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Influence of Apo E Polymorphism on Coronary Artery Disease

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***Abstract*—**The ε_4 allele of the ε_2, ε_3 and ε_4 protein isoform polymorphism in the gene encoding apolipoprotein E (Apo E) has previously been associated with increased cardiac artery disease (CAD); therefore to investigate the significance of this polymorphism in pathogenesis of CAD in Iranian patients with stenosis and control subjects. To investigate the association between Apo E polymorphism and coronary artery disease we performed a comparative case control study of the frequency of Apo E polymorphism in One hundred CAD patients with stenosis who underwent coronary angiography (>50% stenosis) and 100 control subjects (<10% stenosis). The Apo E alleles and genotypes were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). We observed an association between the Apo E polymorphism and CAD in this study. These data suggest that the Apo ε_4 and ε_2 alleles increase the risk for CAD in Iranian population (χ^2 =4.26, p= 0.05, OR=2 and χ^2 =0.38, p=0.53, OR=1.2). These results suggest that ε_4 and ε_2 alleles are risk factors for stenosis.

***Keywords*—**Arterial blood vessels, atherosclerosis, cholesterol.

I. Introduction

APOLIPOPROTEIN E is a plasma protein that serves as a ligand for low density lipoprotein receptors and through its interaction with these receptors participates in the transport of cholesterol and other lipids among various cells of the body [1]. Apo E is an exchangeable protein which acts as ligand for low density lipoprotein (LDL) receptors. It also has a repair function in response to tissue injure. It plays an essential role in lipid metabolism, especially in removal atherogenic remnants of triglyceride rich lipoproteins [1-2] and by reversing cholesterol transport in plasma and intercellular lipid transport within tissues. The human Apo E gene is 3.7 Kb including 4 exons and 3 interons (3-4) and is mapped on the short arm of chromosome 19[3-4]. The mature protein is composed of 299 amino acids i.e. 34 KD with several function domains [4 -7]. The three common isoforms of Apo E2, E3 and E4 are encoded by the Apo ε_2, ε_3 and ε_4 genes, respectively [6] that give rise to different genotypes ($\varepsilon_2/_2$, $\varepsilon_3/_3$, $\varepsilon_4/_4$, $\varepsilon_2/_4$, $\varepsilon_2/_3$ and $\varepsilon_3/_4$). The primary sequence of these proteins is identical except at amino acids 112 and 158, where there can be cysteines (E2), arginines (E4) and arginine at position 158 (E3) [6]. Apo E2 has a lower binding affinity to LDL receptor (1% of the Apo ε_3 binding affinity), whereas the binding affinity of E4 to the LDL receptor is higher [7]. The genetic variations at Apo E have been shown to affect on lipid and lipoprotein levels in the general population [8-9]. The ε_4 isoform is associated with increased levels of total cholesterol (TC) and beta lipoprotein [1] and increased susceptibility [11]. A number of studies have investigated association between genetic susceptibility factor for cardiac heart disease (CHD) or arthrosclerosis in divers ethnic populations [12]. The knowledge of lipid profile may predict the potential victims of cardiovascular disease before its initiation and progression and offer the opportunity for primary prevention. Therefore it is very important to identify factors that may influence blood lipid concentrations. The correlation of the most common Apo E polymorphism with CHD has been extensively investigated in the last three decades [2]. A Meta analysis of 48 diseases [13] showed that the Apo ε allele is a significant risk factor for CHD. On the other hand a trend for the ε_4 allele to be associated with a higher prevalence of target organ damage in patients with mild to moderate hypertension has been proposed [14]. Therefore the current study specially aimed to find whether genetic polymorphism in Apo E gene is a risk factor for CHD in a population from Tehran Iran.

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II. Materials and Methods

A. Study Subjects

The study group consisted of 100 patients (74 males, 25 females, mean age 58.61± 9.35) who were admitted to the cardiology unit of Shahid Rajaee Hospital who had been diagnosed to have arthrosclerosis. The diagnosis was based on the complete physical and clinical examination of patients by the cardiologist followed by investion. For the present study, only patients with arteriosclerosis were included while patients with Alzheimer' disease, pulmonary, renal, hepatic disease, cardiomyopathy congestive heart failure and acute myocardial infarction were excluded.

