RESEARCH ARTICLE

Pharmacogenetic study of ACE, AGT, CYP11B1, CYP11B2 and eNOS gene variants in hypertensive patients from Faisalabad, Pakistan

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Abstract

Objective: To investigate the association of genetic variants of renin angiotensin aldosterone system, endothelial nitric oxide synthase and 11-beta-hydroxylase genes, and the drug efficacy of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker.

Methods: This two time-point study was conducted from April to November 2016 at Allied Hospital, Faisalabad and National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, and comprised of hypertensive patients taking angiotensin-converting enzyme inhibitor and angiotensin receptor blocker who were followed up for 12 weeks. Baseline and follow-up clinical and biochemical parameters were measured for all patients. Total 11 polymorphisms were genotyped by polymerase chain reaction, polymerase chain reaction-restriction fragment length polymorphism and amplification-refractory mutation system-polymerase chain reaction assays. Data was divided into baseline and follow-up groups, while the latter group was further divided into responding and non-responding subgroups on the basis of patient response to angiotensin-converting enzyme inhibitor and angiotensin receptor blocker drugs. Data was analysed using SPSS 20.

Results: Of the 45 patients, 25(55.5%) were females and 20(44.5%) were males. There was a significant reduction in the systolic blood pressure ($p=0.004$) and low-density lipoprotein cholesterol ($p<0.001$) from the baseline to the follow-up. Systolic blood pressure was significantly reduced in the responding group ($p=0.003$), while diastolic blood pressure ($p=0.121$) was not significantly different. There was no effect of angiotensin-converting enzyme, angiotensinogen, 11-beta-hydroxylase, aldosterone synthase and endothelial nitric oxide synthase gene polymorphisms on angiotensin converting enzyme inhibitor and angiotensin receptor blocker efficacy.

Conclusion: Inter-individual response to angiotensin converting enzyme inhibitor and angiotensin receptor blocker was found to be independent of genetic polymorphisms in renin angiotensin aldosterone system, endothelial nitric oxide synthase and 11-beta-hydroxylase genes.

Keywords: Hypertension, Angiotensin converting enzyme inhibitor, Angiotensin receptor blocker, RAAS, Pharmacogenetics. (JPMA 70: 624; 2020) **https://doi.org/10.5455/JPMA.6666**

Introduction

Hypertension (HTN) is a common pathophysiological condition which is also considered "the silent killer" due to its asymptomatic nature in majority of the patients. In spite of impressive developments, clinicians still struggle to attain adequate blood pressure levels in all hypertensive patients owing to their inconsistent response to drugs.

HTN mainly results from up-regulation of renin angiotensin aldosterone system (RAAS), which involves production of vasoactive peptide, angiotensin II (AngII) from angiotensinogen (AGT) by renin and angiotensin-converting enzyme (ACE). Ang II binds to AngII type 1 receptor (AGT1R) and causes hypertension,[1] diabetic nephropathy[2] etc. Moreover, nitric oxide (NO) and aldosterone also help in regulating blood pressure (BP).[3] NO is synthesised by endothelial nitric oxide synthase (eNOS),[3] while aldosterone synthesis and its binding to mineralocorticoid receptor is influenced by aldosterone synthase which is encoded by CYP11B2 gene, and 11-beta hydroxylase which is encoded by CYP11B1 gene.[4]

Antihypertensive drugs ACE inhibitor (ACEi) and angiotensin receptor blocker (ARB) directly affect the AngII and AGT1R. ACEi inhibits the catalytic activity of ACE, and decreases AngII formation, while ARB antagonises AGT1R and blocks the activation of HTN-causing cellular pathways. Even after carefully choosing the drug and its dose, some patients do not respond well to the prescribed drug and require an increased dose or combination therapy.[5] The major underlying reason for this variable drug response is the difference in the genetic makeup of individuals.

