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EVALUATION OF ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM AS A RISK FOR MYOCARDIAL INFARCTION WITH / WITHOUT TYPE 2 DIABETIC IRAQI PATIENTS

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ABSTRACT

Background: Ischemic heart disease (IHD) is the term given to heart problems the lipids in the blood are deposited on the end atrium due to abnormal lipids (metabolism). Due to the higher morbidity rate and mortality rate, IHD has become the most serious cardiovascular disease threatening in Iraqi people. The most common cause of myocardial infarction is atherosclerotic disease of an epicardial coronary artery (or arteries) sufficient to cause a regional reduction in myocardial blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery. It may affect individuals at any age, the most common risk factors include smoking, family history, hypertension, obesity, diabetes, high alcohol consumption, lack of exercise, stress, and hyperlipidemia. Various studies have shown that the polymorphisms angiotensin-converting enzyme ACE (DD) (rs4646994) associated with various heart diseases. **Objective:** To study the correlation between ACE (DD) (rs4646994) gene polymorphism with each of lipid profile and troponin I in hypertensive male Iraqi patients of myocardial infarction with / without type 2 diabetes mellitus. **Materials and Methods:** This case–control study composed of 217 adult males which were classified into three groups: **Group I** comprised 86 patients with myocardial infarction before catheterization with type 2 diabetes mellitus. **Group II** comprised 78 patients with myocardial infarction without T2DM which were admitted in Al-Hussein Medical City Teaching Hospitals / Kerbala Health Directorate - Iraq during 1st, Dec. 2018 to 31, July 2019. **Group III** comprised of 53 apparently healthy individuals as a control group. The risk factors include smoking, family history, hypertension and T2DM. DNA was extracted from whole blood and genotyping was achieved with specific (primers to amplify) the 3 genotypes by using PCR-ARMS techniques. **Results:** The high frequency of D allele in diabetic group act as independent risk factor. The observed data indicated that ACE-DD homotype was higher in T2DM patients as compared to controls. The genotypes for the ACE (DD)(rs4646994) gene band was observed in (190 pb) which indicate the removal a single nucleotide from gene that called homozygotes. **Conclusion:** A positive correlation of angiotensin-converting enzyme (DD) (rs4646994) gene polymorphism in myocardial infarction with and without T2DM for in Iraqi patients were observed.

KEYWORDS: Angiotensin converting enzyme (ACE) ; ischemic heart disease (IHD); type 2 diabetes mellitus (T2DM); myocardium infarction (MI).

INTRODUCTION

Ischemic heart disease 20% of worldwide mortality is the two leading causes of death on a global basis. Ischemic heart disease (IHD) is a large public health problem caused by narrowed heart arteries so, less blood and oxygen reaches the heart muscle and can ultimately lead to heart attack and is associated with a number of modifiable risk factors. The biological parameters of this aggregation are questioned and genetics could. The impact of risk factor confluence on IHD risk by testing whether genetic risk scores associated with these

factors,[1-3] The high prevalence of cardiac risk factors and associated morbidity have been reported in Iraqi adult population and responsible for about one-third or more of all deaths in people order over age 35.[4,5]

Myocardial infarction (MI), also known as a heart attack, occurs when blood flow decreases or stops to a part of the heart, causing damage to the heart muscle. The most common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck or jaw. Often it occurs in the center or left side of the chest and lasts for more than a few minutes. The discomfort may

occasionally feel like heartburn. Other symptoms may include shortness of breath, nausea, feeling faint, a cold sweat or feeling tired. About 30% of people have atypical symptoms.[6]

Most MIs occur due to coronary artery disease. Risk factors include high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet and excessive alcohol intake, among others. The complete blockage of a coronary artery caused by a rupture of an atherosclerotic plaque is usually the underlying mechanism of an MI. MIs are less commonly caused by coronary artery spasms, which may be due to cocaine, significant emotional stress and extreme cold, among others. A number of tests are useful to help with diagnosis, including electrocardiograms (ECGs), blood tests and coronary angiography.[7,8]

The most common cause of MI is atherosclerotic disease of an epicardial coronary artery (or arteries) sufficient to cause a regional reduction in myocardial blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery.[9] It may affect individuals at any age but becomes dramatically more common at progressively older ages, with approximately a tripling with each decade of life and males are affected more often than females, the most common risk factors include smoking, family history, hypertension, obesity, diabetes, high alcohol consumption, lack of exercise, stress, and hyperlipidemia.[10]

