

Original Article

Association between Apolipoprotein E-polymorphism and Ischemic Heart Disease Patients With or Without Type 2 Diabetes Mellitus: A Preliminary Study in Kuwait

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Abstract

Background: We investigated the association between apolipoprotein E polymorphism and ischemic heart disease with or without type 2 diabetes in Kuwait and examined the impact of apolipoprotein E polymorphism in diabetic patients.

Methods: The present study was conducted from January 2005 to June 2006 in the Diabetic Clinic of Al-Amiri and Al-Sabah Hospitals in Kuwait City. Apolipoprotein E polymorphism was assessed in 250 subjects of which 83 were ischemic heart disease patients (41 diabetic and 42 non-diabetic) and 105 were diabetic patients without ischemic heart disease. Results were compared with 62 healthy controls. Apolipoprotein E polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism.

Results: Apolipoprotein E3 allele was the most commonly occurring form. The frequency of apolipoprotein E4 was higher in ischemic heart disease patients with type 2 diabetes (39%) and the non-diabetic (31%) group, but lower in the diabetic (20%) and control groups (16%).

Conclusion: Apolipoprotein E4 allele may be related to the development of ischemic heart disease in patients with or without type 2 diabetes in Kuwait. However, future studies with larger population sizes are needed to establish such relationship.

Keywords: apolipoproteins E; diabetes mellitus, type 2; genotype; Kuwait; myocardial ischemia

Introduction

Apoproteins, found on the surfaces of lipoproteins, provide structural stability and have critical roles in regulating lipoprotein metabolism. In humans, apolipoprotein E (ApoE) is involved in the absorption, synthesis and elimination of cholesterol; removal of chylomicron remnants, and hepatic clearance of dietary fat.^{1,2}

The gene for ApoE is located on chromosome 19. It exhibits genetic polymorphism with three alleles: e2, e3, e4.³ These alleles encode the protein isoforms E2, E3, and E4, respectively. However, for convenience both the protein isoforms and the alleles will be referred to as e2, e3, and e4. Structurally, the three isoforms differ in cysteine-arginine interchanges at two positions in the protein, i.e., in residues 112 and 158. Thus, ApoE3 contains cysteine at 112 and arginine at 158; ApoE2 contains cysteine at both positions; and ApoE4 contains arginine at both positions. ApoE gene is inherited as either homozygous (i.e., e2/2, e3/3 or e4/4) or heterozygous (i.e., e4/2, e4/3, or e3/2).³

The frequencies of the three alleles of ApoE (e2, e3, and e4) vary considerably around the world, but ApoE3 is the most common worldwide.³

The common ApoE polymorphism has an effect on chronic heart disease (CHD), Alzheimer's disease and human longevity.⁴ In the literature, clinical, and postmortem studies have demonstrated a

close relationship between the ApoE4 allele and the occurrence of myocardial infarction and coronary atherosclerosis.⁴

Coronary heart diseases have been related to a high mortality rate. Comparing patients with e3/3 and patients with e2/2 or e2/3 genotypes, Xiang et al. (2003) have demonstrated that the mortality rate of coronary artery disease in patients with e3/4 or e4/4 genotypes was the highest.⁵ Patients with diabetes mellitus have a high probability for developing heart diseases. In a study on patients with type 2 diabetes mellitus (T2DM), it was stated that the ApoE4 allele increased the risk of carotid artery atherosclerosis, particularly in the early stages of diabetes.⁶

There is little information from the Persian Gulf area regarding the distribution of ApoE polymorphism among T2DM and ischemic heart disease (IHD) patients.^{7,8} Hence, this study genotypically describes ApoE in diabetic and/or IHD patients in Kuwait.

Materials and Methods

Sample size and subjects

The study included 250 subjects; of which 188 patients visited the Diabetic Unit at Al-Amiri and Al-Sabah Hospitals in Kuwait between January, 2005 and June, 2006. Of the total, 128 were Kuwaiti and 60 were non-Kuwaiti Arab residents in Kuwait. The controls consisted of 62 healthy subjects; of which 38 and 24 subjects were Kuwaiti and non-Kuwaiti, respectively.

This was a case-control study. The sample size was determined at α -level of 5% and a power of 80% ($\beta=20\%$), using the Epi info™ program. Sample size was estimated to be 250 according to the prevalence of T2DM and IHD in Kuwait as follows: 105 in group I (T2DM patients), 42 in group II (IHD patients), 41 in group III (T2DM with IHD patients), and finally, 62 subjects in group IV (control group).

