Genetic Polymorphism of Angiotensin Converting Enzyme and Angiotensin II Type 1 Receptors and Their Impact on the Outcome of Acute Coronary Syndrome

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Abstract

This study aims to investigate the impact of ACE (rs4343) and AT1R (rs 5182) genetic polymorphisms on the outcome of acute coronary syndrome (ACS) in patients on captopril. 250 participants with ACS were included in this study (Group 1 (120) participants on captopril 25 mg twice daily and Group 2 (130) participants received no captopril (control study)). Participants were genotyped for ACE (rs4343) and AT1R (rs5182) polymorphisms and the phenotype was determined. ACE polymorphism (rs 4343) GG and GA genotypes are more related to STEMI (OR=1.7, 1.5 respectively) and NSTEMI (OR=3, 3.8 respectively), and they were more prone to have Percutaneous Coronary Intervention after ACS attack (OR=11.6, 14.1 respectively)

AT1R (rs 5182) CT genotype is mildly associated with STEMI (OR=1.1), but also prone to have PCI after ACS attack (OR=1.6) while TT genotype has a risk to get less improvement (OR=1.8).

Key Words: ACE; AT1R; genetic polymorphism; Captopril; ACS; RAAS.

Introduction

Acute coronary syndrome (ACS) is a life-threatening situation that causes high rates of morbidity and mortality despite of advances in the diagnosis and management [1,2]. The coronary heart disease (CHD) related deaths in Iraq have reached 32,582 (18.50%) of total deaths according to the WHO data published in 2017 [3-5]. ACS is a multifactorial condition that results from an interaction among environmental and genetic factors[6]. The defect in combination of gene-gene and gene-environment interactions causes such conditions[7]. Single-nucleotide polymorphism (SNP) is generally used to determine the genetic variables that are associated with the ACS. Their association with the disease is established when the occurrence is statistically significant between cases and control[8,9].

The renin-angiotensin-aldosterone system (RAAS) is the crucial hormonal system which controls the hemodynamic stability by regulating the blood pressure, fluid volume, and sodium-potassium balance[10]. Angiotensin 2 is the primary effector of RAAS which is generated by the cleavage of angiotensin 1 by the angiotensin converting enzyme (ACE). ACE gene rs4343 (A2350G) polymorphism is associated with the increased ACE activity among healthy individuals as well as in various pathological conditions[11]. Angiotensin 2 is the most crucial component of the RAAS in vasoconstriction and sodium retention. Majority of the pressure, inflammatory, and proliferative actions of angiotensin 2 are carried out by the AT1R expression in the myocardium[12].

To improve the treatment outcomes and prevention of adverse drugs events, the cardiac pharmacogenomics was developed. Previously, many genetic variants have modified drug metabolism, transport, and targets and they could be used in order to predict an individual's response towards the treatment[13].

Despite of advances in cardiovascular pharmacology, there is significant inter-individual variability in drug response regarding both efficiency and safety profiles. Drug-gene associations are crucial factor which determines the spectrum of response to therapy[14].

ACE inhibitors are major drug classes which provide better life expectancy and improved life quality to many patients suffering from hypertension and/or cardiovascular disorders[15]. The first ACE inhibitor (Captopril) developed in this group was used mainly against hypertension, congestive heart failure, post-myocardial infarction, and preservation of renal function in diabetic nephropathy. Uncertainty regarding response to equivalent doses of captopril among individuals is due to genetic variations in the ACE and AT1R gene.

ACE activity is hugely affected by the genetic polymorphism at the ACE gene among hypertensive patients. Such incidence or factors might explain certain results regarding the effect of ACE activity in blood pressure regulation and cardiovascular complications[16].

Identification of genetic variations among individuals is a crucial way to differentiate the ACE-inhibitor responders and non-responders[17].

The American College of Cardiology, AHA and the American Society of Hypertension have identified that the treatment of patients suffering from hypertension and CAD should include the discussion of genetic polymorphisms which might affect blood pressure response to antihypertensive agents, and the determination of the genetic variants might become useful in order to find the appropriate antihypertensive agents that are effective for reduction of blood pressure and cardiovascular complications[18].

Based on our knowledge, this study is the first pharmacogenomics-based research in Iraq reporting the genotype and allele frequency of ACE and AT1R genes and their impact on captopril potential on ACS outcome in Arab Iraqi population of Mid-Euphrates.

