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Research Article

The Impact of CYP 2C9 rs1799853 and rs1057910 Polymorphism on Plasma Losartan Metabolic Ratio in a Sample of Iraqi Hypertensive Patients

Ali Hassan Ijam[1]*, Bahir Abdul-Razzaq Mshimesh[1], Ahmed Sahib Abdulamir[2], Shokry Faaz Alsaad[3]

[1]*Department of Pharmacology and Toxicology/ College of Pharmacy, Mustansiriyah University, Baghdad, Iraq;* [2]*Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq;* [3]*Department of Cardiology, Babylon Medical College, Babylon University, Babylon, Iraq*

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Abstract

Background: The challenge associated with interindividual diversity in CYP2C9 enzyme activity is primarily related to genetic variations among individuals. Polymorphisms in the *CYP2C9* gene can lead to different enzyme activity, affecting how individuals metabolize drugs. The understanding of interindividual diversity in CYP2C9 enzyme activity has implications for personalized medicine. ***Objective***: To examine the impact of *CYP2C9* gene polymorphisms (rs1799853 and rs1057910) on the losartan metabolism in Iraqi hypertensive patients. ***Methods***: This prospective interventional study was conducted on a sample of hypertension patients from Babylon governorate, Iraq. All patients received 100 mg of losartan once daily. After 4 weeks, blood samples were obtained for genetic analysis and measuring losartan and its carboxylic acid (LCA) metabolite levels. The plasma losartan-to-LCA ratio is used as an indirect determinant of CYP2C9 activity within *CYP2C9* SNP genotypes. ***Results***: Two major allelic polymorphisms, *CYP2C9* rs1799853 (T allele 15.5%) on exon 3 and rs1057910 (C allele 8.5%) on exon 7, have been identified among the patients. Plasma losartan/E3174 metabolic ratio was significantly higher in patients with a CT genotype of rs1799853 SNP (1.65) than in patients with a CC wild-type genotype (1.03). The losartan/E3174 metabolic ratio in heterozygous mutant AC genotypes of rs1057910 (1.18) was also higher than in those with wild-type AA genotypes (1.15); however, these differences are not statistically significant. ***Conclusions***: The rs1799853 SNP variant, but not the rs1057910 SNP variant, significantly impacts CYP2C9 metabolic activity. The plasma losartan/E3174 metabolic ratio appears to be a practical and reliable measure for CYP2C9 activity.

Keywords: *CYP2C9* polymorphism, Hypertension, Losartan, Metabolic ratio.

تأثير تعدد الأشكال rs1799853 CYP 2C9 و rs1057910 على نسبة استقلاب لوسارتان في البلازما في عينة من مرضى ارتفاع ضغط الدم العراقيين

الخلاصة

الخلفية: يرتبط التحدي المرتبط بالتنوع بين الأفراد في نشاط إنزيم CYP2C9 في المقام الأول بالاختلافات الجينية بين الأفراد. يمكن أن يؤدي تعدد الأشكال في جين CYP2C9إلى نشاط إنزيم مختلف، مما يؤثر على كيفية استقلاب الأفراد للأدوية. إن فهم التنوع بين الأفراد في نشاط إنزيم CYP2C9 له آثار على الطب الشخصي. **الهدف**: دراسة تأثير تعدد الأشكال الجيني rs1799853 CYP2C9 و rs1057910 على استقلاب لوسارتان لدى مرضى ارتفاع ضغط الدم العراقيين. **الطريقة**: أجريت هذه الدراسة التدخلية المستقبلية على عينة من مرضى ارتفاع ضغط الدم من محافظة بابل، العراق. تلقى جميع المرضى 100 ملغ من اللوسارتان مرة واحدة يوميا. بعد 4 أسابيع، تم الحصول على عينات الدم للتحليل الجيني وقياس اللوسارتان ومستويات مستقلب حمض الكربوكسيل. تستخدم نسبة اللوسارتان إلى LCA في البلازما كمحدد غير مباشر لنشاط CYP2C9 ضمن الأنماط الجينيةCYP2C9 SNP . **النتائج**: تم تحديد اثنين من تعدد الأشكال الأليلية الرئيسية ، rs1799853 CYP2C9أليل T 15.5% على exon 3 و rs1057910 أليل8.5 C % على exon 7 ، بين المرضى. كانت نسبة استقلاب لوسارتان E3174/ في البلازما أعلى بشكل ملحوظ في المرضى الذين يعانون من النمط الجيني CT من rs1799853 SNP (1.65) مقارنة بالمرضى الذين يعانون من النمط الجيني من النوع البري (1.03) CC وكانت نسبة استقلاب لوسارتان E3174/ في الأنماط الجينية الطافرة غير المتجانسة للتيار المتردد من rs1057910 (1.18) أعلى أيضا من تلك الموجودة في أولئك الذين لديهم أنماط وراثية من النوع البري (1.15) AA ؛ ومع ذلك، فإن هذه الاختلافات ليست ذات دلالة إحصائية. **الاستنتاجات**: يؤثر متغير rs1799853 SNP ، ولكن ليس متغير rs1057910 SNP، بشكل كبير على نشاط استقلاب CYP2C9 يبدو أن نسبة استقلاب لوسارتان E3174/ في البلازما هي مقياس عملي وموثوق لنشاط CYP2C9 .

