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Biochemical and ultrasonographic markers in fetal surveillance

Биохемијски и ултрасонографски маркери у надзору фетуса

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SUMMARY

Introduction/Objective Fetal growth restriction (FGR) is associated with increased fetal and neonatal mortality and morbidity.

The study objective was to investigate the correlation of maternal blood biochemical markers routinely determined in the first and second trimester screening and ultrasound fetal surveillance parameters in the prediction of fetal growth and condition in singleton pregnancies.

Methods In the first trimester we measured serum levels of beta subunit of human chorionic gonadotropin (βHCG) and pregnancy-associated plasma protein A (PAPP-A). In the second trimester we measured values of chorionic gonadotropin (HCG), alpha feto-protein (AFP), unconjugated estriol (E3) and inhibin A, also examined ultrasonographic biometric fetal parameters, amniotic fluid index (AFI) and Doppler resistance indexes. FGR was defined as ultrasonographically determined fetal weight and growth parameters below the 10th percentile for the gestational age. Obtained biochemical and ultrasonographic parameters were correlated.

Results Study included 104 singleton pregnancies. βHCG in the first trimester negatively correlated with fetal growth in the second and third trimester, and the second trimester AFI. Increased PAPP-A correlated positively with elevated resistance index in medial cerebral artery, lower biophysical profile scores, and intermediate type of non-stress test. Lower values of E3 were associated with FGR. Elevated serum AFP levels were linked to oligoamnion in the third trimester. There was no correlation of inhibin A levels with fetal condition.

Conclusion First and second trimester biochemical markers of pregnancy (βHCG, PAPP-A, HCG, AFP and E3) in combination with ultrasonographic biophysical parameters of fetus have predictive value for fetal growth and development.

Keywords: pregnancy; biochemical markers; ultrasound; fetal growth restriction

САЖЕТАК

Увод/циљ Застој у расту плода (*FGR*) је повезан са повећаним феталним и неонаталним морталитетом и морбидитетом. Циљ студије је био да се испита корелација биохемијских маркера из крви мајке који се рутински користе у скринингу првог и другог триместра трудноће и ултрасонографских параметара феталног надзора у предикцији феталног раста и стања у једноплодним трудноћама.

Методе У првом триместру, мерили смо серумске нивое бета субјединице хуманог хорионског гонадотропина (*βHCG*) и протеина плазме повезаног са трудноћом (*PAPP-A*). У другом триместру мерили смо *HCG*, алфа фето-протеин (*AFP*), некоњуговани естриол (*E3*) и инхибин А и проценили ултрасонографске биометријске феталне параметре, индекс амнионске течности (*AFI*) и Доплер индексе резистенције. *FGR* је дефинисан као рестрикција раста фетуса испод 10-ог перцентила за дату гестацијску доб. Добијени биохемијски и ултрасонографски параметри су затим корелисани.

Резултати Студија је обухватила 104 труднице са једноплодном трудноћом. Вредности *βHCG* у првом триместру су имале негативну корелацију са растом фетуса током другог и трећег триместра, као и са *AFI* у другом триместру. Повећана вредност *PAPP-A* позитивно је корелирала са повишеним индексом резистенције у медијалној церебралној артерији, нижим резултатима биофизичког профила и нон-стрес тестом интермедијарног типа. Ниже вредности E3 биле су повезане са рестрикцијом раста фетуса. Повишени нивои *AFP* у серуму били су повезани са олигоамнионом у трећем триместру трудноће. Није постојала корелација инхибина А са феталним стањем.

Закључак Биохемијски маркери првог и другог триместра трудноће (*βHCG, PAPP-A, HCG, AFP* и *E3*) у комбинацији са ултрасонографским биофизичким параметрима фетуса имају предиктивну вредност за процену раста и развоја фетуса.

