Apolipoprotein E polymorphism - a risk factor in Romanian pregnant women with preeclampsia

Influence on serum lipoprotein levels, the degree of severity and perinatal outcome of preeclampsia

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Abstract

Objectives. *to determine the influence of the APOE genotypes on blood lipid levels, the degree of severity and perinatal outcome of preeclampsia.* ***Study design.*** *the APOE genotypes were performed in 101 pregnant women, 51 preeclamptic and 50 healthy pregnant women using PCR-FRLP methods. We compared genotype and allele frequencies between women with preeclampsia and normal pregnant women by Fisher's exact test two-sided. We calculated odds ratio (OR) and 95% confidence intervals (95% CI). The significance of results was defined as p <0.05.* ***Results.*** *Different distribution of the APOE genotypes in pregnant women with preeclampsia compared to normal pregnant women was observed. Higher frequency of ε3/ε4 genotypes was found in pregnant women with PIH, mild and severe preeclampsia compared to normal pregnant women. Higher TG and LDL-C levels and lower HDL-C levels in women with the ε4 allele as compared to women with the ε3/ε3 genotype was observed. Preeclamptic women with the ε4 allele delivered earlier neonates with lower birth weight compared to preeclamptic women with ε3/ε3 genotype. The study is of interest in part due to the novelty of the methods applied for the ε4 allele determination.* ***Conclusions:*** *We observed a significant association of the ε3/ε4 genotype with PIH and severe preeclampsia. The ε4 allele represents a risk factor for dyslipidemia and influence the perinatal outcome of preeclampsia.*

Keywords: *APOE genotypes, PCR-RFLP, preeclampsia, lipoproteins levels, Romania*

Introduction

Preeclampsia represents a pregnancy complication with unknown etiology, with increased maternal and fetal morbidity and mortality risk[1,2,3]. Some of the risk factors for preeclampsia involve oxidative stress associated with endothelial dysfunction[4,5,6]. These could be caused by dyslipidemia with higher triglyceride (TG) and triglyceride rich lipoprotein (LDL-C) levels and lower HDL-C levels. Preeclamptic women with an altered lipid profile are at increased risk to develop cardiovascular diseases later in life probably because of the higher risk to develop metabolic syndrome associated with hypertension and lipid profile alteration[7,8,9]. APOE is implicated in the normal catabolism of triglyceride rich lipoproteins, being a component of chylomicrons, very-low-density lipoproteins (VLDL), and high-density-lipoproteins (HDL)[10]. Thus, APOE could be a marker for dyslipidemia[11,12]. The APOE gene is located on chromosome 19q13.2 and produces apolipoprotein E. There are three APOE isoforms - E1, E2 and E3, coded by three alleles, epsilon 2 (ε2), epsilon 3 (ε3) and epsilon 4 (ε4). These three alleles combine and produce six genotypes, three homozygous genotypes (ε2/ε2, ε3/ε3, ε4/ε4) and three heterozygous genotypes (ε2/ε3, ε2/ε4, ε3/ε4)[13]. The ε2 allele (less cholesterol binding) has Cys at position 112 and 158, the ε3 allele (a more common allele) has Cys at 112 and Arg at 158, and the ε4 allele has Arg at both positions[14]. These six genotypes have different effects on lipid and lipoprotein levels. In most populations, the ε3 is the normal allele. The ε2 allele has a lower affinity for the APOE receptor, which means delayed clearance of apoE-rich chylomicron remnants, overexpression of LDL receptors and increased clearance of LDL particles, all of these changes being associated with lower LDL-C levels[15]. The ε4 allele is associated with high LDL-C levels[10,13,16,17,18]. Eichner in 2002 demonstrated that the APOE genotypes were associated with lipoprotein concentrations[15]. Dallongeville in 1992 showed that patients carrying the ε2 allele have lower HDL-C levels than those carrying the ε3 allele[19]. The results from the MONICA Project and the Scandinavian Simvastatin Survival Study showed a higher risk for cardiovascular diseases and Alzheimer's disease in patients positive for the ε4 allele[14,20,21]. A higher incidence of atherosclerosis was found in patients with this allele[22]. The genetic basis of preeclampsia is

not very clear, but the APOE gene could be associated with this pregnancy disorder. Moreover, the APOE gene is expressed in the placenta[23].

