VIA MEDICA

ORIGINAL PAPER / OBSTETRICS

Ginekologia Polska
2018, vol. 89, no. 3, 142–146

ISSN 0017–0011

DOI: 10.5603/GP.a2018.0024

Evaluation of soluble concentration Fas and Fas ligand in maternal and cord blood 3rd trimester of pregnancy

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ABSTRACT

Objectives: The the study was to estimate the concentrations of antiapoptotic sFas and pro-apoptotic FasL in the serum of pregnant women in the third trimester of pregnancy and in the cord blood serum of neonates from vein and arteries separately. The correlation could be crucial for evaluation of apoptosis process intensity in placenta and the role of fetal blood circulation system on distribution of sFas and FasL.

Material and methods: The study group consisted of 28 pregnant women in physiological pregnancy, between 38––41 weeks. Vein blood was taken from maternal elbow vein and umbilical cord, separately from vein and arteries. The research was done by sets for sFas and FasL from R&D Systems Elisa kit.

Results: In arterial and vein cord blood there were much more lower concentrations of sFas than in maternal blood-arterial cord blood 3351.78 pg/mL, vein cord blood 3351.78 pg/mL versus maternal blood 5769.62 pg/mL ($p < 0.001$). No difference was found in sFas concentrations between cord arterial and vein blood sera. Statistical difference was found between mean concentration of Fas ligand in maternal blood serum (71.36 pg/mL) and arterial cord blood serum (164.57 pg/mL) $p < 0.05$ ($p = 0.001$). Cord arterial blood serum showed much higher concentrations of FasL than maternal blood serum. No difference was found between cord arterial and vein blood sera concentrations of FasL: 164.57 pg/mL vs. 170.00 pg/mL ($p = 0.701$).

Conclusions: Obtained results suggest no influence of sFas and FasL production on fetal organism apoptosis. Lowering of sFas concentration in fetal blood could mean the increase of apoptosis in fetal organism compared to maternal. Higher concentration of FasL in cord blood than in mothers suggests higher apoptosis intensification in fetal circulation and no influence of blood flow across placenta on its concentration.

Key words: sFas, FasL, cord blood, pregnancy

Ginekologia Polska 2018; 89, 3: 142–146

INTRODUCTION

Soluble Fas and Fas ligand are molecules that take part in apoptotic processes. Apoptosis is complicated and multifactor process which by cytotoxic path causes cell death to eliminate destroyed, old or mutated cells. The process in physiological conditions constitutes anti-cancer defense that is lowered in individuals suffering from cancerous diseases [1, 2, 4, 5]. Apoptosis is necessary in remodeling of maternal deciduas, to maintain proper maternal–fetal immune tolerance and trophoblast invasion into spiral arteries. In the course of pregnancy modulation of immune response occurs to prevent pregnancy from rejection of allogenic graft. Immunomodulation could be connected to changes in apoptosis intensification and could cause lowering this processes [1–4]. In the course of pregnancy increase of vascular bed capacity and growing need of oxygen transporting molecules could cause slowing down apoptosis processes in bone marrow. This fact allows longer life of red blood cells, protecting pregnant women from anemia [3, 5].

Fas/APO-1/ CD 95 is membrane receptor from tumor necrosis factor (TNF) family because of death domain presence. To initiate apoptosis process it is necessary to incorporate to Fas molecule of Fas ligand-FasL/CD95L/. System Fas/FasL is crucial in surviving of cells, especially neoplastic cells, in promotion and protection of cancerous stem cells and in the immune elimination of virus-infected and cancerous

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cells. Higher levels of Fas/FasL were observed in individuals suffering from immune system and cancerous diseases [6, 7]. It has been shown that the stress-induced expressions of Fas and FasL are dependent on the activation of various signaling molecules such as p53, nuclear factor-kappa B, specificity protein-1, and early growth response factors. Cytotoxic effect as defragmentation of DNA chain is depended on the cell cycle phase and is strongest presented in indifferent cells and could be observed in cord blood due to presence of stem cells [6, 7].

Activation of Fas/FasL system not only induces apoptosis but also protects trophoblast from maternal immune system. Fas/FasL system protects migration of activated leucocytes between mother and fetus, conversely soluble Fas-sFas could act by other mechanism by slowing down apoptosis, protecting different cells and activated lymphocytes T from apoptosis [6]. Presence of FasL is high in immunopriviledged tissues and cells as leucocytes and placenta. One of the most important factors that influences the increase of proapoptotic processes is membrane Fas receptor [7].

Soluble Fas [sFas] is formed in the process of alternative folding of Fas mRNA. As the effect new protein molecule is formed that contains deletion or incompetent trans-membrane domain. In addition sFasL is proteolytic product of FasL disintegration and its concentration may be in proportion to intensity of apoptotic processes [8]. We could hypothesize that high concentration of sFas correlates with decrease of apoptotic process. Probably to increase this process higher concentrations of FasL are needed because of less affinity to this ligand.