Кључне речи: трудноћа; биохемијски маркери; ултразвук; интраутерусни застој раста

INTRODUCTION

Fetal growth restriction (FGR) is a progressive deviation from the growth curve below the 10th percentile for the particular gestational week. The incidence of this disorder is 4 do

8%, in general population of pregnant women. It is considered pathological when followed by oligohydramnion - reducing the amount of amniotic fluid (the amniotic fluid index - AFI below the value of 50 mm) and pathology of fetal Doppler findings [1]. FGR is associated with increased fetal and neonatal mortality and morbidity generally, while the greatest risk of poor perinatal outcome in fetuses with growth restriction is in the cases of superimposed hypertensive disorders. Fetal hypoxemia in these pregnancies is very often associated with subsequent polycythemia, hypercapnia and neonatal acidosis, lower levels of fetal glycemia, decreased glycogen reserves, decreased concentration of essential amino acids, increased fetal triglyceride concentration due to mobilization from fat reserves, as well as hypoinsulinemia [2].

One of the very common causes of growth restriction is the placental factor because placental structure and function affect the transport and exchange of gases and nutrients, as well as the products of metabolism at the level of uteroplacental circulation [3]. Ultrasound fetal measurements and other markers present the most common diagnostic method for prediction and diagnosing of FGR. Another proposed way of predicting fetal growth is placental assessment in terms of its volume and structure. In case of an incomplete trophoblastic invasion of the spiral arteries, the change from high to low resistance flow in maternal compartments does not happen which can cause preeclampsia and FGR. Therefore, the Doppler ultrasound examination is an additional useful non-invasive method for the assessment of the interaction between fetal and maternal hemodynamic compartment [4,5].

Morphological changes in trophoblasts and placenta also affect changes in levels of synthesis and secretion of different biochemical placental markers, that are also part of screening in pregnancy [6,7]. Screening of the first and second trimester of pregnancy is successfully applied in everyday clinical practice for early prediction and detection of fetal chromosomopathies. Furthermore, recent investigations proposed biochemical maternal

screening as useful in prediction the risk of adverse fetal and maternal outcome. Some data imply that different biochemical markers could be useful for early detection of different fetal complications including FGR and preterm birth [8].

The aim of our study was to investigate the correlation of maternal blood biochemical markers routinely determined in the first and second trimester screening and ultrasound fetal surveillance parameters in the prediction of fetal growth and condition in singleton pregnancies.

METHODS

Healthy pregnant women who conceived naturally and had regular pregnancy check-ups in Clinic of Obstetrics and Gynecology University Clinical Center of Serbia, were prospectively recruited in the study, during a three-months period (2018 year). All investigated women signed informed consent for the study, according to the Declaration of Helsinki. The study confirms to the legal standards and was approved by the Clinic Ethic Committee.

During the first pregnancy examination for every pregnant woman, we took detailed general medical, socio-epidemiological and obstetric history (age, cigarette smoking, method of conception, hereditary and chronic illnesses, parity, gestational complications and outcomes of previous pregnancies such as hypertension, diabetes and pregnancy loss, gestational weeks of deliveries, Apgar score of previously born children). All pregnancies were dated by last menstrual period (LMP) and fetal crown-rump length (CRL) measured by the ultrasound. We also measured nuchal translucency (NT) according to Fetal medicine Foundation and Double test screening method.

Investigated women were regularly checked-up at least once per trimester throughout the pregnancy in our Clinic. They underwent regular screening for chromosomal abnormalities of first and second trimester. All adverse pregnancy outcomes (miscarriage before 20th

gestational week) were noted and those women were excluded from the study. Moreover, exclusion criteria for this study were also: confirmed fetal genetic disorders and malformations, as well as severe chronic diseases of the mother that could influence pregnancy course and outcome: chronic hypertension, systemic lupus eritematosus, chronic kidney diseases, type 1 diabetes mellitus, disorders of thyroid gland.

