Apolipoprotein E, C1 and B Gene Polymorphisms in a Sample of Patients with Coronary Heart Disease in the Kuwaiti Population

Suzanne A. Al-Bustan^a Moussa Alkhalaf^b Ibrahim Al-Rashdan^{c, d}
Salwa Al-Otaibi^a Eman Al-Baker^a David Balding^e Majed A. Alnaqeeb^a

^aDepartment of Biological Sciences, Faculty of Science, and Departments of ^bBiochemistry and ^cMedicine, Faculty of Medicine, Kuwait University, and ^dChest Hospital, Al-Sabah Hospital Complex, Kuwait; ^eDepartment of Epidemiology and Public Health, Imperial College London, London, UK

Key Words

Apolipoproteins · Genetic polymorphisms · Coronary heart disease · Myocardial infarction

Abstract

Objectives: The objective of this study was to investigate the possible association of clinical variables and apolipoprotein (APOE, APOC1 and APOB) polymorphisms with the development of myocardial infarction (MI) and coronary heart disease (CHD) in Kuwaitis. **Subjects and Methods:** APOE, APOC1 and APOB genotypes were determined by polymerase chain reaction followed by restriction fragment length polymorphism in 143 Kuwaiti CHD patients with (n = 88) and without (n = 55) MI and in 122 controls matched for gender and age. Statistical and genetic analyses of the genotype, allele and haplotype frequencies, as well as regression analyses of genetic and clinical variables were done. **Results:** There was a statistically significant association between CHD and medical history of diabetes mellitus ($p < 0.001$), hypertension ($p < 0.01$), high cholesterol ($p < 0.05$) and family history of CHD ($p < 0.001$). A highly significant association ($p < 0.001$) was found, with an adjusted odds ratio of 9.32, for family history and the development of MI. No significant differences were found for allele or genotype frequencies between CHD patients and controls. **Conclusion:** The strong effect of family history suggests a major genetic component for the development of CHD in Kuwaitis, but this

association does not appear to be related to the APO genes studied here. The results in this study encourages future research into these and other polymorphisms and their potential association with MI and CHD in the Kuwaiti population.

Copyright © 2009 S. Karger AG, Basel

Introduction

Coronary heart disease (CHD) is one of the leading causes of death worldwide. Extensive studies have demonstrated that the interaction between various environmental factors and certain genetic polymorphisms leads to CHD. Diabetes, hypertension and obesity have been well documented as risk factors for CHD. During the past decades, scientists have been focusing on the identification and characterization of several genetic polymorphisms that may dispose certain individuals to CHD in the presence of 1 or more risk factors such as hypertension and diabetes. Lipids are well known to play a major role in the development of diabetes, hypertension and subsequently CHD. Therefore, attention is growing in trying to characterize genes responsible for lipid transport and metabolism. The majority of these genes form apolipoproteins, which are lipid-binding proteins that circulate in the bloodstream and are responsible for the transport of triglycerols, phospholipids, cholesterol and cholesteryl esters.

Table 1. Allele and genotype frequencies for APOE, APOCI and APOB in controls and CHD patients (n = 265)

	CHD			Controls (n = 122)
	MI+ (n = 88)	MI- (n = 55)	total (n = 143)	
APOE genotype				
E3/E3	72 (81.8)	42 (76.4)	114 (79.7)	98 (80.3)
E2/E3	2 (2.3)	1 (1.8)	3 (2.1)	2 (1.6)
E2/E2	4 (4.5)	4 (7.3)	8 (5.6)	9 (7.4)
E4/E3	8 (9.1)	7 (12.7)	15 (10.5)	9 (7.4)
E4/E2	2 (2.3)	0 (0.0)	2 (1.4)	3 (2.5)
E4/E4	0 (0.0)	1 (1.8)	1 (0.7)	1 (0.8)
APOE allele				
E4	0.057	0.082	0.066	0.057
E2	0.068	0.082	0.074	0.094
E3	0.875	0.836	0.860	0.849
APOCI genotype				
H1/H1	71 (80.7)	38 (69.0)	109 (76.2)	93 (76.2)
H1/H2	15 (17.0)	14 (25.5)	29 (20.3)	26 (21.3)
H2/H2	2 (2.3)	3 (5.5)	5 (3.5)	3 (2.5)
APOCI allele				
H1	0.892	0.818	0.864	0.869
H2	0.108	0.182	0.136	0.131
APOB genotype				
I/I	46 (52.3)	38 (69.0)	84 (58.7)	71 (58.2)
I/D	37 (42.0)	14 (25.5)	51 (35.7)	42 (34.4)
D/D	5 (5.7)	3 (5.5)	8 (5.6)	9 (7.4)
APOB allele				
I	0.733	0.818	0.766	0.754
D	0.267	0.182	0.234	0.246

