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Archives of Applied Science Research, 2016, 8 (3):4-10
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

ACE Gene Insertion/Deletion Polymorphism in Coronary Artery Disease in the Saudi Population

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ABSTRACT

Genetic factors play a pivotal role in the development and pathogenesis of coronary artery disease (CAD). The angiotensin-converting enzyme (ACE) gene insertion (I)/deletion (D) polymorphism is one of the various genetic factors associated with CAD. In this study, we investigated the association between the ACE gene I/D polymorphism and CAD in the Saudi population. A total of 210 CAD patients were studied, while 103 age-matched healthy adults were used as control subjects. ACE I/D polymorphism was detected by electrophoresing the amplified PCR ACE product on an agarose gel, and several biochemical and behavioral markers were monitored.In the CAD group, we determined that the DD genotype frequency was 65.7%, whereas 27.6% of the patients carried the ID genotype, and 6.7% carried the II genotype. Within the control group, 57.3% carried the DD genotype, 25.2% carried the ID genotype, and 17.5%carried the II genotype. The odds ratio (OR) of the ACE genotype ID versus the II genotype at a 95% confidence interval (CI) was 2.87 (1.24-6.63, p <0.01), and the OR of the DD versus the II genotype at a 95% CI was 3.01 (1.40-6.44, p <0.004). Our results provide a significant association between the ID and DD ACE polymorphisms in CAD patients in the Saudi population.

***Keywords*:** ACE, allele, coronary artery disease, gene polymorphism, Saudi population

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INTRODUCTION

Coronary artery disease (CAD) is a multifactorial disease for which hyperlipidemia, hypertension, diabetes mellitus (DM) and family history are important risk factors. Angiotensin converting enzyme (ACE) plays an essential role in two physiological systems, one leading to the production of the vasoconstrictor, angiotensin II and the other to the degradation of the vasodilator, bradykinin. The wide distribution and multifunctional properties of these two peptides suggests that ACE could be involved in various pathophysiological conditions [1]. The discovery that ACE levels are under genetic control ushered in a new era of investigation, however most studies focused on an insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene as a marker for a functional polymorphism. Dzau (1994) published his remarks of the role of renin-angiotensin system in cardiovascular physiology and reported that ACE-1 is also involved in many pathological conditions including vasoconstriction, coronary thrombosis, heart failure and ventricular remodeling [2]. Recently, many single nucleotide polymorphisms (SNPs) were detected in the gene and the search for the locations of functional polymorphism became a hot topic for many investigators. Nevertheless, association studies on the I/D polymorphism and the clinical outcomes continued,

mostly with conflicting results. The ACE gene, located at chromosome 17q23 contains a polymorphism in intron 16 of an insertion (I) or deletion (D) of a 287 pair Alu repeat sequence which results in three genotypes as II, DD and ID [3,4] The relation between ACE gene polymorphism (DD genotype) and CAD first has been documented by Cambienet al., [5]. Since then numerous studies in the past 15 years have been conducted to evaluate the relationship [6,7,8,9,10] A large body of information showed a strong association between ACE D allele with the risk of CAD [7, 11] in different populations. However, some studies have shown negative associations [8, 12, 13, 14, 15]. In the present investigation we assessed the ACE genotype distribution in patients with angiographically documented CAD of Saudi population.

MATERIALS AND METHODS

Study Population

The study population recruited consisted of 210 patients (147 males and 63 females, aged between 31-89, mean 61.22±10.71 years) who had confirmed coronary angiography after their diagnosis of CAD in King Khalid University Hospital (KKUH), Riyadh, Saudi Arabia. As a Routine procedure, an informed written consent was obtained from all patients as well as control individuals, the control group consisted of age-matched 103 individuals (58 males and 45 females) with no history of CAD. Some patients and controls had history of diabetes, hypertension and other general illnesses. Included subjects were of unrestricted age and gender who gave written informed consent to draw blood at the time of angiography or at the time of screening for research deoxyribonucleic acid (DNA) extraction to be used in studies approved by the hospital's institutional review board and was conducted in accordance with the guidelines set by the ethics committee of College of Medicine and Research Centre (CMRC) of King Saud University, Riyadh, Saudi Arabia. All subjects enrolled in this study were Saudi residents with similar dietary pattern. Key demographic data of subjects were recorded including age and gender lipid profile. Assessment of CAD was made by review of angiograms by the patient's cardiologist.

