

Angiotensin-I-Converting Enzyme (ACE) Insertion/Deletion Polymorphism in Egyptian Patients with Coronary Artery Disease

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ABSTRACT

Angiotensin-I-converting enzyme (ACE) insertion/deletion (I/D) polymorphism has been associated with the genetic susceptibility to coronary artery disease (CAD) and also with the lipid profile in different populations. One hundred and eight Egyptian patients with CAD and one hundred forty three unrelated healthy subjects were included in the study. ACE I/D polymorphism was detected by polymerase chain reaction. We investigated the association between I/D polymorphism of the ACE gene and the presence of CAD as well as its association with hypertension in the patients. Serum levels of lipoprotein (a), Lp (a), and interleukin-6 (IL-6) were estimated in the patients as well as in the healthy subjects. Increased frequency of D allele and DD genotype as well as decreased frequency of I allele and ID genotype were found in CAD patients compared to healthy subjects ($P = .004$, $OR = 0.419$, 95% CI (.2 - .8), for DD genotype), ($P = .008$, $OR = 0.475$, 95% CI (0.27-0.84), for D allele). Higher levels of both Lp (a) and IL-6 were observed in CAD patients compared to control subjects ($P = .0012$, $P = .0346$, respectively). No association was observed in ACE gene polymorphism with diabetes mellitus or hyperlipidemia. However, there is a trend for DD genotype to develop more hypertension than ID genotype ($P = 0.08$, $OR = 2.88$). In conclusion, the I/D polymorphism of the ACE gene (carrying the D allele) is an independent risk factor for CAD in the studied Egyptian population.

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1. INTRODUCTION

Cardiovascular disease (CVD) refers to any disease of the heart and blood vessels. The most common ones are diseases of the heart muscle, strokes, heart attacks, heart failure and heart disease caused by high blood pressure. The renin-angiotensin system (RAS) has been shown to play a key role in the regulation of blood pressure and influence the cardiovascular system and several genes belonging to this system have been associated with coronary artery disease (CAD). According to its degrees of severity, CAD can be classified into chronic CAD (such as stable angina, unstable angina and variant angina) and acute CAD (e.g., myocardial infarction). One of the most intensively investigated genetic polymorphisms is the insertion/deletion (I/D) alleles of the angiotensin-I-converting enzyme (ACE) gene (Freitas et al., 2008).

The ACE is widely distributed in endothelial tissue, where it catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor and vascular smooth muscle growth stimulant and the breakdown of the vasodilator bradykinin to kinin degradation products (Indra and Annapoorani, 2008). By playing a pivotal role in these two cardiovascular hormonal regulatory systems, ACE has an important impact on cardiovascular structure and function.

The ACE gene, located on chromosome 17, has been cloned and found to be characterized in humans by a major insertion/deletion polymorphism consisting of the presence (insertion "I") or absence (deletion "D") of a 287-base pair (bp) alu repeat sequence within intron 16 (Ganesan et al., 2011; Bai et al., 2012). The I allele or a D allele forms 3 possible genotypes: II, ID and DD (Zhou et al., 2012).

As early as 1992 the relation between ACE gene polymorphism and cardiovascular diseases was reported by Cambien et al. Since then numerous studies have been done to evaluate this relationship (Acarturk et al., 2005; Alarcon et al., 2006; Freitas et al., 2008; Kim, 2009).

In 1963, Berg introduced a new class of lipoproteins named lipoprotein (a) abbreviated as Lp (a). This new lipoprotein appeared to be associated with the occurrence of coronary heart disease in men. Lp (a) can be considered as a genetically determined variant of low density lipoprotein (LDL), which is higher in density and size. The danger of Lp (a) lies in the fact that it is 10 times more atherogenic than LDL (Solfrizzi et al., 2006; Fayyaz et al., 2009).