Figures in parentheses are percentages. There were no significant differences ($p > 0.05$) as determined by the χ^2 test.

Apolipoprotein E (APOE) is a 299-amino-acid plasma glycoprotein synthesized in several organs including the liver [1] and plays an important role in the transport of lipids. It is associated with chylomicrons, high-density lipoproteins and, along with triglycerides, forms very-low-density lipoproteins [2]. APOCI, a constituent of triglyceride-rich lipoproteins, and high-density lipoproteins serve as cofactors in enzymatic reactions involving lipid metabolism [3]. The human APOE and APOCI genes are tightly linked in a 45-kilobase (kb) region of chromosome 19 [4, 5]. The wild-type APOE allele, E3, differs at 1 amino acid in exon 4 from each of the next 2 most common alleles: at residue 112 from cysteine to arginine (APOE4), or at residue 158 from arginine to cysteine (APOE2) [5, 6]. Several studies have conflicting findings with regard to the association of APOE polymorphisms and CHD [7–10].

A polymorphism at the APOCI gene locus, an *HpaI* restriction site, has been described as an insertion of CGTT at 317 bp from the 5' transcription initiation site [11]. The *HpaI*-negative allele (H1) has been reported to have a higher frequency in healthy individuals [12, 13]. The APOB gene is located on chromosome 2 and comprises 29 exons >43 kb [14]. A polymorphic segment in the promoter region codes for a signal peptide with 2 common alleles, a 27-amino-acid insertion allele (I) and a 24-amino-acid deletion allele (D) [15].

Few studies have attempted to characterize genetic polymorphisms and their association with CHD in Arabs [16, 17]. CHD is highly prevalent in Kuwait [18], a possible reflection of the high prevalence of diabetes (14.8%) [19], obesity (67.5% men and 72.9% women) [20] and hyperlipidemia (50%) [21]. In the last few decades, CHD has become the leading cause of death in Kuwaitis, with a 33% increase in mortality rate [18]. The objective of the present study was to characterize the APOE, APOCI and APOB polymorphisms in Kuwaiti CHD patients and controls in order to determine their possible associations with the development of myocardial infarction (MI) and/or CHD. In addition, the potential interaction between certain minor alleles at these loci with known risk factors, diabetes, hypertension and family history of CHD, was investigated in MI and CHD patients.

Subjects and Methods

Only Kuwaitis (n = 256) were included in the study. Of these, 143 were patients and 122 controls. The study was reviewed and approved by the ethics committee, Kuwait University, Safat, Kuwait. Both patients and controls gave informed consent. Of the 143 patients, 91 were males and 52 females, with documented CHD by either history of MI, history of angioplasty of 1 or more coronary vessels, or history of coronary artery bypass grafting, who were referred to Al-Sabah Chest Hospital for treatment. The mean age was 60.8 ± 12.1 years. The patients were classified according to the presence (MI+, n = 88) or absence (MI-, n = 55) of MI, confirmed by electrocardiographic documentation and cardiac enzyme elevation. For each patient, any medical history of diabetes, hypertension, dyslipidemia and or hypercholesterolemia was recorded in addition to family history of CHD as clinical variables. Lipid levels and profiles were not documented because many of the CHD patients were managed with medication for a period of time. A control group (n = 122, 65 males and 57 females), matched as far as possible to the patients' gender and age (mean age 57.1 ± 13.0 years), was recruited from individuals referred to Al-Amiri Hospital, Safat, Kuwait, for routine physical check-up. The controls were known to be free from CHD, based on electrocardiograms and biochemical data. All clinical variables (table 1) analyzed in this study were documented for both groups based on their diagnosis, medical files and interview.

