Association of Angiotensin-Converting Enzyme Insertion/Deletion Gene Polymorphism with High Blood Pressure Patients: A Case-Control Study

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Abstract: The angiotensin-converting enzyme (ACE) is a protein-coding gene also known as peptidyl dipeptidase A or carboxycathepsin. ACE is a central component of the renin-angiotensin system, which controls blood pressure by regulating fluid volume in the body. The study involved (300) people aged (30-45) years who were distributed into two groups: the control group (G1) involved (150) healthy individuals (75 males and 75 females), and the second group (G2) included (150) HBP patients (75 males and 75 females). Serum was used to estimate HbA1c, FBS, TC, TG, HDL-c, LDL-c, and VLDL, and BMI and age were considered. Also, a polymorphism study was conducted based on PCR I/D of the ACE gene and examined their correlation with high blood pressure. Results revealed that the levels of TG, TC, and VLDL were significantly higher in the HBP patients (G2) as compared with the control (G1) (p-value < 0.05). Also, results revealed that the overall genotype of the ACE I/D gene was significantly different between the HBP patients (G2) as compared with the control (G1) for the genotype DD ($\chi 2 = 10.174$, p-value = 0.012), D allele ($\chi 2 = 9.708$, p-value = 0.002), and ID & II compared to the DD genotype ($\chi 2 = 9.934$, p-value = 0.002). As a result, it can be concluded that increasing TG, TC, and VLDL levels in serum, as well as Genotype DD, the D allele, and ID & II levels, may be used as an early detection marker of high blood pressure (HBP) disease.

Keywords: Hypertension, HBP, Angiotensin-converting enzyme, ACE.

Introduction

High blood pressure (HBP) is a serious disease with persistent elevation in arterial hypertension. It is considered a multifactorial condition with multiple genetic and environmental causes. High blood pressure is a global problem that affects (15 – 20 %) of all countries [1]. High blood pressure is a chronically heavy systemic or diastolic blood pressure in the systemic arteries. Ventricular contraction results in systolic blood pressure (SBP), the highest in blood pressure (Bp) [2]. Diastolic blood pressure (DBP) is the blood pressure remaining during ventricular stimulation and the lowest blood pressure. The concept of BP corresponds to the contrast (in mmHg)) between systolic and diastolic pressure, while normal pressure is the mean arterial pressure (MAP) during one cardiac period [3]. The blood pressure resulting from high blood pressure is (140 mm Hg or higher) and the diastolic pressure is constant (90 mm Hg or higher), while the average blood pressure of a stable adult is (120 mm Hg systolic and 80 mm Hg diastolic) [4]. Cardiac output and peripheral artery resistance measure blood pressure. Blood pressure may occur due to some environmental modifications in these processes, such as sodium consumption and genetic influences [5]. Most of the complications from High blood pressure include headaches that are unbearable, nosebleed, fatigue, or confusion are symptoms of a nosebleed, problems of vision, pain in the chest, breathing problems, an irregular pulse is a condition that occurs while the heartbeat is irregular, urine with blood [6], the pain in your stomach, throat, or ears is excruciating, nervousness, dizziness, sweating, sleeping problems, flushing of the face [7]. High blood pressure (HBP) causes sudden death, coronary artery failure, myocardial infarction, coronary heart disease (CHD), cardiovascular disorders, and left ventricular hypertrophy (LVH), which is associated with elevation likelihood of the target organ damage being infected (TOD), T2DM, heart failure (HF) and chronic renal disease (CKD) [8]. Genetic differences may have a significant effect on essential High blood pressure (HBP) emergence, which shows a significant risk factor for advanced renal injury, stroke, ischemic heart attacks, and peripheral vascular disease [9]. Angiotensin-converting-enzyme inhibitors (ACE inhibitors) are a class of medication used primarily for the treatment of high blood pressure and heart failure. They work by causing relaxation of blood vessels as well as a decrease in blood volume, which leads to lower blood pressure and decreased oxygen demand from the heart [10]. ACE inhibitors inhibit the activity of the angiotensin-converting enzyme,

