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MTHFR POLYMORPHISM IN PATIENTS OF CORONARY ARTERY DISEASE FROM HARYANA: DOES A1298C POLYMORPHISM ASSOCIATE WITH DISEASE?

Medical Science

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ABSTRACT

Background and Aim: Coronary artery disease is a multifactorial disease caused by various risk factors including genetic factors. MTHFR A1298C polymorphism is one of the important genetic factor which has clinical contribution. Due to lack of information on associations of MTHFR A1298C polymorphism with CAD, we aimed to assess the risk of MTHFR A1298C polymorphism with CAD in patients from Haryana
Materials and Methods: This study included 97 CAD patients (56 males, 41 females) and 93 healthy controls (males 51, females 42) between 21-50 years of age. The risk factors, lipid profile and clinical parameter were recorded. DNA was isolated from blood and MTHFR A1298C polymorphism was screened by PCR-RFLP.
Results: No significant difference ($p>0.05$) in the distribution of MTHFR A1298C polymorphism is observed in CAD patients as compared to healthy controls. No significant difference ($p>0.05$) in the distribution of MTHFR A1298C polymorphism among young age (≤40 years) CAD patients demonstrate no independent association of MTHFR A1298C polymorphism with the early onset of the disease. No significant difference ($P>0.05$) in the incidence of AA, AC and CC is observed in CAD patients with positive family history.
Conclusion: The MTHFR A1298C polymorphism does not associate with the development of CAD in patients from Haryana and therefore, rule out the possibility of independent risk factor for the development of CAD. The incidence of MTHFR A1298C polymorphism does not differ among CAD patients with age, sex and positive family history.

KEYWORDS

Coronary artery disease, MTHFR A1298C, Family history, Haryana.

INTRODUCTION

Coronary artery disease (CAD) usually occurs as a result of narrowing and/or blocking of arteries due to atherosclerosis and is the leading cause of mortality and morbidity across the globe including India (1, 2, 3). The atherosclerosis progresses by building up plaque on the inner wall of the arteries and have grave consequences like angina, stroke and heart attack due to restriction in the blood flow (4). The risk for the development of CAD is multi-factorial and; the risk factors like hypertension, diabetes, smoking, obesity and dyslipidemia are well established risk factors (5,6). The aged and elderly people are more susceptible for development of CAD which act as an independent risk factor and may have synergistic effect due to the presence of other risk factors like hypertension, diabetes, smoking, obesity and dyslipidemia etc. (7). Male are on higher risk for development of CAD than females at young age. However, post menopausal female are at same risk for development of CAD as of male (4). Besides that the factors like positive family history and genetic mutations are also reported to be associated with CAD (6).

In past decade, the associations of genetic risk factors has been explored worldwide by investigating various candidate genes and among them the polymorphism and mutations in the Methylenetetrahydrofolate reductase (MTHFR) gene are known for their connections with the progression of the CAD but the results are still uncertain and varies in different epidemics and ethnicity (8,9). Due to change in the life style and economic status, the incidence of CAD particularly in young aged peoples has increased in India and in Haryana state (3,5). In a previous study, we have reported the need of genetic screening in the in the young aged CAD patients from Haryana as probable risk factors for the development of CAD (5). Therefore, in efforts to delineate the role of genetic factors in development of CAD among young people, the polymorphism screening in MTHFR has been put to task. There are >40 SNPs (single neucleotide polymorphism) have been screened in the MTHFR gene but C677T and A1298C polymorphisms has been reported to have important clinical contribution (10). However, this report will be limited to the impact of MTHFR A1298C polymorphism on CAD as it has not been explored much.

MTHFR catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and; the methyl cycle and methylation reactions are essential for the imprinting of paternal methylated DNA which is dependent on the gene expression and activity of the enzyme (10). The MTHFR A1298C polymorphism changes the amino acid glutamate to alanine which results in reduction of enzyme activity. The MTHFR polymorphism may affect the 5-methyl-tetrahydrofolic acid and reduce the conversion of homocysteine to methionine. The studies have been conducted to assess the associations of A1298C polymorphism in CAD and its impact on homocysteine concentration (9, 10, 11). However, limited information is available regarding associations of A1298C polymorphism as a risk for CAD from India and no information from the North Indian State Haryana.

