ORIGINAL ARTICLE

ASSESSMENT OF CORRELATION BETWEEN MIRNAS-21-3P AND -210-3P EXPRESSION IN MATERNAL AND UMBILICAL CORD PLASMA AND FETAL WEIGHT AT BIRTH

DOI:10.36740/WLek202102111

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ABSTRACT

The aim: To determine the degree of correlation of mass of the fetus and the level of mir-21, mir210 in maternal blood and umbilical cord blood of the fetus in uncomplicated gestation.

Materials and methods: 60 pregnant women with a single baby pregnancy in the third trimester (37-40 weeks) were examined. They all were given a general clinical, obstetric and the level of miRNA21-3p and miRNA210-3p were determined in the whole blood of pregnant women (before labor) and in fetal blood obtained from the umbilical artery at birth. The level of miRNAs was determined by the TaqMan method.

Results: After examining maternal and fetal plasma samples, we were able to determine 49 samples of hsa-miR210-3p and hsa-miR21-3p from maternal plasma, 44 samples of hsa-miR210-3p and 37 samples of hsa-miR21-3p from the cord blood, which is a satisfactory result of more than 50%. Subsequently, between the results obtained and the birth weight of the fetus Pearson's correlation coefficient was studied. According to the results obtained, we found no correlation between fetal mass and hsa-miR210-3p level in maternal plasma (r-0,068674), low positive correlation of fetal mass with hsa-miR21-3p level in maternal plasma (r-0,212181), an average positive correlation with the level of hsa-miR21-3p in umbilical cord blood (r- 0.363374) and a high positive correlation with hsa-miR210-3p in umbilical cord blood (r-0.528616).

Conclusions: Determination of the level of hypoxic miRNAs, in particular hsa-miR210-3p in the umbilical cord blood of the newborn may be a marker of the functional status of the placenta, which programs the normal development of the fetus.

KEY WORDS: miRNA, Hypoxia, pregnancy, fetal programming, newborn

Wiad Lek. 2021;74(2):236-240

INTRODUCTION

In the mid-80s (1980-ies), the results of a research by D. Barker were published that laid the basis of prenatal programming theory. The essence of the problem is that the weight of a child at birth can be a marker of further development of its numerous diseases in the adulthood, namely non-insulin-dependent diabetes mellitus, hypertension, cancer, obesity, as well other pathological states that might even determine the life expectancy of an individual [1].

It was considered that the potential for prenatal development, in particular fetal mass, was formed due to one's genetics. However, according to the literature data as of now, approximately 20% of its variability is due to maternal factors, and much less contribution is made by genetic variants of a fetal genome [2]. Pathology in course of a pregnancy, stress, influence of mother's nutrition and other environmental factors become those triggers that alter gene expression and determine its phenotype. That is, gene reprogramming without changing the structure of DNA determines the structural and functional features of the fetus development and the prognosis of its further state of health.

The main mechanism that mediates changes in the trajectory of growth and formation of the fetus is hypoxia. It has a direct physiological effect on the development of the fetus, but it can also indirectly modulate it through interference with a placenta, as well as via angiogenesis and hematopoesis processes. It is hypoxia that determines which way it would go: normal or pathological. Low oxygenation activates reactions that are regulated by HIF1α (hypoxia-induced factor 1α), at least many of them do. It is involved in cellular control of the utilization and delivery of O_2, its inhibition of growth and development through alteration of gene expression. Also, it promotes anaerobic metabolism pathways [3].

In this regard, the study of non-invasive molecular markers of hypoxia is rising up as a promising area of a fetal medicine. These studies should promote better understanding the pathophysiology of intrauterine hypoxia and fetal programming, as well as to identify its new markers, more accurately predicting the short-term and long-term complications, and monitoring and choosing rational tactics for managing this pregnancy.

New markers of fetal programming include the class of microRNAs that are small non-coding RNA molecules of 18–25 nucleotides (total length's average of 22), which are involved in transcriptional and posttranscriptional regulation of gene expression. In human organism approximately 2000 of them have been determined. Some of them can be allocated from the tissues only, and that fact

complicates studying them. The rest are excreted into the bloodstream in the form of membrane microvesicles or necrotic nanoparticles and are available for determination.

