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Original Article

Correlation between MTHFR gene polymorphism and homocysteine levels for prognosis in patients with pregnancy-induced hypertension

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Abstract: Objective: This research was designed to probe into the correlation between MTHFR gene polymorphisms and homocysteine levels in regard to the prognosis of pregnancy-induced hypertension. Methods: A total of 180 patients with pregnancy-induced hypertension who were admitted in the gynecology and obstetrics department of our hospital were collected as the observation group, and 180 normal pregnant women were selected as the control group. The homocysteine (Hcy) level, polymorphism expression of methylenetetrahydrofolate reductase (MTHFR) gene C677T locus and A1298C locus and the correlation between the different gene loci, Hcy level and pregnancy outcome were observed. Results: The Hcy level in the observation group was 18.1±6.2 (100 mmol/L) which was higher than that in the control group (8.6±3.9 mmol/L) (P<0.001). The C677T polymorphism of the MTHFR gene, and the proportion of CC in the observation group was lower than that in the control group, while that of CT and TT in the observation group was significantly higher (P<0.001). The T allele in the observation group was higher than that in the control group, while the C allele was lower than that in the control group (P<0.001). Hcy of TT type in pregnancy-induced hypertension group was higher than that in CC and CT groups (P<0.05). The incidence of adverse pregnancy outcomes in pregnancy-induced hypertension patients was obviously higher than that in normal control group (P<0.01). The incidence of TT type adverse pregnancy outcomes in MTHFR gene C677T polymorphism in patients with gestational hypertension was significantly higher than that in CC and TC groups (P<0.01). Conclusion: The Hcy level in pregnancy-induced hypertension patients and the proportion of CT and TT in the MTHFR gene C677T locus rose; having the TT-type increased the incidence of abnormal pregnancy, which may be related to the increase of Hcy level.

Keywords: Pregnancy-induced hypertension, MTHFR gene polymorphism, homocysteine, correlation, prognosis

Introduction

Pregnancy-induced hypertension (PIH) is a unique disease that occurs in women during pregnancy. Most of the cases of this disease occur after 20 weeks of pregnancy, and its morbidity accounts for 9.4% of all pregnant women. It is an important factor leading to poor outcomes and death [1]. During the occurrence and development of PIH, pregnant women may have elevated blood pressure, systemic edema and proteinuria, which may further lead to retinopathy, abnormal coagulation function and even multiple organ failure [2-4]. At the moment, the pathogenesis of pregnancy-induced hypertension is complicated, but the role of vascular endothelial injury in the occurrence and development of pregnancy-induced hypertension has been recognized [5, 6]. Previous studies have shown that homocysteine (Hcy) is relevant to vascular endothelial injury, and its level increases in patients with pregnancy-induced hypertension. It is considered that Hcy level plays an important role in the occurrence and development of pregnancy-induced hypertension, and the expression of Hcy in human body is regulated by methylenetetrahydrofolate reductase (MTHFR) gene. The decrease of MTHFR activity can easily increase the concentration of homocysteine in the blood, causing vascular endothelial injury and abnormal vascular endothelial dysfunction, leading to

Table 1. qRT primer sequence numbers

Primer	Forward primers 5'-3'	Reverse primers 5'-3'
MTHFR gene C677T locus	ACAATGTTTAATCCGGTGCCT	CCTCACCTGGATGGGAAAGAT
MTHFR gene A1298C locus	TGAAGAAAGTGTCT	TGAAGCAAGTGTCT

Note: MTHFR: methylenetetrahydrofolate reductase.

the disorders of the coagulation system and fibrinolysis system, and making the body more prone to be in a hypercoagulable state [7]. With the improvement of research technology, more and more clinical attention has been paid to the role of human genes in the pathogenesis of diseases. Genetics has become a target for the diagnosis and treatment of related diseases. To improve new ideas, previous studies have shown that the MTHFR gene polymorphism is closely related to early spontaneous abortion [7]. However, there are regional and population differences in the distribution of MTHFR 6770T and 1298A>C gene polymorphism in different regions and ethnic groups in North and South China [8]. Based on this, this research we included pregnant women in our hospital in the Hainan Province to study the correlation between MTHFR gene polymorphism and Hcy expression and its influence on the prognosis of patients with pregnancy-induced hypertension. The report is as follows.