On every examination we measured pregnant woman height and weight, and calculated their Body Mass Index (BMI), made clinical examination, took a detailed laboratory analysis. Moreover, in the first trimester at the time for mandatory Double test screening (11 to 14 gestational weeks) we determined levels of beta subunit of human chorionic gonadotropin (βHCG) and pregnancy-associated plasma protein A (PAPP-A). In the second trimester at the time of Triple test screening (16 to 19 gestational weeks), values of chorionic gonadotropin, (HCG), alfa feto protein (AFP) and unconjugated estriol (E3) were measured. In the case of indication for more detailed screening test we also performed Quadriple (Q) test, and measured values of inhibin A.

For the purpose of biochemical analyses, we used 10 milliliters of maternal blood, and it was drawn by venipuncture into nonheparinized tubes, for centrifuge process lasting 15 minutes. For results interpretation we used a reference software program SsdwLab 5 and a BRAHMS KRYPTOR analyzer, applying fluorocytometric immunoassay method. The measured serum concentrations (IU/L) of biochemical markers were converted into multiples of median (MoM) and adjusted for appropriate gestational week. We registered different categories of values from extremely low values below 0.5 MoMs and extremely high values above 2 MoMs. A value of 1 MoM represents the middle of the distribution. It is suggested that PAPP-A, βHCG and E3 should not be below 0.5 MoM while AFP should not be over 2 MoM to avoid adverse perinatal outcomes [9].

Fetal condition monitoring included antenatal ultrasound examinations in the period of combined screening of the first and biochemical screening in the second trimester, then control examinations every 4 to 6 weeks (at least once in each trimester). All gestational complications (gestational diabetes, hypertension, bleeding, contractions, premature membrane rupture, etc.) were regularly noted. In case of gestational complications, surveillance parameters were assessed according to the protocols for monitoring of high-risk pregnancies [10, 11].

In the first trimester ultrasound biometrics implied the crown rump length (CRL) for the precise pregnancy dating, also measured nuchal translucency (NT), while in later pregnancy we performed complete fetal biometry to see if there are the sings of growth restriction. We monitored the fetal biophysical profile (BFP) from the 28th week of gestation. Each ultrasound parameter was evaluated with grade from 0 to 2 - respiratory movements of the fetus, fetal movements with registration of flexion and extension, fetal tone, amount of amniotic fluid (by measuring amniotic fluid index-AFI) and the largest pocket of amniotic fluid (below 2 and over 2).

The pathological finding of fetal BFP was set according to current standards 6 and below, while values of 8 were considered as good fetal condition [12]. Interpretation of the cardiotographic monitoring (non-stress test - NST) was performed according to the International Federation of Gynecology and Obstetrics criteria and divided into normal, intermediate and pathological record [13]. Normal NST means that the baseline is form 110-150 beats per minute, reactivity with adequate accelerations (at least two) for 30 minutes of monitoring, and changes in basal frequency with 5 up to 25 per minute. Intermediate NST record means basal frequency from 100 to 110, or from 150 to 170 beats per minute, with saltatory (over 25 beats) or silent (5 and under 5 beats per minute) type of oscillations. Pathological record means- basal frequency is around 150-170/min with reduced variability where the silent type of oscillations is registered or the sinusoidal type of variability.

Further ultrasound examination included measuring the resistance index in the umbilical artery (RiAu) and in middle cerebral artery (RiCm). The normal finding of the resistance index in the umbilical artery is 0.55-0.65, and in the middle cerebral artery 0.75-0.85 [14,15]. Pathological findings in Doppler sonography were increased resistance in the umbilical artery (more then 0,65 measured in the resistance index of the umbilical artery) and reduced resistance in the medial cerebral artery (below the 0,75, called the "brain sparing phenomenon") [14,15]. After the 28th gestational week the amniotic fluid index (AFI) was determined to assess the sufficiency of amniotic fluid quantity. We measured the amount of amniotic fluid in the second and third trimesters by the classic way, by measuring all four quadrants of amniotic fluid. We chacked the values also by measuring the deepest vertical fluid pocket. Based on the sum of the values, we obtained AFI for that gestational age. We compared the obtained values with the percentiles of the amount of amniotic fluid through nomogram tables.

