ASSOCIATION OF MTHFR (C677T) AND ACE (I/D) POLYMORPHISMS WITH HYPERTENSION AND RESPONSE TO TREATMENT AMONG EGYPTIAN PATIENTS

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Hypertension is a continuous rising of systolic blood pressure or a diastolic blood pressure more than 140 mmHg, 90 mmHg, respectively, or both (Chobanian *et al*., 2003). Hypertension is considered as a major public health problem affects the population worldwide. Development of Hypertension is believed to be largely controlled by complex, multifactorial, as well as genetic risk factors (Shaughnessy, 2001). A numerous number of candidate genes and polymorphisms have been implicated in hypertension study as well as determinants risk factors (Agarwal, 2005). Recently, meta-analysis of wide genome association studies were done in large numbers of patients and identified various genetic loci linked not only with Blood Pressure (BP) variation (Levy *et al*., 2009; Newton-Cheh *et al*., 2009) but also in the individualization of the response to treatment (Bayramoglu *et al*., 2015; Fontana *et al*., 2015). Several studies reported the putative role of a mutation at nucleotide C677T in the methylenetetrahydrofolate reductase (MTHFR) gene (Xueqing *et al*., 2007). MTHFR gene is specifically involved in the conversion of the 5, 10-methylenetetrahydrofolate into 5-methylenetetrahydrofolate, which in turn catalyze the re-methylation of homocysteine to methionine (Risch and Merikangas, 1996; Slager and Schaid, 2001; Nakayama *et al*., 2003). As a result, plasma homocysteine (hyperhomocysteinemia) accumulate in individuals suffering from this mutation in MTHFR gene and are likely to develop cardiovascular disorders in particular the hypertension, due to the reduction of the MTHFR enzyme activity (Kang *et al*., 1988; Moat *et al*., 2001; Guilland *et al*., 2003). Another enzyme, Angiotensin-converting enzyme (ACE), a key player zinc-metallopeptidase of the rennin-angiotensin system (RAS) is widely distributed in human body (Salem and Batzer, 2009). The ACE catalyzes the conversion of angiotensin I to the active peptide, angiotensin

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II (a potent vasoconstrictor and aldosterone stimulating peptide), and also stimulates the degradation of bradykinin which is implicated in the control of systemic blood pressure and various acute and chronic effects on the cardiovascular disorders (Wang and Staessen, 2000). ACE polymorphism consisted of the insertion (I allele) or deletion (D allele) of a 287 bp Alu repeat sequence resulting in three different genotypes, DD, II homozygote and ID heterozygote (I/D, rs4646994) (Rigat *et al*., 1990). This ACE (I/D) polymorphism was found to account, for about 20-50% of the inter-individual variation in ACE activity, with the highest ratio of DD genotype over II, and ID genotypes but still 50-80% of the variation arising from other factors (Danser *et al*., 2007; Tsantes *et al*., 2013). Recent studies have been carried out to investigate the association between the ACE I/D polymorphism and hypertension of various populations and conflicting results have been reported with substantial evidence suggesting the positive association of ACE DD genotype variant with hypertension (Zarouk *et al*., 2012; Ali *et al*., 2013; Ji *et al*., 2010).

Hypertension is a common widespread disorder among Egyptian population especially in areas characterized by having high rate of consanguinity plus a high accumulation rate of familial diseases such as, diabetes and obesity. In spite of this fact, few data were -so far- published concerning the genetic background of Egyptian subjects in terms of their susceptibility to hypertension. Limited data are available to assess the relationship between the MTHFR, ACE variants polymorphism and hypertension in our population, and the association of the responsive ability of antihypertensive drug based on the genetic variation of these genes. Thus, the aim of this study was to determine whether the MTHFR (C667T) and (ACE) gene polymorphisms are involved as genetic risk factors for hypertension in Egyptian population and to evaluate the responsive ability of antihypertensive drugs depending on the genetic variations of these genes in the Egyptian patients.

MATERIALS AND METHODS

Materials

All participants provided written informed consent. This study included a group of 36 hypertensive individuals (men and women) with age mean of (49.58 year) referred to Cairo University from Internal Medicine Department, Gezeret Alwarak hospital, Cairo, Egypt. Hypertension was defined as systolic blood pressure (BP) >140 mmHg, diastolic BP >90 mmHg. The drugs used were ACE inhibitors in 21 patients, beta-blockers in 14 patients, and diuretics in one patient. Secondary hypertension was minimized using detailed health questionnaire and clinical evaluation, and none of the patients exhibited symptoms of cardiac or renal failure. A group of 14 non-hypertensive individuals (seven men and seven women) were recruited with systolic and diastolic blood pressures below 140 and 90 mmHg respectively at rest and recumbent position.

