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Apolipoprotein-E Genotyping in Patients with Coronary Heart Disease

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Abstract: The aim of the study was to establish apolipoprotein E genotype in the patients with coronary heart disease (CHD) who are inhabitants of the north-east of Turkey.

The study involved 106 patients (70 male, 36 female; ages ranging from 37 to 69 years) who are local people and were angiocardiographically diagnosed for CHD.

The risk factors such as smoking, alcohol and obesity were determined and serum triglyceride, total cholesterol, LDL and HDL were measured in the subjects. Each patient sample was apolipoprotein E genotyped, which was achieved by PCR amplification of the 227 bp region followed by Cfo I digestion to release specific band patterns.

Subjects with CHD had more hypertension than the control (20.8% vs 7.7%, p: 0.038, z:-2.074), whereas risk factors such as smoking, obesity, alcohol and sedentary life-style were not more frequent in patients with CHD than in healthy controls.

Distribution of apolipoprotein E genotypes among the patients was 5.7% E4/E4 (n=6), 22.6% E4/E3 (n=24), 62.3% E3/E3 (n=66), 7.5% E3/E2 (n:8), 0.94% E2/E2 (n:1) and 0.94% E4/E2 (n:1). The control group exhibited 1.9% E4/E4 (n:1), 9.6% E4/E3 (n:5), 61.5% E3/E3 (n:32), 19.2% E3/E2 (n:10), 3.8% E2/E2 (n:2) and 3.8% E4/E2 (n:2). In our study, the most common apolipoprotein E genotype obtained was E3/E3, either in patients with CHD or in the healthy control group. Therefore, the difference between the groups was not significant (62.3% and 61.5%, p: 0.92 respectively). The genotype E3/E3 of apolipoprotein E in patients with CHD was, however, significantly high, as compared to healthy controls (22.6% vs 9.6%; p: 0.0476, z:-1.98). The frequency of the E3/E2 genotype was significantly higher in the control group than in patients with CHD (19.2% vs 7.5%; p: 0.03, z:-2.16).

Mean total cholesterol levels in CHD patients were 175.4±29.4 mg/dl, while they were 164.4±28.6 mg/dl in the control group (t: 2.26, p: 0.026). Mean LDL-C levels were 107.9±24.6 mg/dl in patients and 99.5±23.6 mg/dl in the control group (t: 2.04, p: 0.043). There were no significant differences between the two groups regarding triglyceride and HDL-Cholesterol. On the other hand, the levels of total cholesterol and LDL-C in patients with CHD were significantly different according to apolipoprotein E genotypes.

In conclusion, according to our study, the E4/E3 genotype was the most common genotype in patient with CHD. This finding, at least in part, could explain one of the important risk factors for coronary heart disease in subjects in eastern Turkey.

Key Words: Apolipoprotein E Genotypes, Coronary heart disease

Introduction

Lipoproteins are an important determinant of atherosclerotic vascular disease in man. The serum concentration and metabolism of lipoproteins are largely modulated by apolipoproteins and it has therefore been hypothesized that genetic variations in apolipoproteins are associated with variations in the susceptibility to coronary heart disease (1,2).

There is a significant effect of apolipopotein E on plasma cholesterol levels and it is responsible by itself for 10% variations in cholesterol levels among individuals within the population (3). Polymorphism of apolipoprotein E has recently received increasing attention.

Three common alleles, E2, E3 and E4, determine the six apolipoprotein E genotypes E2/E2, E2/E3, E2/E4, E3/E3, E4/E3 and E4/E4 (4). High cholesterol and LDL metabolisms are associated with apolipoprotein E polymorphism such that subjects with the genotypes E4/E3 have higher concentrations. Patients with CHD have an increased risk of all manifestations of atherosclerotic vascular disease (5).

The aim of the study was to establish apolipoprotein E genotype in the patients with CHD who are inhabitants of the north-east of Turkey and to find out the relation between apolipoprotein E genotype and the disease.

Materials and Methods

In the study, we selected 106 patients with CHD who were admitted to the cardiology department of University hospital, (aged 37-69; 36 female, 70 male) and 52 healthy controls (aged 40-65; 20 female, 32 male) who had asymptomatic CHD and normal electrocardiography from the eastern part of Turkey. The controls were completely representative of the non-CHD population of the study area. None of the control or CHD patients were receiving hypolipidemic drugs and none had a significant impairment in renal function as assessed by serum creatinine concentration. There was no diabetes mellitus or other disease which might affect the lipid profiles.

Thirty patients had a history of myocardial infarction according to their medical records, and the remaining subjects were assessed as having unstable angina pectoris (UAP) according to WHO criteria (6). According to coronary angiography reports, 32 patients had one coronary artery disease and 58 patients exhibited two or more coronary artery lesions.

Serum cholesterol, LDL, HDL and triglyceride concentration were determined from fresh serum samples drawn after a 12-hour overnight fast.

The apolipoprotein E genotype was determined by PCR amplification of the 227 bp region followed by CfoI digestion to release the specific band pattern.