Objectives

1. To compare APOE genotype/allele frequencies in pregnant women with preeclampsia and in normotensive pregnant women and to determine the levels of significance of the association between different types of preeclampsia and apolipoprotein E genotypes in Romanian pregnant women from Northern Transylvania.

2. We also aimed to check the relationship between the APOE genotypes and blood lipid and lipoprotein levels in pregnant women with preeclampsia, to determine whether the ε2, ε3, ε4 alleles of the APOE gene in pregnant women with preeclampsia were associated with perinatal outcome, gestational age at delivery and birth weight in this cohort.

Materials and methods

Studied groups

This study was performed in the Department of Medical Biochemistry, University of Medicine and Pharmacy Cluj-Napoca. The cases with and without preeclampsia were selected in the Department of Obstetrics and Gynecology, Clinic I, Cluj-Napoca between October 2009 - January 2010 after obtaining written informed consent of the subjects to participate in the study.

The APOE genotypes were determined in 101 pregnant women of >20 weeks gestation, 51 cases of preeclampsia and 50 healthy pregnant women. The Ethics Committee of the Faculty of Medicine, University of Medicine and Pharmacy Cluj-Napoca, approved this study.

The diagnosis of preeclampsia, based on the clinical examination of the pregnant women, was defined as hypertension ≥140/90 mmHg on two occasions six hours apart, together with proteinuria ≥300 mg/24h collection and edema. The group of pregnant women with preeclampsia included 17 (33.33%) pregnant women with PIH, 22 (43.14%) pregnant women with mild preeclampsia and 12 (23.53%) pregnant women with severe preeclampsia, classified according to ACOG practice bulletin[24]. The normotensive pregnant women were matched for age and body mass index- BMI with the women with preeclampsia. For the present study, only primiparous pregnant women were included, while multiparous women were excluded. The demographic characteristics of the pregnant women and newborns are presented in table 1.

Methods

Polymerase Chain Reaction

Two ml venous blood obtained from both groups, women with preeclampsia and normotensive pregnant women, were collected into EDTA containing tubes. Genomic DNA extraction was done using a ZR genomic DNA II kit according to the manufacturer's protocol.

The amplification of the fragment of interest was performed using the polymerase chain reaction (PCR). PCR amplification involves the participation of DNA, the forward and reverse oligonucleotide primers, the thermostable Taq DNA polymerase enzyme, deoxynucleotides, $MgCl_2$ and a 10X reaction buffer. Using the forward 5'-AGACGCGGGCACGGCTGTCCAAGGA-3' and reverse 5'-CCCTCGCGGGCCCCGGCCTGGTACAC-3' primers, we amplified in a thermal cycler BioRad a 244 bp product[17,25]. All the PCR reagents were from Fermentas. PCR was conducted in a reaction mix containing 20 ng genomic DNA, 200 μM deoxynucleotides (dNTPs - dATP, dCTP, dGTP and dTTP), 0.2 μM primers, 2.0 mM $MgCl_2$ and 0.625 U Taq DNA polymerase. PCR amplification involved 35 cycles of genomic DNA denaturation at 94°C for 30 seconds, annealing of the primers at 65°C for 30 seconds, and extension of the primers at 72°C for 1 minute. The 35 cycles were preceded by a denaturation step for 2 minutes at 94°C and were followed by an elongation step for 10 minutes at 72°C.

Enzymatic digestion

The PCR amplified products were digested with the HhaI restriction enzyme (Fermentas) using the restriction fragment length polymorphism (RFLP) methods. Restriction digestion was carried out in a reaction mix containing 6 μl PCR products, 2 U restriction enzyme and 1 μl restriction digestion buffer (X10 concentrate) in a final volume of 10 μl. The reaction mix was incubated for 3 hours at 37°C. The genotypes were determined by electrophoresis on 3% agarose gel stained with 10 mg/ml ethidium bromide solution and visualized on UV light. The fragment sizes after HhaI digestion were as follows: ε2/ε2: 91 bp and 83 bp; ε3/ε3: 91 bp, 48 bp; ε4/ε4: 72 bp, 48 bp; ε3/2: 91 bp, 83 bp and 48 bp; ε4/ε2: 91 bp, 83 bp, 72 bp and 48 bp; ε4/ε3: 91 bp, 72 bp and 48 bp. Each of the genotypes associate fragments of 36, 18 or 16bp, but because these are too small it can't be visible in the agarose gel (Figure 1)[17,25].