The present work aims at studying the association, if any, between the insertion/deletion (I/D) polymorphism of the ACE gene with hypertension in dyslipidemic Egyptian patients with CAD and investigating the impact of multiple complications such as diabetes mellitus, hyperlipidemia with CAD on the polymorphism of ACE gene.

2. SUBJECTS AND METHODS

2.1 Patients

The studied population consists of 108 (73 male and 35 female) patients aging 34 - 75, (mean 53.54 ± 9.1 years). The cases have been selected to be diagnosed as chronic ischemic heart disease (IHD) and individuals having virus C or B were excluded from the study. When categorized according to the status of being hypertensive, hyperlipidemic or diabetic, we had the following: among the total study population, only 66 individuals had hypertension, 56 individuals were with hyperlipidemia and 46 individuals were diabetic. All cases were obese, while only the male cases were smokers.

2.2 Controls

The control group included 143 healthy participants aging between 37 - 58 years, (mean 45.3 ± 7.2 years). None of them had any signs or symptoms suggesting CAD. Based on medical history and cardiovascular questionnaire, all control subjects were free from any other diseases.

A blood sample was obtained from each patient who agreed to participate in the study. The study was also approved by the local Ethics Committee and informed consent was obtained from each participant. Fasting whole blood samples (5ml) were collected from the subjects under investigation by vein puncture using sterile disposable plastic syringes. The blood was divided into two parts, one part delivered to plain tubes and allowed to clot for 10-15 minutes, centrifuged and the separated serum is used to analyze the level of glucose, cholesterol, triglycerides, HDL-cholesterol. The remaining serum is aliquoted into two parts and packed in two clean dry eppendorf tubes and stored at -20°C until other analysis could be performed; namely: lipoprotein(a) and interleukin 6 by ELISA technique.

The second part of the whole blood was taken in EDTA coated tubes for extraction of DNA and then the PCR technique on these DNA samples has been applied.

2.3 Biochemical Analyses

Total cholesterol and triglyceride were determined by standard enzymatic methods. High-density lipoprotein cholesterol was assayed by selective precipitation with phosphotungstic acid-magnesium chloride and LDL cholesterol using the formula of Tunali and Yanardag, (2006). Lp (a) was determined by the Enzyme Linked Immunosorbent Assay using the Assay Max Human Lp (a) kit (USA) by the method of Wild et al., 1997. Interleukin-6 was estimated by The RayBio® Human IL-6 ELISA kit (USA) (Bauer and Herrmann., 1991).

2.4 Determination of Genotypes

Genomic DNA was isolated and purified from whole blood (from tubes containing EDTA) with the use of Fermentas spin columns (Canada), Angiotensin converting enzyme (ACE) genotypes were classified as II, ID and DD. Angiotensin converting enzyme gene D/D polymorphism was determined by polymerase chain reaction (PCR).

The reactions were performed according to the method of Rigat et al. (1990). The sense nucleotide primer was 5'- CTGGAGACCACTCCCATCCTTTCT-3' and the antisense primer was 5'-GATGTGGCCATCAATTCGTGAGAT-3'.

Polymerase chain reactions were performed in a final volume of 25 µl containing 200 µM of each dNTP, 2 mM of MgCl₂, 1× of PCR buffer, 50 pmol of each primer, 1 µg DNA and 1 U of Taq polymerase. Amplification was performed as follows: initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 2 min and extension at 72°C for 3 min. The PCR products were visualized by electrophoresis in a 2% agarose gel with ethidium bromide and the sizes of fragments were estimated by comparison with previously known size markers. The polymorphism detected by PCR was evident as a 490-bp fragment in the presence of the insertion (I allele) and as a 190-bp product in the absence of the insertion (D allele) and documented with a gel documentation system.