Table 2. Frequency of clinical variables in the CHD patients (n = 143) and matched controls (n = 122)

	CHD			Controls
	MI+	MI–	total	
Medical history				
Hypertension	57 (64.8)	41 (74.5)	98 (68.5)**	62 (50.8)
Diabetes mellitus	55 (62.5)	44 (80.0)	99 (69.2)***	40 (32.8)
High cholesterol	46 (52.3)	23 (41.8)	69 (48.3)*	41 (33.9)
Family history				
CHD	38 (46.3)	16 (30.2)	54 (40.0)***	15 (12.7)
Hypertension	24 (29.3)	15 (28.3)	39 (28.9)	43 (36.4)
Diabetes mellitus	30 (36.6)	23 (43.4)	53 (39.3)	41 (34.7)
High cholesterol	8 (9.8)	7 (13.2)	15 (11.1)	13 (11.1)

Figures in parentheses are percentages. Some patients had more than 1 clinical variable. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, for difference between controls and CHD using Fisher's exact test.

Genetic Analysis

A sample of 5 ml blood was collected from each volunteer. Total genomic DNA was extracted from the 5 ml of whole blood using proteinase K digestion and salting-out extraction according to the method described by Miller et al. [22]. Genotyping of the APOE gene locus was performed by polymerase chain reaction (5 ng DNA) followed by restriction fragment length polymorphism (PCR-RFLP) with the *HhaI* restriction enzyme analysis based on the PCR method of Wenham et al. [23], and the products were resolved as described by Al-Bustan et al. [24]. Similarly, APOCI polymorphisms were typed by DNA amplification followed with *HpaI* restriction digest, as described by Klasen et al. [12]. APOB genotypes were analyzed by direct amplification of the promoter site as described by Xu et al. [25]. For quality control of the PCR-RFLP reactions, positive controls for the presence of the restriction site, confirmed by sequencing, were run in every reaction to ensure that no mistyping had occurred. The APOE2 homozygotes in excess of Hardy-Weinberg equilibrium (HWE) expectations were all confirmed by sequencing the PCR products amplified from these samples, using standard sequencing protocols on the ABI 3130xl Gene Analyzer.

Statistical Analysis

Allele and genotype frequencies for all the samples were determined at all 3 loci and tested for HWE using the GENEPOP software (Dos version 3.1) [26]. GENEPOP was also used both to implement the G-like test for population differentiation, by comparing cases and controls, and to confirm linkage disequilibrium between APOE and APOCI. Haplotype frequencies were estimated using the EM algorithm implemented in the Haplo.stats package of R. The clinical variables were analyzed using the statistical package SPSS (version 13.0). Association between pairs of qualitative variables was assessed by Fisher's exact test. Clinical variables and genotypes were compared across groups (MI+, MI–, controls) and for both sexes by the χ^2 test and/or Fischer's exact test. In addition, regular multinomial logistic regression analysis, employing logit link for the outcome variable, was carried out to compute adjusted odds ratios (AORs) and their 95% confidence intervals for

different factors in CHD patients with or without MI, after controlling for confounding. Power calculation on the sample size in this study was done by STATA software (version 8.0) to show if the sizes of the total sample and of the subgroups are sufficient to detect statistical differences in genotype distribution of APOE4.

Results

Genotype frequencies were in HWE at APOCI ($p > 0.05$) and APOB ($p > 0.05$), but not at APOE ($p < 0.001$). In addition, there was no significant difference in genotype or allele frequencies between CHD patients and controls (table 1; G-like test, $p > 0.05$), nor when CHD patients were divided into MI+ and MI–. APOE and APOCI were confirmed to be in strong linkage disequilibrium ($p < 0.0001$). Haplo.stats analysis showed that of the 6 possible haplotypes (data not shown), the most frequent (82.7%) was E3-H1 while no other haplotype gave a frequency estimate $>5\%$. There was no significant association of any haplotype to MI+ or to CHD.