Genomic DNA was isolated from blood (7). Amplification of the apolipoprotein E target sequence was performed by using the 5-primer TCCAAGGAGCTGCAGGCGGCGCA and 3-primer ACAGAATTCGCCCCGGCCTGGTACACTGCCA. This primer pair amplifies a 227 bp region of DNA that spans both apolipoprotein E polymorphic sites. About 15 ml of PCR products were run on 3% agarose gel.

The major band was seen to correspond to a molecular weight of approximately 227 bp. 15 µl of amplification products was subjected to restriction by CfoI. The digested products were electrophoresed, stained with ethidium bromide and viewed and photographed under UV light.

SSPS 6.1 software was used for statistical analysis.

Results

Table 1 lists the characteristics of the study groups. Subjects with CHD had more hypertension than the control subjects, whereas risk factors such as smoking, obesity and alcohol were not frequent in subjects with CHD compared to the healthy controls.

Table 1. Characteristics of control subjects and subjects with CHD.

	Controls	CHD	p Values
No of Subjects	52	106	-
Sex (F/M)	29/23	54/62	P (NS)*
Mean (SD) Age (years)	47.9±6.4	49.8±7.4	p (NS)**
No. (%) of Smokers	24 (46.2)	50 (47.2)	p (NS)*
No. (%) of Obesity (a)	16 (30.8)	26 (24.5)	p (NS)*
No. (%) of Alcohol users	4 (7.7)	7 (6.6)	p (NS)*
No. (%) Hypertension (b)	4 (7.7)	22 (20.8)	P 0.038
No (%) Sedentary life style	20 (38.4)	48 (42.3)	P (NS)*

Statistical analysis within the table were made with *Chi-square test and **Unpaired Student's t test

a. Subjects who have Body mass index > 30 kg/m^2 were considered as obese (8).
b. Subjects who have blood pressure > 140/90 mmHg were considered as hyperactive (9).

Table 2 shows the distribution of apolipoprotein E genotypes among the patients and healthy controls. The most common apolipoprotein E genotype both in CHD patients and in the control group was E3/E3 and the difference was not significant (62.3% and 61.5%, p: 0.92 respectively). The frequency of the E4/E3 genotype was much higher in subjects with CHD than in the control group and the difference was statistically significant (22.6% and 9.6%; p: 0.0476, z:-1.98 respectively). The E3/E2 genotype appeared to be more in control than in

Table 2. Apolipoprotein E Genotypes in Control and CHD.

GENOTYPES	CONTROL (n:52) No (%)	CHD (n:106) No (%)
E4/E4	1 (1.9)	6 (5.7)
E4/E3	5 (9.6)	24 (22.6)*
E3/E3	32(61.5)	66 (62.3)
E3/E2	10 (19.2)	8 (7.5)**
E2/E2	2 (3.8)	1 (0.94)
E4/E2	2 (3.8)	1 (0.94

*p:0.0476 compared to control group
**p:0.03 compared to CHD group

CHD patients (19.2% and 7.5%; p: 0.03, z:-2.16 respectively).

Table 3 represents the mean serum lipid concentration in the two groups. The mean total cholesterol and LDL-C levels were significantly higher in patients with CHD than in the control group (for total cholesterol t: 2.26, p: 0.026; for LDL-C t: 2.04, p: 0.043). There was no significant correlation between the two groups regarding triglyceride and HDL-C. The relation between apolipoprotein E genotypes and serum lipid levels in both groups is indicated in Table 4.

The total cholesterol values determined among patients with CHD were significantly different in the apolipoprotein E genotypes obtained within the group (chi square 21.26 and p: 0.0007). Similary, LDL-C levels were also different among apolipoprotein E genotypes obtained in the patient group (chi square 16.6 and p: 0.0053).

Discussion

The most common isoforms of apolipoprotein E (E2, E3, E4) affect the level of lipids and their profiles (11,12). Likewise, it has been shown that apolipoprotein E2 is associated with lower plasma cholesterol and apolipoprotein E4 has a contrary effect (11-14). On the other hand, some studies have shown a significant

Table 3. Mean Levels of Plasma Lipids According to Apolipoprotein E Genotyping in Patients with CHD.

CHD (n=90)	E2/E2	E3/E2	E3/E3 (n=20)	E4/E3 (n=32)	E4/E4 (n=38)
Total- C mg/dl > 200 n (%)	-	-	126.5 ± 37.2 0 (0)	181.3 ± 41.7 10 (31)	180.2 ± 61.4 14 (37)
LDL-C mg/dl > 130 n (%)	-	-	59.1 ± 39.9 0 (0)	125.8 ± 39.6 11 (34)	118.5 ± 53.7 13 (34)
HDL-C mg/dl- < 35 n (%)	-	-	30.3 ± 6.6 14 (70)	25.4 ± 6.4 23 (72)	28.5 ± 5.7 29 (76)
TG mg/dl > 200 n (%)	-	-	150.5 ± 65.1 8 (40)	159.3 ± 50.5 12 (38)	160.1 ± 62.5 16 (42)

Table 4. Mean Levels of Plasma Lipids According to Apolipoprotein E Genotyping in Patients with Healthy Controls.