Materials and methods

General data

A total of 180 patients with pregnancy-induced hypertension admitted to the gynecology and obstetrics department in our hospital from July 2017 to December 2020 were collected as the observation group. The observation group was divided into poor and normal pregnancy groups according to the pregnancy outcomes. Among 1082 normal pregnant women in the same period, 180 patients who were age matched were considered as the control group. The ages were 20 to 42 years old, with an average age of 25.2±5.8 years. All the above-mentioned pregnant women signed the informed consent form. This research has been approved by the Ethics Committee of our hospital.

Inclusion and exclusion criteria

The inclusion criteria of the observation group were as follows: (1) those who meet the diagnostic criteria of pregnancy-induced hypertension (stages I and II) [9]; (2) aged between 18 and 45 years old; (3) all the women involved are primiparas; (4) singleton pregnancy; (5) gestational age ≥20 weeks; (6) patients have good compliance.

The exclusion criteria were as follows: (1) multiple pregnancy; (2) those with severe heart, liver, kidney and other diseases; (3) those with gestational diabetes; (4) severe coagulation disorders; (5) those with a history of hypertension; (6) there was hyperhomocysteinemia in the past. The control group included normal primiparas and singleton pregnancies in the same period.

Methods

Two tubes of venous blood from the elbow were collected from each subject after admission, and the collected blood samples were stored in ethylene diamine tetraacetic acid sterile tube (Labware Technology (Shenzhen) Co., Ltd., item number: 1186 d). They were stored in a freezer at 4°C for 15 min, and then serum and plasma were separated by a centrifuge with centrifugal force of 1106.8 (xg). Finally, they were stored in a freezer at -80°C and detected by an automatic biochemical analyzer (Hitachi 7600-020 E). Hcy was detected by fluorescence real-time quantitative polymerase chain reaction (q-PCR). In this research, ABI 7500 real-time fluorescence quantitative PCR instrument (ABI Applied Biosystems, USA) and Trizol kit (Molecular Research Center, USA) were used. The upstream and downstream primers were designed and provided by Guangzhou Ruibo Biotechnology Co., Ltd. Then, the polymorphism of C677T and A1298C of MTHFR gene was determined by RT-PCR using kit (Fernentas, Canada) and dual fluorescence quantitative PCR. The primer sequence numbers were shown in **Table 1**.

Outcome measures

Main outcome measures: The correlation between polymorphism of C677T and A1298C of the MTHFR gene and pregnancy outcomes in the pregnancy-induced hypertension group was observed. Pregnancy outcome refers to the adverse reactions of pregnant women dur-

Table 2. Comparison of general information of patients in both groups (n/%, $\bar{x} \pm sd$)

Items	Observation group (n = 180)	Control group (n = 180)	χ^2/t	P
Age (year)	25.8±5.8	25.1±5.0	1.226	0.221
BMI (kg/m^2)	27.21±5.43	27.33±5.52	0.208	0.835
Systolic blood pressure (mmHg)	148.3±10.8	115.6±7.3	33.651	<0.001
Diastolic blood pressure (mmHg)	98.2±7.2	75.4±6.3	31.971	<0.001
Pregnancy times (n)			0.229	0.633
First time	156	159		
Again	24	21		
Week of pregnancy (weeks)	25.2±4.3	25.8±4.8	1.249	0.212
Hypertension grading (n)				
Mild preeclampsia	98			
Severe preeclampsia	82			
Accompanying symptoms (n)				
Edema	79			
Proteinuria	65			
Hematuria	54			
Abnormal kidney function	49			

ing pregnancy. Incidence of adverse reactions = number of adverse reactions/total number of cases ×100%.

The correlation between polymorphisms of the MTHFR gene at C677T and A1298C, and Hcy level in the pregnancy-induced hypertension group was observed.

Secondary outcome measures: The Hcy levels were observed.

Polymorphism expression of the MTHFR gene at the C677T locus and A1298C locus in this study population was assessed.