* ***Corresponding author***: Ali H. Ijam, Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq; Email: ahzajam84@uomustansiriyah.edu.iq

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INTRODUCTION

Losartan potassium is the first selective angiotensin receptor blocker approved for clinical use in treating hypertension. It binds selectively and competitively with high affinity to the angiotensin-1 receptor, which stops the physiological effects that angiotensin II causes [1]. Studies conducted in vitro and in vivo have demonstrated that the cytochrome P450 2C9 enzyme primarily carries out the metabolism of losartan. Losartan metabolism produces a biologically active metabolite known as E-3174. [2]. The CYP2C9 enzyme is encoded by *the CYP2C9* gene. The highly polymorphic nature of *the CYP2C9* gene is of significant importance in contributing to the

variability in enzyme activity for many substrates, hence leading to variations in the capacity to metabolize medications among genetically susceptible individuals [3]. Levels and activity of each CYP enzyme differ from one individual to another due to genetic and/or environmental factors [4]. Genetic mutations can cause reduced CYP enzyme activity by either preventing the synthesis of a CYP enzyme or resulting in the synthesis of an inactive, unstable, or catalytically compromised form, leading to the phenotypes of poor or intermediate metabolizers. This mostly occurs due to exposures to an environmental factor (like an inflammatory process or infectious disease) that inhibits CYP enzyme expression or to chemicals that inhibit or inactivate a preexisting CYP enzyme [5,6]. Gene duplication that overexpresses a CYP enzyme, exposure to medications that stimulate cytochrome P450 production, or stimulation of an already-existing enzyme by a xenobiotic can all lead to increased CYP enzyme activity [7]. Single nucleotide polymorphisms in the cytochrome gene locus cause the majority of differences in CYP activity [8], and the observed impact of these SNPs appears to involve modifications in the enzyme's affinity for its substrate and/or alterations in V-max, leading to varying degrees of impairment in oxidative metabolism, which may depend on the specific substrate involved [9]. We have identified approximately 85 CYP2C9 alleles in the regulatory and coding regions of CYP2C9 to date [10]. The most common types of these alleles are CYP2C9*2 (R144C rs1799853) and CYP2C9*3 (I359L rs1057910), which are much less active than the wild-type allele (CYP2C9*1) [11]. According to our knowledge, a limited number of clinical studies have been conducted in Iraq to investigate the influence of SNPs rs1799853 and rs1057910 within the *CYP2C9* gene on the CYP2C9 metabolic activity in hypertensive patients. Therefore, by measuring the losartan/E3174 metabolic ratio, an indirect method for determining CYP2C9 enzymatic activity, the current research study aimed to determine whether the presence of these SNPs in CYP2C9 encoding genes could influence the CYP29 enzymatic activity to metabolize losartan.

METHODS

Study design

The present study is a prospective interventional clinical trial designed with an eligible, convenient sample to examine the potential impact of *CYP2C9* gene polymorphisms (namely, rs1799853 and rs1057910) on the CYP29 enzymatic activity to metabolize losartan.

Patients selection

The participants (male and female) were recruited from the private clinic of an internist according to the inclusion and exclusion criteria of the study listed below and diagnosed as hypertensive patients at stage I or II according to the ESH (2018) guideline [12]. These patients were selected during the period from the first of June 2022 to the end of February 2023. A total of 272 hypertensive patients were excluded; 172 of them were excluded due to incompliance, loss of contact, refusal to participate, severe hypotension, use of other antihypertensive drugs, and missing the date of sampling. Only 100 patients completed the study.

Inclusion criteria

Patients, both males and females, diagnosed with essential hypertension had mean systolic blood pressure (SBP) between 140 and 180 mmHg, and mean diastolic blood pressure (DBP) between 90 and 110 mmHg, with an age range of approximately 25 to 80 years.

Exclusion criteria

We excluded any condition that could potentially interfere with the study protocol or aim, such as patients with known or suspected secondary hypertension, those with BP measurements less than 180/110 mmHg, and patients with other cardiovascular diseases like myocardial infarction, atrial fibrillation, heart failure, and stroke. Additionally, we excluded patients with metabolic or endocrine disorders (such as diabetes and thyroid disease), autoimmune diseases, malignant tumors, psychiatric disorders, renal or liver dysfunction, pregnant and breastfeeding women, alcoholic patients, women on contraceptive, and those requiring other antihypertensive drugs instead of losartan.

