Research Article

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The association of methylene tetrahydrofolate reductase (MTHFR) A1298C gene polymorphism, homocysteine, vitamin B12, and folate with coronary artery disease (CAD) in the north of Iran

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Abstract

Background: We pursued to find out the possible association of Methylene tetrahydrofolate reductase (MTHFR) A1298C gene polymorphism, blood homocysteine, vitamin B12, and folate with Coronary artery disease (CAD) in the study population in Guilan, north of Iran.

Material and Methods: Ninety patients with CAD and 76 healthy controls were evaluated. MTHFR A1298C polymorphism and its genotype frequency, the plasma level of homocysteine, vitamin B12 and folate were evaluated by using ARMS-PCR, ELISA, and Chemiluminescence methods, respectively.

Results: The frequency of genotypes, A, AC and CC in CAD were 40, 35.6, 24.4%, respectively which was significantly different ($p=0.016$) from the control group that were 26.3, 57.9 and 15.8%, respectively. The serum level of vitamin B12 and folate in genotype A1298C were not statistically significant between two groups ($p>0.05$), however, the plasma homocysteine in patients with CAD was

remarkably higher than the control group ($p<0.001$). Additionally, in CAD patients the plasma level of homocysteine in the AC genotype was significantly higher than the control subjects ($p=0.005$).

Conclusion: It is thus concluded that MTHFR A1298C gene polymorphism is associated with CAD. It seems that the AC genotype of MTHFR A1298C polymorphism might have a protective effect on CAD.

Keywords: A1298C; coronary artery disease; homocysteine; MTHFR; polymorphism.

Introduction

Nowadays, a lot of people have died (about 18 million deaths in 2017) due to cardiovascular diseases (CVDs), which is at least 9% of the deaths worldwide [1]. CVDs include different types of circulatory diseases in which the coronary artery disease (CAD) is among the commonest. CAD, a polygenic and multifactorial condition with various genetic polymorphisms, kills almost seven million people per year. This disease is originated from myocardial ischemia that leads to cardiac muscle tissue death followed by arresting cardiac and possible mortality [2].

Homocysteine is synthesized from methionine through several steps process. There are two possible fates for Homocysteine including conversion to cysteine or conversion back into methionine by tetrahydrofolate (THF) [3–5]. Homocysteine plays a role in the atherosclerotic pathway through several mechanisms, that is, hypercoagulability, oxidative stress, so that all of them will correspond to CVD, yet there is no complete and clear report about the mechanisms of vascular failure induced via hyperhomocysteinemia [6].

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Methylene tetrahydrofolate reductase (MTHFR) is an enzyme that plays a crucial role in homocysteine metabolism, which produces 5-methyltetrahydrofolate. This metabolite is one of the main forms of folate in the circulatory system. It is also a carbon donor in the homocysteine to methionine conversion in the pathway of remethylation [7]. If the function of this enzyme is impaired, the plasma total homocysteine (tHcy) may increase. It has been shown that the increased level of plasma homocysteine is an independent risk for CAD patients [3, 4]. The blood concentration of homocysteine depends on the genetically regulated level of essential enzymes and the intake of some biochemical elements like folate, and vitamin B12. Elevated levels of serum homocysteine are a known risk factor for CAD patients [8].

It has been reported that the relationship between CAD and Hcy level might be influenced by several factors such as dietary habits, smoking, and living [9]. Several studies have been performed in the field of CAD in the Iranian population [10–12]. Additionally, there is some evidence implying that by increasing the homocysteine level to 10%, the risk of CAD may increase [13].

MTHFR gene is located on chromosome 1 (1p36.3) short arm. Thirty-four rare mutations and nine common variants of MTHFR gene have been found including ARG184TER (rs121434294) [14], C677T (rs1801133) [15–17] and A1298C (rs1801131) [18, 19]. The MTHFR A1298C is a point mutation in exon 7 [20]. This transversion leads to alanine be substituted by glutamate at codon 429 which is located on the regulatory site of the protein [21, 22]. The activity of MTHFR is reduced by the polymorphism, yet its effect is lower than the C677T transition [23].

