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The impact of angiotensin II type 1 receptor (ATR1) gene polymorphism (A1166C) on cardiovascular and renal complications in Iraqi hypertensive patients treated with candesartan

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ABSTRACT— Angiotensin II is the most vasoactive peptide and a powerful blood vessels constrictor of renin-angiotensin-aldosterone system (RAAS). It affects arterial musculature, increases peripheral resistance, and raises blood pressure (BP). The majority of angiotensin II's effects are mediated by the angiotensin II type 1 receptor (ATR1). To investigate the impact of A1166C single nucleotide polymorphism (SNP) of ATR1 gene on cardiovascular and renal complications in Iraqi hypertensive patients treated with candesartan. Ninety-two patients with essential hypertension taking 8 mg/day candesartan from not less than three months were recruited for the investigation from Imam Hussein Teaching Hospital and from private clinic in Karbala province, Iraq. Blood pressure, heart rate and echocardiography reports were recorded. The analysis of biochemical parameters such as serum creatinine, urea, angiotensinogen, angiotensin II and some electrolytes levels were done. The tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used for genotyping of the A1166C SNP. The distribution of A1166C SNP in the hypertensive patients was 54.35% AA, 40.22% AC and 5.43% CC. There was no association between A1166C and echocardiogram, biochemical or BP parameters ($P>0.05$) except heart rate; patients with AA genotype have higher heart rate (88.32±10.64, $P=0.005$) than patients with AC and CC genotype. The CC genotype of A1166C is the less frequent than other two genotypes of this SNP in Iraqi hypertensive patients. The A1166C SNP has neither an effect on hypertension complication nor on the patients' response to candesartan in Iraqi hypertensive patients.

KEYWORDS: Essential hypertension, Iraqi hypertensive patients, angiotensin II type 1 receptor, A1166C, candesartan.

1. INTRODUCTION

Hypertension is defined as an abnormally high level of systemic arterial blood pressure (BP) that persists over time. Hypertension becomes more common as people get older [1]. In a small percentage of cases, hypertension is caused by a specific cause, but in the vast majority of cases (≈90 %), it is a disease that caused by various factors such as genetic, environmental, behavioral factors or interaction between these factors [2]. Blood pressure (BP), fluid volume, and sodium/potassium balance are all controlled by the renin-angiotensin-aldosterone system (RAAS). Because of this, any change in the RAAS molecules causes atrial hypertension [3]. Angiotensin II (Ang II) is a vasoactive peptide of the RAAS, and it has a significant impact on cardiovascular health. It binds to two receptor subtypes, Ang II type 1 and type 2 (ATR1 and ATR2) receptors, but the most of Ang II's known effects are mediated by ATR1, such as vascular contraction and pressure response [4]. Using genome-wide association, researchers identified over one

hundred SNP associated with BP phenotypes, and plausible novel mechanisms of BP regulation and potential therapeutic targets [5]. The human ATR1 gene is 55 kb long and is found on chromosome 3q21-25. There are five exons and four introns in this gene. The ATR1 SNP (A1166C; rs5186) is located in the 3′ untranslated area of this gene and results in adenine (A) or cytosine (C) base at the 1166 position (A/C transversion) [6].

1.1 Survey Point

Our study aims to investigate the ATR1 gene SNP effect in the development of essential hypertension (EH) complications in a sample of Iraqi patients. Having knowledge of the role of this genetic variation might help to predict Bp response to angiotensin receptor blockers therapy (candesartan) in individual patients.

1.2 Study determination and figuring

This cross-sectional study was performed on ninety-two patients suffering from EH recruited from Imam Hussein Teaching Hospital and from private clinic in Karbala city, Iraq. The scientific and ethical committees of the College of Pharmacy at the University of Kerbala, as well as the Karbala Health Directorate and Administration of the Imam Hussein Teaching Hospital, all approved the study's protocol. In addition, consents were obtained after explaining the nature and the goal of the study to patients. Patients were chosen based on the following criteria: they were taking candesartan 8 mg daily for not less than three months and their ages were more than 25 years. Patients with secondary hypertension, pregnant women, metabolic or endocrine disorders, or any other severe illness were excluded from the study. All the patients were not on any other antihypertensive medications.

The blood pressure was measured using a mercury sphygmomanometer. Pulse oximeter was used to determine heart rate. Echocardiogram used for cardiac assessment, all done for each patient by a cardiologist.

Blood sample (5mL) was taken from each patient for DNA extraction, genotyping, and biochemical analysis. The biochemical parameters, angiotensinogen and angiotensin II (Bioassay technology laboratory, china) were assessed using enzyme-linked immunoassay technique. While serum urea, creatinine, sodium, potassium and chloride (Abbot, Germany) were assessed using spectrophotometry technique.