This suggestion was investigated by Nagata et all., but the relationship between the membrane and soluble forms of Fas and FasL (sFa, sFasL) is still unknown. Authors suggested that soluble Fas has a suppressive effect on Fas/FasL-mediated apoptosis [9, 14].

Soluble Fas inhibits Fas ligand before getting to receptor and slowed down Fas dependent apoptosis. SFas in late pregnancy could probably be synthesized in fetal cells and transferred by fetal cord blood into placental bed. Low concentration of soluble Fas in first trimester of pregnancy as immunotolerance phenomenon could be changed in late pregnancy [9, 10]. Knowledge that placental apoptosis increases due to pregnancy development suggests that the best time to evaluate sFas and FasL concentrations we can expect in the late pregnancy [11]. There is only few existing data describing value of sFas and FasL in maternal blood and cord blood in third trimester of pregnancy [8–11]. Different than physiological concentrations of those molecules could indicate the risk of placental dysfunction or immunotolerance failure [10, 11].

It is still unclear if in late pregnancy we can expect increased risk of allograft rejection or placental dysfunction caused, for example, by high concentration of FasL or lowering concentration of sFas. Different concentrations of sFas in fetal circulation from cord artery to vein could give an answer of its potential role and distribution in feto-placental unit and its origin

Aim fo the study

The aim of the study was to estimate concentration of two molecules antiapoptotic sFas and pro-apoptotic FasL in serum of pregnant women in third trimester of pregnancy and in cord blood serum of neonates from vein and arteries separately. The correlation could be crucial for evaluation of apoptosis processes intensity in placenta and the role of fetal blood circulation system on distribution of sFas and FasL.

MATERIAL AND METHODS

The study was conducted in High Risk Pregnancy Unit Medical University Lodz in 2014–2015. The study was approved by University Bioethics Board — approval number RNN/149/09/KE. Patients written consent was obtained from every patient.

The study group consisted of 28 pregnant women in third trimester of uncomplicated pregnancy and proper fetal development. Maternal age was between 24–38 years and the proportion of primiparous women to multiparous as about 2:1. All pregnancies are at term. Gestational age was estimated using antenatal ultrasound scan at 12–14 weeks. All patients included to the study delivered by elective caesarian section because of non perinatal indications.

Vein blood was taken from 2 to 4 hours before caesarian section from elbow vein. Umbilical cord blood was taken separately from vein and from arteries after neonate was separated, from placental part of umbilical cord.

The volume of blood samples was no less than 5 mL each. The samples were placed in tubes without anticoagulation factor and stored in room temperature for about 60 minutes — to complete coagulation. After formation of clots samples were centrifugated for 15 minutes RCF 389. After centrifugation supernatant of blood samples was replaced into Eppendorf tubes then frozen and stored at –70°C until the measurement procedure.

The research was done by R&D Systems Elisa kit according recommended by producer schemes. SFas was measured by Fas Elisa kit (catalog number — DFS00). The minimum detectable dose (MDD) of sFas ranged from 31.2 to 2,000 pg/mL. The sensitivity was 20 pg/mL. The serum concentration of Fas was expressed in pg/mL.

FasL was measured by FasL Elisa kit (catalog number — DFL00). The minimum detectable dose (MDD) ranged from 15.6 to 1,000 pg/mL. The sensitivity was 8.05 pg/mL. The serum concentration of FasL was expressed in pg/mL.

Statistical analysis

Data base was prepared on calculation spreadsheet program Microsoft Excel 2014.Statistical calculations were done using statistics software SPSS 14.0 and MS Excel 2014. In the study group the results of measured parameters were estimated by arithmetic means, standard deviation, maximal and minimal values. Data comparisons were analyzed by one-way ANOVA . *P* value < 0.05 was considered to indicate statistical significance.

Study group

The study group consisted of 28 healthy pregnant women in 3rd trimester of pregnancy. Mean age of patients was estimated at 32.17 (sd 4.23) years, minimum age was 24 years and maximum 38 years. The shortest gestational age was 38 weeks, the longest was 41 weeks. Mean gestational age was 39.1 weeks (sd 0.71).

RESULTS

In study group concentration of sFas was measured in maternal blood serum and in umbilical cord right after delivery by cesarean section, separately for arterial and vein blood serum. The results are presented in pg/mL on the graph below (Fig. 1).

Mean value of sFas in maternal blood was 5769.62 pg/mL (sd = 457.72). Minimal value 5183.51, maximal 6857.92 pg/mL. In cord blood mean value of sFas obtained from vein was 3251.38 pg/mL (sd = 672.31), minimal concentration 2244.03 maximal 4349.45 pg/mL. The result of mean sFas concentration obtained from arterial cord vessels was 3351.78 pg/mL (sd = 1172.20), minimal value was 1812.98 and maximal 5647.66 pg/mL.

