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DETERMINATION THE GENOTYPE DD IN ANGIOTENSIN CONVERTING ENZYME GENE IN POLYMORPHIC SITE (G2350A) AS PREDICTOR FOR HYPERTENSION AMONG IRAQI POPULATION

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ABSTRACT

Objective: Many research studies included the polymorphic loci in Angiotensin Converting Enzyme (ACE) among Iraqi population but not with hypertension, therefore our study was to detect the contribution of (ACE) G2350A [I/D] gene Polymorphism in Iraqis patients diagnosed with hypertension. **Methods:** From the October 2016 till March 2017, a total of 132 hypertension patients (97 males /35 females), age between (45-89), (98 patients were positive to family history of hypertension while 34 patients were negative to family history of hypertension), the control group included 132 healthy individual (54 males /78 females), age between (15-77), (59 patients were positive to family history of hypertension while 73 patients were negative to family history of hypertension), 3 ml venous blood were collected in order to DNA extraction. One SNP G2350A in (ACE) gene was genotyped in both cases and control. **Results:** the results showed distributions of genotypes among patients and control as follow, the number of cases they carried the (DD) genotype were 44 while control were 21 (P= 0.012), while the number of cases they carried (II) genotype were 38 while control were 47 (P= 0.45), furthermore the number of cases they carried the (ID) genotype were 55 while control were 64 (P= 0.31), the allele frequencies were as follow (D) allele in cases were 69 and 53 in control (P= 0.27). Furthermore the (I) allele frequency was as follow, 63 in cases and 79 in control (P= 0.29). **Conclusion:** we concluded from the current research that the Angiotensin Converting Enzyme (ACE) G2350A [I/D] gene polymorphism associated with hypertensive patients in Iraqi population.

KEYWORD: Hypertension, Angiotensin Converting Enzyme (ACE) gene polymorphism, SNP.

INTRODUCTION

Hypertension (HTN) consider major health problem, characterized by increasing systolic blood pressure (SBP) > 140 mmHg, and diastolic blood pressure (DBP) > 90 mmHg.[1-3] (HTN) also cause of development of many diseases like coronary heart disease, congestive heart failure, peripheral vascular disease, stroke and renal diseases.[4] Many genes variants contribute in hypertension,[5,6] It's well known a Renin-angiotensin-aldosterone system (RAAS), it's have important function known in regulating blood pressure (BP).[7] The role of (RAAS) genetic polymorphism has been studied previously to determine the linkage of (RAAS) variants and (HTN).[8] The most important gene in (RAAS) is (ACE), it's consider key in (RAAS), therefore it's very important to study the contribution of the (ACE) gene polymorphisms in the (HTN) patients.[9] The purpose of the current research is to detect the linkage between angiotensin converting enzyme gene polymorphisms in SNP G2350A [I/D] and hypertension in Iraqi population.

MATERIAL AND METHODS

Cases and controls selection

From October 2016 till March 2017, a total of 132 patients were diagnosed with hypertension according to the American Society of Hypertension, (97 males /35 females), age between (45-89) mean ± SD (67.92±8.176), (98 patients were positive to family history of hypertension while 34 patients were negative to family history of hypertension), and the systolic blood pressure mean ± SD (177.0±10.01) while the diastolic blood pressure mean ± SD (106.2 ± 10.10), the control group included 132 healthy individual (54 males /78 females), age between (15-77) mean ± SD (41.46±14.50), (59 patients were positive to family history of hypertension while 73 patients were negative to family history of hypertension), and the systolic blood pressure mean ± SD (124.2±5.909) while the diastolic blood pressure mean ± SD (81.02± 6.412). The study was recruited at Baghdad teaching hospital, medical city, Baghdad, Iraq, and the project was approved by Institutional Ethical Committee.

DNA Extraction

4 ml blood withdrawal from both patients and control in EDTA tubes and stored as whole blood at -20°C in order to DNA isolation. Also, the DNA extraction done according to the (Sambrook et al) method.[10]

Primers design and Genotyping of Angiotensin Converting Enzyme (ACE) G2350A [I/D]

We genotyped one SNP G2350A [I/D] in angiotensin converting enzyme gene in both cases and control in our study. The SNP that selected previously was magnified by conventional polymerase chain reaction (PCR), allele specific was our technique in this procedure. Primers were designed by online programme (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Tow forward primers I-allele specific primer was F1:5-ATCCCGGAAATATGAAGACCTGTT-3 and D-allele specific primer: F2:5-GTCCCGGAAATATGAAGACCTGTT-3, while the common reverse was 5-CAGCAGGGACTCACCATTGA-3, after the amplification the DNA fragments separating on 2% agarose gel and visualized on UV-transilluminator our fragment size was 163 bp for each allele as showed in the figure 1.

Statistical analysis of data

The statistical analysis of data was performed by SPSS version 22 to detect the association of genotypes with hypertension among patients and control using Fisher's Exact test, also the allele frequencies were done. Odds ratio were calculated for genotypes among patients and control. The confidence interval (CI) at 95% was done to explain the amount of uncertainty related with samples.[11,12] The significance at value $P < 0.05$.