Statistical indicators

The collected data were statistically processed by SPSS 17.0 statistical software. The measurement data were expressed by the mean ± standard deviation ($\bar{x} \pm sd$). Those that were consistent with a normal distribution and variance homogeneity were analyzed by independent-samples t-test and expressed by t. While those that did not conform to a normal distribution and variance homogeneity were assessed by rank sum test and represented by Z. At the same time, the pregnancy outcomes were evaluated by multivariate Logistic regression. The counting data were examined by Pearson chi-square test, expressed in chi-square. P<0.05 denotes that the difference is statistical remarkable.

Results

Comparison of general data

The systolic blood pressure and diastolic blood pressure in the observation group were higher than those in the control group (P<0.001), and the other data had no statistical difference (P>0.05, **Table 2**).

Comparison of Hcy levels between both groups

The Hcy level in the observation group was 18.1±6.2 (100 mmol/L), higher than that in the control group (8.6±3.9 mmol/L) (P<0.001, **Figure 1**).

Comparison of polymorphism of C677T locus of MTHFR gene between both groups

In the polymorphism of the C677T locus of the MTHFR gene, compared with the control group, the proportion of CC in the observation group was lower, while that of CT and TT was higher (P<0.001). Besides, the T allele in the observation group was higher but the C allele was lower (P<0.001, **Table 3**).

Comparison of polymorphism of MTHFR gene A1298C locus between both groups

There was no difference in polymorphism of the A1298C locus of the MTHFR gene between the two groups (P>0.05, **Table 4**).

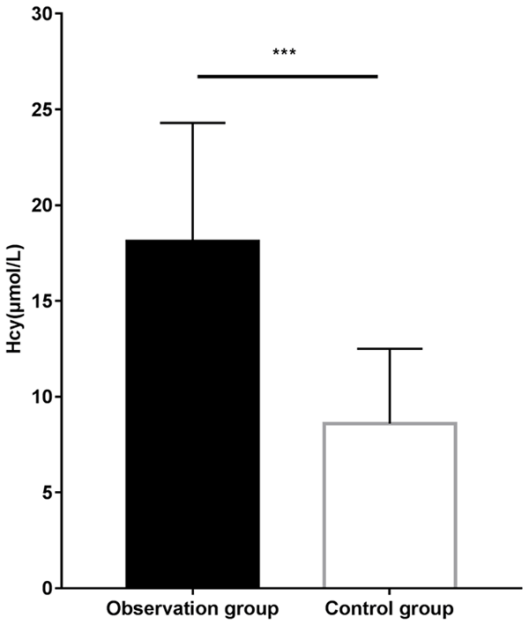

Figure 1. Comparison of Hcy levels between both groups. Compared with control group, ***P<0.001. Hcy: homocysteine.

Relationship between MTHFR gene polymorphism and Hcy level in patients with pregnancy-induced hypertension

The Hcy of the TT type in the pregnancy-induced hypertension group was higher than that in CC and CT groups (P<0.05, **Table 5**).

Comparison of pregnancy outcomes between pregnancy-induced hypertension patients and normal pregnant women

The incidence of premature delivery, uterine inertia, neonatal asphyxia, fetal distress and postpartum hemorrhage in pregnancy-induced hypertension patients was remarkably higher than that in normal control group (P<0.01, **Table 6**).

Comparison between polymorphism of C677T locus of MTHFR gene and pregnancy outcome of pregnant women with gestational hypertension

The incidences of adverse pregnancy outcomes such as TT preterm delivery, uterine atony, neonatal asphyxia, fetal distress and postpartum hemorrhage in the MTHFR gene C677T polymorphism in patients with gestational hypertension were significantly higher than those in the CC and TC groups (P<0.01, **Table 7**).

Comparison between polymorphism of A1298C locus of MTHFR gene and pregnancy outcome of pregnant women with gestational hypertension

There was no marked difference in the incidence of adverse pregnancy outcomes among the different genotypes of MTHFR gene A1298C polymorphism in patients with gestational hypertension (P>0.05, **Table 8**).