It has been postulated that the activity of MTHFR is reduced by almost 35% by the polymorphism of A1298C [24]. The polymorphism of A1298C is less investigated with variable results in different populations [5, 25]. Several reports suggested that the variant “C” allele leads to higher levels of CAD, and other investigations have reported that 1298AA [26] and 1298AC genotypes are associated with CAD [24].

The influences of MTHFR polymorphisms and variations in the plasma level of homocysteine on the pathogenesis of CAD are still controversial [14, 27]. Furthermore, since the limited knowledge on MTHFR A1298C polymorphism from Iranian CAD patients [10–12], we pursued to find out the possible association of this polymorphism, blood Homocysteine, vitamin B12, and Folate with CAD in a population from north of Iran.

Materials and methods

Patients

Seventy-six healthy controls and 90 patients with diagnosed CAD referring to Heshmat Educational & Remedial Center (Rasht, Iran) as the research population. CAD was confirmed by coronary angiography and the patients were divided into three subgroups according to the at least 50% stenosis of one to three coronary arteries. Both groups were matched for gender and age. Subjects were excluded from the research if they had a known history of folate and vitamin B12 supplementation or deficiency, cancer and malignancy that used related drugs for therapy, epilepsy and patients on anticonvulsant therapy (like Phenobarbital, Carbamazepine, Lamotrigine, etc.), renal failure (Creatinine > 1.5 mg/dL) and kidney transplantation that may affect the plasma concentration of Hcy [5, 25]. The ethics committee of Hamedan University of Medical Sciences approved this cross-sectional study (Hamedan, Iran; No: IR.Juus.REC.1396.64). All subjects gave written informed consent.

DNA extraction and genotyping

Fasting blood samples were taken using EDTA tubes and anticoagulant-free tubes. Plasma and serum samples were immediately separated and kept at –20 °C until further analysis. The extraction of DNA was done by a special PCR preparation Kit (Roche Company). The DNA band analysis was performed by electrophoresis on 1.5% agarose gel. In order to determine the MTHFR genotype, the ARMS-PCR method (Allele-specific method) was utilized. The primer sequences were as follow: (for allele A) forward 5' GGA GCT GAC CAG TGA AGA 3' and reverse 5' TGT GAC CAT TCC GGT TTG 3'; (for allele C) forward 5' CTT TGG GGA GCT GAA GGA 3' and reverse 5' AAG ACT TCA AAG ACA CTT G 3' [28]. The PCR program was initiated at 94 °C for 1 min, followed by 35 cycles of 93 °C for 10 s, 64 °C for 10 s, and 72 °C for 20 s, and a final extension at 72 °C for 1 min. The PCR products were separated by 3% (w/v) agarose gel electrophoresis [29].

Measurement of the plasma homocysteine

Blood Hcy was measured by an Axis® Homocysteine (EIA) kit that was based on competition among two groups of S-adenosyl-L-homocysteine (SAH). In the test sample, Hcy was first converted to SAH by using a hydrolase and adenosine. After the addition of the monoclonal anti-SA antibody, the unbound antibody was removed and the secondary rabbit anti-mouse antibody conjugated with horseradish peroxidase (HRP) was added. The peroxidase activity was determined by adding the substrate, and the optical density was measured by spectrophotometer.

Assay of the serum vitamin B12

The determination of B12 in human serum was done according to ARCHITECT (B12 assay kit, Abbott, Ireland). The B12 present in the pre-treated serum sample binds to the intrinsic factor bound microparticles. In the second step, B12-acridinium labeled conjugate was added and the obtained chemiluminescent reaction was calculated.

Assay of the serum folate

This was based on the ARCHITECT instruction kit (Abbott, Ireland). In brief, Folate was released from folate binding protein (FBP). One volume of the prepared serum was added into a reaction vessel, followed by the addition of FBP coated paramagnetic microparticles and diluents. Folate present in the serum binds to the FBP and after that pterotic acid-acridinium labeled conjugate was added. After the addition of pre-trigger and trigger solution, the resulting chemiluminescent reaction was measured as relative light units (RLUs) by the ARCHITECT i System optics.