Materials and Methods

Study Population

This case control study was conducted at the Coronary Care Unit (CCU), AL-Sader Teaching Hospital, Al-Najaf, and Al-Diwanyah Teaching Hospital, Al-Diwanyah, Iraq, for the period between January 2016 to March 2018. The study was approved by the ethical committee in the College of Medicine, Kufa University, Iraq. Two hundred and fifty participants were included in this study. Participants were aged between 30-60 years, clinically diagnosed with ACS according to diagnostic algorithms for the acute coronary syndrome [(19)].

The cases included 125 patients with 77 men and 48 women kept on captopril 25 mg twice daily dosage upon CCU admission in addition to the traditional treatment of ACS.

The control had 125 patients with 76 men and 49 women matched with cases for age and gender diagnosed with ACS and prescribed traditional treatment of ACS. The patients with diabetes mellitus, renal or hepatic impairment, pregnancy, heart failure, captopril contraindication (including hypersensitivity, angioneurotic edema, bilateral renal artery stenosis, captopril administration before CCU admission, mental disorders) were excluded from the study.

DNA extraction and Genotyping

Genomic DNA from blood samples was extracted by using genomic DNA mini extraction kit (Frozen Blood) Geneaid. USA, and performed according to the company's instructions.

The PCR-RFLP method was used for genotyping the SNPs. Primer sequences, PCR profiles, and digestion enzymes are presented in Table 1. The PCR was performed by taking 5µl of DNA template+1.5µl of forward primer+1.5µl of the reverse+12µl of PCR water placed in standard Accu Power PCR PreMix kit that contained Taq DNA polymerase, dNTPs, Tris-HCl pH-9.0, KCl, $MgCl_2$ and loading dye). After digestion, PCR products were separated on a 1% agarose gel to determine the genotypes.

Assessment of plasma angiotensin 2 level: Plasma angiotensin 2 levels were measured using human ANG2 ELISA kit (Wuhan EIAab Science Co., Ltd, China) Catalogue No: E-EL-H0326, sensitivity 18.75 pg/ml, detection range 31.25-2000 pg/ml both for cases and for control.

Statistical analysis: Mean ± standard error (SE) were measured for quantitative variables. Chi-square test was used for the statistical representation of categorical variables (gender, types of ACS, and the clinical outcome). Student t-tests and one-way ANOVA tests were used to compare the study groups.

Genetic equilibrium test using the Hardy-Weinberg equilibrium (HWE) for assessment of genotype and allele frequencies by the chi square test was also performed. The significance level for statistical tests was 0.05 ($p<0.05$), Odd's ratio (OR) at 95 % confidence intervals (CI) was determined. For HWE; P-value >0.05 is considered to be consistent with HWE.

Results

Demographic and laboratory data of participants are presented in Table 1, no significant difference in demographic and clinical variables was noticed between cases and control groups, except the plasma angiotensin 2 level which is highly significant (P-value=0.0001).

Table 1. Demographic and laboratory data (overall subjects).

Variable	Cases n=125	Control n=125	P-value
Age (years)	49.3 ± 0.7	49.5 ± 0.6	0.8
Mean pulse rate (beat/min) on admission	82.3 ± 0.7	80.8 ± 0.5	0.1
Mean pulse rate (beat/min) on discharge	71.1 ± 0.4	71.2 ± 0.4	0.9
Mean systolic blood pressure (mmHg) on admission	139.28 ± 1.1	140.9 ± 0.9	0.3
Mean systolic blood Pressure (mmHg) on discharge	115.8 ± 1.1	115.9 ± 1.1	0.950
Mean diastolic blood pressure (mmHg) on admission	86.97 ± 1.1	87.5 ± 0.9	0.734
Mean diastolic blood pressure (mmHg) on discharge	67.2 ± 1.01	68.1 ± 0.9	0.5
Angiotensin 2 by ELISA (pg/ml)	0.9 ± 0.05	55.02 ± 1.5	0.0001

Data are represented as Mean ± SE.

The genotype and allele frequencies of ACE (rs4343) and AT1R (rs5182) genes

The genotype and allele frequencies of ACE (rs4343) and AT1R (rs5182) genes were not significantly differed between cases and control groups as all were consistent with the HWE (Tables 2).

Table 2. Genotype and allele frequencies of ACE (rs4343) & AT1R (rs5182).

