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Clinical study of serum HCY and MTHFR gene C677T polymorphism in patients with uremia

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Abstract: This study aims to elucidate the relationship between serum homocysteine (HCY) levels, methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism, and the prevalence of H-type hypertension and chronic kidney disease (CKD) in uremic patients in the Jiangjin region of Chongqing city. A cohort of 180 uremic patients admitted to our hospital between January 2020 and December 2023 was selected. The patients were categorized into three groups: H-type hypertension CKD group (n=90), common hypertension CKD group (n=43), and normal blood pressure CKD group (n=47). MTHFR C677T gene polymorphism was detected using Sanger sequencing, and HCY levels were measured. The frequencies of the CT and TT genotypes and the T allele were significantly elevated in the H-type hypertension group compared to the common hypertension and normal blood pressure groups ($P < 0.05$). The serum HCY level in the TT genotype group was higher than in the CC and CT genotype groups. Multiple linear regression analysis demonstrated a positive correlation between serum HCY levels and creatinine ($P<0.005$) and a negative correlation with glomerular filtration rate (eGFR) ($P<0.005$). No significant correlation was found between MTHFR genotype and serum creatinine (Scr) or eGFR ($P>0.05$). In conclusion, the MTHFR C677T gene mutation is associated with elevated serum HCY levels and H-type hypertension in uremic patients. Elevated HCY levels are a risk factor for CKD development, although the gene mutation itself may not be directly implicated in the onset and progression of chronic kidney disease.

1. Introduction

The disease burden caused by Chronic Kidney Disease (CKD) is rapidly increasing and has become a growing public health problem worldwide, becoming the fifth leading cause of death worldwide[1]. About 50% of patients with End Stage Renal Diseases (ESRD) may die from cardiovascular events [2]. High Hcy is an independent risk factor for cardiovascular and cerebrovascular diseases. Uremia patients are often accompanied by renal hypertension, which is

difficult to be controlled. Hypertension accompanied by high Hcy is at greater risk, and the two coexist and have a synergistic effect, which significantly increases the risk of stroke, myocardial infarction and other diseases [3]. By detecting serum HCY level and MTHFR gene polymorphism in uremia patients of Han ethnic group in Jiangjin area of Chongqing, this study explored the correlation between Hcy level and H-type hypertension in uremia patients, as well as its role in the occurrence and development of chronic kidney disease, so as to provide evidence for the prevention and treatment of uremia.

2. Data and methods

2.1 Cases collection and grouping

A total of 180 uremia patients collected from the outpatient and inpatient departments of our hospital from January 2020 to December 2023 were selected. All of them were Han ethnic group, including 85 males and 95 females, aged 15-93 years old, with an average age of (60.43±13.09) years old. In this study, uremia patients were divided into three groups according to differences in blood pressure and Hcy level, namely, H-type hypertension CKD group (90 cases), normal hypertension CKD group (43 cases) and normal blood pressure CKD group (47 cases). There was no statistical significance in age and gender between patients ($P > 0.05$), and there was no comparability. Inclusion criteria: The diagnosis of uremia was in accordance with the Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Diseases formulated by the American Kidney Foundation in 2012 [4]. For patients who have hemodialysis treatment for more than 3 months, they can receive routine hemodialysis treatment, is expected to survive more than 6 months; Those patients are all informed and give consent to the study, and signed the consent document. This study is approved by the Medical Ethics Committee. Exclusion criteria: recent use of drugs affecting Hcy, such as folic acid, methotrexate, vitamin B6, vitamin B1, etc. patients are complicated with tumor and infected persons; patients are unable to complete the interview. The diagnostic criteria for hypertension are systolic blood pressure≥140 mmHg and/or diastolic blood pressure>90 mmHg twice on different days. The diagnostic criterion for elevated Hcy was fasting Hcy level≥15 μmol /L.

2.2 Specimen collection and testing

At least 3-5 ml of fasting peripheral venous blood was collected from patients in the early morning before dialysis, thoroughly mixed with a tube of ethylenediamine tetraacetic acid anticoagulant and frozen at -80℃ for genomic DNA extraction. One tube of centrifuged serum was stored for biochemical and serum Hcy detection.

The Hcy level was detected by enzyme cycle method and biochemical analyzer.

2.3 Analysis of MTHFR gene C677T polymorphism

MTHFRC677T was genotyped by Sanger sequencing. Primers used online Primer3 software design (http://bioinfo.ut.ee/primer3-0.4.0/). The PCR products were purified by SAP (from Promega) and EXO I(from Epicentre) and then sequenced with ABI's BigDye3.1 kit. The sequencing reaction was purified by alcohol and then samples were prepared on ABI3730. ABI3730XL sequencer was used to analyze the sequencing files using Polyphred software, and the results were sorted out after manual proofreading of the records.