Random were included study as control (64 males, 36 females mean age 53.45± 9.35). Control subjects were also similarly evaluated the confounding risk factors included smoking and alcohol consumption, dislipidemia and family history of arthrosclerosis. In the present study, evaluation of the contribution of confounding risk factors of the

development of arthrosclerosis was based on the individual's personal history findings.

B. Genetic Analysis

Leucocytes extracted following standard protocols [15] DNA was amplified by PCR in a DNA cycler (0005.416model T-cy grady) using oligonucleotide primer forward (5́-ACAGAATTCGCCCCGGCCTGGTACAC3́) and reverse (5-TAAGCTTGCCACGGCTGCCAAGCA 3) (.company) as described by Hixson et al [16]. The PCR condition included on initial step of 95º C for 30 miń, followed by 33 cycles (95º C 30 S , 55º C 30 S and 70º 1 min) and by a final extension (70º C, 7 min) with 94º C hold according to a protocol described by Hixson et al [16]. Electrophoresis of amplified products (244 bp) was performed on 10% polyacrylamide gel. After PCR implication 5 units of Hha1 enzyme (New England Biolabs) was added directly to each reaction mixture for digestion of Apo E sequence of PCR product (over night, 37ºC)[16]. Each reaction mixture was loaded onto a 10% polyacrylamide gel and electrophoresed. After electrophoresis, digested fragments were visualized by UV illumination. The size of Hha I fragments were estimated by comparison with known DNA (Fermenta, Gene Ruler 50bp DNA Ladder). Statistically analysis: Allele frequencies were deduced from genotype frequencies, and the Hardy–Weinberg equilibrium was analyzed through the chi-square test. $p<0.05$ was considered significant. Quantitative information was expressed as mean standard deviation. All calculations were performed by using the SPSS 11.5 program. To evaluate the association of Apo E polymorphism with arthrosclerosis: Multiple logistic regressions were used with maximum likelihood estimation of the regression coefficients and their standard error. Multivariate adjusted odd ratios calculated for Apo ε_4 allele ($\varepsilon_4/\varepsilon_4$ and $\varepsilon_3/\varepsilon_4$), ε_2 allele ($\varepsilon_2/\varepsilon_2$, $\varepsilon_2/\varepsilon_3$ and $\varepsilon_2/\varepsilon_4$) and taking allele ε_3 ($\varepsilon_3/\varepsilon_3$) as reference. For each odds ratios we calculated two tailed p values and 95% confidence interval 1.2to 3.8, $p\leq0.05$ in all.

III. Results

In this study we identified three Apo alleles ε_2, ε_3 and ε_4 and six genotypes $\varepsilon_4/\varepsilon_4$, $\varepsilon_3/\varepsilon_4$ $\varepsilon_2/\varepsilon_2$, $\varepsilon_2/\varepsilon_3$, $\varepsilon_2/\varepsilon_4$ and $\varepsilon_3/\varepsilon_3$. In study population for both men and women, allele frequencies did not deviate from Hardy-Weinberg equilibrium. The distribution of Apo E genotypes in patient subjects differed significantly fro control group. As it shown in Table I, the prevalence of six genotypes $\varepsilon_4/\varepsilon_4$, $\varepsilon_3/\varepsilon_4$ $\varepsilon_2/\varepsilon_2$, $\varepsilon_2/\varepsilon_3$, $\varepsilon_2/\varepsilon_4$ and $\varepsilon_3/\varepsilon_3$ in patient and control 30%, 4%, 8%, 18%, 6%, 34% and 15%, 6%, 12%, 8%, 8% 51% respectively. It observed that the prevalence of $\varepsilon_3/\varepsilon_4$ was 1.5 fold high in patient subjects (4% Vs 6%) when compared with controls (χ^2 =0.42, p=0.156, OR=0.58). While prevalence $\varepsilon_2/\varepsilon_3$ and $\varepsilon_4/\varepsilon_4$ genotypes were higher in patients (18% Vs 8% and 30% Vs 15%) than in controls (χ^2 =4.3, p=0.036, OR= 2.52) and (χ^2= 6.4, p= 0.01, OR= 1.86) respectively. The frequency of $\varepsilon_2/\varepsilon_4$ (χ^2=0.3, P=0.57, OR=0.49) and $\varepsilon_2/\varepsilon_2$ (χ^2 = 0.89, p=0.346, OR= 0.57) in control was 1.3 and 1.5 fold high when compared with patient subjects (6% Vs 8%) and (8% Vs 12%) respectively. Statistically significant difference was not found between patients and controls (32% Vs 28%) with respect to ε_2 and allele frequency (χ^2 =0.38, p=0.53, OR=1.2) while ε_3 allele frequency was found to be much more prevalent in patients (34% Vs 51%) than in control (χ^2=5.9, P=0.015, OR=0.44). As it shown in Table II the prevalence of ε_4 allele in patient subjects (34% Vs 21%) is higher than controls (χ^2 =4.23, p=0.04, OR=2).