2.5 Statistical Analysis

Statistical analysis was performed with the statistical discovery software JMP 9. Quantitative data are presented as mean and SD (for normally distributed data), medians, and lower and upper quartiles (for skewed variables). Qualitative data are presented as frequencies. The Shapiro–Wilk test was used to determine whether random samples came from normal distribution. Non-normally distributed data have been logarithmically transformed. The Chi-square test with Yates' correction and Fisher exact test were used to compare categorical variables. One-way analysis of variance (ANOVA) test was used to compare variables with a normal distribution, whereas the Kruskal–Wallis analysis of variance (ANOVA) test was used to compare continuous variables with a distribution other than normal. A binary logistic regression model was used to test for the association of ACE I/D genotypes as well as lipid profile with diabetes mellitus status (0=non-diabetic, 1=diabetic), hypertension status (0=non-hypertensive, 1=hypertensive) and hyperlipidemia status (0=without hyperlipidemia, 1=with hyperlipidemia) by calculating the odds ratios (95% confidence interval) and corresponding P values. Significance was accepted at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the laboratory characteristics of the patients with CAD and healthy subjects. Patients with CAD were older, had a significantly higher concentration of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and atherogenic index than healthy subjects.

Patients with CAD have significantly higher levels of serum lipoprotein (a) as well as interleukin 6 when compared with healthy subjects. Both normolipidemic and hyperlipidemic coronary artery disease patients have significantly higher concentration of Lp (a) compared with healthy subjects. Interleukin-6 level is significantly higher in hyperlipidemic group than in healthy group but there is a statistically non-significant increase in its level in normolipidemic group in comparison with healthy group (Table 2).

The genotype distribution of the ACE/I/D polymorphism is in agreement with Hardy-Weinberg expectations. The genotype analysis shows an increased frequency of DD genotype (82.5% in patients versus 66.4% in healthy controls, $P = 0.004$, OR = 0.419, 95% CI = 0.2 – 0.8) in addition to an increased frequency of D allele (91.3% versus 83.2%, $P = 0.008$, OR = 0.475, 95% CI = 0.27 – 0.84) (Table 3).

Table1. Demographic characteristics of both coronary artery disease (CAD) cases and healthy volunteers

Parameter	Control (n=143) Mean $\pm$ SD	CAD cases (n=108) Mean $\pm$ SD Median(Q1 – Q3)	P-value
Age (years)	45.3 $\pm$ 7.2	53.54 $\pm$ 9.1	0.01*
Total cholesterol (mg/dl)	128.4 $\pm$ 22.99	215.5 $\pm$ 63.06	< 0.0001*
Triglyceride (TG) (mg/dl)	43 $\pm$ 6.6	173.9 $\pm$ 104.6 148 (94 - 227)	< 0.0001*
HDL (mg/dl)	38.72 $\pm$ 5.28	47.54 $\pm$ 24.98 41.6 (28.8 – 4702)	0.101
VLDL (mg/dl)	8.6 $\pm$ 1.32	34.35 $\pm$ 20.7 29.4 (18.8 – 45.0)	< 0.0001*
LDL (mg/dl)	68.08 $\pm$ 32.68	132.05 $\pm$ 63.36	< 0.0001*
Atherogenic index	2.35 $\pm$ 0.63	5.28 $\pm$ 4.26 3.84 (2.49 – 6.87)	0.0049*

SD= standard deviation, Q₁ = first quartile, Q₃ = third quartile. Only non-normally distributed data are expressed as Median (Q₁ – Q₃).

Table2. Serum levels of lipoprotein (a) and interleukin 6 in coronary artery disease (CAD) patients and healthy subjects

Parameter	Control Mean $\pm$ SD Median (Q1–Q3) n = 30	CAD cases Mean $\pm$ SD Median(Q1–Q3) All (n = 59)	Normolipidemic (n = 24)	Hyperlipidemic (n = 35)
Lipoprotein(a) (ng/ml)	2.67 $\pm$ 0.53	5.68 $\pm$ 3.91 4.34 (2.87-7.97)	5.02 $\pm$ 2.9 4.34 (2.98-7.13)	6.09 $\pm$ 4.35 5.72(2.71-7.74)
P-value		0.0012*	0.0012*	0.0013*
Interleukin 6 (pg/ml)	9.25 $\pm$ 3.95	14.66 $\pm$ 10.69 11.04 (8.24-17.77)	14.55 $\pm$ 11.15 11.04(8.24-14.84)	16.7 $\pm$ 13.4 11.04(8.24-21.82)
P-value		0.0346*	0.066	0.029*

