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## THE NATURE OF DISTRIBUTION OF FETAL EXTRACELLULAR DNA DURING PHYSIOLOGICAL PREGNANCY.

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### ABSTRACT

**Introduction.** The analysis of fetal extracellular DNA in the blood of pregnant women can serve as one of the advanced and new directions of a non-invasive prenatal diagnosis. Concentration of extracellular DNA of a mother and a fetus in the process of pregnancy development is still unclear and not completely studied. **The objective of the study** is to analyze literature data and identify new markers in non-invasive prenatal diagnosis during pregnancy period. **Material and methods.** In the process of the present research pregnancies were examined in the

dynamics of gestation. They had been under observation at the Maternity Complex №9 of Tashkent from 2014 to 2016. The research involved pregnant women depending on their gestational age, who were divided into 3 groups: the 1st group (27 pregnant with physiological course of gestation without fetal abnormalities in the I trimester), the 2nd group (29 women with the II trimester of pregnancy) and the 3rd group (21 pregnant in the III trimester of gestation). They were examined in the dynamics of gestation and had been under observation at the Maternity Complex №9 of Tashkent from 2014 to 2016. Pregnancy period ranged from 5 to 12 weeks in the 1st trimester (n=27), from 15 to 19 weeks in the 2nd trimester (n=29) and from 27 to 38 weeks in the third trimester (n=21) of pregnancy. The average age of women concluded  $27,0 \pm 0,5$  years. **Results and discussion.** The total concentration of extracellular DNA fraction in the plasma of pregnant women in the I trimester made  $22.6 \pm 3,2$  ng/ml, in the II trimester -  $10.8 \pm 9,1$  ng/ml and in the III trimester of pregnancy was  $74.3 \pm 4,0$  ng/ml. Fetal DNA is present on the cell surface of the mother's blood, but the main part of the fraction of DNA associated with the surface of the cells, presented DNA of maternal origin. The mechanisms, which observes the distribution of extracellular DNA in the blood of pregnant women, is unknown till today. **Conclusion.** The research establishes that the basic number as the maternal (over 90%) and fetal DNA (over 60%) is due to the surface of blood cells. During physiological pregnancy, the concentration of extracellular DNA is changing and there is a tendency to decreased levels of maternal and fetal extracellular DNA during the II trimester of pregnancy. For enhancing the informational content for noninvasive diagnostic of pathological states at different periods of pregnancy, it would seem appropriate to use the fraction of the fetal extracellular DNA associated with the surface of maternal blood cells.

**Key words:** maternal DNA, fetal DNA, pregnancy, extracellular DNA, gestation.

## Introduction

One of the advanced and new directions of a non-invasive prenatal diagnosis can be the analysis of fetal extracellular DNA in the blood of pregnant women.

In 1997, for the first time the presence of fetal extracellular DNA in plasma and serum of pregnant women was demonstrated [1, 2, 3]. This discovery has led to the intensive study of fetal DNA as a potential marker for the noninvasive prenatal diagnostic [4, 5, 6].

Biological basis, which increases in the concentration of extracellular DNA of a mother and a fetus with the development of pregnancy, remains still unclear [7, 8, 9]. There is an opinion that increase of fetal extracellular DNA concentration in maternal circulation occurs due to the possible reduction of DNA allotment from the mother's body, the reason of which can be various physiological changes of the

functions in the women's excretory organs during the pregnancy [10, 11]. It is proved [13] that in the pregnant women blood plasma circulating DNA molecules are longer than in plasma of non-pregnant women. The fetal DNA molecules are generally shorter than in maternal DNA, and are more fragmented.

