

## LEVELS OF CELL-FREE DNA AND DNASE I ACTIVITY IN COMPLICATED AND NORMAL PREGNANCIES

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Placental pathology is accompanied by the activation of apoptosis in the trophoblast and the subsequent increase in the concentrations of microvesicles containing placental (or fetal) DNA accumulating in the maternal blood. Fragments of fetal DNA stimulate the release of nuclear and/or mitochondrial DNA fragments by neutrophils. Therefore, one can expect that complicated pregnancies will be characterized by the dramatic elevation of total cell-free DNA (cfDNA) levels in maternal plasma. The aim of this work was to study the dynamics of plasma cfDNA concentrations and the activity of DNase I, an enzyme involved in the elimination of cfDNA from the bloodstream, in nonpregnant and pregnant women. Our study recruited 40 healthy nonpregnant women, 40 women with uncomplicated pregnancies and 35 women with the intrauterine growth restriction (IUGR) of the fetus. We did not observe the elevation of the total cfDNA concentrations in the patients with complicated pregnancies. Moreover, cfDNA concentrations in their plasma were even lower (though this difference was statistically insignificant) than in healthy pregnant and nonpregnant women. The median values of cfDNA concentrations in the group of healthy nonpregnant women were 75.5 ng/ml; in the group of healthy pregnant women, 78.0 ng/ml; and in the patients with IUGR, 42.1 ng/ml. At the same time, we observed a significant increase in DNase I activity in the plasma of women with IUGR. The median DNase I activity in the groups of healthy pregnant and nonpregnant women was 3.0 and 3.4 IU/ml, respectively. In patients with different grades IUGR of the fetus this parameter was as high as 6.3 IU/ml ( $p < 0.001$ ). Increased DNase I activity in the plasma of women with complicated pregnancies indirectly suggests a transient elevation of circulating cfDNA levels. Our study shows that the high level of activity exhibited by the cfDNA elimination system impedes the analysis of cfDNA concentrations in complicated pregnancies and skews its results. However, if cfDNA, DNase I activity and the cfDNA/DNase I ratio were all taken into account, it could be possible to develop a tool for the monitoring of cell death in the mother throughout the entire pregnancy.

**Keywords:** cfDNA, DNase I, pregnancy, preeclampsia, IUGR

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## УРОВЕНЬ ВНЕКЛЕТОЧНОЙ ДНК И АКТИВНОСТЬ ДНКАЗЫ I ПРИ НОРМАЛЬНОЙ И ОСЛОЖНЕННОЙ БЕРЕМЕННОСТИ

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При патологии плаценты происходит активация апоптотических процессов в трофобласте, что сопровождается повышением в кровотоке матери концентрации микровезикул, содержащих плацентарную ДНК (или ДНК плода). Фрагменты ДНК плода стимулируют выброс нейтрофилами участков ядерной и/или митохондриальной ДНК. Таким образом, при осложненной беременности следует ожидать значительного увеличения общей концентрации внутриклеточной ДНК (вкДНК) в плазме матери. Целью работы было исследование совместных изменений концентрации вкДНК и активности одного из компонентов системы элиминации вкДНК из кровотока — фермента ДНКазы I в плазме небеременных и беременных женщин. В исследовании принимали участие 40 здоровых небеременных женщин, 40 беременных женщин с нормально протекающей беременностью и 35 пациенток с внутриутробной задержкой роста плода (ВЗРП). Мы не обнаружили повышения уровня суммарной вкДНК у пациенток с осложненной беременностью. Более того, концентрация вкДНК в плазме пациенток была даже ниже (статистически незначимо), чем соответствующие показатели в плазме здоровых беременных и небеременных женщин. Так, значение медианы концентрации вкДНК в группе здоровых небеременных женщин составило 75,5 нг/мл, в группе здоровых беременных женщин этот показатель достигал 78,0 нг/мл, у пациенток с ВЗРП он был равен 42,1 нг/мл. В то же время мы обнаружили достоверное повышение активности ДНКазы I в плазме женщин с ВЗРП. Медиана активности ДНКазы I в группах здоровых беременных и небеременных женщин составила соответственно 3,0 и 3,4 IU/мл. У пациенток с ВЗРП различной степени тяжести этот показатель достигал 6,3 IU/мл ( $p < 0,001$ ). Повышенная активность ДНКазы I в плазме женщин с патологией беременности косвенно свидетельствует о транзитном повышении у них уровня циркулирующей вкДНК. Полученные результаты показывают, что высокий уровень активности системы элиминации вкДНК затрудняет и искажает результаты анализа концентрации вкДНК при беременности, особенно при патологии. Однако если учитывать три показателя — концентрацию вкДНК, активность ДНКазы I и отношение вкДНК/ДНКазы I, то в перспективе можно разработать систему мониторинга уровня гибели клеток в организме матери на протяжении всего периода беременности.

