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Research Article

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A Comparative Study to illustrate the Effects of Beta-1 Adrenergic Receptor Allelic Polymorphism on Disease Outcome in Iraqi Patients with Acute Coronary Syndrome (on Metoprolol Therapy)

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Abstract

Objective: The response to metoprolol therapy in patients with acute coronary syndrome (ACS) is variable and a substantial element of this variability is linked to variation in CYP2D6 enzyme and adrenoreceptor (ADRB1) alleles, genotype polymorphism, affecting the phenotype in terms of both rate of metabolism of the active metoprolol (and hence its metabolic ratio) and the responsiveness of ADRB1 receptors to bound metoprolol. This study aims to investigate different clinically relevant allele variants (allele frequencies) of ADRB1 genes: (Arg389Gly: Gly/Gly, Gly/Arg, Arg/Gly) and to determine whether a specific genotype of Beta-1 adrenergic receptor genes (based on genetic polymorphism "allelic types" and combination) have impact on metoprolol effectiveness (clinical outcome) in Arabic Iraqi patients with ACS.

Methods: Two-hundred and fifty patients with ACS were enrolled in this study and divided into 2 study groups: Group 1 (125 ACS patients receiving metoprolol) and Group 2 (125 ACS patients received no metoprolol therapy). Two millilitres of venous blood samples were collected and stored at -20 C for DNA extraction. Urine samples were also collected to assess the metabolic ratio using High-performance liquid chromatography (HPLC).

Results: There were significant variations in the distribution of the Iraqi patients with respect to CYP2D6 allelic polymorphism in comparison with nearby, Western and Eastern countries and that significant difference contributed to patients' outcome in terms of morbidity and mortality in respect to variable genotypes and phenotypes.

Conclusion: These findings suggest that individualization of metoprolol in patients with ACS is essential to improve patients' outcome.

Keywords: ADRB1 polymorphism; Metoprolol; Acute coronary syndrome

Introduction

Acute coronary syndrome (ACS) is a series of cardiac problems including myocardial ischemia (unstable angina) and myocardial infarction (with or without ST segment elevation) [1]. The cardiac pain in those with unstable angina can be either a new pain feeling, getting worse (i.e., more severe, of longer duration, or more frequent than previous attacks of angina), or happening at rest, in the absence of serologic evidence of myocyte death (i.e., serum levels of troponin and the creatine kinase isoenzyme (CK-MB) are not elevated) [2]. A non–ST elevation myocardial infarction (MI) is said to be present in patient having cardiac chest pain with myonecrosis confirmed by serologic evidence without a detectable ST elevation. Non-ST elevation ACS (NSTE-ACS) is a term that is used to describe both non-ST elevation MI and unstable angina as they are typified by the absence of ST elevation. On the other hand, patients with acute-onset chest pain, cardiac muscle necrosis evident by serological test, and ST segment elevation that is persistent for (>20 minutes) are said to possess an ST segment elevation MI [3].

In 2016, the American Heart Association (AHA) announced that 15.5 million subjects ≥20 years of age in the USA are suffering from coronary heart disease (CHD) [4], whilst the recorded rate increases with age for both genders and it has been observed that approximately every 42 seconds, an American subject will show a clinical evidence of MI. The age-standardized death incidence has declined by 22% since 1990, Although the absolute numbers of CVD mortality rates have significantly increased over the same period, mainly due to a change in age demographics and reasons of death worldwide [5].

It is agreed that beta blockers should be not be given till the ACS patient is clinically stable. β-blocker can reduce blood pressure, heart rate as well as improve muscles contractility—where myocardial oxygen requirements is decreased at rest and during exercise [5,6]. Nowadays metoprolol is the most commonly used beta blocker in acute coronary syndrome. The heart muscles have receptors responding to both α-adrenergic and β-adrenergic inputs. These can be subdivided into subgroups, α1, α2-AR and β-AR subgroups, with 3 well-marked subtypes. The guanine nucleotide regulatory protein (G protein)/adenylyl cyclase (AC)) is the signal transduction pathways for β-AR which regulate cardiac muscles [7].

Previous studies showed that a mutation in β-AR genes ((like single-nucleotide polymorphisms (SNPs)), may produce significant pathophysiological changes [8]. For example, SNPs in β1-AR causes signalling and agonist-mediated changes [9-11]. There is a clear prevalence of SNPs in ADRB1 and ADRB2 to the genetic background of the population being studied. Hence, patient response to β-blocker therapy might be variable due to these inter-ethnic variations. Previous studies showed that lower sensitivity to β-blocker therapy is common in African Americans. While, there is a higher sensitivity to β-blockers in Chinese population with similar doses [12,13]. For that reason, this

case control study was designed to investigate the correlation between ADRB1 genetic polymorphism and outcome in Iraqi mid-Euphrates ACS patients treated with metoprolol.