Methods

Field investigation

The epidemiological questionnaire was distributed for general demographic characteristics, medical history, family history, and other factors including smoking, consanguinity, and blood sugar for all individuals being studied. Physical examinations were performed to obtain height, weight, systolic blood pressure (SBP) and diastolic blood pressure (DBP) of participants. The body mass index (BMI) was calculated as follows: BMI = weight (kg)/height^2 (m^2).

Biochemical Analysis

A 5 mL fasting EDTA-anticoagulated peripheral venous blood sample was collected from each subject. Serum was subsequently separated by centrifugation and followed by automated biochemical analysis to measure levels of blood glucose (FBG), total cholesterol (TC), triglycerides (TG).

Genetic analysis of MTHFR (T677C) and ACE (I/D) genes polymorphism

A 5 ml of fasting peripheral venous blood was obtained using disodium EDTA vacutainers. DNA samples were isolated from the peripheral blood leucocytes by Quick-gDNA MiniPrep (Zymo Research Corp, CA, USA) according of the manufacture instructions and the DNA concentration was estimated using the stander spectrophotometer analysis. Primers and PCR product size for the MTHFR and ACE I/D polymorphism analysis were summarized in Table (1). The PCR products were visualized on 2% agarose gels with a Sizer™ -100 DNA Marker (iNTRON Biotechnology, Korea), and images were captured using a gel documentation system.

Statistical analysis

Data were analyzed using SPSS Win statistical package software version 18.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as median or mean ± stander deviations and range. Categorical data were described as frequency and percentage. Parameters like age, sex, smoking, family history between cases and controls were done using independent ‘t’ test. All numerical values were tested by student’s t test or ANOVA test. Pearson Chi square test was applied to check the association between the selected variables. P vales less than 0.05 were considered statistically significant difference.

RESULTS AND DISCUSSION

The present case–control study investigated the distribution of the C677T polymorphism of MTHFR and ACE polymorphism in 36 patients with hypertension and 14 normal control individuals. Demographic and clinical characteristics of patients and controls are shown in Table (2). The mean systolic blood pressure (SBP), diastolic blood pressure (DBP), consanguinity, and Diabetes mellitus were significantly higher in hypertensive than in normal controls; while mean age, sex ratio, family history, cholesterol levels,

triglyceride were similar in both groups ($p > 0.05$). Compared to controls, total hypertensive cases showed significantly higher frequency of MTHFR mutant allele 677T carriage (TT + CT) (62% vs. 50%) with a lower frequency of the normal 677CC genotype (38.3% vs. 50%, $p = 0.004$) (Table 3, Fig. 1A). While the frequency of ACE polymorphism in this study, among hypertensive and normal cases were 41% vs 28.5% in ACE DD genotype, to be 52.5% vs 64% in DI and 5% vs 7% in II compared to controls. The frequency of ACE DD polymorphism with the hypertensive cases was significant ($p < 0.05$) (Table 3, Fig. 1B).

No association or significant difference were observed between MTHFR C677T polymorphism with respect to gender ratio, age, obesity, blood pressure, frequency of smoking habits, family history of hypertension, cholesterol level, triglyceride, or fasting blood sugar Table (4). Conversely only DD genotype of ACE gene polymorphisms was observed to be associated with hypertension, obesity, and diabetes millets Table (4). MTHFR gene has been studied more frequently in relation to homocysteine and folate metabolism, with regard to a variety of disorders including CVD and Hypertension (Javed *et al*., 2012). MTHFR and the hypertension risk have been discussed by several investigators with conflicting results (Ilhan *et al*., 2008; Cevik *et al*., 2014; Heux *et al*., 2004; Qian *et al*., 2007; Kosmas *et al*., 2004; Yang *et al*., 2014; Markan *et al*., 2007). Many previous studies have implicated that the MTHFR variants C677T and A1298C have been a risk factor for Essential Hypertension (Ilhan *et al*., 2008). Markan *et al*. (2007) reported the same observations in the Indian patients. Yang *et al*. (2014) also reported in a meta-analysis a good association of MTHFR C677T mutation with the risk of hypertension between Asians, Chinese, and Caucasians subjects. Similar findings were obtained in the population of Turkey (Cevik *et al*., 2014). On the other hand, many authors reported that MTHFR C677T polymorphism was independent factor of essential hypertension in different ethnic populations (Ilhan *et al*., 2008; Heux *et al*., 2004; Qian *et al*., 2007) as well as of diastolic hypertension manifested in pregnant women (Kosmas *et al*., 2004). In our study the frequency of mutant MTHFR CT/TT are higher and statically different in the cases group than control while the frequency of wild type are much higher in the control than in patients. Moreover the MTHFR C677T gene polymorphism showed no significant association with other parameters such as age, sex, family history, TG, Cholesterol, Diabetes, smoking habit and hypertension. So our results on Egyptian patients were in agreement with those who failed to show any relationship of MTHFR C677T polymorphism and hypertension.