**Ключевые слова:** вкДНК, ДНКазы I, беременность, преэклампсия, ВЗРП

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The presence of DNA in human blood plasma and serum was discovered as early as 1948, a few years before the structure of this molecule was figured out [1]. This type of DNA went by the name of circulating or cell-free DNA (cfDNA) [2]. In 1997 fetal cfDNA was detected in the blood plasma of a pregnant woman [3]. The discovery of fetal cfDNA in maternal plasma and serum inspired the development of noninvasive methods of prenatal screening for genetic abnormalities in the fetus [4]. Today, it has become possible to sequence the entire fetal genome from cfDNA circulating in the maternal blood [5].

Cell-free DNA analysis is not solely used to screen for genetic defects in the fetus; among its other applications is monitoring for pregnancy complications, such as preeclampsia and miscarriage [6–8]. Here, the idea is not to look for an individual mutant gene but to measure total cfDNA concentrations in maternal blood or the concentration of fetal cfDNA alone. The primary source of fetal cfDNA in maternal blood is thought to be necrosis and/or apoptosis of placental cells [9].

It is important to note that while taking these measurements, researchers tend to ignore the regulatory processes causing cfDNA concentrations to decline. Growing cfDNA levels associated with increased cell death signal the cfDNA elimination system to activate and clear excess cfDNA from the bloodstream. Dropping cfDNA concentrations are observed in patients with chronic conditions accompanied by increased cell death, such as cardiovascular disorders [10] and occupational exposure to radiation [11].

Previously, we investigated the dynamics of cfDNA concentrations and the activity of DNase I, an enzyme involved in the elimination of cfDNA from the bloodstream, in the plasma of nonpregnant women and women with normal and complicated pregnancies [12]. Our findings were not consistent with the literature. Firstly, cfDNA levels in the women with normal and complicated pregnancies did not exceed the values demonstrated by the controls (according to the literature, in both healthy and complicated pregnancies cfDNA concentrations are expected to surge [13]). Secondly, the level of DNase I activity was twice as high in the participants with complicated pregnancies. This phenomenon had not been described previously by other researchers. Considering the obtained results, we decided to conduct another research study with a special focus on the clinical characteristics of healthy nonpregnant and pregnant women.

## METHODS

The study recruited 1175 women aged 22 to 40 (the mean age was  $32 \pm 4$  years) residing in Moscow, Russia, and coming from the same social stratum. Inclusion criteria varied depending on the group: group 1 included healthy nonpregnant female volunteers (medical students and clinical residents;  $n = 40$ ); group 2 consisted of healthy women > 37 weeks of uncomplicated pregnancy ( $n = 40$ ) who had previously given birth to healthy children with no signs of hypoxia or underweight; group 3 consisted of women with complicated pregnancies, miscarriages, placental insufficiency, intrauterine growth restriction of the fetus (IUGR), chronic hypoxia of the fetus, or thin uterine scars (> 30 weeks of gestation,  $n = 35$ ). Exclusion criteria were not applied. Blood samples of healthy nonpregnant women were collected between days 10 and 15 of their menstrual cycles. This study was part of the PhD dissertation and was approved by the Ethics Committee of Pirogov Russian National Medical Research University (Protocol 159 dated November 21, 2016). The recruited women gave informed consent to participate.