Ethical Approval

This study was conducted after review and approval of the Institutional Review Board of the Ethics Committee at KKUH, and all subjects gave written informed consent prior to participation.

Biochemical analysis

Blood samples for the glucose and lipid measurements were withdrawn from the patients and the control subjects after an overnight fast. The plasma glucose concentration was measured by the glucose oxidase method using a Biotrol Kit (BIOTROL, USA) on a Bayer opera analyzer (Bayer Diagnostics (Siemens), Germany). Serum total cholesterol was measured using the Biotrol commercial Kit, HDL cholesterol was determined with a commercial Randox Kit (Randox Laboratories Ltd., United Kingdom), LDL cholesterol was calculated by the formula of Friedwald, and triglyceride determination was made by the method of Lipase/Glycerol Kinase UV endpoint on the opera analyzer.

DNA Extraction

Genomic DNA was extracted from peripheral blood specimens, which were in tubes containing EDTA, which performed as an anticoagulant, using QIAamp DNA isolation Kit fromQIAGEN (Germany).

Determination of ACE I/D Polymorphism

Two oligonucleotide primers, sense: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and antisense: GAT GTG GCC ATC ACA TTC GTC AGT T-3' based on the flanking sequence of the insertion/deletion region on the ACE gene were used to amplify the corresponding DNA fragments (490 bp and 190 bp for the insertion and deletion alleles, respectively) by polymerase chain reaction (PCR). The reaction was performed in a standard 25 µL final volume using the isolated genomic DNA as template according to the following protocol: initial denaturation at 95 ^{0}C for 5 min; 35 cycles of denaturation at 95 ^{0}C for 30 s, annealing at 55 ^{0}C for 45 s, and extension at 72 ^{0}C for 90 s; and final extension for 5 min. The PCR products were separated by electrophoresis on 2.0% agarose gel and visualized by ethidium bromide staining. Gel electrophoresis of the PCR product gave a 190-bp fragment in the absence (D) and a 490-bp fragment in the presence of an insertion (I). Thus,for homozygote's, the amplicons showed 190/190-bp fragments for deletion/deletion (DD) or 490/490- bp fragments for insertion/insertion (II), while heterozygote showed both 490- and 190-bp fragments (ID) [4].

Direct Sequencing of DNA fragment

Sequencing of the PCR product was performed in KFSHRC, Riyadh, KSA, using 3730XL DNA Analyzer (Applied Biosystem, USA) using the same PCR forward or reverse primer. Nucleotide sequences were determined in both directions and the sequences were analyzed to determine the insertion and deletion. (The Accession Number of the ACE I/D sequence in CAD Saudi Patients is KJ140509 in GenBank).

Statistical Analysis

Measurement data were summarized by mean ± standard deviation (S.D.), and compared with two-sample *t*-test. A chi-square analysis was used to evaluate the allelic and genotypic frequencies that were calculated from the observed genotypic counts and to assess Hardy–Weinberg expectations. The same methodology was applied to comparisons between allelic and genotypic frequencies. Associations were determined as odds ratios (ORs) and 95% confidence intervals (CIs). (The odds of carrying a specific allele are defined as the frequency of subjects in whom it occurs divided by the frequency of subjects in whom it does not occur. The odds ratio for CAD is the odds of allelic carriage in the diseased [CAD] group divided by the odds in the control group. Univariate logistic regression was used to determine adjusted ORs for the genetic markers, conditioned on the major CAD risk factors. (*P* value <0.05 was considered statistically significant). Statistical analyses were performed with the Statistical Package for Social Sciences for Windows, version 20 (SPSS, Inc, Chicago, IL, USA).

RESULTS

The characteristics of the 313 study subjects (210 CAD patients and 103 control subjects) are presented in Table 1. The plasma fasting blood sugar (FBS), triglyceride (TG), total cholesterol (TC), and low-density lipoprotein-cholesterol (LDL-c) levels were significantly higher in the CAD group ($p < 0.0001$) compared to the normal control group, and HDL-c was lower in the CAD patients than in the controls. No statistically significant association was determined between the ACE genotype distribution and the lipid profile concentration (Table 2).