ACE is a key enzyme in the vascular system which mediates conversion of Angiotensin I to Angiotensin II, a powerful vasopressor. Several authors have extensively studied genes which encoding components of RAS, such as Angiotensinogen, ACE, Angiotensinogen II type-1

receptor and Renin (-5,434 and -5,312) as genetic determinants of hypertension (Manunta, 2002; Thiol and Weber, 2002). With the same consent of MTHFR gene, the link between ACE D/D allele and hypertension has given contradicted results world-wide. It was confirmed that, ACE D/D allele has a positive association with hypertension in African Americans, Japanese, and Chinese (Duru *et al*., 1994; Chiang *et al*., 1996; Morise *et al*., 1994; Nakno *et al*., 1998; Morshed, 2002). However many other studies failed to show any association (Alaatin, 2002; Maguchi, 1996; Pamies *et al*., 1999; Dazida *et al*., 2001). It has been hypothesized that these inconsistencies in the results could be due to the variations in background of the population characteristics. The results of this study are consistent with previous report, which highlights the positive association between ACE (DD) allele and hypertension. The responsive ability of the studied cases to the antihypertensive drugs was assessed, the significance response was found with ACE DD genotype when the patients received ACE inhibitors drugs while the other allelic variants were not associated specifically with the type of the drugs used Table (5). Moreover there were no significant differences in the Ace-inhibitors treatment doses, frequencies, age, sex and associated diseases between the ACE polymorphism variants (Table 6).

In conclusion, these results suggested that the MTHFR C677T polymorphism is not a risk factor for hypertension, and only the DD genotype of ACE (I/D) polymorphism is strongly associated with hypertension. Hence the DD genotype is the best candidate for treatment with ACE inhibitors. However, more information about the genetics of hypertension can be obtained by performing the study over a large population of Egyptian patients.

SUMMARY

Hypertension, a well-known epidemic health disease, is risk factor for various cardiovascular, peripheral vascular and renal diseases. Renin angiotensin system (RAS) being the most important pathogenic mechanism of hypertension is mediated by a key component; the angiotensin converting enzyme (ACE). Moreover, Mutations in the methylenetetrahydrofolate reductase gene (MTHFR) have been established to be associated with the risk of cardiovascular disease as well as hypertension. This case-control study was conducted out to investigate the potential relationship of ACE (I/D) and MTHFR (C677T) gene polymorphisms with hypertension susceptibility and the responsive ability of antihypertensive drugs in Egyptian hypertensive patients. Thirty six patients suffering of high blood pressure were compared with age and sex matching 14 control cases. MTHFR (C677T) and ACE (I/D) polymorphisms were genotyped by polymerase chain reaction (PCR). The demographic and clinical features of patients and control showed no particular significance ($p > 0.05$) except for the consanguinity and the obesity. For MTHFR polymor-

phism frequency, total hypertensive cases showed significantly higher frequency rate for the mutant allele 677T compared to controls with a lower frequency of the wild type 677CC genotype. Whereas the mutant 677TC+TT genotypes were not significantly associated with the hypertension risk when compared to the wild genotype among the case group. For ACE gene polymorphism, also showed only higher frequency rates of DD allele. Interestingly, ACE DD genotype showed significant association with blood pressure, obesity and diabetes. Finally in response of the antihypertensive drugs, we found that, the best responsive group is DD genotype group when treated with the ACE inhibitors. These result suggested that the MTHFR polymorphism was not associated with hypertension while the ACE DD genotype may be associated with essential hypertension and considered as a potent risk factor for hypertension and moreover it is the best responsive group when treated with ACE inhibitors in Egyptian patients.

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Table (1): Primer sequences used in the study.

Primer	Sequence	*Product size*
MTHFR-*F*	5'-TGC TGT TGG AAG GTG CAA GAT-3'	226 bp
MTHFR-R wild	5'-GCG TGA TGA TGA AAT CGG-3'	
MTHFR-R Mut	5'-GCG TGA TGA TGA AAT CGA-3'	
ACE -F	5'- CTG GAG ACC ACT CCC ATC CTT TCT-3'	I = 490 bp
ACE -R	5'- GAT GTG GCC ATC ACA TTC GTC AGA T -3'	D = 190 bp

F= Forward, R= Reverse. I= Insertion, D= Deletion

Table (2): Demographic and clinical parameters of the studied groups.

