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Methylene Tetrahydrofolate Reductase Gene Polymorphisms and Risk of Myocardial Infarction

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ABSTRACT

Methylenetetrahydrofolate reductase (*MTHFR*) enzyme is one of the chief players in metabolism of circulating forms of homocysteine (Hcy). Any variation in the *MTHFR* gene can affect *MTHFR* enzymatic activity and is associated with serious cardiovascular profile like Myocardial Infarction (MI). The present study is designed to evaluate the association of *MTHFR* (C677T and G1793A) gene polymorphisms with MI in Jammu region. For the study purpose a total of 109 individuals were recruited (49 cases with MI and 60 unrelated healthy controls). Genotyping was done by PCR-RFLP technique and further data was statistically analysed. The frequencies of variant *MTHFR* alleles were higher in patient group than in controls (677T=12.24% vs 1.67% and 1793A=16.33% vs 15%). Logistic regression analyses have shown that *MTHFR* C677T polymorphism was significantly associated with the development of MI whereas *MTHFR* G1793A polymorphism was not associated with the disease in our study population. The haplotype T-G was giving approximately 8.23-fold risk [OR=8.23 (1.79-37.74), p=0.001] and C-G is conferring 2 folds protection [OR=0.5 (0.26-0.96), p=0.03] towards MI outcome. Based on measure of linkage disequilibrium (LD), the two *MTHFR* variants (C677T & G1793A) were in complete LD (D'=1, r2=0.02) in patients and in controls (D'=0.99, r2=0). The obtained data proved the association of *MTHFR* polymorphisms with the progression of MI severity in the population of Jammu region of the Union territory of Jammu and Kashmir State. Although a study comprising of large sample size is required to reach on a final conclusion.

KEY WORDS: C677T, G1793A, MI, *MTHFR*, POLYMORPHISM.

INTRODUCTION

Recent progresses in the field of genetics and genomics have provided substantial benefits to clinical medicine including facilitation of characterization of disease pathogenesis and diagnosis. Genetics has offered effective approaches to the identification of complex diseases like Myocardial Infarction (MI). It is an important clinical problem because of its large contribution to mortality. MI is multifactorial in origin and involves interplay of various environmental exposures viz. smoking, sedentary lifestyle, altered blood lipid levels, obesity, family history, hypertension and genetic profile. All these exposures in concert are responsible for the commencement of immuno-inflammatory cascade leading to endothelial damage, atherosclerosis, thrombus formation, blockage of arteries and finally event of MI. Elevated circulating levels of homocysteine (Hcy) is a well known predictor for complex cardiovascular phenotypes such as MI (Kaur et al., 2016; Raina et al., 2016a).

Homocysteine [COOH-CH(CH2CH2SH)-NH3] is a homologue of the amino acid cysteine and is

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synthesised in the body from dietary methionine. Methylenetetrahydrofolate reductase (*MTHFR*) is a folate and vitamin B12-dependent enzyme which is involved in conversion of Hcy back to methionine. Thus, maintaining plasma Hcy levels (Iqbal et al., 2011). There are two well characterised single nucleotide polymorphisms (SNP) at *MTHFR* locus viz. C to T transition at nucleotide position 677 in exon 4(rs1801133) and G to A transition at nucleotide position 1793 in exon 11 (rs2274976) which have been identified for bringing up alteration in *MTHFR* enzyme activity and disturbance in Hcy metabolism (Frosst et al., 1995; Raddy et al., 2002). These polymorphisms are associated with reduced *MTHFR* enzyme activity by about 70% and 40% in homozygotes and heterogzygotes, respectively (Mao et al., 2008; Kour et al. 2016b Jakó and Sinkó (2017).

Previous reports on *MTHFR* gene polymorphisms have shown that the said gene is significantly associated with CVD in Jammu region (Raina et al., 2016a; Raina et al., 2016b) but the polymorphisms have not been studied in MI so far. Hence, in the present investigation we have evaluated the association of *MTHFR* C677T and *MTHFR* G1793A gene polymorphisms with MI in population of Jammu region (J&K).

MATERIAL AND METHODS

Study population and Ethical Approval: A total of 109 individuals, 49 patients with MI and 60 healthy unrelated, control individuals were recruited for the present study. The patients were enrolled from the Department of Cardiology, ASCOMS, Sidhara, Jammu (J&K) and from private clinics. Sampling for controls was done from University premises. Physically healthy individuals with no history of CAD, hypertension, diabetes, thyroid problem, any form of cancer and other major medical conditions were included as controls. The present study design was approved by institutional Ethical Committee, University of Jammu. A brief health questionnaire covering different parameters such as gender, height, weight, smoking and physical inactivity, along with a consent form was duly filled by every subject enrolled for study. Diagnosis of MI was based on WHO criteria which included clinical history, ECG changes indicating myocardial damage and elevation of biochemical markers (Report of the Joint International Society and Federation of Cardiology, 1979).