If AFI was below 5 cm (below the fifth percentile) oligohydramnion was diagnosed, while AFI greater than 25 indicated polyhydramnion (above the nighty percentile) [16].

For the purpose of this study as the main parameter in prenatal assessment of fetal condition we considered fetal growth. Fetal growth is obtained by computer generation of measured values of ultrasound biometry - biparietal diameter, head circumference, abdominal circumference or abdominal circumference and femur length. The individual parameters measured by ultrasound measurements together provide information on whether the size of the fetus corresponds to the given gestational age or whether there is restriction or acceleration of fetal growth.We also used percentiles of fetal growth in the nomogram tables.

Fetal growth restriction (FGR) was defined as ultrasonographically determined fetal weight and growth parameters below the 10th percentile of those expected for the gestational age. Finally, upon birth, study authors noted the birth-weight and Apgar score of the child, as

well as the gestational week (GW) of delivery (prematurity was considered if delivery occurred before the 37th gestational weeks).

Data were analyzed using methods of descriptive (number, percent, mean, standard deviation) and analytical statistics and applying the SPSS 20 software. The strength of correlation of maternal blood biochemical markersand ultrasound fetal surveillance parameters in the prediction of fetal condition, was assessed using Spearman's rho correlation coefficient. In this study, Pearson's Chi square test (χ^2) was applied in order to assess the significance of the difference in the ultrasound indicators of the fetal condition. Statistically significant differences were considered below 0.05 ($p < 0.05$).

Written consent was obtained from all patients for all procedures as well as the study.

RESULTS

Study included 104 pregnant women with average age of 30.54+/-4.93 years. Majority of examined fetuses had an appropriate level of growth and development (from the 10th to the 90th percentile) assessed by ultrasound during the second and third trimesters of pregnancy. In the second trimester of pregnancy, amount of amniotic fluid below the 10th percentile had 18,2 % of fetuses, and in the third trimester 30,7 % of fetuses (Table 1).

In the third trimester, most fetuses had a biophysical profile value 8, 15 fetuses had BFP 6, 2 fetuses rated BFP 4 (Table 1). Besides the more frequent (21.2%) pathological Resistance index in umbilical artery (RiAu), other evaluated Doppler parameters were normal in most fetuses in the third trimester of pregnancy (Table 1).

Table 2 shows the results of correlations of fetal biometry, fetoplacental circulation and oxygenation with the maternal blood biochemical markers of first trimester screening (βHCG and PAPP-A) during the Double test. Values of βHCG in the I trimester negatively correlated with fetal growth during the II and III trimesters as well as the amount of amniotic fluid in the

II trimester. In fetuses whose mothers had elevated βHCG levels in the first trimester, intrauterine fetal growth restriction was more frequently registered in the second and third trimesters. A significant correlation was observed between PAPP-A values, RiCm values and NST in our study. We registered different categories of values from extremely low values below 0.5 MoMs and extremely high values above 2 MoMs. When PAPP-A was above the reference range in the first trimester, over the 2 MoMs, the RiCm was more frequently elevated, fetuses had lower biophysical profile scores, which was often followed by some kind of pathological findings on the non-stress test.

Results in Table 3 show correlations of fetal biometry, fetoplacental circulation and oxygenation with the biochemical markers of second trimester biochemical screening. Elevated E3 values in the second trimester correlated positively with fetal growth in the second trimester of pregnancy, i.e., lower values of unconjugated estriol correlated with intrauterine fetal growth restriction. Elevated serum AFP levels in the second trimester correlated with the lower values of AFI in the third trimester of pregnancy. Regarding fetal oxygenation and circulation parameters, no correlations were observed with Triple and Q test screening parameters.

