

Original Article

MTHFR gene polymorphism and homocysteine levels in spontaneous abortion of pregnant women

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Abstract: Objective: This research explored the expression of MTHFR gene polymorphism and homocysteine (Hcy) in spontaneous abortions in pregnant women. Methods: Eighty-two spontaneous abortion patients treated in our hospital were selected prospectively, and 82 age-matched healthy and normal delivery women were included. The peripheral venous blood of the two groups was obtained, and the differences between MTHFR gene polymorphism and Hcy levels were analyzed. Results: The Hcy levels in spontaneous abortion patients were higher than those in the healthy control group ($P < 0.05$). In the common mutation (C677T) of MTHFR gene polymorphism, the distribution of MTHFR C677T (TT, CC, CT) genotyping of both groups was different, but the Hcy levels in the observation group were higher than those in the normal control group ($P < 0.05$). In addition, logistic analysis manifested that the Hcy levels were higher than normal and MTHFR C677T (TT) mutant was an independent risk factor for spontaneous abortion of pregnant women. Conclusion: MTHFR C677T (TT) is associated with the occurrence of hyperhomocysteinemia, and its high expression often indicates that the risk of spontaneous abortion is greatly increased, which may provide a new research direction for maternal health.

Keywords: Spontaneous abortion, gene polymorphism, MTHFR C677T, Hcy levels, pregnant woman

Introduction

Spontaneous abortion is one of the common diseases in obstetrics and gynecology. It's also the main influencing factor of aristogenesis, which refers to the natural termination of pregnancy when the pregnant woman is not interfered with by external factors, the gestation period is not more than 28 weeks, and the fetus is less than 1000 g [1-3]. Current studies have shown that the incidence of spontaneous abortion in primiparas exceeds 10%, and the causes include genetic, environmental, anatomical, immune and unknown factors [4-6]. Some studies have revealed that unexplained causes account for 40-70% of spontaneous abortion. In recent years, it shows a different upward trend. It can be seen that improving the etiological research of spontaneous abortion is of great significance to improve the health care of the perinatal period [7, 8].

Recent studies have manifested that spontaneous abortion is relevant to placental artery

thrombosis, and there is a close internal relationship between blood hypercoagulability and spontaneous abortion [9, 10]. At the same time, previous literature has confirmed that homocysteine (Hcy) is directly related to blood hypercoagulability, and the abnormal expression of Hcy is directly relevant to folate metabolism disorder caused by 5, 10-methylenetetrahydrofolate reductase (MTHFR) [11, 12]. The relationship between genetic polymorphisms caused by mutation of MTHFR gene and different cysteine levels is important to improve the etiology of spontaneous abortion [13, 14]. Based on this, this research explored the relationship between MTHFR gene polymorphism and cysteine levels in spontaneous abortions of pregnant women, in order to provide a new direction for clinical research.

Materials and methods

General data

Eighty-two spontaneous abortion patients treated in our hospital from January 2018 to

Correlation between MTHFR gene polymorphism and homocysteine level

December 2020 were prospectively selected as the observation group, and 82 age-matched healthy and normal delivery women who underwent physical examination in the outpatient department during the same period were included as the control group. Inclusion criteria are as follows: 1. First abortion; 2. Aged 21-40; 3. The deformity of reproductive system and the abnormality of physique after relevant detection are eliminated; 4. Vaginal and cervical clinical infection is excluded. Exclusion criteria are as follows: 1. Those with family genetic history; 2. The gestational age is less than 12 weeks, or the embryo stops developing by ultrasound diagnosis; 3. There are other genetic diseases; 4. Patients with blood system diseases; 5. Those who have a history of treatment of coagulation dysfunction; 6. Patients with tumor. In the control group, previous spontaneous abortion, stillbirth, intrauterine restriction, and premature birth, were excluded. B-ultrasound confirmed that fetal development was consistent with the first year of pregnancy, and all the selected subjects knew about this research and signed an informed consent form. This research was approved by the Ethics Committee of our hospital.

Methods

Determination of serum cysteine: A total of 3-5 mL fasting peripheral venous blood was obtained from two groups of subjects. The same amount was taken from the control group on the day of physical examination, and an appropriate amount of anticoagulant was added. Then, it was centrifuged at a speed of 3,000 r/min. Finally, the supernatant was separated and stored at -80°C for later use. The serum content of homocysteine (Merck, USA) was detected by a Hitachi 7600 automatic biochemical analyzer. This research proved that fasting plasma total Hcy >1 µmol/L was hyperhomocysteinemia.

