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Research Article

METHYLENETETRAHYDROFOLATE REDUCTASE C677T GENE POLYMORPHISM AND PREDISPOSITION TO ESSENTIAL HYPERTENSION

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Abstract- Dysregulation of homocysteine pathway due to genetic alterations causes hyperhomocysteinemia induced persistent increase in blood pressure and potentially contributes to susceptibility of hypertension. Methylenetetrahydrofolate reductase (MTHFR) gene is a key modulator of homocysteine pathway and C677T SNP of this gene is linked to the progression of essential hypertension (EH). The present study was designed to investigate the association of MTHFR C677T polymorphism with predisposition to Essential Hypertension. The study was carried on 100 clinically confirmed patients with EH and 100 unrelated sex matched healthy control individuals. Different non-genetic parameters such as smoking status, alcohol intake, sedentary behaviour, family history and diet pattern were evaluated in the study subjects. Genotyping was carried out by polymerase chain reaction followed by restriction fragment length polymorphism method (PCR-RFLP). Logistic regression analysis was carried out to find association between the MTHFR genotypes/alleles and EH. A higher prevalence of non-genetic risk factors was observed in patients (smoking: 35%, physical inactivity: 32%). The genotypic frequency of homozygous wild CC and heterozygous CT genotype was 92% & 8% in patients whereas it was 99% & 1% in controls, respectively. The frequencies of CC vs. CT, CC vs. CT+TT and C vs T differed significantly between the two groups (p=0.02) studied/ analysed. The present study showed a significant association of MTHFR 677CT genotype and MTHFR 677T allele with the predisposition of EH in our population of Jammu region of Jammu & Kashmir State.

Keywords- EH, MTHFR, Homocysteine, SNP

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Introduction

Essential or primary hypertension is a vascular condition and is defined as the persistent elevation of blood pressure (BP), usually above 140/80 mmHg without any apparent cause. It is a complex disease and constitutes 90-95% of hypertensive cases [1]. However, the development and susceptibility of EH is greatly influenced by factors like genetics, race, gender, diet, lifestyle, obesity and psychology, the "Hypertensinogenic factors". Hypertension (HTN) accounts for 9.4 million deaths worldwide every year [2]. It contributes to the burden of cardiovascular diseases, stroke, chronic kidney diseases, diabetes and casualty and disability. Globally, the overall prevalence of HTN in adults aged 25 and above was 40%, being highest in the African region at 46% and the lowest prevalence rate of 35% in Americans [3]. The total observed estimates of HTN prevalence in India was 40.8% in urban and 17.9% in rural India [4].

Most of the traditional research with regard to genetics of EH focuses on the Renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system and vascular-endothelial system due to their direct involvement in regulation of blood pressure [5-9] whereas homocysteine (Hcy) metabolic pathway is an emerging target [10]. Although, Hcy pathway has not a direct control over BP, but any genetic alteration in this pathway can lead to hyperhomocysteinemia, which further promotes atherogenesis and vasoconstriction. All these events form basis of sustained BP or so called HTN. The most extensively studied genetic variation contributing to hyperhomocysteinemia is C to T single nucleotide polymorphism (SNP) at nucleotide position 677 of Methylenetetrahydrofolate reductase (MTHFR) gene which causes an alanine (A) to valine (V) substitution at codon 222 within the catalytic region of the MTHFR protein. The MTHFR 677T variant is associated with the reduced enzyme activity by about 70% and 40% in homozygotes and heterogzygotes, respectively resulting in elevation of plasma homocysteine [11]. This SNP reduces the production of an obligatory co- substrate 5-methyltetrahydrofolate (5-MTHF) from 5,10-methylenetetrahydrofolate (5,10-MTHF), for conversion of homocysteine to methionine. Taking into account the role of Hcy pathway genes in modification of BP, we performed a study to investigate the role of the MTHFR C677T SNP with predisposition to EH.