an important component of the renin-angiotensin system which converts angiotensin I to angiotensin II and bradykinin [11]. ACE inhibitors decrease the formation of angiotensin II, a vasoconstrictor, and increase the level of bradykinin, a peptide vasodilator [12]. This combination is synergistic in lowering blood pressure, while reducing the glomerular hemodynamics, by stressing the glomerular capillaries [13]. Frequently prescribed ACE inhibitors include benazepril, zofenopril, perindopril, trandolapril, captopril, enalapril, lisinopril, and ramipril [14]. ACE gene polymorphisms (I/D) have a positive correlation with high blood pressure in elderly people with high sodium chloride, meaning that it serves as a link indicating the potential genetic risk of salt allergy [15]. Active angiotensin (ACE) acts in the vessels, which binds to the Ang II (a vasoconstrictor) by transcription factor-1 (ATF-1) in the plasma membrane and helps arterial contractions in the blood vessels, leading to an improvement in systolic and diastolic blood pressure, by transporting the protein metalloproteinase (A dipeptide Carboxypeptidase), as the proteinase activity in ACE inhibits the activity of bradykinin, a potent vasodilator [16]. Angiotensin II is aids arterial constriction in the blood vessels, which leads to high blood pressure, and increases the improvement of the extremities by controlling systemic blood pressure and the balance of fluid electrolytes [17]. Bradykinin acts as a peptide vasodilator, controlling blood pressure and cardiovascular homeostasis, via the active vasodilator and peptide [18]. The study aims to reveal the ACE I/D gene polymorphism and its role in the pathophysiology of HBP in Al-Diwaniyah city in Iraq.

Material and Methods

Study Population

The study population consisted of (300) people of both genders (males and females), non-smokers and non-obese, their ages ranged between (30-45) years, who attended Al-Diwaniyah Teaching Hospital in Al-Diwaniyah, Iraq. The study population consisted of 150 patients with HBP (G2), and 150 healthy controls (G1). The gender of the people in both groups(G1 and G2) was 75 of them males and 75 of them females.

Extraction of DNA

Genomic DNA was extracted from whole blood using a DNA Extraction Kit (Bioneer/Korea), were equal to or less than 20 μg/ml. The concentration of extracted DNA

and its purity were estimated by measuring the absorbance at A 260 nm and A 280 nm by the Nanodrop device. The concentration of the DNA samples was (50 ng/µl), and the purity of the DNA samples (1.8 µg/ml).

PCR Amplification

Isolated DNA is amplified with T-ARMS primers, as shown in Table (1).

Table 1. The name and sequence and melting point of prepared ACE (I/D) gene polymorphism.

Gene Name	SNP Name	Sequence(5'−>3')	Peak	Tm (°C)
ACE	I/D	F 5- CTGGAGACCACTCCCATCCTTTCT-3	490 (II)	56
		R 5- GATGTGGCCATCACATTCGTCACGAT -3	190 (DD)	
		F 5- CTG GAG ACC ACTCCC ATC CTT TCT -3 R 5- GAT GTGGCC ATC ACA TTC GTC AGA -3	190 & 490 (I/D)	

F: Forward, R: Reverse, I: Insertion, D: Deletion.

The prefixes were designed by the Korean company Pioneer for all genes used in this study as shown in Table (1). All were dried and prepared with high purity H2O (Bioneer, Korea) according to the manufacturer's instructions, and all were split and kept at - 20 °C. The PCR products were run on 2% agarose gel electrophoresis. The different fragments obtained were homozygous (II) genotypes (490 bp); heterozygous (ID) genotypes (190 & 490 bp); and homozygous (DD) genotypes (190 bp).

Results and Discussion

Sample Clinical and Biochemical Features of Study Groups

These features studied the parameters of the two groups: control group (G1) and the high blood pressure patients group (G2) and gave the values shown in Table (2).

Table 2. The clinical and biochemical features of (G1 and G2) groups.

