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Invasive prenatal diagnosis tests in the practice of modern obstetrics: the perspectives of gene expression assessment

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Abstract

Invasive prenatal diagnosis tests in the practice of modern obstetrics: the perspectives of gene expression assessment.

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The purpose of this study was to evaluate the prospects for the use of gene expression analysis for early diagnosis on the prenatal stage. There was shown that gene expression analysis is a sensitive and highly specific method characterizing physiological adaptive processes. There was discussed the expedience of the further researches could be focused on the assessment of gene expression in the biological tissues samples obtained during invasive prenatal screening tests.

Key words: prenatal diagnosis, gene expression, obstetrics.

In recent years, the arsenal of diagnostic methods used in obstetrics was supplemented by the invasive tests involves obtaining biological material directly from the structures of fetoplacental complex. Currently they use the following types of invasive prenatal diagnostic tests: amniocentesis, cordocentesis, placentocentesis, chorionbiopsy and fetal tissue biopsy [1, 2]. Each of these methods has its own indications and limitations. Thus, chorionbiopsy could be conducted by the vaginal or abdominal access and allow to diagnose chromosomal aberrations and gene mutations in the gestational age of 11-12 weeks. The disadvantage of this method is its low specificity associated with the phenomenon of "placental mosaicism" - nonidentity of the genome of embryonal and chorion cells. Moreover, in 2-3% of cases it could be complicated by the cases of fetal loss, infection and bleeding. Performed in the later stages of pregnancy placentocentesis runs the risk of placental abruption and threatened abortion or premature birth, and infection of the fetoplacental complex with premature rupture of membranes [1, 3, 4].

One of the most commonly used methods of prenatal diagnosis is amniocentesis, which along with the diagnosis of genetic and chromosomal diseases allows to define a number of important biochemical parameters, which are markers of the degree of fetal lung maturity (the content of lecithin and sphingomyelin), hypoxia, immunological disorders. Possible complications of the method include the termination of pregnancy, amniotic fluid leakage, infectious complications, bleeding from the genital tract. However, the likelihood of the complications is less than at chorionic biopsy [3, 4].

After 20th week cordocentesis could be applied for diagnostic purposes. This test can be used both for diagnosis and for intrauterine transfusion. Less commonly they use fetal tissue biopsy [1, 5].

In the order of the Ministry of Health of Ukraine № 417 "The methodical aid for the management of out-patient obstetric care" from 15.07.2011 is not stipulated the necessity of pre-screening ultrasound determination of the pregnancy term for timely referral to prenatal screening. In accordance with the order all pregnant women at high risk for the occurrence of fetus congenital and hereditary diseases should be directed to medical genetic counseling [6]. According to current clinical guidelines using in Ukraine, prenatal diagnosis is carried out in several stages. The first stage - counseling couples planning a pregnancy or patients already having pregnancy. Stage II - prenatal diagnosis of chromosomal abnormalities two-stage fetal malformations of the heart and its main arteries, as well as a wide range of genetic syndromes (includes screening ultrasound and biochemical tests). Biochemical markers in prenatal

diagnosis for multiple pregnancies are nonspecific. Stage III - pregnant women at high risk of perinatal congenital anomalies performed invasive diagnostic test. Phase IV is related for developing tactics of the pregnant woman and the prognosis for the fetus [6, 7].

In the absence of high risk factors for fetal congenital and hereditary diseases pregnant should be informed of the need for prenatal screening stage I. It includes ultrasound screening (in the period from 11 weeks and 1 day to 13 weeks and 6 days) and one biochemical screening (determination of β -hCG and PAPP-A in the multiple of median (MoM)) followed by calculation of the risk of the fetus chromosomal and other congenital abnormalities.

The second stage of prenatal screening includes biochemical "double" test at the second trimester of pregnancy. There are determination of plasma concentrations of AFP and pregnant β -hCG or "triple" test the second trimester - determination of AFP, β -hCG and unconjugated estriol, or "quadruple" the second trimester test - definition of AFP beta-hCG, unconjugated estriol, and inhibin A in the tests of the combination with the II ultrasound screening. On the effectiveness of biochemical screening marker in determining the parameters affect the timing of the study, the use of biochemical analyzers and laboratory kits of poor quality, lack of unified methodological approaches in the laboratory service, and low levels of diagnostic ultrasound. Qualitatively spent a combined prenatal screening I trimester makes it unnecessary to conduct biochemical screening II trimester [7].