Study protocol

During the process of patient screening for essential hypertension, the selected individuals were interviewed using a specialized data sheet that was particularly developed for the purpose of this study. The blood pressure, age, gender, height, weight, and body mass index (BMI) were assessed for each individual and documented with other information, including their name, smoking status, dietary habits, level of physical activity, and family history of hypertension. All patients enrolled received 100 mg of losartan once daily. After 4 weeks of losartan therapy, a volume of 5 ml of venous blood was obtained from each patient. Out of this, 1.0 ml of venous blood was transferred into an EDTA tube to be utilized for genetic analysis. The remaining 4 ml of venous blood was placed in another EDTA tube and processed for centrifugation within 15 minutes for plasma separation. The separated plasma was then used to measure the levels of losartan and losartan carboxylic acid metabolites.

DNA extraction and primer optimization

The DNA extraction was carried out from a blood sample using the ReliaPrep® Blood gDNA Miniprep System, a procedure developed by Promega [13]. The Quantum Fluorimeter was employed to assess the concentration of extracted DNA, enabling the evaluation of both the quantity and quality of the samples for subsequent applications. The primers utilized in this study were specifically developed for

the purpose of polymerase chain reaction (PCR) amplification and subsequent DNA sequencing. These primers were made to target two distinct regions within the *CYP2C9* gene: region 1 (intron 1, exon 2, exon 2, exon 3 and some of intron 3) and region 2 (intron 6 , exon 7 and some of intron 7 and their sequences (Table 1).

Table 1: Primer sequences

Primer	Sequence 5`-3`	Annealing Temp (°C)	Product size (bp)
CYP2C9-F1	GCCTGTGTGGCTGAATAAA	63	990
CYP2C9-R1	CTGGTGACATGTTCTGGAATAG		
CYP2C9-F2	TTCAGCCTATGTGTGTCTTTAT		942
CYP2C9-R2	CTAAGAGTAGCCAAACCAATCT		

F1: forward primer for region one, F2: forward primer for region two, R1: reverse primer for region one, R2: reverse primer for region 2.

Prior to conducting PCR, primer optimization was performed to determine the optimal annealing temperature at which the primer would effectively bind to the DNA template.

PCR and DNA sequencing technique

The DNA samples were subjected to amplification using the conventional PCR technique. Each reaction consisted of 12.5 µL of master mix (Promega, USA), 1 µL of each primer (10 Pmol), 3 µL of DNA template, and the remaining volume was made up to 25 µL using nuclease-free water. The PCR settings utilized for the amplification of the *CYP2C9* gene are listed in Table 2.

Table 2: Program Protocol for polymerase chain reaction

Steps	Temp (°C)	Time (min)	cycle
Initial/denaturation	95	5.0	1
Denaturation	95	0.5	30
Annealing	63	0.5	
Extension	72	0.5	
Final extension	72	7.0	
Holding	4	10.0	1

The PCR products were sent for Sanger sequencing by using an automated DNA sequencer (ABI3730XL, Macrogen Corporation, Korea). Genius software was used for the analysis of the data.

Determination of losartan and its metabolite (E-3174)

Losartan and its carboxylic acid metabolite concentrations in human plasma have been measured using the HPLC method in conjunction with fluorescence detection. Among the components that made up the high-performance liquid chromatography system were a SHIMADZU LC-20AD prominence pump, a SHIMADZU DGU-20A5 prominence degasser, a KNAUER D-14163, Germany 20-microliter loop injector system, a fluorescence detector model RF-10AXL (SHIMADZU, JAPAN) and the column oven CTO-10AS VP/SHIMADZU. The apparatus was also controlled and the data was analyzed by LC LabSolutions SHIMADZU software version 1.25 SP4. The procedure of sample preparation was done by the liquid-liquid extraction method, as described by Yang *et al.* (2012) [14]. The frozen plasma samples were thawed and thereafter allowed to equilibrate to ambient temperature. A 0.4-mL plasma sample was placed into a 10-mL polyethylene plain tube. The tube contained 100 microliters of methanol, 100 microliters of 6% formic acid, and 50 microliters of an internal standard (IS) working solution. The IS working solution consisted of 1 microgram/milliliter of valsartan in methanol. The mixture was thereafter subjected to vortex mixing for a duration of 30 seconds in order to achieve sufficient homogenization. Subsequently, a volume of 5 mL of diethyl ether was introduced into the mixture, followed by vortex mixing for a duration of 5 min. The resulting mixture was then centrifuged at a force of 2100 g (4330 RPM) for a period of 10 min. The organic phase is then transferred to a clean tube and subjected to evaporation in a vacuum oven at ambient temperature. The reconstitution of the residue was carried out by adding 100 microliters of mobile phase. Subsequently, the mixture was subjected to sonication using an ultrasonic bath for a duration of 5 minutes. Following this, centrifugation was performed at a force of 11200 g (10000 RPM) for 5 min. The resulting supernatant was then introduced into the chromatographic system by injecting a volume of 20 microliters. The prepared samples were injected into an HPLC system with chromatographic conditions as described by Daneshtalab *et al.* (2002) [15]. Column: KromaPhase C18 (5 µm, 250 * 4.6 mm) (SCHARLAU, SPAIN), column temperature set at 30 °C. Detection: A fluorescence detector has been setup with a wavelength of excitation of 270 nm and an emission wavelength of 370 nm. Flow rate: 1 ml/min. Injection volume: 20 microliters. Mobile phase: The isocratic mobile phase used in this study comprised a combination of phosphate buffer with a concentration of 0.01M and a pH value of 2.1, along with acetonitrile in a volumetric ratio of 65:35. This mobile phase was made on a daily basis and subjected to filtration using a 0.45-millimeter filter. The phosphate buffer solution used as the mobile phase was created by dissolving 119.9 mg of sodium dihydrogen phosphate in 100 ml of double-distilled water (DW). The pH of the buffer was then adjusted to 2.1 using 85% orthophosphoric acid.