MicroRNAs regulate virtually all biological and metabolic processes in the body, including the development of normal and pathological pregnancy. Determining them from the bloodstream of the mother, as well as from placenta, amniotic fluid and umbilical cord blood allows the researcher to use them as markers of pregnancy miscarriage, preeclampsia, gestational diabetes, macrosomia, delayed fetal development, etcetera. Yet the results are quite controversial due to relatively high costs of microRNA determination, different methodological approaches to their determination, small number of studies undertaken and, thus, the small number of the results obtained.

Among the first chronologically determined and most studied were hypoxic micro-RNAs, specifically mir-21 and mir-210. They circulate, lack tissue-specificity and allow a comprehensive assessment of the body's response to low O_2 concentrations during pregnancy.

When summarizing the available literature data, it is often noted that the presence of miR-21 and mir-210 miRNAs in maternal blood correlates with the degree of fetal hypoxia in utero, and thus they are considered as its markers [4]. However, we did not find neither any works on parallel study for both mother and fetus, nor the study on the presence or absence of relation with the mass of the fetus.

THE AIM

Therefore**,** the issue of our study was to determine the degree of correlation of mass of the fetus and the level of mir-21, mir210 in maternal blood and umbilical cord blood of the fetus in uncomplicated gestation.

MATERIALS AND METHODS

60 pregnant women with a single baby pregnancy in the third trimester (37-40 weeks) were examined.

Inclusion criteria: full-term pregnancy (37-42 weeks), single fetal pregnancy, absence of extragenital pathology, inflammation or preeclampsia, nonsmoking, satisfactory condition of the fetus, agreement of patient.

Exclusion criteria: pregnancy period <37 weeks or> 42 weeks, multi fetal pregnancy, presence of extragenital pathology, inflammation or preeclampsia, smoking, fetal distress, disagreement of patient.

They all were given a general clinical, obstetric and the level of miRNA21-3p and miRNA210-3p were determined in the whole blood of pregnant women (before labor) and in fetal blood obtained from the umbilical artery at birth. The material was a heparinized blood.

The level of hsa-miR-21 and hsa-miR-210 was determined by the TaqMan method.The total RNA was isolated from the blood using the mirVana PARIS method (Ambion, USA) in accordance with the manufacture's protocols. The concentration of RNA was measured with a spectrophotometer NanoDrop ND1000 (NanoDrop Technologies, USA). MiRNAs were identified by a reverse transcription and PCR in real time. The reverse transcription was conducted with High Capacity cDNA Reverse Transcription Kit (Applied Byosystems, USA), with specific primers for every miRNA and 10 ng of total RNA. The quantitative PCR in real time was conducted using TaqMan MicroRNA Assays (Applied Biosystems, USA): U6 snRNA (as endogenous control), assay ID 002438 (hsa-miR21-3p), assay ID 000512 (hsa-miR210-3p). The temperature conditions were as follows: initial denaturation 95°C – 10 min; 45 cycles 95°C – 15 s and 60°C – 60 s. The level of miRNA was determined using the formula ($2^{\Delta Ct}$), normalized according U6 snRNA and is expressed in relative units. Amplification was carried out with 7500 Fast Real-time PCR (Applied Byosystems, USA).The data received was analyzed using 7500 Fast Real time PCR software.

Statistical Analysis. All values are presented as arithmetic mean ± standard deviation (S.D.) Data in all groups were analysis of variance was performed using one-way ANOVA. The software used was Excel 2007 and SPSS Statistics 17.0. Data obtained were analyzed using Microsoft Office 2007 *и* Statistica 6 (StatSoft Inc., USA) programs.

The experimental results received were compared by determining the correlation coefficient between levels of microRNA in womens blood, umbilical blood and newborn weight.