Parameters	Groups		T-test	p-value
	G1-Control Mean ± SD	G2- HBP Mean ± SD		
FBS (mg/dl)	98.13 ± 9.96	99.87 ± 12.01	0.182	0.857
HbA1c (mmol/mol)	4.80 ± 0.59	4.85 ± 0.49	0.274	0.786
TG (mg/dl)	146.53 ± 36.32	254.13 ± 16.25	2.41	0.023*
TC (mg/dl)	181.87 ± 15.16	217.33 ± 50.9	2.018	0.053*
HDL-C (mg/dl)	44.15 ± 7.92	42.53 ± 9.29	0.507	0.616
LDL-C (mg/dl)	100.40 ± 16.6	117.37 ± 49.82	1.189	0.245
VLDL (mg/dl)	30.47 ± 15.51	48.87 ± 4.25	2.475	0.020*
BMI (kg/m^2)	26.83 ± 4.01	29.34 ± 3.45	1.835	0.077
Age (year)	35.20 ± 10.85	41.60 ± 7.56	1.874	0.71

FBS, fasting blood sugar; HbA1C, glycated hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol; VLDL-C, very-low-density lipoproteins cholesterol; BMI, body mass index. The data are expressed as mean ± SD. P-value < 0.05 is considered significant (determined by T-test).

Table (2) compares the mean ± SD values for the two groups of hypertension patients (G2) and the control (G1). The mean ± SD values represent the rate of measurements calculated for the two groups of high blood pressure patients (G2) and the control (G1) at the p-value ≤ 0.05. When comparing the hypertension group (G2) which represents hypertension patients, and the control group (G1) which represents control (healthy people) in Table (2). It was found that the two selected groups were different with respect to the mean values of each of the parameters (TG , TC , and VLDL), as the values were significantly higher in the high blood pressure patients group (G2) when compared with the control group (G1) and after verifying the results, it was found that they have a correlation significant when comparing the biochemical and clinical properties of the two groups (G2 and G1), as their P-value was influential (p-value < 0.05) in the parameters (TG , TC , and VLDL), which given mean ± SD values in G2 (254.13 ± 16.25, 217.33 ± 50.9, 48.87 ±

4.25) respectively. High blood pressure may result from high cholesterol and triglycerides in the patient, so physicians do tests for the parameters (TC, TG, HDL, LDL, and VLDL) for the patient with high blood pressure because they are influencing factors. Groups (G2 and G1) did not show differences with respect to the rest of the other parameters (FBS, HbA1c, HDL, LDL, BMI, Age), which gave mean ± SD values in G2 (99.87 ± 12.01, 4.85 ± 0.49, 42.53 ± 9.29, 117.37 ± 49.82, 29.34 ± 3.45, 41.60 ± 7.56) respectively. When compared to their values in the group (G1), there was no significant correlation for these values because the difference between their values in the two groups (G2 and G1) was close and there was no significant correlation between them, the p-value of these parameters were (0.857, 0.786, 0.616, 0.245, 0.077, 0.71) respectively, so it is considered non-influential because (p-value > 0.05), these results consistent with the finding by [19], [22], and [16]. Group (G2) indicates that the mean values of the parameters (FBS, HbA1c, LDL, BMI, Age) were higher than the average value of the normal range in the group (G1) except for (HDL), which falls within the range less than the normal range for the group (G1). High blood pressure is caused by dyslipidemia, as high triglycerides (TG) contribute to reaching the artery walls, which increases the risk of stroke, heart attacks, and heart disease due to hypertension [8]. High triglycerides (TG) increase the value of systolic and diastolic pressure, which causes a rise in high blood pressure and thus increases the risk of developing atherosclerosis, as the accumulation of triglycerides on the walls of veins and arteries loses their elasticity and makes them harden, which causes enlargement of cardiac muscle when trying to pump blood to the body [23]. Observed from the Table (2) that the value of Mean ± SD calculated for the parameter (TG) in the group of high blood pressure patients (G2) was (254.13 ± 16.25) and the value of the statistical hypothesis test (T-test) was (2.41), which indicates that the correlation was very large between the parameter (TG) and the group of high blood pressure patients (G2), where the p-value was influential (p-value = 0.023), and this indicates that the parameter (TG) has a significant relationship associated with the group (G2) that represents high blood pressure patients (p-value < 0.05), these results consistent with the finding by [24-26,16,20]. Cholesterol is present in all cells of the body and contributes to building new cells and producing steroid hormones. A high level of cholesterol in the blood causes an increase in the fatty deposits formed within the walls of the blood vessels, which causes narrowing of the bloodstream