Comparison of general data between poor and normal pregnancy groups

According to the pregnancy outcomes, pregnant women with pregnancy-induced hypertension were further divided into poor and normal pregnancy groups. The incidence of systolic blood pressure, diastolic blood pressure, severe preeclampsia and abnormal renal function in the poor pregnancy group were higher than those in normal pregnancy group. The polymorphism of the C677T locus of the MTHFR gene was different (all P<0.05, **Table 9**).

Multivariate logistic regression analysis of pregnancy outcomes

Taking the pregnancy outcome as the dependent variable, the variables with differences in univariate analysis were selected. Systolic blood pressure, diastolic blood pressure, hypertension grade, abnormal renal function and polymorphism of the C677T locus of the MTHFR gene were regarded as independent variables systolic blood pressure (systolic blood pressure ≥148.3 mmHg (mean value) is the limit, diastolic blood pressure ≥98.2 mmHg (mean value) is the limit). After variable screening by stepwise forward (Ward) method, multivariate Logistic regression analysis revealed that the polymorphism of the C677T locus of the MTHFR gene was an independent factor leading to poor pregnancy outcomes (P<0.01, **Tables 10** and **11**).

Discussion

Hypertension during pregnancy is a common disease of pregnant women. As pregnancy hypertension becomes aggravated, it will cause organ damage and poor pregnancy outcomes [10, 11]. At present, the pathogenesis of pregnancy-induced hypertension is still vague. With the development of molecular biology technol-

Table 3. Comparison of polymorphisms at the C677T locus of the MTHFR gene between the two groups of patients (n/%)

Group	Observation group (n = 180)	Control group (n = 180)	χ^2	P
C677T genotype frequency (n/%)			26.931	<0.001
CC	82 (45.56)	130 (72.22)		
CT	76 (42.22)	36 (20.00)		
TT	22 (12.22)	14 (7.78)		
Allele frequency (n/%)			22.894	<0.001
C	240 (66.67)	296 (82.22)		
T	120 (33.33)	64 (17.78)		

Note: MTHFR: methylenetetrahydrofolate reductase.

Table 4. Comparison of polymorphisms at the A1298C locus of the MTHFR gene between the two groups of patients (n/%)

Group	Observation group (n = 180)	Control group (n = 180)	χ^2	P
A1298C genotype frequency (n/%)			1.049	0.592
AA	106 (58.89)	104 (57.78)		
AC	68 (37.78)	66 (36.67)		
CC	6 (3.33)	10 (5.55)		
Allele frequency (n/%)			0.282	0.595
A	280 (77.78)	274 (76.11)		
C	80 (22.22)	86 (23.89)		

Note: MTHFR: methylenetetrahydrofolate reductase.

Table 5. Correlation between MTHFR gene polymorphisms and Hcy levels in patients with gestational hypertension ($\bar{x} \pm sd$)

	Hcy (μmol/L)	Z	P
C677T genotype frequency (n/%)		3.031	0.049
CC (n = 82)	16.8±6.3		
CT (n = 76)	16.8±6.6		
TT (n = 22)	20.4±6.9*,#		
A1298C genotype frequency (n/%)		0.048	0.953
AA (n = 106)	17.5±6.5		
AC (n = 68)	17.8±5.7		
CC (n = 6)	17.6±7.0		

Note: Compared with CC group, *P<0.05; compared with CT group, #P<0.05. MTHFR: methylenetetrahydrofolate reductase.

Table 6. Pregnancy outcomes in patients with hypertension during pregnancy compared with normal pregnant women (n/%)

Group	Observation group (n = 180)	Control group (n = 180)	χ^2 value	*P* value
Preterm birth	41 (22.78%)	7 (3.89%)	27.788	<0.001
Weak contractions	36 (20.00%)	5 (2.78%)	26.452	<0.001
Neonatal asphyxia	27 (15.00%)	7 (3.89%)	12.992	<0.001
Intrauterine distress	32 (17.78%)	11 (6.11%)	10.357	<0.001
Postpartum hemorrhage	31 (17.22%)	9 (5.0%)	13.613	<0.001
Other	20 (11.11%)	7 (3.89%)	6.767	0.009

ogy, the research on genetic pathogenesis of pregnancy-induced hypertension patients has been deepening.