Materials and Methods

Study population: A total of 200 individuals, 100 patients with EH and 100 healthy unrelated, age & sex matched controls were enrolled from the outpatient department of ASCOMS, Sidhara, Jammu (J&K) for the present study. The present study design was approved by Animal and Human Experimentation Ethical Committee (AHEEC), University of Jammu. A brief health questionnaire covering different parameters such as gender, height, weight, diet pattern, smoking, alcohol intake, salt consumption, family history of HTN, etc. along with a consent form was duly filled by every subject enrolled for study.

Inclusion and Exclusion criteria: Essential hypertensive subjects were defined as per criterion given by JNC7 [12]: systolic blood pressure (SBP) of ≥140 mm Hg and/or diastolic blood pressure (DBP) of ≥90 mm Hg, or those currently receiving

anti-hypertensive therapy for more than three months. Patients suffering from secondary hypertension or having an existing medical condition like diabetes, ischemic heart disease, myocardial infarction, renal or hepatic disease were excluded.

Sample collection and biochemical analysis: 2 millilitres of peripheral blood sample was collected from each study subject in EDTA coated vials. Total cholesterol (TC), triglycerides (TG), high- density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined by routine biochemical assays on the automated biochemical analyser (Roche, Cobas CIII).

Genomic DNA extraction and MTHFR genotyping: Genomic DNA was extracted from stored blood samples by phenol-chloroform-isoamyl method [13]. The genotyping of MTHFR C677T polymorphism was done by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique. The PCR was performed by using forward primer: *5'-TGA AGG AGA AGG TGT CTG CGG GA-3'* and reverse primer: *5'AGG ACG GTG CGG TGA GAG TG-3'* to generate amplicon of 198bp. The PCR conditions are presented in [Table-1]. The amplified product was given restriction digestion with *HinfI* restriction enzyme (New England Biolabs) and the digested fragments were visualized on 4% agarose gel. The 198, 175 & 23 bp bands were obtained for heterozygous variant, 175 & 23 bp for homozygous variant and 198 bp for wild type.

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. Continuous variables were compared between the groups by using two-tailed student's *t*-test. Allelic frequencies were calculated by the gene - counting method. Hardy-Weinberg equilibrium (HWE) and the genotypic as well as allelic distribution of the C677T polymorphism of MTHFR gene was analyzed using Pearson's goodness of fit chi-square (χ^2) test.

Table-1 *Shows the reaction condition for PCR amplification of MTHFR C677T SNP.*

S. No.	Steps	Temperature	Time
1.	Pre-denaturation	94°C	2 min
2.	Denaturation	94°C	30 sec
3.	Annealing	62°C	60 sec
4.	Initial primer extension	72°C	30 sec
5.	Final primer extension	72°C	7 min
40 cycles at 4°C			

Results

The general characteristics of the study participants are presented in [Table-2] whereas the physiometric, anthropometric and biochemical characteristics were given in [Table-3]. The prevalence of smoking, alcohol intake, physical inactivity and positive familial background for HTN was high in patients as compared to controls. The levels of systolic blood (SBP), diastolic blood pressure (DBP), pulse pressure (PP), pulse rate (PR), body mass index (BMI) waist-hip ratio (WHR) and biochemical variables were higher in the diseased group than in healthy controls ($p<0.05$–0.001).