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Table 1. Distribution of the studied subjects according to different groups, gender and nationality.

Group Nationality and gender	DM		IHD		DM+IHD		Control		
	n	%	n	%	n	%	n	%	
Kuwaiti	Male	40	47.1	8	40.0	13	56.5	18	47.4
	Female	45	52.9	12	60.0	10	43.5	20	52.6
	Subtotal	85	100	20	100	23	100	38	100
Non-Kuwaiti	Male	8	40.0	12	54.5	10	55.6	12	50.0
	Female	12	60.0	10	45.5	8	44.4	12	50.0
	Subtotal	20	100	22	100	18	100	24	100
Total		105			42			41	
									62

DM=diabetes mellitus; IHD=ischemic heart disease.

Groups I and III were randomly selected from attendants of the Diabetic Unit in Al-Amiri and AL-Sabah Hospitals, Kuwait. Group II were randomly chosen patients who attended the Cardiology Outpatient Department at the same hospital. Finally, as controls, group IV consisted of healthy blood donors who visited the same hospital.

Clinical examination

Patients were reported as IHD because they had a history of myocardial infarction according to medical records or were assessed with unstable angina pectoris according to the World Health Organization (WHO) criteria.⁹

We performed a stress test for all negative subjects (non-ischemic), including control (healthy) subjects. All admission files were carefully reviewed for any reports by consulting doctors for any heart disease signs or symptoms, including: duration, sudden, chronic, or any other detailed information regarding recent hospitalizations and/or laboratory tests. Those files were considered as the non-ischemic group.

Ethical clearance

The study was approved by the Ethical Committee of the Ministry of Health and the College of Health Sciences, Kuwait.

Study tool

Each subject was interviewed and a signed informed consent was taken. Age, gender, and nationality were recorded.

Sample collection and DNA extraction

Blood samples were collected as follows: 5 mL of blood from a peripheral vein were withdrawn according to the Vacutainer® System with a disposable vacuum device (Vacutainer) and stored in tubes that held 0.1% EDTA. Samples were maintained at 4°C until DNA extraction. The Qiagen DNA Extraction Kit was used for DNA purification and extraction from blood. DNA extraction was performed according to the manufacturer's procedure (Qiagen, USA).

Genotyping

For PCR-RFLP genotyping, two primers were used in the amplification: upstream primer E2mut (5' ACT GAC CCC GGT GGC GGA GGA GAC GCG TGC) and downstream primer E3 (5' TGT TCC ACC AGG GGC CCC AGG CGC TCG CGG).¹⁰ Reaction mixtures were incubated at 94°C for 3 min, subjected to 40 cycles of amplification (94°C, 10 sec; 65°C, 30 sec; 72°C, 30 sec) and incubated at 72°C for 7 sec. Restriction digests containing 10 µL amplification reaction and either 2.5 U *AflIII* or 1.5 U *HaeII* were incubated at 37°C overnight. The products of digestion were analyzed on a 4% agarose gel.

Digestion of 318 base pair fragments produced six genotypes: e2/2, e2/3, e2/4, e3/3, e3/4, and e4/4. Homozygote (e2/2, e3/3, and e4/4) and heterozygote (e2/3, e2/4, and 3/4) genotype amplifications resulted in 231/232-bp fragments.

Statistical analysis

All analyses were carried out with the Statistical Package for Social Sciences, SPSS version 17. ApoE genotype frequencies were calculated by genotype counting. Comparison among the four groups was done by the likelihood ratio Chi square test ($LLR\chi^2$). The level of significance was set at 5% for all tests in this study.

Results

The overall response rate was 95.06%, with the following response rates per group: group I (95.45%), II (93.33%), III (95.35%), and VI (95.38%).

Distribution of studied subjects according to group, gender, and nationality are shown in Table 1. It was found that group I (n=105) included 48 males and 57 females. Group II (n=42) consisted of 20 males and 22 females. Group III (n=41) had 23 males and 18 females. Finally, group IV (n=62) consisted of 30 males and 32 females. Out of the total studied subjects, 166 (66.4%) were Kuwaitis and 84 (33.6%) were non-Kuwaitis.

Table 2 shows the distribution of studied subjects according to genotyping of ApoE polymorphism by group and nationality.

