Original paper

Association of *MTHFR* C677T Gene Polymorphism with Ischemia in Iraqi Population Undergoing Coronary Angiography

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Abstract

Background: According to the last WHO report in 2014, Iraq is ranked as 22 out of 172 country in coronary artery disease (CAD) as the most leading cause of death. Iraq has an age standardized death rate of 187.65 due to CAD i.e., more than 187 per 100000 Iraqi people die due to CAD, which represent the first cause of death. To our knowledge, there is no Iraqi study concerning the association of MTHFR C677T gene polymorphism with sever stenosis.
Objective: To study the link between C677T MTHFR gene polymorphism and coronary artery disease in individuals who had undergone coronary angiography in Iraqi people after we classify them into those with and without ischemia.
Materials and Methods: population of the study comprised 150 patients (aged 50.4 ± 6.4 year) and 150 control subjects (aged 49.2 ± 4.6 year) that undergone to angiography for coronary vessels. We regarded angiography as positive when there is more than 70% reduction in the diameter of coronary vessels.
Measurements of the participant's sugar and lipid profile were all carried out on fasting blood samples without anticoagulant by standard enzymatic assays. The *MTHFR* C677T polymorphism detection was carried out by PCR-RFLP method.
Results: There is a significant statistical relationship between the MTHFR genotype and the presence of coronary artery disease (P< 0.001). In addition to statistically significant difference among the three genotypes CC, CT, TT regarding BMI, cholesterol level, triglycerides level, and VLDL (P < 0.05)
Conclusion: MTHFR polymorphisms (CT, TT) genotype have been found to be risk factor for coronary artery disease in Iraqi population.
Key words: coronary artery disease, MTHFR C677T polymorphism, coronary angiography, Iraq

Introduction

Coronary artery disease (CAD) is the most important leading cause of morbidity and mortality universally [(1)]. In both developing and developed countries CAD is a major public health problem which has induced considerable concerns about its increasing prevalence in the medical community worldwide [(2)].
Coronary artery disease is a multi-factorial disease with both environmental and genetic determinants. The etiology of CAD is still not completely understood but it has been demonstrated that individual susceptibility to this disease are associated with variations in some genes[(3, 4)]. One of these gene is Methylenetetrahydro-folatereductase (MTHFR), its' polymorphism lead to elevation in the level of homocysteine. Homocysteine is an amino acid precursor which is essential as an intermediate product in methionine metabolism [(5)]. Increase homocysteine level is an independent risk factor for coronary atherosclerosis [(6)]. Vitamins B6, B12 or

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folate deficiency was considered as one of the causes of increase in plasma homocysteine. Another cause is defect in homocysteine metabolism due to deficiency of one of the three enzymes which is essential in homocysteine metabolism: cystathionine β-synthase, methyltetrahydrofolate homocystei-nemethyl transferase, and methylenetetrahydrofolate reductase (MTHFR). Although, numerous meta-analyses have shown the involvement of MTHFR in CAD (7and 8), but there are also some conflicting results [9, 10 and 11]. In our research we explored the association of MTHFR C677T polymorphism with CAD in Iraqi patients.

This study main aim was to evaluate the association between C677T MTHFR polymorphism and coronary artery disease in Iraqi individuals who had undergone coronary angiography after we classify them into those with and without ischemia.

Material and methods

This case control study was carried out in Kufa College of Medicine, biochemistry department. The study included 150 patients that undergone to coronary angiography (in the cardiology center in Al-Sader teaching hospital in Al-Najaf city) which revealed stenosis more than 70%. Control group consists of 150 individuals which also undergone to coronary angiography but the angiography revealed normal result. The period of collection of samples was from January to April, 2017. Phenotypic data contained body mass index (BMI), and lipid profile. All the patient gave their written informed consent for taking part in this study. The study was approved by Ethical Committee of college of medicine, Kufa university. Genotyping of rs1801133 polymorphism was carried out by PCR-RFLP. DNA was extracted from whole blood, genotyping was achieved with specific primer to amplify fragment for digestion with restriction enzyme. (HINF1), followed by electrophoresis on agarose gel. Statistical analysis was carried out using SPSS program. Continuous data were presented as mean±SD, while number and percentage was used for presenting qualitative data.

Results

The demographic characteristics (age, gender, BMI, lipid profile, hypertension, smoking) of the participants have been described in table 1. The amplicon size of amplification product of MTHFR gene was 198bp. The digestion of MTHFR gene product indicated one (198bp), two (179, 22bp) or three (198, 179, 22bp) bands for those with wild type (CC), homozygous (TT) and heterozygous (CT) genotypes respectively. Genotyping frequencies of rs1801133 polymorphism were found to be consistent with Hardy-Weinberg equilibrium (Hardy-Weinberg equilibrium results in cases and controls P-values were 0.207&0.507 respectively) as shown in table 2.

Distribution of MTHFR genotypes in study groups show significant difference between cases and control groups as shown in table 3.

