Tropical Journal of Pharmaceutical Research April 2019; 18 (4): 851-855
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i4.24

Original Research Article

Association of CYP2C19*2 and *17 genetic variants with hypertension in Pakistani population

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Sent for review: 19 May 2019 *Revised accepted: 13 March 2019*

Abstract

Purpose: *To investigate the association of *2 and *17 single nucleotide polymorphisms (SNPs) of CYP2C19 gene with hypertension in Pakistani population.*
Methods: *The study was conducted on 527 hypertensive patients and 530 unrelated healthy controls from selected regions of Pakistan. DNA was extracted from leukocytes and all patients and controls were genotyped for two SNPs (rs4244285 and rs12248560) of CYP2C19 gene by allele specific polymerase chain reaction (AS-PCR).*
Results: *Multi-allelic polymorphism in CYP2C19 identified four distinct phenotypes known as ultra-rapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM) and poor metabolizer (PM) in hypertensive patients and controls. For CYP2C19*2 polymorphisms, overall wild type and mutant allele frequency were 75 and 25 % in hypertensive patients, and 64.2 and 35.8 % in controls. For CYP2C19*17 polymorphisms, the overall wild type and mutant allele frequency were 66.6 and 33.4 % in hypertensive patients and 75.6 % and 24.4 % in controls. Significant difference in allele frequencies for CYP2C19*2 and *17 was demonstrated between hypertensive and non-hypertensive subjects.*
Conclusion: *To the best of our knowledge, this is the first report on CYP2C19 frequencies in hypertensive Pakistani patients. The finds should help clinicians to determine a suitable optimal dosage of some drugs in order to reduce side effects.*

Keywords: *CYP2C19, Genotyping, Single nucleotide polymorphism, Adverse drug reactions, Pakistani population*

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INTRODUCTION

Cytochrome P450 2C19 (CYP2C19), an important enzyme of the cytochrome P450 family, is involved in the metabolism of several drugs. Inter-individual variation in treatment response is due to genetic polymorphisms in CYP2C19 [1]. PM of *CYP2C19*2* sustain omeprazole for a longer time period which helps in stronger inhibition of gastric acid secretion for longer interval [2]. Psychiatric patients having CYP2C19*17 allele show 42 % lower escitalopram serum levels showing high risk of treatment failure [3]. On the other hand,

CYP2C19*17 allele carriers have reduced risk of breast cancer occurrence [4].

US FDA has issued a "Black Box" to Clopidogrel in March 2009, recommending CYP2C19 genotyping before prescribing Clopidogrel and using guidelines available for Clopidogrel drug monitoring [5]. Another study reported that PM of CYP2C19 had higher levels of Gliclazide suggesting CYP2C19 role in Gliclazide clearance [6]. CYP2C19 PM genotype in cirrhotic patients having hepatitis C virus is associated with higher risk of developing hepatocellular carcinoma [7]. Previous studies revealed that CYP2C19 rs10509676 variant homozygote (AA genotype) was associated with HTN [8]. The present study was carried out to explore the association of *2 and *17 alleles of CYP2C19 and HTN and how it's affecting recent treatment regimes.

EXPERIMENTAL

Subjects

The study group included 1057 subjects, consisting of 527 hypertensive patients and 530 controls from representative regions of Pakistan. The study was conducted in compliance with the Declaration of Helsinki [9] and was approved by the Ethical Committee and Institutional Review Board (IRB) of Quaid-i-Azam University, Islamabad (#IRB-QAU-97). A written informed consent was obtained from all subjects before participation in the study.

Genotyping

Blood samples (5 ml in tubes containing EDTA) were drawn from the subjects and kept at 4 ^{o}C until DNA extraction from peripheral blood leukocytes by phenol chloroform method. CYP2C19 alleles CYP2C19*2 (c.G681A; rs4244285) and CYP2C19*17 (c.C806T; rs12248560) were genotyped by Allele-specific (AS-PCR) and the primer data is given in supplementary data (Table 2 and 3). In each 20 µL PCR reaction, 1X PCR buffer (2.5 µL) (Fermentas), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM each of forward and reverse primers, 0.2 units of *Taq* DNA polymerase (Fermentas) and 100 ng of DNA samples were used.