Common variant association studies have linked approximately 60 genetic loci to coronary risk. Large-scale gene sequencing efforts and functional studies have facilitated a better understanding of causal risk factors.[11]

Various gene polymorphisms including (serine/ threonine kinase 39 gene, tumor suppressor gene (*P53*) codon 72, C282Y mutations of (HFE) gene) have been studied in hypertensive patients with various heart attack.[12-14]

Angiotensin converting enzyme (ACE I/D) gene polymorphism was first reported by Rigat et al (1990). It is characterized by the presence or absence of a 287 bp Alu repeat sequence in intron 16 of the ACE gene and the most common genetic variation related to the RAS system is the insertion / deletion (ACE I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene. In general, variant D is more frequent in African or Caucasians and variant I is more frequent in the Asian population.[15] ACE gene is expressed in bone marrow cells and encodes angiotensin converting enzyme (ACE). It converts angiotensin I to active peptide angiotensin II, which stimulates proliferation of hematopoietic stem cells.[16]

Single nucleotide polymorphisms (SNPs) of RAS genes such as angiotensin-converting enzyme (ACE) are known to be associated with cardiovascular diseases.[17]

ACE gene is the most extensively studied gene, and I/D polymorphism of ACE gene is found to be strongly associated with the activity of ACE (120). DD genotype in ACE carry a significantly increased risk of myocardial infarction.[18] Association of angiotensin-converting enzyme (ACE) (rs4646994) D allele and susceptibility of coronary artery disease (CAD).[19]

The aim of the presented work is to study the correlation between ACE (DD) (rs4646994) gene polymorphism with each of lipid profile and troponin I in hypertensive ischemic heart disease (myocardial infarction) of Iraqi male with type 2 diabetes mellitus / without type 2 diabetic patients.

MATERIALS AND METHODS

The current case-control study comprised of 217 subjects classified into three groups. Group I composed of 86 myocardial infarction patients with type 2 diabetes mellitus. Group II composed of 78 myocardial infarction patients without type 2 diabetes mellitus, Group III composed of 53 apparently healthy individuals without any diseases as a control group.

The samples were collected from Cardiology center, Al-Hussein Teaching Hospital and Al-Zahraa Teaching Hospital, Al-Hussein Medical City, Kerbala Health Directorate / Kerbala – Iraq from the 1st Dec. 2018 to 31, July 2019. The biochemical investigations were performed in researches laboratory, department of biochemistry, college of medicine, university of Kerbala during the mentioned period. The exclusion criteria include: (1) female, (2) T1DM type, (3) patients treated with catheterization, (4) children.

The inclusion criteria include adult male selected patients diagnosed with one of the following diseases, MI with type 2 DM, MI without type 2 DM before preforming catheterization, with or without risk factor: smoking, family history , hypertension and T2DM.

All cases complete a detailed questionnaire that included information about age, sex, family history, drug history, medical history and other relevant information. Informed consent has been taken from all subjects. Kerbala Medical College Ethical Committee has approved the study protocol.

Five milliliters of blood was drawn from vein puncture from all individuals participated in this study. The collected blood was divided into three parts:[1] One ml of blood that used for molecular analysis, collected in EDTA containing tube and used for DNA extraction, then was analyzed directly to obtain high purity of DNA.[2] One ml placed in EDTA containing tube for analyzing HbA1c test.[3] Three ml of blood placed in gel tube for biochemical analysis. The DNA was extracted from whole-blood samples using the genomic DNA extraction Kit (Geneaid Biotech Ltd. UK).Then DNA

concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U.K.).

Genotyping for SNP ACE (I/D)(rs4646994) gene was performed by the polymerase chain reaction-

Amplification Refractory Mutation System (PCR-ARMS) method. For ACE (I/D) (rs4646994) gene using thermo cycler (Biometra, Germany). The primer sequence of ACE (I/D) (rs4646994) gene was:

Table (1): Primer sequence for alleles of ACE (DD)(rs4646994) gene.

