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Effect of CYP2C9 rs2860905 Polymorphism on the Efficacy of Losartan in Pakistani Hypertensive Patients

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ABSTRACT

Hypertension is a multifactorial disease characterized by high blood pressure in blood vessels. It is caused by the dysregulation of renin angiotensin aldosterone system (RAAS). It is a major risk factor for other metabolic diseases including cardiovascular diseases, diabetes, and others. Various RAAS targeting drugs such as losartan are prescribed as the first line therapy to treat hypertension. However, their efficacy varies among individuals owing to genetic variations in drug binding substrates or drug metabolizing enzymes, which convert the prodrug to active drug. CYP2C9 gene encodes for an enzyme which metabolizes losartan. Various studies have concluded that genetic variations in CYP2C9 affect the response of losartan due to the variation in its metabolism. Thus, the current study aimed to check the effect of CYP2C9 rs2860905 G>A polymorphism on the efficacy of losartan. For this purpose, a total of 48 subjects were selected and genotyped for rs2860905 polymorphism using in-house developed tetra-ARMS-PCR. The subjects were divided into responding (n=34) and non-responding (n=14) groups on the basis of their blood pressure after treatment with losartan. Statistical analysis demonstrated that rs2860905 GG genotype was more prevalent in the responding group as compared to the non-responding group (50% vs. 36%). Multinomial regression analysis showed that the carriers of GA or AA genotype did not respond to losartan treatment efficiently as compared to those of GG genotype. However, these results could not achieve statistical significance. To conclude, CYP2C9 rs2860905 G>A polymorphism does not affect the efficacy of losartan in Pakistani hypertensive subjects.

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Keywords: CYP2C9, drug efficacy, genetic variation, losartan, rs2860905, polymorphism

1. INTRODUCTION

Hypertension is the paramount cause behind cardiovascular diseases [1]. It may force individuals towards leading a disability-adjusted life and may even cause death [2]. Due to its silent manifestation, it remains uncontrolled and even undiagnosed in many individuals. Hence, it is termed as a 'silent killer' [3]. According to the European [4] and US Guidelines [5], hypertensive individuals should aim to maintain <140/90 mmHg. More than 116.4 million individuals already suffer from hypertension and the number is expected to rise to 1.5 billion individuals by 2040 [6]. Studies have also confirmed that genes not only play their role in the manifestation of hypertension but also affect the response to antihypertensive drugs [7]. The efficacy of these drugs is influenced by the variants of their respective drug metabolizing genes. Hypertension is a multifactorial and multi-genic disease. So, diet, physical activity, and genomic profile of an individual can contribute to the manifestation of this disease [8]. The inter-individual variability of hepatic metabolism can result in inefficient control of blood pressure in hypertensive patients. Pharmacogenetic knowledge of antihypertensive drugs and their respective hepatic drug-metabolizing enzymes can help physicians to prescribe the most effective drugs to their patients [9].

Renin-angiotensin-aldosterone system (RAAS) is a hormone system that is directly responsible for the regulation of blood pressure and systemic vascular resistance [10, 11]. When renal blood flow is low, an active enzyme known as renin is released by the kidneys into the blood stream. This enzyme converts angiotensinogen (produced from liver) to angiotensin I that which is an inactive hormone precursor [12]. Angiotensin I is further converted into an active peptide namely angiotensin II through the angiotensin converting enzyme found in the vascular endothelial cells of lungs [13]. Angiotensin II binds with angiotensin type 1 receptor (AT1R) that initiates a cascade of reactions leading to vasoconstriction and hence elevates blood pressure. Over activation of AT1R due to any pathophysiological condition subsequently leads to hypertension and cardiovascular diseases. Hypertension can be effectively managed by blocking AT1R [14].

Angiotensin II receptor blockers are widely prescribed for hypertension management due to their high efficacy, specificity to AT1R without disturbing the useful functions of AT2R, and manageable withdrawal rates and side effects as compared to other anti-hypertensive drugs [15]. ARBs drew even more attention during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, since it was suggested that patients taking ARBS had better prognosis and less severe COVID-19 related outcomes [16]. United S Food and Drug Administration (FDA) has approved eight ARBs so far. Losartan was the first ARB and it was patented in 1986. It was approved as a medicine in 1995 in the U.S. It was the ninth most prescribed drug in the U.S. in 2020, surpassing 54 million prescriptions (The Top 300 of 2020, 2020). Losartan is safely prescribed to individuals suffering with diabetes, cardiac, or kidney issues. It is orally administered and has the bioavailability of approximately 33% after undergoing first-pass metabolism. During metabolism, it is converted into an active metabolite E-3174, whose pharmacological activity is 40 times higher than that of losartan [17].

