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Soluble Fas ligand in maternal and umbilical cord blood in pregnancies complicated by preeclampsia

Preeclampsia is characterised by an increase in vascular tone, hypertension, enhanced platelet aggregation and coagulation and proteinuria. It results in increased perinatal morbidity and mortality and unfavourable perinatal outcomes. It seems that the immunological system plays a very important role in pregnancy complicated by preeclampsia, but the etiology of this disease is uncertain. One of the progress mechanisms of this disorder is the abnormal activation of the maternal immunological system in relation to the fetus as an allograft. Also increased trophoblast apoptosis was observed in the placenta of preeclamptic pregnant women and it is possible that this biological phenomen is important for the pathogenesis of the preeclampsia and mediated through a soluble factor(s) present in maternal blood (4).

The Fas/Fas ligand system is one of the main apoptotic pathways controlling placental apoptosis (8). The Fas/FasL pathway of apoptosis is abnormally activated in diseases associated with impaired immune tolerance or chronic inflammation. Fas ligand inhibits T cell function in immune-privileged organs and may be associated with maintenance of the immune-privileged status (1). Furthermore, the interaction of Fas with its ligand (FasL), induces apoptosis. It is possible that preeclampsia may be related to impaired maternal-fetal tolerance and may be associated with abnormal activation of Fas/FasL pathway.

The aim of this study was to analyse the maternal and umbilical cord soluble Fas ligand (sFasL) in pregnancies complicated by preeclampsia with appropriate-for-gestational-age weight infants and in normotensive pregnancies. The study was given the approval of the Board for Supervising Ethics in Medical Experiments at the Medical University of Lublin.

PATIENTS AND METHODS

The study was carried out on 17 preeeclamptic delivering patients in the third trimester of pregnancy with severe preeclampsia (group P) with appropriate-for-gestational-age weight infants. Preeclampsia was determined by increased blood pressure >140 mm Hg systolic and >90 mm Hg diastolic in women who were normotensive before 20 weeks' gestation accompanied by proteinuria defined as the urinary excretion of more than 0.3 g protein in 24-hour specimen. Severe preeclampsia was defined as blood pressure >160/110 mmHg on at least 2 occasions 6 hours apart, proteinuria > 5 g in a 24-hour urinary protein excretion, or HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) and when hypertension and proteinuria were associated with one or more of the following clinical manifestations: renal abnormalities (oliguria, creatinine, increased urine acid), hematologic abnormalities (thrombocytopenia, microangiopathic hemolysis), hepatocellular dysfunction (elevated liver enzymes, right-upper quadrant pain) or neurologic symptoms (headache, visual disturbances, seizures).

The control group consisted of 12 healthy normotensive patients with singleton uncomplicated pregnancies, without any renal, heart and vascular diseases and normal laboratory tests (group C). All arterial blood pressure measurements in the control group were normal and did not exceed 135/85 mmHg. None of the patients from this group suffered from proteinuria. All patients in the study were non-smokers.

Maternal and umbilical serum soluble FasL concentrations were estimated using a sandwich ELISA assay (human sFas Ligand kit BMS Vienna, Austria). Data were expressed as mean +/– SD and were statistically analyzed with the computer program "Statistica 5.0" using the Shapiro-Wilk test for normal distribution of data, and equality of variance by Levene test and, subsequently Student's t-tests, or (in unequal variance) the Cochran-Cox test (absence of normal distribution and non-parametric data), the Mann-Whitney U test and Kruskal-Wallis test in independent samples and the t-test or Wilcoxon rank sum test for data dependent samples in one group of patients. The level of statistical significance was established as $p<0.05$.

RESULTS

There were no statistically significant differences in gravidity, parity and maternal age in patient profiles between groups. Creatinine and urea levels were normal in all patients. None of the patients from control group suffered from proteinuria

There are no statistically significant differences in gestation age and in birth weight of infants. The mean birth weights of infants were 3354.17 +/– 785.92 g in group P and 3041.11 +/– 524.87 g in the control group. The mean gestation age at birth was 37.63 +/– 3.33 weeks in preeclamptic patients versus 37.97 +/– 2.36 weeks in the control group respectively.

Systolic and diastolic blood pressure and mean arterial blood pressure were higher in the study group in comparison with the control group. These differences were statistically significant ($p<0.001$). The mean systolic blood pressure values were 161.56 +/– 12.74 mmHg in group P versus 108.24 +/– 9.18 mmHg in the control group. The mean diastolic blood pressure values were 112.00 +/– 9.78 mmHg in the study group P versus 68.93 +/– 7.38 mmHg in the control group.

