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The Interdependence of Fetal and Maternal Extracellular DNA in Pregnant Women with Fetal Malformations

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ABSTRACT

*One of the promising and quite new directions of non-invasive prenatal diagnostics can be the analysis of extracellular fetal DNA in the blood of pregnant women. **Objective:** determination of prognostic significance of fetal DNA level in prognosis of fetal pathology. **Material and Methods:** seventeen pregnant women with fetal pathology in the second trimester were examined in the dynamics of gestation in obstetric complex No. 9 in Tashkent. Control group included 29 pregnant women with physiological course of gestation and without pathology. **Results:** the concentration of fetal DNA in plasma of pregnant women with fetal malformations in the second trimester exceeds the indicators of pregnant women without fetal pathology in 1.8 times. The total concentration associated with the surface of cells of fetal DNA in 2.3 times higher than in women without fetal pathology. The maternal DNA in fetal pathology was significantly decreased (in 3.1 times) in the fraction associated with the surface of blood cells, whereas the level of the mother's DNA in plasma from pregnant women with fetal abnormalities was lower in 1.7 times, in comparison with pregnant women without fetal pathology. The analysis of correlation between fetal and maternal extracellular DNA showed that this relationship is higher in the plasma and in the fraction associated with ionic interactions than in the fraction of extracellular DNA associated with cell surface proteins as in the physiological course of pregnancy, as in fetal abnormalities. **Conclusions:** the obtained results allow concluding that to increase the informative value of non-invasive diagnosis of fetal pathology at different stages of pregnancy, it seems appropriate to use fractions of fetal and maternal extracellular DNA associated with the surface of blood cells of the mother.*

Introduction. In the last time, due to the high incidence of viral infections among the population, interest in viruses and their role in the genesis of reproductive losses is growing. In acute viral infections, intrauterine infection of the fetus and placental damage often occur [1]. In recent years, there has been an increase in the incidence of acute respiratory viral infections (ARVI) in pregnant women, reaching 35.6% [2], which adversely affects the course and outcome of pregnancy.

There is very inconsistent evidence that ARVI in the mother may increase the risk of certain congenital malformations in children such as hydrocephalus, esophageal atresia

or anophthalmia/microphthalmia [3, 4]. There are cases of increased risk of anencephaly because of flu epidemic in the United States and Asia [5, 6].

A case-control study of congenital developmental anomalies in Hungary demonstrates the relationship between ARVI in the mother in the second and third months of pregnancy and congenital malformations in the child, such as cleft lip or palate, neural tube defect and cardiovascular malformations [7].

A case-control study, involving 363 neonates with neural tube defect and 523 healthy children, revealed that an increased risk of this disease was associated with ARVI in the mother [8]. In a number of case-control trials, the association between diseases in the mother and high risk of congenital defects [9]. In the literature, there is insufficient information about the course of gestation and complications from the fetus, depending on the duration of pregnancy and the severity of course of the infectious process.

The significance of the level of fetal DNA as a predictive marker in pregnant women with fetal pathology remains practically unexplored that determines the urgency of the problem and necessity for targeted research in this direction.

The need for developing new methods for non-invasive diagnosis of fetal development disorders is dictated by the following reasons. On the one hand, genetic analysis of fetal material obtained by invasive procedures reflects the true chromosome status of fetal cells with high accuracy; on the other hand, such manipulations involve a risk of abortion. The available non-invasive methods of prenatal diagnosis of chromosomal pathology are safe for maternal and fetal health; however, their informativeness does not exceed 60% [10, 11, 12].

One of the promising and quite new directions of noninvasive prenatal diagnostics can be the analysis of extracellular fetal DNA in the blood of pregnant women. In 1997, for the first time, the presence of fetal extracellular DNA in the serum of pregnant women was determined [13, 14].

This discovery stimulated intensive study of fetal DNA as a marker for non-invasive prenatal diagnosis. However, until now, the reason for the increase in the concentration of extracellular fetal DNA in the blood of women bearing fetuses with intrauterine growth disorders remains unclear. Obtaining new data on the peculiarities of DNA circulation in fetal developmental anomalies will make it possible to evaluate the possibility of analyzing extracellular fetal DNA as a marker of non-invasive prenatal diagnosis.