## Fetal development

Fetal development was evaluated by ultrasonography: a few biometric measurements were taken, including the biparietal diameter, thoracic and abdominal circumferences, and femur length. If the measured sizes were showing a 2-week lag behind the population average [14], the patient was diagnosed with grade I IUGR; a 2–4-week lag, grade II IUGR; more than a 4-week lag, grade III IUGR. The final diagnosis was established postpartum based on the newborn's weight. The reference interval lay between the 75<sup>th</sup> and 25<sup>th</sup> percentiles; grade I IUGR corresponded to the 2<sup>th</sup>–10<sup>th</sup> percentiles; grade II, to the 10<sup>th</sup>–3<sup>th</sup> percentiles; grade III was below the 3<sup>th</sup> percentile [15]. We also calculated the weight-to-height ratio: the value of >60 suggested normal growth; grade I IUGR corresponded to the range from 55 to 60; grade II IUGR, to 50–55; grade III IUGR was below 50. Fetal cardiac function was evaluated by cardiocotography (CTG) and Doppler ultrasonography of the uterine, umbilical and fetal blood flows. Ultrasonography was performed on Voluson 530 MT (Kretztechnik; Austria) and Voluson E8 (General Electric; USA) equipped with three different transducers: RIC 5–9 D (4–9 MHz), C1–5D (2–5 MHz), and RAB 4–8 D (2–8 MHz). CTG was done using the GE Corometrics (250CX) fetal monitor (USA).

## Special assays

Fragments of circulating cfDNA were isolated from 1 ml of heparinized blood plasma samples by phenol extraction. Blood cells were pelleted by centrifugation at 400 g for 10 min. The obtained plasma was mixed with 10% sodium lauroyl sarcosinate, 0.2 M EDTA and a standard 0.075 mg/ml RNase solution (Sigma; USA) and incubated for 45 min. Then it was treated with a standard 0.2 mg/ml proteinase K solution (Promega; USA) and left to sit for 24 h at 37 °C. Following 2 cycles of washing with a saturated phenol solution, the DNA fragments were pelleted by adding two volumes of ethanol and 2 M ammonium acetate. Then the pellet was washed twice with 75% ethanol, dried and dissolved in water. CfDNA concentrations were measured by fluorescence on LS-55 (Perkin Elmer; USA) using the Pico Green fluorescent probe (Sigma; USA).

DNase I activity in blood plasma was measured using a technique proposed in [16]. Briefly, the substrate for DNase I (Sintol, Russia) is a 30 b.p. long double-stranded oligodeoxyribonucleotide described by the formula R6G - ACC CCC AGC GAT TAT CCA AGC GGG - BHQ1. The sequence of a model substrate is not critical. On its 5'-end the oligonucleotide is tagged with fluorescent 5(6)-carboxyrhodamine; on its 3'-end it contains the fluorescence quencher BHQ1. As the endonuclease continues to hydrolyze phosphodiester bonds, the emitted fluorescent signal intensifies. In our experiment, we added 10 µl of blood plasma to 90 µl of the solution for DNase I containing 10 mM HEPES, pH 7.5, 20 mM MgCl<sub>2</sub>, 5 mM CaCl<sub>2</sub>, and 3 pM of the oligonucleotide. The reaction went on for 1 h at 37 °C. Fluorescence was recorded using a plate reader (EnSpire; Finland). DNase I activity was calculated from a calibration curve showing a correlation between fluorescence enhancement and the concentration of a standard DNase I sample (Sigma; USA) in the solution. DNase I activity was expressed in arbitrary units: 1 unit = 1 ng/ml, i.e. it shows an increase in the substrate fluorescence resulting from the activity of DNase I taken at a concentration of 1 ng/ml (1 h 37 °C). At least three parallel measurements were taken for each sample. The relative standard error of measurement was 5%.

The data were processed in StatPlus 2007 (Statistical Graphics Corp.; USA); the Mann–Whitney U-test was applied.

## RESULTS

In group 2 consisting of women with uncomplicated pregnancies fetal cardiac function scored from 7 to 10 points on the Fisher scale; the aortic blood flow was 180 to 260 ml/min; the umbilical blood flow was 86 to 140 ml/min per 1 kg of the fetus's weight. In group 3, 28 of 35 women demonstrated poorer fetal cardiac function (4 to 7 points on the Fisher scale); the aortic blood flow was 120 to 174 ml/min; the umbilical blood flow was 60 to 86 ml/min per 1 kg of the fetus's weight. The data obtained from the patients with grades I, II and III IUGR are presented in Table 1. The most pronounced impairment of fetal cardiac function was observed in the patients with grades II and III IUGR. In the participants with grades II IUGR, circulation disorders manifested as uteroplacental and fetoplacental blood flow abnormalities. Grade III IUGR was characterized by fetoplacental blood flow disorders, such as the absent or reversed diastolic flow in the umbilical artery or aorta, and the abnormal uterine blood flow. In the patients with grades II and III IUGR fetal cardiac function scored less than 7 points on the Fisher scale; their diastolic flow in the umbilical artery or aorta was either reversed or absent and the uterine blood flow was abnormal.