TABLE I
PREVALENCE OF APO E GENOTYPES IN CARDIAC HEART DISEASE AND CONTROLS

Genotype	Patient	Control	χ^2	P value	OR
$\varepsilon_3/\varepsilon_3$	34	51	5.9	0.015	0.4
$\varepsilon_4/\varepsilon_4$	30	15	6.4	0.01	1.86
$\varepsilon_3/\varepsilon_4$	4	6	0.42	0.156	0.58
$\varepsilon_2/\varepsilon_3$	18	8	4.3	0.036	2.52
$\varepsilon_2/\varepsilon_2$	8	12	0.89	0.346	0.57
$\varepsilon_2/\varepsilon_4$	6	8	0.3	0.57	0.49

TABLE II
PREVALENCE OF APO E ALLELES IN CARDIAC HEART DISEASE AND CONTROLS

Alleles	Patient	Control	χ^2	P value	OR
ε_3	34	51	5.9	0.015	0.44
ε_4	34	21	4.23	0.04	2
ε_4	32	28	0.38	0.53	1.2

ε_3= ($\varepsilon_3/\varepsilon_3$), ε_2 = ($\varepsilon_2/\varepsilon_2$, $\varepsilon_2/\varepsilon_3$ and $\varepsilon_2/\varepsilon_4$) ε_4=($\varepsilon_4/\varepsilon_4$ and $\varepsilon_3/\varepsilon_4$)

IV. Discussion

Studies conducted in different parts of the globe reveal that gene frequencies at Apo E locus are highly heterogeneous between the populations. The ε_3 is the most common form of the gene in most of the population [17-18]. In a population-based study Venkutaramana et al [19] reported that the allele frequencies in Indian population 85%-92% for ε_3 allele, 3.9% for ε_4 allele and 3.5% for ε_2 allele. In the present study Apo ε allele frequencies in the control group of Tehran population are 34%, 34% and 32% for ε_4, ε_3 and ε_2 respectively which are not comparable with the study of Venkutaramana et al and others [19]. The prevalence of $\varepsilon_2/\varepsilon_2$, $\varepsilon_2/\varepsilon_3$,$\varepsilon_2/\varepsilon_4$, $\varepsilon_3/\varepsilon_3$, $\varepsilon_3/\varepsilon_4$, and $\varepsilon_4/\varepsilon_4$ in Korean adults were 0.3%, 10.3%, 0.6%, 75.3%, 12.5% and 0.4% for men and 0.6%, 9.1%, 1.0%, 72.9%, 15.3% and 0.9% for women respectively. The reasons for these discrepancies could be genetic heterogeneity and gene environment interactions in different ethic population. It is well known that the ε_4 allele of Apo E is associated with increased prevalence of arthrosclerosis and cardiac heart disease (CHD)[20-21]. However there are controversial results concerning the association between Apo E genotypes and some cardiovascular risk factors. Some studies have suggested that high blood pressure may be associated with the presence of the ε_4 allele [22-24], while others have found its association with ε_2 allele [25]. However no association was found in few studies [25]. In this study we evaluated the distribution of Apo E genotype and alleles in angiogaraphically defined CAD patients and control subjects,