Table 2. Distribution of the studied subjects according to genotypes and nationality.

Alleles	DM				IHD				DM+IHD				Control			
	K		NK		K		NK		K		NK		K		NK	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
e 3/3	57	67.1	16	80.0	12	60.0	13	59.1	12	52.2	9	50.0	28	73.7	18	75.0
e 3/4	5	5.9	1	5.0	5	25.0	5	22.7	2	8.7	2	11.1	6	15.8	3	12.5
e 4/4	13	15.3	2	10.0	1	5.0	2	9.1	7	30.4	5	27.8	0	0.0	1	4.2
e 2/4	2	2.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.6	0	0.0
e 2/3	2	2.4	0	0.0	2	10.0	2	9.1	1	4.3	0	0.0	1	2.6	2	8.3
e 2/2	6	7.1	1	5.0	0	0.0	0	0.0	1	4.3	2	11.1	2	5.3	0	0.0
Total	85	100	20	100	20	100	22	100	23	100	18	100	38	100	24	100

DM=diabetes mellitus; IHD=ischemic heart disease; K=Kuwaiti; NK=non-Kuwaiti.

Table 3. Distribution of the studied subjects according to prevalent genotype and nationality.

Allele groups	DM		IHD		DM+IHD		Control	
	K	NK	K	NK	K	NK	K	NK
e 3/3	57	16	12	13	12	9	28	18
e 3/4+e 4/4	18	3	6	7	9	7	6	4
e 2/2+e 2/3+e 2/4	10	1	2	2	2	2	4	2
Total	85	20	20	22	23	18	38	24
LLR $\chi^2_{(2)}$	1.548		0.022		0.069		0.085	
P-value	0.461		0.989		0.966		0.958	

DM=diabetes mellitus; IHD=ischemic heart disease; K=Kuwaiti; NK=non-Kuwaiti.

e3/3 was the most common encountered allele in all study groups (66%), while e2/4 was the least prevalent (1.2%). The allele distribution was as follows: e4/4 (12.4%), e3/4 (11.6%), e2/2 (4.8%), and e2/3 (4%).

Individuals were classified into the following 3 groups: 1) ApoE2 carrying the e2/2, e2/3, or e2/4 genotypes; 2) ApoE3 carrying the most common e3/3 genotype; and 3) ApoE4 carrying either the e3/4 or e4/4 genotypes. Table 3 shows the distribution of studied subjects according to prevalent groups and nationality. Among the study groups, ApoE3 allele was the most common. In descending order, the ApoE3 allele was higher in group III (39%) followed by group II (31%), but lower in groups I (20%) and IV (16%).

However, there were no statistically significant differences in distribution of the allele groups and nationality, as shown by the different P values of the LLR χ^2 -test (Table 3). Among Kuwaitis, there was no statistical association between the different groups and the alleles group for both Kuwaitis (LLR $\chi^2_{(6)}$ =4.965; P=0.548) and non-Kuwaitis (LLR $\chi^2_{(6)}$ =5.490; P=0.483).

The distribution of studied Kuwaiti subjects according to alleles' groups by group and gender is listed in Table 4. There were no statistically significant differences of the distribution of alleles' groups and gender among the different study groups. Among male Kuwaitis, there was no statistical association between the alleles group and the group of subjects (LLR $\chi^2_{(6)}$ =2.717; P=0.843) as was the case with female Kuwaitis (LLR $\chi^2_{(6)}$ =2.829; P=0.830). Moreover, there were no statistically significant differences between both sexes in each study group, when the alleles' groups were considered.

Table 5 shows the distribution of alleles' groups and gender among the different study groups. Among non-Kuwaiti males, there was no statistical association between alleles group and the group of subjects (LLR $\chi^2_{(6)}$ =8.531; P=0.202). Also, among non-Kuwaiti females, there was no statistical association between the group of subjects and alleles group (LLR $\chi^2_{(6)}$ =1.507; P=0.959). In addition, there were no statistically significant differences between both sexes in each study group, when the alleles' groups were considered, as shown by the different P values of the LLR χ^2 test.