Table 1. Characteristics of the control and CAD patients

Characteristic	Controls *n*=103	CAD group *n*=210	*p* value
Age, years			
Mean ± SD	46.6016.69	61.2210.71	
Range	(20.0-78.0)	(31.0-89.0)	*p*< 0.0001
Gender			
Male, %	58(56.3%)	147(70%)	
Female, %	45(43.7%)	63(30%)	*p*< 0.0001
FBS, mmol/L			
Mean ± SD	4.480.66	8.083.53	
Range	(3.21-7.10)	(3.3-20.6)	*p*< 0.0001
TG, mmol/L			
Mean ± SD	1.110.28	1.871.23	
Range	(0.53-1.94)	(0.57-8.70)	*p*< 0.0001
TC, mmol/L			
Mean ± SD	3.810.56	4.161.03	*p*< 0.001
Range	(3.01-7.11)	(0.77-7.5)	
HDL-c, mmol/L			
Mean ± SD	1.240.38	1.150.98	
Range	(0.76-2.15)	(0.52-10.7)	*p*= 0.2302
LDL-c, mmol/L			
Mean ± SD	1.650.61	2.370.88	
Range	(0.86-4.5)	(0.59-5.89)	*p*< 0.0001

The data are represented as the mean ±SDs for all of the quantitative traits. Student's t-test and theχ2 test were used to compare the values of the controls and the CAD patients.

Table2. Plasma TG, TC, HDL-c, and LDL-c concentrations of various ACE genotypes in the CAD patient group

Genotype	TG (mmol/L)	TC (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)
ACE				
II	2.061.11	4.010.93	0.990.21	2.220.78
ID	1.821.20	4.170.94	1.221.10	2.350.80
DD	1.871.27	4.181.08	1.150.99	2.400.93
***p*value**	0.774	0.853	0.668	0.794

Diabetes mellitus, dyslipidemia, hypertension, and smoking were selected as potential risk factors. The major CAD risk factor frequencies are summarized in Table 3. Diabetes mellitus, dyslipidemia, hypertension, and smoking were more frequent in the CAD patient group than in the controls. Diabetes mellitus, dyslipidemia, hypertension, and smoking were confirmed as CAD risk factors (odds 22.29, 9.41, 23.57, and 3.55, respectively, $p < 0.0001$).

Table 3. CAD risk factors in the patients and control subjects

Parameter	CAD *n* = 210	Control *n* = 103	OR	95% CI	*p**
Diabetes mellitus					
Diabetic	137 (65.2%)	8 (7.8%)			
Non diabetic	73 (34.8%)	95(92.2%)	22.29	10.26-48.39	0.0001
Dyslipidemia					
Positive	121 (57.6%)	13 (12.6%)			
Negative	89 (24.4%)	90 (87.4%)	9.41	4.95-17.90	0.0001
Hypertension					
Hypertensive	155 (73.8)	11 (10.7%)			
Normotensive	55 (26.2)	92 (89.3%)	23.57	11.74-47.32	0.0001
Smoking					
Smoker	83 (39.5)	16 (15.5%)			
Nonsmoker	127 (60.5)	87 (84.5%)	3.55	1.95-6.48	0.0001

** p< 0.05; CI = confidence interval.*

The genotype frequencies did not deviate from the Hardy-Weinberg expectations in the controls or the CAD group (Table 4). The genotype distributions for the ACE genotype within the CAD group (n = 210) were as follow: 138 individuals were DD (65.7%), 58 (27.6%) carried the ID genotype and 14 (6.7%) carried the II genotype. Within the control group (n = 103), 59 individuals displayed the DD genotype (57.3%), 26 (25.2%) carried the ID genotype, and 18 (17.5%) carried the II genotype. The ACE allele distribution demonstrated that the CAD patients had a reduced II frequency and higher DD and ID frequencies compared with the control group (Table 4).

Table 4. ACE genotype distributions in Saudi CAD and healthy patients.

Genotype	Groups		Total (*n*= 313)
	Controls (*n*= 103)	CAD patients (*n*= 210)	
DD	59 (57.3%)	138 (65.7%)	197 (62.94%)
ID	26 (25.2%)	58 (27.6%)	84 (26.84%)
II	18 (17.5%)	14 (6.7%)	32(10.22%)

TheX^2test was used to compare genotype distributions between the control and CAD patients.

The ACE gene polymorphism allele frequency is summarized in Table 5. The I and D alleles were significantly higher in the CAD group compared with the controls.

Table 5. ACE allele frequencies in Saudi CAD and healthy patients

Alleles	Groups		Total	*P* value
	Controls (*n*= 103)	CAD patients (*n*= 210)		
D	144 (69.90%)	334 (79.52%)	478 (76.36%)	**0.005**
I	62 (30.10%)	86 (20.48%)	148 (23.64%)	
Total	206	420	626	

The X^2test was used to compare allele frequencies between the control and CAD patients.

