Apolipoprotein E gene polymorphism and left ventricular function in Iranian patients with thalassemia major

Left ventricular (LV) failure is the main cause of death in thalassemia. Iron overload in patients with thalassemia leads to the formation of oxygen free radicals. Of the various apolipoprotein E (apoE) alleles, apoE4 is the least efficient in conditions of oxidative stress in comparison with apoE2 and apoE3. Our results showed that apoE4 is a genetic risk factor for LV dysfunction in thalassemia.

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Left-sided heart failure is the most common presentation of cardiac involvement in blood transfusion-dependent thalassemic patients and is the result of iron deposition. ApoE acts as scavenger of free radicals; iron chelation is probably another mechanism of its antioxidant activity. The antioxidant and iron binding activities of the major alleles rank E2>E3>E4.[1] The E4 allele was reported to be a genetic risk factor for LV failure in patients with homozygous ?-thalassemia.[2,3] In this study, we investigated the role of the apoE gene polymorphism on LV function in thalassemic patients from southern Iran. We enrolled 202 patients with thalassemia major (age ≥10 years) selected mainly on the basis of their echocardiographic findings. All patients were receiving blood transfusions to maintain the hemoglobin level above 9 g/dL and subcutaneous deferoxamine for at least five nights per week. According to the echocardiographic findings, patients were divided into three groups: (i) patients with no cardiac impairment; (ii) patients with LV dilatation but normal LV systolic function; and (iii) patients with LV systolic dysfunction. LV dilatation was defined as a LV end diastolic diameter (LVEDD) above the 90th percentile for the patient's body surface area.[4] The criterion for LV dysfunction was a shortening fraction (SF) less than 28%. Patients with systemic or endocrine disorders that might affect heart function were excluded from the study. The patients' classification was based on the study by Economou-Petersen *et al.*[1] but with some modifications. One hundred and ninety-eight healthy unrelated subjects (95 men and 103 women, aged 23.7±5.2 years) from the same geographic region were included as a control group. ApoE genotyping was performed as described previously.[5] Analysis of quantitative variables among groups was carried out using one-way ANOVA, Tukey post hoc analysis. The χ^2 test or Fisher's exact test was used to compare apoE allele frequencies. p values <0.05 were considered statistically significant. The Central Medical Ethics Committee of ACECR approved the study.

Table 1 presents the basic clinical, hematologic and echocardiographic characteristics of the patients, the results of apoE genotyping are shown in Table 2. A comparison of the patient and control groups, showed that both E3/E4 genotype and E4 allele frequencies were higher in group 3 than in the controls (p<0.05, OR=2.97, 1.06<OR<8.32 and p<0.01, OR=3.01, 1.15<OR<7.69, respectively). Other values were not statistically different between the patient and control groups. LV end systolic diameter and interventricular septum thickness were greater in E3/E4 patients than in E3/E3 patients (p<0.05 and p<0.01, respectively).

As shown in Table 1, the basic clinical and hematologic characteristics did not differ significantly between the three groups of patients, but those in groups 2 and 3 had developed some degree of LV (LV dilatation preceding LV dysfunction and overt LV dysfunction). These differences could arise from different genetic susceptibility loci.

Table 1. Basic clinical, hematologic and echocardiographic characteristics in the three groups of patients.

Characteristic (mean ± 1SD)*	*Group 1 (n=135)*	*Group 2 (n=38)*	*Group 3 (n=29)*	*p value (Among groups)*	*p value* (Between groups)*
Age (years)	16.5±4.7	18.1±6	16.5±4	NS	
Sex (M/F)	62/73	20/18	10/19	NS	
Body surface area (m^2)	1.26±0.2	1.26±0.2	1.21±0.1	NS	
First transfusion (months)	23±28	18±20	19±26	NS	
First chelation (years)	5.2±4	6.4±5	5.4±4.2	NS	
Hemoglobin (g/dL) (mean in the preceding year)	9.7±0.7	9.5±0.8	9.6±0.6	NS	
Units of blood transfused (mean in the preceding 7 years)	127±22	131±24	141±28	NS	
Serum ferritin (ng/mL) (mean in the preceding 5 years)	3257±1254	3219±1247	3431±1074	NS	
LVEDD (mm)	43.8±4.1	50.1±33	48.3±5.6	<0.001	<0.001 <0.001 <0.05
LVESD (mm)	27.3±4.2	32.1±3.7	35.7±5.6	<0.001	<0.001 <0.001 <0.01
IVS thickness (mm)	7.7±1.8	7.1±1.5	8.7±2.2	<0.01	NS <0.01 <0.01
Ejection fraction (%)	66.8±7	66.2±9.5	50.1±7.4	<0.001	NS <0.001 <0.001
Shortening fraction (%)	36.1±4.7	37±7	24±3.6	<0.001	NS <0.001 <0.001
LVPW thickness	7.5±3.6	7±1.5	8±2.1	NS	
RVEDD (mm)	17.2±5.7	17.4±6	19±6	NS	