and affects the flow of blood in the arteries and veins, which increases the risk of hypertension [27]. Cholesterol binds to certain proteins made up of lipoprotein, which facilitates the movement of cholesterol into the blood vessels. High levels of cholesterol cause atherosclerosis, which is a dangerous accumulation of cholesterol and fatty deposits on the walls of the arteries, which causes an obstruction to blood flow and leads to a heart attack or to stop the transfer of blood to the brain (Stroke) [26]. Observed from the Table (2), that the value of mean ± SD calculated for the parameter (TC) in the group of high blood pressure patients (G2) was (217.33 ± 50.9) and the value of the statistical hypothesis test (T-test) was (2.018), which indicates that the correlation was very large between the parameter (TC) and the group of high blood pressure patients (G2), where the p-value was influential (p-value = 0.053), and this indicates that the parameter (TC) has a significant relationship associated with the group (G2) that represents high blood pressure patients (p-value < 0.05), these results consistent with the finding by [28]. Changes in fat metabolism lead to insulin resistance and elevated blood pressure due to higher VLDL levels and lower lipid metabolism. VLDL lipoprotein contains the largest amount of triglycerides that bind to proteins in the blood and that cause cholesterol particles to accumulate in the arteries, which leads to narrowing of blood vessels and causes hypertension because of blocking blood flow within those vessels [23]. Observed from the Table (2), that the value of Mean ± SD calculated for the parameter (VLDL) in the group of high blood pressure patients (G2) was (48.87 ± 4.25) and the value of the statistical hypothesis test (T-test) was (2.475) which indicates that the correlation was very large between the parameter (VLDL) and the group of high blood pressure patients (G2), where the p-value was influential (p-value = 0.020), and this indicates that the parameter (VLDL) has a significant relationship associated with the group (G2) that represents high blood pressure patients (p-value < 0.05), these results consistent with the finding of [29].

Investigation between the Association of ACE I/D Gene Polymorphisms and Risk of High Blood Pressure (G2) as Compared with Control (G1)

These studies examined the association between ACE I/D gene polymorphism with the risk of high blood Pressure as compared with control. The study was based on the results obtained from the genotyping. The statistical analysis between the allele frequencies and

genotype distributions of ACE I/D gene Polymorphisms in the two groups (Control G1, and High blood pressure patients G2) was confirmed by the descriptive statistical at (p-value < 0.05).

Amplification of ACE I/D Gene Polymorphism

The angiotensin-converting enzyme is a metallopeptidase zinc enzyme, which catalyzes the conversion of angiotensin I (ACE1), (low in aldosterone) into a physiologically active peptide angiotensin II (ACE2) and bradykinin [30]. Angiotensin II is a potent vasopressor and aldosterone-stimulating peptide that controls blood pressure and fluid-electrolyte balance. This angiotensin-converting enzyme (ACE) also inactivates the vasodilator protein (Bradykinin). ACE2 acts as a vasoconstrictor to stimulate the action of aldosterone, by water reabsorption, therefore ACE2 causes blood vessels to narrow, and decreases liquid blood flow, which leads to increased blood pressure [31]. The ACE gene consists of the primers (I, D, and ID), and when performing a PCR assay for these primers and for a set of eight DNA samples, Gel documentation showed that the primers (II) give the same bands that appeared at (490 bp), also Gel documentation showed that the primers (DD) give the same bands that appeared at (190 bp). While Gel documentation showed that the primers (DD) give the same bands that appeared at (190 bp, and 490 bp). Amplification of ACE I/D gene polymorphism is shown in Figures (1 and 2). The alleles distribution for ACE I/D gene in the two groups is as follows:

The Genotype of the ACE I/D Gene in the Control Group (G1)

The genotype frequencies and allele distributions of I/D polymorphisms of ACE gene for the Control (G1) group that are shown in Table (3) are calculated from Figure (1).