SD= standard deviation, Q₁ = first quartile, Q₃ = third quartile. Non-normally distributed data are expressed as Median (Q₁ – Q₃). P-value versus control.

Table 3. Angiotensin converting enzyme (ACE) gene polymorphism in both coronary artery disease (CAD) cases and healthy volunteers

	Control n (%)	CAD casesn (%)	OR (CI 95%)	p-value
Genotype	48 (33.6)	18 (17.5)	Ref.	
ID	95 (66.4)	85 (82.5)	0.419 (0.2 – 0.8)	0.004* ¶
DD				
Allele frequency				
I	48 (16.8)	18 (8.7)	Ref.	
D	238 (83.2)	188 (91.3)	0.475 (0.27 – 0.84)	0.008* ¶¶

I: insertion polymorphism. D: deletion polymorphism. ID: insertion-deletion polymorphism.

Statistics: OR = odds ratio, CI = confidence interval, p-value: ¶ DD versus ID, ¶¶ D versus I.

A logistic regression model (Table 4) was used to adjust the odds ratio for hypertension and the ACE I/D genotypes, while controlling for other predictive variables. There is a trend for D

allele to be an independent risk factor in hypertensive CAD patients (odds ratio 2.88, $P=0.08$). A significant association is observed in age and LDL level with hypertension. However, no association has been observed between hypertension and either of hyperlipidemia, total cholesterol, triglycerides, Lp (a) and IL6.

A logistic regression analysis (Table 5) with diabetes mellitus as a dependent variable and the ACE I/D genotypes and other predictor parameters has been performed, There is no evidence that the ACE I/D genotypes can be regarded to be an independent risk factor for diabetes mellitus. However, age and hypertension have association with diabetes mellitus.

Table 6 shows that no association is found in the ACE I/D genotypes with hyperlipidemic CAD patients while triglycerides and LDL were correlated with hyperlipidemia.

The hypertensive group having DD genotype has a statistically significant increase in all lipid profile parameters except HDL-cholesterol (which has a significant decrease) and total cholesterol (which has non-significant decrease) in comparison with normotensive group having DD genotype. However the group with ID genotype in hypertensive patients has a significant decrease in triglycerides, VLDL cholesterol, LDL cholesterol ($P = 0.0001$) and a statistically significant increase in HDL cholesterol compared with ID genotype group without hypertension (Table7).

The group of hypertensive patients with DD genotype shows increased values of both glucose and creatinine and decreased values of lipoprotein(a) when compared with normotensive group. Similarly, the ID group in hypertensive patients has a significant decrease in the level of LP(a) Table(8).

3.2 Discussion

Beside very well known risk factors, genetic factors play a role in the development of coronary artery disease. Genetic factors differ in various populations. Among these, ACE gene polymorphism has been paid extensive attention and proposed as a coronary artery disease risk factor. ACE gene polymorphism determines the serum and tissue ACE activity which is high in subjects with DD genotype (Malik et al., 1997). ACE by causing high angiotensin II (Ang II) and low bradykinin levels may increase the risk of coronary artery disease (CAD) (Cambien et al., 1992). Angiotensin II increases the macrophage derived growth factor and platelet derived growth factor which have a role in the genesis of atherosclerosis. Furthermore, Ang II leads to LDL- C oxidation and stimulates neutrophil, macrophage and T-lymphocytes (Keidar et al., 1993). ACE decreases nitric oxide release via the bradykinin-kallikrein system and causes endothelial dysfunction which has also an important role in the genesis of atherosclerosis. Homozygous deletion subset of the ACE I/D polymorphism are associated with deteriorated endothelial function (Mulder et al., 2003).