PCR amplification of CYP2C19*2 region was carried out by initial denaturation at 95 ^{o}C for 5 min, followed by 30 cycles of denaturation at 94 ^{o}C for 1 min, annealing at 62 ^{o}C for the amplification of "A" allele-specific DNA fragment and 60 ^{o}C for "G" allele-specific DNA fragment for 40 seconds, extension at 72 ^{o}C for 1 min followed by final extension at 72 ^{o}C for 5 min. The thermal profile for the amplification of CYP2C19*17 was similar to CYP2C19*2 amplification except annealing at 58 ^{o}C for both alleles. Once DNA amplification was done, the PCR products were run on 2 % agarose gel in order to confirm the genotype of patients. The phenotype was inferred theoretically from the genotype.

Statistical analysis

Association between the CYP2C19*2 and CYP2C19*17 variants and HTN was determined using SPSS version 20.0 [10]. The level of significance for all the results was set at $p < 0.05$. Chi-square test was used to test the significance of the Hardy-Weinberg equilibrium of the polymorphism of the two genes in the entire data of the patient and the control groups.

RESULTS

The distribution of CYP2C19 genotypes and allele frequencies for the *2 and *17 SNPs within the hypertensive patient and control groups are presented in Table 1. Distribution of genotypes for the *2 polymorphism in hypertensive patients was 60 % homozygous wild type (CYP2C19*1/*1), 10 % homozygous mutant (CYP2C19*2/*2), 30 % heterozygous (CYP2C19*1/ *2); and in controls 40 % homozygous wild type (*1/*1), 20 % homozygous mutant (*2/*2) and 40 % heterozygous (*1/*2). For CYP2C19*2 polymorphisms, overall wild type and mutant allele frequency was 75 and 25 % in hypertensive patients and 64.2 and 35.8 % in controls.

Similarly distribution of genotypes for the *17 polymorphism was 40 % homozygous wild type (*1/*1), 10 % homozygous mutant (*17/*17) and 50 % heterozygous (*1/*17/, *2/*17); and in controls 60 % homozygous wild type (*1/*1), 10 % homozygous mutant (*17/*17) and 30 % heterozygous (*1/*17/, *2/*17). Whereas for CYP2C19*17 polymorphisms, the overall wild type and mutant allele frequency was 66.6 % and 33.4 % in hypertensive patients and 75.6 % and 24.4 % in controls. On analyzing the data it came out that, out of 527 cases registered, 48 % were males and 52 % were females. In our case frequency of primary and secondary hypertension were (70 % and 30 %) respectively. Our results shows that among cases, 97 % were married, 50 % had a positive family history of hypertension, 55 % were obese, 20 % were smokers, 60 % were non-working, 40 % are taking normal diet and 60 % undergoes normal sleep. Cases were stratified according to the

association of other complications into 5 subgroups including Gp I: hypertension with diabetes (30 %); Gp II: hypertension with cardiac complication (10 %); Gp III: hypertension with renal complications (10 %); Gp IV: hypertension with other diseases (10 %); Gp V: no disease (30 %). According to medication details, 60 % of the patients received monotherapy while 40 % patients received combination therapy; 15 % patients did not take any medication. Regarding drug classes, CYP2C19*2 is more prevalent in patients taking angiotensin converting enzyme (ACE) inhibitor (30.35 %), followed by beta blocker (BB) (20.68 %), Calcium channel blockers (CCB) (18.49 %), angiotensin receptor blocker (ARB) (16.41 %) and Diuretic (12.50 %). Whereas, CYP2C19*17 shows highest frequency in ARB (30.14 %) followed by CCB (28.47 %), Diuretic (25 %) BB (24.13 %) and ACE inhibitor (2.75 %). However, CYP2C19 (*2/*2) mutant allele was more frequent in cardiac patients (26 %) followed by other diseases (20 %), renal patients and patients who did not take any medication (19 %) and diabetics (16 %). Moreover CYP2C19 (*17/*17) allele showed high prevalence in Diabetics (22 %) followed by Cardiac patients (21 %), patients not taking any medication (20 %), patients having other diseases (19 %), patients on renal diseases (18 %). Finally, the distribution of alleles and genotypes of CYP2C19*2 and *17 showed a significant difference between hypertensive patients and normal controls ($p \leq 0.05$) which suggests that CYP2C19*2 and *17 are associated with hypertension in the Pakistani population studied.

DISCUSSION

CYP2C19*2 allele frequency in the present study population is (35.8 %). This result was consistent with those reported by Asian populations (14-39 %) [11], in India (40.2 %) [12], Philippines (39 %) [11], Chinese-Han (37 %) [13], Lure (35 %) [14], Hakka (31 %) [15] and Japan (30 %) [16].