MTHFR detection: Altogether 3 mL blood samples were gotten from two groups of subjects, and then stored in a refrigerator at -80°C for later use after EDTA anticoagulation. For the reliability of test results and the simplicity of operation, this experiment used the blood genomic DNA extraction kit provided by a biochemical reagent company (Suzhou Taitong Gene Testing Equipments Co., Ltd., China) to

extract the DNA from the peripheral blood of the subjects. Simultaneously, the related genes were amplified by polymerase chain reaction (PCR). Finally, the amplification products were treated by restriction endonuclease HinfI. The natural genotypes of MTHFR include CC, CT, and TT.

Data statistics

All the data were analyzed by SPSS 22.0 statistical analysis software, and the measurement data were expressed by mean ± standard deviation ($\bar{x} \pm sd$), and an inter-group comparison was made by independent t-test. The counting data were represented by number/percentage (n, %) and those between groups were compared by F-test. The rates between groups were assessed by Chi-square test, and the related risk factors of spontaneous abortion were evaluated by multivariate logistic regression analysis. P<0.05 was considered a significant difference.

Results

Comparison of baseline data between two groups of subjects

There was no significant difference between the two groups in age, gestational age, BMI, hypertension and diabetes (P>0.05), so the two groups were comparable (**Table 1**).

The Hcy levels in the peripheral blood of spontaneous abortion pregnant women were higher than those of normal parturients (P<0.05). This preliminarily indicated that Hcy might participate in the pathophysiological process of spontaneous abortion (**Figure 1**).

MTHFR gene polymorphism detection results and gene frequency distribution of two groups of subjects

There were marked differences in the distribution of three genotypes of MTHFR677T between the two groups (P<0.001). The expression frequency of homozygous mutant TT in the observation group was higher than that in the control group ($\chi^2=20.922$, P=0.000). This preliminarily indicated that the mutation of MTHFR677T gene might be intrinsically related to spontaneous abortion (**Table 2**).

Correlation between MTHFR gene polymorphism and homocysteine level

Table 1. Comparison of general information of two groups of research subjects

Group	Observation group (n=82)	Control group (n=82)	χ^2/t	P
BMI (kg/m ²)	26.84±3.21	26.05±3.19	1.581	0.116
Age (years)	27.6±10.6	28.0±11.2	0.252	0.801
Hypertension (n)	5	2	0.597	0.440
Diabetes (n)	1	3	0.256	0.613
Pregnancy history (n)			0.266	0.606
First	26	21		
Repeated	22	24		
History of gynecological surgery (n)			0.085	0.771
Surgery	6	6		
Laparoscopic surgery	1	3		

Note: BMI: body mass index.

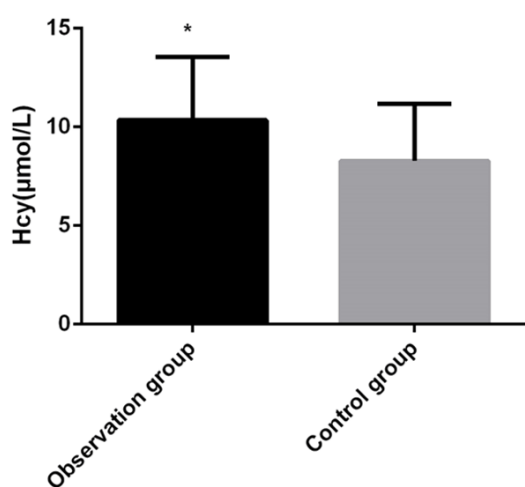


Figure 1. Comparison of peripheral blood Hcy levels between the two groups of subjects. Compared with the control group, *P<0.05. Hcy: homocysteine.

Comparison of Hcy levels among three MTHFR677T genotypes

Among the three genes of MTHFR677T in both groups, the frequency of T gene was higher than that of C allele (P<0.001), and the Hcy levels in peripheral blood of TT homozygote were higher than those of CC wild type and CT heterozygote. This suggested that the mutation of T allele was related to Hcy expression to some extent (Table 3; Figure 2).

Multivariate logistic regression analysis of spontaneous abortion

Multivariate logistic regression analysis showed that increased Hcy levels and TT mutation in MTHFR677T were independent risk factors

for abortion in pregnant women (all P<0.001, Table 4).

Discussion

Recently, the morbidity of spontaneous abortion has been increasing year by year, but the etiology has not been thoroughly explained [15, 16]. At the moment, placental pathological analysis shows that placental artery thrombosis may be a risk factor for abortion of pregnant women. Previous studies have confirmed the direct correlation between the overexpression of cysteine and the existence of blood hypercoagulability and thrombosis [17, 18].

