N.A. Bebyakova, A.A. Kuba, N.A. Fadeeva, A.V. Khromova

INTERACTION OF OESTRADIOL AND VASOACTIVE FACTORS IN WOMEN WITH rs 2070744 POLYMORPHISM OF ENDOTHELIAL NO-SYNTHASE GENE

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The aim of this study is the identification of interaction of oestradiol and vasoactive endothelial factors in women with polymorphism T-786C of endothelial nitric oxide synthase (eNOS) gene during follicular and luteal phase of the menstrual cycle. Sample size was 116 women with average age 19,6 years (95% CI 18,4 – 22,4), being born and living in the circumpolar region. Genotyping of T-786C polymorphism of eNOS gene was performed by pyrosequencing. The peripheral vascular tone was evaluated by tetrapolar reography. Concentration of nitric oxide (NO) was measured by Griss reaction. The level of oestradiol and endothelin-1 (ET-1) was measured by ELISA (enzyme-linked immunosorbent assay). Index ratio vasodilator to vasoconstrictor was calculated (NO/ET-1). «SPSS statistics» (StatSoft, USA) was used for statistical analyses. Our study found that the frequency of T allele was 0,67, C allele – 0,33 and C/C genotype was the rarest. The highest concentration of NO was found in women with C/C genotype during follicular phase. The concentration of NO in luteal phase in women with different genotypes of *eNOS* gene was similar. The level of ET-1 in women with T/T and C/T genotypes was normal during follicular and luteal phase, but in women with C/C genotype the level of ET-1 was above the normal reference range during follicular and luteal phase. The study of peripheral vascular tone found that C/C genotype was correlated with higher level of index of peripheral resistance (IPR) during follicular phase after dosed physical test, higher level of IPR was before and after dosed physical testduring luteal phase. The concentration of oestradiol in women with T/T and C/T genotypes was higher during luteal phase, in women with C/C genotype the level of oestradiol was higher in follicular phase. Correlation analysis between oestradiol and NO determined the moderate correlation (r=0,302; p=0,05) in C/T genotype, the strong positive association in C/C genotype during follicular phase (r=0,755, p=0,03) and luteal phase, and no association in T/T genotype. Thus, it was revealed that the presence of both mutant alleles C was correlated with imbalance of vasoactive factors (higher level of vasoconstrictors), leading to higher vascular peripheral tone. Also it was found that the association between oestradiol and NO manifested only in case of presence of mutant allele C of polymorphism T-786C gene of *eNOS* in genotype, that can approve protective function of oestradiol in relation to NO synthesis and can be confirmed by higher level of NO in women with C/C genotype during follicular phase of cycle.

Keywords: oestradiol, nitric oxide, endotheline-1, polymorphism T-786C gene of *eNOS*.

Introduction. Nowadays genetic predictors of cardiovascular pathologic processes, including endothelial disfunction (ED),are widely studied.ED is accompanied by decrease of nitric oxide (NO) synthesis and active local synthesis of ET-1 [1, 17]. Endothelial nitric oxide synthase (*eNOS*) is the endothelial isoform of NOS, which regulates synthesis of NO in endothelium cellsand is encoded by *eNOS* gene [4]. Some single nucleotide polymorphisms of *eNOS* gene were identified, for example T-786C polymorphism which plays a specific role in the development of ED [5, 7, 8].

Expression of eNOS gene is controlled by different factors, including estrogens. In women estrogens increase the concentration of NO in blood by means of genomic and non-genomic mechanisms of expression [9, 10, 13, 14].

It was established that concentration of estrogens influences on endothelium-related vasodilatation in women [6]. Most studies about mechanisms of ED leading to cardiovascular diseases are performed in menopausal and postmenopausal women with different pathological conditions, chronic endocrine disorders, diseases of reproductive system, in women taking modern medication [2, 3].So the association between women sex hormones and vasoactive endothelial factors in healthy women of reproductive age without confirmed cardiovascular pathology with polymorphism of *eNOS* gene is not studied.