Concentrations of cfDNA in the healthy nonpregnant participants (Table 2) varied from 11 to 123 ng/ml (the median value was 75.5 ng/ml); in uncomplicated pregnancies the figures ranged from 2 to 347 ng/ml (the median value was 78 ng/ml),

and in IUGR this interval was from 1.2 to 595.7 ng/ml (the median value was 42.1 ng/ml). The Mann–Whitney U-test did not reveal significant differences between the groups ( $p > 0.05$ ). Nine samples (22.5%) representing groups 2 and 3 had cfDNA concentrations falling above the reference interval established for nonpregnant women (123 ng/ml). Interestingly, unlike the nonpregnant participants, the pregnant women demonstrated a wider variability of cfDNA concentrations. The variation coefficient was 0.42 for group 1, 0.87 for group 2, and 1.37 for group 3.

Perhaps, a decline in cfDNA concentrations was largely caused by the increased activity of the components constituting the system of cfDNA elimination from the bloodstream. One of the factors affecting cfDNA elimination is the activity of DNase I in blood plasma, an enzyme responsible for cfDNA hydrolysis. In our study the activity of this enzyme (Table 2) in the nonpregnant participants varied from 1.1 to 5.9 IU/ml (the median value was 3 IU/ml); in normal pregnancies, between 0.6 and 14.8 IU/ml (the median value was 3.4 IU/ml); in IUGR, between 3.9 and 14.3 IU/ml (the median value was 6.3 IU/ml). The Mann–Whitney U-test did not reveal any significant differences between groups 1 and 2 ( $p > 0.05$ ). However, the group of patients with complicated pregnancies significantly differed from the group of healthy nonpregnant ( $p < 10^{-7}$ ) and healthy pregnant women ( $p < 10^{-5}$ ) in terms of DNase I activity in blood plasma. So, the blood plasma of pregnant women with IUGR shows higher levels of DNase I activity in comparison with healthy pregnant and nonpregnant women. In 18 (51.4%) of 35 pregnant women from group 3 DNase I activity was high; in contrast, high DNase I activity is not typical for nonpregnant

**Table 1.** Characteristics of patients with IUGR of the fetus

	Patients with grade I IUGR <i>n</i> = 11	Patients with grade II IUGR <i>n</i> = 13		Patients with grade III IUGR <i>n</i> = 11		
				<i>p</i> 1		<i>p</i> 1
Blood flow in the aorta	220 (154; 254)	150 (126; 180)	0.0002	122 (120; 142)	0.0001	0.0005
Blood flow in the umbilical vein	110 (82; 120)	80 (64; 86)	0.0001	64 (60; 68)	0.0001	0.0002
Reversed diastolic flow in the umbilical artery or aorta	–	1 (7.7%)	1	6 (54.5%)	0.0124	0.0233
Preterm labor	–	3 (23.1%)	0.2228	4 (36.3%)	0.0902	0.6591
Preeclampsia	–	3 (23.1%)	0.2228	7 (63.6%)	0.0039	0.0953
Scores on Fisher scale	8 (7; 9)	6.5 (6; 7)	0.0001	5 (4.5; 6)	0.0001	0.0002
Gestational age, weeks	40 (38; 41)	38 (36; 41)	0.0397	37 (30; 40)	0.0008	0.0084
Weight of the fetus, g	2,790 (2,630; 3,030)	2,520 (2,350; 2,650)	0.0001	1,720 (600; 2,270)	0.0001	< 0.0001
Height of the fetus, cm	48 (47; 51)	47 (46; 50)	0.0221	43 (29; 47)	0.0002	0.001

**Note:** results are presented as median values (the maximum value ÷ the minimum value); *p*1 is the *p* value indicating the difference from the group of pregnant patients with grade I IUGR; *p*2 is the *p* value indicating the difference from the group of pregnant patients with grade II IUGR.