1.3 Genotyping

The Genomic DNA was extracted using G-spinTM Total DNA extraction kit (Intron, Korea). To detect the A1166C SNP, tetra ARMS-PCR was used. The primers were designed using primer1 program and purchased from Bioneer Company, Korea. The primers sequences were: inner forward primer GCTGTCCACACTGGCTCACA, inner reverse primer GGAAGACTGGCTGCTCCCTTAC, outer forward primer GATACTAAGTCCTAGGGCCAGAGCC, outer reverse primer CACCTGAAGCAGCCGTTTGT. The PCR reaction mixtures included 4 µL of DNA sample, 1 µL of outer forward primer, 1 µL of outer reverse primer, 1 µL of inner forward primer, 1 µL of inner reverse primer, 4.5 µL of nuclease free water and 12.5 µL of GoTaq® G2 Green Master Mix (Promga, USA). The PCR reaction components were mixed at room temperature, centrifuged for 10 s at 2000 x g in a micro centrifuge for mixing then placed in thermocycler (Techne, England). The program of PCR was: Initial denaturation at 94°C for 3 minutes, followed by 30 amplification cycles (denaturation at 94°C for 30 seconds, annealing at 65°C for 45 seconds, extension at 72°C for 55 seconds, and final extension at 72°C for 5 minutes). Horizontal electrophoresis using 1.5 % w/v agarose gel which is pre-stained by 5 µL ethidium bromide was used to detect the amplified DNA fragments.

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1.4 Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences software version 20 (SPSS, San Diego, California, USA). The information was represented as mean and standard deviation. Fisher's exact was used to investigate differences in data expressed as percentages when the cell count was <5. The Chi square test was used to determine examine differences in genotype groups expressed as a percent when cell count was >5. To look for differences between genotype groups, the analysis of variance (ANOVA) test was used. All statistical procedures and tests were carried out with a significance level of P value <0.05.

2. FINDINGS AND DISCUSSION

2.1 The demographic characteristics and genotyping of ATR1 A1166C SNP of the hypertensive patients

The Demographic characteristics of hypertensive patients participated in the study are illustrated in Table 1.

Table 1 The demographic characteristics of the hypertensive patients (N = 92)

Demographic features (N = 92)			
Data represented by Mean ± SD		Data represented by percentage	
Age (years)	52.39±10.67	Male Patients	46 (50%)
BMI (kg\m²)	31.04±5.45	Female patients	46 (50%)
Duration of hypertension (years)	7.26± 6.76	Smokers	13 (14.1%)
Duration of treatment (years)	4.21±3.65		

SD: Standard Deviation, BMI: Body mass index. N: Number of the study subjects.

Genotyping of A1166C was done using tetra ARMS-PCR, the amplified DNA fragments were visualized on 1.5% agarose gel after horizontal electrophoresis was performed. The wild type (AA) showed two DNA fragments; 497 bp and 354 bp, the heterozygous mutant type (AC) showed three DNA fragments; 497bp, 354 bp and 188 bp and the homozygous mutant type (CC) showed two DNA fragments; 497 bp and 188 bp (Figure 1). The size of PCR amplicon was determined by comparing with DNA ladder 100 - 1500 bp.

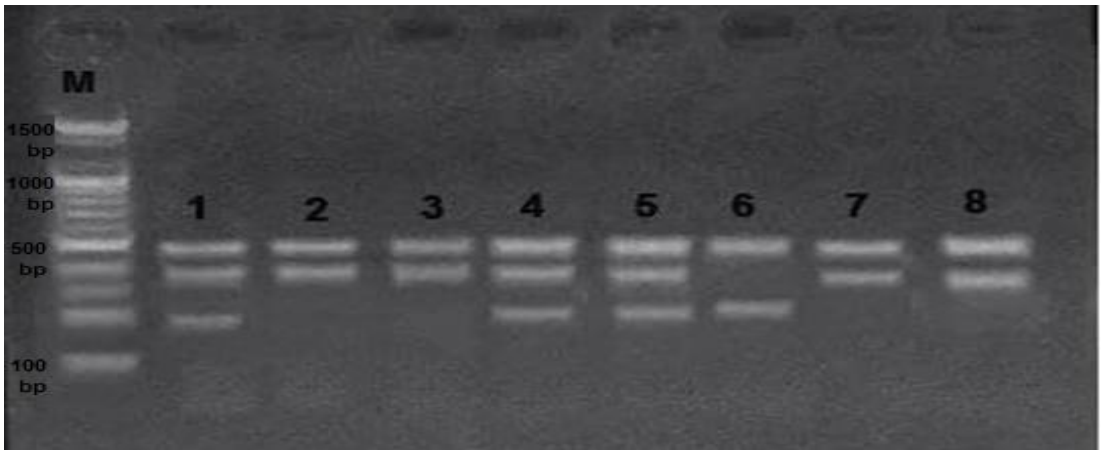

Figure 1 Agarose gel 1.5% (w/v) electrophoresis for the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) products of A1166C SNP pre stained with 5 µL ethidium