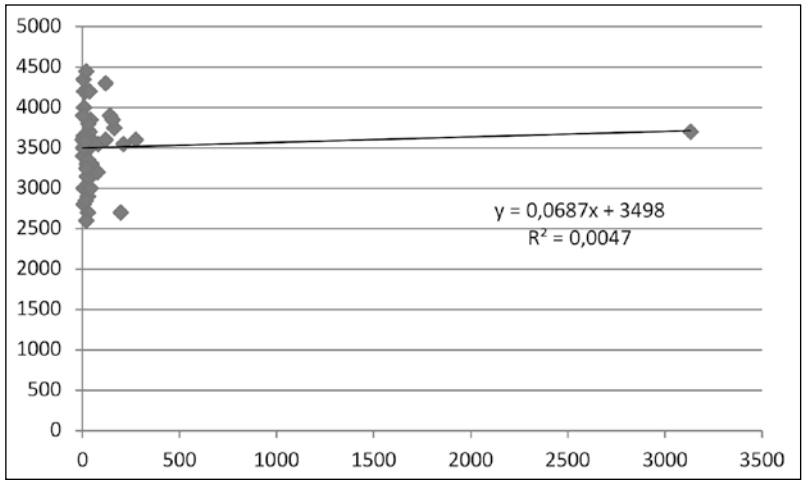

Fig. 1. Correlation between miRNAs-210-3p expression in maternal plasma and fetal weight at birth.

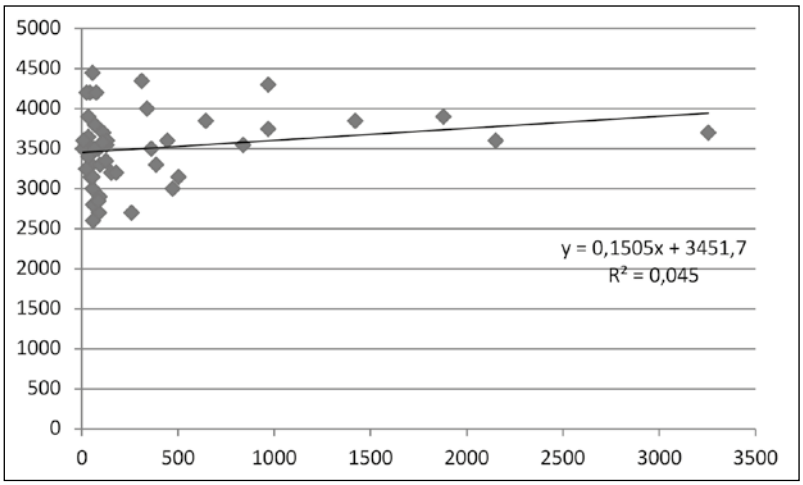

Fig. 2. Correlation between miRNAs-21-3p expression in maternal plasma and fetal weight at birth.

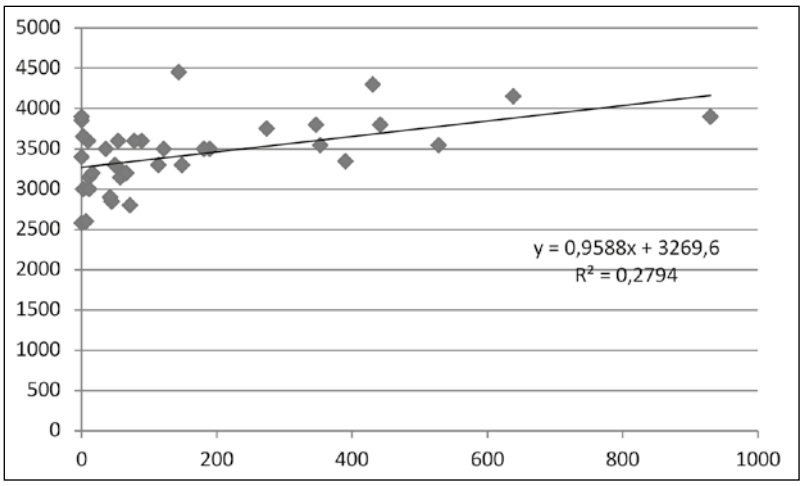

Fig. 3. Correlation between miRNAs-210-3p expression in umbilical cord plasma and fetal weight at birth