and found these polymorphisms as risk factors for atherosclerosis. As it shown, the distribution of $\varepsilon_4/\varepsilon_4$, $\varepsilon_2/\varepsilon_3$, $\varepsilon_3/\varepsilon_3$, genotypes and ε_3 and ε_4 alleles in patients group were significantly different from control group. It is suggested that the ε_4 allele and $\varepsilon_2/\varepsilon_3$ genotype of Apo E may be less efficient at retarding the oxidation of LDL than others. As it shown in Tables 1 the prevalence of $\varepsilon_3/\varepsilon_3$ genotype was 1.5 fold high in control group when compared with patients (51% Vs 34%, p=0.015) whereas prevalence of $\varepsilon_4/\varepsilon_4$ and $\varepsilon_2/\varepsilon_3$ genotypes was 2 and 2.25 fold high in patient group than control subjects (30% Vs 15%, p= 0.01and 18% Vs 8%, p=0.036). This finding suggests that the prevalence of $\varepsilon_4/\varepsilon_4$ and $\varepsilon_2/\varepsilon_3$ genotypes may be risk factors in this complex disease. The frequency of ε_3 allele was 1.5 fold high in control group when compared to patient (51% Vs 34%, p= 0.015, χ^2 = 5.9, OR=0.44), while statistically difference was found between patients and controls with respect to ε_2 allele frequency (32% Vs 28%, p= 0.53, χ^2=0.38, OR=1.2). It is suggested that ε_2 allele may be a risk factor for CAD disease in Iranian population. A significant difference was found between the prevalence of ε_4 allele in patient group as comparison with control subjects (34% Vs 21%, p=0.04, χ^2 =4.23, OR=2). These results showed an evidence of an association between the ε_2 and ε_4 alleles and CAD. This finding is accordance or different to some studies that performed in different population with coronary artery disease. These findings are accordance to the results of two meta-analysis [26a]. The results of these study showed that the odd ratios (ORs) for coronary heart disease (CHD) in ε_2 and ε_4 alleles versus persons had with ε_3 allele. Compared with those who had the ε_3 allele, the pooled ORs for CHD among carriers of ε_4 allele were 1.3 in the classic random effects model and 1.42 in the bayesian hierarchical random effect mode. These two model showed that no evidence of association between the $\varepsilon_2$allele and CHD risk (Ors= 0.93 and 0.98) respectively [26]. They showed a similar estimates for each of $\varepsilon_2/\varepsilon_2$, $\varepsilon_2/\varepsilon_3$, $\varepsilon_2/\varepsilon_4$, $\varepsilon_3/\varepsilon_4$ and $\varepsilon_4/\varepsilon_4$ genotypes compared with $\varepsilon_3/\varepsilon_3$ genotype in both classic random- effects model and a bayesian hierarchical random effect model. They showed that persons with $\varepsilon_3/\varepsilon_4$ and $\varepsilon_4/\varepsilon_4$ genotypes had higher risk for CHD (ORs =1.41 and 1.36) respectively than those with the $\varepsilon_3/\varepsilon_3$ genotype, whereas there was no evidence of any association between CHD risk and $\varepsilon_2/\varepsilon_2$, $\varepsilon_2/\varepsilon_3$ genotypes (ORs = 0.43, 1.04 and 1.11) respectively. The results of our study with respect to ε_4 allele carriers are accordance to two meta analysis studies results.