The odds ratios of the ACE genotype II *vs.* the ID, the DD, and the DD+ID genotypes (95% CI) were2.87 (1.24-6.63, p<0.01), 3.01 (1.40-6.44, p<0.004), and 2.96 (1.40-6.23, p<0.004), respectively; hence, there was a significant association between the ID and DD ACE polymorphisms and CAD in the Saudi population (Table 6).

Table 6. CAD odds ratio associations with ACE genotypes

	OR	95%CI	*p**
ACE genotypes			
ID vs. II	2.87	(1.24-6.63)	0.01
DD vs. II	3.01	(1.40-6.44)	0.004
DD vs. ID	1.05	(0.60-1.82)	0.87
DD vs. ID and II	1.43	(0.88-2.32)	0.15
DD and ID vs. II	2.96	(1.40-6.23)	0.004

**p<0.05 CI = confidence interval.*

DISCUSSION

Coronary artery disease (CAD) is a multifactorial disorder with genotype and environmental interactions having an important role in its development. CAD is the leading cause of heart failure. The role of the rennin–angiotensin–aldosterone system in heart failure is well known and angiotensin converting enzyme (ACE) has a major role in this system [16]. ACE is also involved in many pathological conditions including vasoconstriction, coronary thrombosis and ventricular remodeling [2]. Niemic et al found a positive association between total high cholesterol, high LDL or overweight/obesity and the DD genotype, and also found this association, concerning dyslipidemia and obesity [19]. Obesity and hypercholesterolemia have been shown to play a role in ACEgene expression [17]. The presence of DD genotype increases the risk of developing CAD when associated to each of classical risk factors: hypertension, obesity, diabetes and hyperlipidemia considered as total cholesterol ≥200 mg/dl, TG ≥150 mg/dl, LDL cholesterol ≥130 mg/dl and HDL cholesterol ≤40 mg/dl [18].

The association between ACEI/D polymorphism and Type 2 diabetes have been rather controversial. Some studies have affirmed a clear association between DD genotype and the disease in Caucasians [20] while others have excluded this hypothesis [21].

A significant association of the ACE gene D allele with essential hypertension was documented in the African-American, Chinese, and Japanese populations [22, 23, 24, and 25]. On the other hand, the I allele was associated with high blood pressure in an Australian population with strong evidence of familial hypertension [26]. It has been suggested that the population heterogeneity in the association of ACE I/D polymorphism with essential hypertension may be due to significant variations of population backgrounds [27]. Smoking may increase ACE levels by means of a nicotine enhancement of ACEgene expression [28]. A large population based study [1] found a positive association between the D allele of the I/D polymorphism and carotid artery thickness among smokers: individuals carrying only one of the risk factors did not show significant differences in artery thickness when compared to non-smokers with II genotype, while carriers of both risk factors had significantly higher artery thickness. Niemic et al, found a synergistic effect due to smoking and ACEI/D DD join presence, [19]. As this study was performed in Saudi population, we may compare it to the other studies in the Middle East as an area with more racial and geographical and life style relationships. Previous study performed on Iranian male population by Shafiee et al [16], demonstrated DD genotype distribution was 54 % and 48.66 % in healthy subjects and patients suffering from CAD, respectively. In Turkish population ACE I/D polymorphism has been studied in several cardiovascular diseases, although the results varied in different types of the disease, for example Akbulut et al [29] found no significant relationship between DD genotype frequency and ischemic heart failure in CAD patients. On the other hand, Berdeli et al [30] reported a significant correlation between DD genotype and premature CAD in Turkish population. Isbir et al [31] reported that the greater frequency of deletion allele (D) in the CAD group than in the control subjects was significant. Bas¸ar et al [32] carried out a study on Turkish patients with peripheral vascular disease on Western part of the country and suggested that the ACE- ID genotype may be a risk factor for the disease and Tanriverdi et al [33] reported that the presence of D allele of ACE gene polymorphism is a risk factor for increased carotid intima-media thickness (IMT) which is an early sign of atherosclerosis. In Pakistani population, the frequency of the ACE-I/I genotype was significantly higher in hypertensive patients, aged 20–40 years, than in normotensive controls of the same age group, whereas no overall significant differences were observed between the II, ID and DD-ACE genotypes.