Materials and Methods

Two-hundred and fifty adults were included in this study (127 males and 123 females), who were clinically diagnosed with ACS and they were randomly divided into 2 study groups: Group 1 which had 125 participants with ACS for whom metoprolol therapy was prescribed (metoprolol succinate 100 mg once daily). The diagnosis of ACS was based the ACC/AHA criteria in 2015. Group 2 (the control group) included the remaining 125 participants who were also diagnosed with ACS but they were not taking due to the presence of contraindications to this medication. This study was reviewed and approved by the Human Research Ethics Committee, Faculty of Medicine, University of Kufa.

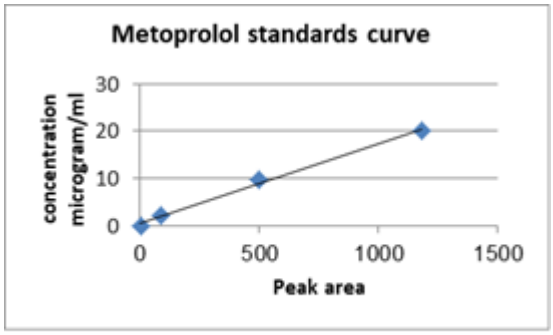
Two millilitres of venous blood samples were aspirated from each participant and transferred into an EDTA tube and then stored at -20 C for DNA extraction. Urine samples were also collected from group1 (taking metoprolol) only. The urine samples for each patient were taken, 8 hours after metoprolol dose and samples then stored at -20 C up to the time for HPLC analysis [14]. The oxidation capacity of metoprolol was expressed as urinary metoprolol to alpha-hydroxy metoprolol (M/HM) ratio (metabolic ratio: MR). Poor metabolizer phenotype was considered when the urinary MR is greater than 12.6 [15].

The polymerase chain reaction (PCR) for ADRB1 primers, originally designed by Feng et al. 2015 [16]. These primers were provided by (Bioneer Company, Korea). Genomic DNA extraction was also performed using Genomic DNA mini extraction kit (Frozen Blood) Geneaid. USA, Table 1.

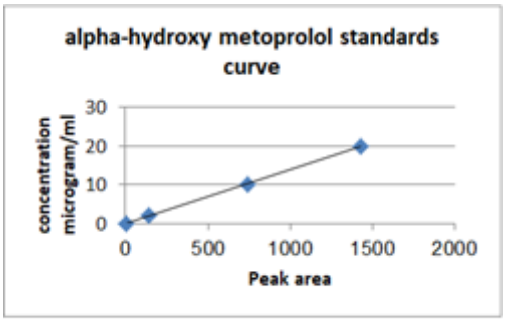
	SNP	Sequence	Amplicon
ADRB1	rs1801253	ACGCTGGGCATCATCATGGGC	332bp
		CATCGTCGTCGTCGTCGTCC	

Table 1: The Multiplex PCR primers with their sequence and amplicon size.

Metoprolol and its major metabolite (alpha-OH metoprolol) were chemically assessed in urine samples using high-performance liquid chromatography (HPLC) according to Godbellon & Duval method [17]. The standard curves for metoprolol and alpha-hydroxy metoprolol were conducted by injecting 3 injections of each concentration (metoprolol and alphahydroxy metoprolol) then a peak area was plotted against the concentration as shown in Figure 1 (A and B).



A



B

Figure 1: Metoprolol (A) and Alpha-hydroxy Metoprolol (B) standard curve estimation.

The study was conducted at Al-Sadder Teaching Hospital and the Department of Pharmacology and Therapeutics/College of Medicine/ Kufa University, at Al-Najaf province and Al-Diwaniyah Teaching Hospital at Al-Diwaniyah province, Iraq and extended from January 2017 through January 2018.

Statistical analysis

Statistical analyses were performed using SPSS (version 22). Single-sample Kolmogorov-Smirnov test to determine the normality of distribution. Mean ± SE were used for continuous variables, while median was used for non-normal distribution. Chi-squared test used for categorical variables, while independent t-test for continuous variables with normal distribution. Statistical significance was considered when P value ≤ 0.05.

Results

Table 2 shows the demographic characteristics of the all participants in this study (Table 2).

