Original Article

Apolipoprotein E4 allele and the risk of left ventricular dysfunction in thalassemia major

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Background: Left ventricular (LV) failure is the main cause of death in thalassemia. Iron overload in these patients leads to formation of oxygen free radicals. Apolipoprotein (ApoE) E4 allele is the least efficient in oxidative stress condition compared with apoE2 and apoE3 alleles. This study was performed to determine the association of three different ApoE alleles with LV dysfunction in thalassemia major patients in southern Iran.

Methods: The present study comprised 202 patients with thalassemia major divided into three groups according to echocardiographic findings: Group 1 (n=135) had no cardiac impairment; Group 2 (n=38) exhibited LV dilatation but normal LV systolic function and Group 3 (n=29) showed LV systolic dysfunction. DNA was obtained from all patients and 198 healthy control subjects for ApoE genotyping.

Results: Frequency of both apoE3/E4 genotype and apoE4 allele in Group 3 were higher than the control group with corresponding values of $P<0.05$, Odds Ratio=2.97, $1.06<OR<8.32$ and $P<0.01$, $OR=3.01$, $1.15<OR<7.69$, respectively and confidence Interval of 95%. There were no differences observed between controls and patient groups in relation to other genotype and allele frequencies. Interventricular septum thickness and LV end diastolic diameter in apoE4/- patients were more than those of apoE3/E3 patients.

Conclusion: ApoE4 allele increases the risk of LV impairment in thalassemia major.

Keywords: Thalassemia; Left Ventricular dysfunction; Apolipoprotein E

Introduction

Thalassemia is a monogenic disorder affecting globin chain synthesis. Iran is one of the countries located at the thalassemia belt[1] with more than 20000 registered thalassemic patients[2]. Heart failure is the main cause of death in thalassemia. Left-sided heart failure is the most common presentation (83%) of cardiac involvement in these patients[3]. In the blood transfusion dependent patients, iron deposition leads to serious myocardial injuries. The major impact of iron deposition is visible on ventricular function[4]. Hydroxyl radical formation via the Fenton and Harber-Weiss reactions lead to damage of sub-cellular structures[5]. Apolipoprotein E (ApoE) is a well known lipid transport protein. Its gene, located at chromosome 19q13.2 has three major alleles including E2, E3 and E4[5]. ApoE alleles are associated with increasing risk of central nervous system and cardiovascular disorders such as Alzheimer's disease, dementia, atherosclerosis, coronary heart disease and stroke[6-8].

ApoE4 allele has been reported as a genetic risk factor for LV failure in homozygous β thalassemia[9,10] and LV systolic dysfunction in elderly[11]. ApoE acts as scavenger of free radicals. Antioxidant and iron binding activities

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of major alleles of ApoE are different (ranked E2>E3>E4)[12]. Iron chelating is also considered as another probable mechanism of ApoE anti-oxidant activity too[13].

Herein, we describe, the role of ApoE gene polymorphism on LV function in patients with β thalassemia major in Fars Province, southern Iran.

Materials and Methods

The present study was performed on 202 patients, 92 males and 110 females, with major thalassemia with the minimum age of 10 years and approved by the Central Ethics committee of Iranian Academic Center for Education, Culture and Research (ACECR). Selection of the patients was mainly based on their echocardiographic records.

They were followed by Cooley's Anemia Center of Dastgheib Hospital and evaluated for cardiac cares in Fatemeh-Zahra Heart Hospital affiliated to Shiraz University of Medical Sciences. Each patient received blood transfusion every 2 to 3 weeks to maintain hemoglobin level above 9 g/dl. Iron chelation by subcutaneous injection of deferoxamine for at least five nights per week was prescribed for all patients.

Transfusion therapy, the hemosidrosis level, the mean pretransfusion hemoglobin level, units of blood transfused and mean serum ferritin level at 6-months interval were evaluated during the last 1, 7 and 5 years of follow-ups for each patient. Cardiac evaluation included medical history, clinical chest X-ray (CXR), electrocardiography (ECG) and M-mode echocardiographic studies. According to echocardiographic findings, patients were divided into three groups based on Economoupetersen *et al* study[9] with some modifications. Group 1 included patients with no cardiac impairment; group 2, with LV dilatation but normal LV systolic function; and group 3, with LV systolic dysfunction. LV dilatation was defined as LV end diastolic diameter (LVEDD) higher than 90 percentile for the patient's body surface area[14].