2.4 Statistical analysis

SPSS 20.0 statistical software was used for statistical analysis. Hardy-Weinberg law of genetic balance was used to detect population representativeness of samples. Measurement data were expressed as ($x \pm s$), and T-test was used for comparison between groups. The statistical data were expressed as [n (%)], and $\chi 2$ test was used for the two comparisons. Binary logistic regression was used for multivariate analysis, and $P<0.05$ was considered statistically significant.

3. Results

3.1 Results of MTHFRC677T gene amplification

The electrophoretic results of prim-specific PCR products were the expected bands, which were verified as target sequences by Sanger sequencing (see Figure 1). Three genotypes were detected by Sanger sequencing MTHFRC677T genotype analysis, and the distribution was CC type 65 cases, CT type 80 cases, TT type 35 cases.

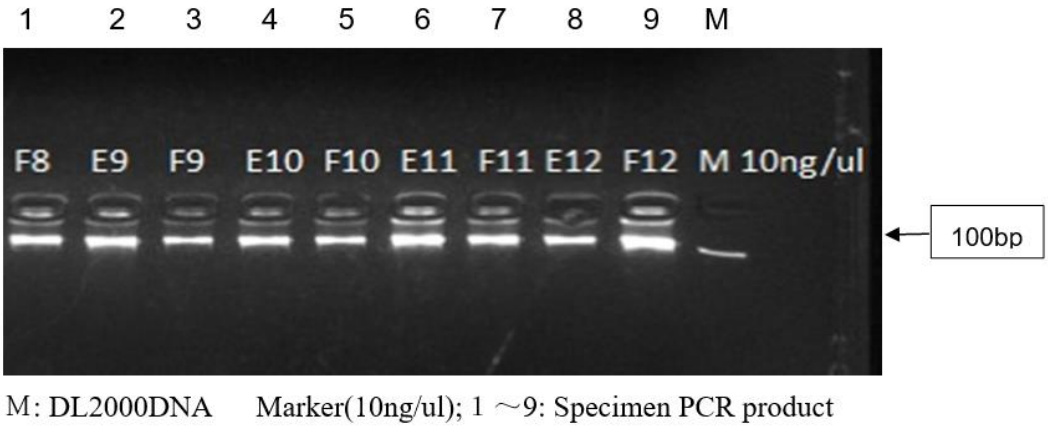

Figure 1 Electrophoretic image of MTHFR gene amplification product

3.2 Genotyping of three groups of patients

Table 1 Polymorphism ratio of MTHFR C677T in three groups (n= number of cases)

Group	n	Genotype			Allele	
		CC	CT	TT	C	T
H type hypertension group	90	25(27.8%)	38(42.2%)	27(30%)	44 (48.9%)	46 (51.1%)
Common hypertension group	43	23(53.5%)	17(39.5%)	3(7%)	31.5(73.3%)	11.5(26.7%)
Normal blood pressure group	47	17(36.2%)	25(53.2%)	5(10.6%)	29.5(62.8%)	17.5(37.2%)
χ^2		1.6	8.43	30.40	1.83	9.67
P		0.449	0.015	0.000	0.425	0.003

The results of three groups showed that the distribution frequency of MTHFR C677T genotype in the two groups was consistent with Hardy-Weinberg genetic balance ($\chi 2=1.322$, $P=0.25$). The comparison of polymorphism frequencies of MTHFR C677T among the three groups is shown in Table 1. Except for CC genotype, CT and TT genotype frequencies were significantly different among the three groups ($P < 0.05$). The frequency of CT and TT genotype and the frequency of T allele in

H-type hypertension group were significantly higher than those in ordinary hypertension and normal blood pressure groups, and the differences were statistically significant (P<0.05).

3.3 Comparison of serum Hcy concentration between different genomes

Serum Hcy concentration was (16.78 ±5.99) μmol/L in CC genotype group, (19.82±6.84) μmol/L in CT genotype group and (28.27±11.95) μmol/L in TT genotype group. The serum Hcy concentration in TT genotype group was higher than that in CC genotype group and CT genotype group, and the difference was statistically significant (P<0.01). The serum Hcy concentration in CT genotype group was higher than that in CC genotype group, and the difference was statistically significant (P<0.01), as shown in Table 2.

Table 2 Comparison of serum Hcy concentration between different genomes

Group	Number of Cases	Serum Hcy concentration (μmol/L)
CC	65	16.78 ±5.99 a
CT	80	19.82 ±6.84b
TT	35	28.27 ±11.95c
F		24.842
P		0.000

Note: Compared with CT group, a $P < 0.05$; Compared with TT group, b$P < 0.05$; The comparison between CC group and TT group showed that c$P < 0.05$.