The associations between clinical variables and CHD are summarized in table 2. There were no significant differences ($p > 0.05$) in the genotype, allele and clinical variables between males and females (data not shown). Medical history of diabetes mellitus, hypertension and high cholesterol were significantly ($p < 0.05$) associated with CHD. In addition, family history of CHD was highly significant ($p < 0.01$), with a frequency of 40.0% in CHD patients compared with 12.7% in controls. Multinomial regression analysis of MI+ and MI– patients and controls revealed highly significant ($p < 0.01$) unadjusted

Table 3. UORs and AORs comparing MI+ and MI- CHD patients with controls

	MI+ (n = 99)	MI- (n = 55)
UOR		
History of diabetes	3.42 (1.93–6.06)**	8.20 (3.83–17.55)**
History of hypertension	1.78 (1.01–3.13)*	2.83 (1.40–5.72)*
Family history of CHD	5.93 (2.96–11.9)**	2.97 (1.34–6.60)
APOE: E4 (+)	1.08 (0.45–5.58)	1.43 (0.55–3.67)
APOCI: H2 (+)	0.77 (0.39–1.51)	1.44 (0.71–2.91)
APOB: D (+)	1.27 (0.73–2.21)	0.62 (0.32–1.22)
AOR for family history		
With all variables	9.32 (3.78–23.0)**	3.70 (1.32–10.3)*
With APOE4	6.65 (3.01–14.7)**	3.85 (1.53–9.72)*
With APOE (E4) + APOCI (H2) + APOB (D)	6.67 (3.01–14.8)**	3.77 (1.49–9.51)*
AOR for all risk factors ¹		
With APOE: E4 (+)	1.66 (0.48–5.80)	1.05 (0.25–4.37)
With APOCI: H2 (+2)	0.62 (0.20–1.92)	1.74 (0.55–5.55)
With APOB: D (+)	1.33 (0.67–2.66)	0.62 (0.27–1.41)

AOR values are based on multinomial regression analysis adjusting for confounding variables. * $p < 0.05$, ** $p < 0.001$, significance level. ¹ Variables are given in table 2.

odds ratios (UORs) for diabetes mellitus 3.42 (1.93–6.06) and family history of CHD 5.93 (2.96–11.9), as well as statistical significance ($p < 0.05$) for hypertension UOR 1.78 (1.01–3.13) in MI+ patients. MI- patients had a higher UOR for diabetes mellitus and hypertension (table 3).

Discussion

The present study describes the association of known risk factors for CHD and their possible relation to the alleles at the APOE, APOCI and APOB gene loci in the Kuwaiti population. Although no statistical significance was found between the minor alleles APOE4, APOCI *HpaI* insert allele and APOB signal peptide deletion allele and CHD, generally, and MI, specifically, several significant findings were revealed. The statistically significant association between the medical history of diabetes mellitus, hypertension ($p < 0.01$), high cholesterol and family history ($p < 0.001$) with CHD in MI+ as well as in MI- patients (table 2) confirmed the established reports that diabetes mellitus increases the risk of CHD 3- to 4-fold [27]. In the present study, there was no statistical difference in the prevalence of diabetes between MI+ and MI-; however, the AOR estimate (table 3) was higher in MI- (2.83) than in MI+ patients (1.78). MI has been shown to be more extensive in CHD patients with diabetes, and

these have a lower survival rate compared with non-diabetic patients [28].

The significant association between hypertension and CHD of this study is in agreement with the Framingham study [29] that demonstrated an increased risk of CHD with blood pressure as well as with several other studies including those that explored the possible role of APOE4 allele [27, 30]. Given that multinomial regression analysis gave a higher UOR for hypertension in MI- compared with MI+ patients (table 3), it appears that both diabetes mellitus and hypertension played an important role in the development of CHD without the manifestation of MI, unlike in patients with MI in which other variables appeared to add to the risk of developing the disease. This is evident by the highly significant association of family history of CHD in MI+ (UOR 5.93, $p < 0.001$) compared with MI- patients (UOR 2.94, $p > 0.05$). Similarly, the odds ratio adjusted for confounding variables (AOR) was much higher for MI+ (AOR 9.32, $p < 0.001$) than for MI- patients (AOR 3.70, $p < 0.05$). These data further confirmed the reported role of family history as an independent risk factor for CHD [30].

