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MTHFR (ALA 222 VAL) POLYMORPHISM AND AMI IN PATIENTS WITH TYPE II DIABETES MELLITUS

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ABSTRACT

The prevalent Ala222Val single nucleotide polymorphism of the MTHFR gene has been shown to be associated with type II diabetes. The objective of the present study was to find out whether there is genetic predisposition for development of acute myocardial infarction in type II diabetes mellitus among South Indian Tamil population. PCR-based restriction enzyme analysis was performed in DNA isolated from 120 acute myocardial infarction patients with diabetes mellitus and 100 non diabetic healthy individuals with no documented cardiovascular diseases. The results indicate that the MTHFR 677TT genotype is absent in both case and controls. The MTHFR 677CT genotype was observed among 32 (26.7 %) cases and 20 (20%) controls and the MTHFR 677CC genotype among 88 (73.3%) cases and 80 (80%) controls. The allelic frequencies were in accordance to Hardy Weinberg equilibrium. There was no statistical difference in genotype distribution between cases and controls. In conclusion, we suggest that the analysis of MTHFR genotyping for C677T polymorphism alone need not be considered to find out whether there is genetic predisposition for development of acute myocardial infarction in type II diabetes mellitus among South Indian Tamil population.

KEY WORDS

MTHFR gene, Type II diabetes, Polymorphism, Genetic predisposition, Acute Myocardial Infarction.

INTRODUCTION

Diabetes mellitus is a complex, multifactorial and polygenic disease and it is a major life threatening health problem all over the world. Patients with diabetes mellitus (DM) have 2-6 fold increase in the prevalence of cardiovascular disease (1). Acute myocardial infarction is a cardiac disease which occurs due to sudden occlusion of coronary artery due to prolonged ischemia. Ozmen et al found a high concentration of homocysteine in patients with Diabetes mellitus and there are also studies that report an independent association between homocysteine and cardiovascular disease in patients with Diabetes mellitus (2,3,4)

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Previous study conducted among Tamil population suggests hyperhomocysteinemia as a significant risk factor for acute myocardial infarction (AMI) among Tamil population (5). Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme in the remethylation cycle of homocysteine metabolism. Cobalamin, riboflavin and folate are the vitamins involved in the remethylation of homocysteine to methionine. It has also been reported that simultaneous presence of decreased serum cobalamin status, hyperhomocysteinemia and MTHFR mutant genotypes (C677T and A1298C) might lead to an increased risk for the occurrence of acute myocardial infarction among South Indian Tamil population (6).

The single nucleotide polymorphism in the MTHFR gene results in substitution of Alanine by Valine (Ala222Val) thereby reducing the activity of MTHFR causing hyperhomocysteinemia (7). The C677T polymorphism is thought to decrease the binding affinity of FAD to MTHFR and may increase the rate of dissociation of FAD from the enzyme, leading to the dissociation of the active dimer into monomers (8, 9,10).

The C677T polymorphism results due to a common C to T transition at nucleotide 677 of the MTHFR gene (8). Occurrence of mutant genotype has become the focus due to its role in the pathogenesis of various diseases including premature atherosclerosis (8, 11), coronary artery disease (12) and complications of diabetes mellitus (13). As Diabetes mellitus is a major risk factor for acute myocardial infarction, it has been hypothesized that the MTHFR C677T SNP might be associated with AMI in individuals with diabetes mellitus. This study has been designed to find out the association between MTHFR C677T SNP and AMI in individuals with Type II diabetes mellitus.

MATERIALS AND METHODS

Fresh human blood was collected in EDTA coated tubes from 120 AMI patients (48-65 years) with diabetes mellitus, with no other conventional cardiovascular risk factors (hypercholesterolemia, hypertension, past history of heart disease, family history and unhealthy lifestyles such as smoking and alcoholism) and similar age and sex matched 100 non-diabetic healthy individuals with no cardiovascular risk factors. The collected blood was stored in –20°C till analysis. DNA isolation was carried out according to Sambrook and Russel from the frozen blood (14).

DNA was isolated from all the 220 study subjects. PCR-based restriction enzyme analysis was performed in DNA isolated from 120 AMI patients with diabetes mellitus and 100 non - diabetic healthy individuals with no acute AMI.

PCR analysis(6): PCR amplification of a 198bp sequence of the MTHFR gene was performed as previously described. Approximately 120 ng of genomic DNA was incubated in a total reaction volume of 50μL containing both the forward and reverse primers for the MTHFR C677T (Ala222Val) SNP, using 2.5 U Taq DNA polymerase (Bangalore Genei, India). Amplification for the MTHFR C677T SNP was performed with an initial denaturation step at 93°C for 2 min in a thermal cycler (Eppendorf India Limited). The PCR amplification conditions were as follows: 34 cycles consisting of 1 min denaturation at 92°C, 1 min annealing at 64°C and 1 min extension at 72 °C. The final cycle included a 10-min extension step at 72°C.

Restriction enzyme analysis: The MTHFR C677T SNP creates a *Hinf*I (Fermentas Life Sciences, Germany) restriction enzyme recognition sequence. The SNP was detected by digestion of PCR amplified products with *Hinf* I for 3 hrs at 37°C. Restriction fragment size analysis was performed by visualization of digested PCR products after separation by gel electrophoresis in a 10% polyacrylamide gel.

Statistical analysis: The allele frequencies were calculated by allele counting. Pearson Chi-square (χ^2) test was performed to find the statistical significance between the genotypes.