Losartan is metabolized by an enzyme CYP2C9 which is a member of the superfamily of enzymes Cytochrome P450. This enzyme is encoded by the *CYP2C9* gene in human beings. *CYP2C9* has multiple genetic variants with varying frequencies in various ethnicities have been identified so far [18, 19]. These polymorphisms can reportedly affect the efficacy of losartan [20]. In this study, the aim was to investigate the effect of polymorphism rs2860905 of *CYP2C9* gene on the efficacy of losartan.

2. MATERIALS AND METHODS

2.1. Study Subjects

In this study, a total of 48 patients taking losartan were recruited from DHQ Teaching Hospital, Sargodha and some private clinics after informed consent. Blood samples were drawn and collected in EDTA (ethylenediaminetetraacetic acid) containing vacutainers and stored at -20^{o} C. This blood was used to extract DNA from leukocytes using organic phenol-chloroform-isoamyl alcohol method. A questionnaire about demographic details, disease history, medicine history, and dietary habits was filled by all enrolled subjects. Blood pressure of each patient was measured in the sitting position by placing an inflatable cuff on the upper arm. All the patients taking losartan were included in the cohort. Individuals

suffering with cancer or any chronic infectious disease such as AIDS or hepatitis were excluded. All the patients were well informed about the aim of the study and gave written informed consent. All the procedures and protocols conformed to the Declaration of Helsinki.

2.2. Genotyping

Primers were designed for Tri-Amplification Refractory Mutation System-Polymerase Chain Reaction (Tri-ARMS-PCR) by using primer1 software (http://primer1.soton.ac.uk/primer1.html) and analyzed for quality parameters including GC content, melting temperature, hairpin loop, heterodimer, and homodimer by using the freely available online tool Oligoanalyzer (https://www.idtdna.com/pages/tools/oligoanalyzer). *In silico* PCR of USCS genome browser (https://genome.ucsc.edu/cgi-bin/hgPcr) was used to assess the specific amplification of designed primers and product size was re-confirmed. Three primers [Forward (CATTGAGGACCGTGTTCAAGAGG), Reverse for G_allele (AAATGAACCTTTTATACCCACACTGGAC), and Reverse for A_Allele (AAATGAACCTTTTATACCCACACTGGAT)] were finalized and synthesized by Macrogen Korea.

Tri-ARMS-PCR assay was optimized for specific and efficient genotyping of *CYP2C9* rs2860905 G>A polymorphism. PCR reaction mixture was prepared in two different tubes for each sample. Forward primer was added in both tubes, with one tube containing reverse G_Allele primer and the other tube containing reverse A_allele primer. Amplification was done at 62.4ºC in Bio-Rad T100^{TM} Thermal Cycler. The amplified product was resolved in ethidium bromide containing 1.5% agarose gel and analyzed under MERADD (ICCC) GelDoc 100. Each allele showed a band of 280bp. The appearance of band one sample showed the homozygous sample, while amplification in both samples showed heterozygous sample (Figure 1).

2.3. Statistical Analysis

On the basis of blood pressure values, the samples were divided into responding and non-responding groups. Chi-square test, t test, and multinomial regression test were applied to achieve the above-mentioned objectives. These tests were applied by using SPSS (version 20).