Table-2 *General Characteristics of the study participants*

Variables	Patients			Controls		
	Males (n=50)	Females (n=50)	Total (N=100)	Males (n=50)	Females (n=50)	Total (N=100)
Age (yrs.)	56.86±16.56	54.02±14.28	55.44±15.45	44.52±13.04	41.9±10.14	43.21±11.69
Smoking : *Yes*	27 (54%)	8 (16%)	35 (35%)	19 (38%)	2 (4%)	21 (21%)
No	23 (46%)	42 (84%)	65 (65%)	31 (62%)	48 (96%)	79 (79%)
Alcohol intake: *Yes*	20 (40%)	-	20 (20%)	14 (28%)	-	14 (14%)
No	30 (60%)	50 (100%)	80 (80%)	36 (72%)	50 (100%)	86 (86%)
Physical activity: *Yes*	22 (44%)	10 (20%)	32 (32%)	36 (72%)	23 (46%)	59 (59%)
No	28 (56%)	40 (80%)	68 (68%)	14 (28%)	27 (54%)	41 (41%)
Diet: *Veg.*	13 (26%)	23 (46%)	36 (36%)	18 (36%)	23 (46%)	41 (41%)
Non-veg.	37 (74%)	27 (54%)	64 (64%)	32 (64%)	27 (54%)	59 (59%)
Salt consumption: *Excess*						
Average/low	15 (30%)	16 (32%)	31 (31%)	6 (12%)	5 (10%)	11 (11%)
	35 (70%)	34 (68%)	69 (69%)	44 (88%)	45 (90%)	89 (89%)
FH of HTN: *Yes*	18 (36%)	17 (34%)	35 (35%)	7 (14%)	10 (20%)	17 (17%)
No	32 (64%)	33 (66%)	65 (65%)	43 (86%)	40 (80%)	83 (83%)

Table-3 *Physiometric, anthropometric and biochemical variables among study subjects*

Variables	Patients			Controls			p-value
	Males (n=50)	Females (n=50)	Total (N=100)	Males (n=50)	Females (n=50)	Total (N=100)	
SBP (mmHg)	146.24±19.12	142.06±23.76	144.15±21.56	120.98±4.18	119.5±5.04	120.24±4.67	**<0.0001***
DBP (mmHg)	91.12±8.46	88.64±10.98	89.88±9.83	80.96±5.32	79.78±4.28	80.37±4.84	**<0.0001***
Pulse pressure (PP)	55.12±14.08	53.42±18.12	54.27±16.17	40.02±5.06	39.72±3.10	39.87±4.18	**<0.0001***
Pulse rate (PR)	82.94±11.59	86.3±18.96	84.62±15.72	74.36±5.34	73.04±3.03	73.70±4.37	**<0.0001***
BMI	24.75±4.42	24.66±5.62	24.71±5.03	22.16±3.78	22.88±4.42	22.52±4.11	**0.0009***
WHR	1±0.08	0.99±0.10	1±0.09	0.95±0.06	0.96±0.06	0.95±0.06	**<0.0001***
TC (mg/dl)	158.60±53.12	157.25±43.61	157.93±48.35	135.15±33.78	121.5±32.61	128.32±33.74	**<0.0001***
TG (mg/dl)	180.58±77.68	167.26±53.53	173.71±66.68	122.37±22.91	116.56±27.09	119.46±25.13	**0.0005***
HDL (mg/dl)	33.85±10.21	37.44±9.14	35.68±9.81	50.03±7.63	52.51±6.29	51.27±7.07	**<0.0001***
LDL (mg/dl)	114.68±55.78	111.57±40.43	113.13±48.50	77.78±21.33	74.52±21.28	76.15±21.56	**<0.0001***

Sex wise comparison showed that smoking and consumption of non-vegetarian diet was more common in male subjects of both groups, whereas physical inactivity and vegetarianism was much more prevalent in females. Alcoholism was completely lacking in female subjects of the present study. The genotypic and allelic frequencies are summarized in [Table-4]. There was complete absence of mutant TT genotype in the study population, whereas the female subjects of the control group were devoid of heterozygous CT genotype. Overall, there was a higher frequency of wild CC genotype in the study population. The genotypic frequencies were in accordance with HWE in both groups (patients: χ^2= 0.17, p=0.9 and controls: χ^2=0, p=0.6). The association of MTHFR C677T polymorphism with risk of EH can be obtained by calculating odds ratio (OR) [Table-3]. There was a significant difference in frequencies of

CC vs. CT as well as CC vs. CT+TT genotypes between the patients and controls (OR=8.6, p=0.02). OR for C vs. T allele showed that the 'T' allele of MTHFR gene was adding 8-folds risk which was significant for the development of EH in our study population.