Genotyping of MTHFR gene polymorphisms: Genotyping of *MTHFR* C677T and *MTHFR* G1793A polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously (Raina et al., 2016b; Raddy et al., 2002). Statistical analysis: Statistical analysis was carried out by using SPSS (Chicago, IL, USA) software version 21. Clinical characteristics of all the subjects were expressed as mean ± SD. Allelic frequencies were calculated by the gene-counting method. Hardy-Weinberg equilibrium (HWE) and the genotypic as well as allelic distribution of both polymorphisms of MTHFR gene were analyzed using Pearson's goodness of fit chi-square ($\chi 2$) test. The odd-ratios (OR) for *MTHFR* polymorphisms were calculated with 95% confidence interval (CI) under different genetic models. SHEsis software (Shi and He, 2005) was used to calculate the haplotype frequencies and Linkage disequilibrium (LD) pattern. A p-value of <0.05 was considered as statistically significant.

Table 1. Showing genotypic and allelic distribution of MTHFR gene polymorphisms in study participants.

Genotypes/ alleles/	MI Cases					Controls (N=60)
MTHFR C677T	IWMI (n=21)	AWMI (n=10)	STEMI (n=13)	NSTEMI (n=5)	Total (N=49)	
CC	16 (76.19%)	7 (70%)	11 (84.62%)	4 (80%)	38 (77.55%)	58 (96.67%)
CT	4 (19.05%)	3 (30%)	2 (15.38%)	1 (20%)	10 (20.41%)	2 (3.33%)
TT	1 (4.76%)	0	0	0	1 (2.04%)	0
C	36 (85.71%)	17 (85%)	24 (92.31%)	9 (90%)	86 (87.76%)	118 (98.33%)
T	6 (14.29%)	3 (15%)	2 (7.69%)	1 (10%)	12 (12.24%)	2 (1.67%)
MTHFR G1793A						
GG	15 (71.43%)	7 (70%)	9 (69.23%)	4 (80%)	35 (71.43%)	42 (70%)
GA	5 (23.81%)	2 (20%)	4 (30.77%)	1 (20%)	12 (24.49%)	18 (30%)
AA	1 (4.76%)	1 (10%)	0	0	2 (4.08%)	0
G	35 (83.33%)	16 (80%)	22 (84.62%)	9 (90%)	82 (83.67%)	102 (85%)
A	7 (16.67%)	4 (20%)	4 (15.38%)	1 (10%)	16 (16.33%)	18 (15%)

RESULTS AND DISCUSSION

These 49 cases of MI were further categorised into different subtypes according to ECG patterns and location of infarct. It was found that the inferior wall myocardial infarction (IWMI) was prominent type of heart attack occurring in 21 patients followed by ST- Elevation Myocardial Infarction (STEMI) in 13 patients, Anterior Wall Myocardial Infarction (AWMI) in 10 patients and Non-ST Elevation Myocardial Infarction (NSTEMI) in 5 patients only. The percentage distribution of both *MTHFR* C677T and *MTHFR* G1793A polymorphisms are summarised in Table 1. It was observed from the data that the wild CC genotype was the most prevalent one followed by heterozygous CT genotype and then the variant homozygous TT genotype. There was a considerable increase in CT genotype in patient group in judgement to controls (20.41% vs 3.33%). The prevalence of TT-genotype was higher in MI patients (2.04%) whereas there was complete absence of variant genotype in healthy controls. Overall, the frequency of variant T-allele was significantly higher in patients i.e. 12.24% than in controls i.e. 1.67%.

The prevalence of genotypes/ alleles were also studied in different MI categories and it was observed that risk allele (T) was highly prevalent in IWMI patients (14.49%) followed by AWMI (15%), NSTEMI (10%) and then, STEMI (7.69%). As regards of *MTHFR* G1793A polymorphism, the genotype distribution pattern in both MI patients and control individuals showed that the percentage of wild GG- genotype was higher (71.43% vs 70% respectively) in comparison to heterozygous GA-genotype (24.49% vs 30%% respectively) and risk AA-genotype (4.08% vs 0% respectively). Additionally, the allele percentage of the variant A- allele in MI patients was almost comparable with the control group (16.33% vs 15% respectively). The order of prevalence of A-allele in different MI subtypes was as follows: AWMI (20%), IWMI (16.67%), STEMI (15.38%) and NSTEMI (10%). The observed frequencies of *MTHFR* polymorphisms were in concordance with HWE in both study groups.