**Table 2.** Concentrations of plasma cfDNA and the activity of DNase I in healthy nonpregnant women and women with normal and complicated pregnancies

	Healthy nonpregnant women <i>n</i> = 40	Healthy pregnant women <i>n</i> = 40		Pregnant women with IUGR of the fetus <i>n</i> = 35		
				<i>p</i> 1		<i>p</i> 1
Concentration of cfDNA, ng/ml	75.5 (11.0; 123.0)	78 (2.0; 347.0)	0.59	42.1 (1.2; 595.7)	0.316	0.258
DNase I activity, IU/ml	3 (1.1; 5.9)	3.4 (0.6; 14.8)	0.088	6.3 (3.9; 14.3)	< 0.001	< 0.001
cfDNA/DNase I ratio	20.5 (6.5; 101.8)	25.9 (0.3; 112.4)	0.889	8.4 (0.1; 152.7)	< 0.001	0.002

**Note:** results are presented as median values (the maximum value ÷ the minimum value); *p*1 is the *p* value indicating the difference from the group of healthy nonpregnant women *p*2 is the *p* value indicating the difference from the group of healthy pregnant women.

healthy women. In group 2 increased DNase I activity was observed for only 4 (10%) of 40 pregnant women ( $p = 0.0002$ ; Fisher's exact test applied).

Table 3 shows the correlation between cfDNA concentrations and the level of DNase I activity. The group of healthy nonpregnant women demonstrated a moderate but statistically significant negative correlation between these two parameters ( $R = 0.37$ ;  $p < 0.05$ ). The pregnant women, especially those with complicated pregnancies, demonstrated a weak correlation between cfDNA levels and DNase I activity.

The subgroups of patients with different grades IUGR did not differ significantly in terms of the studied parameters. However, cfDNA concentrations and the ratio of cfDNA to DNase I activity strongly tended to grow with the severity of IUGR (Table 4). The analysis revealed that only 4 (16.7%) of 24 patients with grades I and II IUGR had high cfDNA concentrations not observed in nonpregnant women, whereas there were as many as 6 women (54.5%;  $p = 0.041$ , Fisher's exact test applied) in the subgroup of 11 patients with grade III IUGR who had elevated cfDNA levels. Moreover, when comparing the cfDNA/DNase I ratio between the patients with different grades IUGR and the controls, significant differences were observed only for the patients with grades I and II IUGR ( $p < 0.001$ ). The patients with grade III IUGR had the same cfDNA/DNase I ratio as the healthy pregnant and nonpregnant participants (Tables 2, 4).

## DISCUSSION

Concentrations of maternal cfDNA strongly correlate with the amount of placental cfDNA [17]. It is known that only a small fraction of maternal cfDNA circulating in blood comes from solid organs such as the liver or kidneys; the rest originates from hematopoietic cells. For example, differentiating erythroblasts are a stable source of low molecular weight cfDNA fragments. Rapid elevation of cfDNA levels in the circulation in pathology or following physical effort is caused by the activation of neutrophils that release extracellular traps containing nuclear and/or mitochondrial DNA [18].

CfDNA has an impact on many cells in the body. Circulating DNA can contribute to oxidative stress, stimulate the synthesis of anti-inflammatory cytokines and induce aseptic inflammation [19]. CfDNA-containing extracellular traps released by activated neutrophils attract platelets and significantly increase the risk of thrombosis [18]. The body defends itself against the negative impact of excess cfDNA by activating the system of

its elimination from the bloodstream. A part of this system is the activity of endonucleases present in human blood. DNase I is the main blood endonuclease that hydrolyzes phosphodiester bonds in DNA strands. Accumulation of single-strand breaks entails double-strand breaks, thus producing low molecular weight cfDNA fragments. These fragments can be easily eliminated through the kidneys. Healthy nonpregnant women participating in our study demonstrated a moderate but statistically significant negative correlation between the amount of cfDNA in blood plasma and the activity of DNase I. It looks like this activity is the major factor responsible for the elimination of cfDNA fragments from the bloodstream. Women with uncomplicated pregnancies and patients with IUGR showed no correlation between DNase I activity and cfDNA levels.