It is assumed that fetal DNA enters the mother's blood by the transportation through the placenta of fetal cells, which are rapidly destroyed by the immune system of the mother, and as a result of the lysis of placenta cells, and direct release of fetal DNA into the mother's blood [14, 15]. Fetal DNA is already determined in the first weeks of the fetal development (in 80% of pregnant women it was found on the 28th day after conception) [14]. These data show that fetal DNA appears in the maternal blood before the time of the circulatory system formation of the fetus. Nowadays, many studies confirm that the source of fetal extracellular DNA in the maternal bloodstream are trophoblast cells [15]. Modern technologies allow to apply new knowledge in the practice of medicine [16, 17, 18, 19]. Thus, the analysis of the literature of recent years shows that the further study of fetal nucleic acids in the mother's blood is necessary for identification of new markers in non-invasive prenatal diagnostics during the pregnancy period.

**Materials and methods of study.** In the process of the present research pregnancies were examined in the dynamics of gestation. They had been under observation at the Maternity Complex №9 of Tashkent from 2014 to 2016. Depending on the gestational age pregnant women were divided into the following groups: the 1<sup>st</sup> group involved 27 pregnant women with physiological course of gestation and without fetal abnormalities in the first trimester, the 2<sup>nd</sup> group consisted of 29 pregnant women in the II trimester and the 3<sup>rd</sup> group consisted of 21 pregnant in the third trimester of gestation. Pregnancy age in women ranged from 5 to 12 weeks in the I trimester (n=27), from 15 to 19 weeks in the II trimester (n=29) and from 27 to 38 weeks in the III trimester (n=21). The average age of women was  $27,0 \pm 0,5$  years.

Information on pregnancy outcomes in women involved in the research was analyzed after the childbirth. The analysis of the study included pregnant women without complications during gestation and fetal abnormalities.

A method of measuring the concentration of fetal DNA in the mother's blood lies in performing of a quantitative real time PCR. So that to evaluate the features of circulating extracellular DNA in the blood of pregnant women at different stages of pregnancy, the level and proportion of the fractions freely circulating in plasma and associated with the cell surface, was analyzed. The material sampling was performed with the informed consent of the pregnant women involved in the research.

**Results and discussion.** The concentration of the total fraction of extracellular DNA (DNA of the mother and the fetus) in the plasma of pregnant

women in the first trimester was  $22.6 \pm 3.2$  ng/ml, in the second trimester -  $10.8 \pm 9.1$  ng/ml and in the III trimester of pregnancy -  $74.3 \pm 4.0$  ng/ml. The concentration of extracellular DNA, eluted from the cell surface was  $467.2 \pm 16.3$  ng/ml,  $192.1 \pm 3.5$  ng/ml and  $387.5 \pm 11.6$  ng/ml in the I, II and III trimesters of pregnancy, respectively (Table 1).

**Table 1****The concentration of extracellular DNA in the blood of pregnant women**

Pregnancy period (weeks)	The concentration of DNA in plasma, (ng/ml)	The concentration of DNA, associated with the surface of blood cells, (ng/ml)
9 $\pm$ 1 (n=27)	22,6 $\pm$ 3,2	467,2 $\pm$ 16,3
17 $\pm$ 0,2 (n=29)	10,8 $\pm$ 9,1*	192,1 $\pm$ 3,5*
30 $\pm$ 0,4 (n=21)	74,3 $\pm$ 4,0* ▼	387,5 $\pm$ 11,6* ▼

Note: \* - compared with patients in the 1st trimester of gestation (P<0,05);

▼ – compared with patients in the second trimester ( P<0,05).

When comparing concentrations both of circulating and associated with the cell membrane extracellular DNA at different stages of the normal pregnancy, changes in the level of fractions during the pregnancy period ( $p > 0.05$ ) were identified. On the other hand, the differences in the concentrations of extracellular DNA in plasma between the trimesters of pregnancy, has been noted previously [5, 11]. The research revealed that, there was a tendency for decrease of DNA level in plasma and the concentration of DNA, associated with the surface of blood cells during the II trimester of pregnancy (Table 1). This process is connected to such physiological changes in the body of pregnant woman as hemodilution.