RESULTS AND DISCUSSION

After restriction digestion with *Hinf* I, the following fragment sizing patterns were observed by the gel electrophoresis: the 677 C/C genotype results in no cleavage of the amplified 198bp fragments, the 677C/T genotype results in 3 fragments of 198,175 and 23bp. MTHFR 677T/T genotype which will result in complete cleavage of the 198bp fragment into 175 and 23bp was not observed in any of the study subjects.

Genotype and allelic frequencies for the C677T SNP of the MTHFR gene in AMI patients with diabetes mellitus and non diabetic healthy individuals with no documented cardiovascular diseases were calculated. Genotype frequencies obtained between the AMI patients and controls did not differ significantly from the frequencies predicted on the basis of the Hardy–Weinberg law of population genetics ($p>0.05$).

When the prevalence of the MTHFR C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene among South Indian Tamil population was determined and the association of the mutant allele with AMI was evaluated, no association was found between genotype distribution and AMI (15). This study was conducted to find out association between MTHFR C677T SNP and AMI in individuals with type II diabetes mellitus.

The results indicate that the MTHFR 677TT genotype is absent in both case and controls. The MTHFR 677CT genotype was observed among 32 (26.7 %) cases and 20 (20%) controls and the MTHFR 677CC genotype among 88 (73.3%) cases

Table 1: MTHFR C677T Polymorphism - Genotype and Allele Frequency in AMI Patients and Controls

Genotype/Allele	Total (n=220)	Diabetic Patients (n=120)	Controls (n=100)
GENOTYPES (C677T)			
CC	168	88 (73.3%)	80 (80%)
CT	52	32(26.7%)	20(20 %)
TT	—	—	—
ALLELES			
C	0.88	0.86	0.90
T	0.12	0.14	0.10

Table 2: Prevalence of the MTHFR gene C677T mutation in different populations (17)

Country	Ethnic population	No. of subjects	Genotype frequency			T allele
			C/C	C/T	T/T	frequency
India	SouthIndians(Tamil) Present study	100	80	20	0	0.10
Indonesia	Javanese	68	57	11	0	0.81
Japan	Mongolian	244	96	116	32	0.37
Australia	Caucasians	225	88	113	24	0.36
Brazil	Amerindian	129	77	42	10	0.24
Canada	Caucasians	414	172	183	59	0.36
Canada	Inuit	174	155	17	2	0.06
China	Mongolian	121	51	53	17	0.35
England	Caucasians	222	96	97	29	0.34
Holland	Caucasians	503	224	234	45	0.32
Korea	Mongolian	124	33	82	9	0.40
Ireland	Caucasians	1309	600	568	141	0.32
Italy (North)	Caucasians	130	42	71	17	0.40
Italy (South)	Caucasians	431	130	223	78	0.44
Mexico	Mexican	250	44	119	87	0.59
South Africa	African	107	85	22	0	0.10
Sub-Sahara	African	301	263	38	0	0.63
USA	African-American	496	363	127	6	0.14
USA	Hispanics	169	63	71	35	0.42

and 80(80%) controls (Table 1). There is no deviation in the "T" allele frequency (0.1) observed in the present study to that established in the previous study (0.1) which was conducted among the same south Indian Tamil population (15). As allele frequencies are population specific, there is variation in allelic frequencies in different populations (16) (Table 2).

A study conducted in Sweden by Zetterberg et al indicated that the MTHFR polymorphisms may have a major impact on foetal survival (18). Another study from India reported the impact of MTHFR variants on foetal viability and gender. They identified that high mortality was observed in individual with one "T" allele (19). Similarly, several other studies conducted among other populations including Canadians (20), have also concluded that increase in mutant allele in MTHFR causes decrease in foetal viability. Thus the absence of MTHFR 677 TT genotype in the study population may be explained by the fact that the occurrence of the 677TT genotype may be deleterious and it might have its impact on foetal viability as described previously (19).

In the light of the above data, we conclude that the presence of mutant TT genotype may be deleterious and this might be the reason for the absence of TT genotype (2 mutant alleles) among the study population in the present study.

Maeda et al found an association between mutant homozygous genotype for MTHFR C677T and Diabetic retinopathy in individuals with type II diabetes mellitus (21). Similarly, Ksaizek et al have also found that the MTHFR C677T mutation in the MTHFR gene predisposes type 2 diabetes patients to the development of diabetic retinopathy. They observed a high (13.5%) prevalence of mutant genotype in DM patients when compared to the controls (9.5%) (22).

A study relating the MTHFR gene C677T mutation and left ventricular hypertrophy (LVH) suggested the MTHFR mutant genotype as a possible risk factor for the development of LVH in the type II diabetes mellitus (23). Agullo-Ortuno et al found an association between homocysteine levels and diabetic complications such as macroangiopathy, retinopathy and nephropathy in type I diabetes, whereas such an association was not seen among type II diabetic subjects (24).

Another study conducted among Israeli Jewish population with

type 2 diabetes mellitus suggested that folate supplementation in diabetic patients with the C677T mutation and low-normal serum folate may prevent the onset or retard the progression of diabetic nephropathy in type II diabetic patients (25).The MTHFR C677T mutation of MTHFR gene was found to be common in Chinese population and MTHFR C677T gene polymorphism associated with a predisposition to increased plasma homocysteine levels may represent a genetic risk factor for diabetic nephropathy in Chinese type 2 diabetic patients (26).

In conclusion, as there was no statistical difference in genotype distribution between cases and controls, we suggest that the analysis of MTHFR genotyping for C677T polymorphism alone need not be considered to find out whether there is genetic predisposition for development of acute myocardial infarction in type II diabetes mellitus among South Indian Tamil population.

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