Table 4. Logistic regression analysis for association of ACE I/D and other predictor variables with hypertension

Variables	β	SE	χ^2	Odds ratio	95% CI lower	95% CI upper	P value
Age(years)	0.0497	0.0235	4.4962	1.0510	0.9587	1.1522	0.0340*
Gender(male)	-0.2241	0.3481	0.4147	0.6387	0.1632	2.4997	0.5196
Diabetes mellitus	1.1479	0.3670	9.7848	9.9326	2.3568	41.8606	0.0018*
Hyperlipidemia	-0.0408	0.3134	0.0169	0.9217	0.2697	3.1491	0.8965
<i>ACE I/D genotype</i>							
DD genotype	0.6203	0.3903	2.5257	3.4574	0.7487	15.9655	0.1120
D allele	0.5304	0.3049	3.027066	2.8889	0.8744	9.5446	0.0819
TC	-0.0352	0.0224	2.4792	0.9654	0.8843	1.0539	0.1154
Triglycerides	0.0098	0.0087	1.2829	1.0099	0.9761	1.0448	0.2574
LDL	0.0429	0.0204	4.4442	1.0439	0.9638	1.1307	0.0350*
LP(a)	0.0728	0.1044	0.4856	1.0755	0.7142	1.6195	0.4859
IL6	0.0067	0.0364	0.0343	1.0068	0.8729	1.1612	0.8530
Creatinine	0.6521	2.5005	0.0680	1.9196	0.0001	34689.9861	0.7943

Table 5. Logistic regression analysis for association of ACE I/D and other predictor variables with diabetes mellitus

Variables	β	SE	χ^2	Odds ratio	95% CI lower	95% CI upper	P value
Age(years)	0.2016	0.0779	6.695816	1.2233	0.9014	1.6603	0.0097*
Gender(male)	-0.31092	0.31400	1.044707	0.5266	0.1539	1.8013	0.3067
Hypertension	1.07818	0.35060	9.862001	9.0033	2.2842	35.4868	0.0017*
Hyperlipidemia	-0.35964	0.29963	1.389861	0.4928	0.1519	1.5982	0.2384
<i>ACE I/D genotype:</i>							
DD genotype	-0.47306	0.41295	1.399004	0.3779	0.0754	1.8953	0.2369
D allele	-0.2554	0.2923	0.763734	0.6000	0.1908	1.8867	0.3822
TC	-0.0368	0.0264	1.949045	0.9638	0.8691	1.0689	0.1627
Triglycerides	0.0216	0.0111	3.759305	1.0218	0.9782	1.0675	0.0525
LDL	0.0391	0.0248	2.490569	1.0399	0.9436	1.1461	0.1145
LP(a)	-0.0604	0.0948	0.405392	0.9414	0.6492	1.3651	0.5243
IL6	-0.0454	0.0654	0.482098	0.9556	0.7394	1.2350	0.4875
Creatinine	-4.1217	3.6205	1.295981	0.0162	0.0000	23643.9705	0.2549

Table 6. Logistic regression analysis for association of ACE I/D and other predictor variables with hyperlipidemia