Table 1: Basic clinical and hematological characteristics in three groups of patients*

Characteristic (mean ± 1SD**)	Group 1 (N=135)	Group 2 (N=38)	Group 3 (N=29)
Age (Years)	16.5±4.7	18.1±6	16.5±4
Sex (M/F)	62/73	20/18	10/19
Body Surface Area (m^2)	1.26±0.2	1.26±0.2	1.21±0.1
Age at the first blood transfusion (months)	23±28	18±20	19±26
Age of the chelation treatment beginning (years)	5.2±4	6.4±5	5.4±4.2
Hemoglobin (g/dl)	9.7±0.7	9.5±0.8	9.6±0.6
Units of blood transfused (n)	127±22	131±24	141±28
Serum ferritin (ng/ml)	3257±1254	3219±1247	3431±1074

*Any of these characteristics were not significantly different among groups
** SD: Standard Deviation

Table 2: M-mode echocardiographic evaluation of the patients

Echocardiographic indices (mean±1SD)	Group 1 (N=135)	Group 2 (N=38)	Group 3 (N=29)	P Value (Among groups)
LVEDD (mm)	43.8±4.1	50.1±33	48.3±5.6	<0.001
LVESD (mm)	27.3±4.2	32.1±3.7	35.7±5.6	<0.001
IVS thickness(mm)	7.7±1.8	7.1±1.5	8.7±2.2	<0.01
EF (%)	66.8±7	66.2±9.5	50.1±7.4	<0.001
SF (%)	36.1±4.7	37±7	24±3.6	<0.001
LVPW thickness (mm)	7.5±3.6	7±1.5	8±2.1	NS
RVEDD (mm)	17.2±5.7	17.4±6	19±6	NS

Abbreviations: LVEDD: left ventricular end diastolic diameter; LVESD: left ventricular end systolic diameter, IVS: interventricular septum; LVPW: left ventricular posterior wall; RVEDD: right ventricular end diastolic diameter, EF: (left ventricular) ejection fraction, SF: (left ventricular) shortening fraction, NS: not statistically significant.

Criterion for LV dysfunction was shortening fraction (SF) of less than 28%. Patients with systemic or endocrine disorders that might affect heart function were excluded from the study. Control group, checked by a physician, consisted of 198 healthy unrelated volunteers (95 males and 103 females) with mean age of 23.7 years±5.2 SD from the same geographic region.

Genomic DNA was extracted from whole blood. Polymerase chain reaction combined with restriction fragment length polymorphism (PCR-RFLP) and polyacryl amide gel electrophoresis were used for apoE genotyping as described previously[15].

Analysis of quantitative variables among groups was carried out by ANOVA-one way, Tukey post hoc analysis with P <0.05 considered significant. ApoE allele frequencies in patient and control groups were compared using X^2 test. However, Fisher's exact test was used whenever X^2 test was not reliable.

Table 3: ApoE genotype and ApoE allele frequencies for patients and controls*

		Group 1 (N=135)	Group 2 (N=38)	Group 3 (N=29)	Control group (N=198)
ApoE genotype (%)	E2/E2	0	0	0	0
	E2/E3	9.6	15.8	3.45	11.6
	E2/E4	0.7	2.6	0	1
	E3/E3	75.6	73.7	72.4	78.8
	E3/E4	12.6	7.9	20.7	8.1
	E4/E4	1.5	0	3.45	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

*Only E3/E4 genotype and E4 allele frequencies in group 3 were different from those of controls while other comparisons did not show any significant differences.

Results

Of 202 patients, 135 subjects were included in Group 1, 38 in group 2 and 29 in group 3. There were no significant differences found among three groups in regard to basic clinical and hematological characteristics of the patients (Table 1). The results of echocardiographic evaluation of the patients are shown in Table 2. As seen, left ventricular end diastolic diameter (LVEDD) and left ventricular end systolic diameter (LVESD) indices differed among the three groups, while interventricular septum (IVS) thickness, ejection fraction (EF) and SF were not significantly different between groups 1 and 2. Also, RVEDD and left ventricular posterior wall (LVPW) thickness did not differ among the groups. Table 3 shows the ApoE genotype and ApoE allele frequencies in three groups of patients and control group. A comparison of each patient group with the control group, showed that the frequencies of both E3/E4 genotype and E4 allele in group 3 were significantly higher than those of control group with respective values of $P<0.05$, $OR=2.97$, $1.06<OR<8.32$ and $P<0.01$, $OR=3.01$, $1.15<OR<7.69$ at CI=95%. Other values were not statistically different between the patient and control groups.

ANOVA of echocardiographic indices among ApoE genotypes showed that LVESD in patients with E3/E4 genotype was higher compared with those of E3/E3 genotype as the most frequent genotype (32.5±4.9 against 28.9±5.4, $P<0.05$). In addition, IVS thickness in the E3/E4 was more than those of E3/E3 and E2/E3 patients (8.8±2.2 against 7.5±1.7 mm, $p<0.01$ and 7.1±1.9 mm, $p<0.05$, respectively. IVS thickness in the E4/-patients was more than those of E3/E3 and E2/E3 patients ($P<0.01$ and $P<0.05$, respectively). A higher LVESD was observed in the E4/-patients than E3/E3 ones ($P<0.05$). By analysis of covariance, association of ApoE alleles with LV function was independent of age and gender.

Discussion

Iron deposition is the main cause of cardiac complications in thalassemia major. The deposition of iron is greatest in the ventricular walls[4]. In regard to basic clinical and hematological characteristics (Table 1), no significant differences were found between three groups of patients, while those included in group 2 and group 3 developed some degrees of LV dysfunction. It should be considered that LV dilatation usually precedes LV dysfunction. These differences can arise from diversities in loci of genetic susceptibility. For these patients, susceptibility can be expressed as more tendency to iron uptake and iron deposition as this is the case with hereditary hemochromatosis[16] and/or more vulnerability to iron overload, for instance, ApoE4 allele carriage[9,10].