Standard calibration curves preparation

For the preparation of the calibration curve for the detection of losartan and its metabolites, four different standard mixes of 2000/1000, 1000/500, 200/100 and 20/10 ng/ml of losartan/losartan carboxylic acid, respectively, were prepared, and each standard mix contained 500 ng/ml of the drug valsartan as an internal standard. The calibration curves were developed by plotting the ratios of the peak areas (relative peak area) (LOS/IS; E-3174/IS) versus the concentration of the standard mix of each one and calculating the linear regression equation (y=a+bx) (Figure 1, A and B).

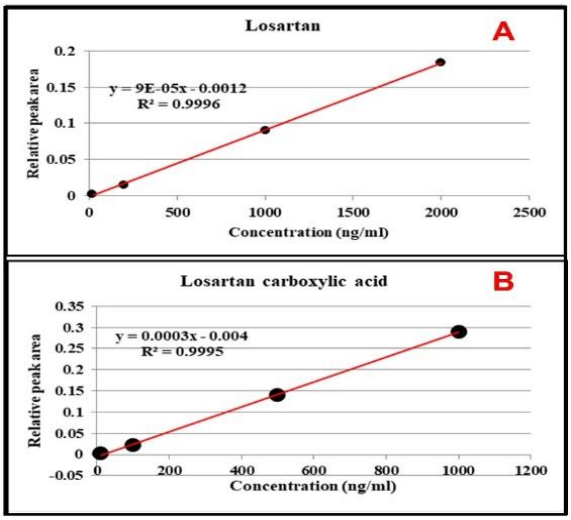

Figure 1: The calibration curves of losartan (A); The calibration curves of losartan carboxylic acid (B).

Calculation of CYP2C9 enzyme activity

The ratio of drug to metabolite, where the parent chemical is the numerator and the metabolite is the denominator, was employed as a means to assess CYP2C9 activity in plasma. The evaluation of CYP2C9 activity was conducted by determining the plasma losartan-to-losartan carboxylic acid (LCA) ratio. A larger ratio is indicative of diminished CYP2C9 activity, while a lower ratio indicates increased CYP2C9 activity [16].

Ethical consideration

This research began after getting approval from the scientific committee at the University of Mustansiriyah, College of Pharmacy. Also, the agreement of the Babylon Health Directorate was achieved according to the Ethical Committee of the Iraqi Ministry of Health (73 on June 14, 2022). All participants in this study sign a written consent.

Statistical analysis

IBM SPSS 26.0 saved and processed the data. Numerical data were expressed as mean±SD, whereas categorical variables were expressed as percentages and frequencies. A one-way ANOVA test was used as a statistical test for the changes in the numerical variables (losartan, losartan carboxylic acid metabolite and losartan metabolic ratio) across different genotypes. The Chi square ($\chi 2$) test was used for the categorical variable and to test the deviation from the Hardy-Weinberg equilibrium (HWE). *p*-values < 0.05 were considered significant.

RESULTS

For the study, a total of 272 hypertensive patients were screened. Among them, 100 patients completed the study. The distribution of gender was 48 males (48%) and 52 females (52%). The age range was between 18 and 80 years, with a mean of 50.58±11.28 years. More than half of the patients were obese, with a mean body mass index (BMI) of 32.21±6.42 kg/m^2. Smoking was a habit among 14% of the patients, and 86 % were non-smokers. The mean systolic and diastolic blood pressure were 154±10.31 mmHg and 93.85±5.02 mmHg, respectively. The patient's demographic and clinical characteristics are shown in Table 3.

Table 3: Demographic and clinical characteristics of patients (n=100)

Characteristics	Value
Age (years)	50.58±11.28
Gender	
Male	48(48)
Female	52(52)
Height (m)	1.68±0.09
Weight(kg)	90.69±17.46
BMI (Kg/m^2)	32.21±6.42
Smoking habits	
Smoker	14(14)
Not smoker	86(86)
SBP (mmHg)	154±10.31
DBP (mmHg)	93.85±5.02

Values were expressed as frequency(%) and mean±SD. SBP: systolic blood pressure; DBP: diastolic blood pressure.

Losartan, its carboxylic acid metabolite, and the internal standard (valsartan) were well separated by the HPLC method with fluorescence detection. The average retention times of losartan, E-3174 and valsartan were 6, 10, and 12.5 min, respectively (Figure 2).

