Journal of Hainan Medical University

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Correlation of methylenetetrahydrofolate reductase polymorphism with Hcy metabolism and inflammatory response in patients with recurrent cerebral infarction

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ARTICLE INFO

Article history:
Received 12 Jun 2017
Received in revised form 19 Jun 2017
Accepted 3 Jul 2017
Available online 14 Jul 2017

Keywords:
Recurrent cerebral infarction
Methylenetetrahydrofolate reductase
Homocysteine
Inflammatory response

ABSTRACT

Objective: To study the correlation of methylenetetrahydrofolate reductase (MTHFR) polymorphism with Hcy metabolism and inflammatory response in patients with recurrent cerebral infarction. **Methods:** 40 patients with recurrent cerebral infarction who were treated in Yulin Third Hospital between December 2013 and December 2016 were selected as recurrent group, 58 patients with primary cerebral infarction were selected as primary group, and 60 healthy volunteers were selected as control group. Peripheral blood MTHFR gene C677T polymorphism and serum levels of Hcy metabolism indexes and inflammatory response indicators were determined. **Results:** CC genotype constituent ratio of recurrent group was significantly lower than that of primary group and control group while CT genotype and TT genotype constituent ratio were significantly higher than those of primary group and control group; serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in recurrent group and primary group were significantly higher than those in control group while VitB12 and FA levels were significantly lower than those in control group; serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in recurrent group were significantly higher than those in primary group while VitB12 and FA levels were significantly lower than those in primary group. Serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in patients with CC genotype were significantly lower than those in patients with CT genotype and TT genotype while VitB12 and FA levels were significantly higher than those in patients with CT genotype and TT genotype; serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in patients with CT genotype were significantly lower than those in patients with TT genotype while VitB12 and FA levels were significantly higher than those in patients with TT genotype. **Conclusion:** MTHFR gene C677T polymorphism is closely related to the recurrence of cerebral infarction, and allele C mutation to T will affect Hcy metabolism and aggravate inflammatory response.

1. Introduction

Cerebral infarction is a clinically common cerebrovascular disease with high disability and fatality rate. Atherosclerosis is a pathological basis for cerebral infarction and patients with a history of cerebral infarction are thought to be the high-risk group for cerebral infarction. In recent years, the recurrence of cerebral infarction has received more and more attention, and the prognosis of recurrent cerebral infarction is even worse than that of primary cerebral infarction, but the mechanism of cerebral infarction recurrence is not fully clear. Hyperhomocysteinemia is closely related to many kinds of cardiovascular and cerebrovascular diseases[1,2], methylenetetrahydrofolate reductase (MTHFR) is the key enzyme that catalyzes the homocysteine (Hcy) metabolism, and the reduction of its catalytic activity can cause the accumulation of Hcy. Current research suggests that the mutation of 677 base of MTHFR gene from cytosine to thymine will reduce the activity of the transcription products and thus affects the metabolism of Hcy[3,4]. In the following study, the correlation of MTHFR gene

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Fund Project: Special Scientific Research Project of Education Department of Shaanxi Provincial Government No: 16JK1852.

C677T polymorphism with Hcy metabolism and inflammatory response in patients with recurrent cerebral infarction was specifically analyzed.

2. Subject information and research methods

2.1 General information of subjects

40 patients with recurrent cerebral infarction who were treated in Yulin Third Hospital between December 2013 and December 2016 were selected as recurrent group, 58 patients with primary cerebral infarction were selected as primary group, all patients were diagnosed with cerebral infarction by head CT and MRI scanning, and the patients who took vitamin B12, folic acid and other drugs in the last 3 months, and the patients who were complicated by infectious diseases, blood system diseases, malignant tumor and autoimmune disease after admission were ruled out. Recurrent group included 25 men and 15 women that were 46-67 years old; primary group included 35 men and 23 women that were 45-68 years old. 60 healthy volunteers who received physical examination during the same period were selected as control group, including 36 men and 24 women that were 40-65 years old. There was no significant difference in the general data of the three groups (P>0.05).

