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Detection of the Association between ACE Gene Polymorphism and Lipid Profiles Level in Some Iraqi Hypertensive Patients

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ABSTRACT

Angiotensin-converting enzyme (ACE) plays a central role in the control of hypertension. ACE insertion/deletion (I/D) polymorphism has been reported to affect lipid profiles. There has not been any study on the influence of ACE I/D gene polymorphism on lipid levels in Iraqi hypertensive patients. The present study was therefore aimed at examining the association between ACE I/D polymorphism with hypertension and its effects on lipid levels in some Iraqi patients. Blood specimens were obtained from seventy hypertensive patients (43 males and 27 females) and forty healthy individuals with identical ages. The blood samples were divided into two parts; serum was recovered from the first part and used for estimating lipid levels. The second part of the blood samples was utilized for extracting genomic DNAs which were used to detect ACE I/D polymorphism. The results of the lipid profile analyses indicated a significant increase ($p<0.01$) in the treatment group compared with control group. The frequencies and odds ratio values of mutant D allele and DD genotype were significantly high ($p<0.01$) in the experimental group in contrast to the control group. Also, high levels of the low-density lipoprotein and triglycerides were correlated with the presence of D allele in the treatment group ($p<0.05$). Conclusively, the DD genotype and D allele can be considered as indicators of hypertension and disorders of lipid profiles in Iraqi population.

***Keywords*:** ACE gene polymorphism, Hypertension, Iraqi population, Lipid profiles

Introduction

Hypertension is a condition in which arterial blood pressure increases slowly within a month or a year.[1] It is the significant hazard factor for cardiovascular maladies.[2] Hypertension is a typical, polygenic and complex issue resulting from the interactions between a few risk factors and environmental components.[3] The disease incidence has risen worldwide in both developed and developing countries.[4] Different genetic and environmental factors play an essential role in the incidence of high blood pressure.[5]
Hyperlipidemia is traditionally define as a condition in which the levels of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) in the plasma exceed an arbitrary normal limit, while high density lipoprotein cholesterol (HDL-C) level is low.[6] Hypertension and hyperlipidemia are important risk factors for cardiovascular disease and are the cause of many deaths in most countries with simple and moderate-income.[7] Hyperlipidemia is progressively essential in hypertension patients that have not been examined. Some studies have revealed that the TC, TG and lipoproteins may be irregular in patients with hypertension compared to normal people.[8] Many clinical tests have accurately shown that hyperlipidemia and hypertension are the most important risk factor for coronary artery disorder and cardiovascular.[9]
Differences in blood lipid levels and lipoprotein are considered key adjustable factors for the cardiovascular disease and have been recognized as independent risk factors for hypertension.[10]
Renin-Angiotensin System (RAS) is the main physiological controller of hypertension.[11] RAS plays the role of mediator in controlling blood pressure and body fluid status.[12] In RAS, angiotensin conversion enzyme (ACE) is the main enzyme.[13] It stimulates the conversion of inert angiotensin I to dynamic angiotensin II which is well-known to be involved with vascular hypertrophy, disruption of bradykinin, strong vasoconstriction and promotes atherosclerosis.[14] ACE is a glycosylated protein, expressed mainly in different tissues such as lung, kidneys, testis, duodenum and placenta.[15,16] Also, ACE that is expressed in endothelial cells especially cells of circulatory system degrades bradykinin.[17] ACE converts angiotensin I to angiotensin II essentially in the pulmonary circulation[18] Researchers revealed that low ACE level plays an essential role in the normal function of endothelial cells of cardiovascular system.[19] Genes that encode RAS proteins are considered as susceptibility factors for hypertension.[20] The ACE gene consists of 26 exons and 25 introns and is located on chromosome 17q23.3. Insertion or deletion (I/D) polymorphism includes the presence or absence of the Alu sequence of 287 bp in the intron sixteen of the ACE gene.[21] ACE I/D polymorphism (rs4646994) influences ACE levels. Individuals with II genotype have low levels of ACE compared with the DD genotype.[22] ACE II genotype is associated with a significant decrease in hypertension, in contrast to the DD genotype.[23] ACE I/D polymorphism indirectly affects the regulation of lipids.[24] ACE is connected with some cardiovascular abnormalities such as myocardial infarction,[25] cardiovascular diseases,[26] strokes and coronary heart disease.[27] Some studies have evaluated the relationship between ACE I/D polymorphism and hypertension in North Indian,[28] Chinese,[29] and Brazilian population.[21] However, few studies have assessed the relationship between ACE and lipid profiles in the Saudi population that determine the ACE I/D genotypes with familial hypercholesterolemia.[30] Similarly, in the Kuwaiti population that was carried out on Type 1 diabetic children.[31] To the best of our knowledge, there was no any study on the

