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Relation between the Angiotensin II Type 1 Receptor (AGTR1)-521C/T Gene Polymorphism and Blood Pressure

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Abstract

Angiotensin II sort one (AT1) receptor mediates the constriction and growth-promoting result of angiotensin in humans. It has been reported that a polymorphism of the AT1 receptor gene (a C/T at position-521) The single nucleotide polymorphism (SNP) -521C/T is located within the promoter region of the agtr1 gene, may be associated with essential hypertension (HT). The objective of this study is the analysis of C-521T polymorphism in angiotensin II type 1 receptor (AGR1) in Baghdad patients with essential hypertension as well as controls. We examined 50 patients with essential hypertension and 50 normotensive patients. In order to identify the C-521T angiotensin II type 1 receptor variant, we used the following methods: DNA extraction, PCR amplification and enzymatic digestion of the PCR product using *Ssp* I restriction endonuclease enzyme and the PCR product sent to Macrogene Company for DNA sequencing. In the study groups, the -521C/T variant was found more frequently in control subjects (68%), than in hypertensive patients (60%). We identified (30%) heterozygotes in the hypertensive group compared with (20%) in the control group and (27%) homozygotes in the hypertensive group compared with (24%) in the control group. No association was detected between angiotensin II type 1 receptor (AGTR1)-521 C/T gene polymorphism and blood pressure.

Keywords: Hypertension; Renin angiotensin system; AGTR1 gene (C-521T)

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Introduction

Hypertension is one of the most challenging health problems in the world. It has been estimated that, globally, almost one billion individuals have hypertension [1]. By 2025, that number is estimated to increase by 60%, which means that 1.56 billion people could be hypertensive [2]. Statistics of the World Health Organization for 2012 showed that hypertension is the leading cause of mortality of worldwide (responsible for 13% of global deaths) [3,4].

The statistics referred by the annual report of the Iraqi Ministry of the year (2008-2013) showed that the number of patients infected with the high blood pressure in Iraq was (3,184,348), and the number of infected people in Baghdadas shown in the table (Table 1).

The renin-angiotensin system (RAS), sometimes referred to as the renin-angiotensin-aldosterone system, plays an important role in the regulation of blood pressure [5]. Upon a decrease in blood pressure, renin is released mainly by juxtaglomerular cells and acts on angiotensinogen (*agt*), cleaving off the decapeptide angiotensin-I (Ang I), the latter is then converted by angiotensin-converting-enzyme (ACE) to angiotensin-II (Ang II), an octapeptide, Ang II is an active vasopressor which causes constriction of arteriolar smooth muscle In addition to its direct actions which lead to an increase in blood pressure, Ang II acts on the adrenal cortex to cause the release ofthe sodium-retaining hormone aldosterone [6].

The human gene for angiotensin II type 1 receptor (*agtr1*) located at chromosome 3q21, has a length of >55 kb and is composed of five exons and four introns, a single nucleotide polymorphism (SNP) has been described that in which there is either an adenine (A) or a cytosine (C), or base (A/C) transversion in position 1166 in the 3' untranslated region of the gene [7].

The *agtr1* gene -521C/T polymorphism is associated with an increased risk for hypertension [8]. The single nucleotide polymorphism (SNP) -521C/T is located within the promoter region of the *agtr1* gene [9]. The aim of this study is the analysis of -521C/T polymorphism in the AGTR1 gene in Iraqi patients with essential hypertension as well as controls. This is the first such study carried out in Iraq, initiated to investigate the role of genetic factors in the pathogenesis of hypertension.

Materials and Methods

The current study included 50 EH patients (41 males, 9 female) with

mean age of 20±70 years old. Also 50 healthy subjects (26 males, 24 female) matching in age and gender (20±70 years old) were included as control group. The patients were selected from the Alyarmok hospital, Baghdad. A written consent was obtained from all subjects acceptive to anticipate within the study for each the clinical part and the genetic study. All subjects under study were subjected to thorough history taking and clinical examination with special emphasis on BP and BMI.

Essential hypertension was diagnosed in individuals with systolic blood pressure (SBP) >140 mmHg and/or a diastolic blood pressure (DBP) >90 mmHg, for at least three consecutive blood pressure measurements. Blood pressure was measured using a mercury sphygmomanometer after a rest for at least 15 min in quiet condition and the pressure was determined as the average of the three measurements. The normotensive controls were healthy people with a negative history of cardiovascular disease and with an SBP <140 mmHg and DBP <90 mmHg measured on 3 separate occasions, also all study subjects were non diabetic with normal renal functions.