Parameter	Cases (N = 36)	Control (N = 14)	F/X^2	*P-value*
Age *Mean* ± *SD*	49.58± 16.1	44.71± 5.99	16.78	0.28
Male *range*	17 (47.2%)	7 (50%)	0.27	0.77
Female *range*	19(52.8%)	7 (50%)	21.35	0.24
Family history *range*	20 (55.5%)	7 (50%)	4.07	0.69
Smoking *Range*	7 (11%)	4(28.5%)	4.37	**<0.05**
Obesity Range	15(41)	1(7%)	1.83	**<0.001**
Consanguinity range	9 (25%)	2 (14%)	8.37	**<0.05**
SBP *Mean* ± *SD*	153.3±23.4	119.29±11.58	2.52	**<0.001**
DBP *Mean* ± *SD*	104.9±15.27	80.36±8.19	0.57	**<0.001**
Cholesterol *Mean* ± *SD*	171.17±48.04	159.3 ±30.79	3.78	0.39
Triglycerides *Mean* ± *SD*	166.4±84.4	139.8±76.7	5.39	0.307

P: cases vs. controls, bold: p significant <0.05, F: Variance of the group means (numerical)
X^2: chi square (Categorical)

Table (3): Genotyping and allele frequency of MTHFR C677T and ACE gene polymorphisms among hypertensive Egyptians case compared to control.

Parameter	Cases (N = 36)	Control (N = 14)	F/X^2	*P-value*
MTHFR polymorphisms				
CC (Wild)	14 (38%)	7(50%)	0.034	<0.001
TC+TT (Mutant)	22 (62%)	7(50%)	2.39	<0.01
ACE polymorphisms				
DD	15(41%)	4 (28.5)	3.32	<0.001
ID	19(52.5%)	9 (64%)	1.41	0.17
II	2(5%)	1(7%)	0.17	0.42

P: cases vs. controls , bold : p significant <0.05, X^2: chi square (percentage)

Table (4): Association between the genetic polymorphisms and parameters between the hypertension patients.

Parameters	MTHFR		*P-value*	*ACE*			*P-value*
	CC (n=14)	TT/TC (22)		*DD (n=15)*	*ID (n=19)*	*II (n= 2)*	
Age *Mean ± SD*	49.5 ± 16.1	51.8 ± 15.9	0.85	51.87 ± 15.9	47.42 ± 16.2	53 ± 24.0	0.71
Male *range*	9 (64%)	9 (25%)	<**0.01**	8 (53%)	10 (52%)	-	0.87
Family history *range*	20 (55.5%)	7 (50%)	0.56	8 (53%)	12 (63%)	-	0.42
Smoking *Range*	4 (36%)	3 (8%)	<**0.00**	4 (26%)	3 (16%)	-	<**0.01**
Obesity Range	5 (35)	11 (30%)	0.46	4 (26%)[1]	11 (57%)[2]	1 (50%)[2]	<**0.01**
Consanguinity range	9 (25%)	2 (14%)	<**0.05**	2 (13%)	7 (36%)	-	<**0.01**
Diabetes range	4 (36%)	7 (19%)	<**0.01**	6 (40%)	4 (21%)	1 (50%)	<**0.01**
SBP *Mean ± SD*	152.8 ± 20.5	153.6 ± 25.6	0.97	186.3 ± 18.8	161.3 ± 18.8	160. ± 42.4	<**.001**
DBP *Mean ± SD*	93.6 ± 8.4	99.1 ± 18.2	0.24	103 ± 8.99	106 ± 19.15	95 ± 7.07	<**.01**
Cholesterol *Mean ± SD*	175.1 ± 54.9	168.2 ± 44.8	0.68	169.1 ± 45.9	165.2 ± 30.8	175.5 ± 23.2	0.29
Triglycerides *Mean ± SD*	165 ± 84.4	166 ± 84.34	0.99	167 ± 44.6	166 ± 74.5	144.5 ± 21.9	0.93

Groups bearing different numbers are significantly different from each other at P<0.05
Bold numbers show statistical significant difference.