Angiotensin converting enzyme gene primers	
Forward	5' CTG GAG ACC ACT CCC ATC CTT TCT 3'
Revers	5' GAT GTG GCC ATC ACA TTC GTC AGAT 3'

Primers were taken in a lyophilized state. The units of a lyophilized primer are known as a mass in Pico-moles. The subsequent steps were done for the reconstitution and dilution of the primers:

The tube was centrifuged at 10000 rpm for 5-10 min before de-capping. The chosen volume from nuclease free water were added according to the manufacturer to obtain a 100 p-moles /µL (master stock). The primers

were re-mixed by suitable vortex. Ten microliters of the master stock were transported to a 0.5 mL eppendorf tube that contained 90 µL of nuclease free water to get a 10 pmoles/µL (working stock). The master stock and working stock were kept at -20 °C. The working stock was warmed up and kept on ice for use in PCR and then stored at -20 °C after each use. The PCR program for ACE (DD) (rs4646994) gene show in Table (2).

Table (2): The program of PCR for three genotype of ACE (DD) (rs4646994) gene.

Type of cycle	Temperature C°	Time	No. of cycles
Initial denaturation	95	5 min	1
Denaturation	95	30 sec	35
Annealing	60	30 sec	
Extension	72	40 sec	
Final extension	72	5 min	1
Hold	4	10 sec	

Mean and standard deviation (M ± SD) were statistically measured. All data were analyzed using statistical IMB analysis system SPSS software X27.

RESULTS

The total male patients included in this study were (164) of myocardial infarction with / without type II diabetes mellitus, the mean ± SD of their age was (56 ± 9) years while for the control group, the mean ± SD of 53 individuals was (46 ±18) years as shown in Table 3. The age of patients was distributed widely between different age groups adult and elderly. Most patient whom had MI with T2DM were of the elderly category (>60 years).

The young adult category (20-39 years) rarely had any of the studied diseases. In correlation between the age and the tested for diseases these results were shown to be highly significant (P value <0.001).

The aging of the population worldwide will result in increasing numbers of elderly patients, among whom heart disease is the leading cause of death. Changes in cardiovascular physiology with normal aging and prevalent comorbidities result in differences in the effects of common cardiac problems as well as the response to their treatments.[20]

Table (3): Age groups distribution among all collected samples.

Sample groups	Age groups,\ years			Total
	20-39	40-59	>60	
Control	28	5	20	53
MI with T2DM	9	31	46	86
MI without T2DM	1	57	20	78
Total	38	92	58	217

***P* value <0.01**

Normal aging is associated with a decreased compliance of the central arteries due to a number of age-related changes in the structural components in the artery. Older people have increased amounts of collagen in the arterial

wall, and the collagen fibers have more permanent cross-linkages with other collagen fibers due to the non-enzymatic effects of advanced glycation end-products (AGE).[21]

Various molecular and biochemical studies have been performed on Iraqi patients including the genetic polymorphism and their relations with various biomarkers in sera of hypertention and ischemic heart diseases.[22] Fig. 1, indicate the genotypes of angiotensin converting enzyme gene polymorphism in myocardial infarction with / without T2DM. Angiotensin converting enzyme, ACE (II, ID and DD) (rs4646994) gene polymorphisms was for the homozygous and heterozygous. Genetic studies of the renin-angiotensin system have indicated that the ACE gene polymorphisms are associated with cardiovascular disease as indicated in this study, see Tables 4 and 5.
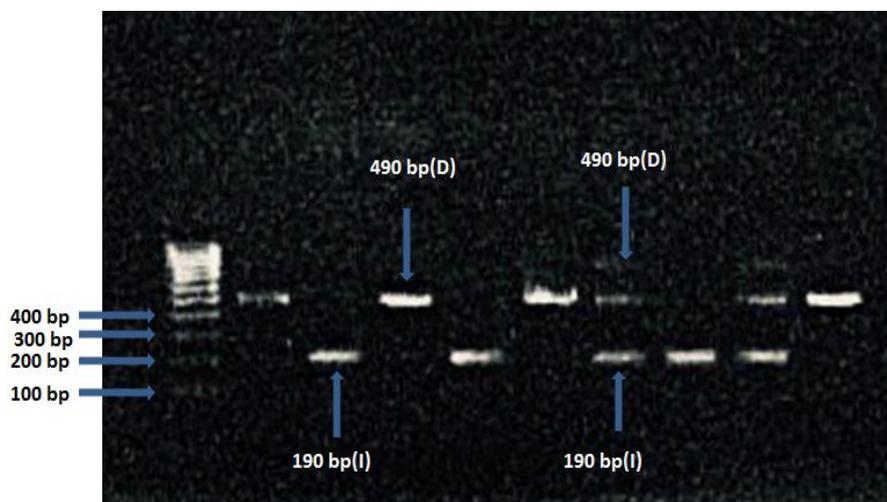

Figure (1): Genotypes for the ACE (II, ID and DD) (rs4646994) gene polymorphisms of homozygous and heterozygous individuals.