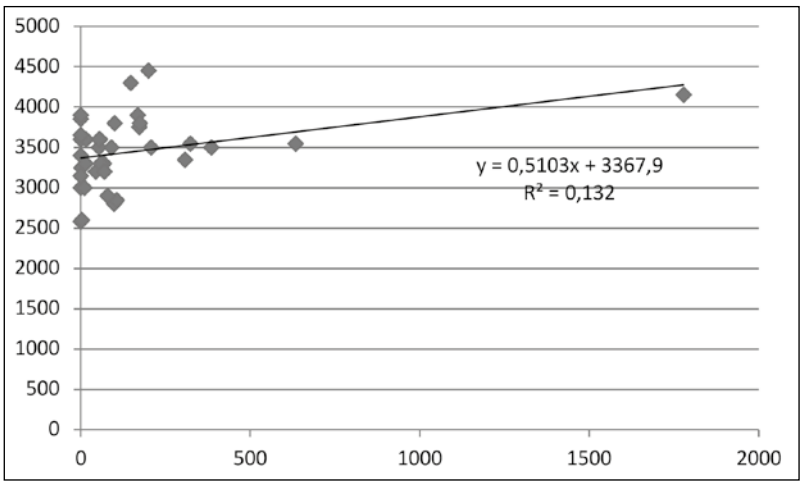

Fig. 4. Correlation between miRNAs-21-3p expression in umbilical cord plasma and fetal weight at birth.

RESULTS

The study was conducted in 60 pregnant women. The mean age of pregnant women was 28.32 years ± 4.6 years. 55 women (91.7%) of these were carrying their first child, 5 women (8.3%) had already given birth before. Vaginal delivery – 58 women (96.7%), and 2 (3.3%) underwent caesarean section because of the clinical mismatch between the size of the fetus and pelvis of the mother.

After examining maternal and fetal plasma samples, we were able to determine 49 samples of hsa-miR210-3p and hsa-miR21-3p from maternal plasma, 44 samples of hsa-miR210-3p and 37 samples of hsa-miR21-3p from the cord blood, which is a satisfactory result of more than 50%. Subsequently, between the results obtained and the birth weight of the fetus Pearson's correlation coefficient was studied. The results obtained are presented in Figgs 1-4.

According to the results obtained, we found no correlation between fetal weight and hsa-miR210-3p level in maternal plasma (r-0,068674), low positive correlation of fetal weight with hsa-miR21-3p level in maternal plasma

(r-0,212181), an average positive correlation with the level of hsa-miR21-3p in umbilical cord blood (r- 0.363374) and a high positive correlation with hsa-miR210-3p in umbilical cord blood (r-0.528616).

DISCUSSION

The relevance and prospects of epigenetic studies, as well as the widespread use of PCR (polymerase chain reaction) in clinical practice and the focus of research on the simplification of microRNA determination make it possible to conduct this study. But the questions regarding its appropriateness arise. The presence of medium and high correlation between hypoxic miRNAs and its mass in the umbilical cord blood of the newborn only, and the negative result of the study of maternal blood have no prognostic value for us with respect to fetal weight. But on the other hand, it allows answering the certain pathogenetic questions.

The data obtained by us contradicts the literature data. In particular, Whitehead C. et.al. determined the miRNAs of placental origin in maternal blood. According to their results, the level of hypoxic miRNAs in maternal blood flow, in particular miR-210, correlates with the acidic status of fetuses with intrauterine growth restriction (IUGR) in acute fetal hypoxia in the second period of labor, and chronic hypoxia with premature delivery of fetuses with IUGR [5]. However, the investigated miRNAs do not have rigid tissue specificity, and according to the study, it is not possible to clearly identify whether they belong to a mother or a fetus. Therefore, their level in the circulation of a mother is an aggregated value for the maternal organism and the feto-placental complex, largely reflecting the condition of the pregnant woman and the pathology present.

The umbilical cord artery reflects the state of placental microcirculation and its functioning more precisely. Thus, the present correlation of angio-microRNA (hsa-miR210-3p hsa-miR21-3p) in the umbilical cord blood with the newborn's mass may indicate the degree of placental dysfunction, which is key factor in terms of pregnancy outcome and subsequent prognosis for the baby.