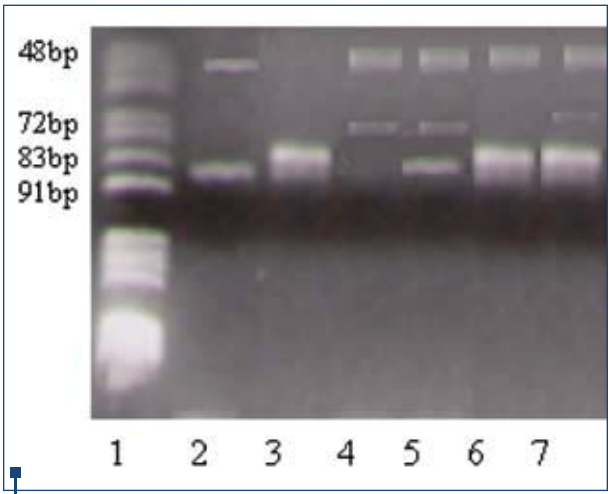

Figure 1. APOE genotypes after enzymatic digestion of the amplified fragments with HhaI restriction enzyme; Lane 1. pBR HaeIII Digest DNA molecular marker; Lane 2: ε3/ε3 genotype - fragments of 91 and 48bp; Lane 3: ε2/ε2 genotype - fragments of 91 and 83bp; Lane 4: ε4/ε4 genotype - fragments of 72 and 48bp; Lane 5: ε4/ε3 genotype- fragments of 91, 72 and 48bp; Lane 6: ε3/2 genotype - fragments of 91, 83 and 48bp; Lane 7: ε4/ε2 genotype- fragments of 91,83,72 and 48bp

Serum analysis

The serum lipid and lipoprotein levels, tryglicerides (TG), high density lipoproteins (HDL-C), low density lipoproteins (LDL-C) were determined with AMP Diagnostics kit using colorimetric methods. The normal references range was 65-140mg/dl for tryglicerides, 35-70mg/dl for HDL-C and <130mg/dl for LDL-C.

Statistical analysis

Systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), TG, LDL-C, HDL-C were expressed as mean ± SD. We analyzed the differences in lipid and lipoprotein levels between the two groups using Student's t test. APOE genotype and allele frequencies were calculated in both groups, pregnant women with and without preeclampsia. We performed statistical procedures using the statistical software package SAS 6.12 for Windows (SAS Institute Inc., Cary, NC, USA). We compared genotype and allele frequencies between these groups by Fisher's exact test two-sided. In order to test for differences in the prevalence of the mutated genotypes, an exploratory method was used and we calculated 95% confidence intervals (95% CI). To test the association between preeclampsia and different variables, lipid and lipoproteins levels and APOE genotypes, odds ratio were also performed. The significance of results was defined as $p < 0.05$.

Results

There were no statistical significant differences in maternal age and BMI between the two groups, but there were statistically significant differences in SBP/DBP, gestational age at delivery and birth weight. The same observation was true for SBP/DBP, gestational age at delivery and birth weight in the subgroups of women with PIH, mild and severe preeclampsia as compared to normal pregnant women (Table 1).

In our study, pregnant women with preeclampsia had higher TG and LDL-C levels and lower HDL-C levels than normal pregnant women, but in a normal range for LDL-C and HDL-C. On the other hand, by analyzing different types of preeclampsia, our study confirms higher TG and LDL-C levels and lower HDL-C levels in all types of preeclampsia as compared with normal pregnant women (Table 2).