Several studies have reported drug-gene association for

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HTN treatment.[6,7] However, this association is not conclusive due to variable response to antihypertensive drugs. The current study was planned to expand the genetic panel and genetic variants of other genes in order to evaluate the efficacy of ACEi and ARBs in lowering BP with respect to polymorphisms in eNOS, CYP11B1 and RAAS genes.

Materials and Methods

This two time-point study was conducted from April to November 2016 at the Allied Hospital, Faisalabad and National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, and comprised of hypertensive patients of either gender, aged ≥35 years and taking ACEi or ARB drugs. The sample size was calculated by using an online calculator[8]. Parameters used to calculate the sample size were 95% confidence interval (CI), confidence level of 10% and population size about 0.7 million adult hypertensives among the population of Faisalabad, Pakistan. Using non-probability consecutive sampling, subjects meeting the inclusion criteria cited above were recruited from the Allied Hospital, Faisalabad. This study was approved by the institutional (National Institute for Biotechnology and Genetic Engineering) ethics review committee. All the patients were taking the lowest possible doses of ACEi (lisinopril or perindopril) or ARB (losartan or valsartan) as per their treating physicians' prescription. The drug dose was later increased for some patients according to their response to the drug, and the treatment was followed up for 12 weeks. At the end of the 12th week, there were dropouts due to relocations, wrong contact numbers and non-compliance to medicine and death. Remaining patients represented the final sample size.

For genetic and biochemical analysis, 5ml blood samples were taken from all patients at the baseline and at the end of the follow-up period. Clinically important biochemical parameters were measured on Clinical Chemistry Analyzer Microlab 300 (Merck Inc.). For genetic study, deoxyribonucleic acid (DNA) was isolated from the white blood cells (WBCs) by using organic method.[9] Polymorphisms of ACE (rs4340) and eNOS (rs61722009) genes were genotyped by polymerase chain reaction (PCR) assay using previously reported primers.[10,11] Genetic analysis of rs699 (AGT) and rs1799998 (CYP11B2) were done by PCR-restriction fragment length polymorphism (PCR-RFLP) using enzymes and primers reported earlier.[12] Other polymorphisms of AGT (rs4762, rs5049, rs5051), CYP11B1 (rs6410, rs6387) and eNOS (rs1799983, rs2070744) genes were genotyped by an in-house-developed amplification refractory mutation system (ARMS)-PCR assay.[13] Due to preferential amplification of ACE (rs4340) D allele, to avoid the mistyping of ID genotype as DD genotype, an I allele-specific primer[14] was used which indicated 4% mistyped samples.

Data was analysed using SPSS 20. All continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were expressed as frequencies and percentages. The data of biochemical and clinical parameters was divided into baseline and follow-up groups. The data of follow-up group was further divided into responding and non-responding groups on the basis of patient response to ACEi or ARB to lower BP. All the variables were checked for normality of distribution. All polymorphisms were tested for Hardy-Weinberg Equilibrium (HWE) by using chi-square test.[15] To evaluate the difference in the mean values of clinical and biochemical parameters between baseline and follow-up groups, Wilcoxon signed rank test or paired t test was used for non-normally or normally distributed parameters, respectively. Kruskal Wallis test for more than 2 groups was used to compare non-normally distributed variables. Chi square test was used to find the association between drug response and diabetes mellitus, smoking and ischaemic heart disease (IHD). However, due to violations in the assumptions of chi-square, Fisher's Exact test with Freeman-Halton extension for 2X3 contingency table was calculated by using an online resource[16] for the association between genotypes and drug response. Chi square test was also used to calculate odds ratio (OR).

Results

Of the 100 patients initially enrolled, 55(55%) were lost to follow-up, and the remaining 45(45%) represented the

Table-1: Comparison of baseline and follow-up biochemical and clinical parameters.