2.2 MTHFR gene C677T polymorphism detection

3 mL of peripheral blood was taken from the recurrent group and primary group of patients after admission, 3 mL of peripheral blood was taken from the control group of volunteers during physical examination, whole blood genomic DNA extraction kit was used to isolate genomic DNA in peripheral blood, MTHFR gene C677T loci primers were designed, PCR amplification was conducted, and the MTHFR gene C677T polymorphism of amplification products were judged by biochip reader, including CC genotype, CT genotype and TT genotype.

2.3 Serum Hcy metabolism index and inflammation index detection

3 mL of peripheral blood was taken from the recurrent group and primary group of patients after admission, 3 mL of peripheral blood was taken from the control group of volunteers during physical examination, the blood was centrifuged to separate serum, electrochemical luminescence kits were used to determine Hcy, VitB12 and FA levels, and enzyme-linked immunosorbent assay were used to determine HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels.

2.4 Statistical methods

SPSS 19.0 software was used for statistical processing of the data in the study, measurement data among three groups were by variance analysis, count data were by chi-square test, and P<0.05 was judged as statistical significance in differences of processing results.

3. Results

3.1 MTHFR gene C677T polymorphism distribution of three groups of subjects

MTHFR gene C677T polymorphism CC genotype, CT genotype and TT genotype constituent ratio of recurrent group were (8/40), (13/40) and (19/40) respectively; MTHFR gene C677T polymorphism CC genotype, CT genotype and TT genotype constituent ratio of primary group were (30/58), (13/58) and (15/58) respectively; MTHFR gene C677T polymorphism CC genotype, CT genotype and TT genotype constituent ratio of control group were (52/60), (5/60) and (3/60) respectively. After statistical analysis, CC genotype constituent ratio of recurrent group was significantly lower than that of primary group and control group while CT genotype and TT genotype constituent ratio were significantly higher than those of primary group and control group.

3.2 Serum Hcy metabolism indexes of three groups of subjects and their relationship with MTHFR gene C677T polymorphism

Analysis of serum Hcy metabolism indexes Hcy (μmol/L), VitB12 (μmol/L) and FA (ng/mL) levels in three groups of subjects was as follows: serum Hcy levels in recurrent group and primary group were significantly higher than that in control group while VitB12 and FA levels were significantly lower than those in control group; serum Hcy level in recurrent group was significantly higher than that in primary group while VitB12 and FA levels were significantly lower than those in primary group. Differences in pair-wise comparison of serum Hcy, VitB12 and FA levels were statistically significant among three groups of subjects (P<0.05).

Analysis of the relationship of MTHFR gene C677T polymorphism with serum Hcy metabolism indexes Hcy, VitB12 and FA in recurrent group and primary group was as follows: serum Hcy level in patients with CC genotype was significantly lower than that in patients with CT genotype and TT genotype while VitB12 and FA levels were

Table 1.
Comparison of serum Hcy metabolism indexes among three groups of subjects.

Groups	n	Hcy	VitB12	FA
Recurrent group	40	26.49±3.42$^{*\#}$	293.41±36.48$^{*\#}$	5.98±0.75$^{*\#}$
Primary group	58	18.12±2.41*	365.62±44.59*	8.12±1.03*
Control group	60	7.93±0.94	621.28±78.49	11.38±1.59

*: compared with control group, P<0.05; $^{\#}$: compared with primary group, P<0.05.

Table 2.
Relationship between MTHFR gene C677T polymorphism with serum Hcy metabolism indexes.

Groups	n	Hcy	VitB12	FA
CC genotype	38	14.59±2.03	477.43±62.39	9.49±1.25
CT genotype	26	22.38±3.29^{a}	333.57±47.96^{a}	7.03±0.88^{a}
TT genotype	34	33.17±4.52^{ab}	232.32±33.12^{ab}	4.68±0.68^{ab}

^{a}: compared with CC genotype, P<0.05; ^{b}: compared with CT genotype, P<0.05.

Table 3.
Comparison of serum inflammation indexes among three groups of subjects.