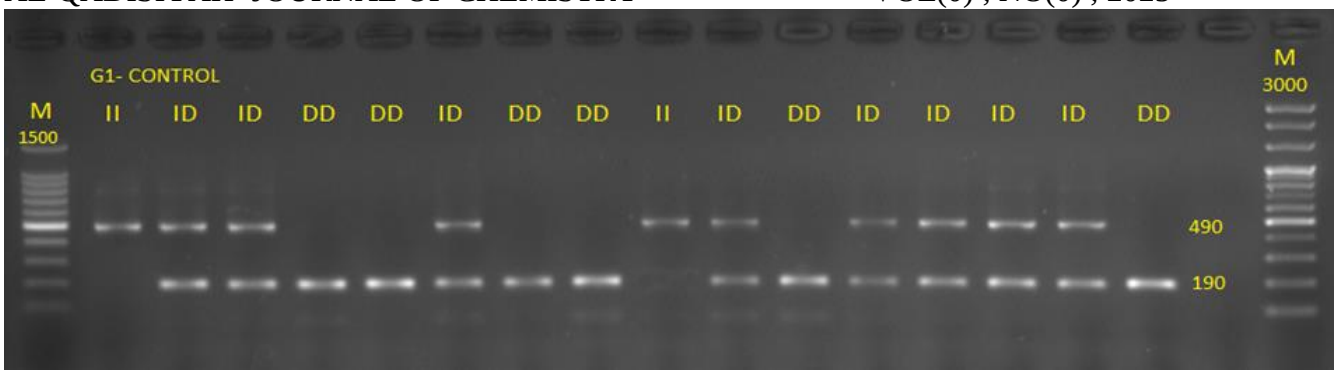

Figure 1. Gel electrophoresis of ACE (I/D) gene polymorphism amplified with a specific pair of primers using conventional PCR of control group (G1). M: DNA ladder (100-1500 bp). The PCR products were stained with safe stain dye. homozygous DD genotypes (490 bp); heterozygous ID genotypes (190 bp); homozygous II genotypes (190 and 490 bp).

The Genotype of the ACE I/D Gene in the High Blood Pressure Group (G2)

The genotype frequencies and allele distributions of I/D polymorphisms of ACE gene for the Hypertension (G2) group that are shown in Table (3) are calculated from Figure (2).

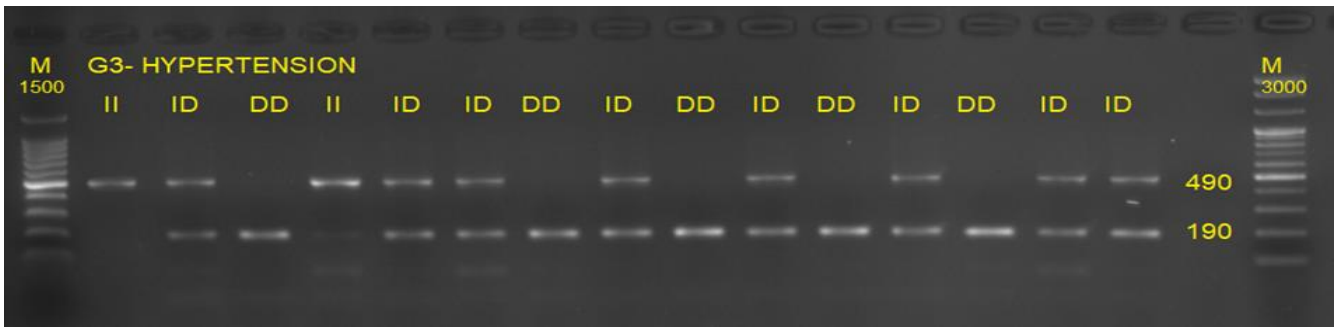

Figure 2: Gel electrophoresis of ACE (I/D) gene polymorphism amplified with a specific pair of primers using conventional PCR of high blood pressure group (G2). M: DNA ladder (100-1500 bp). The PCR products were stained with safe stain dye. homozygous DD genotypes (490 bp); heterozygous ID genotypes (190 bp); homozygous II genotypes (190 and 490 bp).