In a study by Bhavani et al [27] the prevalence of genotypes and allele frequencies among hypertension patients and controls were identified. They showed that prevalence of $\varepsilon_3/\varepsilon_4$ genotypes was 1.5 fold high in patients when compared to controls (14.5% Vs 10.0 %, $p \leq 0.05$) while prevalence of $\varepsilon_2/\varepsilon_3$ genotype was high in controls than in patients (6.5% Vs 4.3%. They showed that statistically significant difference was not found between patients and controls with respect to ε_2 and ε_3 allele frequencies, while ε_4 allele frequency was found to be more prevalent in patients (12.16%) than in controls (5.75%, χ^2 = 10.87, p= 0.05). They found that this allelic association should higher relative incidence of ε_4 allele (χ^2=9.13, p=0.05) as compared to other alleles and also in case with family history of hypertension (χ^2= 6.79, $p \leq 0.05$). Analysis of the apolipoprotein E gene polymorphism in large Caucasian population by Hubacle et al [28], the carrier of mutant allele Arg 136 / Ser in C Zech region was identified. They estimated that the population frequency of this Apo E mutation is very low. They suggested that this mutation in subjects not necessarily connected with elevated lipid in all cases. In present study the mutant allele (Arg 136 Ser) in Iranian population was not found. The results of a study by Merho [29] showed that Apo E polymorphism had a significant effect on lipid levels in Koreans, that the association between the Apo E allele type and HDL-C was modified by age in women, and that the association between the Apo E allele and triglyceride levels was modified by smoking status in men [29]. These findings high light the important effect of gene-environment interaction on lipid levels. They showed that the carrier of the Apo ε*/ ε_2 (ε_2) allele had a significant total cholesterol and LDL-C concentrations than carrier of the Apo ε*/ε_3 (ε_3) or ε*/ε_4 (ε_4) alleles, regardless of gender [29]. It is proposed that the context dependency of Apo E polymorphism and associated effects have been demonstrated suggesting other genetic components to be equally important, and lifestyle including dietary habits are now recognized factors that can either mask or expose an effect of a specific Apo E genotype [30-32]. It is suggested that the association between Apo E polymorphism and different lipoproteins (Triglyceride-HDL-C and LDL-C) levels are not entirely similar among different populations. Gene – environment interaction may contribute to the discrepancies observed between studies. Previous studies have shown that HDL-C levels vary with physical activity, alcohol consumption and diet [33-34]. Reznik et al [35] showed that the association between and Apo E polymorphism postprandial triglyceride clearance was modified by age, bodyweight and triglyceride pool level. As shown by Alessandro [36] in young adults, the Apo ε_4 allele and cigarette smoking act synergistically increasing an individual's propensity to have a cerebral ischemic event. However the mechanism underlying of Apo E polymorphism to CHD risk is not completely understood and deserves further investigation. Although the impact of Apo E polymorphism on plasma levels of total and low density lipoprotein cholesterol, apolipoprotein B and apolipoprotein E is well established, the triglycerides, high –density lipoprotein cholesterol, apolipoprotein A-1 and lipoprotein (a) remain equivocal [37-39]. It has been suggested that the ε_4 allele is related to HDL-C and LDL-C levels, while the potential antiatherogenic of the ε_2 involving lower levels of LDL-C may be offset by accumulation of atherogenic large very low density lipoprotein cholesterol and remnant rich lipoproteins [37-39]. Beyond the effect on lipid metabolism, Apo E genotypes may also affect CHD risk though antioxidative, inflammatory and immune activities [37-39].

In conclusion, our results support the notion that a significant association of ε_4 allele is observed with coronary heart disease (CHD) in addition to the other well known risk factors and positive family history. Carriers of ε_4 allele form a high risk group showing greater susceptibility to CHD while the ε_2 allele has no effect. Further, this observation interplay between genetic and environmental factors must be thoroughly considered in order to evaluate the etiological role

of Apo E in CHD. There is convincing evidence that the relationship between Apo E genotype and plasma lipoprotein lipid levels is context –dependent, being significantly influenced by age [40] and sex [41-42]. Some evidence [43-44] also indicates that the responses of plasma lipoprotein – lipid levels to different lipid lowering interventions may be affected by an individual's Apo E genotype, indicating the significance of gene-environment interactions.

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