Controls (n=52)	E2/E2 (n=6)	E3/E2 (n=10)	E3/E3 (n=18)	E4/E3 (n=9)	E4/E4 (n=9)
Total- C mg/dl > 200 n (%)	143.1 ± 46.8 0 (0)	152.2 ± 45.7 0 (0)	140.9 ± 56.1 0 (0)	175.3 ± 58.2 3 (33)	172.9 ± 57.5 3 (33)
LDL-C mg/dl > 130 n (%)	82.0 ± 51.6 0 (0)	95.7 ± 48.7 1 (10)	79.9 ± 46.9 0 (0)	120.4 ± 57.8 3 (33)	124.8 ± 37.7 3 (33)
HDL-C mg/dl < 35 n (%)	30.8 ± 6.6 2 (33)	30.0 ± 7.25 4 (40)	28.3 ± 7.1 12 (67)	26.6 ± 9.6 6 (67)	26.8 ± 7.0 7 (78)
TG mg/dl > 200 n (%)	150.0 ± 70.0 2 (33)	148.0 ± 72.9 3 (30)	155.9 ± 77.9 3 (33)	170.2 ± 45.6 3 (33)	184.1 ± 43.3 3 (33)

Plasma concentrations (mean ± SD) of lipids (mg/dl) in CVD patients as a function of apolipoprotein-E polymorphism. Cut-off values were accepted for total-C >200 mg/dl, LDL-C >130 mg/dl, HDL-C< 35 mg/dl and TG > 200 mg/dl (10). Statistical evaluations were performed according to these values.

increase in the frequency of the E4 allele in survivors of myocardial infarction or in patients with angiographically verified coronary heart disease (15). Furthermore, the allele E4 has been observed to increase the risk of myocardial infarction at an early age (16).

The apolipoprotein alleles frequency in Turkey is 74.2% E3/E3, 12.9% E4/E3, 10.6% E3/E2, 1.1% E4/E4, 0.8% E4/E2 and 0.4% E2/E2 according to the Turkish Heart Study (17). In our study, although the E3/E3 genotype was the most common, there was no significant correlation between the CHD group and the control group. The genotype of E4/E3 in patients with CHD was more common than in the to healthy controls (p: 0.047). Our results (Table 2) were consistent with other group work regarding the E4 allele and coronary artery disease (5, 15-20). On the other hand, besides the frequency of E3/E2 genotype being much lower in CHD group than in the control group (Table 2; 7.5% and 19.2%, p: 0.03 respectively), serum total cholesterol and LDL-C levels appeared to be much higher than in the control group (Table 4). It might, however, be difficult to interpret for regional distribution of the apolipoprotein E genotype and to use it as a risk factor for CHD, since the number of subjects studied was not high enough.

Why should people with the E4 allele be particularly susceptible to coronary heart disease? It seems that the risk for coronary heart disease could be related to the impact of the apolipoprotein E genotypes or serum lipid and lipoprotein concentration. Apolipoprotein E participates in the conversion of VLDL remnants to LDL, and this metabolic pathway is particularly effective in subjects with the E4 allele, which might result in a higher rate of production of LDL in these subjects (21). Furthermore, the HDL subfraction containing apolipoprotein E is considered to play an important part in the reverse cholesterol transport (22).

High LDL concentrations in people with the E4 allele seem to be related to the particularly effective absorption of the cholesterol in these subjects, which may be one explanation for the differences in LDL concentrations among people with the various apolipoprotein E genotypes (23).

In our study, high levels of total cholesterol (> 200 mg/dl) and LDL-C (> 130 mg/dl) which are accepted risk factors for CHD, were more frequent in patients than in the healthy controls (Table 3).

It is known that there is no direct relation between apolipoprotein E alleles and HDL-C. It has, however, been reported that HDL-C levels could be lowered in cases of overrepresentation of apolipoprotein E (17). In our study, the level of HDL-C was under the expected values (> 35 mg/dl) in both the CHD and control groups (Table 3).

Although an association between an increase in serum TG levels and CHD incidence is disputed, it has been proposed that there is a relation between increased serum TG level and CHD risk (1,6,11). There was also no considerable difference between the CHD and control groups in terms of serum triglyceride concentration.

Subjects with CHD had more hypertension and were less physically active (in other words had a sedentary life-style) than the control subjects, whereas no differences in other cardiovascular risk factors (smoking, alcohol consumption and obesity) were found between the groups of CHD and healthy controls. This finding may support the idea that the susceptibility to coronary heart disease cannot be explained by these classic cardiovascular risk factors alone.

According to our study results, the more frequent appearance of the apolipoprotein E4/E3 genotype in patients with CHD, using also that genotype as a risk factor for CHD in the region and association of apolipoprotein E distribution with total LDL-C levels that is a risk factor for CHD patients, may be useful for understanding the regional profile of the disease related to apolipoprotein E genotypes and lipid profiles.

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