In this research, the Hcy level in patients with pregnancy-induced hypertension increased, suggesting that there was abnormal metabolism. Previously, research has shown that vascular endothelial injury plays a vital role in the pathogenesis of pregnancy-induced hypertension and cardiovascular and cerebrovascular diseases. Hcy is an amino acid relevant to vascular endothelial injury [12]. It has been reported that it is at a high level *in vivo*. Under oxidation conditions, more hydrogen peroxide and superoxide ion free radicals are produced. These produced substances can easily cause damage to

Table 7. Comparison of MTHFR gene C677T locus polymorphism with pregnancy outcomes in pregnant women with gestational hypertension (n/%)

Group	CC (n = 82)	TC (n = 76)	TT (n = 22)	χ^2	P
Preterm birth	13 (28.05%)	17 (22.37%)	11 (50.00%)	11.511	0.003
Weak contractions	15 (18.29%)	12 (23.68%)	9 (40.91%)	7.003	0.003
Neonatal asphyxia	10 (12.20%)	9 (11.84%)	8 (36.36%)	8.976	0.011
Intrauterine distress	12 (14.63%)	11 (14.47%)	9 (40.91%)	9.175	0.001
Postpartum hemorrhage	13 (15.85%)	8 (10.53%)	10 (45.45%)	14.798	0.001
Other	7 (8.54%)	6 (7.89%)	7 (31.82%)	11.312	0.003

Note: MTHFR: methylenetetrahydrofolate reductase.

Table 8. Comparison of MTHFR gene C677T locus polymorphism and pregnancy outcomes with pregnant women with gestational hypertension (n/%)

Group	AA (n = 106)	AC (n = 68)	CC (n = 6)	χ^2 value	*P* value
Preterm birth	24 (22.64%)	15 (22.06%)	2 (33.33%)	0.401	0.818
Weak contractions	21 (19.81%)	14 (20.59%)	1 (16.67%)	0.059	0.971
Neonatal asphyxia	16 (15.09%)	10 (14.71%)	1 (16.67%)	0.018	0.991
Intrauterine distress	18 (16.98%)	12 (17.65%)	2 (33.33%)	1.342	0.511
Postpartum hemorrhage	18 (16.89%)	12 (17.65%)	1 (16.67%)	0.143	0.931
Other	13 (8.54%)	7 (7.89%)	0 (0.00%)	0.939	0.625

Note: MTHFR: methylenetetrahydrofolate reductase.

the vascular endothelium and promote the formation of vascular smooth muscle [7]. The high level of Hcy in human body results in an increase in the production of mercaptolactone compounds through spontaneous decomposition. Under the combined action of Hcy and retinol in human body, platelet aggregation in the human body increases, making thrombosis easier to occur. Meanwhile, Hcy at a high level can inhibit the function of the vascular endothelium and reduce the release of nitric oxide from the vascular endothelium, thus affecting the contraction and relaxation function of blood vessels [13]. A recent study of patients with pregnancy-induced hypertension has also revealed that Hcy level was higher than that of normal people, consistent with the results of this research [14].

We further studied the causes of increased Hcy in patients with pregnancy-induced hypertension, and examined the related loci of the MTHFR gene. It was found that the TT-type Hcy level was increased in polymorphisms of the C677T locus of the MTHFR gene, while the polymorphism of the A1298C locus had no obvious correlation with Hcy level. Research has shown that the MTHFR gene plays a crucial role in regulating metabolism in the human body and participates in the occurrence of many diseases [15]. Mutations in related gene sites of folate metabolism in the MTHFR gene can lead to folate absorption disorder, bringing about the increase of Hcy level in human body [16]. It has also been revealed that the C677T and A1298C loci of the MTHFR gene are the most common polymorphic site. Previous studies have signified that the mistranslation of C and T at the C677T locus of the MTHFR gene leads to abnormal MTHFR gene function, and mutation of the C677T locus of the MTHFR gene is also related to the occurrence of pregnancy-related diseases [17]. In this research, the ratio of CT and TT in the C677T locus of the MTHFR gene in patients with pregnancy-induced hypertension was higher than that in the control group, suggesting that the mutation rate of the C677T locus in patients with pregnancy-induced hypertension increased. It was found that the ratio of the T allele of the C677T locus in patients with pregnancy-induced hypertension was increased, which was in line with the results of this research [18]. A1298C is another mutation site of the MTHFR gene. Some studies have shown that A1298C mutation can also lead to abnormal Hcy metabolism and increased plasma Hcy level [19, 20]. Research has also revealed that