Genotyping was carried out by PCR amplification of peripheral blood genomic DNA extracted using Blood genomic mini spin kit (Bioneer) followed by restriction enzyme digestion, For SNP-521 polymorphism analysis, DNA was amplified using the forward primer, 5′-CGT GAT GTC TTT ATC TGG TTT TG-3′ and the reverse primer 5′-CGA ACT TTG GTA ATA CAG TTG TGG-3′, PCR was performed in a 20 µl total volume Primer forward 1 µl (10PM), Primer reverse 1 µl (10 PM), Template DNA 5 µl, and 13 D .W .A total of 35 PCR cycles with denaturation at 94℃ for 1 min, annealing for 1 min at 62℃ and extension at 72℃ for 1 min. were conducted. An initial DNA denaturation at 94℃ was carried out for 5min and final extension at 72℃ was carried out for 5 min each. 10 µl of amplified products was mixed with 0.5 *Ssp I* enzyme, 2 µl of enzyme buffer and 7.5 free nucleases deionized distilled water then incubate for 4 h in 37℃ [10,11]. All digestion mixtures were loaded to the well in 3% agarose gel stained with 2.5 µl ethidium bromide. The following fragment size patterns were observed by agarose gel electrophoresis. The PCR product sent to the Macrogene Company for DNA sequencing.

Results

The result of genotype to AGTR1 gene of people controls and HTN in patients through RFLP technique showed in the table (Table 2). No association was detected between angiotensin II type 1 receptor (AGTR1)-521 C/T gene polymorphism and blood pressure.

SNP-521 in agtr1 gene was detected as 271 bp band (Figure 1), to diagnose the alleles of SNP-521. PCR products were subjected to *Ssp* I restriction enzymes. The following fragment sizing patterns were observed by agarose gel electrophoresis:

- **Wild type:** CC (271bp) were digested in two fragments (174bp, 97bp) (Figure 1, Lane 2 and 3).
- **Heterozygous:** CT, *Ssp*I restriction enzymes to show three fragments in agarose gel electrophoresis (271 bp,174 bp and 97 bp) (Figure 1, Lanes 6,7 and 8).
- **Homozygous:** TT (271) PCR fragments weren't digested (Figure 1, Lane 4 and 5).

The below table summarized the results of the PCR product sequence and showed that there are 10 SNP were detected insamples of the *agtr1*gene (Table 4).

Discussion

The renin-angiotensin-aldosterone system is implicated in the pathogenesis of hypertension, cardiac hypertrophy and coronary heart disease. The major biologically active product of the renin-angiotensin system is angiotensin II, it plays an important role in the progression of cardiac and renal diseases. Most of the effects of angiotensin II are mainly mediated by the angiotensin II type 1 receptor (AGTR1), including vascular contraction, press or responses, renal tubular sodium transport, and aldosterone secretion [10]. The promoter region of the agtr1 gene is a regulatory region to the level of gene transcription, when mutation occurring within this region it will lead to a change in gene expression. In this study mutation at -521 C/T in the promoter region of the agtr1 gene and relationship with some HTN patient was detected. The results of genotype at C-521T site of agtr1 gene between hypertensive patients and apparently healthy control individuals were shown in the table (Table 3). The CC genotype frequency was higher in apparently healthy subjects 56(56%) than in hypertensive patients, 43(43%). There weren't differences between control subjects and hypertensive patients as related with TT allele's frequencies. The CT genotype frequency was higher in hypertensive patients 30(30%) than in apparently healthy control subjects 20(20%). So, heterozygous CT genotype frequency in patients' group was at risk for incidence of hypertension.

The results of this study agree with the results of Xun Z, et al. (2000) studied some mutation in C521T, G2228A and C1424G in the agtr1 gene promoter in Caucasian people, they found no association of three polymorphic sites in the agtr1 gene promoter with hypertension and Bei et al observed the absence of any association between -521 C/T and hypertension through the study of Tibetan population [11,12]. While Gong HT, et al. (2013) studied some mutation by the Chinese population and found that the variables 1166A/C, 573T/C, -810A/T, and -521C/T polymorphisms of the agtr1 gene was associated with high blood pressure and the work of other studies to determine the response of these variables on antihypertensive treatment in agtr1 gene, no significant reduction in blood pressure was found in individuals with the 1166A/C, 573T/C, or -810A/T polymorphisms of the agtr1 gene but -521C/T variation was associated with an antihypertensive effect patients with the agtr1 -521CC genotype had a significant reduction in beat pressure level compared to those carrying the T cistron [8]. Another study done by Henderson S, et al. (2004) found that the T allele in -521C/T increased the risk of EH in African Americans (AAs) [10].

Several explanations may account for the lack of association between-521C/T agtr1 promoter and hypertension: First, the regulation of the agtr1 promoter and, subsequently, the distribution or density of the receptor protein may be without effect on blood pressure regulation. This appears to be highly unlikely given the robust data of molecular, physiological and pharmacological studies that document the critical role of the receptor for fluid and blood pressure homeostasis [13]. Second, the regulation of the agtr1 promoter may be without major functional importance. This appears to be unlikely as well given that the transcription level of the agtr1 gene that has been found to be tightly regulated in several cardiovascular disorders. Moreover, inhibition of the receptor dose dependently decreases blood pressure suggesting that the number of functionally active receptor sites translates directly to functional activity of the system [14]. Perhaps the most likely explanation for the lack of association may be that the single base pair exchange in the agtr1 promoter region that we studied, have no strong effect on the regulation of the agtr1 promoter. Therefore, the variant may be without any functional relevance, and thus without

effect on blood pressure, or Ethnic groups play a major role in the incidence disease regarding their genetic material and genetic variation which may affect the gene expression pressure.

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