Materials and Methods

Pregnant women were examined in the dynamics of gestation in obstetric complex No. 9 in Tashkent. The main group consisted of 17 pregnant women with fetal pathology in the second trimester. Control group included 29 pregnant women with physiological course of gestation and without pathology. The average age of women was 26.3 ± 0.5 years.

The method for measuring the concentration of fetal DNA in the mother's blood was to carry out quantitative real-time PCR. In order to evaluate the features of circulation of extracellular DNA in the blood of pregnant women in the second trimester of pregnancy, the levels and ratio of fractions of freely circulating in plasma and cell-bound DNA were analyzed. The material was taken with the informed consent of the pregnant women participating in the study.

Results and Discussion

We conducted analysis of correlation between fetal and maternal extracellular DNA in the blood of pregnant women bearing healthy fetuses and those with violations of

intrauterine development in the second trimester of gestation. The study of the relationship of fetal and maternal DNA in blood fractions is necessary in the light of the fact that the successful detection of some fetal loci depends on the amount of fetal DNA found among DNA molecules of maternal origin [15]. Thus, for example, in most cases it is not possible to identify the DNA replicas inherited by the fetus from the father in the fraction that circulates in the DNA plasma. At the same time, when using samples obtained by electrophoretic separation of maternal and fetal DNA, the loci inherited by the fetus from the father were determined in all the cases analyzed [12].

Analysis of the level of fetal extracellular DNA freely circulating in plasma and associated with the cell surface during normal pregnancy showed that most fetal DNA (more than 60%) is bound to the surface of blood cells of the mother (Table 1).

The mechanisms by which such a distribution of extracellular DNA is observed in the blood of pregnant women is currently unknown [12, 16]. It is likely that a higher concentration of extracellular DNA on the surface of blood cells, compared to plasma, is due to the presence of a certain degree of affinity for membrane structures and circulating DNA, as demonstrated previously [17, 18].

The study of fetal DNA levels circulating and bound to the surface of cells in the blood of pregnant women showed that the concentration of fetal DNA in the plasma of pregnant women with fetal malformations in the second trimester was 77.9 ± 1.6 copies/ml in the mother's plasma that is 1.8-fold higher than those of pregnant women without fetal pathology. Whereas, the concentration of the total cell-bound fetal DNA was 156.2 ± 4.5 copies/ml, which is 2.3-fold higher than the values of women without fetal pathology (Table 1).

Table 1.

The concentration of extracellular fetal DNA (copies/ml) in the blood of pregnant women with fetal malformations in the second trimester of gestation

Study groups	The level of plasma DNA	The mother's DNA bound to the surface of blood cells		Total bound DNA
		II	CSP	
Pregnant women with fetal pathology (n = 17)	77.9 ± 1.6	65.9 ± 3.2	90.3 ± 1.5	156.2 ± 4.5 ■
Pregnant women without fetal pathology (n = 29)	43.3 ± 1.4	$31.4 \pm 5.2^*$	$36.5 \pm 2.6^*$	$67.9 \pm 3.7^*$ ■
	$p \leq 0.05$	$p < 0.01$	$p < 0.01$	$p < 0.001$

Note: * – differences between the level of fetal DNA in pregnant women with and without fetal pathology; ■ – differences between total bound fetal DNA and its plasma concentration ($P < 0.001$); (II - ion interactions, CSP – cell surface proteins).

It should be noted that in the group of women with fetal malformations, the concentration of total bound fetal DNA was 2 times lower relative to its concentration in the plasma.

Consequently, the concentration of fetal DNA reflects the level of proliferative / apoptotic changes occurring in the placental tissue and, thus, can serve as a marker of impaired fetal development. Indeed, in addition to the cases of miscarriage of fetuses with pathology, a significant increase in the level of fetal DNA is observed in such complications of pregnancy as preeclampsia [19], premature births [20], and placenta accreta [21].

It is not excluded that the changing nature of the distribution of extracellular DNA between plasma and formed elements in women's blood is also observed in such complications of pregnancy as undeveloped pregnancy, preeclampsia, as in our cases, the gestation of fetuses with malformations [15, 19, 20, 22].

