Archives of the Balkan Medical Union
vol. 53, no. 2, pp. 205-209
June 2018

ORIGINAL PAPER

EXAMINATION OF THE LIPID PROFILE IN PREGNANT OBESE WOMEN, CONSIDERING THE ALLELE STATUS OF THE GENES OF ANGIOTENSIN-CONVERTING ENZYME (ACE I / D) AND PLASMINOGEN-1 (PA-1 4G / 5G) ACTIVATOR-INHIBITOR

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Received 14 Feb 2018, Accepted 02 May 2018

Abstract

Changes in the lipid profile in pregnant women, with and without obesity, have been studied depending on the angiotensin-converting enzyme (ACE) gene I/ D polymorphism and the 4G5G polymorphism of the plasminogen activator inhibitor type I (PAI-1). The combination of the D-allele of the ACE gene and the 5G allele of the PAI-1 gene is a risk factor of increasing total cholesterol in pregnant women, regardless of the obesity class, and of increasing triglycerides in pregnant women without obesity. The presence of the D-allele of the ACE gene in the haplotype, regardless of the combinations of the genotypes of the PAI-1

Résumé

L'analyse du profil lipidique des femmes enceintes avec de l'obésité selon le statut allélique des gènes de l'enzyme de conversion de l'angiotensine (ECA I/D) et de l'inhibiteur d'activation du plasminogène 1 (RA-1 4G/5G)

Les modifications survenues dans le profil lipidique des femmes enceintes avec ou sans obésité ont été étudiées selon le polymorphisme I / D du gène de l'enzyme de conversion de l'angiotensine (ECA) et du polymorphisme génique 4G5Gde l' inhibiteur d'activation du plasminogène de type I (RAI-1). La combinaison entre

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gene, is a risk factor of increasing triglycerides in pregnant women with obesity.

Keywords: pregnancy, obesity, lipids, ACE gene polymorphism, PAI-1gene polymorphism.

l'allèle D et l'allèle du gène RAI-1 est un facteur de risque pour l'augmentation de la teneur totale en cholestérol chez les femmes enceintes quel que soit le degré d'obésité et pour l'augmentation du taux de triglycérides chez les femmes enceintes sans l'obésité. La présence de l'allèle D du gène de l'ECA dans les haplotypes indépendamment de combinaisons des génotypes du gène RAI-1 constitue un facteur de risque pour l'augmentation des triglycérides chez les femmes enceintes obèses.

Mots-clés: la grossesse, l'obésité, les lipides, le polymorphisme du gène ECA, le polymorphisme du gène RAI-1.

Introduction

Considering the high incidence of complications of pregnancy, labor, metabolic changes, and neonatal morbidity in obese women[1], these are not features of the morphotype; they should be considered as a syndrome with systemic changes in the functions of the central nervous system and the endocrine organs, at the membrane-cellular level.

Recent studies have confirmed a relationship between the discoordination of metabolism in mother's obesity and the placental dysfunction. The impaired development of spiral arteries and hypercoagulation are among the causes of this pathology[2-8]. Obesity, dyslipidemia, disorders in glucose metabolism and development of insulin resistance are important factors for cardio-metabolic risks, including those during pregnancy, which affect the cardiovascular and hemostatic prognosis of the pregnancy for the mother and the fetus, both in the childbirth and in the postpartum period.

In this case, secondary hypercholesterolemia is considered to be a possible compensatory response during the 2nd-3rd trimesters of pregnancy, even in women without obesity, who do not require treatment[2]. The presence of family dyslipidemia indicates the possible genetic determinism of this process[3,4]. However, in Ukraine, such studies are extremely rare, mainly done in patients with cardiovascular diseases[5,6].

The connection between the polymorphism of genes that determine the activity of RAAS, procoagulation and fibrinolytic potential due to lipid metabolism in pregnant women has not been studied. Particular attention, in our opinion, should be given to the stratification of clinical and genetic markers associated with changes in fat metabolism in the first trimester of pregnancy with obesity[7], to identify high-risk groups for the development of dyslipidemia.