The amplification product of the ACE gene polymorphism (I/D) is three alleles, the values of whose bands were calculated from the Figures (1, and 2), and those values are shown in Table (3).

Table 3. Size of bands of ACE (I/D) gene polymorphism**.**

Genotype		No. of bands	Size of bands (bp)
Homozygous	II	1	490
Heterozygous	ID	2	190, 490
Homozygous	DD	1	190

There are three alleles in the ACE (I / D) gene, and they are homozygous II, heterozygous ID, and homozygous DD. When the bands appear at 490 bp, (190 bp, and 490 bp) and 190 bp, respectively. Based on the research from these alleles, the percentage of all alleles was calculated for control (G1), high blood pressure patients (G2), as shown in Figures (1 and 2), respectively.

Polymorphisms ACE (I/D)

When studying the Figures (1 and 2) of comparison of genotype distribution and allele frequencies that appeared of ACE gene, it was found that this gene has two types of alleles: I and D, where the percentage of allele D was greater than the percentage of allele I in all groups (Control, and hypertension patients), it was found that the percentage of allele D was 142 (71%), and 112 (61%) in Control, and high blood pressure patients, respectively. While the percentage of allele I was 58 (29%), and 88 (44%), in control, and high blood pressure patients, respectively, as shown in Figure (3).

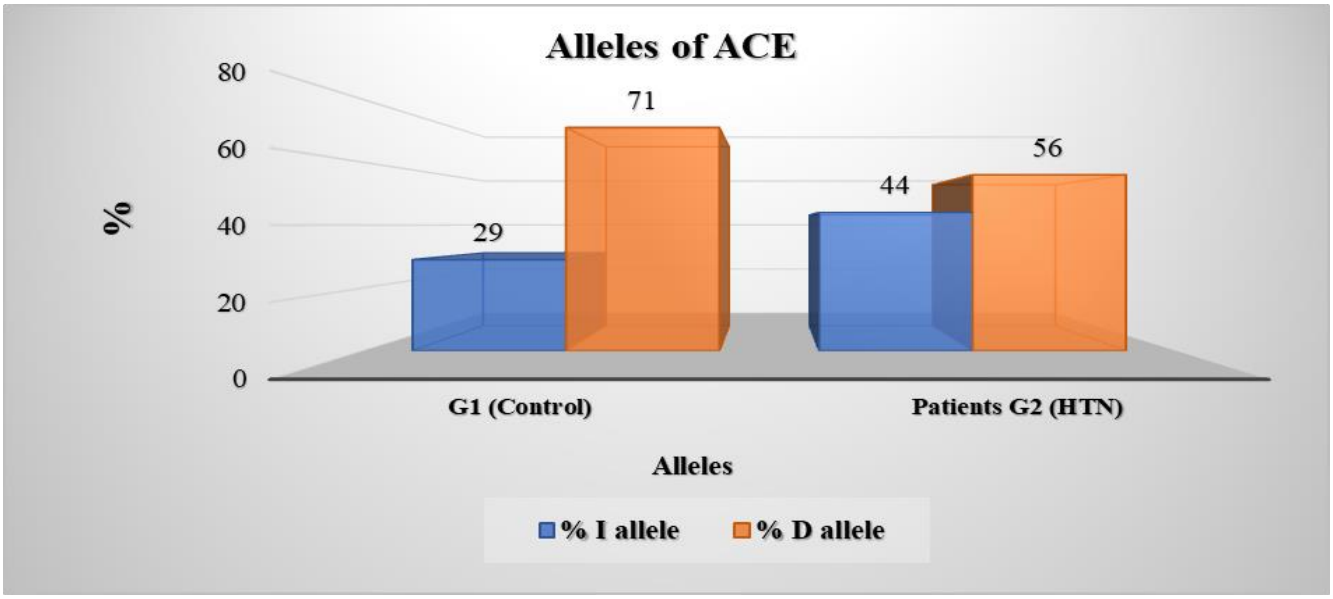

Figure 3. The percentage of alleles I/D of ACE in blood samples of control, and hypertension patients.