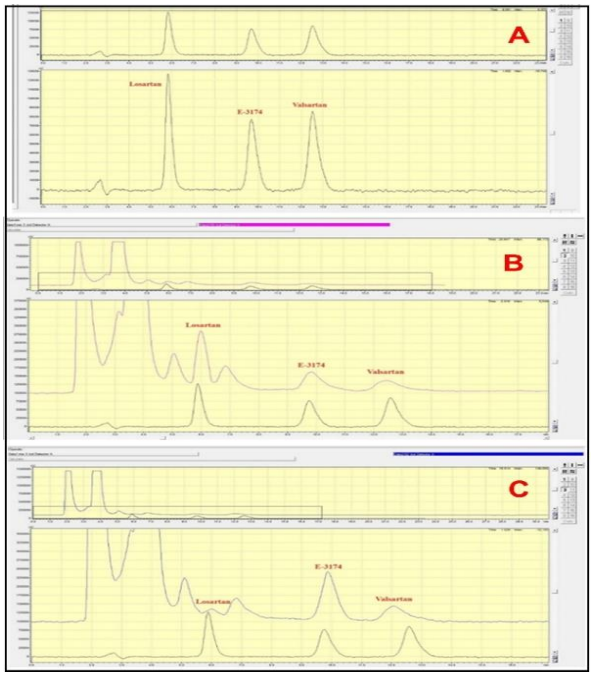

Figure 2: Chromatograms of losartan, E-3174(losartan metabolite) and internal standard valsartan. (A) standard mix solution shows retention time of each analyte. (B) plasma sample in patient with low metabolic state. (C) plasma sample in patient with high metabolic state.

The average plasma losartan and losartan carboxylic acid metabolite (E-3174) among the studied hypertensive patients were 178.68±104.79 and 219.58±153.19, respectively. The overall metabolic ratio of losartan among the studied hypertensive patients was 1.15±1.09 (Table 4).

Table 4: Plasma concentration of losartan, E-3174 and metabolic ratio among 100 patients with essential hypertension

Variables	Value
Losartan conc. (ng/ml)	178.68±104.79
E-3174 conc. (ng/ml)	219.58±153.19
Losartan/E-3174 ratio	1.15±1.09

Values were expressed as mean±SD.

Along with the analysis of two specific regions of the *CYP2C9* gene, the two major allelic polymorphisms *CYP2C9* rs1799853 on exon 3 and rs1057910 on exon 7 have been identified among the 100 hypertensive patients (Table 5).

Table 5: Genotype and allele frequency of the identified single nucleotide polymorphisms

SNPs		
Genotype frequency	Allele frequency	HWE *p*-value
rs1799853		
CC 73(73)	C 169(84.5)	
CT 23(23)	T 31(15.5)	0.67
TT 4(4)		
rs1057910		
AA 86(86)	A 183(91.5)	0.06
AC 11(11)	C 17(8.5)	
CC 3(3)		

Values were expressed as frequency (%). HWE: Hardy-Weinberg Equilibrium; SNPs: single nucleotide polymorphism; C: cytosine; T: thymine; A: adenine.

There were no statistically significant differences (p>0.05) in demographic and clinical characteristics between the rs1799853 and rs1057910 SNP genotypes of the hypertensive patients enrolled in this study (Tables 6 and 7). Regarding the rs1799853 SNP, the losartan concentrations in patients with the rs1799853 CC wild-type genotype (179.06±102.87ng/ml) did not significantly (p>0.05) differ from the rs1799853 CT and TT mutant genotypes (181.93±112.40 and 153.02±121.70 ng/ml, respectively). Also, the E3174 concentrations in patients with the rs1799853 CC wild-type genotype (229.27±162.63 ng/ml) did not significantly (p>0.05) differ from the rs1799853 CT and TT mutant genotypes (165.63±92.99 and 353.02±167.18 ng/ml, respectively).

Table 6: Association of *CYP2C9* rs1799853 single nucleotide polymorphism genotype with demographic and clinical characteristics

Characteristic	rs1799853 SNP (n=100)			*p*-value
	CC (n=73)	CT (n=23)	TT (n=4)	
Age (years)	49.78±11.28	53.39±11.21	49.00±11.91	0.396
BMI (kg/m^2)	31.53±6.20	33.78±7.28	35.52±1.44	0.197
Gender				
Male	37(50.7)	10(43.5%)	1(25)	0.536
Female	36(49.3)	13(56.5)	3(75)	
SBP (mmHg)	154.38±10.45	152.93±9.99	153.12±11.79	0.832
DBP (mmHg)	94.28±5.27	92.93±4.30	91.25±2.20	0.308

Values were expressed as frequency (%) and mean±SD. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; n: number of patients; CC: wild type genotype; CT: heterozygous mutant genotype; TT: homozygous mutant genotype.