Genotype	Cases n (%)	Control n (%)	Odd ratio, 95% CI	p-value
		ACE (rs4343)		
GG	60 (48%)	62 (49.6%)	0.9 (0.4-2.03)	0.8
GA	50 (40%)	49 (39.2%)	0.9(0.4-2.2)	0.9
AA	15 (12%)	14 (11.2%)	1	
G allele	17(68%)	17(69.2%)	0.9 (0.6-1.4)	0.8
A allele	8(32%)	7(30.8%)	-	
		AT1R (rs5182)		
Genotype	**Cases n (%)**	**Control n (%)**	**Odd ratio, 95% CI**	**p-value**
CC	70 (56.0%)	70 (56.0%)	1	
CT	45 (36.0%)	44 (35.2%)	1.02 (0.6-1.7%)	0.9
TT	10 (8.0%)	11 (8.8%)	0.9 (0.4-2.3)	0.8
C allele	185 (74%)	184 (73.6%)	1	
T allele	65 (26%)	66 (26.4%)	0.9(0.7-1.5)	0.9

Relation of different genotypes of ACE gene (rs4343) with the type of ACS.

GG genotype had 1.7 folds increment in STEMI risk while GA genotype had 1.5 folds. Both GG and GA genotypes had more than 3-folds increased risk to have NSTEMI, while UA occurred non-significantly between cases and control groups Table 3.

Table 3. Comparison of relation of different genotypes of ACE gene (rs4343) & AT1R gene (rs5182) with the type of ACS.

ACE gene (rs4343)						
TYPE	**GG**		**GA**		**AA**	
	Case N (%)	**Control N (%)**	**Case N (%)**	**Control N (%)**	**Case N (%)**	**Control N (%)**
STEMI	46 (49.5%)	45 (48.4%)	33 (35.5%)	38 (40.9%)	14 (15.1%)	24 (12.9%)
	OR=1.7 (0.8-3.8)		OR=1.5 (0.7-3.3)		1	
	P=0.2		P=0.3			
NSTEMI	8 (44.4%)	8 (44.4%)	9 (50%)	7 (38.9%)	1 (5.6%)	3 (16.7%)
	OR=3 (0.3-35.3)		OR=3.8 (0.3-45.6)		1	
	P=0.4		P=0.3			
UA	6 (42.9%)	9 (64.3%)	8 (57.1%)	4 (28.6%)	0 (0%)	1 (7.1%)
	OR ---		OR---		1	
	P=0.7		P= 0.3			

AT1R gene (rs5182)						
TYPE	**CC**		**CT**		**TT**	
STEMI	**Case N (%)**	**Control N (%)**	**Case N (%)**	**Control N (%)**	**Case N (%)**	**Control N (%)**
	49 (52.7%)	50 (53.8%)	38 (40.9%)	35 (37.6%)	6 (6.5%)	8 (8.6%)
	1		OR=1.1(0.6-2.02)		OR=0.7 (0.2-2.4)	
			P=0.7		P=0.6	
NSTEMI	12 (66.7%)		11 (61.1%)		2 (11.1%)	
	1	OR=0.4 (0.06-2.3)	OR=1.8 (0.3-12.1)	5 (27.8%)	4 (22.2%)	2 (11.1%)
		P=0.3	P=0.5			
UA	9 (64.3%)		9 (64.3%)		5 (35.7%)	
	1	OR=1.3 (0.3-6.2)	OR--	4 (28.6%)	0 (0%)	1 (7.1%)
			P=0.5			
		P=0.8				

Relation of different Genotypes of AT1R gene (rs5182) with the type of ACS

No risk noticed among TT genotype carriers to have increased STEMI, while among CT the risk increased by 1.1-folds. For NSTEMI 1.8-folds risk increased among TT genotype carriers but no risk detected among CT, for UA CT carriers have 1.3 increased risk Table 3.

Relation of different genotypes of ACE gene (rs4343) with the clinical outcome

The number and percentage of cases carrying GG, GA genotypes significantly improved over the control, however no difference in the risk between GG and GA genotypes compared to the AA genotype.

No significant difference in number and percent of patients getting HF or arrhythmias in cases and control groups (Table 4).

Table 4. Comparison of relation of different Genotypes of ACE gene (rs4343) with the clinical outcome.