The Figures (1 and 2) of genotype distribution observed for ACE (I/D) (Intron 25 I > D) also showed that there are three types of polymorphisms: II, ID, and DD. The values of the percentages of the first polymorphism II were 11% and 19% for Control, and high blood pressure patients, respectively. The values of the percentages of the second polymorphism ID were 36%, 50% for control, and high blood pressure patients, respectively. The values of the percentages of the third polymorphism DD were 53%, and 31% for control, and high blood pressure patients, respectively (Figure 4). The ID genotype was the major genotype at hypertension patients in I/D (ACE). The values of percentage of I/D alleles, II, ID, and DD genotypes of ACE in Figure (4) were calculated from the Figures (1 and 2).

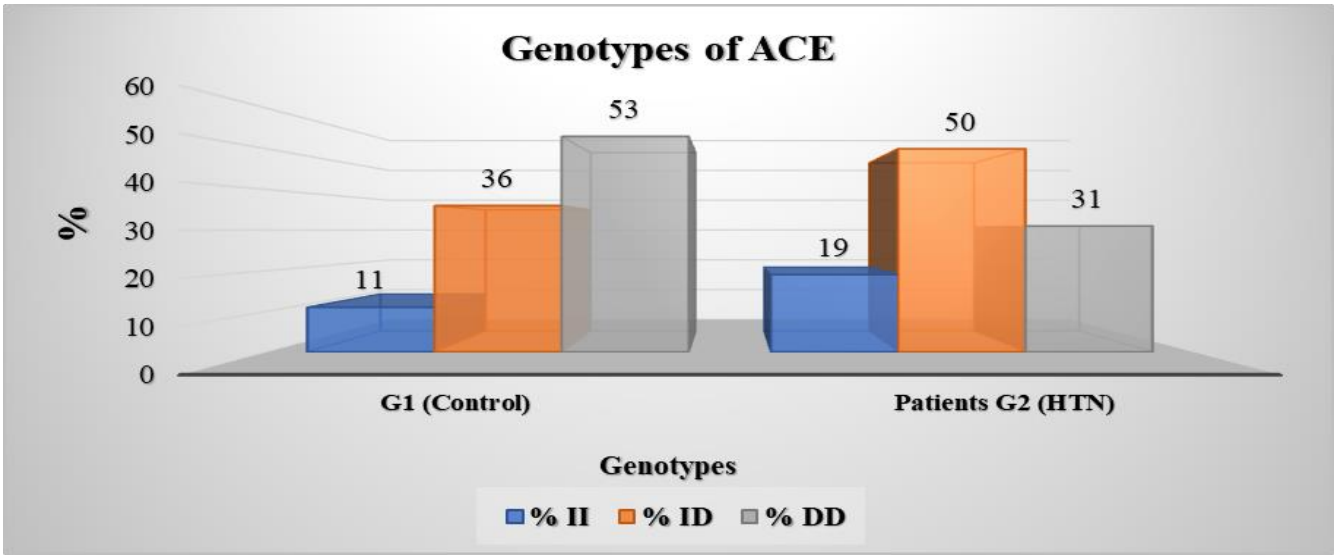

Figure 4. The percentage of II, ID, and DD genotypes of ACE in samples of Control, and hypertension patients.