Placental pathology is accompanied by apoptosis in the trophoblast with the subsequent increase in the concentration of microvesicles with placental cfDNA in the maternal blood stream. Trophoblast microvesicles, in turn, stimulate the release of web-like nuclear and mitochondrial DNA strands (netosis) by neutrophils. Therefore, one can expect that complicated pregnancies will be characterized by the dramatic elevation of total cfDNA levels in maternal plasma. However, in the course of this work we did not observe an increase in total cfDNA concentrations in pregnant women with IUGR. Moreover, plasma cfDNA concentrations were lower (insignificantly, though) in complicated pregnancies than in healthy pregnant and nonpregnant women. At the same time, we observed a statistically significant increase in DNase I activity exerted in the plasma of pregnant women with IUGR, which indirectly suggests a transient elevation of circulating cfDNA levels in patients with complicated pregnancies. Perhaps, a substantial increase in total cfDNA levels circulating in the plasma of pregnant women with IUGR triggers the activation of protective mechanisms, of which DNase I is particularly important, promoting elimination of excess cfDNA from the bloodstream. The majority of our patients with grades I and II IUGR had moderate placental flow defects (Table 1) that led to a moderate and possibly transient increase in cfDNA levels rapidly eliminated from the blood stream by activated DNase I. Those patients had low cfDNA concentrations, highly active DNase I and a low cfDNA/DNase I ratio. The majority of patients with grade III IUGR underwent rapid accumulation of cfDNA in plasma which the elimination mechanisms failed to handle. Those patients had increased cfDNA concentrations, highly active DNase I and a high cfDNA/DNase I ratio. Besides, excess cfDNA in the circulation

**Table 3.** Spearman's correlation between cfDNA concentrations and DNase I activity

	<i>R</i>	<i>p</i>
Healthy nonpregnant women	-0.39	< 0.05
Healthy pregnant women	-0.28	> 0.05
Women with complicated pregnancies	-0.18	> 0.05

**Table 4.** Levels of cfDNA and DNase I activity in patients with different grades IUGR

	Patients with grade I IUGR <i>n</i> = 11	Patients with grade II IUGR <i>n</i> = 13	Patients with grade III IUGR <i>n</i> = 11			
			<i>p</i> <sub>1</sub>	<i>p</i> <sub>1</sub>	<i>p</i> <sub>2</sub>	
Concentration of cfDNA, ng/ml	34.7 (8.0; 160.7)	76 (7.8; 251.1)	0.817	103.5 (1.2; 595.7)	0.212	0.354
DNase I activity, IU/ml	5.7* (4.1; 13.1)	7.5* (3.9; 14.3)	0.183	5.2* (3.9; 10.0)	0.793	0.271
cfDNA/DNase I ratio	5.2* (0.6; 28.2)	10.1* (0.8; 36.4)	0.817	18.5 (0.1; 152.7)	0.325	0.271

**Note:** results are presented as median values (the maximum value ÷ the minimum value; *p*<sub>1</sub> is the *p* value indicating the difference from the group of patients with grade I IUGR; *p*<sub>2</sub> is the *p* value indicating the difference from the group of patients with grade II IUGR; \* represents  $p < 0.01$  indicating the difference from the groups of healthy nonpregnant and pregnant women.

aggravates placental flow defects and increases the risk of poor pregnancy outcomes.

Our study demonstrates that high activity of the cfDNA elimination system impedes the analysis of cfDNA concentrations in pregnancy, especially if the latter is complicated, and skews results. This could be the reason why the literature is very controversial on the dynamics of cfDNA concentrations in pregnancy. However, if all of the three parameters (cfDNA concentrations, DNase I activity and the cfDNA/DNase I ratio) are taken into account, the development of a tool for cell death monitoring throughout the entire pregnancy becomes possible (such tests could be done once in a trimester, for instance). These parameters provide information on cell death and the performance of the cfDNA elimination system composed of DNase I and other components that have a role in pregnancy. If such tests revealed increased DNase I activity during a certain

week of pregnancy plus elevated cfDNA levels, one could infer the increased rates of cell (specifically, placental) death. High cfDNA concentrations in combination with increased DNase I activity indicate insufficient clearance of cfDNA from the body and, therefore, pathology in the stages when ultrasonography is unable to detect IUGR due to the lack of visible signs.

## CONCLUSIONS

We have studied the dynamics of cfDNA concentrations and the activity of DNase I in the blood plasma of healthy nonpregnant women and women with normal and complicated pregnancies. We have shown that plasma cfDNA concentrations alone are not a reliable marker of IUGR in the last trimester. However, if cfDNA levels and DNase I activity are measured in combination, they can offer valuable information on the development of IUGR.

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