On considering the non-availability of information from Haryana on genetic risk factors in young aged patients without any well known risk factors (hypertension, diabetes, smoking, obesity), there is need to screen the genetic mutations and polymorphism in such CAD patients and therefore, the present study has been designed to investigate the role of MTHFR A1298C polymorphism as a risk for development of CAD in patients from Haryana with respect to the factor like the age, sex and family history. However, to exclusively assess the role of MTHFR A1298C polymorphism on the development of CAD, the patients with known risk factors hypertension, diabetes, smoking and obesity have been excluded from this study.

MATERIAL AND METHOD

Study design:

In this study, the 97 CAD patients and 93 age / sex matched healthy

controls who have normal ECG were recruited on the basis of inclusion / exclusion criteria. The subjects were recruited from the OPDs & ward of Department of Cardiology, PGIMS, Rohtak after due consent from the subjects. The known risk factors (Hypertension, Diabetes, Smoking, Obesity) , family history of CAD , clinical parameters and biochemical investigations (Blood sugar, Lipid profile- Total-cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol and triglycerides) were recorded on a predesigned proforma. The positive risk factors were assigned as per the criteria mentioned in our previous study (5). Out of 641 CAD patients whose performa were filled up and screened, only 97 could be recruited following inclusion/ exclusion criteria.

The DNA was isolated from one ml blood of CAD patients and control followed by screening for MTHFR polymorphism. The molecular investigations were done in the Department of Biotechnology & Molecular Medicine, PGIMS Rohtak and Centre for Medical Biotechnology, M.D. University, Rohtak.

Inclusion & Exclusion criteria

The patients/controls within the age of 21-50 years were included to exclude the possibility of age related risk factor of CAD. The CAD patients and the controls with the past history of hypertension, diabetes, smoking and obesity were excluded. The CAD patients with positive family history were included because the family history may be associated with persistence of genetic mutations or polymorphism. This facilitates us to limit the study for the assessment of genetic factor MTHFR A1298C polymorphism as independent risk factor for CAD.

DNA Extraction and detection of MTHFR A1298C polymorphism by Restriction fragment length polymorphism (RFLP):

The DNA was isolated by phenol/chloroform methods from the blood as described elsewhere with slight modification (12). The MTHFR A1298C polymorphism was detected by PCR-RFLP methods. First, the PCR was performed using forward (5'- CTT TGG GGA GCT GAA GGA CTA CTA-3') and Reverse (5'- CAC TTT GTG ACC ATT CCG GTT TG -3') primers for 35 cycles. The amplified PCR product of 163 bp was detected by agarose gel electrophoresis. The PCR products of 163 bp were digested with *MboII* (MBI Fermentas) restriction enzyme for 4 hrs and analyzed on polyacrylamide gel (12%). The genotype was assigned as described elsewhere (13).

Briefly, the fragments of 56, 31, 30, 28, and 18 bp base pairs (bp) represent the AA (homozygous), fragments of 84, 31, 30, and 18 bp represent CC (homozygous) and, fragments of 84, 56, and 30 bp represent AC (heterozygous) genotype (Figure-1). Mainly, the absence of fragment of 84 bp and presence of 56bp represent AA, absence of fragment of 56 bp and presence of 84 bp represent CC and, the presence of both 84 bp and 56 bp fragments represent AC genotype.

Statistical analysis

The Chi-square between percentages, Chi-square and Chi-square trend, wherever applicable, were performed using SPSS software. The p value < 0.05 was considered as statistically significant. The categorical variables have been presented as number and percentage.