In this regard, it seems appropriate to study the features of circulation of extracellular DNA of the mother and fetus to assess the potential use of this marker for non-invasive prenatal diagnosis, as well as for monitoring the course of pregnancy.

The level of extracellular DNA of the mother in the second trimester of gestation in fetal pathology showed low DNA values relative to the mother's DNA without fetal development pathology (Table 2). Thus, the level of maternal DNA was significantly reduced (in 3.1 times) in the fraction bound to the surface of blood cells. While the level of mother's DNA in plasma in pregnant women with fetal pathology was only in 1.7 times lower, in comparison with the indices of pregnant women without fetal pathology. This, in turn, affected the difference of the total bound DNA relative to its plasma concentration ($P < 0.001$). So, if in women without fetal pathology the total bound extracellular DNA of the mother exceeded the values of DNA in plasma in 8.4 times, then in fetal pathology the difference was observed to a lesser degree (in 4.6 times).

It should be noted that changes in the extracellular mother DNA were of opposite nature to changes in fetal DNA in fetal pathology, the values of which exceeded the parameters of women with physiological course of gestation.

Table 2.

The concentration of extracellular maternal DNA (copies/ml) in the blood of pregnant women with fetal malformations in the second trimester of gestation

Study groups	The level of plasma DNA	The mother's DNA bound to the surface of blood cells		Total bound DNA
		II	CSP	
Pregnant women with fetal pathology (n = 17)	160.2 ± 3.5	33.0 ± 2.1	705.5 ± 4.4	738.5 ± 2.6■
Pregnant women without fetal pathology (n = 29)	272.4 ± 4.9*	95.7 ± 1.8*	2193.5 ± 4.9*	2289.2 ± 4.8*■

Note: * – differences between the level of maternal DNA in pregnant women with and without fetal pathology; ■ – differences between total bound fetal DNA and its plasma concentration ($P < 0.001$); (II - ion interactions, CSP – cell surface proteins).

The analysis of correlation between fetal and maternal extracellular DNA in plasma and on the surface of red blood cells of pregnant women without fetal pathology in the second trimester showed that the ratio in the physiological pregnancy is higher in plasma and in the fraction associated with ionic interactions (II) than in the fraction of extracellular DNA bound to cell surface proteins (CSP). The analysis of correlation between fetal and maternal extracellular DNA in plasma and on the surface of blood cells of pregnant women with intrauterine fetal development disorders showed a similar correlation with only a larger difference.

Thus, the obtained results allow to conclude that to increase the informative value of non-invasive diagnosis of fetal pathology at different stages of pregnancy, it seems

appropriate to use fractions of fetal and maternal extracellular DNA associated with the surface of blood cells of the mother.

It is shown that in such developmental disorders, the concentration of the mother's DNA is significantly reduced, in comparison with the norm, in the fraction of DNA bound to the surface of blood cells. Since fetal cells are considered as the main source of extracellular fetal DNA in the mother's blood, it should be noted that the fetal DNA concentration, which reflects the level of proliferative/apoptotic changes occurring in placental tissue, can serve as a marker of disturbances in the course of gestation [22]. However, the use of fetal DNA in combination with other markers in programs for screening pregnant women increases the information content of detection of Down syndrome in the fetus [20]. Thus, the analysis of the level of extracellular DNA in the blood of pregnant women, including in combination with the assessment of the nature of the distribution of DNA between the plasma and the surface of the blood cells, can be successfully used as an additional non-invasive marker of fetal development pathology and gestation disorders.

Conclusions

1. The concentration of fetal DNA in plasma of pregnant women with fetal malformations in the second trimester exceeds the indicators of pregnant women without fetal pathology in 1.8 times. The total concentration associated with the surface of cells of fetal DNA in 2.3 times higher than in women without fetal pathology.

2. The maternal DNA in fetal pathology was significantly decreased (in 3.1 times) in the fraction associated with the surface of blood cells, whereas the level of the mother's DNA in plasma from pregnant women with fetal abnormalities was lower in 1.7 times, in comparison with pregnant women without fetal pathology.

3. The analysis of correlation between fetal and maternal extracellular DNA showed that this relationship is higher in the plasma and in the fraction associated with ionic interactions than in the fraction of extracellular DNA associated with cell surface proteins as in the physiological course of pregnancy, as in fetal abnormalities.

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