DISCUSSION

In this study we found the negative correlation of βHCG values of the I trimester with fetal growth of the II and III trimesters as well as the amount of amniotic fluid in the II trimester. In fetuses whose mothers had elevated βHCG levels in the first trimester, intrauterine fetal growth restriction was more frequently registered in the second and third trimesters as well as AFI below the 50th percentile in the second trimester of pregnancy. In some literature data βHCG values over the 90th percentile was linked to fetal growth disturbance [17]. In other large studies βHCG values of I trimester below the 5th percentile was correlated with growth restriction below the 10th percentile [18]. According to some data βHCG values above 4.0

MoM were associated with low birth weight and hypertensive disorders in 22.5% of pregnant women. βHCG values, over 10 MoM were found in 92% of cases with adverse perinatal outcomes in terms of severe fetal growth restriction and neonatal complications, placental abruption as well as severe hypertensive disorders [19]. Authors reported an association of elevated second-trimester HCG values with growth restriction, which we did not establish [20].

In our study we did not confirm the connection between values of PAPP-A and fetal growth and AFI. Contrary, some authors found a significant degree of association between low PAPP-A values and the risk of intrauterine fetal growth restriction in as many as 73% of pregnancies that ended before 37 weeks of gestation and in 46% of term pregnancy terminations [21]. According to literature data, the value of PAPP-A below the 5th percentile for gestational age is significantly correlated with premature birth and intrauterine fetal death. In studies assessing a combination and interaction of several factors, including βHCG values as well as parity, age, smoking and increased BMI it was shown that measuring values before and after the 13th week of gestation during pregnancy screening gives similar results in prediction of pregnancy outcome [22]. On the other hand, in our study when PAPP-A was above the referral range in the first trimester, the RiCm was more frequently elevated, end-diastolic block occurred, children had lower biophysical profile scores, and the non-stress test was more often of the intermediate type. Recent research has mainly found that the first trimester biophysical markers such as uterine artery Doppler could be used in combination with biochemical markers for the prediction of perinatal outcome. On the other hand, other studies found that faulty parameters of flow through umbilical and middle cerebral artery between 35 and 37 weeks may imply on fetal growth restriction, preeclampsia and fetal hypoxia, all as a consequence inadequate placentation [23]. When biochemical markers with changes in hemodynamics were analyzed, extremely decreased PAPP-A values below the 3rd percentile were registered in pregnant women with increased systolic-diastolic ratio in the umbilical

artery, end-diastolic block and diastolic flow reversal [24]. In previous investigations low values of PAPP-A around 0.45 MoM are reported in correlation with fetal growth restriction, and usually in such cases elevated AFP is registered in the second trimester, which is usually explained by the presence of a placenta of smaller dimensions and its morphological damage [25]. Adequate secretion of all placental markers is affected by the invasion and structure of trophoblast while compromising trophoblast circulation causes the change in the concentration of these markers. For these reasons, in the case of placental hypo perfusion, PAPPA levels are reduced, resulting in intrauterine fetal growth restriction [25].

In our study elevated E3 values in the second trimester correlated positively with fetal growth in the second trimester of pregnancy, i.e., lower values of unconjugated estriol were linked to growth restriction. According to data from the literature, E3 values are reduced in both intrauterine fetal growth restriction and in pregnancies with reduced amniotic fluid [26].

Furthermore, our results show that elevated serum AFP levels in the second trimester are associated with oligoamnion in the third trimester of pregnancy. The data of our study regarding the second trimester markers partially agree with the results of research by other authors, which is that AFP values over 2 MoM were correlated with oligoamnion, for which we found an association in the third trimester of pregnancy, as well as the correlation of lower values of unconjugated estriol with fetal growth. In a large study of over 60,000 singleton pregnancies, where the Triple test was routinely performed, elevated AFP values above 2.5 MoM were found to be closely associated with gestational hypertension, miscarriage, preterm birth, intrauterine growth restriction, oligoamnion and placental abruption [27]. Low AFP values below 0.25 MoM have been associated in the literature with more frequent intrauterine fetal death [28].