Aim: to determine the association between oestradiol and vasoactive factors (NO and ET-1) in women with T-786C (rs 2070744) polymorphism of *eNOS* during follicular and luteal phase of menstrual period.

Materials and methods: Sample size was 116 women with average age 19,6 years (95% CI 18,4 – 22,4), being born and living in the circumpolar region. Criteria for including: women with constant regular ovulatory cycle(measured by rectal temperature). Criteria for excluding: women with acute and chronic diseases, with hormone therapy [11].Examination was carried out on 5-7 day (follicular phase) and on 19-21 day (luteal phase) of cycle.

The study was approved by ethics committee of Northern State Medical University, Arkhangelsk city. All examined participants were students of this university.

Genotyping of T-786C polymorphism of *eNOS* gene was performed by pyrosequencing (Russia, ApmliSens, *"Тоноскрин"* "Arterial hypertension" kit). Hemodynamic examination was carried out on empty stomachon mornings, dosed physical test (the Martin-Kushelevsky test) was used for estimation of cardiovascular system. Evaluation of peripheral vascular tone was performed by means of tetrapolar reography with calculating index of peripheral resistance (IPR). The concentration of serum NO was measured by Griss reaction (USA, «R&D Systems, kit «Total NO/Nitrite/Nitrate»). Serum oestradiol level was measured by enzyme-linked immunosorbent assay (Russia, "Вектор-Бест"). Serum ET-1 level was evaluated beenzyme-linked immunosorbent assay (Austria, "BIOMEDICA GRUPPE"). Index

BEBYAKOVA Nataliya A. – Doctor of Biological Sciences, Professor, Head of the Department of Medical Biology and Genetics, Northern Medical State University, Arkhangelsk, nbebyakova@mail.ru, ORCID 0000-0002-9346-1898; SPIN 6326-5523; **KUBA Anastasia A.** – lecturer - researcher, Department of Medical Biology and Genetics, Northern Medical State University, Arkhangelsk, ana-kuba@yandex.ru, ORCID 0000-0001-5707-1836; SPIN3908-3099; **FADEEVA Nataliya A.** – lecturer - researcher, Department of Medical Biology and Genetics, Northern Medical State University, Arkhangelsk, tascha1811@rambler.ru, ORCID 0000-0001-7588-4929; SPIN 1375-7904; **KHROMOVA Anna V.** – Candidate of Medical Sciences, Associate Professor, Dean of the Faculty of Preventive Medicine and Medical Biochemistry, Northern Medical State University, Arkhangelsk, medbio@nsmu.ru, ORCID 0000-0001-6440-4061; SPIN 6279-5445

Oestradiol and vasoactive endothelial factors in women with polymorphism T-786C (rs 2070744) *eNOS* gene depending on menstrual phase. Medianvalues (Q1;Q3)

Genotypes	Phase					
	Follicular phase			Luteal phase		
	T/T	C/T	C/C	T/T	C/T	C/C
NO.mcmol/l	13.713* (11.706;15.719)	15.53▲ (13.97;17.098)	18.239*▲ (13.21;23.262)	16.804 (12.148;21.459)	16.470 (14.709;18.231)	15.582 (12.386;18.77)
ET-1. fmol/ml	0.62* (0.362;1.00)	0.84▲ (0.39;1.66)	1.24*▲ (0.28; 2.670)	1.227* (0.726;1.728)	0.987▲ (0.758;1.217)	2.51*▲ (0.160;4.87)
NO/ ET-1	22.476 (13.11;28.01)	19.063 (14.40;25.22)	14.84 (8.49;25.412)	19.49 (13.66;25.33)	21.77 (16.96;26.57)	11.15 (6.15;16.15)
Oestradiol. nmol/l	0.218 (0.208;0.228)	0.268 (0.234;0.303)	0.341** (0.13;0.543)	0.308 (0.252;0.364)	0.362 (0.288;0.437)	0.269* (0.166;0.37)

Comments. Significant differences ($p<0.05$): ** - for C/C genotype in follicular and luteal phase; * - between C/C and T/T genotypes; ▲ – between C/C and C/T genotypes.

ratio vasodilator NO to vasoconstrictor ET-1 was calculated (concentration NO/ concentration ET-1). «SPSS statistics» (StatSoft, USA) was used for statistical analyses.