Gardemann et al. have shown a relation between the presence of CAD and ACE- D allele in a large case-controlled study [7]. Furthermore, DD genotype of the ACE gene has been reported as a risk factor for the development of early atherosclerosis also in carotid arteries in a Chinese population [34]. There are some other studies in which ACE I/D gene polymorphism has not been found to be associated with the prevalence of CAD [12, 36]. In a sample

in European population with the lowest risk ACE- I/D polymorphism have been reported not to be associated with an increased risk of CAD or myocardial infarction (MI) [8]. It has been suggested that the different results from the studies may be due to either the population-specific effect in relation to the ACE gene polymorphism or the lack of the documentation of CAD by means of coronary angiography [6, 12].

An association of the ACE DD genotype with the presence of CAD has been found in Australian population [6]. On the other hand van Bockxmeer reported that ACE I/D polymorphism did not play a role in the development of CAD or MI in Western Australian Caucasian population [37]. Another study showed that no association between ACE I/D polymorphism and CAD in 463 Caucasians [38].

CONCLUSION

We can state that the ACE I/D polymorphism patterns vary from population to others, and we can conclude that the ACE gene I/D polymorphism carrying the D alleles an independent risk factor for CAD in the studied Saudi population.

Acknowledgments
The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group project number RGP-VPP-173.

Conflict of Interests
The authors declare that they have no competing interests.

REFERENCES

[1] F. A. Sayed-Tabatabaei, B. A. Oostra, C. M. Isaacs, Duijn van and J. C. M. Witteman, *Circulation Research*,**2006**. 98 (9): 1123-1133.
[2] V. J. Dzau, *Journal of Hypertension*,**1994**. 12 (1): 3-10.
[3] M. G. Mattei, C. Hubert, F. Alhenc-Gelas, N. Roeckel, P. Corvol and F. Soubrier, *Cytogentics and Cell Genetics*,**1989**. 51: 1041-1045.
[4] B. Rigat, C. Hubert, P. Corvol and F. Soubrier, *Nucleic Acids Research*,**1992**. 20 (6): 1433-1438.
[5] F.Cambien, O. Poirier, L. Lecerf, A. Evans, J. P. Cambou, D. Arveiler, G. Luc, J. M. Bard, L. Bara and S. Richard, *Nature*,**1992**. 359: 641-644.
[6] X. L. Wang, R. M. McCredie and D. E. Wilcken, *ArteriosclerosisTrombosis and Vascular Biolology*, **1996**. 16: 115-119.
[7] A. Gardemann, M. Fink, J. Sticker, Q. D. Nguyen, J. Humme, N. Katz, H. Tillmanns, F. W. Hehrlein, M. Rau and W. Haberbosch, *Atherosclerosis*, **1992**. 139 (1): 153-159.
[8] J. Ferrieres, J. B. Ruidavets, J. Fauvel, B. Perret, D. Taraszkiewicz, J. Fourcade, M. Nieto, H,. Chap and J. Puel, *Atherosclerosis*, **1999**. 142 (1): 211-216.
[9] J. E. Eichner, V. J. Christiansen, W. E. Moore, S. T. Dunn and E. Schechter, *Atherosclerosis*, **2001**. 154(3): 673-679.
[10] E. Acarturk, G. Attila, A. Bozkurt, O.Akinar, S. Matyar and G. Sedaoglu, Journal of *Biochemistry and Molecular Biolology*,**2005**. 38(4): 486-490.
[11] N. %5Akar, O.Aras, K. 33%5Omürlü and S. %5Cin,*Scandinavian Journal of Clinical and Laboratory Investigation*, **1998**. 58 (6): 491-495.
[12] W. Friedl, F. Krempler, B. Paulweber, M. Pichler and F. A. Sandhofer, *Atherosclerosis*,**1995**. 112(2): 137-143.
[13] M. Pfohl M, M. Koch, s. Prescod, K. K. Haase, H. U. Haring and K. R. Karsch, *European Heart Journal*,**1999**. 18: 1318-1325.
[14] B. Agerholm-Larsen, B. G. Nordestgaard and A. Tybiaerg-Hansen, *ArteriosclerosisTrombosis and Vascular Biolology*,**2000**. 2: 484-492.
[15] I. Canavy, M. Henry, P. E. Morange, L. Tiret, O. Poirier, A.Ebagosti, M. Bory and I. Juhan-Vague, *Thrombosis and Haemostasis*,**2000**. 83 (2): 212-216.
[16] S.Shafiee, M. Firoozrai, S. Salimi, H. Zand, B. Hesabi and A. Mohebbi, *Pathophysiology*,**2010**. 17 (3): 163-167.
[17] K. Gorzelniak, S. Engeli, J. Janke, F. C. Luft and Sharma, *Journal of Hypertension*,**2002**. 20 (5): 965-973.