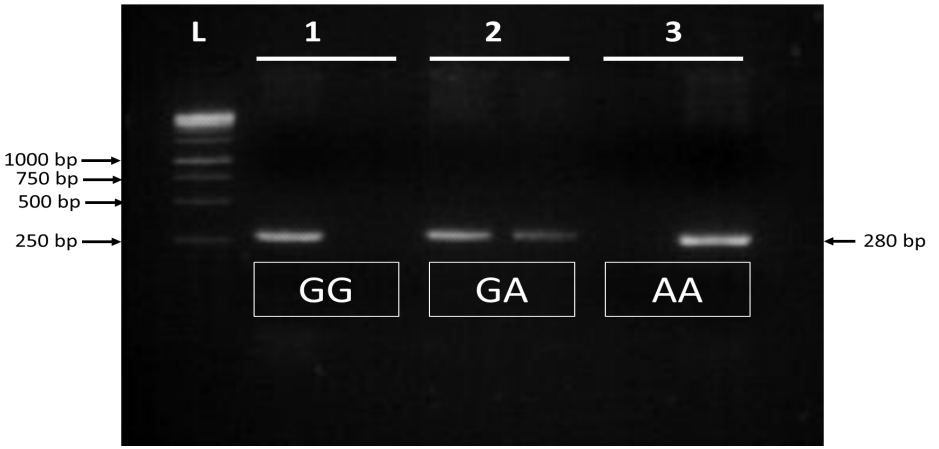

Figure 1. Amplification and Genotyping of *CYP2C9* rs2860905 G>A polymorphism

3. RESULTS

The statistical analysis of anthropometric parameters demonstrated that the number of females was higher in both study groups. The subjects of the non-responding group were older (62±17 vs. 53±10, p=0.034) and had higher BMI (30±7 vs. 28±5, p=0.44) than the individuals of the responding group. The analysis of clinical parameters revealed that cardiovascular diseases and the family history of cardiometabolic diseases was more prevalent in the non-responding group; however, these differences were not significant. Obesity status and the duration of hypertension was also insignificantly different between both groups. Although, significant differences were observed for systolic blood pressure (SBP) (p<0.001) and diastolic blood pressure (DBP) (p=0.026). This significance was obvious because the patients' response to the antihypertensive drug was assessed on the basis of SBP and DBP. Dietary habits including salt intake, glucose intake, vegetable and meat consumption, and fat type were almost similar in both groups (Table 1).

Table 1. Comparison of Anthropometric, Clinical, and Dietary Parameters of Responding and Non-responding Groups

Parameter	Responding (n=34)	Non-responding (n=14)	Significance
Anthropometric Parameters			
Age	53±10	62±17	0.034

Parameter		Responding (*n*=34)	Non-responding (*n*=14)	Significance
Gender	Male	24%	29%	0.71
	female	76%	71%	
BMI		28±5	30±7	0.44
Clinical Parameters				
SBP		130±10	152±7	<0.001
DBP		83±8	89±7	0.026
Obesity	Normal	30%	30%	0.82
	Overweight	40%	30%	
	Obese	30%	40%	
Duration of Hypertension	<= 1 year	28%	38%	0.72
	1-5 year	54%	54%	
	6-10 year	13%	8%	
	11-15 years	5%	0%	
Cardiovascular Disease		12%	28%	0.16
Family History		35%	43%	0.62
Smoking Status		18%	7%	0.35
Dietary Habits				
Salt Intake	More	0%	0%	0.31
	Medium	0%	0%	
	Less	19%	14%	
	Very less	81%	79%	
	none	0%	7%	
Glucose Intake	More	0%	0%	0.09
	Medium	0%	14%	
	Less	28%	29%	
	Very less	59%	36%	
	none	13%	21%	
Vegetables	More	45%	36%	0.75
	Medium	55%	64%	
	Less	0%	0%	
	Very less	0%	0%	
Meat	More	13%	0%	0.12
	Medium	63%	46%	
	Less	20%	46%	
	Very less	0%	8%	
	None	4%	0%	
Fats	Oil	67%	89%	0.19

Parameter	Responding (*n*=34)	Non-responding (*n*=14)	Significance
Banaspati ghee	33%	11%	

Chi-square test was applied to check the association of rs2860905 with the response to antihypertensive drug losartan, which is a substrate of CYP2C9 encoded drug metabolizing enzyme (Table 2). Descriptive analysis revealed that in the responding group rs2860905, GG genotype was more prevalent as compared to AG and AA genotypes (50% vs. 35% and 15%, respectively). Similarly, the frequency of G allele in the responding group was almost double than A allele (68% vs. 32%). Whereas, in the non-responding group, the frequency of heterozygous rs2860905 GA was higher, followed by GG and AA genotypes. Although G allele was more prevalent in the non-responding group as well; however, the difference in the frequencies of A allele and G allele was only 8%, which is less pronounced than the difference observed in the responding group (36%). Similarly, though there were differences in the prevalence of genotypes and alleles between responding and non-responding groups; still, these differences could not achieve statistical significance. Chi-square test revealed that rs2860905 does not affect the blood pressure reducing potential of losartan ($\chi^2 = 0.86$, p=0.65). The analysis of rs2860905 alleles also complemented these findings ($\chi^2 = 0.96$, p=0.35).