bromide. M: 100-1500 bp DNA marker, lanes 1, 4, 5 show heterozygous mutant type (AC), Lanes 2, 3, 7 and 8 show the wild type (AA), lanes 6 show homozygous mutant type (CC).

2.2 Association between ATR1 A1166C SNP and demographic characteristics

There was no statically significant association between ATR1 A1166C SNP and demographic characteristics ($p>0.05$) in hypertensive patients as shown in table 2. this may be due to randomization in selection patients (any patient had the study criteria had been taken in the study). In our study this result is important since the objective of the study is to see the impact of A1166C SNP of ATR1 gene on hypertension complications, so any differences in the association between genotypes and measured parameters (echo, BP and biochemical) if exist may be attributed to the genetic differences instead of demographic features differences between patients.

Table 2 Association of demographic characteristic between different genotype of ATR1 A1166C SNP

Demographic parameters		**Patient Genotype (N=92)**			**P value**
		AA (N=50)	**CC (N=5)**	**AC (N=37)**	
Age (years)		53.82±11.18	49.8±12.22	50.81±9.72	0.371
BMI ($kg/m^{2)}$		30.25±5.77	31.57±5.55	32.04±4.93	0.31
Duration Of Hypertension (years)		7.48±7.47	6±5.15	7.14±6.03	0.89
Duration Of treatment (years)		4.39±4.17	2.66±1.98	4.18±3.03	0.605
Gender	Male	23(50%)	2(4.3%)	21(45.7%)	0.609
	Female	27(58.7%)	3(6.5%)	16(34.8%)	
Smoking	Yes	7(53.8%)	0(0%)	6(46.2%)	0.895
	No	43(54.4%)	5(6.3%)	31(39.2%)	

Data is represented as mean ± standard deviation and percentage, N denoting the number of subjects, BMI denoting body mass index

2.3 The distribution of A1166C SNP in hypertensive patients

The distribution of A1166C SNP among the patients was 54.3% wild genotype (AA), 40.22% heterozygous mutant genotype (AC) and 5.4% homozygous mutant genotype (CC). This result is incompatible with [7] who studied on Lebanese hypertensive patients and founded that genotype distribution of A1166C SNP was 25% AA, 52% AC and 23%CC. Also our result is incompatible with [8] who studied on Egyptian hypertensive patients and founded that genotype distribution of A1166C SNP was 66.3% AA, 25.3% AC And 8.4%CC.

Table 3 Distribution of A1166C SNP in the hypertensive patients

Genotypes			Allele frequency		Hardy–Weinberg equilibrium X^2 test
			A	C	
Symbol	Frequency	%	0.745	0.255	0.302 P= 0.86
AA	50	54.35			
AC	37	40.22			
CC	5	5.43			
Total	92	100			

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2.4 Association between ATR1 gene polymorphism and blood pressure parameters

In There was no significant difference between the hypertensive patients with AA, AC and CC genotypes of A1166C SNP regarding the BP parameters (systolic BP, diastolic BP and mean atrial pressure), except the heart rate which was significantly higher in patients with AA genotype (Table 4). Since the mean of Bp parameters were within the normal range, candesartan is good drug in controlling Bp regardless to the A1166C genotypes. It can be concluded that A1166C is not responsible of the resistance to candesartan in Iraqi hypertensive patients. These findings agree with [9] who showed that response to ACE inhibitors and ARBs was independent of genetic polymorphisms in RAAS. But they are not agree with [10] who showed that ATR1 gene polymorphism predicts response to losartan, where the C allele showed greater BP response.

Table 4 The association between A1166C SNP and blood pressure parameters

Blood pressure Parameters	Patient Genotype (N=92)			P value
	AA (N=50)	CC (N=5)	AC (N=37)	
Systolic blood pressure (mmHg)	139.2±19.7	126±20.75	139.46±23.24	0.399
diastolic blood pressure (mmHg)	82.7±11.66	70±7.07	83.38±12.36	0.06
Mean atrial pressure	101.56±13.23	88.8±11.28	102.11±14.72	0.126
Heart rate (beats per minute)	88.32±10.64	75±7.81	81.84±12.08	0.005

The data is represented as mean±standard deviation, N denoting the number of the study subjects.