The D genotype had a band in (190 pb) that remove a single nucleotide from gene and it was called homozygotes, I genotype had a band in (490 pb) that insertion a single nucleotide from gene that called homozygotes while, ID genotype had two band I and D in the same gene that mean in the same gene had insertion and deletion single nucleotide it's called heterozygotes, so we see two band in the same location differ in speed of migration as indicated in Fig. 1.

Table (4): Distribution of ACE I/D (genotypes and alleles) in myocardial infarction patients with T2DM as compared with control.

ACE (II,ID and DD) (rs4646994) genotype	MI with T2DM N = 86	Control N = 53	Odds ratio	CI 95%	*P value*
DD (Ref)	27	5	-	-	-
II	45	29	3.48	(1.2 – 10.07)	≤ 0.05
ID	14	19	7.3	(2.26 – 23.8)	≤ 0.01

Table (5): Distribution of ACE I/D (genotypes and alleles) in myocardial infarction patients without T2DM as compared with control group.

ACE (II,ID and DD) (rs4646994) genotype	MI without T2DM N = 78	Control N = 53	Odds ratio	CI 95%	*P value*
DD (Ref)	10	5	-	-	-
II	66	29	0.88	(0.28 – 2.8)	0.8
ID	2	19	19	(3.11 – 116.1)	≤ 0.01

Table 6 and 7 below indicate the association between some biomarkers investigated with the angiotensin converting enzyme gene DD allele (rs4646994) in T2DM patient with ischemic heart diseases. As shown in both in Tables 6-8, only VLDL-C and TG indicate a highly significant *P value*.

Table (6): Biochemical characteristics in relation to DD allele of angiotensin converting enzyme (rs4646994) gene in MI with T2DM as compared with control.

Biomarker D/D allele	Control Mean ± SD	MI with T2DM Mean ± SD	P value
HDL-C	40 ± 17	36 ± 12	0.6
LDL-C	101 ± 20	128 ± 33	0.1
VLDL-C	15 ± 4	30 ± 8	≤ 0.01
TG	73 ± 20	196 ± 83	≤ 0.01
HbA1c	5±0.5	8±0.7	≤0.01

*Past3(t-test)

Table (7): Biochemical characteristics in relation to DD allele of angiotensin converting enzyme (rs4646994) gene in MI without T2DM as compared with control group.

Biomarker D/D allele	Control Mean ± SD	MI without T2DM patients Mean ± SD	P value
HDL-C	40 ± 17	34 ± 6	0.37
LDL-C	101 ± 20	110 ± 39	0.66
VLDL-C	15 ± 4	21 ± 4	≤ 0.05
TG	73 ± 20	99 ± 17	≤ 0.05
HbA1c	5±0.5	5±0.3	≤0.01

* Past3(t-test)

Table (8): Biochemical characteristics in relation to DD allele of angiotensin converting enzyme (rs4646994) gene in MI patients with T2DM as compared with MI without T2DM.

Biomarker D/D allele	MI with T2DM Mean ± SD	MI without T2DM Mean ± SD	P value
HDL-C	36 ± 12	34 ± 6	0.69
LDL-C	128 ± 33	110 ± 39	0.177
VLDL-C	30 ± 8	21 ± 4	≤ 0.01
TG	196 ± 83	99 ± 17	≤ 0.01

* Past3 (t-test)

Cardiac troponin are used to identify patients who would benefit from urgent revascularization for acute coronary syndromes and might be used in patients with stable ischemic heart disease to identify those at high risk for cardiovascular events who might also benefit from prompt coronary revascularization. The cardiac troponin concentration was an independent predictor of death from cardiovascular causes, myocardial infarction, or stroke in patients who had both type 2 diabetes and stable ischemic heart disease.[23]