Statistical analysis

Statistical analysis was employed by SPSS software version 21.0. A chi-square test and Kruskal–Wallis test followed by Mann–Whitney was used to analyze biochemical parameters. The considered statistically significant was p-value less than 0.05. Both CAD and control groups were in line with the Hardy–Weinberg equation.

Results

Analysis of agarose gel electrophoresis of the A1298C polymorphisms was done for the genotyping of two alleles (A/C). The two expected bands were observed on the gel: 117 and 77-bp bands.

The study population was investigated in terms of A1298C mutations as well as their relationships with the levels of homocysteine, B12, and folate. 54.2% of the study population were male (32 and 58 for control and CAD groups, respectively) and 45.8% were female (44 and 32 for control and CAD groups, respectively). The age range of the study population was 40–70 years old.

The frequency of A1298C genotypes in CAD cases and controls are listed in Table 1. The frequency of genotype distribution was significantly different between the two groups ($p=0.016$). The genotype frequencies of AA, AC and CC in CAD were 40, 35.6 and 24.4%, respectively and in control group were 26.3, 57.9 and 15.8%, respectively.

The effects of B12, Folate, and Hcy normality in A1298C genotypes were determined through the Shapiro test. The

levels of Hcy, B12, and folate in each of the A1298C genotypes (AA, AC, and CC) in the CAD and control groups have been shown in Table 2 and Figure 1. Only the Hcy concentration (that was measured with ELISA) in the AC genotype was statistically significant ($p=0.005$). The above biochemical parameters were also compared in the CAD and control groups. These findings showed that only Hcy concentration was significant between the two groups ($p<0.001$). Although the concentration of B12 and folate were different in the CAD and control groups, no significant difference ($p>0.05$) was observed between them.

The coronary stenosis in A1298C genotypes for CAD subjects is presented in Table 3. The vein thrombosis percentage in the AC genotype was the highest (71.9%), while the lowest percentage was seen in CC genotype (31.8%) ($p=0.026$).

Discussion

CAD is a leading cause of death worldwide. The World Health Organization (WHO) statistics show that ischemic heart disease was responsible for nearly nine million deaths in 2016. Diet and genetics are suggested to play an important role in the risk of CAD. The status of CAD in developing countries is worse with increasing trends of mortality [30]. Generally, it is estimated that at least 50% of people have a susceptibility to CAD is owing to genetic backgrounds [31]. Some studies have disclosed that polymorphisms of the genes are involved in the folate/homocysteine pathway as risk factors for CAD lately [32].

Many studies have shown the association of MTHFR (s1801133) gene variety with CAD. MTHFR plays an important role in homocysteine metabolism, and elevated homocysteine has been considered as an independent risk factor to CAD. Moreover, SNPs in MTHFR can act as a biological marker in order to predict the susceptibility to CAD [33]. It was suggested that MTHFR gene polymorphisms could influence the prognosis of recurrent hard cardiac events in patients who underwent MI [34]. Heidari et al. suggested that there is a significant relationship between

Table 1: Distribution of genotype and allele frequencies of MTHFR A1298C polymorphism in cases and controls.

Genotypes/allele	Cases (n=90)	Controls (n=76)	χ^2	Odds ratio	95% CI	P-value	P-value*
AA	36 (40%)	20 (26.3%)	3.45	1.81	0.963–3.62	0.063	0.016
AC	21 (35.6%)	44 (57.9%)	8.28	0.401	0.214–0.751	0.004	
CC	22 (24.4%)	12 (15.8%)	1.90	173	0.790–3.71	0.169	
A	0.58	0.55					
C	0.42	0.45					

*Chi-square test.

Table 2: Homocysteine, folate, and vitamin B12 concentrations according to MTHFR A1298C gene polymorphisms in CAD and controls. The data represented as mean $\pm$ SD.