Table 1 Maternal and newborn characteristics in the studied groups, women with preeclampsia and normal pregnant women

Characteristics	Preeclampsia N = 51	PIH N= 17	Mild PE N= 22	Severe PE N= 12	Control women N= 50
Age, years	28.16 ± 4.34	28.65 ± 4.14	27.63 ± 4.58	28.42 ± 4.46	28.04
Maternal weight, kg	66.25 ± 14.81 **p= 0.03**	62.76 ± 10.03	70.23 ± 18.98 **p<0.01**	63.92 ± 10.32	60.92 ± 9.68
Maternal height, m	1.65 ± 0.05	1.65 ± 0.05	1.66 ± 0.05	1.64 ± 0.06	1.67 ± 0.06
BMI, Kg/m²	24.22 ± 5.2	23.16 ± 4.18	25.34 ± 6.41	23.68 ± 3.78	22.48 ± 9.95
SBP, mmHg	153.92 ± 16.65 p<0.01	144.71 ± 10.67 p<0.01	147.73 ± 2.29 p<0.01	178.33 ± 14.2 p<0.01	126.2 ± 4.69
DBP, mmHg	102.84 ± 10.16 **p<0.01**	97.06 ± 7.51 **p<0.01**	100 ± 3.09 **p<0.01**	116.25 ± 10.25 **p<0.01**	75.4 ± 4.83
Pregnancy, no (%) Single Twin	49 (96.08%) 2 (3.92%)	17 (100%) -	21 (95.45%) 1 (4.55%)	11 (91.67%) 1 (8.33%)	49 (98%) 1 (2%)
Maternal complications, no (%) Eclampsia HELLP syndrome	1 (1.96%) 2 (3.92%)	- -	- 1 (4.55%)	1 (8.33%) 1 (8.33%)	- -
Gestational age at delivery, weeks	35.24 ± 4.54 **p<0.01**	34.24 ± 4.24 **p<0.01**	37 ± 4.11 **p<0.01**	33.42 ± 4.87 **p<0.01**	38.64 ± 4.86
Mode of delivery, no (%) Cesarean section Vaginal birth	34 (66.67%) 17 (33.33%)	10 (58.82%) 7 (41.18%)	16 (72.73%) 6 (27.27%)	12 (100%) -	10 (20%) 40 (80%)
Apgar score at 5 minutes	7.9 ± 2.42 **p<0.01**	7.53 ± 2.79 **p<0.01**	8.68 ± 0.95 **p=0.001**	7 ± 3.35 **p<0.01**	9.54 ± 0.78
Birth weight, grams	2620.19 ±1045.6 **p = 0.002**	2620.19 ±1045.6 **p<0.01**	3136.36 ± 851.22	2120.833 ±954.52 **p<0.01**	3155.8 ± 506.22

physical characteristics are expressed as mean ± SD; PIH - pregnancy induced hypertension; PE - preeclampsia; SBP - systolic blood pressure; DBP - diastolic blood pressure; in order to analyze the differences between the two groups we used Student's t test

Table 2 Serum TG, HDL-C and LDL-C levels depending on the severity of preeclampsia

Lipids	Preeclampsia	p	PIH	p	Mild PE	p	Severe PE	p	NP women
TG mg/dl	253.33 ± 40.81	< 0.01	247.41 ± 4.75	0.01	252.68 ± 43.39	< 0.01	262.92 ± 30.13	< 0.01	214.4 ± 31.15
HDL-C mg/dl	44.9 ± 10.61	< 0.01	43.09 ± 10.05	< 0.01	48.6 ± 10.09	< 0.01	40.68 ± 10.87	< 0.01	62.67 ± 19.95
LDL-C mg/dl	104.44 ± 31.89	< 0.01	102.29 ± 28.14	0.02	95.49 ± 32.3	NS	123.88 ± 29.97	< 0.01	81.74 ± 34.59
LDL-C:HDL-C ratio	2.49 ± 1.03	< 0.01	2.48 ± 0.86	< 0.01	2.11 ± 0.94	0.006	3.2 ± 1.1	< 0.01	1.44 ± 0.76
TG:HDL-C ratio	5.98 ± 1.76	< 0.01	6.56 ± 1.74	< 0.01	5.44 ± 1.58	< 0.01	6.87 ± 1.87	< 0.01	3.82 ± 1.64

PIH - pregnancy induced hypertension; NP women- normal pregnant women; TG - triglycerides; HDL-C - high density lipoprotein; LDL-C - low density lipoprotein; PE - preeclampsia; p < 0.05 - statistical significance - p less than 0.05; in order to analyze the differences between the groups of women with different types on preeclampsia and normal pregnant women we used Student's t test