Groups	*n*	HMGB1	sCD40L	YKL-40	Lp-PLA2	MMP-9
Recurrent group	40	15.51±1.93*#	0.58±0.08*#	0.31±0.04*#	42.51±5.59*#	223.41±27.94*#
Primary group	58	10.28±1.44*	0.35±0.06*	0.17±0.02*	26.54±3.59*	135.42±16.49*
Control group	60	3.41±0.54	0.18±0.02	0.09±0.01	11.32±1.58	84.51±9.39

*: compared with control group, $P<0.05$; #: compared with primary group, $P<0.05$.

Table 4.
Relationship between MTHFR gene C677T polymorphism with serum inflammation indexes.

Groups	*n*	HMGB1	sCD40L	YKL-40	Lp-PLA2	MMP-9
CC genotype	38	9.32±1.16	0.27±0.04	0.18±0.02	22.51±2.93	127.95±14.58
CT genotype	26	13.36±1.93[a]	0.42±0.08[a]	0.28±0.05[a]	35.53±4.58[a]	186.59±22.31[a]
TT genotype	34	17.69±2.32[ab]	0.67±0.09[ab]	0.46±0.08[ab]	47.59±6.38[ab]	286.59±35.28[ab]

[a]: compared with CC genotype, $P<0.05$; [b]: compared with CT genotype, $P<0.05$.

significantly higher than those in patients with CT genotype and TT genotype; serum Hcy level in patients with CT genotype was significantly lower than that in patients with TT genotype while VitB12 and FA levels were significantly higher than those in patients with TT genotype. Differences in pair-wise comparison of serum Hcy, VitB12 and FA levels were statistically significant among patients with three genotypes ($P<0.05$).

3.3 Serum inflammation indexes of three groups of subjects and their relationship with MTHFR gene C677T polymorphism

Analysis of serum inflammation indexes HMGB1 (pg/mL), sCD40L (ng/mL), YKL-40 (ng/mL), Lp-PLA2 (ng/mL) and MMP-9 (ng/mL) levels in three groups of subjects was as follows: serum HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in recurrent group and primary group were significantly higher than those in control group; serum HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in recurrent group were significantly higher than those in primary group. Differences in pair-wise comparison of serum HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels were statistically significant among three groups of subjects ($P<0.05$).

Analysis of the relationship of MTHFR gene C677T polymorphism with serum inflammation indexes HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 in recurrent group and primary group was as follows: serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in patients with CC genotype were significantly lower than those in patients with CT genotype and TT genotype; serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in patients with CT genotype were significantly lower than those in patients with TT genotype. Differences in pair-wise comparison of serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels were statistically significant among patients with three genotypes ($P<0.05$).

4. Discussion

Hyperhomocysteinemia is an independent risk factor for various cardiovascular and cerebrovascular diseases that has received more and more attention in recent years[5]. Hcy is produced during methionine cycle metabolism, and then metabolized with the VitB12 and FA as coenzyme and the methyl donor as material. The product encoded by MTHFR gene is a class of flavoprotein, which plays an important role in the metabolism of Hcy; this protein catalyzes the methylenetetrahydrofolate reduction into methyltetrahydrofolate and provides methyl for Hcy metabolism[6]. When the catalytic activity of the MTHFR gene is reduced, the metabolic process of Hcy will be affected and the Hcy level will increase. MTHFR gene exon 677 locus is the common mutation locus, which is prone to mutations from cytosine to thymine and causes C677T polymorphism[7,8]. In order to define the correlation between MTHFR gene 677 base polymorphism and recurrence of cerebral infarction, the distribution of MTHFR gene C677T polymorphism was analyzed in the study, and the results showed that CC genotype constituent ratio of recurrent group was significantly lower than that of primary group and control group while CT genotype and TT genotype constituent ratio were significantly higher than those of primary group and control group. It means that the decrease of MTHFR gene 677 base wild-type genotype CC and the increase of mutant-type genotypes CT and TT are closely related to the recurrence of cerebral infarction, and combined with the biological function of MTHFR gene expression products, it is speculated that MTHFR gene CC genotype mutation to CT genotype and TT genotype will cause the reduced catalytic activity of MTHFR expression products, and then participate in cerebral infarction recurrence.