In this study, the strong role of family history with CHD may reflect the role of genetic polymorphisms, which is more evident in the MI+ than in the MI- patients. Although this study did not find any significant association ($p > 0.05$) between the 3 APO loci and either

MI or CHD, the strong association of family history does suggest a major genetic component for the development of CHD in the Kuwaiti population, especially in the MI+ patients. Moreover, it appears that the 3 polymorphisms, APOE4, APOCI (*HpaI* insert) and APOB (signal peptide deletion), may play a secondary role in the development of CHD, as evident by the increased AOR (table 3) with family history in MI+ (9.32, $p < 0.001$) and MI- patients (3.70, $p < 0.05$). There has been controversy regarding the association of APOE4 with CHD. Several studies have shown APOE4 to be an independent predictor of CHD [8, 16, 27, 30], while others did not find such an association [7, 9, 10]. There has been only 1 study of an Arab population, which reported an association between APOE4 and coronary artery disease [16] yet failed to find an association between family history and CHD. It is difficult to assess with great certainty the role of APOE4 in the development of MI and CHD in the Kuwaiti population as the genotypic and allele frequencies were not in HWE ($p < 0.001$) in all CHD patients as well as in the controls. There was an excess of E2/E2 genotypes in the sample studied which may have caused the deviation from HWE. Sequencing all the homozygote (E2/E2) samples was reconfirmed, and hence, the result was not a genotyping error. It is possible that the excess E2/E2 genotypes could reflect the high prevalence of dyslipidemia in the Kuwaiti population [21]. Moreover, this result was not related to a possible bias in the sampling of patients and controls since the genotypes and allele frequencies for the other 2 gene loci analyzed, APOCI and APOB, were found to be in HWE ($p > 0.05$), suggesting randomized sampling. It is possible that these results were obtained simply by chance or the presence of APOE null alleles in the samples analyzed. The Kuwaiti population is heterogeneous [24] with evidence of admixture, as previously reported in a study that investigated APOE polymorphisms in the Kuwaiti population from different ethnic background and found them to be in HWE [24] for all 3 alleles. In addition, APOE4 frequencies in this study, 6.6% for CHD patients and 5.7% for the matched controls (table 1), differed from those reported by Al-Shammri et al. [31] who observed a higher frequency (9%) of the APOE4 in Kuwaiti healthy volunteers and a 12% frequency in patients with multiple sclerosis [31]. The differences in these results could be due to underlying ethnic bias in the samples collected for the controls that may have masked the effect of APOE polymorphism [14]. In this study, the association between lipid levels, APOE polymorphisms and CHD was not investigated because lipid level data prior to treatment and management for the samples analyzed were not available. Although the APOE4

variant binds normally to the low-density lipoprotein receptors, it has been shown to be associated with higher cholesterol concentrations and the concomitant increased risk for coronary artery disease [27]. However, there was a statistical difference ($p < 0.05$) between the patients (48.3%) and matched controls (33.9%) with regard to medical history of high cholesterol (table 2). Moreover, the mean age of the patient samples was 60.8 years. Therefore, it could be that the APOE genotypes and allele frequencies were not in HWE for the patient samples, since the sample comprised mostly the older group, and possibly, those carriers of APOE4 have been excluded from the sample pool by death. Gerdes et al. [7] reported an increased mortality risk from CHD for carriers of the APOE4 allele.

It is also worth pointing out that the *HpaI* insert allele of the APOCI gene was not associated with CHD, as previously reported [32]. However, the deletion allele of the signal peptide in the APOB gene locus had a higher frequency in CHD, and AOR in MI+ compared with MI- patients could indicate the possible role of APOB signal peptide and its relation to lipid transport in the development of CHD. Few studies have examined the genotype frequency of the APOB signal peptide polymorphism with regard to CHD.