Table 7: Association of *CYP2C9* rs1057910 single nucleotide polymorphism genotype with demographic and clinical characteristics

Patient's characteristic	rs1057910 SNP (n=100)			*p*-value
	AA (n=86)	AC (n=11)	CC (n=3)	
Age (year)	50.03±11.18	53.45±9.33	55.67±21.22	0.471
BMI (kg/m^2)	31.86±6.06	35.86±8.36	28.65±5.40	0.093
Gender				
Male	42(50.7)	3(43.5)	3(25)	0.075
Female	44(49.3)	8(56.5)	0(75)	
SBP (mmHg)	154.30±10.55	151.81±9.88	153.33±2.88	0.752
DBP (mmHg)	93.54±5.02	94.53±4.71	100.00±0.00	0.080

Values were expressed as frequency (%) and mean±SD. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; n: number of patients; AA: wild type genotype; AC: heterozygous mutant genotype; CC: homozygous mutant genotype.

Regarding the rs1057910 SNP, the losartan concentrations in patients with the rs1057910 AA wild-type genotype (174.97±105.41 ng/ml) do not significantly (p>0.05) differ from the rs1057910 AC and CC mutant genotypes (189.01±99.81 and 246.88±115.90 ng/ml, respectively). Also, the E3174 concentrations in patients with the rs1057910 AA wild-type genotype (220.22±154.67 ng/ml) did not significantly (p>0.05) differ from the rs1057910 AC and CC mutant genotypes (183.96±96.71 and 331.76±268.80 ng/ml, respectively). The plasma losartan/E3174 metabolic ratio used as an assessment of the CYP2C9 enzyme activity was significantly higher (p<0.05) in a group of patients with a CT genotype of rs1799853 SNP (1.65±1.82) than in patients with a CC wild-type genotype (1.03±0.69). Moreover, the losartan/E3174 metabolic ratio in heterozygous mutant AC genotypes of rs1057910 (1.18±0.66) was also higher than in those with wild-type AA genotypes (1.15±1.14); however, these differences are not statistically significant (Table 8).

DISCUSSION

CYP2C9 is the focus of pharmacogenetic research for genotype-based drug therapy, as it is one of the most prevalent hepatic CYP enzymes and catalyzes the metabolism of many clinically significant drugs [17]. Inter-individual variability in CYP2C9 enzyme activity is observed, and it can have significant implications for drug response and toxicity. Understanding the interindividual variability in CYP2C9 activity is crucial for personalized medicine and optimizing drug therapy [6]. Previous research indicates that the process of losartan transforming into E3174 is strongly influenced by the activity of CYP2C9. Additionally, the genetic variations of the *CYP2C9* rs1799853 and rs1057910 SNPs may be significantly associated with the pharmacokinetics of losartan and its active metabolite. However, the correlation between variations in the *CYP2C9* gene and the pharmacokinetic characteristics of losartan has yielded inconclusive results, possibly due to differences in *CYP2C9* allele frequency between

ethnic groups as well as differences in sample sizes in these studies [18].

Table 8: plasma losartan, losartan carboxylic acid concentration and losartan metabolic ratio among different genotypes of identified SNPs of *CYP2C9* gene

Genotypes	Plasma losartan (ng/ml)	Plasma E-3174 (ng/ml)	Metabolic ratio (MR)
rs1799853 C>T			
CC	179.06±102.87	229.27±162.63	1.03±0.69
CT	181.93±112.40	165.63±92.99	1.65±1.82
TT	153.02±121.70	353.02±167.18	0.52±0.34
p-value	0.879	0.044	0.025
rs1057910 A>C			
AA	174.97±105.41	220.22±154.67	1.15±1.14
AC	189.01±99.81	183.96±96.71	1.18±0.66
CC	246.88±115.90	331.76±268.80	1.06±0.68
p-value	0.481	0.335	0.988

Values were expressed as mean±SD. CC, AA: wild type genotypes; CT, AC: heterozygous mutant genotypes; TT, CC: homozygous mutant genotypes.