Genotypes of MTHFR A1298C	Homocysteine ($\mu\text{mol/L}$)				Folate (ng/mL)				Vitamin B ₁₂ (pg/mL)			
	CAD	Controls	P-value*	P-value**	CAD	Controls	P-value*	P-value**	CAD	Controls	P-value*	P-value**
AA	17.30 $\pm$ 6.22	14.16 $\pm$ 6.22	0.06	0.001	10.43 $\pm$ 4.98	12.08 $\pm$ 4.31	0.209	0.432	254.78 $\pm$ 113.43	291.40 $\pm$ 171.79	0.713	0.491
AC	18.71 $\pm$ 13.67	12.61 $\pm$ 5.43	0.005		10.22 $\pm$ 3.86	10.58 $\pm$ 4.74	0.780		284.56 $\pm$ 133.11	318.18 $\pm$ 235.39	0.780	
CC	16.33 $\pm$ 5.67	16.25 $\pm$ 10.64	0.264		10.82 $\pm$ 4.15	9.55 $\pm$ 2.75	0.879		232.82 $\pm$ 133.65	298.33 $\pm$ 188.10	0.368	

*MannWhitney U test.

**KruskalWallis.

MTHFR gene polymorphisms with CAD, specifically in the Iranian population [35]. It has demonstrated that MTHFR gene polymorphism may be associated with a higher risk for the development of premature CAD in Indians [36]. Yu et al. suggested that there is a significant correlation between MTHFR genetic polymorphism and the development of CAD in Han Chinese [37]. It has been suggested that the MTHFR C-A helotype is a protective haplotype for CAD in the Chinese Han population [38]. In addition, MTHFR genotype and allelic frequencies were not different in the coronary artery lesions (CALs) group compared to the controls in the Korean population [39].

Many studies were indicated that the association between serum concentration of Hcy, B12, and Folate with the risk of CAD. Lin and colleagues showed that high plasma homocysteine levels had a direct effect on the risk of CAD

independent of MTHFR C677T genotypes [40]. It was proposed that increased plasma homocysteine levels might be an increase in atherosclerotic vascular disease. Hyperhomocysteinemia is now suggested as an independent risk factor for CAD. Mild hyperhomocysteinemia is quite prevalent in the general population [41]. It was documented that there is a significant connection between homocysteine plasma levels and the frequency as well as the progression of CAD [42]. In 2004, Lee and colleagues showed that vitamin B6 supplementation with vitamin B6 is less effective than the combination of folic acid and vitamin B12 in lowering the plasma level of Hcy in CAD patients [43].

In this study, MTHFR A1298C polymorphism was significantly associated with CAD. We have also shown that the plasma level of Homocysteine was dramatically higher in CAD patients compared to control subjects. The

Figure 1: Scattergram of homocysteine levels in CAD and control groups according to genotypes of MTHFR A1298C.

Table 3: The coronary stenosis percentage according to A1298C genotypes in CAD patients.

Genotype A1298C		Number of thrombosed vessel			Total	P-value*
		1	2	3		
AA	Count	9	13	14	36	0.026
	% Within genotype A1298C	25%	36.1%	38.9%	100.0%	
AC	Count	3	6	23	32	
	% Within genotype A1298C	9.4%	18.8%	71.8%	100.0%	
CC	Count	5	10	7	22	
	% Within genotype A1298C	18.9%	32.2%	48.9%	100%	
Total	Count	17	29	44	90	
	% Within genotype A1298C	48.9%	32.21%	18.9%	100%	

*Qui-square test.

vein thrombosis percentage in AC genotype carriers was the highest (71.9%), while the lowest percentage was for CC genotype (31.8%). It is also suggested that this genotype had a higher Hcy serum concentration than AA and CC genotypes in the CAD group.

Several limitations might be included in this study. One of them is the small sample size of CAD patients. Furthermore, ethnic variation, treasury different genetic, family types, and other factors that we could account might give these results.

Thus, it is concluded that the MTHFR A1298C gene polymorphism is associated with CAD. It seems that the AC genotype of the gene polymorphism might have a protective effect on CAD. However, further studies in larger populations including other genetic and environmental factors are required to achieve a conclusion.

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