Variables	β	SE	χ^2	Odds ratio	95% CI lower	95% CI upper	P value
Age(years)	0.0117	0.0217	0.290440	1.0118	0.9293	1.1015	0.5899
Gender(male)	0.1032	0.2821	0.133774	1.2292	0.4068	3.7135	0.7146
Diabetesmellitus	-0.3776	0.3003	1.581565	0.4699	0.1448	1.5247	0.2085
Hypertension	-0.0727	0.3123	0.054248	0.8646	0.2542	2.9407	0.8158
ACE I/D genotype							
DD genotype	0.0014	0.3399	0.000016	0.9973	0.2631	3.7803	0.9968
D allele	-0.1498	0.2988	0.251123	0.7412	0.2297	2.3916	0.6163
Triglycerides	0.0110	0.0031	12.317270	1.0110	0.9987	1.0235	0.0004*
LDL	0.0724	0.0251	8.298817	1.0750	0.9742	1.1863	0.0040*
LP(a)	0.1868	0.1610	1.346554	1.2054	0.6414	2.2653	0.2459
IL6	-0.0066	0.0716	0.008453	0.9934	0.7504	1.3151	0.9267
Creatinine	5.6423	4.1211	1.874502	282.0967	0.0000	2925769350	0.1710

Table 7. Lipid profile according to ACE genotype for coronary artery disease (CAD) patients suffering from hypertension

Genotype (n)	Total cholesterol (mg/dl) mean $\pm$ SD	Triglycerides(mg/dl) mean $\pm$ SD Median (Q ₁ - Q ₃)	HDL (mg/dl) mean $\pm$ SD Median (Q ₁ -Q ₃)	VLDL (mg/dl) mean $\pm$ SD Median (Q ₁ -Q ₃)	LDL (mg/dl) mean $\pm$ SD Median (Q ₁ -Q ₃)	Atherogenic index mean $\pm$ SD Median (Q ₁ -Q ₃)
<i>Hypertensive</i>						
DD (54)	215.11 $\pm$ 64.31	183.96 $\pm$ 118.37	52.04 $\pm$ 34.13	36.79 $\pm$ 23.67	123.87 $\pm$ 67.11	5.12 $\pm$ 4.74
p ₁	0.974	137(100.5-221.5) < 0.0001*	41.6(28.8-67.2) < 0.0001*	27.4(20.1-44.3) 0.0023*	131.6(89.4-160.2) < 0.0001*	3.99(1.9-6.7) < 0.0001*
ID (9)	214.89 $\pm$ 77.92	160.00 $\pm$ 94.58	42.31 $\pm$ 14.56	32.00 $\pm$ 18.92	140.58 $\pm$ 75.50	4.58 $\pm$ 2.48
p ₂	0.082	117(91.5-223.5) < 0.0001*	44.8(27.2-54.4) < 0.0001*	23.4(18.3-44.7) 0.0011*	122.4(83.9-169.1) < 0.0001*	3.55(2.65-6.76) < 0.0001*
p	0.993	0.568	0.407	0.568	0.510	0.743
<i>Normotensive</i>						
DD (31)	217.10 $\pm$ 60.60	159.77 $\pm$ 86.73	48.74 $\pm$ 25.36	31.95 $\pm$ 17.35	140.94 $\pm$ 55.68	5.11 $\pm$ 4.07
		153.5(76-243.2)	41.6(30.4-67.2)	30.7(15.2-48.6)	143.8(92.7-177)	3.5(2.6-6.81)
ID (9)	217.50 $\pm$ 66.14	193.63 $\pm$ 122.23	48.00 $\pm$ 25.73	38.73 $\pm$ 24.45	130.78 $\pm$ 74.68	5.63 $\pm$ 5.09
		148(99-273)	35.2(32-51.2)	29.6(19.8-54.6)	169(130.8-176.2)	5.4(3.1-8.4)
p	0.908	0.3434	0.934	0.3434	0.765	0.708

SD= standard deviation, Q1 = first quartile, Q3 = third quartile. Non-normally distributed data are expressed as Median (Q1 – Q3). P = significant probability for comparison between DD and ID groups within each group. P₁ = significant probability for DD in hypertensive versus DD in normotensive groups. P₂ = significant probability for ID in hypertensive versus ID in normotensive groups.