ACE gene (rs4343)						
Outcome	**GG**		**GA**		**AA**	
	Case N (%)	Control N (%)	Case N (%)	Control N (%)	Case N (%)	Control N (%)
Improved	26 (50.0%)	24 (66.7%)	12 (23.1%)	10 (27.8%)	14 (26.9%)	2 (5.6%)
	OR=0.2(0.03-0.8)		OR=0.2 (0.03-0.9)		Reference	
	P=0.02		P=0.04			
PCI	27 (44.3%)	28 (41.2%)	33(54.1%)	28 (41.2%)	1 (1.6%)	12 (17.6%)
	OR=11.6 (1.4-95.2)		OR=14.1 (1.7-115.6)		Reference	
	P= 0.2		P=0.01			
HF	3 (50%)	3 (33.3%)	3 (50%)	6 (66.7%)	0 (0%)	0 (0%)
	OR --		OR---			
	P=1		P=0.8			
Arrhythmia	4 (66.7%)	7 (58.3%)	2 (33.3%)	5 (41.7%)	0	0
	OR---		OR----			
	P=0.8		P=0.7			
AT1R gene (rs5182)						
Outcome	**CC**		**CT**		**TT**	
	Case N (%)	Control N (%)	Case N (%)	Control N (%)	Case N (%)	Control N (%)
Improved	31 (59.6%)	21 (58.3%)	13 (25%)	12 (33.3 %)	8 (15.4%)	3 (8.3%)
	Reference		OR=0.7 (0.3- 1.9)		OR= 1.8 (0.4-7.6)	
			P=0.5		P=0.4	
PCI	33(54.1%)	40(58.8%)	26(42.6%)	20(29.4%)	2(3.3%)	8(11.8%)
	Reference		OR= 1.6 (0.7-3.3)		OR=0.3 (0.06- 1.5)	

			P= 0.2		P= 0.1	
HF	4(66.7%)	4(44.4%)	2(33.3%)	5(55.6%)	0(0%)	0(0%)
	Reference		OR=---		OR=---	
			P=0.7		P=1	
Arrhythmia	2(33.3%)	5(41.7%)	4(66.7%)	7(58.3%)	0(0%)	0(0%)
	Reference		OR=---		OR =---	
			P=0.8		P=0.7	

Relation of different Genotypes of AT1R gene (rs5182) with the clinical outcome

No significant difference between CT, TT genotypes get improved in cases and control groups as compared with CC genotype, TT genotype has 1.8 folds increased risk to get less improvement. No significant difference in number and percent of patients underwent PCI in cases and control groups among TT, CT genotypes as compared with CC genotype, however CT genotype has 1.6 folds increased risk to have PCI post ACS, no significant difference in number and percent of patients getting HF or arrhythmias in cases and control groups (Table 4).

Relation of different Genotypes of ACE gene (rs4343) with plasma Angiotensin 2 level in pg/ml

There was a highly significant difference in mean plasma angiotensin 2 level between different ACE genotypes (GG,GA,AA) in cases and control groups. (See appendix table 2) with GG,GA genotypes had higher plasma Angiotensin 2 levels in cases and control(GG the high, GA the intermediate, AA the low).

Relation of different Genotypes of AT1R gene (rs 5182) with plasma Angiotensin 2 level in pg/ml

There was a highly significant difference in mean plasma angiotensin 2 level between different AT1R genotypes in cases and control groups. (see appendix table 3) with CC, CT genotypes had higher Angiotensin 2 levels in cases and control, CT + TT genotypes combination carried higher plasma concentration of angiotensin 2 (the risk genotypes) more than CC (the protective genotype).

Discussion

Acute coronary syndrome is a focused health concern for being both prevalent and portends a poor prognosis. It is the common subtype of CAD which carries a higher morbidity and mortality rate with significant impact and burden[20]. Based on recent WHO data published in 2017, coronary heart disease related deaths in Iraq have reached 32,582 (18.50%) of total deaths [20].

The best proxy for the ACE I/D genotype is the ACE rs4343 polymorphism in which A allele marking insertion and G allele marking deletion of rs4343[21].

The significant variability in response to ACEI treatment between individuals could be partially due to the involvement of genetic determinants. The G allele causes the increased activity of the renin-angiotensin system, as is associated with the increased activity of the ACE enzyme[22].

In present study, the genotype frequency of GG, ID, II for cases were 60 (48.0%), 50 (40.0%), 15 (12.0%) respectively with GG, ID alleles of ACE gene being dominant over II with D allele frequency of 0.68 and I allele frequency of 0.32 (Table 1). Where as in the control group the genotype frequency of GG, ID, II were 62 (49.6%), 49 (39.2%), 14 (11.2%) respectively with GG, ID alleles of ACE gene being dominant over II with G allele frequency of 0.69 and I allele frequency of 0.3 (Table 1).