RESULTS:

The incidence of MTHFR A1298C polymorphism among the CAD patients and healthy controls has been depicted in Table -1. The polymorphism of A1298C has been denoted as AA, AC and CC genotypes. The incidence of AA, AC and CC genotype among CAD patients and healthy controls are found to be comparable to each other and no significant difference has been observed ($p>0.05$). Similarly, the allelic frequency of A and C among the CAD patients and healthy controls are comparable to each other without any significant difference ($p>0.05$) (Table-1). These results demonstrate that the transition of MTHFR 1298 A to C does not associate with development of CAD in the patients.

No age associated difference has been observed in the distribution of MTHFR A1298C genotypes in CAD patients. Among the various age groups (21-30 years, 31-40 years and 41-50 years), the differences in the incidence of genotype AA, AC and CC is found to be statistical non-significant ($p>0.05$). Among the young aged CAD patients ($\leq$40 years), the non-significant difference ($p>0.05$) in the distribution of genotypes of MTHFR A1298C has been observed (Table-2). These results rule out the possibility of early onset of the diseases in the young patients due to MTHFR A1298C polymorphism. Also, no gender associated difference in the incidence of genotype AA, AC and CC is observed among CAD patients in this study (Data not shown).

The positive family history is observed in nearly 50 % of the CAD patients but no statistical significant difference ($p>0.05$) is observed in the incidence of positive family history among CAD patients of various MTHFR 1298 genotypes (Table-3). These results suggest that the positive family history may be contributing for the development of CAD but it is independent of the presence of MTHFR A1298C polymorphism.

The HDL-cholesterol, VLDL-cholesterol and triglycerides level of the CAD is found to be significantly higher than healthy controls but no significant difference is observed in the level of the total cholesterol and LDL-cholesterol level among them. However, lipid profile of the CAD patients among different genotypes (AA, AC and CC) is found to be comparable without any significant difference (Data not shown).

DISCUSSION:

MTHFR gene encodes the methylenetetrahydrofolate reductase enzyme which play a major role in maintaining the homocysteine level. The MTHFR polymorphisms have been reported to be associated many diseases like osteoporosis, diabetes, psychiatric disorder, Alzheimer, dementia, cancer and CAD etc. However, the reports on the associations of MTHFR gene polymorphism as a risk for CAD are still inconsistent and inconclusive worldwide. The MTHFR A1298C polymorphism has been reported as one of the clinically important polymorphism which may have associations with the disease pathology. This study has been aimed to investigate the risk of development of CAD due to MTHFR A1298C gene polymorphism in the CAD patients without any known history of modifiable risk factors like hypertension, diabetes, Smoking and obesity. This strategy has permitted us to exclusively investigate the role of genetic risk factor on the development of the disease. Therefore, associations of CAD with MTHFR A1298C polymorphism has been investigated with respect to age, sex and family history among CAD patients from Haryana.

The MTHFR A1298C polymorphism forms three genotypes AA, AC and CC and; the incidence of these genotypes has been found to be comparable without any significant difference among CAD patients and healthy controls. The allelic frequency of A and C has also been found comparable among CAD patients and healthy controls in this study. These results suggest that the transition of MTHFR 1298 A to C do not confer as an independent risk factor for development of CAD. The A1298C polymorphism is not much documented and the results vary in different population. The MTHFR A1298 C polymorphism has been reported to reduce the activity of MTHFR by 35% (14). The high frequency of CC genotype has been reported in Indian population with potential impact on hyperhomocysteinemia (15). Similarly, a European study shows the significant associations of A1298C polymorphism with CAD (16). However, the Indian studies have reported the increased variants of MTHFR A1298C polymorphism in CAD patients but not associated with risk for CAD (10,14,17). Some studies even reported the protective effect of AC genotype on CAD rather a risk factor (18, 19). These findings suggest no associations of MTHFR A1298C polymorphism as risk factor for CAD. Our finding are in consistent with these reports and suggest that MTHFR A1298C polymorphism does not associate as a risk factor for the development of CAD in patients from Haryana.