Catalytic product of MTHFR gene has significant influence on Hcy metabolism in vivo, and the blocked Hcy metabolism can cause hyperhomocysteinemia and increase the risk of a variety of cardiovascular and cerebrovascular diseases. Hcy has a variety of biological activities in the body, it can significantly activate inflammation and oxidative stress response, promote the inflammatory cell activation and infiltration in vascular endothelium, and also cause lipid to have peroxidation and form ox-LDL, and inflammatory cells become foam cells after devouring ox-LDL and then directly participate in the formation of the artery atheromatous plaque[9,10]. In addition, Hcy can also influence the clotting process and activate platelets and clotting factor V, which promotes the thrombosis and accelerate cerebral infarction[11,12]. In the study, analysis of Hcy metabolism in patients with cerebral infarction showed that serum Hcy levels in recurrent group and primary group were significantly higher than that in control group while VitB12 and FA levels were significantly lower than those in control group, and the changes in above Hcy metabolism indexes in recurrent group were more significant than those in primary group. This indicates that abnormal Hcy metabolism is closely related to the occurrence and recurrence of cerebral infarction. In order to further clarify whether MTHFR gene C677T polymorphism affected the metabolism of Hcy, above serum Hcy metabolism indexes in cerebral infarction patients with different MTHFR genotypes were analyzed in the study, and the results showed that serum Hcy level in patients

with CC genotype was significantly lower than that in patients with CT genotype and TT genotype while VitB12 and FA levels were significantly higher than those in patients with CT genotype and TT genotype; serum Hcy level in patients with CT genotype was significantly lower than that in patients with TT genotype while VitB12 and FA levels were significantly higher than those in patients with TT genotype. This shows that the catalytic activity of products encoded by wild-type homozygote CC genotype is stronger, and the Hcy level is lower in the body; the catalytic activity of products encoded by mutant-type CT genotype and TT genotype are lower, and the catalytic activity of products encoded by mutant-type homozygote TT genotype is more significantly lower.

High Hcy level in patients with cerebral infarction can significantly activate the process of inflammation, and HMGB1, sCD40L, YKL-40, Lp-PLA2, MMP-9 and so on are the inflammation indexes closely related to disease progression in patients with cerebral infarction. HMGB1 is the late inflammatory response-associated mediator, which promotes the activation of macrophages and the improvement of phagocytosis[13]; sCD40L is a member of the tumor necrosis factor superfamily, which is involved in the regulation of inflammatory cell adhesion and platelet activation[14]; YKL-40 is a new pro-inflammatory factor and promotes the activation of multiple inflammatory cells[15]; Lp-PLA2 and MMP-9 are the inflammatory mediators secreted by activated macrophages, which have a significant effect on the stability of atheromatous plaque. In the study, analysis of the degree of inflammation in patients with cerebral infarction showed that serum HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in recurrent group and primary group were significantly higher than those in control group, and the changes in above inflammation indexes in recurrent group were more significant than those in primary group. This indicates that the abnormal activation of the inflammatory response is closely related to the occurrence and recurrence of cerebral infarction. In order to further clarify whether MTHFR gene C677T polymorphism affected the inflammatory response process, above serum inflammation indexes in cerebral infarction patients with different MTHFR genotypes were analyzed in the study, and the results showed that serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in patients with CC genotype were significantly lower than those in patients with CT genotype and TT genotype; serum Hcy, HMGB1, sCD40L, YKL-40, Lp-PLA2 and MMP-9 levels in patients with CT genotype were significantly lower than those in patients with TT genotype. It means that the inflammatory reaction is weaker in patients with wild-type homozygote CC genotype, and the inflammatory reaction is stronger in patients with mutant-type CT genotype and TT genotype, and the inflammatory reaction activation is more significant in patients with homozygote TT genotype mutation.

MTHFR gene C677T polymorphism is closely related to cerebral infarction recurrence, MTHFR gene 667 base allele C mutation to T can affect the metabolism of Hcy and aggravate the inflammatory response, and the Hcy metabolism disorders and inflammatory reaction activation levels in patients with homozygote TT genotype mutation are more significant than those in patients with heterozygote CT genotype mutation.

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