Left-sided heart failure in homozygous β-thalassemia is associated with the major histocompatibility complex[17].

ApoE4 allele has been reported as a genetic risk factor for LV failure in homozygous β-thalassemia in Greek[9] and Italian[10] populations. In thalassemia major, formation of hydroxyl radicals resulting in iron overload leads to peroxidative damage of membrane lipids and proteins. These injuries also affect the mitochondrial respiratory chain and α-ketoglutarate dehydrogenase complex[18]. ApoE4 allele has the least antioxidant and iron binding

activities[12]. ApoE isoproteins have differential metabolic pathways. E4 allele is catabolized three times faster than E2 allele[8,19,20]. The ApoE gene polymorphism also has a strong effect on the level of its gene product: E2 is associated with higher concentrations of ApoE while E4 is associated with lower concentrations[6,21]. The accumulation within cells of ApoE3 and ApoE2 are two to four-fold higher than that of ApoE4[7,22]. Therefore, the intracellular localization, the major location of iron storage and the formation of free radicals, in relation to ApoE4 is less than those of ApoE3 and ApoE2. Inheritance of E4 allele is associated with higher protein modification by 4-hydroxynonenal[23,24].

Regarding differences of apoE isoforms, major thalassemic patients who have E4 allele are at a higher risk for iron-induced damage of cellular and sub-cellular particles, particularly mitochondria,[5,18] which could result in cell death by necrosis or apoptosis. It is noteworthy that E4 allele induces the *Fas* mediated apoptosis in cardiomyocytes[11,25]. Studies of heart failure in human and animals have led to the hypothesis that progressive LV dysfunction may result, in part, from the continuous loss of cardiomyocytes[11,26]. Death of a significant number of adult cardiomyocytes, either by necrosis or apoptosis, can permanently diminish cardiac performance. Apoptosis detected in the failing heart generally affects scattered individual cells[11,27]. The aging process occurring in the heart is characterized by a significant loss of cardiomyocytes and reactive hypertrophy of the remaining cells[11,28]. It is estimated that the aging process itself contributes to loss of nearly 30% of all LV cardiomyocytes[11,26].

E4 allele may also increase the risk of myocarditis, a pathogenic pathway leading to LV failure, in some of thalassemic patients via accumulation of oxygen free radicals[9,29].

In the present study, we found that E4 allele is a genetic risk factor for LV dysfunction in thalassemia major. As mentioned previously, E4 allele has been reported as a genetic risk factor for LV failure in homozygous β-thalassemia[9,10]. Regarding late onset of symptoms in connection with cardiac involvement in thalassemia,[4] asymptomatic LV systolic dysfunction is generally accepted to be a precursor for heart failure. Reduced EF increases the risk of cardiovascular mortality and hospital admissions for heart failure[11,30]. A study reported from Netherlands, associated E4 allele with LV systolic dysfunction in elderly.[11]

Despite association of E4 allele with LV dilatation in thalassemia,[9,10] such relationship was not found in present investigation. The reason might be due to the exclusion of age limitation for initiation of chelation treatment. This characteristic was the predictor of LVEDD increase in linear regression analysis ($P<0.005$, $\beta=0.233$). However, our study indicated that E4 allele was associated with IVS thickness and LVESD increment. Of three patients with E4/E4 genotype (Table 3), one was 17 years old and included in group 3 (17 years old) but two aged about 12 years and were included in group 1. The reasons could be based on two hypotheses. Firstly, ApoE haplotype variants accounted for the difference[31] and secondly, advanced age was necessary for involvement of E4 allele in LV function. It is noteworthy that parents of each E4/E4 patients in group 1 had consanguineous marriage that increased the probability of haplotype homogeneity.

As E4 allele is reported as genetic risk factor for LV systolic involvement, nevertheless, it must be emphasized that the ApoE do not simply determine the eventual outcome of cardiac status in thalassemia. Other loci such as cytochrome C oxidase, superoxide dismutase and catalase could be of potential relevance to the oxidative damage of organs in β-thalassemia[9,32]. Highly variable manifastations in monogenic disorders such as thalassemia, suggest that the boundaries between monogenic and polygenic disorders might not always be so clear-cut as previously thought[33].

In blood transfusion dependent thalassemic patients, a main cause of cardiac involvement is oxidative damage, resulting from iron overload. In this connection, antioxidant supplements could be helpful to reduce organ damage, especially in ApoE4 carriers.

Effective role of vitamin C and E in the E4 allele carriers have been reported[34,35]. Captopril, an angiotensin-converting enzyme inhibitor, also has a known role in scavenging free radicals[36]. In this regard, its administration might be helpful in thalassemic patients with cardiac involvement particularly E4 carriers.

In conclusion, ApoE4 allele is a genetic risk factor for LV impairment in thalassemia major.

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