There are the following WHO-approved standard indications for referral for invasive prenatal diagnosis [1, 7]:

- Age over 35 years;
- The presence of at least two spontaneous abortions in early pregnancy;
- The presence of child or fetus from a previous pregnancy with Down syndrome, other chromosomal diseases with multiple birth defects;
- Family carrier of chromosomal aberrations;
- Monogenic disease, previously diagnosed in the family or in the close relatives;
- Use before or during early pregnancy some genotoxic pharmacological agents (chemotherapeutic agents, anticancer drugs, and others.);
- Borne viral infections (hepatitis, rubella, toxoplasmosis, etc.);
- Radiation exposure of any spouses prior to the conception.

Along with classical cytogenetic methods in recent years there are widely used proteomic and molecular genetic methods, including the assessment of the presence of gene mutations [8-10]. At the same time, evaluation of gene expression is relatively rare [1, 10].

The purpose of this study was to evaluate the prospects for the use of gene expression analysis for early diagnosis on the prenatal stage.

Material and methods.

RNA isolation was carried out on the basis of the Clinic of Reproductive Medicine "Nadia" from biopsy samples maternal placenta to study gene expression HIF1A (OMIM 603348), eNOS (OMIM 163729), VEGFA (OMIM 192240) and PIGF (OMIM 600153).

For this purpose, the following procedures were carried out sequentially: sampling and holding the biopsy placental RNA isolation, reverse transcription and polymerase chain reaction (PCR) in real time.

Biopsy of the placenta is delivered by the conchotomy. Fragments of the placenta were stored in 10 volumes equivalent RNeasy Lysis Solution (Qiagen, USA, Cat # 69903) at "200C" from the time of sample taking to the investigation.

RNA extraction was performed using a kit RNeasy Blood and Tissue Kit (Qiagen, Germany, # 52304) according to the manufacturer's protocol for isolation of nucleic acids from tissue fragments. The characteristics of the isolated RNA was determined using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA) by determining the A260 / A280 and A260 / A230. The resulting RNA was maintained at -20 °C" and was used for reverse transcription. The reverse transcription was carried out using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA, # 4368814) according to the manufacturer's recommendations [11]. The assessment of gene expression was performed using resynthesized TaqMan® Gene Expression Assay (Applied Biosystems, USA). We used the following test system for genes:

- HIF1A (OMIM 603348): # 4453320 - Hs00153153_m1;
- eNOS (OMIM 163729): # 4453320 - Hs01574659_m1;
- VEGFA (OMIM 192240): # 4453320 - Hs00900055_m1;
- PIGF (OMIM 600153): # 4448892 - hCG1987697;
- GAPDH (OMIM 138400) - the internal control gene: # 4331182 - Hs99999905_m1.

Amplification and detection was performed using PCR of the real-time system 7500 Real-Time PCR System (Applied Biosystems, USA, # 4351105) with SDS 2.0.5 software with the following temperature conditions: 2 min at 50°C; 10 min at 95°C; 15 sec at 95°C, 1 min at 60°C). The analysis of the results was carried out manually by the $\Delta\Delta C_t$ method. All molecular genetic studies have been performed in a certified genetic laboratory NPF "Hope" (Head - Ph.D. Mykytenko D.O.).

Statistical processing was conducted by the software Statistica 10.0 (StatSoft Inc., USA) [12].

The results of the study.

In the analysis of gene expression HIF1A there was found that its minimum value relative to the initial level by the control genome of each specimen in group I was $3.37 * 10^3$, whereas the maximum value of $- 4.56 * 10^5$. Such wide amplitude of the oscillation indicates that even amongst healthy women tissue oxygen supply varies widely. But in the II group the fluctuation range of expression values was at the level $2.52 * 10^2 - 6.90 * 10^7$, and in the III group - from $4.94 * 10^3$ to $5.78 * 10^5$.

Thus, the relative gene expression HIF1A in patients with iron-deficiency anemia was significantly higher (OR = 10.2 (95% CI 8,1-12,4) than in the control group ($p < 0,001$). The presence of placental dysfunction during pregnancy obviously increased gene expression HIF1A, and thus protein production induced by hypoxia factor, compared with the control, to lesser values (OR = 4.1 (CI 95% 2.9-5.3, $p < 0,01$), which can be regarded as a manifestation of adaptation to chronic hypoxia.

In the analysis of gene expression VEGFA we identified certain differences between the groups in terms of the significant growth in the II and III clinical group. But significant differences in gene expression PIGF in pregnant women with different state of ferrokinetics and hemodynamics in the "mother-placenta-fetus" was not found.