Table 2: Showing Logistic regression analysis for *MTHFR* (C677T) polymorphism

Genetic Model	Genotypes/ Alleles	MI (N=49)	Controls (N=60)	OR (95% CI)	p-value
Co-dominant	CC	38	58	1 (Reference)	
	CT	10	2	7.63 (1.58-36.76)	0.004
	TT	1	0	Not possible†	-
Dominant	CT+TT	11	2	Not possible†	-
	CC	38	58	1 (Reference)	
Recessive	TT	1	0	Not possible†	-
	CT+CC	48	60	1 (Reference)	-
Allelic	C	86	118	1 (Reference)	
	T	12	2	8.23 (1.79-37.73)	0.001

†Some genotype combinations were not observed, so it was not possible to calculate odds ratio.

Table 3. Showing Logistic regression analysis for *MTHFR* (G1793A) polymorphism

Genetic Model	Genotypes/ Alleles	MI cases (n=49)	Controls (n=60)	OR (95% CI)	p-value
Co-dominant	GG	35	42	1 (Reference)	
	GA	12	18	0.80 (0.34-1.89)	0.6
	AA	2	0	Not possible†	-
Dominant	GA+AA	14	18	Not possible†	-
	GG	35	42	1 (Reference)	
Recessive	AA	2	0	Not possible†	-
	GA+GG	47	60	1 (Reference)	
Allelic	G	82	102	1 (Reference)	
	A	16	18	1.11 (0.53-2.30)	0.8

†Some genotype combinations were not observed, so it was not possible to calculate odds ratio.

The association of *MTHFR* gene polymorphisms with risk of MI can be obtained by calculating Odds Ratio (OR) [Table-2 and 3]. For *MTHFR* C677T polymorphism, there was a significant difference in frequencies of CC vs. CT genotypes between the MI patients and healthy controls (OR=7.63 (1.58-36.76); p=0.004). OR for C vs. T allele showed that the 'T' allele was conferring approximately 8.23-folds risk, which was statistically significant for the development of MI in our study population. In context to *MTHFR* G1793A SNP, none of the applied genetic models were associated with risk of MI occurrence. Allocation of haplotype frequencies of *MTHFR* C677T and G1793A polymorphism among MI cases and healthy controls is given in Table 4. The haplotype T-G was giving approximately 8.23-fold risk [OR=8.23 (1.79-37.74), p=0.001] and C-G is conferring 2-folds protection [OR=0.5 (0.26-0.96), p=0.03] towards MI outcome. Based on measure of linkage disequilibrium (LD), the two *MTHFR* variants (C677T & G1793A) were in complete LD (D'=1, r^2=0.02) in patients and in controls (D'=0.99, r^2=0) (figure 1).

Table 4. Association of *MTHFR* haplotypes with risk of MI.

Variant MTHFR C677T/ G1793A	MI Cases (N=49)	Controls (n=60)	OR (95% CI)	p-value
C-A	0.163	0.15	1.10 [0.53-2.30]	0.8
C-G	0.714	0.833	0.5 [0.26-0.96]	0.03
T-G	0.122	0.016	8.23 [1.79-37.74]	0.001

Table 5. Showing general characteristics of the study participants

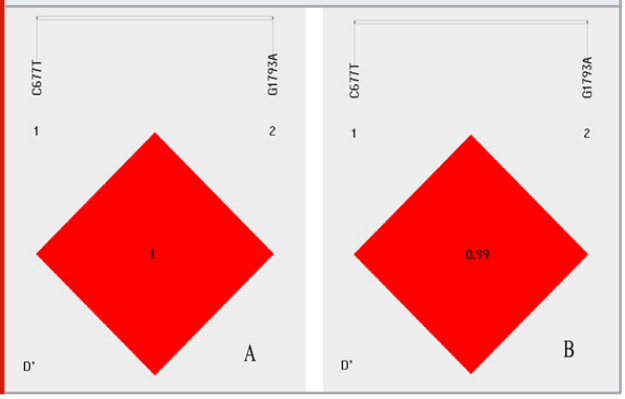
Variables	MI cases (N=49)	Controls (N=60)	OR (95% CI)	p-value
Age (yrs.)	46.75±6.7	48.91±7.4	-	0.1
Sex				
Males	40 (81.63%)	36 (60%)	-	-
Females	9 (18.37%)	24 (40%)	-	-
Blood pressure (mmHg)				
SBP	143.33±20.81	123.05±8.23	-	<0.0001
DBP	87.51±10.44	80.55±4.65	-	<0.0001
Pulse pressure	55.82±16.74	42.50±5.74	-	<0.0001
BMI	23.65±4.58	22.93±4.09	-	0.4
Smoking				
Y	26 (53.06%)	15 (25%)	3.39 (1.51-7.62)	
N	23 (46.94%)	45 (75%)	Ref. (1)	0.003
Sedentary lifestyle				
Y	23 (46.94%)	35 (58.33%)	0.64 (0.30-1.35)	
N	26 (53.06%)	25 (41.67%)	Ref. (1)	0.2