Parameter	Baseline	Follow-up	Significance (p < 0.05)
Number	45	45	-
SBP (mmHg)	151±29	130±22	0.004*
DBP (mmHg)	92±13	86±15	0.092
Glucose (mg/dL)	144±73	149±115	0.225
Uric acid (mg/dL)	6.9±3.4	7.8±2.8	0.116
Albumin (g/dL)	4.0±0.7	4.2±0.4	0.319
Total protein (mg/dL)	6.1±1.0	6.2±0.7	0.483
Creatinine (mg/dL)	1.0±0.6	1.0±0.7	0.703
Urea (mg/dL)	45±32	46±31	0.358
Total cholesterol (mg/dL)	182±60	167±42	0.159
HDL-C (mg/dL)	45±10	49±9	0.255
LDL-C (mg/dL)	75±20	67±10	<0.001*
Triglycerides (mg/dL)	217±138	225±100	0.918

*shows the statistical significance (p<0.05). Wilcoxon signed rank test or paired t test were applied.
SBP; Systolic blood pressure, DBP; Diastolic blood pressure, HDL-C; High density lipoprotein cholesterol, LDL-C; Low density lipoprotein cholesterol.

Table-2: Clinical, drug details and comparison of clinical and biochemical parameters between responding and non-responding groups.

Parameter	Responding	Non-responding	Significance (p<0.05)
Number	30	15	-
Age(years)	54±12	53±13	0.855
Drug details			
ARB	6 (20%)	3 (20%)	1.0
ACEi	24 (80%)	12 (80%)	1.0
Clinical parameters			
SBP(mmHg)	122±19	145±20	0.003*
DBP(mmHg)	83±15	92±14	0.121
Glucose(mg/dL)	143±109	166±133	0.608
Biochemical parameters			
Uric acid(mg/dL)	8.2±3.2	7.4±2.0	0.568
Albumin(g/dL)	4.2±0.5	4.1±0.2	0.955
Total protein(mg/dL)	6.2±0.9	6.0±0.4	0.628
Creatinine(mg/dL)	1.0±0.6	1.1±0.8	0.608
Urea(mg/dL)	38±20	61±43	0.073
Total cholesterol(mg/dL)	166±43	176±41	0.446
HDL-C(mg/dL)	49±9	49±10	0.894
LDL-C(mg/dL)	67±7	66±6	0.644
Triglycerides(mg/dL)	217±104	243±98	0.447
Clinical details			
Diabetes mellitus	9 (30%)	5 (33%)	0.484
Ischaemic heart disease	14 (47%)	8 (53%)	0.545
Smoking	3 (10%)	3 (20%)	0.295

*shows the statistical significance (p<0.05). Mann Whitney U test and Chi square test were applied.
SBP; Systolic blood pressure, DBP; Diastolic blood pressure, HDL-C; High density lipoprotein cholesterol, LDL-C; Low density lipoprotein cholesterol, ARB: Angiotensin receptor blocker; ACEi: Angiotensin converting enzyme inhibitor.

final sample size. Of them, 25(55.5%) were females and 20(44.5%) were males. Among all the parameters, the baseline and follow-up groups indicated a significant difference in systolic blood pressure (SBP) (p=0.004) and low-density lipoprotein-cholesterol (LDL-C) (p<0.001) levels (Table-1).

The SBP of the responding subgroup was significantly less than that of the non-responding subgroup (p=0.003). Although, diastolic blood pressure (DBP) was also reduced in the responding subgroup than the non-responding group, the reduction was not statistically significant (p=0.121). There was higher prevalence of ischaemic heart disease (IHD) in the non-responding subgroup compared to the responding subgroup (Table-2).

Genotypic frequencies of all polymorphisms except rs2070744 were in accordance with HWE (p>0.05). Genotype frequencies of AGT gene polymorphisms rs699 and rs4762 were similar in both groups, and despite the differences in genotypic frequencies, the difference was not statistically significant (Table-3).

Table-3: Genotypic frequencies of responding and non-responding groups.