Association between ACE I/D Gene Polymorphism and Risk of Hypertension (G2) as Compared with Control (G1)

The genotype frequencies and allele distributions of I/D polymorphism of ACE (I/D) for control (G1) and high blood pressure patients (G2) groups are shown in Table (4).The

results revealed a significant association between ACE (I / D) I, D-alleles, and hypertension where $\chi 2$ is 10.174 and (p-value = 0.006 < 0.05). This indicates that there was a significant relationship between this SNP and the risk of hypertension. Also, the results show that the frequencies were 11% for II, 36% for ID, and 53% for DD in G1 (Control). The frequencies were 19% for II, 50% for ID, and 31% for DD in G2 (High blood pressure patients). There was a significant association in I/D polymorphism of ACE between G1 healthy (Control) and G2 (High blood pressure patients) (p-value < 0.05) as shown in Table (4). In G1, the I allele frequency was 58 (29%) and the D allele was 142 (71%) and in G2 the frequencies were 88 (44%) and 112 (56%) for I and D alleles, respectively. Descriptive statistics analyses revealed that the I/D polymorphism, ID, and DD genotypes were compared with the II genotype there was a non-significant difference (p-value = 0.165 > 0.05), while when compared between ID and II genotypes with DD genotype we found there was a significant difference (p-value = 0.003 < 0.05). There was also a statistically significant relationship between the D allele and the I allele (p-value = 0.003 < 0.05), these results are consistent with the finding by [16], and [18]. The genotype frequencies and allele distributions of I/D polymorphism of ACE for Control (G1) and high blood pressure patients (G2) groups that showed in Table (4) calculated from Figures (1 and 2).

Table 4. The genotypes and allele distribution of ACE (I/D) polymorphism in G1 (Control) and G2 (Hypertension).

Polymorphisms ACE (I/D)	**G1 (Control) N=100(%)**	**G2 (HBP) N=100(%)**	**X^2**	**p value**	**OR (95%CI)**	**p value**
II ID DD	11 36 53	19 50 31	10.174	0.006*	1.0^{ref} (1.0^{ref}) 1.244 (0.582-2.931) 2.953 (1.244-7.012)	 0.618 0.012*
I allele D allele	58(29%) 142(71%)	88(44%) 112(56%)	9.708	0.003*	1.0^{ref} (1.0^{ref}) 1.924 (1.272-2.910)	 0.002*
II ID&DD	11 89	19 81	2.510	0.165	1.0^{ref} (1.0^{ref}) 1.898 (0.852-4.229)	 0.113

DD ID&II	53 47	31 69	9.934	0.003*	1.0[ref] (1.0[ref]) 2.510 (1.408-4.473)	0.002*

Based on the values in Table (4), found that individuals carrying the ID genotype of I/D manifested no effect on the increased risk of high blood pressure in comparison with those carrying the II genotype (OR = 1.244, 95%Cl = 0.582 - 2.931, p-value = 0.618 > 0.05), these results are consistent with the finding by [16,18]. While the DD genotype of I/D manifested an increased risk of high blood pressure compared with those carrying the II genotype (OR = 2.953, 95%Cl = 1.272 - 7.012, p-value = 0.012 < 0.05), these results are consistent with the finding by [17,18]. In the dominant model, observed that the ID & DD genotype of I/D showed no effect on increasing risk of high blood pressure compared with the II genotype (OR = 1.898, 95%Cl = 0.852 - 4.229, p-value = 0.113 > 0.05), these results are consistent with the finding by [17]. While in the recessive model, there was showed a significant association found when compared ID & II genotype with DD genotype, where (OR = 2.510, 95%Cl = 1.408 - 4.473, P-value = 0.002 < 0.05), these results are consistent with the finding by [18].

Conclusions

In summary, the study found a great association between parameters (TG, TC, and VLDL) in Iraqi patients with high blood pressure, as their P-value was influential (P-value < 0.05), which showed an increased risk of high blood pressure. Found a great association between ACE gene polymorphisms and high blood pressure; observed that individuals carrying the DD genotype of I/D, the D allele of I/D, and the ID&DD genotype of I/D compared with the DD genotype showed an increased risk of high blood pressure (p-value < 0.05).

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