3.4 Correlation analysis of Hcy, MTHFR C677T genotype and renal function

Multiple linear regression analysis was performed for Hcy and MTHFR C677T genotypes with creatinine (Scr) and eGFR: Using Scr and eGFR as continuous dependent variables and natural logarithm transformation, Hcy and genotype as independent variables. The analysis showed that Hcy was positively correlated with Scr ($P < 0.05$) and negatively correlated with eGFR ($P < 0.05$). There was no significant correlation between MTHFRC677T genotype and Scr and eGFR ($P > 0.05$), as shown in Table 3.

Table 3 Correlation analysis of Hcy and MTHFRC677T genotypes with Scr and eGFR

Independent variable	Scr					eGFR				
	β	SE	*P*	OR Value	95%CI	β	SE	*P*	OR Value	95%CI
HCY<15	1.0	—	—	—	—	—	—	—	—	—
HCY≥15	0.186	0.013	0.016*	1.832	1.263-3.435	-0.124	0.018	0.015*	1.762	1.453-3.476
CC Type	1.0	—	—	—	—	—	—	—	—	—
CT Type	0.014	0.035	0.483	0.965	0.768-1.076	-0.017	0.026	0.325	0.763	0.764-1.068
TT Type	-0.012	0.025	0.465	0.612	0.365-1.254	0.014	0.032	0.432	0.753	0.426-1.314

* represents a significance level of <5%

4. Discussion

Hcy is a product of methionine metabolism in the body, also known as "garbage amino acid", and its own oxidation will also produce a large amount of free oxygen. High Hcy can induce local oxidative stress, interfere with the production of NO, mediate the loss of important endothelial antioxidant system, and increase the concentration of ROS, thus generating oxidative stress, resulting in endothelial cell damage and damage to organs, tissues and cells [5].

In recent years, foreign studies have suggested that high Hcy is an important factor in the

occurrence and development of CKD, as well as a key factor and independent predictor of the occurrence of cardiovascular complications of CKD, thus leading to a decline in the survival rate of patients with CKD[6-7]. A prospective cohort study conducted by domestic Kong et al.[8] in 5917 Chinese middle-aged and elderly population showed that high Hcy level was a risk factor for eGFR decline and could be used to predict kidney injury. The proportion of Hcy increase in patients with CKD is about 36% to 89%, and in patients with ESRD, the proportion can reach 85% to 100%[9]. In this study, the detection results of 180 patients who had undergone blood purification showed that the proportion of Hcy increase was about 70%, which was similar to the domestic references reports. In addition, with the increase of serum Hcy level, the higher the serum creatinine level of patients is, the more obvious the decrease of eGF is, which once again confirms that the high serum Hcy level of uremia patients in this region is significantly correlated with the occurrence and development of H-type hypertension and CKD, and early intervention should be actively carried out.

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the metabolic process of Hcy. Mutation of MTHFR 677C /T gene locus can affect the metabolism of Hcy, resulting in the reduction of heat resistance and activity of the enzyme by about 50%, resulting in the increase of Hcy level and the risk of cardiovascular and cerebrovascular diseases [10]. Most previous studies in patients with hypertension and stroke have suggested that MTHFR C677T gene polymorphism and serum Hcy level are independent risk factors for the occurrence and development of H-type hypertension[11-14]. However, there are few relevant studies in ESRD patients, no relevant reports have been seen in China, and there are controversies in foreign studies. Trovato et al.[15] found that among 630 Italian Caucasian subjects, MTHFR 677 C>T and 1298 A>C occurred less frequently in ESRD patients receiving hemodialysis, suggesting that these gene variants may have a protective effect on renal function progression. This study suggested that the TT gene mutation rate of ESRD patients was about 19.4%, accounting for 30% in the H-type hypertension group, which was significantly higher than 16% in the Italian Caucasian, indicating racial differences. Although the serum Hcy level of TT genotype was higher than that of CC and CT genotype, suggesting that this variant could affect Hcy metabolism and lead to high Hcy level, no correlation was found between the MTHFR 677 C>T genetic variant and Cr and eGFR. This may be due to the fact that we only selected part of the Han ethnic group with uremia in the region for the study, and the sample size was insufficient, which failed to reflect the situation of the entire ethnic group, and the population analysis needs to be further expanded.

In summary, MTHFR gene C677T genetic variation is significantly associated with elevated serum Hcy level and H-type hypertension in uremia patients in Jiangjin area of Chongqing, and high Hcy level is a risk factor for the occurrence of CKD. However, whether MTHFR gene C677T genetic variation is related to the occurrence and development of chronic kidney disease remains to be further explored.

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