Gene	Polymorphism	Genotypic frequency [N (%)]		Association (p< 0.05)
		Responding (n=30)	Non-responding (n=15)	
ACE	rs4340	II=8(27%)	II=5(33%)	0.91
		ID=16(53%)	ID=8(53%)	
		DD=6(20%)	DD=2(14%)	
AGT	rs699	TT=6(20%)	TT=3(20%)	1
		TC=18(60%)	TC=9(60%)	
		CC=6(20%)	CC=3(20%)	
	rs4762	CC=24(80%)	CC=12(80%)	1
		CT=6(20%)	CT=3(20%)	
		TT=0(0%)	TT=0(0%)	
	rs5049	GG=20(67%)	GG=9(60%)	0.54
		GA=10(33%)	GA=5(33%)	
		AA=0(0%)	AA=1(7%)	
	rs5051	AA=7(23%)	AA=2(13%)	0.49
		GA=16(54%)	GA=11(74%)	
		GG=7(23%)	GG=2(13%)	
CYP11B1	rs6410	GG=20(67%)	GG=8(53%)	0.09
		AG=6(20%)	AG=7(47%)	
		AA=4(13%)	AA=0(0%)	
	rs6387	AA=19(63%)	AA=7(47%)	0.19
		GA=6(20%)	GA=7(47%)	
		GG=5(17%)	GG=1(6%)	
CYP11B2	rs1799998	TT=15(50%)	TT=5(33%)	0.59
		TC=12(40%)	TC=8(53%)	
		CC=3(10%)	CC=2(14%)	
eNOS	4b/a	bb=20(67%)	bb=9(60%)	0.23
		ba=6(20%)	ba=6(40%)	
		aa=4(13%)	aa=0(0%)	
	rs1799938	GG=22(73%)	GG=12(80%)	0.24
		TG=8(27%)	TG=2(13%)	
		TT=0(0%)	TT=1(7%)	
	rs2070744	TT=17(57%)	TT=8(53%)	0.57
		CT=4(13%)	CT=4(27%)	
		CC=9(30%)	CC=3(20%)	

Statistical analysis done by Fisher exact test with Freeman-Hilton extension with statistical significance of p<0.05.
ACE: Angiotensin-converting enzyme; AGT: Angiotensinogen; eNOS: endothelial nitric oxidesynthase.

Allelic frequencies of rs699, rs4762, rs5051, rs6410 and rs1799938 were same in the responding and non-responding subgroups and the alleles had no observable effect on the drug response (Table-4).

Discussion

To treat HTN, several antihypertensive drugs like ACEi, ARB, β-blockers, calcium (Ca2+)-channel blockers etc. are used. Among these, ACEi and ARB are recommended as first line therapy to treat HTN in all populations except Africans owing to their low plasma renin levels which makes the ACEi and ARB less effective than other antihypertensive drugs.[17,18] In spite of the beneficial

Table-4: Allelic frequencies and odds ratio (OR) of all polymorphisms for responding and non-responding group.

Gene	Polymorphism	Allelic frequency [N (%)]		Association (p< 0.05)	Odds Ratio (Confidence interval)
		Responding (n=30)	Non-responding (n=15)		
ACE	rs4340	I=32(53%)	I=18(60%)	0.55	1.31 (0.54-3.19)
		D=28(47%)	D=12(40%)		
AGT	rs699	C=30(50%)	C=15(50%)	1	1.0(0.42-2.40)
		T=30(50%)	T=15(50%)		
	rs4762	C=54(90%)	C=27(90%)	1	1.0(0.23-4.31)
		T=6(10%)	T=3(10%)		
	rs5049	A=10(17%)	A=7(23%)	0.45	1.52(0.51-4.50)
		G=50(83%)	G=23(77%)		
	rs5051	A=30(50%)	A=15(50%)	1	1.0(0.42-2.40)
		G=30(50%)	G=15(50%)		
CYP11B1	rs6410	A=14(23%)	A=7(23%)	1	1.0(0.36-2.82)
		G=46(77%)	G=23(77%)		
	rs6387	A=44(73%)	A=21(70%)	0.74	0.45(0.32-2.23)
		G=16(27%)	G=9(30%)		
CYP11B2	rs1799998	C=18(30%)	C=12(40%)	0.34	1.56(0.62-3.89)
		T=42(70%)	T=18(60%)		
eNOS	4b/a	a=14(23%)	a=6(20%)	0.72	0.82(0.28-2.41)
		b=46(77%)	b=24(80%)		
	rs1799938	T=8(13%)	T=4(13%)	1	1.0(0.28-3.63)
		G=52(87%)	G=26(87%)		
	rs2070744	C=22(37%)	C=10(33%)	0.76	0.86(0.34-2.17)
		T=38(63%)	T=20(67%)		