The purpose of our study was to investigate changes in the lipid profile in pregnant women, with and without obesity, depending on the polymorphism of the I/ D gene of ACE, 4G/ 5G of the PAI-1 gene.

Material and methods

The prospective study involved 72 obese pregnant women (experimental group) and 21 virtually healthy pregnant women, without excessive body weight (control group), who signed an informed consent to participate to the study. According to the age and parity of the labor, the groups did not differ significantly: the average age was 24.9 ± 5.31 years.

Venous blood sampling for laboratory and genetic studies was carried out in the 6-10th weeks of pregnancy (embryonic period of gestation). The alleles of the PAI-1 gene and the I/ D polymorphic allele (Alu-repeat in the 16th intron (rs4340)) in the ACE gene were studied by isolating the genomic DNA from the peripheral blood leukocytes.

The polymorphic site was amplified using a polymerase chain reaction (PCR) by means of an amplifier „Amply-4L" (Russia). Fragments of amplified DNA were separated by gel electrophoresis, stained with bromide ethidium, visualized with a transilluminator in the presence of a marker of molecular weights (100-1000 bp).

The values of lipid metabolism were studied with a spectrophotometer („AF", Finland), with a wavelength of 500 ± 20 nm, using BioSystems S.A. reagents. (Spain). In the plasma of venous blood, stabilized with EDTA, taken on an empty stomach after 12 hours of fasting, the following tests were determined: total cholesterol (TC), triglycerides (TG), low and high density lipoproteins (LDL cholesterol and HDL cholesterol). The atherogenic index (AI) was calculated by the formula of A.N. Klimov: AI = (TC-HDL cholesterol) / HDL cholesterol.

All pregnant women have been evaluated by mandatory obstetrical examinations, consultations of specialists (endocrinologist, cardiologist),

general-clinical and biochemical research for TORCH-infection, according to the Order of the Ministry of Health of Ukraine of July 15, 2011, No. 417 „On the organization of ambulatory obstetric and gynecological care in Ukraine".

The statistical processing was performed using application programs MS® Excel® 2003 ™, Primer of Biostatistics® 6.05 and Statistica® 7.0 (StatSoftInc., USA). The reliability of the data obtained was evaluated using the dual-test method by means of Student's *t*-test (distribution by the Kolmogorov-Smirnov test was close to normal); correlation relations – according to Pearson coefficients and Spearman's rank correlation coefficient; the analysis of qualitative characteristics – by the criterion χ^2 (for frequencies less than 5 –the Fisher's exact test was applied). The difference was considered to be reliable at $p < 0.05$.

Results and discussion

Table 1 displays the values of plasma lipids rate in pregnant women in the first trimester, depending on the degree of obesity. Pregnant women with class I obesity were majority in the experimental group – 75.0% (n = 54), 19.4% (n = 14) had class II obesity and 5.55% (n = 4) – class III obesity. There was a reliable increase in the content of TC, TG, LDL cholesterol and the atherogenic index (AI) in pregnant women with obesity classes II-III, compared to those with class I, by 9.0% and 18.8% (p <0.01), 10.8% and 14.4% (p≤0.014), 16.3% and 15.8% (p <0.001), 33, 5% and 44.4% (p <0.001), respectively, and to healthy pregnant women without obesity by 1.5-3.4 times (p <0,001).