[18] A. I. Freitas, I. Mendonca, m. Brion, M. M. Sequeira, R. P. Reis, A. Carracedo and A. Brehm, *BMC Cardiovascular Disorders*,**2008**. 8(15): 1-12.
[19] P. Niemec, I. Zak and K. Wita, *Genetic Test*ing, **2007**. 11(4): 353-359.
[20] J. W. Stephens, S. S. Dhamrait, J. A. Cooper, J. Acharya, G. j. Miller, S. J. Hurel and S. E. Humphries, *Molecular Genetics and Metabolism*,**2005**. 84(1): 83-89.
[21] T. B. Grammer, W. Renner, S. von Karger, B. O. Boehm, B. R. Winkelmann and w. Maerz, *Molecular Genetics and Metabolism*,**2006**. 88(4): 378-380.
[22] K. Duru, S. Farrow, J. Wang, W. Lockbetteb and T. "Kurtz, *American Journal of Hypertension*,**1994.** 7(8): 759-762.
[23] F. T. Chiang, T. H. Chern, Z. P. Lai, C. D. Tseng, K. L. Hsu, H. M. Lo and Y. Z. Tseng, *Journal of Human Hypertens*ion, **1996** 10(12): 823-826.
[24] T. Morise, Y. Takeguchi and R. Takeda, *Lancet*,**1994**. 343: 125.
[25] Y. Nakano, T. Oshima, H. Hiraga, H. Matsuura, G. Kajiyama and M. Kambe, *Journal of Laboratory and Clinical Medicine*, **1998**. 131(6) 502-506.
[26] R. Y. L. Zee, Y. K. Lou, L. R. Griffiths and B. J. Morris, *Biochemical and Biophysical Research Communications*, **1992**. 184(1): 9-15.
[27] J. Barley, A. Blackwood, N. D. Cartere, D. E. Crew, J. K. Cruickshank, S. Jeffreys, A. O. Ogunlesi and G. A. Segnella, *Journal of Hypertension*, **1994**. 12(8): 955-957.
[28] S. Zhang, I. Day and S. Ye, *Atherosclerosis*,**2001**. 154(2): 277-283.
[29] T. Akbulut, T. Bilsel, S. Terzi, F. Ciloglu, S. Unal Dayi, N. Sayar, I. Peker and K. Yesilcimen, *European Journal of Medical Research*,**200l**. 8: 247–253.
[30] A. Berdeli, C. Sekuri, F. S. Cam, E. Ercan, A. Sagcan and I. Tengiz, *ClinicaChemicaActa*,**2005**. 351(1): 87–94.
[31] T. Isbir, H. Yilmaz, B. A˘gachan, M. Aydin and C. S. Isbir, *IUBMB Life*, **1999**. 48: 205–207.
[32] Y. Bas¸ar, N. Salmayenli, M. Aksoy, S. Seckin, M. Aydin and E. Ozkök, *Hormonal and Metabolic Research*, **2007**. 39(7): 534–537.
[33] H. Tanriverdi, H. Evrengul, H. Mergen, C. Acar, D. Seleci, O. Kuru, S. Tanriverdi and A. Kaftan, *Heart and Vessels*,**2007**. 22(1): 1–8.
[34] M. Ismail, N. Akhtar, M. Nasir, S. Firasat, Q. Ayub and S. Khaliq, *Journal of biochemistry and molecular biology*,**2004**.37: 552–555.
[35] J. R. Jeng, *American Journal of Hypertension*,**2000**. 13(1): 111-119.
[36] K. Lindpaintner, M. A. Pfeffe, R.Kreutz, M. J. Stampfer, F. Grodstein, F. LaMotte, J. Buring and C. H. Hennekens, The *New England Journal of Medicine*,**1995**. 332(11): 706-711.
[37] F. M. van Bockxmeer, C. D. Mamotte, V. Burke and R. R. Taylor, *Clinical Science*,**2000**. 99(3): 247-251.
[38] X. Jeunemaitre , F. Ledru, S. Battagli, M. T. Guillanneu, D. Courbon, C. Dumont, O. Darmon, L. Guize, J. L. Guermonprez, B. Diebold and P. Ducimetiere, *Human Genetics*,**1997**. 99(1): 66-73.