Producer suggestion is that in adult human serum sFas concentration started from 4792 to 17150 pg/mL. According to presented extreme values sFas concentration in maternal blood serum samples comprise in those values. In arterial cord blood all samples presented lower values, in opposite in vein cord blood were in 15.4% of results presented normal range and the rest of the results –84.6% were lower than the lowest normal range. That meant lower fetal concentration of this molecule than normal in adults organism.

In arterial and vein cord blood there were much more lower concentration of sFas than in maternal blood-arterial cord blood 3351.78 pg/mL and vein cord blood 3351.78 pg/mL versus maternal blood 5769.62 pg/mL ($p < 0.001$). Statistical analysis was done by Kruskal-Wallis test and U Mann-Whitney test and by ANOVA variance.

Significant difference of sFas value between maternal and cord blood was detected. In maternal blood we observed higher concentration of sFas than found in vein cord blood , p was lower than 0.05 ($p = 0.013$). Variances were not similar.

Arterial cord blood serum concentration of sFas was also significantly lower than in maternal serum. Statistical difference was found in mean sFas concentration comparing maternal blood serum to vein cord blood serum ($p < 0.001$). No difference in sFas concentration was found between neonate cord arterial serum and vein blood serum ($p = 0.98$).

Graph below presents results of Fas ligand concentrations in maternal and cord blood sera (Fig. 2).

Mean value of FasL in maternal blood serum was 71.36 pg/mL (sd = 15.14). Minimal value 52.69, maximal 106.99 pg/mL. In cord blood mean value of FasL obtained from cord vein serum was 170.00 pg/mL (sd = 57.45), minimal concentration 37.93 maximal 261,226 pg/mL. The result of mean FasL concentration in serum obtained from arterial cord vessels was 164.57 pg/mL (sd = 52.82), minimal value was 35.80 and maximal 226.90 pg/mL.

Producer suggests that in adult human serum FasL concentration started from 39.8 to 145 pg/mL. According to presented extreme values FasL concentration in maternal blood samples was in many cases different than expected values. Cord blood serum concentration taken from arterial vessels in 7.7% of cases was lower than producers' suggestion

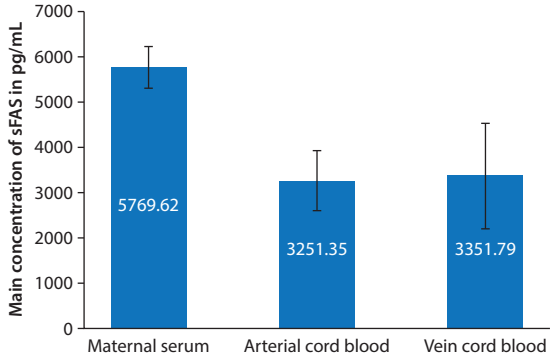

Figure 1. Concentration of soluble Fas in maternal and cord blood sera expressed in pg/mL

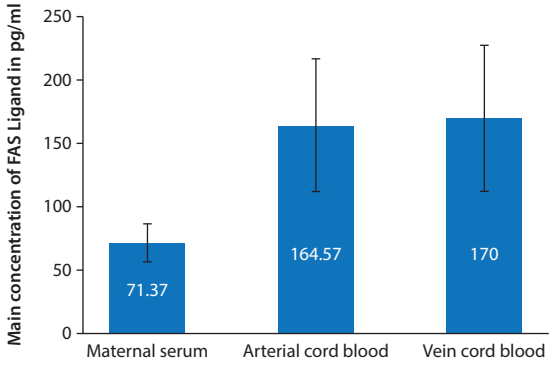

Figure 2. Concentration of Fas ligand in maternal and cord blood expressed in pg/mL

but 61.6% of the results were higher, the rest — 30.7% was between concentrations limits suggested by producer.

Cord vein blood serum in 23.1% of samples presented normal range of concentration, 69.2% results were higher than norms and 7.7% were much lower concentration of FasL.

In cord blood serum taken from arteries and vein more results of high concentration were observed than in maternal blood.

Using analysis of variance ANOVA significance of FasL concentration between maternal and cord blood was estimated. Significance was lower than 0.05 ($p = 0.005$). Variances were not similar. Measuring by non parametric U Mann-Whitney test FasL in maternal blood and comparing to vein cord blood statistical difference was found between mean concentrations $p < 0.05$ ($p = 0.001$). U Mann Whitney test shows statistical difference between mean concentration of Fas ligand in maternal blood serum (71.36 pg/mL) compared to arterial cord blood serum (164.57 pg/mL). Cord arterial blood serum showed much higher concentrations of FasL than in maternal blood serum. No difference was found between cord arterial and vein blood sera concentration of FasL 164.57 pg/mL vs. [170.00 pg/mL] ($p = 0.701$).