The plasma content of TC and TG in pregnant women in the first trimester, depending on the haplotypes of the ACE and PAI-1 genes, is presented in Table 2. The content of TC and TG in the plasma of pregnant women from the control group with a combination of the D-allele of the ACE gene and the 5G allele of the PAI-1 gene (ID / 4G5G, ID / 5G5G, DD / 5G5G) was significantly dominant over those in carriers of the II / 5G5G-haplotype: by 8.7% (p <0.05) than TC, by 16.1%, 17, 2% and 24.1% (p <0.05) more than TG, respectively. A similar trend was observed in the increase of the content of TC in women from the experimental group with ID / 4G5G, ID / 5G5G, DD / 4G5G combinations, compared to the II / 5G5G-haplotype – 11.0%, 12.4% and 9.9% respectively (p <0.05). The level of TG was prevalent in pregnant women with obesity in the presence of a haplotype of the D-allele of the ACE gene, regardless of the combination of PAI-1 gene (ID / 4G5G, ID / 5G5G, DD / 4G4G, DD / 4G5G, DD / 5G5G) over that with II / 5G5G combination – by 6.6-17.9% (p <0.05).

The concentration of HDL cholesterol was significantly lower in homozygous carriers of the D-allele of the ACE gene and the 4G allele of the PAI-1 gene (DD / 4G4G, DD / 4G5G) than in the combination of II / 5G5G by 17.4% and 8.7% (p <0.05). In addition, the homozygous combination of „mutant" alleles in both genes (DD / 4G4G) determined an adverse decrease in anti-atherogenic HDL cholesterol compared to those with a combination of D-allele and a protective 5G allele (ID / 4G5G, ID / 5G5G, DD / 4G5G, DD / 5G5G haplotypes) by 10.5-18.4% (p <0.05).

There was a reliable increase in the atherogenicity index (AI) with D-allele in a haplotype, regardless of the genotypes of the PAI-1 gene, in contrast to those with the combination of II / 5G5G, – by 10.8-26.4% (p <0.05); at the maximum value of this indicator in carriers DD / 4G4G haplotype, compared to obese pregnant women with DD / 5G5G-haplotype (p <0.05).

Therefore, DD genotype of the ACE gene is a risk factor for lipid metabolism in pregnant women without obesity by the level of TG and AI, D-allele – among pregnant women with obesity, with the increase of TC, TG, LDL cholesterol, IA and boundary reduction of HDL cholesterol. 4G / 5G polymorphism of the PAI-1 gene in pregnant women without obesity does not associate with changes in lipid metabolism; pregnant women with obesity and 4G4G genotype belong to the risk group of lowering the anti-atherogenic lipid fraction – LDL cholesterol and an increase in AI. The combination of the D-allele of the ACE gene and the 5G allele of the PAI-1 gene is a risk factor of increasing the content of TC in pregnant women, regardless of obesity, and an increase in TG in pregnant women without obesity. The presence of the D-allele of the ACE gene in the haplotype, regardless of the combinations of the genotypes of the PAI-1 gene, is a risk factor of increasing TG in pregnant women with obesity. Homozygous combination of „mutant" alleles of both genes (DD / 4G4G) determines the adverse reduction of anti-atherogenic HDL cholesterol by 10.5-18.4% (p <0.05) and an increase in AI in obese pregnant women.

Impairment of lipid metabolism in women with obesity is found by many researchers[1,6, 9-13]. Increasing TC, LDL cholesterol, and TG with a decrease in HDL cholesterol are considered to be risk factors of perinatal complications, in particular placental dysfunction[2, 14-19].

Conclusions

1. DD – genotype of the ACE gene is a risk factor of lipid metabolism impairment in pregnant women without obesity by the level of TG and AI, D-allele – among pregnant women with obesity with increasing TC, TG, LDL cholesterol, AI and boundary reduction of HDL cholesterol.