Table 8. Age, glucose, creatinine, Lp(a) and IL6 according to ACE genotype for CAD patients suffering from hypertension

Genotype (n)	Age(years) mean $\pm$ SD	Glucose mean $\pm$ SD Median (Q ₁ – Q ₃)	Creatinine mean $\pm$ SD	Lp(a) mean $\pm$ SD Median (Q ₁ – Q ₃)	IL6 mean $\pm$ SD Median (Q ₁ – Q ₃)
<i>Hypertensive</i>					
DD (54)	54.20 $\pm$ 9.40	136.35 $\pm$ 78.87	1.04 $\pm$ 0.26	5.60 $\pm$ 4.57	14.41 $\pm$ 13.05
P ₁	< 0.0001*	121(88.5-172.5) 0.001*	< 0.0001*	4.3(2.4-6.9) < 0.0001*	9.28(7.84-16.3) 0.203
ID (9)	58.56 $\pm$ 9.62	170.33 $\pm$ 52.62	1.11 $\pm$ 0.30	6.77 $\pm$ 3.38	16.68 $\pm$ 8.19
P ₂	0.264	193(119-209.5) 0.222	< 0.0001*	5.87(3.67-9.77) 0.0001*	12.25(11.04-23.8) 0.522
P	0.207	0.219	0.465	0.486	0.683
<i>Normotensive</i>					
DD (31)	51.03 $\pm$ 9.10	126.60 $\pm$ 79.10	1.01 $\pm$ 0.19	5.26 $\pm$ 3.23	14.25 $\pm$ 11.61
		107(92-136)		5.3(2.74-7.4)	8.76(8.2-20.17)
ID (9)	51.89 $\pm$ 3.86	139.56 $\pm$ 110.87	1.07 $\pm$ 0.17	6.44 $\pm$ 2.98	22.59 $\pm$ 21.61
P	0.787	97.5(89.25-230.25) 0.665	0.388	7.35(3.6-9.67) 0.433	13.2(7.4-15.06) 0.319

SD= standard deviation, Q1 = first quartile, Q3 = third quartile. Non-normally distributed data are expressed as Median (Q1 – Q3). P = significant probability for comparison between DD and ID groups within each group. P₁ = significant probability for DD in hypertensive versus DD in normotensive groups. P₂ = significant probability for ID in hypertensive versus ID in normotensive groups.

It has been shown in Elsammak et al., 2011 that there is a significant increase in the frequency of DD genotype in Egyptian patients with hepatitis C virus when compared to healthy volunteers. Therefore, in the present study we excluded CAD patients with virus C or B in order to avoid the interference which can take place in studying the different genotypes of ACE gene and we found that the D allele and DD genotype showed high frequencies in Egyptian population with CAD compared to healthy controls whereas the frequencies of I allele and ID genotype were significantly low in patients compared to healthy subjects. II genotype did not appear in our sample population, so the DD genotype is a significant predictor of CAD in the population living in Delta area of Egypt. Our finding is in agreement with that of Alarcon et al. (2006) who found that there was an increase in DD genotype frequency in Mexican patients with coronary artery disease and similar results were reported in patients with CAD in Southern Turkey by Acarturk et al. (2005) and Abd El-Aziz et al. (2012). The present results are not in agreement with the results of Shafiee et al. (2010) who report that ACE DD genotype is not associated with increased risk of CAD in the Iranian population.

Lipoprotein (a) [Lp(a)] is an atherogenic particle that structurally resembles a low-density (LDL) particle but contains a molecule of apolipoprotein(a) attached to apolipoprotein B-100 by a disulphide bond. (Comlekci et al., 1997). In the present study we found a significant increase in the serum levels of lipoprotein (a) in total population of CAD patients compared with healthy controls. Similarly, the level of Lp(a) was still significantly increased in both groups (hyperlipidemic and normolipidemic patients) in comparison with healthy group. So, Lp(a) can be considered a significant risk factor in CAD and hyperlipidemia. The present findings agree well with the results of Foody et al. (2000); Rajasekhar et al. (2004) who found that Lp(a) was higher in CAD patients compared to healthy subjects. Gambhir et al. (2008) also found that elevated Lp(a) levels confer genetic predisposition to CAD in young Asian Indians.