In the present study four models of inheritance have been used. The calculated odds ratio was adjusted for BMI, Triglycerides, Cholesterol, HDL, LDL, and VLDL (table 4). In the co-dominant model, the risk of IHD was significantly increased (47 fold) (OR=47, 95% CI: 5.56-397.04, P<0.001) in homozygous genotype (TT) with respect to those of the wild type (CC) after adjustment for BMI and lipid profile.

Although, the (CT) genotype significantly (OR=8.87, 95%CI: 4.24-18.53, P<0.001) raised the risk of IHD by nearly nine-fold.

Dominant and recessive models in table 4 were demonstrated to raise the risk of IHD by 10.68 and 16.30 respectively.

Table 1. Demographic characteristics of the participants

Characteristics	Cases (n=150)	Control (n=150)	P-value
Age (years)	50.4 ± 6.4	49.2 ± 4.6	0.052
Gender (Male)	94 (62.7%)	78 (52.0%)	0.062
BMI (kg/m^2)	28.00±3.00	22.76±2.91	0.001
Triglycerides (mg/dl)	126.7 ± 27.9	122.1 ± 38.6	0.233
Total cholesterol (mg/dl)	188.1 ± 25.0	168.7 ± 23.9	< 0.001
HDL (mg/dl)	51.7 ± 9.6	57.0 ± 15.2	< 0.001
LDL (mg/dl)	110.9 ± 23.4	92.7 ± 25.9	< 0.001
VLDL (mg/dl)	25.4 ± 5.6	24.0 ± 7.5	0.082
Hypertension	32 (21.3%)	26(17%)	0.35
Smoking	30 (20.0%)	36 (24.0%)	0.403

n: number, BMI: body mass index, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low-density lipoprotein.

Table2. Hardy-Weinberg equilibrium results for MTHFR gene polymorphism genotypes in cases and controls.

Study group	X^2	P-value
Cases (n=150)	1.59	0.207
Controls (n=150)	0.44	0.507

X^2:Chi square

Table3. Distribution of MTHFR genotypes in study groups

MTHFR Genotype	Cases N (%)	Controls N (%)	Total	P-value
CC	65 (43.3%)	133 (88.7%)	198 (66.0%)	P< 0.001
CT	62 (41.3%)	16 (10.7%)	78 (26.0%)	
TT	23 (15.3%)	1 (0.7%)	24 (8.0%)	
Total	150 (100%)	150 (100%)	300 (100%)	

Table 4. Results of genotype and allele frequency of MTHFR gene polymorphism in cases and controls

Characteristics	Cases (n=150)	Controls (n=150)	OR (95% CI)	OR* (95% CI)	P-value
Co-dominant model**					
CC (reference)	65	133			
CT	62	16	7.93 (4.3-14.8)	8.87 (4.2-18.5)	<0.001
TT	23	1	47.06 (6.2-356.2)	47.00 (5.6-397.0)	<0.001
Dominant model					
CT+TT	85	17	10.23 (5.6-18.6)	10.68 (5.2-22.0)	<0.001
Recessive model					
CC+CT (reference)	127	149			
TT	23	1	26.98 (3.6-202.6)	16.3 (2.1-127.7)	0.001
Additive model					
2(TT)+CT	108	18			
Frequency of T allele	0.36	0.06			0.001

CI: Confidence Interval, n: number, OR: Odds Ratio, OR: Adjusted Odds Ratio for BMI, Triglycerides, Cholesterol, HDL, LDL, and VLDL.*

The minor allele (T) frequency in cases was found to be 0.36, while that of control group was found to be 0.06. Biochemical characteristics of IHD cases according to MTHFR gene of rs1801133 polymorphism in the co-dominant and dominant models

were as shown in tables 5 and 6. A significant association between raised BMI, cholesterol, triglycerides and VLDL with MTHFR rs1801133 polymorphism has been found in the co-dominant model (table 5). In the dominant model there were significant association between the gene polymorphism and BMI ($p<0.001$), such association was didn't seen with another biochemical parameters such as cholesterol, triglycerides, LDL, HDL and VLDL (table 6). The genetic power of the study was calculated to be 100% according to OSSE, http:/osse.bii.a.star.edu.sg/.

Discussion

Coronary artery diseases (CAD) are a common cause of morbidity and mortality in Iraq [12]. According to latest WHO data "published in May 2014" deaths due to Coronary heart disease in Iraq were found to be 18.60% of total deaths [12]. Studies in Iraq pointed to high incidence of Coronary heart disease in young people which increase suspicion of genetic impact in these diseases [13]. S. K. Shaikhow et al in their study performed in Kurdistan Iraq; showed that the Premature coronary artery disease is alarming in the country [14].

MTHFR gene mutation 'the gene of our study' had significant association with stenosis of arteries, this hypothesis mentioned by several researches [15, 16 and 17]. In the present study we found a significant difference in the frequency of genotypes and alleles of MTHFRC677T between cases and control.