Following the analysis of the APOE genotypes, we suggest a different distribution of these in women diagnosed with preeclampsia compared to women with normal pregnancies (ε2/ε3 - 13.73% vs 22%, ε3/ε3 - 56.86% vs 64%, ε3/ε4 - 21.57% vs 6%, ε4/ε4 - 7.84% vs 4%). None of the women with preeclampsia had the ε2/ε2 or ε2/ε4 genotypes. At the same time, the frequency of the three alleles, ε2, ε3 and ε4, was different in the two groups (preeclamptic women vs. normal pregnant women, ε2-6.86% vs 14%, ε3-74.51% vs 78%, ε4-18.63% vs 8%).

The APOE genotypes' distribution was also different between the different types of preeclampsia. Thus, a higher frequency of ε3/ε4 genotypes was found in women with PIH (17.65%), mild (24.82%) and severe preeclampsia (33.33%) compared to normal pregnant women (6%). In contrast, a lower frequency of ε2/ε2 genotypes was found in all preeclamptic women compared to normal pregnant women (Table 3).

Pregnant women with the ε4 allele had a statistically increased risk to develop PIH (OR 4.14, p = 0.013) and severe preeclampsia (OR 4.43, p = 0.019) (Table 4).

In order to check the association of lipid and lipoprotein levels with the apo E genotypes, we divided the subjects into three groups: individuals with the ε2 allele, individuals with ε3/ε3 genotypes, and individuals with the ε4 allele. The analysis of the lipid profile according to APOE genotypes revealed the following results: statistically significant higher TG (mg/dl, 271.79 ± 30.33 vs. 248 ± 40.89, p= 0.039) and LDL-C levels (mg/dl, 136.73 ± 20.01 vs 93.26 ± 29.38) and lower HDL-C levels (mg/dl, 39.64 ± 9.11 vs 46.5 ± 11.46) in preeclamptic pregnant women with the ε4 allele as compared to preeclamptic pregnant women with the ε3/ε3 genotype. Preeclamptic pregnant women with the ε4 allele delivered at 32.36 ± 4.38 weeks compared to 35.34 ± 4.64 weeks for preeclamptic pregnant women with ε3/ε3 genotype. Also, neonates of preeclamptic women with ε4 allele had a birth weight of 2114.286 ± 1081.92 grams compared to 2851.034 ± 964.64 grams for neonates from preeclamptic women with ε3/ε3 genotype (Table 5).

Discussions

In normal pregnancy, there are physiological changes in the lipid profile because of hormonal variations, but lipid levels come down to those before the pregnant state in 6-8 weeks in the postpartum period[26]. Perturbation of lipid metabolism with increased triglycerides and decreased HDL-C determines increased oxidative

Table 3 Distribution of APOE genotypes and alleles frequency depending on the severity of preeclampsia

	APOE genotypes						APOE alleles		
	ε2/ε2	ε3/ε4	ε2/ε4	ε3/ε3	ε2/ε3	ε4/ε4	ε2	ε3	ε4
Normal pregnancy (N = 50)	1 (2%)	3 (6%)	1 (2%)	32 (64%)	11 (22%)	2 (4%)	14 (14%)	78 (78%)	8 (8%)
Preeclampsia (N = 51)	-	11 (21.57%)	-	29 (56.86%)	7 (13.73%)	4 (7.84%)	7 (6.86%)	76 (74.51%)	19 (18.63%)
PIH (N = 17)	-	3 (17.65%)	-	9 (52.94%)	2 (11.76%)	3 (17.65%)	2 (5.88%)	23 (67.65%)	9 (26.47%)
Mild PE (N = 22)	-	4 (24.82%)	-	13 (59.09%)	5 (18.18%)	-	5 (11.36%)	35 (79.55%)	4 (9.09%)
Severe PE (N = 12)	-	4 (33.33%)	-	7 (58.33%)	-	1 (8.33%)	-	18 (75%)	6 (25%)

PIH - pregnancy induced hypertension; PE - preeclampsia

Table 4 APOE genotypes and alleles - association with different types of preeclampsia