Characteristic	Group 1	Group 2	Total	P
Number of cases	125	125	250	---
Age				0.494†

Mean ±SD	52.49 ± 6.29	53.04 ± 6.45	52.76 ± 6.37	NS
Range	36-60	36-60	36-60	
Gender				
Male	90	89	179	0.888* NS
Female	35	36	71	
Diagnosis				
NSEMI	45	44	89	0.964* NS
SEMI	63	65	128	
UA	17	16	33	
PR-Admission	82.83 ± 6.59	82.67 ± 7.31	82.75 ± 6.95	0.856† NS
PR-Discharge	75.58 ± 11.42	76.09 ± 13.83	75.84 ± 12.65	0.754† NS
DBP-Admission	79.96 ± 9.95	79.24 ± 10.00	79.60 ± 9.96	0.569† NS
DBP-Discharge	73.95 ± 6.16	73.39 ± 11.52	73.67 ± 9.22	0.631† NS
SBP-Admission	126.40 ± 14.16	125.12 ± 13.60	125.76 ± 13.87	0.467† NS
SBP-Discharge	116.67 ± 7.10	115.19 ± 16.50	115.94 ± 12.68	0.359† NS
ECG Findings:				
Inferior MI	26	30	56	0.285*¥ NS
ST depression	40	35	75	
Lateral MI	28	33	61	
Anterior MI	3	0	3	
Septal MI	6	2	8	
Normal	22	25	47	

Table 2: Demographic characteristic of study groups (Iraqi patients with ACS).

Group 1: Patients with ACS on metoprolol treatment; Group 2: Patients with ACS with no metoprolol treatment; SD: Standard deviation; NS: not significant; † Independent samples t-test; *Chi-Square test; ¥ > 20% of cells have expected count >5.

While Table 3 explains the genotype frequency of ADRB1 in both study groups. The prevalence rate of CC genotype in all patients enrolled in the present study was 39.2% (Group 1 was 40.8% and Group 2 was 37.6%). The CG genotype rate, (on the other side), was 46.4% (Group 1 was 46.4% and Group 2 was 46.6%). The genotype rate for GG was 14.4% (Group 1 was 12.8 and in Group 2 was 16%).

ADRB1 genotype	Group 2	Group 1	Total
	n=125	**n=125**	**n=250**
CC	47 (37.6%)	51 (40.8%)	98 (39.2%)
CG	58 (46.4)	58 (46.4%)	116 (46.4%)
GG	20 (16.0%)	16 (12.8%)	36 (14.4%)

Table 3: Genotype frequency of ADRB1.

In table 4 The allele frequency of ADRB1 was demonstrated. The prevalence rate of allele C in all patients with ACS was 62.4% (Group 1 was 64% and Group 2 was 60.8%), whereas the prevalence rate of allele G in all patients was 37.6% (Group 1 was 36% and Group 2 was 39.2%).

ADRB1 genotype	Group 2 n=250	Group 1 n=250	Total n=500	χ2	P
C	152 (60.8%)	160 (64.0%)	312 (62.4%)	0.546	0.460
G	98 (39.2%)	90 (36.0%)	188 (37.6%)		

Table 4: Allele frequency of ADRB1.

In table 5, Hardy Weinberg Equilibrium was shown. The observed and expected count of homozygous CC genotype, heterozygous CG genotype and homozygous GG genotype were 98, 116, 36 and 97.3, 117.3, 35.3 respectively. The differences were not statistically significant (P=0.8) suggesting that the sample included in the present study was representative for the population of the mid- Euphrates region concerning ADRB1 wild and variant genotypes.

Genotypes	Observed count	Expected count	χ2	P
Homozygote reference (CC)	98	97.3	0.031	0.859 NS
Heterozygote (GC)	116	117.3		
Homozygote variant (GG)	36	35.3		

Table 5: Hardy Weinberg Equilibrium.

Table 6, shows the percentage of the participants in Group 1 who experienced some of the major side effects of metoprolol side effects divided as per their ADRB1 genotype. No significant difference was found in the prevalence rate of bradycardia and hypotension among patients with GG, CG and CC genotypes (P>0.6).

Metoprolol side effect	GG N=16	CG N=58	CC N=51	Total	P
Hypotension	6	14	18	38	0.604
Bradycardia	6	14	18	38	0.604

Table 6: Metoprolol side effect according to ADRB1 genotype in patients with ACS.

Table 7 compares the association between their ADRB1 genotypes and their disease outcome.

Genotype (No.)	Outcome					P (favorable vs non-favorable)
	Favorable	Non-favorable				
	Improve	PCI	Arrhythmia	HF	Died	
CC (51)	34 (66.7%)	13 (25.5%)	3 (5.9%)	1 (2.0%)	0 (0.0%)	Reference
GC (58)	34 (58.6%)	23 (39.7%)	1 (1.7%)	0 (0.0%)	0 (0.0%)	0.387
GG (16)	6 (37.5%)	5 (31.3%)	0 (0.0%)	3 (18.8%)	2 (12.5%)	0.038

Table 7: The association between the outcome of Iraqi patients with ACS and ADRB1 genotype.

Figure 2 shows the results of the DNA that extracted from whole blood samples using "Genomic DNA mini extraction kit".