The gender wise difference in the distribution of MTHFR A1298C genotypes (AA, AC and CC) has not been observed among the CAD and healthy controls of this study. Also, no age associated difference was observed in distribution of AA, AC and CC genotype among the CAD patients of this study. No significant difference in the frequency of various A1298C genotypes among CAD patients in the age of $\leq$40 years shows that presence of A1298C genotypes do not associate with age in this study. These results suggest that the MTHFR A1298C polymorphism does not associate as an independent risk factor for the development of early onset of CAD.

The high incidence of positive family history has been observed among CAD patients of this study because these patients do not have risk factors like hypertension, diabetes, smoking and obesity. However, the non-significant differences on the incidence of positive family history among CAD patients with various A1298C genotypes rule out the possibility of any independent association of MTHFR A1298C polymorphism with family history. No association of

MTHFR A1298C polymorphism with the lipid profile among CAD patients has been observed in this study.

In summary, the presence of MTHFR A1298C polymorphism does not appear to be an independent risk factor for the development of CAD. The comparable incidence of various A1298C polymorphism among CAD patients of different age groups and young patients (≤40 years), rule out the possibility of early onset on the CAD due to this genetic variation. The A1298C polymorphism does not seems to be associated with the positive family history of CAD in this study. However, this report specifically focused on exclusive analysis of MTHFR A1298C as risk for CAD and therefore does not explain the interaction with other CAD risk factors. Nevertheless, the finding needs to validated by conducting a study having large sample size to completely rule out the associations of MTHFR A1298C polymorphism with CAD.

In conclusion, the presence of MTHFR A1298C polymorphism does not associate as an independent risk factor for the development of CAD in patients from Haryana. It also does not associate with the early onset of the disease. The incidence of MTHFR A1298C polymorphism is independent of age, sex and positive family history in this study.

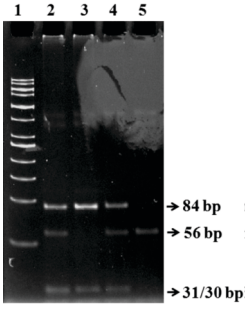

Figure1: The representative image of polyacrylamide gel electrophoresis showing the PCR product digested by *MBoII* for detecting the MTHFR polymorphism. Lane 1 -50 bp DNA ladder, Lane 2 &4 – AC, Lane 3- CC and Lane 5 –AA polymorphism of MTHFR 1298.

Table: 1 Incidence of MTHFR A1298C polymorphism among CAD patients and healthy controls.

MTHFR A1298C polymorphism	CAD n=97 (%)	Healthy controls n=93 (%)	P Value
AA	47 (48.45%)	40 (43.01%)	>0.05
AC	45 (46.39%)	49 (52.69%)	>0.05
CC	5 (5.15%)	4 (4.3%)	>0.05
Allele Frequency			>0.05
A	71.64 %	69.35%	>0.05
C	28.36 %	30.65%	>0.05

Table: 2 Age-wise distribution of MTHFR A1298C polymorphism in CAD patients.

Age	MTHFR 1298 AA (n=47)	MTHFR 1298 AC (n=45)	MTHFR 1298 CC (n=5)	*P Value*
21-30 (Years)	11 (23.40%)	13 (28.89%)	2 (40%)	>0.05
31-40 (Years)	20 (42.55%)	19 (42.22%)	2 (40%)	
41-50 (Years)	16 (34.05%)	13 (28.89%)	1 (20%)	
≤40 years	31 (65.95%)	32 (71.11%)	4 (80%)	>0.05

Table: 3 Family history of the Coronary artery disease in the study subjects as provided by the patients.

MTHFR A1298C polymorphism	Family history Yes/No	Family history (%)	*P Value*
AA (n=47)	22/25	46.80 %	>0.05
AC (n=45)	24/21	46.66%	
CC (n=5)	2/3	66.66%	
CAD (n=97)	48/49	49.48%	

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Conflict of Interest: There are no conflicts of interest.

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