The molecular and genetic aspects of adolescent girls anomalous uterine bleeding: the role of endothelial dysfunction syndrome

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Abstract
The objective of the study is to assess NOS3 and ESR1 gene polymorphism in adolescent girls born with low birth weight (LBW) and suffered by anomalous uterine bleeding (AUB). A total 95 adolescent girls were studied including 32 born with LBW and AUB; 36 girls with normal birth weight and AUB; and 27 healthy girls. Single allele gene polymorphism NOS3 786T>C, 894G>T, ESR1 351A>G and 397T>C was studied. The existence of polymorphous allele C gene NOS3 786T>C (for homozygote OR = 2.03; 95% CI: 1.12–3.68; \( p = 0.04 \)) was detected in LBW girls with AUB. It was suggested that intrauterine programming of endothelial dysfunction syndrome could play a significant role in the development of AUB in adolescent girls born with LBW.

Introduction
The problem of developmental origins of diseases, including intrauterine programming, is one of the most interesting and intriguing. The impact of maternal pregnancy and labor peculiarities on the development of reproductive health of the adolescent girls was demonstrated earlier [1–3]. It was shown that the menstrual abnormalities and anomalous uterine bleeding demonstrated more frequently in girls born from mothers who suffered by preeclampsia, pregnancy termination threat and diagnosed fetal intrauterine growth restriction during the pregnancy [2,4,5]. It was suggested that the trigger of above-mentioned obstetrical complications could be an endothelial dysfunction syndrome [6–8].

The role of NO synthase (NOS3) genetic polymorphism in the development of preeclampsia and placental dysfunction was demonstrated [9,10]. We suggested that gene polymorphism which could regulate the endothelial function may be inherited by LBW girls from mothers with pregnancies complicated by preeclampsia and placental dysfunction. Endothelial dysfunction in these girls could be phenotypically realized in AUB. These bleedings could be a result of endothelial growth factors dysbalance, changes in vascular tone, angiogenesis and endometrial local hemostasis [11]. The study of endothelial NO synthase gene polymorphism reveals functionally interrelated polymorphisms 894G/T (rs1799983) and 786 T/C (rs2070744) [12]. The significant role of these polymorphisms demonstrated in cardiovascular diseases (ischemic heart disease, myocardial infarction, preeclampsia and stroke) and some other diseases (diabetes) [12,13].

It is well known that estrogens are important regulators of the endothelial function. These hormones can activate and release NO, prostacycline, hyperpolarizing endothelial factor and decrease the release of the vasoconstricting factors (endothelin I and angiotensin II) [10,14–16]. Estrogens impact could be realized via specific nuclear and membrane nongenomic receptors which are located in vascular smooth muscle cells and endothelium. There are two estrogen receptors ERα and ERβ which are the parts of nuclear steroid hormone receptors family, ERα plays the most significant role in the endothelium signal transmission [17].

There is limited information on molecular mechanisms of these polymorphisms that influence on receptors activation. ESR1 PvuII polymorphism could impact ESR1 gene expression through changes in transcriptional factors binding, and could influence the ESR1 splicing [18]. Some vascular protective effects of estrogens could be mediated through nongenomic mechanisms, including activation ways: MAPK (mitogen-activated protein kinase) and phosphatidylinositol-3-kinase. That is the way of NO (NOS3) endothelial synthase activation in combination with genomic effect of estradiol on NO synthesis [19,20].

Several aspects of intragenomic interaction of polymorphic NOS genes and role of estradiol receptors genes in number of diseases, including pathology of reproduction, are still unknown. This problem needs further clarification.

The objective of this study is to assess NOS3 and ESR1 genes polymorphic associations in adolescent girls with different birth weight suffering from anomalous uterine bleeding.

Materials and methods
A total 95 adolescent girls were studied. There were three groups. Group 1 consisted of 32 girls with AUB who were born with low
birth weight (2367.3 ± 34.6 g). Group 2 consisted of 36 girls suffered from AUB and normal birth weight (3436.4 ± 54.3 g, p < 0.001). Control group consisted of 27 healthy girls with normal birth weight (3416.0 ± 177.2 g, p < 0.001). The main causes of low birth weight of the Group 1 girls were maternal preeclampsia and placental dysfunction.

Mean gestational age at the moment of birth for all girls was 37 weeks and more. All groups were comparable according to maternal parity, gestational age and mode of delivery. All girls and their parents gave informed consent for participation in the study. The information on girls’ birth weight and length was obtained from medical documentation related to birth certificate kept by the girls’ parents.

Gene polymorphism study

Genomic DNA was extracted from peripheral blood samples using standard techniques. DNA amplification was performed using “Probe-GS-Genetics” (DNA Technologies, Russia) diagnostic kit from 0.5 ml of blood with EDTA (to prevent coagulation). DNA amount was measured by “KBM” (DNA Technologies, Russia) kit for PCR reactions. The sample of 1.0 ng of genomic DNA was used for each reaction. Allele-specific PCR method was used for the samples genotyping according to allele variants of genes in real time with assessment of the melting curves of amplification products. The PCR analysis was performed automatically using detector amplifier “DT-96” (DNA Technologies, Russia).

Statistical analysis was performed by “Statistica-7.0” package. Data were compared using Student’s t-criterion. Nonparametric variables were assessed using χ² Pearson’s criterion. The odds ratio (OR) was determined with 95% confidence interval (95% CI). Null hypothesis was rejected at p < 0.05. The Hardy–Weinberg law test on genotype distribution was performed by χ² criterion using Hardy–Weinberger equilibrium program.