The result of the analysis showed that the main part of extracellular DNA fraction (over 90%) is linked to the surface cells of maternal blood (Table 1). It is possible that the changing nature of extracellular DNA distribution between plasma and formed elements in the blood of women is observed in such complications of pregnancy as non-developing pregnancy, preeclampsia and pregnancy cases with fetus congenital abnormalities [7, 13, 14]. In this regard, it is reasonable to study the peculiarities of maternal and fetal extracellular DNA circulation during various states of gestation to access the potential use of this marker for the non-invasive prenatal diagnostic and monitoring of the pregnancy.

At this stage, by applying the PCR real time method the level of fetal and maternal extracellular DNA circulating freely in the plasma and associated with the cell surface was analyzed in the process of normal pregnancy; as well as the link of DNA concentration with the pregnancy period was evaluated. The evaluations of

the average concentrations of fetal and maternal DNA at normal pregnancy is presented in the Table 2 and 3.

**Table 2**

**The concentration of fetal extracellular DNA (c/ml) in the blood of pregnant women at physiological course of gestation, without fetal abnormalities**

The gestational age (weeks)	The concentration of fetal DNA in the plasma	Fetal DNA, associated with the surface of maternal blood cells		Total associated fetal extracellular DNA
		IIs	PCS	
I trimester (n=27)	32,6±1,7▼	26,5±2,8▼	57,2±3,3▼	83,7±3,1▼■
II trimester (n=29)	43,3±1,4*	31,4±5,2*	36,5±2,6*	67,9±3,7*■
III trimester (n=21)	64,7±6,3*▼	36,1±1,4*▼	88,3±2,5*▼	124,4±1,9*▼■

Note: \* - differences with regard to patient's data in the 1st trimester of gestation ( $P < 0.05$ );

▼ - differences in patient's data in the second trimester ( $P < 0,05$ );

■ - differences in total associated fetal DNA relatively to its concentration in the plasma ( $P < 0,001$ ); (II - ionic interactions; PCS - proteins of the cell surface).

Thus, the research reveals that, fetal DNA is present on the cell surface of the maternal blood, but the main part of DNA fraction associated with the surface of the cells, is presented by DNA of maternal origin.

From the obtained data it is seen that the concentration of fetal DNA in the fraction (Table 2), associated with the surface of cells by ionic bonds, is comparable to those in maternal plasma, and slightly higher level of extracellular DNA was observed in the fraction, bounded with proteins of the cell surface (PCS). Moreover, this type of distribution of the total fraction of extracellular DNA in the blood of pregnant women, the basic amount of fetal DNA (over 60%) is also associated with the surface of maternal blood cells.

The mechanisms that have led to extracellular DNA distribution in the blood of pregnant women are remain unknown today [4, 9]. The research establishes that the basic number of maternal (over 90%) and fetal DNA (over 60%) is connected with the surface of blood cells.

Table 3.

**The concentration of maternal extracellular DNA (c/ml) in the blood of pregnant women at physiological course of gestation, without fetal abnormalities**

The Gestational age (weeks)	The concentration of maternal DNA in the plasma	Extracellular DNA, associated with the surface of maternal blood cells		Total associated maternal extracellular DNA
		IIs	PCS	
I trimester (n=27)	232,5±4,6	134,2±2,9	3521,0±5,7	3655,2±5,9■
II trimester (n=29)	272,4±4,9	95,7±1,8*	2193,5±4,9*	2289,2±4,8■
III trimester (n=21)	978,1±2,7* ▼	344,9±7,1* ▼	3735,2±6,4 ▼	4080,1±6,7■

Note: \* - differences regarding patient's data in the 1st trimester of gestation (P<0,05);

▼ – differences regarding patient's data in the II trimester (P<0,05);

■ - the differences of the total associated DNA regarding to its concentration in the plasma (P<0,001); (ionic interactions; cell surface proteins).