Vein cord blood serum presents higher FasL concentrations than observed in those obtained from maternal blood serum.

DISCUSSION

Analysis of the soluble Fas [sFAS] results obtained from maternal blood serum shows that all results were comparable with standards showed by producer. We didn't find higher results than 6851 pg/mL and this observation pointed that expected values of sFas concentration in pregnant women is much lower than high normal concentrations.

If we compare our results to those obtained by Velore and all our results were much higher than in healthy non pregnant women and in third trimester of pregnancy [11, 12].

This observation is coherent with results of Nagata suggested that soluble Fas has a suppressive effect on Fas/FasL--mediated apoptosis [9, 12]. Maybe we could expect lower intensification of apoptosis for example in placenta. In one observation levels of sFas and FasL were calculated in second trimester of pregnancy in amniotic fluid and authors found no differences between small for gestational age (SGA), large for gestational age (LGA) and appropriate for gestational age (AGA) pregnancies of amniotic fluid concentrations of sFas and FasL [13]. This could suggest no dependence of sFas and Fas L on fetal weight. Ashton suggested the crucial role of Fas/FasL role in trophoblast invasion, spiral artery remodeling and placental development [14].

In our study we found that parameters of apoptosis were higher in cord blood than in maternal blood. In fetal organism the process of multiplication of cells has higher level than apoptosis that allows fetal growth but in third trimester of pregnancy the process of multiplication of cells is low. We can suggest that this takes place because of processes of cells hypertrophy and probably all newly developed cells were unnecessary and destroyed.

An increase of apoptosis in placenta probably is caused by abnormal placentation and the effect we can observe as fetal growth restriction. In our study there were only AGA fetuses, so we could expect no increase of apoptotic processes in maternal blood [15].

We found statistical difference in sFas concentration between maternal blood and cord arterial and vein blood. Higher concentration was found in maternal blood and is possible that it is the protecting mechanism that plays role in placental protection from acceleration of apoptosis. Other observations show different results — higher cord blood sFas concentration measured at time of the delivery than in pregnant women [16]. As we think this difference could be caused by the way of delivery- spontaneous delivery or elective caesarean section.

There were no differences between arterial and vein blood from umbilical cord. There was no other available data on comparison of arterial/vein ratio of sFas. But we can suggest that fetal blood circulation doesn't demonstrate changes in contact of villi with maternal blood [13–15].

If compared Fas ligand [sFasL] concentrations in serum, different results were seen. In our study we compared concentration of Fas ligand [FasL] in maternal and cord blood sera. We found difference between maternal blood serum and both arterial and vein cord blood serum. Higher level of FasL was observed in maternal blood but we found no difference between cord vein and arterial blood.

Vrachinis et all found increased FasL expression in amniotic fluid in severe and very severe SGA fetuses that reflects the increased apoptosis in the fetus and placenta [13]. This observation could explain lower concentration of FasL in cord blood in uncomplicated pregnancy that protests fetal organism form apoptosis.

Umbilical serum level of FasL in observation of Randhava was significantly higher than maternal serum. There were no suggestions of the reason for this result [16, 17]. The results of Randhava was similar to our results.

The consequences of deregulation of Fas/FasL-mediated apoptosis linked to self-reactivity, immune dysfunction and malignant transformation and placental disfunction [17].

Maternal mean serum levels of sFas and sFasL were measured in preeclamptic pregnancies and compared to healthy pregnant women, showed no differences between compared groups [16, 18, 19].

From clinical point of view measurement of maternal blood FasL could not indicate the intensification of apoptosis process in fetal organism, but evaluation of maternal

to fetal blood proportion of FasL concentration could be helpful to establish normal causality of the relationship that still remains uncertain.

All presented by different authors data were obtained in small groups and further research is needed to evaluate normal concentrations of measured parameters for healthy pregnancy and SGA or preeclamptic patients [19]. There is so far little literature data to confirm either of these results.

CONCLUSIONS

1. The study did not demonstrate significant differences in sFas and FasL concentration in cord arterial and vein blood that suggest no influence of fetal organism on apoptosis by sFas and FasL production
2. Lowering of sFas concentration in fetal blood could mean the increase of apoptosis in fetal organism comparing to maternal.
3. Higher concentration of FasL in cord blood than in mothers suggested higher apoptosis intensification in fetal circulation and no influence of blood flow across placenta on its concentration.
4. Further studies were needed to establish normal values of sFas and FasL levels in early, second trimester and third trimester of physiological and pathological pregnancy.

Conflict of interests

We declare that we have no conflict of interest.

The study was supported by Medical University funds no-503100404/503-11-001.

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