Conclusion

The strong effect of family history suggests a major genetic component for the development of CHD in Kuwaitis, but this association does not appear to be related to the APO genes studied here. As family history was an independent risk factor in the CHD patients sampled in this study, future research into these and other polymorphisms and their potential association with MI and CHD in the Kuwaiti population is recommended.

Acknowledgments

This research was supported and partially funded by Kuwait University Research Administration, Projects SL05/03 and SL01/02, and by the Graduate School at Kuwait University. The authors would also like to acknowledge the General Facility Project (GS01/02) for the use of the ABI 3130xl Gene Analyzer. The authors extend their deepest appreciation and gratitude to all the participants in this study and to the technical assistance provided by Ms. Babitha Annice at the Faculty of Science, Dr. Thanaa Refai and the technical staff at the Clinical Laboratory in Al-Ameri Hospital, and to the technical staff at Al-Sabah Chest Hospital in Kuwait for their assistance with the blood collection.

References

- Mahley RW: Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;240:622–630.
- Siest G, Pillot T, Régis-Bailly A, Leininger-Muller B, Steinmetz J, Galteau M, Visvikis S: Apolipoprotein E: an important gene and protein to follow in laboratory medicine. *Clin Chem* 1995;41:1068–1086.
- Sehayek E, Eisenberg S: Mechanisms of inhibition of apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway. *J Biol Chem* 1991;266:18259–18267.
- Das JK, McPherson J, Burns GAP, Karathanasis SK, Breslow JL: Isolation, characterization, and mapping to chromosome 19 of the human lipoprotein E gene. *J Biol Chem* 1985;260:6240–6247.
- Smith JD: Apolipoprotein E4: an allele associated with many diseases. *Ann Med* 2000;32:118–127.
- Rall SJr, Mahley RW: The role of apolipoprotein E genetic variants in lipoprotein disorders. *J Intern Med* 1992;231:653–658.
- Gerdes L, Gerdes C, Kervinen K, Savolainen M, Klausen IC, Hansen P, Kesäniemi Y, Færgeman O: The apolipoprotein ε4 allele determines prognosis and the effect on prognosis of simvastatin in survivors of myocardial infarction: a substudy of the Scandinavian simvastatin survival study. *Circulation* 2000;101:1366–1371.
- Scuteri A, Bos A, Zonderman A, Brant L, Lakatta E, Fleg J: Is the Apo E4 allele an independent predictor of coronary events? *Am J Med* 2001;110:28–32.
- Kolovou G, Yiannakouris N, Hatzivassiliou M, Malakos J, Daskalova D, Hatzigeorgiou G, Cariolou M, Cokkinos D: Association of apolipoprotein E polymorphism with myocardial infarction in Greek patients with coronary artery disease. *Curr Med Res Opin* 2002;18:118–124.
- Liu S, Ma J, Ridker PM, Breslow JL, Stampfer MJ: A prospective study of the association between ApoE genotype and the risk of myocardial infarction among apparently healthy men. *Atherosclerosis* 2003;166:323–329.
- Xu Y, Berglund L, Ramakrishnan R, Mayeux R, Ngai C, Holleran S, Tycko B, Shachter N: A common *Hpa*I RFLP of apolipoprotein C-I increases gene transcription and exhibits an ethnically distinct pattern of linkage disequilibrium with the alleles of apolipoprotein E. *J Lipid Res* 1999;40:50–58.
- Klasen EC, Talmud PJ, Havekes L, de Wit E, van der Kooij-Meijis E, Smit M, Hansson G, Humphries SE: A common restriction fragment length polymorphism of the human apolipoprotein E gene and its relation to type III hyperlipidemia. *Hum Genet* 1987;75:244–247.