APOE	Preeclampsia N= 51	PIH N= 17	Mild preeclampsia N= 22	Severe preeclampsia N= 12
ε2/ε2 OR, p	-	-	-	-
ε2/ε3 OR, p	0.56 [0.18-1.78], NS	0.63 [0.18-2.21] NS	1.04 [0.27-3.97] NS	-
ε2/ε4 OR, p	-	-	-	-
ε3/ε3 OR, p	0.74 [0.31-1.78] NS	0.63 [0.18-2.21] NS	0.81 [0.26-2.57] NS	0.78 [0.19-3.41] NS
ε3/ε4 OR, p	4.3 [1-21.07] 0.041	3.35 [0.47- 24.37] NS	3.48 [0.58-22.32] NS	7.83 [1.16-57.37] 0.022
ε4/ε4 OR, p	2.04 [0.3-16.98] NS	5.14 [0.61- 50.07] NS	-	2.18 [0.07-35.76] NS
ε2 allele OR, p	0.45 [0.16-1.27] NS	0.38 [0.06-1.93] NS	0.61 [0.16-2.18] NS	-
ε3 allele OR, p	0.82 [0.41-1.66] NS	0.59 [0.23-1.52] NS	0.81 [0.37-1.79] NS	0.79 [0.29-21.5] NS
ε4 allele OR, p	2.63 [1.02-6.96] 0.037	4.14 [1.29-13.38] 0.013	1.32 [0.31-5.46] NS	4.43 [1.15-17.11] 0.019

PIH - pregnancy induced hypertension; OR - odds ratio; NS - non-significant; p- statistical significance - p less than 0.05; we performed statistical procedures using the statistical software package SAS 6.12 for Windows (SAS Institute Inc., Cary, NC, USA); we compared genotype and allele frequencies between women with preeclampsia and normal pregnant women and between different types of preeclampsia and normal pregnancy by Fisher's exact test two-sided

Table 5 Influence of APOE genotypes on serum TG, LDL-C, HDL-C levels and perinatal outcome of preeclampsia

Characteristics	APOE		
	ε2⁺ (N= 8)	ε4⁺ (N= 14)	ε3/ε3 (N = 29)
TG, mg/dl mean ±SD	240.38 ± 50.05	271.79 ± 30.33 **p= 0.039***	248 ± 40.89
LDL-C, mg/dl mean ±SD	88.46 ± 13.08	136.73 ± 20.01 **p<0.01***	93.26 ± 29.38
HDL-C, mg/dl mean ±SD	48.35 ± 6.68	39.64 ± 9.11 **p= 0.04***	46.5 ± 11.46
Gestational age at delivery, weeks mean ±SD	36.38 ± 4.62	32.36 ± 4.38 **p= 0.049***	35.34 ± 4.64
Birth weight, grams mean ±SD	2668.75 ± 1085.71	2114.286 ± 1081.92 **p= 0.04***	2851.034 ± 964.64

**comparison between preeclamptic women with ε4 allele and preeclamptic women with ε3/ε3 genotype; in order to analyze the differences between the groups of preeclamptic women with the ε4+ and the group of preeclamptic women with the ε3/ε3 genotype we used Student's t test.*

stress, contributing to the development of preeclampsia[8,27,17,28,29,30,31,32]. Regarding the lipid profile, our results showed serum TG and LDL-C levels on the one hand and serum HDL-C levels on the other hand being significantly higher and lower, respectively, in women with preeclampsia compared to serum levels in women with normal pregnancies, but in the normal range for LDL-C and HDL-C suggesting the hypothesis that abnormal lipid metabolism plays an important role in preeclampsia. These results can suggest the increased risk of lipid storage in spiral arteries, which would cause endothelial dyfunction, as a result of increased

LDL generation. Also, the LDL-C: HDL-C and TG:HDL-C ratios are significantly increased in all groups of preeclamptic women compared to normal pregnant women. Our results regarding lipid and lipoprotein profiles in preeclampsia are in agreement with those obtained by Belo (2002) and Jayanta (2006)[30,32,33].