The present study aimed to determine the plasma losartan/E3174 metabolic ratio among genotypes of different *CYP2C9* SNPs detected in hypertensive patients. This is the first study, to our knowledge, to report the relationship between *CYP2C9* genotypes and the losartan/E3174 metabolic ratio in an Iraqi population. At baseline, this study found no significant differences between genotypes of the rs1799853 and rs1057910 SNP variants regarding clinical and demographic features (age, gender, BMI, and baseline SBP and DBP). This lack of significant differences may be attributed to the fact that all individuals enrolled in this study met the predetermined criteria for participation. The value of this finding in the current study is important as it aligns with the study's objective of investigating the impact of *CYP2C9* genetic polymorphism on the plasma losartan/E3174 metabolic ratio. Any observed variations in the CYP2C9 enzyme activity, based on SNP genotypes, can be attributed to genetic variations rather than differences in demographic characteristics among the groups. This study found a significant association between the rs1799853 SNP and plasma losartan/E3174 metabolic ratio, where the metabolic activity in heterozygous CT genotype carriers decreased by 60% when compared with the CC wild genotype carriers. The effect of this variant on phenotype can be either by the SNP itself or through linkage to other unidentified SNPs that may indirectly affect the expression of *the CYP2C9* gene [19,20]. Individuals with the homozygous mutant TT genotype exhibit a 49.5% increase in CYP2C9 metabolic activity compared to individuals with the wild-type CC genotype. The observed group differences between the TT and CC genotypes of rs1799853 SNPs may be attributed to the limited sample size of the TT mutant genotype group. Furthermore, the presence of *CYP2C9**2 (rs1799853) is strongly linked to *CYP2C8**3 or variations in the *CYP2C9* promoter. This suggests that there may be a true increase in enzyme activity due to the linkage with high-activity variations in *CYP2C8* or other genes located within the *CYP2C9* gene locus, which includes *CYP2C8, CYP2C18,* and *CYP2C19* [21,22]. Notably, the consequences of the rs1799853 SNP do not always align with the effects of the rs1057910 SNP [23]. The current research findings on rs1057910 genotypes suggest that there was no statistically significant variation in the metabolic ratio of losartan across individuals with different variant genotypes. The *CYP2C9* metabolic activity in heterozygous AC genotype carriers was decreased by 2.5% when compared with the AA wild-type genotype carriers. This difference in metabolic activity between two SNPs may be due to a lesser frequency of mutant AC genotype group (n=11) of rs1057910 SNP carriers compared to mutant CT genotype group (n=23) of rs1799853 SNP carriers. Another possible explanation for these results is that the degree of activity reduction and changes in kinetic parameters caused by *CYP2C9*3* are substrate-dependent. The variation in flexibility of a substrate within the binding pocket of the CYP2C9 enzyme is responsible for the differences in activity reduction and changes in kinetic parameters observed between substrates [24]. Previous studies collectively demonstrated that the effects of the rs1799853 SNP variant were not as evident as those of the rs1057910 SNP. There was a slight, statistically insignificant, increase in plasma losartan/E-3174 ratios in the rs1799853 heterozygous mutant genotype group compared with the wild-type genotype group but a statistically significant increase in these ratios in the rs1057910 heterozygous mutant genotype group compared with the wild-type genotype group [25–27]. This inconsistency with the findings of the present investigation could potentially be attributed to the administration of a single dosage of losartan in those particular studies. The effects that may not be evident with a single dose of a drug can become apparent when the drug is administered over an extended period of time.

Study limitations

There were some limitations in this study. Initially, the sample size is limited, leading to limited statistical power to identify any association. Second, this study selects two common gene polymorphisms. Additional research is required to determine if there are other gene variants that contribute to the variation in CYP2C9 enzymatic activity among individuals.

Conclusion

The rs1799853 SNP variant, but not the rs1057910 SNP variant, has a significant impact on CYP2C9 metabolic activity. The plasma losartan/E3174 metabolic ratio appears to be a practical and reliable measure for CYP2C9 activity. Nevertheless, it is necessary to encourage more extensive studies with a greater number of participants aiming to confirm or refute the findings of the current study.