RESULTS

After correlated the genotypes among patients and control, our results shown the distribution of genotypes, allele frequencies and significance among cases and control, as shown in the table 1, the number of cases they carried the (DD) genotype were 44 while control were 21, the (OR) was 2.10, (95% CI) 1.14 - 3.92 and the (P value) was 0.012, while the number of cases they carried (II) genotype were 38 while control were 47, the (OR) was 0.81, (95% CI) 0.48 - 1.36 and the (P value) was 0.45, furthermore the number of cases they carried the (ID) genotype were 55 while control were 64, the (OR) was 0.78, (95% CI) 0.49- 1.24 and the (P value) was 0.31. Otherwise, the allele frequencies were as follow (D) allele in cases were 69 and 53 in control, (OR) was 1.30, (95% CI) 0.83 - 2.05 and the (P value) was 0.27. Furthermore the (I) allele frequency was as follow, 63 in cases and 79 in control, (OR) was 0.80, (95% CI) 0.52 - 1.23 and the (P value) was 0.29.

Table 1: ACE gene polymorphism and allele frequencies among hypertension patients and their control.

Gene polymorphism (ACE) G2350A [I/D]	Cases		Control		OR	(95% CI)	Significance (Fisher's Exact test)
	No.	%	No.	%			
DD	44	33.3	21	15.9	2.10	(1.14 - 3.92)	P = 0.012*
II	38	28.8	47	35.6	0.81	(0.48 - 1.36)	P = 0.45
ID	50	37.9	64	48.5	0.78	(0.49- 1.24)	P = 0.31
Total	132	100	132	100			
Allele frequencies							
D	69	52.3	53	40.1	1.30	(0.83 - 2.05)	P = 0.27
I	63	47.7	79	59.9	0.80	(0.52 - 1.23)	P = 0.29
Total	132	100	132	100			

*significant at P≤0.05

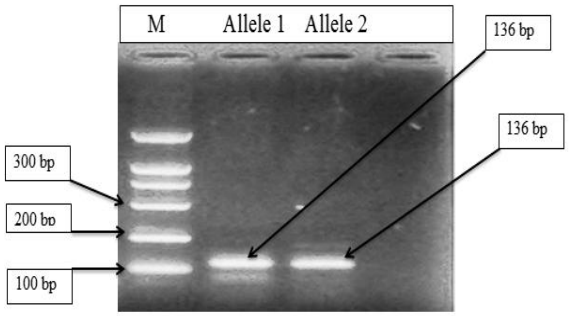

Figure 1: agarose gel electrophoresis for allele specific PCR for angiotensin converting enzyme (ACE) gene polymorphisms. Heterozygous genotype will appear two allele together (I) and (D) allele as shown in the figure, while the homozygous genotype will appear one allele only on gel.

DISSUASION

Regarding findings in our results, we revealed to that, the Angiotensin Converting Enzyme (ACE) G2350A [I/D] gene polymorphism have important role in hypertension development, furthermore the genotype (DD) consider a risk factor to diseases development. It's well known that the renin–angiotensin system (RAS) have crucial role in homeostasis of electrolyte and regulation of blood pressure. As mentioned previously the key enzyme of the RAS is (ACE). The one of important function if ACE is imbalance between forces of vasoconstriction over forces of vasodilation elevates vascular tone and leads to systemic elevation of blood pressure.[13] Many researches discussed the relation of (ACE) gene polymorphisms and hypertension[14-17] Moreover in researches included (ACE) gene polymorphisms in Iraqi population,[6,7,18,19]

but actually we don't found any research studied the relation between (ACE) and (HTN). In recent study by (Zawilla, et al. 2014), they investigated the role of (ACE) gene polymorphism and the association with (HTN) in occupational noise exposure in Egyptian population, their results a proved an association between ACE gene polymorphisms and occurrence of (HTN) in persons that exposure to noise.[13] In Lebanese hypertensive patients the finding by (YB Saab, et al. 2011), revealed that no linkage[20] in (HTN) with the ACE I/D polymorphism. (Martı´nez, et al. 2000) agree with recent study while he studied the role of (ACE) gene polymorphism with (HTN) in Spanish-Mediterranean population, whereas proved there is no association between (ACE) I/D polymorphism and (HTN).[16] Others studies disagreed with our results and failed to obtained the association between (ACE) gene polymorphisms and (HTN).[18,19,21,22] On the other hand, in Bangladeshi population, recent study suggest there is strong association between (ACE) gene polymorphism and (HTN) and the DD genotype was associated with (HTN) in male but not in female.[21] Enhanced the previous study (Martinez-Rodriguez, N, et al. 2013) studied the relation between (ACE) gene polymorphism and (HTN) in Mexican ethnic they suggested there are strong association between (ACE) gene polymorphism and the ''GGATG'' haplotype (HTN) responsible to development of hypertension.[23] Otherwise (Heidari, F., et al. 2015) suggested that, the D allele consider risk factor to development (HTN) in Malay male and the presence DD genotype in hypertensive patients consider good prognosis in response to anti-hypertensive (ACE inhibitors) drugs.[24]

In conclusion, regarding our results we conclude that the Angiotensin Converting Enzyme (ACE) G2350A [I/D] gene polymorphism linked to hypertensive patients with Iraqi population and the DD genotype consider a risk predictor to developing hypertension among individuals in Iraqi population.

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