- Hubacek JA, Pitha J, Adamkova V, Skodova Z, Lanska V, Poledne R: Apolipoprotein E and apolipoprotein CI polymorphisms in the Czech population: almost complete linkage disequilibrium of the less frequent alleles of both polymorphisms. *Physiol Res* 2003;52:195–200.
- Blackhart BD, Ludwig EM, Pierotti VR, Caiati L, Onasch MA, Wallis SC, Powell L, Pease R, Knott TJ, Chu ML, Mahley RW, Scott J, McCarthy BJ, Levy-Wilson B: Structure of the human apolipoprotein B gene. *J Biol Chem* 1986;262:15364–15367.
- Visvikis S, Chan L, Siest G: An insertion deletion polymorphism in the signal peptide of the human apolipoprotein B gene. *Hum Genet* 1990;84:373–375.
- Dzimiri N, Meyer B, Hussain S, Basco C, Afrane B, Halees Z: Relevance of apolipoprotein E polymorphism for coronary artery disease in the Saudi population. *Arch Pathol Lab Med* 1999;123:1241–1245.
- Alkhalaf M, Al-Bustan S, Hamoda H, Abdella N: Polymorphism of p53 codon 72 in Kuwaiti with coronary artery disease and diabetes. *Int J Cardiol* 2005;115:1–6.
- Al-Adsani A, Memon A, Peneva A, Baidas G: Clinical epidemiology of acute myocardial infarction in Kuwait. *Acta Cardiol* 2000;55:17–23.
- Abdella N, Al Arouj M, Al Nakhi A, Al As-soussi A, Moussa M: Non-insulin-dependent diabetes in Kuwait: prevalence rates and associated risk factors. *Diabetes Res Clin Pract* 1998;42:187–196.
- Al-Isa AN: Prevalence of obesity among adult Kuwaitis: a cross-sectional study. *Int J Obes Relat Metab Disord* 1995;19:431–433.
- Akanji AO, Abdullah A, Tahzeeb S: Lipoprotein(a), tissue plasminogen activator and plasminogen activator inhibitor 1 levels in hyperlipidaemic patients in Kuwait. *Eur J Clin Invest* 1997;27:380–386.
- Miller SA, Dykes DD, Polesky HF: A simple salting-out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- Wenham PR, Newton CR, Price WH: Determination of apolipoprotein E genotypes by the amplification refractory mutation system. *Clin Chem* 1991;37:241–244.
- Al-Bustan SA, Alnaqeeb MA, Annice BG, Ibrahim G, Al-Rubaian J, Ahmed A, Refai TM: Apolipoprotein E genotyping among the healthy Kuwaiti population. *Hum Biol* 2005;77:487–498.
- Xu CF, Tikkanen MJ, Huttunen JK, Pietinen P, Butler R, Humphreys S, Talmud P: Apolipoprotein B signal peptide insertion deletion polymorphism is associated with Ag epitopes and involved in the determination of serum triglyceride levels. *J Lipid Res* 1990;31:1255–1261.
- Raymond M, Rousset F: GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 1995;86:248–249.
- Attila G, Acarturk F, Eskandari G, Akpınar O, Tuli A, Kanadasi M, Kayrin L: Effects of apolipoprotein E genotypes and other risk factors on the development of coronary artery disease in Southern Turkey. *Clin Chim Acta* 2001;12:191–196.
- Tarkun I, Cetinarıslan B, Cantürk Z, Tarkun P, Agacdiken A, Komsouglu B: Homocystein concentrations in type 2 diabetic patients with silent myocardial ischemia: a predictive marker. *J Diabetes Complications* 2004;18:165–168.
- Levy D, Kannel W: Cardiovascular risk: new insights from Framingham. *Am Heart J* 1983;116:266–272.
- Ranjith N, Pegoraro RJ, Rom L, Rajput MC, Naidoo DP: Lp(a) and apoE polymorphisms in young South African Indians with myocardial infarction. *Cardiovasc J S Afr* 2004;15:111–117.
- Al-Shammri S, Fatania H, Al-Radwan R, Akanji AO: The relationship of ApoE genetic polymorphism with susceptibility to multiple sclerosis and its clinical phenotypes in Kuwaiti Arab subjects. *Clin Chim Acta* 2005;351:203–207.
- Wang CH, Zhou X, Zhou GD, Tan XD, Han DF, Zheng F, Liu F: Genetic association of apoE and apoCI gene polymorphisms with coronary heart disease. *Zhonghua Liu Xing Bing Xue Za Zhi* 2004;25:982–985.