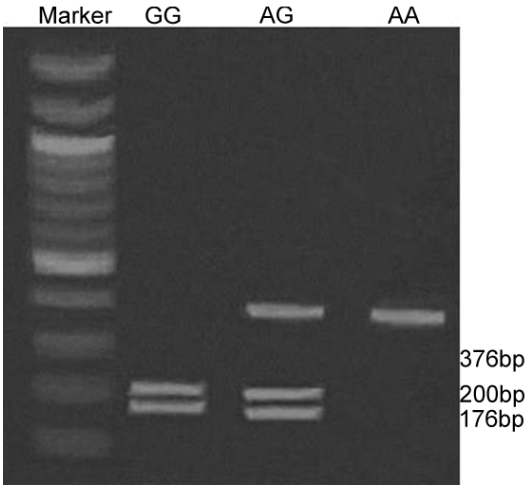

Figure 1. Electropherogram of Genotypes.

hs-CRP at baseline may be a risk factor for peritoneal-related peritonitis.

Genotype and allele frequency comparison

The PCR amplification product was 376 bp, and the Bsh1236I cleavage site 5'...CG↓CG...3' was also a mutation site of -717A/G, and the fragments obtained by digestion were 200 bp and 176 bp, respectively. According to the electrophoresis band analysis, the -7117A/G genotype was 376 bp for the AA band, 376 bp, 200 bp and 176 bp for the AG type, and the GG type bands were 200 bp and 176 bp, respectively (**Figure 1**). The genotype distribution of the three groups was consistent with the Hardy-Weinberg genetic equilibrium law (X^2 values were 1.530, 1.607 and 0.508, respectively, $P > 0.05$).

There was no significant difference in frequency distribution of genotype AA, AG, GG and allele A and G between the peritoneal dialysis group (peritonitis group and non-peritonitis group) and the control group (X^2 value was 1.976 and 1.893, $P > 0.05$), while the frequency distribution of genotype AA, AG and GG in the peritonitis group was different from that in the non-peritonitis group ($P > 0.05$). There was no significant difference ($X^2 = 4.076$, $P > 0.05$), and the frequency distribution of allele A in peritonitis group was higher than that in non-peritonitis group ($X^2 = 4.639$, $P = 0.031$), as shown in **Table 3**.

Genotype and hs-CRP

In order to determine whether the genetic background of the study participants affects the expression of inflammatory factors, we grouped the participants according to the genotype, and further analyzed the relationship between CRP

Table 3. Frequency distribution of CRP -717A > G genotypes and alleles in the three groups

Group	Number	genotypes [n(%)]			alleles [n(%)]	
		AA	AG	GG	A	G
peritonitis group	68	54 (79.41)	12 (17.65)	2 (2.94)	120 (88.24)	16 (11.76)
non-peritonitis group	70	45 (64.29)	20 (28.57)	5 (7.14)	110 (78.57)	30 (21.43)
control group	60	37 (61.66)	19 (31.67)	4 (6.67)	93 (77.50)	30 (25.00)

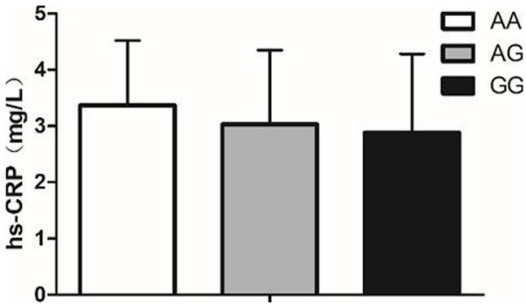

Figure 2. hs-CRP in AA AG and GG genotype groups.