Statistical analysis done by Pearson chi square. Odds ratio were calculated by chi square test with statistical significance of p<0.05.
ACE: Angiotensin-converting enzyme; AGT: Angiotensinogen; eNOS: endothelial nitric oxide synthase.

effects of ACEi and ARB in lowering BP, the inter-individual variability in drug response cannot be ignored.

Several studies have reported strong effect of RAAS gene variants on the efficacy of ACEi and ARB.[19,20] It is well-established that DD genotype of ACE (rs4340) polymorphism increases plasma levels of ACE, but the effect of ACE (I/D) genotypes on drug response is non-conclusive. Some studies report that DD genotype favours the ACEi response,[19,21] while others report the association between II genotype and ACEi[20,22] while several studies came out with no effect of genotypes on ACEi therapy.[23,24] To some extent, the current study also favours the studies done in Malaysia according to which ACE DD genotype favoured the drug response of ACEi,[25] but the results of the current study are not statistically significant.

Polymorphisms of AGT have also shown inconsistent results.[26,27] The present study contradicts the earlier findings which reported the influence of rs699 TT genotype on ACEi function.[7] In the current study, genotypic frequencies of rs699 and rs4762 were same in the responding and non-responding subgroups, which favours the observations that ACEi efficacy is not affected by ACE (I/D) polymorphism rs699 and rs4762.[6]

The third most important component of RAAS is aldosterone. Genetic variants influencing mineral ocorticoid metabolism and aldosterone synthesis (CYP11B1 and CYP11B2) can promote HTN owing to fluid-electrolyte derangements. Although ACEi and ARBs do not directly affect these genes, a study from Europe involving 105 British Caucasian families reported that codominant model for rs6387 was associated with increased aldosterone synthesis, leading to HTN and allelic stratification favoured G allele.[28] Very few studies have focussed on the polymorphisms in CYP11B1/2 genes. Even among those handful studies, association between HTN and CYP11B1/2 gene polymorphisms is contradicting for rs6410, rs6387 and rs1799998[28,29] and the current study found that antihypertensive activity of ACEi and ARBs was independent of rs6410, rs6387 and rs1799998.

NO in blood vessels is synthesised by eNOS. ACEi can indirectly affect NO generation as it helps in reducing BP. The genetic variants of eNOS gene (4b/a, rs1799983 and rs2070744) are widely studied for their association with cardiac diseases,[30] HTN and response to ACEi. Numerous studies have confirmed its association with HTN,[11,31,32] while the association of eNOS polymorphisms with ACEi

response was not reproduced by some studies.[33] Contrary to a Brazilian population study,[33] the current study found that the frequency of rs2070744 TT genotype was nearly same in both subgroups, while the frequency of CC was more in the responding subgroup than the non-responding subgroup. Furthermore, no individual polymorphism showed association with ACEi efficacy.

The current study has its limitations. The findings may have some screening power but cannot be generalised. Further studies with more stringent criteria and large sample sizes are needed to increase the pharmacogenetic value of the current findings.

Conclusion

ACEi and ARBs reduced BP, but no effect of ACE, AGT, CYP11B1/2 and eNOS gene variants was observed for inter-individual variability in RAAS blockades.

Disclaimer: None.

Conflict of Interest: None.

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