**Comparison between groups: 1 with 2, 1 with 3 and 2 with 3, respectively. SD: standard deviation;NS:not significant; LVEDD: left ventricular end diastolic diameter; LVESD: left ventricular end systolic diameter, IVS: interventricular septum; LV: left ventricular; LVPW: left ventricular posterior wall; RVEDD: right ventricular end diastolic diameter.*

ApoE4 has been reported to be a genetic risk factor for LV failure in patients with homozygous β-thalassemia in Greece[2] and Italy.[3] Compared to the apoE2 and apoE3 alleles, the apoE4 allele has the least antioxidant and iron binding activities,[1] lowest turn-over,[6] gene expression level,[7] and accumulation within cells[8] and the highest potential to protein modification by 4-hydroxynonenal.[9] ApoE4 also induces *Fas*-mediated apoptosis in cardiomyocytes.[10]

With regards to differences of apoE isoforms, patients with thalassemia major carrying the apoE4 allele are at a higher risk of iron-induced damage of sub-cellular particles, certainly in mitochondria, which could lead to cell death by necrosis or apoptosis. Progressive LV dysfunction may result from the continuous loss of cardiomyocytes.[10] Previously, E4 was reported to be a genetic risk

Table 2. ApoE genotype and apoE allele frequencies for patients and controls*.

Frequency		*Group 1 (n=135)*	*Group 2 (n=38)*	*Group 3 (n=29)*	*Control group (n=198)*
ApoE genotype, n(%)	E2/E2	0 (0)	0 (0)	0 (0)	0 (0)
	E2/E3	13 (9.6)	6 (15.8)	1 (3.45)	23 (11.6)
	E2/E4	1 (0.7)	1 (2.6)	0 (0)	2 (1)
	E3/E3	102 (75.6)	28 (73.7)	21 (72.4)	156 (78.8)
	E3/E4	17 (12.6)	3 (7.9)	6 (20.7)	16 (8.1)
	E4/E4	2 (1.5)	0 (0)	1 (3.45)	1 (0.5)
ApoE allele (%)	E2	5.2	9.2	1.7	6.3
	E3	86.7	85.5	84.5	88.6
	E4	8.1	5.3	13.8	5.1

**E3/E4 genotype and E4 allele frequencies in group 3 were higher than those of the controls, other values were not statistically different between the patient and control groups.*

factor for LV failure in patients withh homozygous β-thalassemia;[2,3] we have found that it also associated with LV dysfunction. Regarding the late onset of cardiac symptoms in thalassemia, it is generally accepted that asymptomatic LV systolic dysfunction is a precursor of heart failure. While other studies have shown an association of E4 with LV dilatation in thalassemia,[2,3] this was not apparent in our study. Of the three patients with the E4/E4 genotype (Table 2), one (17 years old) was in group 3 but two (12 years old) were in group 1. Two possible reasons for this could be that apoE haplotype variants make a difference and that advanced age is necessary for the effect of E4 on LV function to become manifest. While E4 appears to be genetic risk factor for LV involvement, it must be emphasized that the apoE do not simply determine the end-point of cardiac status in thalassemia.

It has been reported that antioxidants play an effective role in E4 carriers,[11] thus, such compounds might be helpful in thalassemic patients with cardiac involvement, particularly in those carrying the E4 allele.

In conclusion, apoE4 allele is a genetic risk factor for LV impairment in thalassemia major. As the main cause of cardiac involvement in thalassemia is oxidative damage resulting from iron overload, efficient iron chelation treatment is an important part of cardiac care in thalassemia.

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