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influence of ACE I/D gene polymorphism on lipid levels in Iraqi hypertensive patients. Therefore, this research was aimed at evaluating the association between ACE I/D polymorphism and its effects on lipid levels in Iraqi patients with hypertension in Salah Al-din province.

Materials and Methods

Study population
Seventy hypertensive patients (43 males and 27 females) diagnosed by specialist doctors in some private clinics in Salah Al-din province, Iraq volunteered for the study and served as the treatment group. Also, forty subjects of same sexes (ages 40 to 70 years) served as control group.

Ethical approval
Ethical approval for the conduct of the study was obtained from the Department of Biology, College of Sciences, University of Tikrit, Tikrit, Iraq with No. 3/7.

Sample collection
An aliquot of 5 ml blood sample was collected and divided into two: 2 ml was placed in an EDTA tube, stored at -20 ℃ for DNA extraction and the remaining 3 ml was kept in plastic tube for recovery of blood serum.

Lipid profile analyses and detection of ACE I/D polymorphism
The serum was used for lipid profile tests by employing a spectrophotometer and local commercial kits, performed according to the manufacturer's instructions. DNA was extracted as described;[32] concentration and purity were estimated by Nanodrop 2000. PCR was set up with 100 ng of DNA template, a set of primers and 2X GoTaq Green master mix (Promega USA) in a 25 ul reaction volume. The nucleotide sequences of the set of primers included, forward 5'- CTG GAG AGC CAC TCC CAT CCT TTC T-3' and reverse 5' GAC GTC GCC ATC ACA TTC GTC AGA T 3' as reported.[33] The PCR program as shown in Table 1 below.

Table 1: PCR Program

Stage 1	95 ℃	5 min	**1 cycle**
	94 ℃	45 sec	
Stage 2	56 ℃	45 sec	**30 cycle**
	72 ℃	45 sec	
Stage 3	72 ℃	7 min	**1 cycle**

The PCR products were electrophoresed on 2% agarose gel, stained with Redsafe and visualized by Gel Documentation system.

Statistical analysis
Data were analyzed using SPSS version 20 PC. Person's chi-square test was employed to calculate the allelic and genotypic frequencies of odd ratio (OR) and 95% confidence interval (CI) of the patients and control group. P value < 0.05 was considered significant, while p value < 0.01 was highly significant. Lipid levels between patients and control group, and among the ACE polymorphisms were compared using the student's t-test and ANOVA test.

Results and Discussion

Comparison between lipid levels of hypertensive patients and control group
Hypertension is perceived in detail as a significant hazard factor for cardiovascular disease, stroke, diabetes, and renal sicknesses.[34] The disease is also frequently associated with metabolic abnormalities such as lipid profile derangement.[35] In the present study, the results obtained for lipid profile analyses for 110 subjects (40 as control and 70 as hypertensive patients, of which 43 were males and 27 females) are presented in Table 2. There were abnormal levels of all lipids in the treatment group compared with healthy control group. The levels of TC, TG, VLDL and LDL were elevated significantly ($p \leq 0.01$), while HDL-C was observed to be low ($p \leq 0.01$) in the treatment group compared with control. The analyses of lipid profile clearly showed that the TC, TG, VLDL and LDL were higher significantly while the HDL level was lower at $p \leq 0.01$ in the experimental group compared with healthy control group. These variations were associated with obesity and insulin resistance, consequently are the reasons for both hypertension and dyslipidemia.[10] Several studies have shown that hypertensive patients have high levels of the LDL, TG, VLDL and cholesterol.[36] A study conducted in Baghdad province found the same results; high serum TC, TG, VLDL and LDL in patients with hypertension compared with healthy controls.[37] High fatty values are individual's susceptibility factor for hypertensive patients and play a central role in the progression of hypertension pathogenesis. It may increase the risk of heart disease, blood vessels and atherosclerosis.[4] HDL removes excess cholesterol from cells and stopping endothelial dysfunction. Also, Low HDL-C causes endothelial injury that leads to increased hypertension.[8] Statistical analyses of the treatment group according to gender showed no significant difference in the TC, TG, VLDL levels (Table 3). HDL level was significantly lower ($p = 0.006$) in male hypertensive patients (45.534 ± 6.996 mg/dL) than in female subjects (41.111 ± 6.203 mg/dL). Meanwhile, LDL level was higher (p value 0.002) in male patients in comparison with the females.