Results and discussion. All alleles and all genotypes of T-786C polymorphism were determined and were not deviated from Hardy-Weinberg Equilibrium. Our study showed that the frequency of T allele was 0,67, C allele – 0,33 and C/C genotype was the rarest. This frequency of alleles and genotypes of T-786C polymorphism of *eNOS* gene is similar to frequency in European population [TheAl. Lete FRE quency Database, 2021, http://alfred.med.yale/edu].

According to literature C allele is associated with low level of NO, but this study demonstrated that higher level of NO was determined in C/C women during follicular phase in comparison with T/T and C/T women. NO level had no significant difference in women with different genotypes of T-786C polymorphism during luteal phase (Table 1).

The level of ET-1 in women with T/T and C/T genotypes was normal during follicular and luteal phase, but in women with C/C genotype the level of ET-1 was higher than the normal reference range during follicular and luteal phase (more than 2,5 times) (Table 1). High level of ET-1 is the factor of imbalance of vasoactive endothelial factors which is confirmed by low index NO/ET-1 in women with C/C genotype. IPR before and after physical test in T/T and C/T women was normal during follicular and luteal phase, IPR in C/C women was above reference interval after physical test during follicular phase and after and before physical test during luteal phase. Probably imbalance of endothelial factors in these women can explain high level of IPR during luteal phase.

The concentration of oestradiol in women with T/T and C/T genotypes was higher during luteal phase, in women with C/C genotype the level of oestradiol was higher in follicular phase compared with luteal phase (Table 1).Correlation analysis between oestradiol and NO level showed absence of significant difference in women with T/T genotype, moderate correlation in C/T genotype during follicular phase (r=0,302; p=0,05) and luteal phase and the strong positive association in case of C/C genotype (r=0,755, p=0,03).

It was demonstrated that allele C in genotype leaded to lower NO levelin comparing with women with T/T and C/T genotype [15]. But our study showed that the highest NO level was determined in C/C women during follicular phase in comparing with women with C/T and T/T genotypes. Apparently it is associated with compensatory function of oestradiol which increases NO level by means of activation *eNOS* by direct phosphorylation of estrogen receptors and subsequent transport of signal through protein kinase cascades, which activates eNOS [12, 16]. Also the genomic way can increase the expression of *eNOS* gene.

So in this study it was determined that the association between oestradiol and NO level appears only on presence of C allele of T-786C polymorphism. The absence of this association in women with T/T genotype, moderate correlation in C/T women and strong correlation in C/C women supports the hypothesis.

Conclusions:

1. The spread of alleles and genotypes of T-786C polymorphism of *eNOS* gene in circumpolar-women is similar to European population. The frequency of T allele was 0,67, C allele – 0,33 and C/C genotype was the rarest.

2. Peripheral vascular tone in women with C/C genotype was above normal value after dosed physical testduring follicular phase, during luteal phase - before and after dosed physical test. Women with T/T and C/T genotypes had normal value of vascular tone during follicular and luteal phase.

3. C/C genotype was correlated with higher NO level during follicular phase and with imbalance of vasoactive endothelial factors (prevalence of vasoconstrictors during both phases of cycle).

4. Correlation analysis between oestradiol and NO level showed moderate correlation in women with C/T genotype and positive association in women with C/C genotype, which demonstrates the positive influence of oestradiol to production of NO.