Table 1: Genotype (%) among patients and controls

Variable	Patients (n=527)	Control (n=530)	OR	*P*-value	CI
		CYP2C19*2			
Homozygous Wildtype	307 (60%)	231 (40%)	0.55		0.433-0.706
Heterozygous	174 (30%)	219 (40%)	1.42		1.111-1.835
Homozygous Mutant	46 (10%)	80 (20%)	1.85	<0.00001	1.265-2.731
		Alleles			
Wildtype	788 (75%)	681 (64.2%)	0.60		0.502-0.731
Mutant	266 (25%)	379 (35.8%)	1.64	0.000	1.367-1.988
		CYP2C19*17			
Homozygous Wildtype	213 (40%)	332 (60%)	2.47		1.93-3.165
Heterozygous	276 (50%)	137 (30%)	0.31		0.244-0.410
Homozygous Mutant	38 (10%)	61 (10%)	1.67	<0.00001	1.094-2.558
		ALLELES			
Wildtype	702 (66.6%)	801 (75.6%)	1.55		1.282-1.874
Mutant	352 (33.4%)	259 (24.4%)	0.644	0.000005	0.533-0.779

Table 2: Primers for CYP2C19*2

Primer	Sequence (5′-3′)	Length	Melting temp (^{o}C)	Size (bp)	Type of PCR product
2C19*2 F	CAGAGCTTGGCAATATTGTATC	22	57.1	291	Control
2C19*2 R	ATACGCAAGCAGTCACATAAC	21	57.4		
2C19*2 A	GTAATTTGTTATGGGTTCCT	20	52.3		A allele fragment
2C19*2F	CAGAGCTTGGCAATATTGTATC	22	57.1	169	
2C19*2G	ACTATCATTGATTATTTCCCG	21	55.6	202	G allele fragment

Table 3: Primers for CYP2C19*17

Primer	Sequence (5′-3′)	Length	Melting temp (^{o}C)	Size (bp)	Type of PCR Product
2C19*17F	AAGAAGCCTTAGTTTCTCAAG	21	55.5		
2C19*17R	AAACACCTTTACCATTTAACCC	22	56.6	507	Control
2C19*17T	TGTCTTCTGTTCTCAAAGTA	20	52.3	218	T allele fragment
2C19*17 R	AAACACCTTTACCATTTAACCC	22	56.6		
2C19*17 F	AAGAAGCCTTAGTTTCTCAAG	21	55.5		C allele fragment
2C19*17C	ATTATCTCTTACATCAGAGATG	22	54.7	330	

Similarly CYP2C19*17 allele frequency in our Pakistani population is (24.4 %) and were close to other populations namely, Iran (21.7 %) [17], Saudi Arabia (25.7 %) [18], Turkey (24.4 %) [19] and Germany (25.5 %) [20].

HTN is a major public health issue affecting approximately 1 billion people worldwide [21]. Among hypertensive drugs, most commonly prescribed were CCB and BB [22]. Previous studies show that in case of severe kidney patients, ACE inhibitor or ARB alone or in combination should be the first choice of therapy regardless of ethnic background [23]. On the other hand, patients receiving ACE inhibitor as initial treatment instead of CCB are on 51 % increase threat of having stroke [24]. Previous studies in a large trial show that compared to an ACE inhibitor, thiazide-type diuretic plays vital role in improving cerebrovascular heart failure and combined cardiovascular outcomes [25].

CONCLUSION

Assessment of CYP2C19*2 and *17 variants and HTN association has been conducted for the first time among Pakistani population. This findings indicate that the frequency of CYP2C19*2 and *17 allele are high. The findings should be helpful for physicians to determine the correct drug dosage according to individual's metabolic capacity, lead to decreasing adverse drug reactions and improve therapeutic outcomes.

DECLARATIONS

Acknowledgement

Thanks are due to Mr. Amjad Farooq for his technical assistance, and BioMES, Alpha Genomics Private Limited, Islamabad - Pakistan for editing and reviewing the manuscript. Thanks are also due to the patients who participated in the study.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Sana Riaz, Atika Mansoor, Saima Siddiqi, Aneesa Sultan designed all the experiments and revised the paper. Sana Riaz, Muhammad Usman Tareen, Sana Rubab, Ayesha Batool and Anwarullah performed the experiments, Sana Riaz, Atika Mansoor, Saima Siddiqi and Aneesa Sultan wrote the paper.

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