Genetic analysis of ACE gene polymorphism
ACE is an important factor in RAS and it stimulates the conversion of the angiotensin I into angiotensin II.[24] The ACE I/D variants affect ACE action and lead to changes in the RAS. Therefore, ACE I/D polymorphism is associated with changes in RAS system components and may cause obesity and insulin resistance.[13] In our study, ACE gene was PCR-amplified and when the products were separated on agarose gel electrophoresis, the results revealed two bands: 190 bp which represented D allele and 490 bp representing I allele (Fig. 1). The results indicated wildtype (II) with one band (490 bp), heterozygous (ID) with two bands (490 and 190 bp) and mutant homozygous (DD) with one band (190 bp). This may be the first study to detect ACE I/D gene polymorphism in Iraqi patients with hypertension. In this case, hypertensive patients had a significant difference in the genotypic and allelic frequencies. As shown in Table 4, patients had higher mutant DD genotype (68.58%) and allelic frequency (75.72%), while the II genotype was higher (40%) in the control than in the treatment group (17.14%). This indicated that the genetic makeup of the mutant genotype may be associated with hypertension which was reinforced by the high value of the odd ratio of the mutant DD genotype (3.56) and D allele (2.820) in the patient's group.
ACE I/D polymorphism has been studied significantly as a genetic risk element for high blood pressure, but some studies have revealed conflicting results. The ACE I/D genotypes and odds ratio (OR) in the treatment and healthy control groups (Table 5) showed that there were significant variances in the genotypes and allelic frequency between the treatment and control groups at p values (0.022) and (0.005) respectively. The results indicated that mutant DD genotype was more frequent in the experimental group, while II and ID genotypes were low in the control group. The OR of genotype DD (OR=3.56, CI 95% 1.41- 8.96) and allele (OR=2.820, CI 95% 1.572- 5.061) were significantly increased. From these results, it can be considered that D allele was associated with an increased hypertension disease in the Iraqi population. Furthermore, the relationship between lipid profiles and ACE polymorphism, lipid profiles of the genotypes were compared among the treatment group, as shown in Table 5. The results of HDL, TC and VLDL were not significant between all the genotypes. Meanwhile, LDL and TG were significantly higher ($p \leq 0.01$) in DD genotype compared with other genotypes (II and ID). Sometimes, genetic association studies give conflicting results in a similar population. Therefore, we compared our results with other studies conducted in different Arabian countries and found them similar. For instance, hypertension among Saudi subjects had a considerably higher frequency of the mutant D allele (98.3 % vs. 92.4%, $p = 0.028$, OR = 4.8) of the ACE gene.[38] Also, in Lebanon, the ACE I/D variant is common and related to an increased risk of hypertension.[39] Bayoumi and coworkers reported a high rate of D allele in the Arabian and African societies.[40] A study in Kuwaiti on Arabian children with idiopathic nephrotic syndrome found a relationship between the ACE D allele and clinical sign of idiopathic nephrotic syndrom.[41] Meanwhile, other studies did not detect any connection with the DD genotype, such as in Egyptian females.[13] These controversial findings may be caused by several possible factors including association which may be affected by ethnic differences, populations heterogeneity, impact of sample size and genetic-environmental connections.[42,43]

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Table 2: Comparison between lipid levels of hypertensive patients and control group

Parameter	Study population		*P* value
	Patient (N = 70)	Control (N = 40)	
HDL-C (mg/dL)	43.542 ± 5.222	52.05 ± 4.403	0.001**
TC (mg/dL)	222.828 ± 5.513	162.25 ± 3.290	0.001**
LDL-C (mg/dL)	152.414 ± 3.478	71.6 ± 4.755	0.001**
VLDL-C (mg/dL)	40.257 ± 2.678	32.375 ± 5.637	0.009**
TG (mg/dL)	187.357 ± 4.456	75.925 ± 4.074	0.001**

HDL-C: High density lipoprotein cholesterol; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low-density lipoprotein cholesterol; TG: Triglycerides; Values represents Mean ± SD. *p<0.05 significant and **p<0.01 high significant.