Thus, it can be assumed that the described circulation features of extracellular DNA in the blood of pregnant women is observed due to the action of the similar mechanisms, the role of which should be identified. Probably the 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 of the membrane structures and circulating DNA, that was discussed previously [5, 12]. In addition, it is possible that DNA molecules of different lengths, including those in the composition of the nucleoprotein [9] and lipoprotein complexes [8, 18], have different binding capacity to the surface of blood cells. However, it should be noted that molecules of circulating maternal and fetal DNA differ significantly in their size; namely the fragments of maternal DNA have a greater length than fetal DNA [20]. This fact, on the other hand, confirms the possible influence of this mechanism on the features of the circulating DNA of maternal origin.

Circulation features of extracellular DNA during pregnancy should be considered when conducting molecular genetic diagnostic. It was noted that during normal pregnancy the concentration of extracellular DNA changes [21]. The study showed that, there was also some tendency to reduce the level of maternal and fetal

extracellular DNA in the II trimester of pregnancy (Table 4). Apparently, the reason of this is gemodilution - physiological changes in the pregnant body.

**Table 4.**  
**The change in the level of extracellular DNA during pregnancy**

Trimester	The concentration of extracellular DNA from the surface of blood cells			The efficiency of fetal DNA exposure (%)
	Measurement of DNA level, ng/ml	Measurement of DNA level using real-time PCR, copies/ml		
		Maternal DNA	Fetal DNA	
I	467,2±16,3 ▼	3655,2±5,9 ▼	83,7±3,1 ■	94
II	192,1±3,5*	2289,2±4,8*	67,9±3,7*■	67

Note: \* - differences regarding the patient's data in the 1st trimester of gestation (P<0,05);

▼ – differences regarding the patient's data in the second trimester (P<0,05);

■ - differences of the fetal DNA relatively to the maternal DNA (P<0,001).

**Discussion.** According to the stated above, the rapid increase in the level of fetal extracellular DNA is observed only in the last weeks of pregnancy and during a childbirth [3, 5, 6], whereas the present study results shows that the II trimester of pregnancy concluded in average 30 weeks. In those studies, where such link was observed, the gestational age ranged from 35 to 42 weeks [7, 8]. It is interesting to note that the link between the level of fetal DNA and the period of pregnancy in the present research was determined in the fraction of fetal DNA, associated with the surface of maternal blood cells. It is most likely that a significant increase in the level of fetal DNA in the maternal blood in the last weeks of pregnancy is the result of a gradual increase in placental barrier permeability [9]. In order to verify this assumption, the analysis of the concentration of fetal DNA dynamics in the late stages of pregnancy was carried out [10]. It has been determined that a significant correlation between the duration of pregnancy and the level of fetal DNA was observed in the late III trimester, and the growth in the concentration of fetal DNA averaged 29,3% for each subsequent week of pregnancy.

The biological basis for the analysis of the observed changes in the level of maternal and fetal extracellular DNA during pregnancy is still undiscovered today. However, the increase in the level of both maternal and fetal DNA during pregnancy testifies in favor of the fact that mechanisms for releasing fetal and maternal DNA into the bloodstream and their removal from the body may have



similar nature. So, for example, the source of fetal and maternal DNA are the cells of fetal and maternal parts of the placenta, namely, fetal DNA is the product of vital functions of trophoblast cells, whereas maternal DNA are the cells of the decidual membrane [11, 16, 18].

Thus, the analysis results of extracellular DNA level in the blood of pregnant women, in combination with the evaluation of DNA distribution between the plasma and the surface of blood cells, can be successfully applied as an additional noninvasive marker of pathological fetal development and dysfunctions of the pregnancy course.

**Conclusion.** During physiological pregnancy the concentration of extracellular DNA changes and there is a tendency for levels decrease both of maternal and fetal extracellular DNA during the II trimester of the pregnancy. For enhancing the informational content of noninvasive diagnostic of pathological states at different periods of pregnancy, it would seem appropriate to use the fraction of fetal extracellular DNA associated with the surface of maternal blood cells.

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