In this preliminary study, we examined the APOE genotypes in association with preeclampsia and lipids profile. To the best of our knowledge, this is the first report regarding the APOE genotypes in Romanian preeclamptic women. APOE plays an important role in the removal of atherogenic remnants of triglyceride rich lipoproteins[34]. In the adult population, an association between the presence of mutated genes and lipoprotein levels has been demonstrated[12]. Moreover, the ε2 isoform binds with low affinity to the LDL-receptor and determines impaired intestinal cholesterol absorption and defective clearance of chylomicron remnants[17,35]. Results concerning the association of different APOE alelae and preeclampsia are contradictory. In the present study, there were no statistically significant differences regarding the distribution of the ε3/ε3 genotypes in the two groups, preeclamptic vs normal pregnancies patients. Our preliminary study showed that the ε3/ε4 genotype was positively associated with preeclampsia. We found a higher frequency of the ε4 allele and a lower frequency of the ε2 allele in women with preeclampsia compared to normal pregnant women. Therefore, we regard the ε2 alela as a protective factor in time, whereas the ε4 alela as a risk factor for the occurence of preeclampsia.

Our results differ from those obtained by Makkonen (2001) and Francoualin (2002) regarding the association of the APOE genotype with preeclampsia[17,36]. The study made by Nagy (1998) showed a higher risk for severe preeclampsia in women with the ε2 allele[12]. In our study there were no preeclamptic women with ε2 allele. Atkinson in 2003 found a higher risk of preeclampsia associated with the ε3 allele[37]. The distribution of the ε2 allele was in agreement with that obtained by Corbo (1998), who showed a frequency between 2%-14.5% worldwide[38]. Our results are in agreement with those obtained by Chicosi (2000) and JiYing (2009), which showed a relationship between preeclampsia and the APOE- ε4 allele. Also the frequency of the ε3 allele was higher in controls than in preeclamptic women[39,40].

The analysis of the different types of preeclampsia revealed that the frequency of the ε3/ε4 genotype was also increased in PIH and mild preeclampsia. A statistically significant association was found with severe preeclampsia. Higher association was found between PIH and ε4 allele and between severe preeclampsia and ε4 allele. Also, the distribution of the ε3/ε3 genotype was almost similar in pregnant women with and without preeclampsia. A higher frequency of the ε3 allele was found in both cases and controls, which confirms the hypothesis that the ε3 allele is the most common allele in the population.

We also investigated the association of the APOE genotypes with preeclampsia and with lipid and lipoproteins levels.

Given that our study shows no significant differences regarding the distribution of the ε3/ε3 genotype between the two pregnant groups, preeclamptic and with normal pregnancies, we considered it to be a reference genotype. Thus, we tried to establish the influence of the ε2, respectively ε4 alelae upon plasma levels of TG, HDL-C, LDL-C in preeclamptic women, compared to the reference genotype ε3/ε3.

Our preliminary study showed lower TG and LDL-C levels in preeclamptic women with the ε2 allele compared to preeclamptic women with the ε3/ε3 genotype. Thus, the lower LDL-C levels in women carrying the ε2 allele were associated with a reduced risk of preeclampsia. Also, the higher LDL-C and TG levels observed in preeclamptic women with the ε4 allele compared to preeclamptic women with the ε3/ε3 genotype were associated with the risk for preeclampsia. Our results are in agreement with those obtained by Belo in 2002 and JiYing in 2009[33,40]. A significant association was found between the ε4 allele and the pregnancy outcome, gestational age at delivery and birth weight, preeclamptic women with ε4 allele delivered earlier, neonates with lower birth weight as compared to preeclamptic women with the ε3/ε3 genotype.

Conclusions

In this cohort of pregnant women, dyslipidemia may be a possible risk factor for all types of preeclampsia. Our results suggest that the apolipoprotein E genotype could represent a risk factor for preeclampsia in this population. We observed a significant association of the ε4 allele with PIH and severe preeclampsia. Also, the ε4 allele in association with dyslipidemia represents a risk factor for preeclampsia. The ε4 allele could also influence the perinatal outcome of preeclampsia.

Furthermore, the study is of interest in part due to the novelty of the methods applied for the ε4 allele determination.

Further studies are needed to confirm these observations. In the future, in order to prevent preeclampsia, it could also be useful to determine blood lipids and to check if there is an association between lipoprotein concentrations in preeclamptic women and newborn genotypes for APOE and other genes possibly associated with dyslipidemia. ■

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