Table 3: Serum lipid levels of hypertensive patients according to gender

Parameter	Gender		*P* value
	Male (N = 43)	Female (N = 27)	
HDL-C (mg/dL)	45.534 ± 6.996	41.111 ± 6.203	0.006**
TC (mg/dL)	220.674 ± 4.914	230.518 ± 4.553	0.435
LDL-C (mg/dL)	151.372 ± 3.413	156.111 ± 5.875	0.569
VLDL-C (mg/dL)	41.488 ± 5.235	38.407 ± 2.438	0.495
TG (mg/dL)	195.357 ± 6.265	172.703 ± 9.705	0.002**

HDL-C: High density lipoprotein cholesterol; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low-density lipoprotein cholesterol; TG: Triglycerides; Values represents Mean ± SD. *p<0.05 significant and **p<0.01 high significant.

Table 4: Distribution of genotypic and allelic frequencies of ACE I/D gene polymorphism of hypertensive patients and control group

	Patient (N=70)		Control (N=40)				
Genotype	**No.**	**%**	**No.**	**%**	**p value**	**OR**	**(95 % CI)**
II	12	17.14	16	40		1 Ref.	-
ID	10	14.28	6	15	0.022*	2.22	0.63 - 7.83
DD	48	68.58	18	45		3.56	1.41 - 8.96
Alleles	**No.**	**%**	**No.**	**%**	**p value**		
I	34	24.28	38	47.5		1 Ref.	-
D	106	75.72	42	52.5	0.005**	2.820	1.572 to 5.061

*p<0.05 significant and **p<0.01 high significant.

Table 5: Serum lipid levels of hypertensive patients and control group according to ACE I/D polymorphism

	Genotype			
Parameter	**II (N=12)**	**ID (N=10)**	**DD (N=48)**	***P* value**
HDL-C (mg/dL)	42.833 ± 6.454	41.7 ± 3.401	44.104 ± 6.540	0.690
TC (mg/dL)	218.833 ± 7.089	204.2 ± 3.745	227.708 ± 4.256	0.328
LDL-C (mg/dL)	148.25 ± 6.209	136.7 ± 3.578	156.729 ± 4.719	0.005**
VLDL-C (mg/dL)	34.833 ± 4.043	38.5 ± 2.494	42.062 ± 2.023	0.056
TG (mg/dL)	172.181 ± 5.759	163.7 ± 5.501	191.479 ± 7.028	0.001**

HDL-C: High density lipoprotein cholesterol; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low-density lipoprotein cholesterol; TG: Triglycerides; Values represents Mean ± SD. *p<0.05 significant and **p<0.01 high significant.

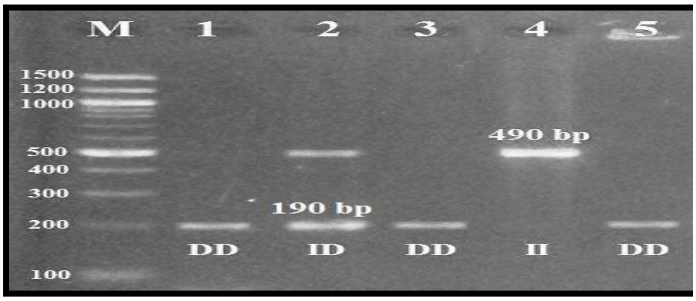

Figure 1: Agarose gel electrophoresis of the ACE Insertion/Deletion gene. Lane M: 100 bp DNA ladder; Lanes 1, 3 & 5: DD mutant homozygote; Lane 2: ID heterozygote; Lane 4: II wildtype homozygote.

Conclusion

The lipid profile level and ACE DD mutant genotype of the gene polymorphism were highly significant in patients with hypertension. Lipid levels of LDL-C and TG were correlated with the ACE I/D genotypes. Meanwhile, I/D polymorphism may be considered as an indicator of hypertension in Iraqi patients. Further research is required for the calculation of lipid profile and ACE levels compared with ACE gene polymorphism in different cities of Iraq, to confirm our findings

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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