Table (5): Association between the genetic polymorphisms and the response to antihypertensive drugs between the hypertension patients

Parameter	Blood Pressure (SBP/DBP)			*P-value*
	ACE-inhibitors (21)	B-blockers (14)	Diuretic(1)	
MTHFR polymorphisms				
CC (Wild)				
Before After	150.4/92.5 135/92.5	153.8/95.3 148/85	151.8/91.8 140/100	0.68
TC+TT (Mutant)				
Before After	151.3/97.1 142 /92.1	156.7/ 103 147/ 102	-	0.59
ACE polymorphisms				
DD				
Before After	180.3/100 128/85	191/103 150/90	210.2/99 160/100	**0.011**
ID				
Before After	154.3/103 139/93	176.4/116 148/91	190.6/115 141/100	0.95
II				
Before After	130/100 130/90	170/.95 160/95	-	0.64

Bold number shows statistical significant difference

Table (6): Ace- inhbitores treatment doses, frquencies, age, sex and associated disseases between ACE polymorphism variaents.

Variables	ACE DD	ACE DI & II
Drug dose & frequencies	Captopril 50 mg 2 times/day Or Enalapril 20 mg once/day	Same
Sex	M 57.14 % F 42.86 %	M 50 % F 50 %
Age mean	45.43	42.64
Associated diseases		
Diabetes Obesity	14.29 % 42.86 %	17.65 % 53.85 %

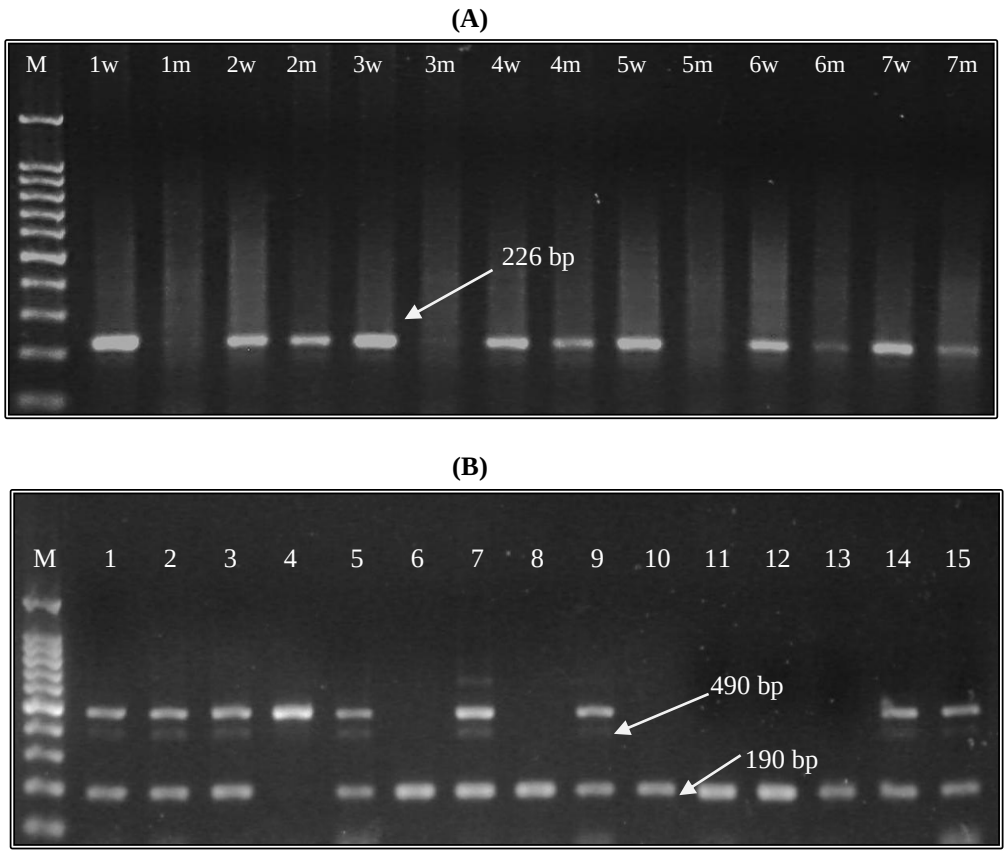

Fig. (1): Representative gel profiles of ACE DI and MTHFR polymorphisms. A Lanes 1,3, and 5 show CC wild MTHFR, while lanes 2, 4, 6, and 7 show CT heterozygous MTHFR, w (wild primer), m (mutant primer).B; Lanes 1, 2, 3, 5, 7, 9, 14, and 15 show DI form; Lanes 6, 8, 10, 11, 12 and 13 show DD form; Lane 4 shows II form. M, Sizer™ -100 DNA Marker (iNTRON Biotechnology, Korea)