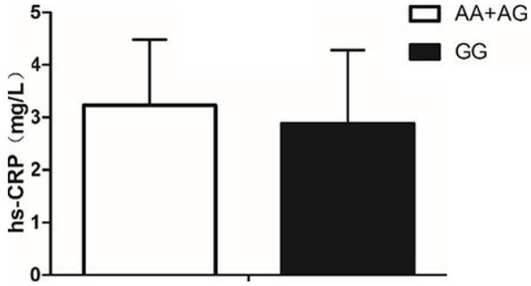

Figure 3. hs-CRP in AA+AG and GG genotype groups.

genotype AA, Ag, GG and hs-CRP concentration. The results showed that there was no significant difference in hs-CRP concentration among the three genotypes (*P > 0.05*), as shown in **Figure 2**. In order to determine whether the alleles of the participants in this study affect the expression of inflammatory factors, we compared the relationship between the concentration of hs-CRP between AA+Ag genotype and GG genotype. The results showed that the serum hs-CRP of individuals with AA+Ag genotype was higher than that of GG genotype, but the difference was not statistically significant (*P > 0.05*), as shown in **Figure 3**.

Discussion

CKD patients show microinflammation with the decline of renal function [8]. Decreasing hs-CRP level can delay the progress of cardiovascular disease and improve survival rate [9]. Similar to the results of previous studies [10, 11], we found that the baseline hs-CRP of peritoneal dialysis patients was higher than that of the control group regardless of whether they had been infected with peritonitis or not. Compared with the non-peritonitis group, the hs-CRP level of peritonitis group was higher. It is suggested that hs-CRP, as an important pro-inflammatory mediator, may partially participate in the progress of chronic kidney disease, and the elevated baseline hs-CRP may be one of the risk factors for peritoneal-related peritonitis. The US Kidney Data System [12] and China multi-center combined cross-sectional survey [13] showed that about 60% of peritoneal dialysis patients had malnutrition manifestations such as anemia and hypoalbuminemia. Our results are similar to the above, and we found that anemia and hypoalbuminemia were more pronounced in patients with previous peritonitis. Clinically, microinflammation is one of the important causes of malnutrition in patients undergoing peritoneal dialysis. Inflammation significantly increases the occurrence of complications such as hypoalbuminemia, anemia and atherosclerotic plaque formation [9, 14]. At present, the research on CRP gene polymorphism mainly focuses on cardiovascular and cerebrovascular diseases. Whether the hs-CRP level is affected by genetics is still inconclusive, and there are few studies on the relationship between hs-CRP and peritoneal peritonitis. Some scholars [7] found that CRP +2147A > G and -717A > G genotypes did not affect CRP levels. However, Kovacs et al. [15] believe that CRP-286 gene polymorphism is associated with CRP levels in patients with coronary heart disease. Hung et al. [16] found that the CRP gene rs2808630 site mutation is associated with hs-CRP levels and proteinuria in non-Hispanic black populations. Our study did not find a correlation between CRP genotypes AA, AG and GG and hs-CRP concentration. We further found that serum hs-CRP levels

in AA+AG individuals were higher than those in GG, but the difference was not statistically significant. We did not find any difference in the frequency distribution of AA, AG and GG genotypes and allele A and G frequencies between the peritoneal dialysis group and the control group. It is possible that the -717A > G locus and other genetic polymorphisms may affect the occurrence of chronic kidney disease in the genetic linkage imbalance. We compared the frequencies of AA, AG and GG genotypes of -717A > G with those of non-peritonitis, the frequencies of allele A in peritonitis group were higher than those in non-peritonitis group. Our results suggest that the A allele may be a susceptibility gene for peritonitis in patients with peritoneal dialysis. Because of the small number of participants in this study, a multicenter prospective study by haploid or increased detection sites will further elucidate the role of CRP gene mutations in the development of peritoneal-related peritonitis.

In summary, there is a microinflammation state in patients with peritoneal dialysis. The peritoneal-related peritonitis may be associated with higher baseline hs-CRP, and the CRP promoter region -717 A allele may be a susceptibility gene for peritonitis. Therefore, patients with peritoneal dialysis who have high levels of hs-CRP and microinflammation should be given special attention and early treatment.

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Disclosure of conflict of interest

None.

Address correspondence to: Xiao-Lan Chen, Department of Nephrology, Affiliated Hospital of Nantong University, No. 20 Xisi Road, Chongchuan District, Nantong 221000, Jiangsu, China. Tel: +86 0513 81160202; Fax: +86 0513 85052236; E-mail: chenxl8448@sina.com

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