Table 2. Genotypic and Allelic Frequencies of *CYP2C9* rs2860905 G>A Polymorphism and their Association with the Response to Antihypertensive Drug

Genotype	Responding (*n*=34)	Non-responding (*n*=14)	Significance
GG	17 (50%)	5 (36%)	$\chi^2 = 0.86$ p=0.65
GA	12 (35%)	6 (43%)	
AA	5 (15%)	3 (21%)	
A allele	22 (32%)	12 (49%)	χ^2 =0.96 p=0.35
G allele	46 (68%)	16 (57%)	

To further analyze the effect of rs2860905 on the efficacy of antihypertensive drug losartan, multinomial regression analysis was carried out. The results of multinomial regression showed that the carriers of AA and GA genotypes are at a higher risk of non-responsiveness to antihypertensive drug therapy as compared to individuals with GG

genotype. Multinomial regression analysis of *CYP2C9* rs2860905 alleles was also parallel to the genotype results and showed that the carriers of A allele incur a higher risk of the reduced efficacy of losartan. However, these results for genotypes and alleles could not achieve statistical significance (Table 3). Thus, it was concluded that *CYP2C9* rs2860905 G>A polymorphism does not significantly affect the drug efficacy of losartan which is a commonly prescribed first line drug to treat hypertension.

Table 3. Multinomial Regression Analysis of *CYP2C9* rs2860905 G>A with Antihypertensive Drug Efficacy

Genotype	Multinomial Regression Analysis Odds ratio (Confidence Interval) *p*-value
AA	1.99 (0.30-13.1) 0.48
GA	1.49 (0.34-6.54) 0.59
GG	Ref
A	1.57 (0.63-3.87) 0.33
G	Ref

4. DISCUSSION

Pakistan is one of the most populated countries in the world with a population of over 220 million (Bureau of Statistics, Government of Pakistan, at www.pbs.gov.pk). According to the 2020 report of World Health Organization (WHO), 37.3% of the Pakistani population is hypertensive and this number is increasing day by day. Hypertension is a major cause of cardiovascular diseases and also the determiner of several other metabolic diseases including diabetes, dyslipidemia, stroke, and many more. To reduce the burden of these cardiometabolic diseases, there is a dire need to control hypertension and keep the blood pressure within the normal range (<140/90 mmHg).

Several studies have reported that polymorphisms in the genes of blood pressure regulating pathways and drug metabolizing enzymes affect the efficacy of antihypertensive drugs. These genetic variations could reduce the activity of drug metabolizing enzymes which convert the prodrug to active drug. Thus, it makes the respective drug used to keep the blood pressure under the threshold value less potent.

CYP2C9 rs2860905 is one such polymorphism which affects the drug efficacy of losartan. The current study aimed to check the allelic and

genotypic frequencies of this polymorphism in Pakistani subjects along with exploring its role in antihypertensive (losartan) drug response. Although several studies have been conducted on various ethnicities of Pakistan regarding the polymorphisms of CYP2C9 gene; however, no study has yet addressed rs2860905 polymorphism for its role in losartan metabolism and blood pressure control. Thus, to the best of our knowledge, the current study is the first study in Pakistan that was conducted to determine the effect of *CYP2C9* rs2860905 G>A in controlling high blood pressure.

CYP2C9 gene polymorphisms have been extensively studied for their role in warfarin (an anticoagulant medicine) efficacy [21–23]. However, their role in the metabolism of losartan (an antihypertensive drug) could not be ignored [24]. CYP2C9*2 (rs799853) and CYP2C9*3 (rs1057910) have been studied widely for their role in determining the efficacy of losartan among various populations [25, 26], while rs2860905 has been commonly studied to determine the warfarin dose. Studies conducted on Caribbean Hispanic population and Han Chinese patients of valvular disease showed that rs2860905 variant is associated with warfarin sensitivity which could help in a more stable dosing of warfarin [27, 28]. A recent study from Taiwan also concluded that *CYP2C9* rs2860905 GG genotype increases the risk of ischemic stroke in the adult population suffering from atrial fibrillation [29]. The current study showed that GG genotype of rs2860905 could be associated with the improved efficacy of losartan in hypertensive subjects. However, these results were not statistically significant. Thus, the study adds a very important and interesting data in the existing literature by showing that rs2860905 polymorphism of CYP2C9 gene does not affect the efficacy of losartan, which is significantly affected by other genetic variants of CYP2C9 gene.

4.1. Conclusion

The current study determined that *CYP2C9* rs2860905 GG genotype or G allele could be associated with the increased efficacy of losartan, although these results were not statistically significant. Thus, on the basis of these results, it was concluded that *CYP2C9* rs2860905 G>A polymorphism does not affect the efficacy of losartan. However, a larger sample size is needed to confirm this association.

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