Discussion

Many studies related to the distribution of genetic polymorphism of ApoE and its impact on different diseases have been carried out in different populations. However, in the literature and to our knowledge, there is no reported data investigating the association of ApoE polymorphism with different chronic non-communicable diseases such as IHD and T2DM in Kuwait. In this study, we have observed that e3 allele was the most frequent, followed by e4 and e2, in the healthy control as well as the other study groups. This agrees with many studies that have been carried out locally^{11,12} in other Persian Gulf populations such as Oman and Saudi Arabia, indicating a high degree of interrelation^{13,14} as well as in various populations worldwide.¹⁵⁻¹⁷

The major finding in the present study was that the T2DM and IHD group had the highest Apo e4/4 genotype. Also, the frequency of Apo e3/4 was the most prevalent among the IHD group, which was in agreement with other recent studies.¹⁸

Table 4. Distribution of the studied Kuwaiti subjects according to prevalent genotypes and gender.

Allele groups	DM			IHD			DM+IHD			Control			Total		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
e 3/3	28	29	57	5	7	12	7	5	12	13	15	28	53	56	109
e 3/4+e 4/4	7	11	18	2	4	6	5	4	9	3	3	6	17	22	39
e 2/2+e 2/3+e 2/4	5	5	10	1	1	2	1	1	2	2	2	4	9	9	18
Total	40	45	85	8	12	20	13	10	23	18	20	38	79	87	166
LLR $\chi^2_{(2)}$	0.614			0.209			0.054			0.038			0.339		
P-value	0.735			0.901			0.973			0.981			0.844		

DM=diabetes mellitus; IHD=ischemic heart disease; M=male; F=female.

Table 5. Distribution of the studied non-Kuwaiti subjects according to prevalent genotypes and gender.

Allele groups	DM			IHD			DM+IHD			Control			Total		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
e 3/3	8	8	16	7	6	13	5	4	9	9	9	18	29	27	56
e 3/4+e 4/4	0	3	3	4	3	7	4	3	7	2	2	4	10	11	21
e 2/2+e2/3+e 2/4	0	1	1	1	1	2	1	1	2	1	1	2	3	4	7
Total	8	12	20	12	10	22	10	8	18	12	12	24	42	42	84
LLR $\chi^2_{(2)}$	4.74			0.038			0.032			0			0.262		
P-value	0.093			0.891			0.984			1			0.877		

DM=diabetes mellitus; IHD=ischemic heart disease; M=male; F=female.

As can be observed, no $\epsilon 2/4$ was found in diabetic and or non-diabetic IHD patients and the presence of an $\epsilon 2/3$ genotype and $\epsilon 2$ allele frequency were not significantly different in T2DM, IHD with or without diabetes patients, and control groups. Recently, Camsari et al.¹⁹ have reported that the distribution of Apo $\epsilon 2/3$ genotype and ApoE2 allele frequency did not differ significantly between the diabetic and non-diabetic atherosclerotic patients. This led to the assumption that ApoE2 allele may not be a particular risk factor in T2DM patients.

We also observed that the frequency of the ApoE4 allele was high among patients with IHD with or without T2DM in Kuwaiti and non-Kuwaiti groups. This data was well-matched with other studies, which have shown that in individuals with the ApoE4 allele, the risk and severity of IHD were greater.²⁰

The genotype frequency differences in this study were of interest since Apo $\epsilon 3/4$ was the most prevalent among the non-diabetic IHD patients. This correlated exactly with previous studies which, in non-diabetic patients, showed the association of the ApoE4 allele with the risk of myocardial infarction and coronary artery disease.²¹⁻²³

Furthermore, Apo $\epsilon 4/4$ in the IHD T2DM patients was high, which was in agreement with other studies which have shown an association of the ApoE4 allele with the risk of cardiovascular disease in both male and female T2DM patients.^{24,25}

In the non-diabetic population, studies have shown that the ApoE4 was associated with higher plasma levels of cholesterol, which may predispose to cardiovascular disease.^{26,27} In the diabetic population, ApoE4 allele was associated with the risk of IHD as mentioned in earlier studies. One study differed from our findings, the results of which showed that the ApoE polymorphism and, notably, the ApoE4 allele were not universal risk factors for cardiovascular diseases.²⁸

Since there is a small amount of updated information from the Persian Gulf region,^{12,15,20} this preliminary study is the first step to consider an association between ApoE polymorphism and IHD patients with or without T2DM in Kuwait.

We conclude that the ApoE4 allele might be associated with increased risk of IHD in diabetic and non-diabetic patients in Kuwait. We recommend further studies with large population sizes to highlight the association of ApoE4 to IHD patients with or without T2DM in Kuwait.

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