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Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Song JC, White CM. Pharmacologic, pharmacokinetic, and therapeutic differences among angiotensin II receptor antagonists. Pharmacotherapy: *J Hum Pharmacol Drug Ther*. 2000;20(2):130-139. doi: 10.1592/phco.20.3.130.34788.
2. Yasar U, Babaoglu MO, Bozkurt A. Disposition of a CYP2C9 phenotyping agent, losartan, is not influenced by the common 3435C> T variation of the drug transporter gene ABCB1 (MDR1). *Basic Clin Pharmacol Toxicol*. 2008;103(2):176-179. doi: 10.1111/j.1742-7843.2008.00283.x.
3. Daly AK, Rettie AE, Fowler DM, Miners JO. Pharmacogenomics of *CYP2C9*: functional and clinical considerations. *J Personalized Med*. 2017;8(1):1. doi: 10.3390/jpm8010001.
4. Zhao M, Ma J, Li M, Zhang Y, Jiang B, Zhao X, et al. Cytochrome P450 enzymes and drug metabolism in humans. *Int J Mol Sci*. 2021;22(23):12808. doi: 10.3390/ijms222312808.
5. Al Omari A, Murry DJ. Pharmacogenetics of the cytochrome P450 enzyme system: review of current knowledge and clinical significance. *J Pharm Pract*. 2007;20(3):206-218. doi: 10.1177/08971900073048.
6. Tracy TS, Chaudhry AS, Prasad B, Thummel KE, Schuetz EG, Zhong XB, et al. Interindividual variability in cytochrome P450–mediated drug metabolism. *Drug Metab Dispos*. 2016;44(3):343-351. doi: 10.1124/dmd.115.067900.
7. Sezutsu H, Le Goff G, Feyereisen R. Origins of P450 diversity. *Philosoph Transact Royal Soc B Biol Sci*. 2013;368(1612):20120428. doi: 10.1098/rstb.2012.0428.
8. Lam YW, Scott SR, (Eds.), Pharmacogenomics: challenges and opportunities in therapeutic implementation, (2nd Ed.), Elsevier; 2019.
9. Zhou SF, Zhou ZW, Huang M. Polymorphisms of human cytochrome P450 2C9 and the functional relevance. *Toxicology*. 2010;278(2):165-188. doi: 10.1016/j.tox.2009.08.013.
10. PharmVar Standards. Available at: https://www.pharmvar.org/genes
11. Wei L, Locuson CW, Tracy TS. Polymorphic variants of CYP2C9: mechanisms involved in reduced catalytic activity. *Mol Pharmacol*. 2007;72(5):1280-1288. doi: 10.1124/mol.107.036178.
12. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, *et al*. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). *Eur Heart J*. 2018;39(33):3021-3104. doi: 10.1093/eurheartj/ehy339.
13. Miniprep-system-low-elution-volume-with-high-yield/Updated. Available at: http://www.promega.com/resources/pubhub/reliaprepblood-gdna
14. Yang L, Guo T, Xia DY, Zhao LS. Pharmacokinetics of losartan and its active carboxylic acid metabolite E-3174 in five ethnic populations of China. *J Clin Pharm Ther*. 2012;37(2):226-231. doi: 10.1111/j.1365-2710.2011.01279.x.
15. Daneshtalab N, Lewanczuk RZ, Jamali F. High-performance liquid chromatographic analysis of angiotensin II receptor antagonist valsartan using a liquid extraction method. *J Chromatogr B*. 2002;766(2):345-349. doi: 10.1016/s0378-4347(01)00507-2.
16. Kvitne KE, Krogstad V, Wegler C, Johnson LK, Kringen MK, Hovd MH, et al. Short-and long-term effects of body weight, calorie restriction and gastric bypass on CYP1A2, CYP2C19 and CYP2C9 activity. *Br J Clin Pharmacol*. 2022;88(9):4121-4133. doi: 10.1111/bcp.15349.
17. Fekete F, Mangó K, Déri M, Incze E, Minus A, Monostory K. Impact of genetic and non-genetic factors on hepatic *CYP2C9* expression and activity in Hungarian subjects. *Sci Rep*. 2021;11(1):17081. doi: 10.1038/s41598-021-96590-3.
18. Park YA, Song YB, Yee J, Yoon HY, Gwak HS. Influence of CYP2C9 genetic polymorphisms on the pharmacokinetics of losartan and its active metabolite E-3174: a systematic review and meta-analysis. *J Personalized Med*. 2021;11(7):617. doi: 10.3390/jpm11070617.
19. Hatta FH, Teh LK, Helldén A, Hellgren KE, Roh HK, Salleh MZ, et al . Search for the molecular basis of ultra-rapid CYP2C9-catalysed metabolism: relationship between SNP IVS8-109A> T and the losartan metabolism phenotype in Swedes. *Eur J Clin Pharmacol*. 2012;68:1033-1042. doi: 10.1007/s00228-012-1210-0.
20. Robert F, Pelletier J. Exploring the impact of single-nucleotide polymorphisms on translation. *Front Genetics*. 2018;9:507. doi: 10.3389/fgene.2018.00507.
21. Yasar U, Lundgren S, Eliasson E, Bennet A, Wiman B, De Faire U, et al. Linkage between the *CYP2C8* and *CYP2C9* genetic polymorphisms. *Biochem Biophys Res Commun*. 2002;299(1):25-28. doi: 10.1016/s0006-291x(02)02592-5.
22. Speed WC, Kang SP, Tuck DP, Harris LN, Kidd KK. Global variation in CYP2C8–CYP2C9 functional haplotypes. *Pharmacogenomics J*. 2009;9(4):283-290. doi:10.1038/tpj.2009.10.
23. Kirchheiner J, Brockmöller J. Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin Pharmacol Ther*. 2005;77(1):1-6. doi: 10.1016/j.clpt.2004.08.009.
24. Takanashi K, Tainaka H, Kobayashi K, Yasumori T, Hosakawa M, Chiba K. *CYP2C9* Ile359 and Leu359 variants: enzyme kinetic study with seven substrates. *Pharmacogenetics Genom*. 2000;10(2):95-104. doi: 10.1097/00008571-200003000-00001.
25. Babaoglu MO, Yasar U, Sandberg M, Eliasson E, Dahl ML, Kayaalp SO, et al. *CYP2C9* genetic variants and losartan oxidation in a Turkish population. *Eur J Clin Pharmacol*. 2004;60:337-342. doi: 10.1007/s00228-004-0785-5.
26. Dorado P, Beltrán LJ, Machin E, Penas-LLedo EM, Terán E, LLerena A, et al. Losartan hydroxylation phenotype in an Ecuadorian population: influence of *CYP2C9* genetic polymorphism, habits and gender. *Pharmacogenomics*. 2012;13(15):1711-1717. doi: 10.2217/pgs.12.160.
27. Sandberg M, Johansson I, Christensen M, Rane A, Eliasson E. The impact of *CYP2C9* genetics and oral contraceptives on cytochrome P450 2C9 phenotype. *Drug Metab Dispos*. 2004;32(5):484-489. doi: 10.1124/dmd.32.5.484.