There was a significant increase in all lipid parameters such as cholesterol, T.G, LDL, and VLDL in CAD patients in comparison with healthy subjects, this result agrees with the result reported by Daghash et al., 2007.

Interleukin-6 (IL-6) is a multifunctional cytokine and plays a central role in modulating the inflammatory response. IL-6 is produced by many cells, including endothelial cells, adipocytes and macrophages, and initiates the production of acute phase proteins such as C-reactive protein (CRP). Epidemiological and clinical studies have demonstrated that elevated concentrations within the normal range are associated with both risk for future myocardial infarction (MI) and increased cardiovascular mortality in healthy and diseased populations (Ljungman et al., 2009).

In the present study there was a highly significant increase in serum IL-6 level in all patients with CAD when compared with healthy controls. When the patients were divided into two groups according to hyperlipidemia, there was no significant difference in IL6 levels between normolipidemic group and control group while a significant increase was persistent in hyperlipidemic group when compared with controls. These results are in agreement with the results of Hudzik et al. (2011) who found that there is a strong correlation between IL-6 and the extent of asymptomatic left ventricular ejection fraction (LVSD) in patients with documented CAD. The later investigators stated that elevated IL-6 concentrations preceded progression to clinically overt heart failure (HF) and the higher the IL-6 concentration the earlier the manifestation of HF symptoms.

The present logistic analysis of association of ACE I/D and other predictor variables with diabetes mellitus demonstrated that age and hypertension are significantly associated with the incidence of diabetes mellitus but diabetes mellitus is not significantly associated with the ACE I/D genotype (Table 5). This result is in agreement with that of Kim (2009) who found no significant difference of glucose levels among ACE I/D genotypes. The logistic analysis of association of ACE I/D and hypertension showed also strong association between hypertension, age, diabetes mellitus and LDL cholesterol. Moreover there was a trend of association of DD genotype with hypertension (Table 4). The present results are in good agreement with Tripathi et al. (2006) and Kim (2009) who found that ACE DD genotype implicate a strong possible role in the hypertensive state.

No association was found between ACE I/D and hyperlipidemia (Table 6), a finding which was in accordance with that of Vargas-Alarcon et al. (2006) who reported an association of ACE I/D with the disease but not with the lipid levels.

The lipid profile according to ACE genotype for CAD patients with hypertension showed that DD genotype in hypertensive patients had more tendency to have higher levels of triglycerides and HDL but lower LDL level compared with normotensive patients. The results confirm those of Cardoso et al. (2008) who found that II genotype was associated with higher HDL cholesterol levels, especially in women.

The present study showed that DD genotype in hypertensive patients had significant increase in glucose level comparing to its level in DD genotype of normotensive patients. These results are not in agreement with those of Cardoso et al. (2008); Mittal et al. (2011) who found a tendency for glucose levels to be higher in II genotype. However the present results are in agreement with that of Bonnet et al. (2008). Lp (a) is significantly higher in both DD and ID genotypes of hypertensive patients in comparison with that of DD and ID genotypes in normotensive patients, a finding which agrees well with the results of Bhavani et al. (2003) who found Lp (a) was significantly higher in hypertensive patients than healthy controls. The finding of a correlation between ACE genotypes and Lp (a) level in Egyptian hypertensive patients has not been previously reported.

4. CONCLUSION

The insertion/deletion polymorphism of ACE gene (carrying D allele) is an independent risk factor for coronary artery disease in the studied Egyptian population and high levels of triglycerides and VLDL-C in DD genotype subjects may indicate a high cardiovascular risk factor of D allele. There is a trend of association of ACE I/D polymorphism with hypertension but not with hyperlipidemia or diabetes mellitus. Lipoprotein (a) and interleukin 6 are considered to be the more important independent risk factors in CAD.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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