Table 9. Comparison of general information between the poor and normal pregnancy groups

Items	Poor pregnancy group (n = 87)	Normal pregnancy group (n = 93)	χ^2/t	P
Age (year)	26.1±6.0	25.4±5.5	0.817	0.415
BMI (kg/m^2)	27.33±5.21	27.12±5.28	0.268	0.789
Systolic blood pressure (mmHg)	152.3±11.1	146.3±10.2	3.779	<0.001
Diastolic blood pressure (mmHg)	99.2±7.5	96.2±7.0	2.776	0.006
Pregnancy times (n)			0.069	0.792
First time	76	80		
Again	11	13		
Week of pregnancy (weeks)	25.7±4.6	25.1±4.3	0.905	0.367
Preeclampsia grading (n)			18.513	<0.001
Mild preeclampsia	33	65		
Severe preeclampsia	54	28		
Accompanying symptoms (n)				
Edema	38	41	0.003	0.956
Proteinuria	34	31	1.018	0.313
Hematuria	28	26	1.373	0.241
Abnormal kidney function	31	18	6.011	0.014
C677T polymorphism of MTHFR gene			19.405	<0.001
CC	25	57		
CT	49	27		
TT	13	9		
A1298C polymorphism of MTHFR gene			1.161	0.560
AA	51	55		
AC	33	25		
CC	3	3		

Note: MTHFR: methylenetetrahydrofolate reductase.

Table 10. Independent variable assignment for multi-factor logistic regression analysis

Factors	Independent variables	Assignment
Systolic blood pressure	X1	≥148.3 mmHg = 1, <148.3 mmHg = 0
Diastolic blood pressure	X2	≥98.2 mmHg = 1, <98.2 mmHg = 0
Hypertension classification	X3	Severe preeclampsia = 1, Mild preeclampsia = 0
Abnormal renal function	X4	Yes = 1, No = 0
C677T polymorphism of MTHFR gene	X5	CT and TT = 1, CC = 0

Note: MTHFR: methylenetetrahydrofolate reductase.

Table 11. Multifactorial logistic regression analysis of poor pregnancy outcome

Factor	β	SE	Wald value	OR value (95% CI)	P
C677T polymorphism of MTHFR gene	1.932	0.712	7.812	7.122 (1.712-21.223)	0.003

Note: MTHFR: methylenetetrahydrofolate reductase.

the A1298C mutation of the MTHFR gene has no correlation with pregnancy-induced hypertension [18]. This research shows that the A1298C mutation of the MTHFR gene has no correlation with pregnancy-induced hypertension patients, which is consistent with the above results.

In this research, the incidence of adverse pregnancy events in patients with pregnancy-

induced hypertension was higher than that in the control group. Previous studies have found that the occurrence of hypertension during pregnancy will lead to placental dysfunction, resulting in an increase in the incidence of adverse pregnancy outcomes [21]. The correlation between adverse pregnancy reactions and the MTHFR gene was further studied. The results showed that the incidence of TT-type abnormal pregnancy at the C677T site was higher than that in CC and CT groups, but there was no obvious correlation between adverse pregnancy outcomes and mutation at A1298C site, which was consistent with previous results [22].

This research still has some limitations. For instance, the sample size is relatively small, which can be expanded for further study. The related mechanism of MTHFR gene polymorphisms and the influencing factors of poor pregnancy in pregnant women with pregnancy-induced hypertension should be explored in depth.

To sum up, the Hcy level of pregnancy-induced hypertension patients and the proportion of CT and TT in the MTHFR gene at C677T locus rise. TT-type increases the incidence of abnormal pregnancy, which may be related to the increase of Hcy level.

Disclosure of conflict of interest

None.

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