We regarded the group which undergone to coronary angiography in al-Najaf cardiac center and had sever stenosis >70% as cases, while the control group had normal coronary angiography. The present study shows association between the TT and CT genotypes for MTHFR gene with the severity of CAD; however, these genotypes appear to be independent risk factors. Several studies about MTHFR gene mutation have been done in the neighboring countries such as Iran [18] and Turkey [19], in addition to many other researches [20 and 21]. All of these studies were consistent our hypothesis (the presence of correlation between MTHFR gene polymorphism to ischemic heart disease). On the other hand, there were researches in Indian [22] and Koreans [23] people demonstrated that no importance of such association with ischemic heart diseases.

Table 5. Biochemical characteristics of IHD cases according to MTHFR gene polymorphism genotype (co-dominant model)

Characteristics	CC (n=65)	CT (n=62)	TT (n=23)	P-value
BMI (kg/m^2)	21.3 ± 1.7	23.9 ± 3.1	25.0±3.0	< 0.001
Cholesterol (mg/dl)	183.8±23.5	188.6±25.2	198.7± 26.1	0.045
Triglycerides (mg/dl)	122.3±24.8	126.4±29.8	140.3± 28.1	0.028
VLDL (mg/dl)	24.5 ± 5.0	25.3 ± 6.0	28.1 ± 5.6	0.028

n: number, BMI: body mass index, VLDL: very low-density lipoprotein.

Table 6. Biochemical characteristics of IHD cases according to MTHFR gene polymorphism genotype (Dominant model)

Characteristics	CC (n=65)	CT+TT (n=85)	P-value
BMI (kg/m^2)	21.3 ± 1.7	24.2 ± 3.1	< 0.001
Total Cholesterol (mg/dl)	183.8 ± 23.5	191.3 ± 25.7	0.067
Triglycerides (mg/dl)	122.3 ± 24.8	130.1 ± 29.8	0.082
VLDL-C (mg/dl)	24.5 ± 5.0	26.0 ± 6.0	0.082
LDL-C (mg/dl)	107.4 ± 21.9	113.6 ± 24.2	0.109
HDL-C (mg/dl)	51.9 ± 8.9	51.6 ± 10.1	0.834

n: number, BMI: body mass index, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low-density lipoprotein.

MTHFR gene mutation of C-to-T substituted at nucleotide position 677 in the coding region of gene, that lead to substitution of amino acid number 222 alanine to valine. This substitution results in prevention Flavin Adenine Dinucleotide (FAD) binding, with loss of folate, and reduced activity of MTHFR enzyme at higher temperatures (thermolabile) that lead to increase level of homocystiene [24]. However, homocysteinemia can be resulted from gene mutation of other enzymes responsible for the metabolism of homocysteine. These enzymes may be 5, 10 methylenetetrahydrofolatereductase (MTHFR), methionine-synthase, and cystathionine β-synthase [25]. Although, the most important and common one is MTHFR 677, which is recorded to associate with mild and moderate increase in the level of homocysteine (13–24μM and 25–60μM respectively) [25].

Homocysteine can lead to development of coronary artery disease "CAD" by different mechanisms for example its effects on endothelium layers of blood vessels and smooth muscle layers with destruction of blood vessel structure and function [26]. However, mechanisms of such effects include increasing in proliferation of smooth muscle of blood vessels, oxidative damage, dysfunction of vascular endothelium, an increase of collagen synthesis and destruction of elastic material of blood vessels [26]. The investigation of the effect of homocysteine on C-reactive protein "CRP" expression on vascular smooth muscle cells "VSMCs", demonstrated that homocysteine has induced protein and mRNA expressions of CRP in (VSMCs) both in vitro and in vivo [27]. An evidenced pathogenesis role of homocysteine in atherosclerosis is given by the findings of such study.

Additionally, Homocysteine has important role in increasing the activity of Hydroxymethyl Glutaryl Co-enzyme-A (HMGCo-A) reductase which is leading to increase synthesis of cholesterol [28]. Cholesterol are independent risk factors for coronary artery disease "CAD"[29-34]. An elevated cholesterol level lead to atherosclerosis so, it is a risk factor for coronary artery disease.

Results of the present study show that serum triglyceride and cholesterol have significant statistical correlations with the diseases at different levels. As mentioned in the result (Table 5), the co-dominant model exhibits a statistically significant difference among the three genotypes CC, TT, CT regarding cholesterol level, VLDL, and triglyceride levels.

There are some limitations of this study included a study of another MTHFR SNPs such as A1298C and investigation its' correlation to ischemic heart disease. In addition to explore the role of polymorphism of other genes such as MPO, Apo lipoprotein E polymorphism, etc and showing its' association with ischemic heart diseases. The genetic aspect of these diseases give us advantage as part of prospective management in the medical future of ischemic heart diseases in the world.

Conclusion

MTHFR polymorphism was common in the population of Iraq and significantly associated with CAD. The frequency of CT and TT genotypes was correlated with CAD. These genotypes may represent a genetic risk factor for CAD.

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