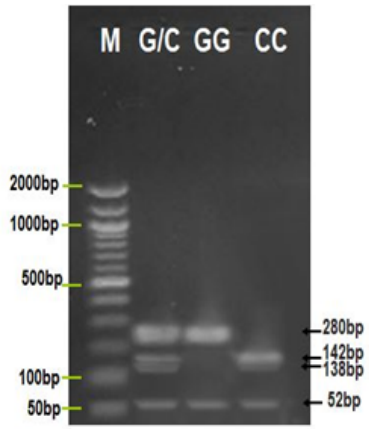


Figure 2: Agarose gel electrophoresis showing the PCR-RFLP product analysis of ADRB1 gene polymorphism using BstNI restriction enzyme.

M: marker (2000-50bp), lane (G/C) heterozygote, Lane (CC) Wild homozygote and Lane (GG) mutant type homozygote.

Discussion

The present study showed that patients with homozygous (CC) genotype accounted for 98 out of 250 (39.2%), patients with heterozygous (CG) genotype accounted for 116 out of 250 (46.4%) and patients with homozygous (GG) genotype accounted for 36 out of 250 (14.4%). These results were consistent with Hardy Weinberg equilibrium; therefore, our sample should correctly reflects the mid-Euphrates population of Iraq. These results are similar to Zoghi et al. 2016, who found that the frequency distribution of ADRB1 alleles in Turkish population was as following; 30.6%, 55.8% and 13.6% for CC, GC and GG genotypes respectively [18]. In addition, findings from our study agrees with Tamilian population study in whom the ADRB1 genotype frequency was as following: 54.8%, 38.8% and 6.4% for CC, GC and GG genotypes, respectively [19].

Gly substitution for the highly functioning Arg389 within the ADRB1 receptor and coupling to the signalling molecules within the cells to increase the Gs protein coupling, thus increasing the activity of the ADRB1 receptor [12]. In the present study, the rate of heart failure and death was more frequent in patients with the homozygous wild genotype CC than both homozygous recessive GG and heterozygous genotypes CG. This was noticeable in both Groups (1 and 2). This may be explained as patients who have Arg389Arg phenotype were recommended to need a higher dose of metoprolol for beta blockade in order to attain a response to metoprolol equivalent to that of Gly389 carriers [20], while, in group 2 where the wild allele C makes the receptor more sensitive to the effect of endogenous catecholamines leading to more rapid heart rate and forceful myocardial contraction at time of anxiety accompanying heart attack despite compromising blood flow, which may lead to catastrophic complication such as sudden death and extensive myocardial infarction with subsequent heart failure [21]. Previous studies conducted on genotype-differing siblings, have noticed that those who were homozygous for Arg389 allele had a higher resting heart rates and diastolic blood pressures as compared to the siblings with Gly389 allele [22]. It is worth to mention that other studies noticed a "no effect" of this polymorphism on hemodynamic (both in resting & exercise) [23]. The variation in response to dobutamine within the Arg389Gly polymorphism in healthy subjects have been shown in one study; those with Arg389 β1AR having greater blood pressure and contractility responses [24]. On the contrary, Parvez Bet al. 2012 observed that subjects who are homozygous for Arg389 may have a more favourable response to metoprolol treatment than subjects who are homozygous for Gly389Gly [25]. In the current study, it was found that the rate of improvement in group of patients on metoprolol therapy was significantly higher than that of patients who did not receive metoprolol therapy and this is similar to the finding of the meta-analysis published by Gong et al. 2017 which described a significant reduction in rate of complications in acute myocardial infarction patients following beta blocker usage with a reduction in the rate of complication by a fraction of 0.32 [26], whereas, in this study, the reduction fraction in the rate of complication was 0.40. Moreover, the present study showed that the rate of arrhythmia and rate of death were significantly higher in those who did not take beta blocker therapy than individuals who received beta blocker therapy.

It is worth to mention that there is no general agreement about when to describe blockers following drugs following heart disease. The American guidelines justify the use of these medications for all patients with acute myocardial infarction (AMI). The European guidelines, on the other side, recommend Class IIa for patients without heart failure or left ventricular failure [5,27]. In general practice, many patients with AMI are directed to take β-blockers for the rest of their life without considering if they have heart complications [28]. This need to be clinically followed-up and confirmed its outcome because the clinical benefits that associated with the use of beta-blockers is variable especially for AMI patients without heart failure [29].

Conclusion

We conclude that individualization of metoprolol in patients with ACS is essential to improve patients' outcome in Iraqi patients with ACS. This is essential due to the significant variations in the distribution of the Iraqi patients with respect to CYP2D6 allelic polymorphism as compared with other countries worldwide. This significant difference contributes to the patients' outcome in terms of morbidity and mortality in respect to variable genotypes and phenotypes.

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