The general characteristics of the study participants are presented in Table-5. The controls were slightly elder than patients, with a mean age of 48.91 years compared to 46.75 years in the patient group. BMI was significantly higher in patients with a mean value of 23.65±4.58 than in controls with mean value of 22.93±4.09, howsoever the values did not reach a statistical significance (p=0.4). The physiometric characteristics viz. systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP) were higher in patients in comparison to controls in a significant manner (p<0.0001). In patient group, 53.06% cases were smokers whereas; among controls only 25% were involved in habit of tobacco smoking. OR analysis revealed that smoking was adding nearly 3.39 folds risk to the progression of MI in our population [OR = 3.39 (1.51–7.62); p = 0.003]. Majority of patients as well as controls were living a sedentary lifestyle and hence, lack of association was reported with this parameter.

The incidence of MI in India is relatively higher being 64.37/1000 people (Rao et al., 2014). The disease etiology involves dual interaction of environmental and genetic factors. Homocysteine is a sulphur containing amino acid made from a common dietary amino acid, methionine that inflicts damage to the inner arterial lining and contributes to coronary heart disease and event of MI in longer duration. Henceforth, genes controlling Hcy metabolism are considered as an emerging candidates for progression of MI. With this background the present

research work was aimed to study *MTHFR* (C677T and G1793A) gene polymorphisms with the risk of MI in population of Jammu region. A high prevalence of CC genotype was reported in the present study in comparison to CT and TT genotypes. In fact, a complete absence of TT genotype was observed in MI subtypes including AWMI, STEMI and NSTEMI as well as in healthy controls. Raina and researchers (2106a and 2016b) have also reported extremely higher frequency of CC genotype and lower prevalence of TT genotype in cardiovascular diseases in Jammu region. The results are consistent with other studies done by Markan et al., 2007; Lakshmi et al., 2011; Iqbal et al. 2011; Matam et al., 2014; Ezzat et al. (2014).

Figure 1: Linkage Disequilibrium (LD plot for MTHFR gene polymorphisms (A) patients (B) Controls.



The *MTHFR* 677T-allele was having a significant role in the aetiology of MI in our population (C vs T: OR=8. 23, p= 0.001). Gülec et al. (2001); Ezzat et al. (2014), Shaker et al. (2014) and Grek et al. (2015) were also in agreement of association of this polymorphism in onset of MI. Contrary to these observations, there were reports on lack of association of *MTHFR* C677T polymorphism with increased risk for MI in different population groups (Angeline et al., 2007; Iqbal et al., 2011; Verdoia et al., 2014; Iqbal et al. 2016).

Rady et al. (2002) reported a functional polymorphism (G1793A) in exon 11 of *MTHFR* gene that results in an arginine to glutamine substitution at codon 594 (R594Q) and is associated with coronary heart diseases. There is scarcity of data establishing a connection of *MTHFR* (G1793A) genotypes and MI. In the present study it was observed that the prevalence of wild (GG) genotype was higher in MI patients than in controls whereas heterozygous (GA) genotype frequency was lower in patient group when compared to healthy controls. The prevalence of variant (AA) genotype was also low in study population and controls were found to be devoid of AA genotype completely. Among different MI subtypes, variant genotype was present only in IWMI and AWMI.

In view of GG-genotype, Rady and co-associates (2002) also reported a lower prevalence of GA and AA-genotypes in their study populations. The results of our study demonstrated that the *MTHFR* (G1793A) was not in association with the development of MI. This is consistent with previous studies which have shown lack of *MTHFR* G1793A polymorphism with cardiovascular diseases (Kebert et al., 2006; Trifonova et al., 2012; Neto et al., 2013).Haplotype analysis was also performed and it has been observed that the haplotype combination T-G was giving approximately 8.23-fold risk whereas C-G was providing 2 folds protection towards MI outcome in population of Jammu region.

CONCLUSION

The results suggested that *MTHFR* gene is an informative candidate which can be used as a potential diagnostic marker for MI. Thus, further studies are necessary to ascertain the relationship between *MTHFR* variants and MI.

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