Figure 2 Below indicate the troponin biomarker in this study in T2DM with and without ischemic heart diseases.
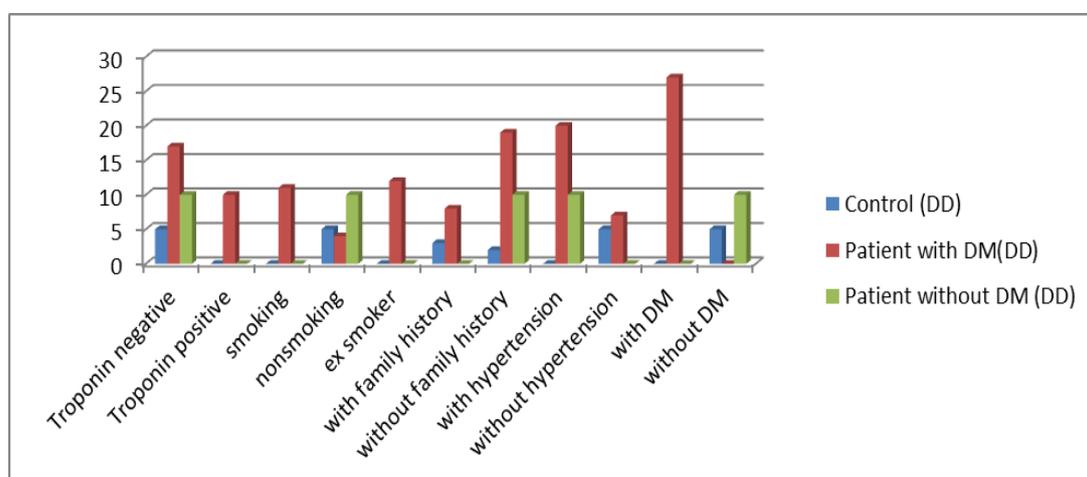
Figure (2): Comparison of result collected from patients of ischemic heart diseases with and without T2DM and control according to DD genotype.

The highest level for troponin negative was observed in figure (1) for patient of IHD with T2DM, whereas in troponin positive biomarker was patient of IHD with T2DM only. For the smokers and ex-smokers they contain only patients of IHD with DM, while non-smokers had the highest percentage of ischemic patients without DM and the lowest for ischemic patients with T2DM.

DISCUSSION

IHD is a polygenic disease that involves complex interactions among several pathophysiological pathways with multiple genes and environmental risk factors.[24] We analyzed several traditional cardiovascular risk factors in patients with CAD. This analysis revealed the diabetes, hypertension, and dyslipidemia have significant effects on the presence of CAD. These results are consistent with studies performed in Egypt, Iran, Spain, and Germany.[25] There are several pathophysiology mechanisms that explain diabetes, hypertension, and dyslipidemia in the contribution of CAD. Abnormal processes such as damage to the arterial wall, metabolic disorders, oxidative stress, and endothelial dysfunction can lead to arterial sclerosis, lesions of the coronary artery, and increase in the risk of development of atheromatous plaque.[26]

The aging of the population worldwide will result in increasing numbers of elderly patients, among whom heart disease is the leading cause of death which is in agreement with our result. Changes in cardiovascular physiology with normal aging and prevalent comorbidities result in differences in the effects of common cardiac problems as well as the response to their treatments.[27] Normal aging is associated with a decreased compliance of the central arteries due to a number of age-related changes in the structural components in the artery. Older people have increased amounts of collagen in the arterial wall, and the collagen fibers have more permanent cross-linkages with other collagen fibers due to the nonenzymatic effects of advanced glycation end-products (AGE),[28] The DD genotype showed band at 190 bp, II genotype showed band at 490 bp while ID genotype showed both bands at 190 and 490 bp as shown in figure.[1]

The ACE DD genotype may contribute to thrombogenic risk or vasoconstriction factors rather than the atherogenic risk factors.[27] Higher lipid levels in older patients with T2DM carrying the D allele,[29] genotypes influence the response of plasma lipids to treatment with fluvastatin. Subjects with the DD genotype had a greater reduction in plasma levels of LDL-C than those with ID or II genotypes. Similarly, the reductions in plasma levels of total cholesterol.[30]