This research manifested a significant difference between the cysteine levels in peripheral blood of spontaneous abortion and normal pregnant women. It preliminarily confirmed that cysteine might be involved in the occurrence and development of spontaneous abortion. It also supported that there was a certain correlation between the increased cysteine levels in peripheral blood and spontaneous abortion. As to the specific mechanisms, in addition to possible vascular embolism caused by thrombosis, fetal nutrition supply is abnormal [19, 20]. Besides, the direct placental cytotoxicity of Hcy causes abnormal embryonic development and direct damage to vascular endothelial cells, resulting in placental arterial dynamic disorder, and finally leads to abortion.

This research revealed that the frequency of T gene expression in pregnant women in the observation group was higher than that in the control group, and the Hcy levels in peripheral blood of pregnant women with MTHFR677T

Correlation between MTHFR gene polymorphism and homocysteine level

Table 2. Comparison of three genotypes of MTHFR C677T between both groups

Group	Genotype			χ^2	P
	Homozygous CC	Heterozygous CT	Mutant TT		
Observation group (n=82)	4	48	30	34.962	0.000
Control group (n=82)	27	50	5		

Table 3. Comparison of C/T gene frequency between two groups of subjects

Group	Allele		χ^2	P
	C	T		
Observation group (n=82)	56	108	26.955	0.000
Control group (n=82)	104	60		

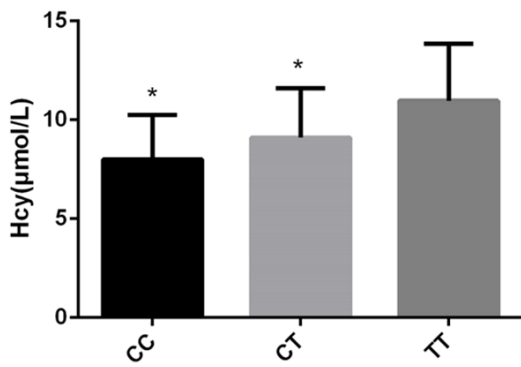


Figure 2. Comparison of Hcy levels of three different genotypes of MTHFR C677T genes. Compared with TT, *P<0.05. Hcy: homocysteine.

(TT) were higher than those with other genotypes (CC/CT), which further confirmed that mutation of the MTHFR gene might lead to abnormal levels of Hcy. The mechanism is as follows: the overexpression of cysteine may be related to the expression defect caused by the mutation of its metabolic enzyme gene, and the genes relevant to cysteine metabolism mainly include MTHFR. Gene analysis showed that it is located on autosomal 1p36.3 with a length of 1980 nucleotides and plays a key role in the biochemical process of homocysteine methylation metabolism to produce cysteine [21]. However, research signified that the C677T base located in exon 4 of MTHFR is a common mutation site, and the activity of MTHFR decreased after the mutation, which led to the accumulation of cysteine and finally led to the high expression of Hcy in peripheral blood [22]. Some literature also shows that the epidemiological survey of European and American population does not reveal a signifi-

cant correlation between MTHFR gene mutation and spontaneous abortion, which may be related to ethnic differences and research groups and methods [23-25].

Logistic analysis manifested that elevated cysteine level and MTHFR C677T (TT) mutant were independent risk factors for spontaneous abortion. This suggested that MTHFR C677T (TT) mutant caused the expression of cysteine in peripheral blood of pregnant women to a certain extent, similar to previous studies [26]. The specific biological pathway is necessary for us to further understand the mechanism of MTHFR C677T (TT) involved in the occurrence and development of spontaneous abortion.

Limitations of this study include: 1. It is a single-center study with a small number of individuals. A multi-center large sample study is required to improve clinical verification; 2. The clinical data are collected simultaneously, and only correlation analysis is performed. Further research is needed to clarify a causal relationship; 3. The specific threshold of the early warning effect of cysteine levels in peripheral blood on spontaneous abortion needs to be studied and improved; 4. It is important to improve the relevant data of minority nationalities in China for exploring the relationship between MTHFR gene mutation and spontaneous abortion.

In general, MTHFR C677T (TT) mutation may lead to abnormal cysteine metabolism and even spontaneous abortion. This may play a crucial role in improving the early identification and treatment of spontaneous abortion.

Disclosure of conflict of interest

None.

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Correlation between MTHFR gene polymorphism and homocysteine level

Table 4. Multivariate logistic regression analysis

Index	Standardized β	OR	95% CI	P
Increased Hcy	1.16	3.19	1.04-4.89	<0.001
MTHFR C677T gene (TT type)	1.05	2.88	1.00-2.45	<0.001

Note: Hcy: homocysteine.

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Correlation between MTHFR gene polymorphism and homocysteine level

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