2.5 Association between ATR1 gene polymorphism and Echocardiograph parameters

There was no significant association ($p>0.05$) between A1166C SNP and Echo parameters (Table 5). These results agree with [11], who had shown that there is no association between the A1166C SNP and left ventricular systolic performance in Greek population. And agree with [12] who founded Lack association of the RAAS Genes Polymorphisms and Left Ventricular Hypertrophy in Hypertension. But our results not agree with [13] who showed that there was a good association between left ventricular hypertrophy occurrence and C allele of the A1166C SNP, and also not agree with [14] who observed that subjects having the homozygous AT1R CC genotype had a significantly lower ejection fraction than subjects carrying the A allele, while Baudin discovered that patients with ATR1 CC or AC genotypes tended to have a lower ejection fraction and increased left ventricular mass [15].

Table 5 The association between A1166C SNP and Echocardiogram parameters

Echocardiogram parameters		Patient Genotype (N=92)			P value
		AA (N=50)	CC (N=5)	AC (N=37)	
Ejection Fraction%		66.6±5.77	67.8±7.22	66..19±7.69	0.869
IVS (mm)		12.04±1.82	11.4±1.67	11.78±2.14	0.699
	Normal	12(44.4%)	2(7.4%)	13(48.1%)	

LVH	Mild	33(60%)	2(3.6%)	20(36.4%)	0.29
	Moderate	5(62.5%)	1(12.5%)	2(25%)	
	Severe	0(0%)	0(0%)	2(100%)	

The data is represented as mean±standard deviation, N denoting the number of the study subjects.

2.6 Association between ATR1 gene polymorphisms and biochemical parameters

There was no significant association ($p>0.05$) between A1166C SNP and renal functions parameters (serum creatinine and urea), there is an agreement between this result and the result of [16] who found that there is no impact of A1166C SNP on renal function in East Asians. This result also agree with [17] who founded that A1166C polymorphisms was not associated with any of renal diseases. The A1166C polymorphism is not located within a coding sequence or a splice location. As a result, it is not associated with a mutation altering the ATR1's encoded amino acid sequence or equilibrium-binding parameters [18]. However, this polymorphism may be linked to another regulatory polymorphism or to elements in the 3′ UTR that affect transcript stability, resulting in changes in transcriptional activity and AT1R expression. [10] hypothesized that people who had the C allele of A1166C SNP had higher renal and systemic angiotensin II activity. This finding is incompatible with our finding that indicates that there are no significant differences between Ang II levels in the patients with AA, AC and CC of A1166C SNP.

Table 6 The association between A1166C SNP and the biochemical parameters

Biochemical parameters	Patient Genotype (N=92)			P value
	AA (N=50)	CC (N=5)	AC (N=37)	
Blood Urea (mg/dl)	30.36±10.14	24.8±8.58	30.32±8.83	0.452
Serum Creatinine (mg/dl)	0.74±0.22	0.64±0.2	0.79±0.28	0.322
Potassium (mmol/l)	4.2±0.46	4.18±0.37	4.16±0.42	0.907
Sodium (mmol/l)	137.74±4.1	139.5±1.92	139.2±3.94	0.275
Chloride (mmol/l)	99.45±5.97	105.75±2.06	101.07±5.79	0.093
Angiotensinogen Level (ng/L)	81.43±51.08	70.84±10.22	91.38±83.43	0.694
Angiotensin 2 Level (ng/L)	22.34±28.46	17.7±13.01	28.7±50.13	0.686

The data is represented as mean±standard deviation, N denoting the number of the study subjects.

According to the findings of this study, The A1166C SNP is not a useful genetic marker for prediction of hypertension complication in the Iraqi hypertensive patients treated with candesartan. In addition, A1166C SNP does not cause resistance to candesartan. Antihypertensive treatment response may still be influenced by the ATR1 gene despite a lack of association. The incompatibility of our results with the other studies could due to the small number of participants in our study, genetic heterogeneity among populations which is the most plausible basis for such disagreement. Future prospective studies to investigate the effect of A1166C SNP and other ATR1 SNPs on anti-hypertensive drug responsiveness with larger sample size are

recommended.

3. Conclusion

The CC genotype of A1166C is the less frequent than other two genotypes of this SNP in Iraqi hypertensive patients. The A1166C SNP have neither an effect on hypertension complication nor on the patients' response to candesartan in Iraqi hypertensive patients.

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