In the analysis of the expression of eNOS gene there were identified certain differences between the groups in terms of a growth by 1.4 folds (OR = 1.4 95% CI 1.1-1.8) in the II clinical group and a sharp decline (10 times - OR = 10.0 95% CI 8.8-11.2) in the III clinical group.

Thus, in the analysis of gene expression HIF1A, eNOS, VEGFA and PIGF established (Fig. 1) that the least pronounced changes were in the group of pregnant females suffering from iron-deficiency anemia and were characterized by PIGF gene expression.

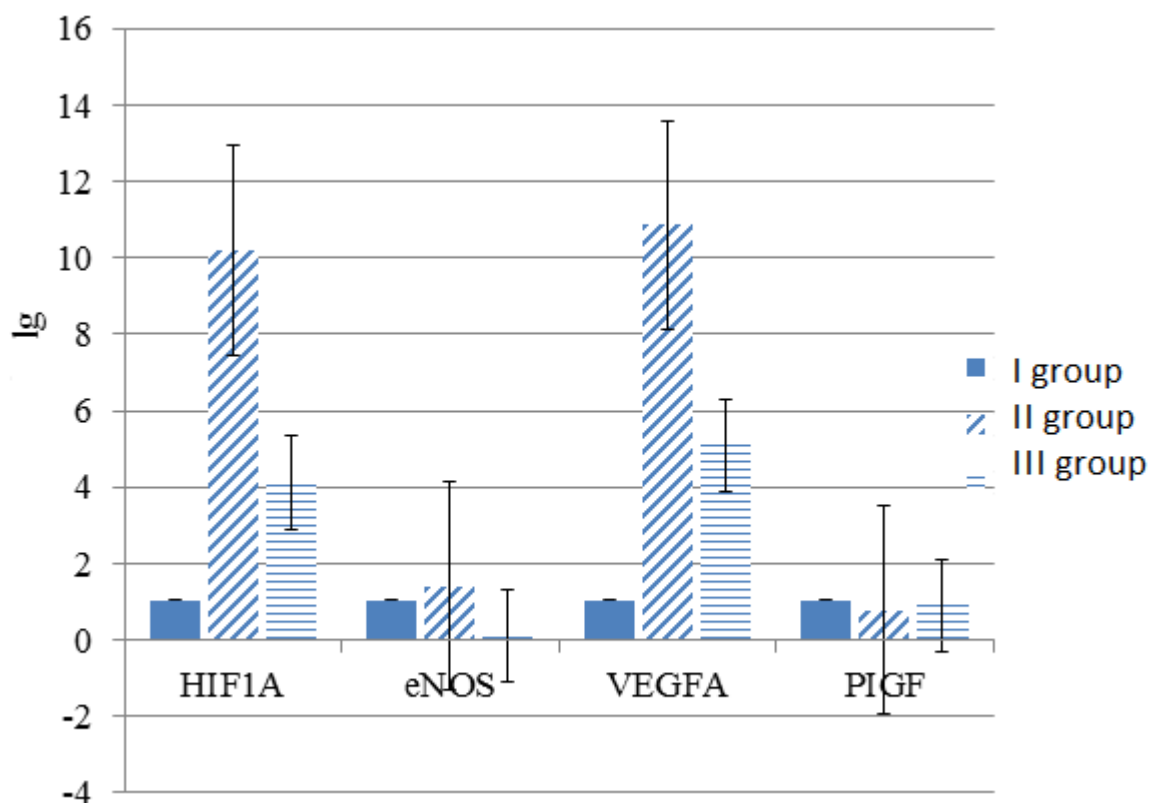


Figure 1. Gene expression in the examined patients.

So, the activity of HIF1A and VEGFA gene expression has increased dramatically, and the expression of eNOS gene depended on the degree of placenta dysfunction. Despite the fact that the activity of gene expression and HIF1A VEGFA increased for iron deficiency anemia, respectively, by 10.2 and 10.9 folds less pronounced increase in HIF1A and VEGFA gene expression. These changes were arising on the background of iron deficiency anemia, and accompanied by paradoxical decrease in expression of eNOS gene. This phenomenon can be explained by the depletion of the body's adaptive capabilities.

Given the fact that in this study the placenta biopsies were obtained in the third stage of labor, assess the level of expression of these genes is not possible. At the same time, applied research method suitable for use in performing invasive procedures for prenatal screening [1, 10].

Conclusions

1. Gene expression analysis is a sensitive and highly specific method characterizing physiological adaptive processes
2. Further researches could be focused on the assessment of gene expression in the biological tissues samples obtained during invasive prenatal screening tests.

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