A significant increase in occurrence of STEMI and NSTEMI among GG and ID genotypes was observed, where as no significance in occurrence of UA was reported (Table 2).

The number and percentage of cases carrying GG, ID genotypes significantly improved when compared with the control (Table 2). This might be partially attributed to the dual action of captopril, that inhibits ACE and reduces the production of vasoconstrictor angiotensin 2, beside the increment in vasodilator peptides such as bradykinin and prostaglandins.

Bleumink et al. reported that the subjects with GG genotype have a relative resistance to ACE-inhibitor therapy when compared to the II genotype, with the ID in an intermediate position, so ACE inhibitors may not affect those with a GG genotype and hence need higher doses [23].

GG genotype have higher chances to get interventional coronary revascularization post ACS attack despite of having early treatment with captopril, however no significant difference in number and percentage of patients underwent PCI between cases and control groups (Table 4).

ACE GG genotype is a crucial factor for ACS basically for acute myocardial infarction, greater ACS severity and improved risk of instant cardiac death, greater risk of presenting with a higher number of stenosed vessels (in all three coronary arteries), infarction of the left anterior descending artery and with anterior wall infarction, the GG genotype conferred a 2.69-fold higher risk of ACS, compared with the II genotype. The GG polymorphism and D allele may influene severity of CAD, in comparison to I allele which have a protective effect. GG genotype and D allele carriers have higher stenosed vessels [24,25].

There was a significant difference in number and percentage of patients underwent PCI between cases and control groups (Table 4), this indicates that ID genotype have greater risk over GG, II genotypes to get interventional coronary revascularization post ACS attack and hence might have captopril resistance and eventually require higher doses.

Based on our results, the mean plasma level of angiotensin 2 in cases treated by captopril was 0.9 ± 0.05pg/ml, where as in control group it was 55.02 ± 1.5 pg/ml. Hence, there was a significant difference in plasma angiotensin 2 level between cases and control groups (Table 1). There are variable data regarding the association of angiotensin II levels with the ACE II, ID, and GG genotypes where some studies have concluded that despite the effect of this polymorphism on the plasma ACE levels, there is no influence in angiotensin II or aldosterone production [26,27]. According to previous studies the highest ACE levels was reported in GG genotype [28-31]. Hence such findings describe the high concentration of plasma angiotensin 2 in GG and ID genotypes in our study. High plasma level of angiotensin II influences higher long-term mortality even with correction of established cardiovascular risk factors.

The SNP rs5182 is located in the 3' region of the AT1R gene and it represents a silent C>T difference in the leucine codon 191 which is associated with the risk of hypertension[32]. Many studies have demonstrated the relation of such polymorphism to several diseases have controversial results. However, Bonnardeaux et al. reported that rs5182 has no significant relation with hypertension[33-35].

The TT genotype with increased STEMI has not been found to be associated with any risk, where as among CT, the risk increased by 1.1-folds. Though for NSTEMI, a 1.8-fold risk increased among TT genotype carriers, there was no risk detected among CT. For UA-CT carriers, 1.3-fold risk increased (Table 3). Hence, CT genotype have higher risk towards STEMI and UA, while TT genotype has higher risk towards NSTEMI.

In the present study, heterozygous CT genotype of rs5182 of AT1R seems to be significantly involved in ACS, while not much data is yet available regarding such involvement. No significant difference between CT and TT genotypes got improved in cases and control groups. However, in comparison to CC genotype, TT genotype has a lower risk towards getting any improvement (Table 4).

CT/CC genotypes are found to be associated with reduced risk of heart attack among those who were treated with ACE inhibitors. The subjects with at least one copy of the C allele might have advantage during therapy with ACE inhibitor [36].

There was a decrease in cardiovascular mortality, non-fatal myocardial infarction, and resuscitated cardiac arrest rate during 4.2 years of follow-up. Perindopril users were significantly associated with the treatment benefit in the AT1RSNP carriers (rs5182, rs275651), and also demonstrated a stepwise reduction of risk with a pronounced treatment benefit [36].

Conclusion

ACE polymorphism (rs 4343) GG and GA genotypes have higher plasma angiotensin 2 level, more related to STEMI and NSTEMI, are more prone to have PCI after ACS attack despite early treatment with captopril thus might have a sort of captopril resistance and may be in need for higher doses. AT1R (rs 5182) CT genotypes is more associated with STEMI more prone to have PCI after ACS attack while TT genotype has a risk to be less improved and have a sort of treatment resistance**.**

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