Regarding fetal oxygenation and circulation parameters, no correlations were observed with Triple and Q test screening parameters in this study. Studies by other authors generally

report lower first-trimester marker values in fetuses with fetal growth restriction. In our study, in the overall sample, elevated marker values correlated with growth restriction, which we can conclude that fetuses with fetal growth restriction also had normal marker values, i.e. and fetuses with elevated markers may have an orderly fetal growth trend by weeks of gestation. Data from a large meta-analysis that included 91 studies showed that placental function markers are isolated and insufficient to anticipate fetal and neonatal birth conditions, and that a combined antepartum approach assessing separately or jointly a number of biochemical markers should be included to get a better prediction of pregnancy outcome [29, 30].

CONCLUSION

Maternal blood biochemical markers routinely determined in the first and second trimester screening (βHCG, PAPP-A, HCG, AFP, E3) in the assessment of the risk of chromosomal abnormalities, correlate well with ultrasound parameters of fetal monitoring that are regularly used in clinical practice (biophysical parameters of fetal surveillance (biophysical profile, Doppler measures of fetoplacental circulation and oxygenation) and, therefore, may be significant indicators of impending fetomaternal complications and good predictors of adverse singleton pregnancy.

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Table 1. Ultrasound parameters of fetal monitoring

Parameters		Number of fetuses	Percent (%)	Pearson's χ^2 test	p values
Fetal growth II trimester	< 5th percentile	1	1	161.139	0.001
	5th to 10th percentile	7	6.7		
	10th to 50th percentile	80	76.9		
	50th to 90th percentile	13	12.5		
	> 90th percentile	0	0		
Fetal growth III trimester	< 5th percentile	1	1	159.400	0.001
	5th to 10th percentile	16	15.4		
	10th to 50th percentile	69	66.3		
	50th to 90th percentile	13	12.5		
	> 90th percentile	1	1		
Amniotic fluid index (AFI) II trimester	< 5th percentile	2	1.9	140.782	0.001
	5th to 10th percentile	17	16.3		
	10th to 50th percentile	76	73.1		
	50th to 90th percentile	6	5.8		
Amniotic fluid index (AFI) III trimester	< 5th percentile	7	6.7	133.000	0.001
	5th to 10th percentile	25	24		
	10th to 50th percentile	63	60.6		
	50th to 90th percentile	4	3.8		
	> 90th percentile	1	1		
Umbilical artery resistance index (RiAu)	pathological	22	21.2	31.360	0.001
	normal	78	75		
Middle cerebral artery resistance index (RiCm)	pathological	5	4.8	81.000	0.001
	normal	95	91.3		
Biophysical profile (BFP)	4	2	1.9	178.160	0.001
	6	15	14.4		
	7	1	1		
	8	82	78.8		
Non-stress test (NST)	normal	81	77.9	108.510	0.001
	intermediate	13	12.5		
	pathological	4	3.8		

Fetal growth II trimester-normal growth for gestational age 50th percentile, extreme values – fetal growth restriction – below the 10th percentile, acceleration growth above the 90th percentile; amniotic fluid index – pathological below the 5cm – oligohydramnion, above the 25 cm polihydramnion; biophysical profile – normal 8, pathological below 8; RiAu index – normal range from 0,55 to 0,65 (approximetly 50th percentile the in the third trimester) above the 0,65 – pathological; RiCm-normal range from 0,75 to 0,85 in the third trimester, above the 0,85 or under the 0,75 – pathological; non-stress test – classification of non-stress test according to the International Federation of Gynecology and Obstetrics recommendations