Results

The mean age of Group 1 girls was 14.1 ± 0.3 years, girls from Group 2 were 13.5 ± 0.2 years old and girls from control group were 14.6 ± 0.4 years old p > 0.05. All groups were comparable by the age. The body mass index (BMI) of Group 1 girls was 24.3 ± 1.8 kg/cm², which was more than BMI in Group 2 (22.1 ± 1.3 kg/cm², p = 0.04) and in control group (20.1 ± 1.7 kg/cm², p = 0.008). The height of Group 1 girls (161.0 ± 1.8 cm) was less than in Group 2 (163.3 ± 1.6 cm, p = 0.03) and of control group (164.0 ± 2.1 cm, p = 0.02).

The age of onset of menarche was different in the girls of all groups. This age was 10.8 ± 0.3 years for girls of Group 1, 11.7 ± 0.2 years for Group 2 and 12.4 ± 0.5 years, p < 0.01 for the girls of control group. In 85.5%, the anomalous uterine bleeding was the actual start of menarche in girls of Group 1 and in 57.4% AUB repeated several times in this group.

The genotyping results demonstrated that alleles and genotype distribution in both (1 and 2) groups correspond to Hardy–Weinberg equilibrium. The possible influence of the genotype peculiarities on the endothelial functional state was assessed according the general, multiple and dominant models. The genotype distribution analysis according to polymorphic gene variant of NOS3 and ESR1 (Table 1) demonstrated significant increase in genotype occurrence frequency containing polymorphic alleles 786C of gene NOS3:786T > G; in homozygote and heterozygote state in Group 1 girls (for homozygote, OR = 2.03; 95% CI: 1.12–3.68; p = 0.046; for heterozygote, OR = 1.68; 95% CI: 1.09–2.60; p = 0.046; Table 1). The frequency of alleles NOS3 and ESR1 genes distribution demonstrated more frequent occurrence of allele 786C of NOS3 gene in girls of Group 1 (NOS3:786T > C: OR = 3.46; 95% CI: 1.16–10.34; p = 0.02; Table 2). The allele polymorphism NOS3 and ESR1 of Group 1 and 2 girls demonstrated no significant difference in polymorphous allele 786C in NOS3 (Table 2).

Recessive model of inheritance reveals difference in polymorphic gene loci NOS3 and ESR1 occurrence in adolescent girls groups. The allele 786C in NOS3 gene in homozygote and heterozygote states was found more frequently in girls of Group 1 (OR = 2.56; 95% CI: 1.01–6.5; p = 0.04; Table 2) and Group 2 (OR = 3.46; 95% CI: 1.16–10.34; p = 0.02; Table 2) than in control group. NOS3:786TT genotype was detected more frequently in the girls of control group (OR = 0.12, 95% CI: 1.04–0.12; p = 0.04; Table 1).

We suggest that polymorphous allele C in locus 786 of endothelial NO synthase is associated with increased risk of AUB in adolescent girls. Also, the dominant model of NOS3 and ESR1 genotypes reveals more frequent occurrence of ESR1:397CC genotype with polymorphous locus 397C in estrogen receptors alpha gene in Group 1 girls (OR = 4.58; 95% CI: 0.97–21.68; p = 0.04; Table 3).

Therefore, polymorphous allele 397C in ESR1 gene additionally increases the risk of anomalous uterine bleedings in low birth weight girls.

Discussion

We suggest that the birth of the girls with small-for-gestational age weight from mothers with endothelial dysfunction could be determined by intrauterine programming changes of endothelial function of these girls. These changes could be realized as anomalous uterine bleeding at the moment of menarche beginning in adolescence. We have studied 786 T > C, 894G > T and ESR1 351A > G (Xbal) and 397T > C (Pvull) gene polymorphism and some endothelial function markers in LBW girls. The analysis of distribution frequencies of NOS3 and ESR1 gene alleles demonstrates that the frequency of NOS3 786T > C gene occurrence significantly higher in LBW girls with AUB. It was demonstrated that allele C existence in 786T > C NOS3 gene promoter would lead to eNOS suppression and activity decrease [13,16,20,21]. These changes in eNOS could decrease NO synthesis and release, which determines an endothelial dysfunction.

Low nitrites blood level and diminished compensatory brachial artery blood flow activation by acetylcholine injection and estrogens were reported in persons with allele C of 786C NOS3 gene [22]. The relationship between existence of polymorphous
which in concert cause AUB.

more fragile and stimulate uncontrolled endometrial angiogenesis,

This process can also increase the VEGF production of oxygen active forms at endothelial dysfunction hypoxia decreases the endometrial blood flow and increases the growth factor (VEGF) synthesis. It was also shown that the vascular cells which are increasing the vascular endothelial tissue hypoxia can trigger the anomalous angiogenesis by endothelial dysfunction can cause a significant decrease in NO-mediated blood flow increase and NO bioavailability [13]. It was suggested that endothelial dysfunction is associated with significant decrease in NO-dependent blood flow increase in girls of control group.

We suggest that AUB in LBW girls born from mothers suffered from preeclampsia and placental dysfunction could be a result of intrauterine programming of endothelial dysfunction associated with NOS3 and ESR1 gene polymorphism which lead to angiogenesis and vascular tone alterations. Further research on molecular and genetic origins of AUB will be essential for prediction and prevention of uterine bleeding in adolescent girls.

Conclusion

We can suggest that such proangiogen reaction could be a part of the compensatory mechanisms to protect optimal endometrial perfusion at this particular condition.

Declaration of interest

The authors report no declaration of interest.

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