Discussion

EH is a complex polygenic disease prevalent in most of the worldwide populations and its manifestation is influenced by the action of several genes in conjecture with epidemiologic (environmental and demographic) factors. In this milieu, the present study is focussed to evaluate the potential role of non-genetic and genetic risk factors in EH. In our study, we found a higher prevalence of smoking, physical inactivity and familial history of HTN in patients. There was a strong association of physiometric parameters (SBP, DBP, PP, PR), anthropometric (BMI & WHR) with the aetiology of EH in the present investigation. The results were in accord with Bhavani et al, Yadav et al, Manimunda et al, Sagare et al and Kishore et al [14-18]. The genetic variants of the Hcy pathway has emerged as a strong candidate for EH phenotype. An elevated level of Hcy, a thiol amino acid, is responsible for 2-3 folds increase risk of EH [19], the possible mechanism being hyperhomocysteinemia induced vascular remodelling and vasoconstriction.

Table-4 *Association analysis of MTHFR C677T SNP with EH*

Genotypes/Alleles	Patients			Controls			Odds Ratio**	95% CI	p-value
	Males (n=50)	Females (n=50)	Total (N=100)	Males (n=50)	Females (n=50)	Total (N=100)			
CC	44 (88%)	48 (96%)	92 (92%)	49 (98%)	50 (100%)	99 (99%)	**1(Ref.)**		
CT	6 (12%)	2 (4%)	8 (8%)	1 (2%)	0	1(1%)	8.6	1.06-70.17	**0.02***
TT	0	0	0	0	0	0	Not possible#	-	-
CT+TT	6 (12%)	2 (4%)	8 (8%)	1 (2%)	0	1	8.6	1.06-70.17	**0.02***
C	0.94	0.98	0.96	0.99	1	0.99	**1(Ref.)**		
T	0.06	0.02	0.04	0.01	-	0.01	8.3	1.03-66.92	**0.02***

* indicates significant p-value; **Odds ratio was calculated for overall patients and controls, #OR was not calculated due to absence of genotypes.

Under genetic component, the present study analysed the role of the MTHFR C677T SNP of Hcy pathway in connection with susceptibility of EH in North Indian population (J&K). The MTHFR 677T-allele was having a significant role in the aetiology EH in our population (C vs T: OR=8. 3, p= 0.02). A strong positive association was observed in the hypertensive subjects of Saudi population [20] and in South West Camerron [21]. There was complete absence of mutant TT-genotype but the heterozygous CT-genotype was significantly adding approximately 8.6 folds increase risk in the development of EH in our population which was consistent with earlier findings done in Indian population [22]. In Morocco population, the homozygous 677TT variant of MTHFR gene is associated with the risk of hypertension [23]. Caucasian population showed a modest significant association between MTHFR C677T SNP and EH [24]. A meta- analysis study declared the MTHFR C677T SNP to be associated with increased risk of EH in different population groups belonging to Asian, Caucasian and Chinese ethnicity [25]. In addition to EH, the MTHFR variation increases the risk of developing adverse CVD phenotypes [11, 26-28]. The frequency of T-allele was higher in male EH patients in comparison to female counterpart in our study. Likewise, it was observed that TT- genotype and T-allele was more prevalent in Spanish male hypertensives [29]. Two independent studies by Nakata et al [30] and Fowdar et al [31] revealed, T-allele to be associated with lower BP levels in Japanese and lack of association of MTHFR 677T variant with EH in Caucasians respectively.

Conclusion

Although, despite having a higher frequency of CC-genotype we declared a significant association of MTHFR 677CT-genotype as well as 677T-allele in predisposition of EH in our studied North Indian population of J&K state. The study can be taken up on a large sample size to further authenticate the results.

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