Table 1. Lipid profile in pregnant women depending on the obesity class

Lipid profile	*Group of control, n=21 (the pregnant without obesity)*	*Obesity classes in the pregnant, n=72*		
		Class I n=54	*Class II, n=14*	*Class III, n=4*
TC, mmol/L	3.68±0.12	5.01±0.08 p<0,001	5.46±0.14 p<0,001 p_1<0,01	5.90±0.44 p<0,001 p_1<0,01
TG, mmol/L	1.0±0.08	1.66±0.02 p<0,001	1.84±0.03 p<0,001 p_1<0,01	1.91±0.15 p<0,001 p_1=0,014
HDL cholesterol, mmol/L	1.28±0.07	0.91±0.01 p<0,001	0.78±0.02 p<0,001 p_1<0,01	0.79±0.10 p<0,001 p_1=0,046
LDL cholesterol, mmol/L	2.34±0.15	3.86±0.06 p<0,001	4.49±0.12 p,p_1<0,001	4.47±0.20 p,p_1<0,001
AI, st. un.	1.92±0.26	4.48±0.12 p<0,001	5.98±0.23 p,p_1<0,001	6.47±0.32 p,p_1<0,001

Notes: 1. TC – total cholesterol; LDL cholesterol/HDL cholesterol – low density lipid cholesterol / high density lipid cholesterol; TG – triglycerides; AI – atherogenic index. 2. p – reliability of differences between the values compared to the control ones; p_1 – reliability of differences between the values compared tothose in the pregnant with class I obesity; p_2 – reliability of differences between the values compared to those in the pregnant with class II obesity.

Table 2. The content of total cholesterol and triacylglycerol in blood plasma of pregnant women depending on haplotypes of genes ACE (I / D) and PAI-1 (4G / 5G)

Groups, n (%)	*Genotype combination of the ACE (I/D) and PAI-1 (4G/5G) genes*					
	***II/4G4G**, n=0 (%)*		***II/4G5G**, n=2 (%)*		***II/5G5G**, n=13(%)*	
	TC, mmol / L	*TG, mmol / L*	*TC, mmol / L*	*TG, mmol / L*	*TC, mmol / L*	*TG, mmol / L*
Pregnant women in the control group, n=6 (6,5)	0		3.72	0.87	3.56±0.14	0.87±0.02
Pregnant women in the experimental group, n=9 (9,7)	0		4.91	1.51	4.82±0.19 p<0,001	1.51±0.02 p<0,001
	ID/4G4G, n=0		**ID/4G5G**, n=15 (%)		**ID/5G5G**, n=9 (%)	
Pregnant women in the control group, n=11 (11,8)	0		3.87±0.12 II/5G5G	1.01±0.02 II/5G5G	3.52±0.15	1.02±0.04 II/5G5G
Pregnant women in the experimental group, n=13 (14,0)	0		5.35±0.22 p<0,001 II/5G5G	1.61±0.04 p<0,001 II/5G5G	5.42±0.25 p<0,001 II/5G5G	1.67±0.07 p<0,001 II/5G5G
	DD/4G4G, n=4(%)		**DD/4G5G**, n=24(%)		**DD/5G5G**,n=26(%)	
Pregnant women in the control group, n=4 (4,3)	0		3.5	1.21	3.63±0.21	1.08±0.12 II/5G5G
Pregnant women in the experimental group,n=50 (54,8)	4.85±0.38	1.73±0.08 II/5G5G	5.30±0.12 II/5G5G	1.78±0.33 II/5G5G	4.99±0.71 p=0,004	1.75±0.10 p<0,001 II/5G5G

Notes: 1. TC– total cholesterol; TG – triglycerides. 2. p – the reliability of differences between the values compared to the control ones for each haplotype separately; the reliability of differences between the values compared to a certain haplotype raised to a power (p≤0,05).

2. The combination of the D-allele of the ACE gene and the 5G allele of the RAI-1 gene is a risk factor of an increase in the content of TC in pregnant women, regardless of obesity, and an increase in TG in pregnant women without obesity.
3. The presence of the D-allele of the ACE gene in a haplotype regardless of the combinations of the PAI-1 gene genotypes is a risk factor of an increase in TG in pregnant women with obesity.
4. Homozygous combination of „mutant" alleles of both genes (DD / 4G4G) determines the adverse reduction of anti-atherogenic HDL cholesterol by 10.5-18.4% (p <0.05) and an increase in IA in obese pregnant women.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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