On the basis of the numerous studies, microRNAs that are also called angio-microRNAs have been found to play an important role in placental angiogenesis. For instance, miR-21, miR-20a and miR-210 have been shown to induce angiogenesis, whereas miR-16, miR-34a and miR-222 inhibit angiogenesis. In the placenta, miR-20a and miR-34a were identified in helix remodeling, miR-16 in the vascular endothelial growth factor signaling pathway, and miR-210 in trophoblast migration and invasion. In the case of imbalance between pro- and anti-angiogenic factors, there may be an increase or decrease in the lumen of the blood vessels, which can lead to abnormal development of the placenta. In addition, oxidative stress is involved in placental vessel dysfunction [6]. The aforementioned miRNAs are involved in fundamental biological processes such as inflammation (miR-21), regulation of the cell cycle (miR-21, miR-210), apoptosis (miR-21), oxidative stress (miR-210) and cellular senescence (miR- 21, miR-210) [7].

Almost all available studies in this field are being conducted with placental tissue and in low-weight fetuses (intrauterine growth restriction). But the results are also quite controversial. For example, Cindrova-Davies T. et. al. determine increased expression of miR-21 in cases of preeclampsia and IUGR [8]. At the same time, the decreased expression of placental *miR-21* in combination with *miR-16* was determined by Maccani, M. A et.al. in children with extremely low weight at birth. Moreover, the simultaneous decrease in their level has a greater prognostic result, than a simple low expression of each of them individually do. That suggests an additive effect [9].

Zhang, J. et al found an increase in the expression of miR – 21 in macrosomal placenta in comparison with control samples [10]. Jiang, H. et. al. suggest that its activation in placenta can cause macrosomia via stimulating cellular differentiation and proliferation [11]. miR-21 regulates adipocyte differentiation and proliferation, and its expression correlates positively with body mass index [12]. That is, the miR-21 study makes it possible to evaluate the complex impact of many factors of mainly placental origin on fetal mass.

A placenta determines the growth and development of the fetus, and the development of placenta is controlled by the level of oxygen tension. Hypoxia-induced factor 1α (HIF1α) plays an important role in the control of placental oxygenation [13]. HIF directly targets vascular endothelial growth factor (VEGF), namely a major regulator of angiogenesis. MiR-210 was one of the first hypoxic miRNAs to be detected as a direct transcriptional target of HIF. Under normoxic conditions miR-210 is expressed at low levels, especially in certain cell types, such as endothelial cells. Under the influence of hypoxia, the transcription of miR-210 is dynamically activated in all known mammalian cell types, primarily through HIF-1α interaction. MiR-210 also reduces the need for cellular energy, causing hypoxic cell growth to be stopped by influencing the transcription factor E2F (E2F3), a cell cycle regulator not directly regulated by HIF transcription [14]. HIF1α not only induces the expression of miRNA -210, but in turn, it has been shown that this miRNA inhibits HIF1α. Hypoxia determines the expression of HIF1α and some of its target genes / miRNA -210 and their association with fetal growth parameters. During normal healthy pregnancy the expression of miR-210 remains at a fairly constant level [15]. Thus, the presence of a high level of correlation of hsa-miR210-3p in the umbilical cord blood of the newborn may be a marker of the functional status of the placenta, which programs the normal development of the fetus.

CONCLUSIONS

Hsa-miR210-3p potentially holds some interest for clinical practice, as possible markers of hypoxia both past and current, as well as its severity and effects.

Determination of the level of hypoxic miRNAs, in particular hsa-miR210-3p in the umbilical cord blood of the newborn may be a marker of the functional status of the placenta, which programs the normal development

of the fetus. But this study provides only partial answers to questions regarding the importance of hypoxia in programming of the fetal development. For a more complete picture, further studies in this area with larger patient and microRNA samples should be performed.

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Conflict of interest:

The Authors declare no conflict of interest.

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Received: 18.03.2020
Accepted: 10.11.2020

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article