Table 2. Correlation of fetal biometry, fetoplacental circulation and oxygenation with the maternal biochemical markers of first trimester screening

Parameters		DT HCG (MoM)	DT HCG category of MoM values	DT PAPPA MoM	DT PAPPA category of MoM values	DT NT (first trimester) MoM
Fetal growth II trimester	ρ	-0.228	-0.214	0.044	0.099	0.120
	p value	0.025	0.035	0.668	0.335	0.239
Fetal growth III trimester	ρ	-0.212	-0.170	0.139	0.130	0.086
	p value	0.037	0.096	0.177	0.205	0.402
Amniotic fluid index II trimester	ρ	-0.280	-0.249	-0.016	-0.002	0.130
	p value	0.006	0.014	0.880	0.985	0.202
Amniotic fluid index III trimester	ρ	-0.082	-0.092	0.144	0.057	0.005
	p value	0.425	0.371	0.162	0.582	0.964
Umbilical artery resistance index (RiAu)	ρ	-0.156	-0.155	0.147	0.102	-0.154
	p value	0.126	0.130	0.152	0.325	0.130
Middle cerebral artery resistance index (RiCm)	ρ	-0.146	-0.158	0.332	0.272	0.007
	p value	0.154	0.122	0.001	0.007	0.946
Biophysical profile (BFP)	ρ	-0.051	-0.027	-0.243	-0.127	-0.033
	p value	0.622	0.792	0.017	0.219	0.747
Non-stress test	ρ	-0.004	-0.107	-0.310	-0.224	0.178
	p value	0.970	0.303	0.002	0.030	0.082

DT – Double test; HCG – human chorionic gonadotropin; PAPPA – plasma protein A , NT – fetal nuchal translucency in the first trimester, MoM – multiple of median, ρ – Spearman's rho correlation coefficient

Table 3. Correlations of fetal biometry, fetoplacental circulation and oxygenation with the maternal biochemical markers of second trimester biochemical screening

Parameters		TT HCG MoM	TT AFP MoM	TT E3 MoM	QT HCG MoM	QT AFP MoM	QT E3 MoM	QT Inhibin A MoM
Fetal growth II trimester	ρ	-0.333	-0.141	-0.164	-0.258	0.258	-0.775	-0.775
	p value	0.152	0.565	0.516	0.742	0.742	0.225	0.225
Fetal growth III trimester	ρ	-0.184	-0.005	0.526	-0.632	-0.316	-0.316	-0.632
	p value	0.451	0.984	0.025	0.368	0.684	0.684	0.368
Amniotic fluid index II trimester	ρ	0.032	-0.278	-0.116	0.447	0.894	-0.894	-0.447
	p value	0.894	0.250	0.647	0.553	0.106	0.106	0.553
Amniotic fluid index III trimester	ρ	0.083	-0.522	0.232	-0.775	-0.775	0.258	-0.258
	p value	0.735	0.026	0.354	0.225	0.225	0.742	0.742
Umbilical artery resistance index	ρ	-0.290	-0.012	0.012	.	.	.	.
	p value	0.229	0.962	0.962	.	.	.	.
Middle cerebral artery resistance index	ρ	-0.105	0.136	0.205	-0.894	-0.447	0.007	-0.894
	p value	0.667	0.590	0.416	0.106	0.553	0.946	0.106
Biophysical profile	ρ	0.017	-0.417	0.011	-0.632	-0.316	-0.316	-0.632
	p value	0.944	0.085	0.965	0.368	0.684	0.684	0.368
Non-stress test	ρ	-0.051	0.173	0.124	0.894	0.447	0.007	0.894
	p value	0.835	0.492	0.624	0.106	0.553	0.946	0.106

TT – triple test; HCG – human chorionic gonadotropin; AFP – alpha fetoprotein; E3 – estriol; Q – quadriple test, MoM – multiple of medians, ρ – Spearman's rho correlation coefficient