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B.M. Gasanova, N. S-M. Omarov, M.L. Polina, N.I. Douglas, T.E. Burtseva, S. S. Sleptsova, P.N. Zakharova

FEATURES OF ANEMIA IN PREGNANT WOMEN OF VARIOUS GENESIS IN ETHNIC SAMPLES

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GASANOVA Bakhtykei M. – PhD in Medical Sciences, Doctoral Candidate of the Department of Obstetrics and Gynaecology, Medical Institute, People's Friendship University of Russia, Moscow, Russia, https://orcid.org/0000-0001-6871-7102; **OMAROV N. S-M.** – Doctor of Medicine, Head of the Department of Obstetrics and Gynecology, Dagestan State Medical University, Dagestan, Russia; **POLINA Miroslava L.** – PhD in Medical Sciences, Gynecologist at the Women's Health Medical Center, Moscow, Russia, https://orcid.org/0000-0003-3883-3967; **DOUGLAS Natalya I.** – Doctor of Medicine, Head of the Department of Obstetrics and Gynecology, Medical Institute, North-Eastern Federal University, Yakutsk, Russia, **SLEPTSOVA Snezhana S.** – Doctor of Medicine, Head of the Department of Infectious Diseases, Phthisiology and Dermatovenerology, Medical Institute of M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia, https://orcid.org/0000-0002-0103-4750, **BURTSEVA Tatiana E.** – Doctor of Medicine, Department of Pediatrics and Pediatric Surgery of the of the North-Eastern Federal University, Institute of Medicine, Yakutsk, Russia, https://orcid.org/0000-0002-5490-2072; **ZAKHAROVA Praskovya N.** – resident of the Department of Obstetrics and Gynecology. Medical Institute, M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia, e-mail: pucca_95@mail.ru

Objective of the Study: to determine in different ethnic samples of pregnant women with anemia of various genesis the factors influencing on its development; perinatal outcomes in anemia of various genesis; features of the production of pro- and antioxidant blood factors.

Material and methods

A prospective cohort study of pregnant women with IDA and ACD was carried out in various ethnic samples. In the Republic of Dagestan, the sample with anemia consisted of 470 women: with IDA (n = 286) and ACD (n = 184). In the sample of pregnant women with anemia in the Republic of Sakha (Yakutia) (n = 284) we also distinguished groups with IDA (n = 186) and ACD (n=98). The control group - 34 healthy pregnant women in the Dagestan population and 42 - in the Yakut one was introduced into the study to compare the indicators in the study of pro- and antioxidant factors.

Research methods included the assessment of a general blood test, serum iron (SI), C-reactive protein (CRP), ferritin, total protein, pro- and antioxidant factors (erythrocyte and blood serum catalase, sulfhydryl groups (SH-groups), ceruloplasmin (CP) and malondialdehyde (MDA) in blood serum), the level of IgG to parasitic infections.

Results

The study showed a variety of risk factors and conditions contributing to the development of anemia on the background of lower iron and lower hemoglobin concentration in various ethnic samples (ecological, biological and social biotopes). True ID was confirmed at low levels of ferritin (100.0%) and serum iron.

Inflammatory diseases of the pelvic organs were twice as common in women with ACD than IDA ($p < 0.05$).

Iron limiting participation for erythropoiesis in ACD was accompanied by an increase (85.6%) or normal serum ferritin level (14.4%), increase in the level of CRP (100,0%), lymphocytes (29.3%), monocytes (22.8%), blood sedimentation rate (ESR)(14.7%).

Pregnant women with true ID were characterized by a balanced increase in the level of pro- and antioxidant factors.

Iron metabolism violations in ACD were accompanied by a pronounced imbalance in the production of hydroperoxides and antioxidant protection factors.

The effect of excess lipid peroxidation products on the placenta in ACD in the Yakut population was accompanied by an evolutionarily accumulated level of endogenous antioxidants (blood plasma and erythrocyte catalase, sulfhydryl groups). A decrease in the compensatory mechanisms of the placenta of pregnant Dagestan women with ACD influenced the higher frequency of morphological and functional immaturity of newborns due to the moderate antioxidant potential. Morphofunctional immaturity of newborns (MFI) in the group with ACD was detected one and