The high frequency of D allele in diabetic group act as independent risk factor. Other population studies reported association of ACE I/D polymorphism with T2DM, thereby demonstrating geographical and racial/ethnic variations of ACE I/D polymorphism with T2DM.[31] According to the observed data, ACE-DD homotype was higher in T2DM patients as compared to controls. Similar results were obtained in other previous study which revealed higher frequency of DD genotype in diabetic patients compared to controls. The positive association of ACE-DD genotype with T2DM was demonstrated in patients with diverse ethnic populations whereas negative association was found in others in which the DD genotype is associated with twice the normal level of serum ACE activity.[32] Furthermore, recent findings, have shown an association between the ACE D allele, diabetes, and the risk of developing cardiovascular disease in European people which is in agreement with our findings.[33]

Renin–angiotensin system (RAS) plays a central role in the regulation of sodium metabolism, vascular tone, blood pressure, renal hemodynamics, and vascular modeling. In diabetes mellitus, activation of the RAS by hyperglycemia may be the key mechanism and effects seem to be amplified with adverse consequences such as atherosclerosis and occlusive microangiopath. Suggestive evidence for this notion is the impressive beneficial effect of pharmacological interference with the RAS in large vessel disease as well as in renal and retinal microangiopathy.[34]

Smoking is a potent risk factor for cardiovascular morbidity and mortality. In IHD, the formation of an occlusive thrombus at the site of rupture of a plaque in the coronary arteries leads to reduced circulation to that part of the myocardium and compromises its contractility, eventually causing heart failure, Smoking is a major risk factor for IHD. The deletion (D) allele of the angiotensin converting enzyme (ACE) gene polymorphism has been associated with hypertension, ischemic stroke and myocardial infarction. Polymorphism and cigarette smoking influence the intima-media thickness (IMT) of the carotid artery. The subjects carrying only one of the risk factors (the D allele or smoking) did not show significant differences in IMT when compared with homozygous nonsmokers.[35]

Both family history group and without family history group had the highest percentage of ischemic patient with DM as compared with the control group. In another study that family history of diabetes has a major public health impact on diabetes in the United States. In spite of the recent interest and focus on genomics and precision medicine, family health history continues to be an integral component of public health campaigns to identify persons at high risk for developing type 2 DM and early detection of diabetes to prevent or delay complications.[36] Prospective study indicated that family history was an independent risk factor for IHD in China. The individuals with family history were at high risk.[37]

The group with hypertension had higher incidence to IHD with T2DM and the lowest for IHD patients without

T2DM. The group without hypertension had the higher level with patient of IHD with T2DM but the lowest with control. In another study ACE gene (DD) is known to be associated with the occurrence of ischaemic stroke through its effect on pathogenesis of atherosclerosis and hypertension.[38] The possible reason for association between DD genotype and hypertension could lie in the structure of ACE and its circulatory levels. Biochemical investigations have proved that individuals with DD genotype contain higher levels of circulatory ACE. However, this association weakens with the increase in age.[39] The levels of circulatory ACE decreases gradually from DD, ID to II. On other hand, structural studies indicated that D allele of ACE contains C-domain (in addition to N-domain), which binds more efficiently to Angiotensin I. So, higher plasma ACE levels and more efficient binding to Angiotensin I increase the circulatory levels of bioactive Angiotensin II, which causes vasoconstriction that reduces arterial elasticity and leads to hypertension,[40] The DD genotype acts synergistically with increased blood pressure to increase the risk of coronary disease. However, it also appears that the ACE I/D polymorphism may be a promoter by itself in the development of hypertension. Multiple maladaptive pathways play roles in this process. Angiotensin converting enzyme D allele carriers have been found to have higher tissue ACE messenger RNA expression, increased concentrations of circulating ACE, and increased bradykinin degradation. These may impair endothelium-dependent vasodilatation that generates damaged blood vessels and contributes to hypertension.[22,41]

The ACE DD individuals and compared II genotypic group, the frequencies of person that carry ACE D allele (DD+ID) was significantly higher in hypertensive groups than controls in patient in Syria and,[42] which is in agreement with our study.

In our study the correlation between lipid profile for DD genotype of Angiotensin Converting Enzyme (rs4646994) gene indicate that the LDL-C had highest level in patient with T2DM. Angiotensin-converting enzyme (ACE) DD (rs4646994) gene predisposes to type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).[43]

CONCLUSION

Angiotensin-converting enzyme (DD) polymorphism was associated with IHD (MI and UA) and type2 DM. Carriers of the homozygous genotype (DD) genotype of (rs4646994) have association and increased risk of development of IHD and the T2DM increased the progression and complication for IHD.

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