Mean concentrations of sFasL in sera were similar between both groups for maternal samples. The mean maternal sFasL values were 208.80 +/– 35.23 pg/mL (ranging from 148.00 to 264.00 pg/mL) in group P compared with 197.71 +/– 23.98 pg/mL (ranging from 156.00 to 254.00 pg/mL) in the control group.

The levels of sFasL were higher in umbilical cord blood in preeclamptic patients than in the control subjects.The mean values were 278.42 +/– 96.97 pg/mL (ranging from 180.00 to 526.00 pg/mL) in group P compared with 198.46 +/– 28.87 pg/mL (ranging from 176.00 to 286.00 pg/mL) in the control group. This difference was statistically significant ($p<0.001$).

Mean serum concentrations of sFas ligand in maternal and umbilical cord sera did not differ in the healthy controls. But in our study group of preeclamptic patients, higher umbilical sFas ligand values were observed in comparison with the maternal blood. This difference was statistically significant in preeclamptic patients ($p<0.02$).

DISCUSSION AND CONCLUSIONS

Apoptosis, programmed cell death was observed in the placenta throughout gestation; however, a higher frequency occurs in the villi in third trimester compared with first trimester, suggesting that placental apoptosis is a process that occurs during normal pregnancy (5, 6).

Fas, also called APO-1 or CD95, is a cell surface receptor that can induce apoptotic cell death in sensitive cells, after the triggering of APO-1 by its ligand. In this study mean concentrations of sFasL in sera were similar between both groups for maternal samples whereas the umbilical levels of sFasL were higher in pregnancy complicated by preeclampsia than in healthy pregnant women. Furthermore, mean serum concentrations of sFasL in maternal and umbilical cord sera did not

differ in the healthy controls. However, higher umbilical sFas ligand values were observed in comparison with the maternal blood in preeclamptic patients.

Kuntz et al. (3) observed higher not only umbilical, but also maternal sFas ligand concentrations in preeclamptic pregnancies. They concluded that expression is altered in gestation complicated by preeclampsia and speculated that activation of the Fas/FasL pathway mediates associated pathologic processes in affected women and their infants.

Strand et al. (7) observed that CD95 ligand (FasL) derived from the placenta acts systemically and is a primary cause of liver damage in HELLP syndrome. They showed that blocking of CD95L can reduce liver cell apoptosis, indicating that such a strategy may have therapeutic advantages. Liver dysfunction was accompanied by increased sFasL levels as well as kidney damage (9, 10). Apoptosis plays an important role in the hepatocellular damage observed in acute rejection and also in HCV reinfection (10). Thus, increased plasma sFasL may play an important role also in liver and renal disorders observed in preeclampsia.

Neale et al. (4) observed that serum from preeclamptic women reduced trophoblast viability in H8 trophoblast cells culture, and this phenomenon was enhanced by treatment with anti –Fas antibody. Treatment with a blocking anti-Fas ligand antibody reversed this effect. Serum from normal pregnant women did not affect trophoblast cell viability (4).

Hsu et al. (2) suggested that preeclampsia might be a disorder of maternal immune intolerance partly associated with elevated circulating levels of soluble Fas that protect maternal immune cells from apoptosis and subsequently lead to an increased apoptosis in trophoblast.

These findings concerning similar maternal and umbilical levels of sFasL in healthy controls and higher umbilical levels of sFasL in pregnancy complicated by preeclampsia may suggest imbalance in apoptosis mediated by the Fas/FasL system in preeclamptic pregnancy in fetal compartment and may indicate the disturbances between programmed death and life cell decision in this pregnancy complication. It is possible that lack of the increased levels of soluble FasL in maternal blood may result from increased used (action).

Further studies are necessary to evaluate the role of the Fas/FasLsystem in preeclamptic pregnancies not only with soluble, but also with membrane bound form of Fas ligand molecule.

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SUMMARY

The aim of this study was to analyse the maternal and umbilical cord soluble Fas ligand (sFasL) in pregnancies complicated by preeclampsia with appropriate-for-gestational-age weight infants. The study was carried out on 17 preeeclamptic delivering patients in the third trimester of pregnancy with severe preeclampsia. The control group consisted of 12 healthy normotensive patients with singleton uncomplicated pregnancies. Maternal and umbilical serum sFasL concentrations were estimated using a sandwich ELISA assay. Mean concentrations of sFasL in sera were similar between both groups for maternal samples. The levels of sFasL were higher in umbilical cord blood in preeclamptic patients than in the control subjects. Mean serum concentrations of sFas ligand in maternal and umbilical cord sera did not differ in the healthy controls. But in the study group of preeclamptic patients, higher umbilical sFas ligand values were observed in comparison with the maternal blood. These findings may suggest imbalance in apoptosis mediated by the Fas/FasL system in preeclamptic pregnancy, especially in fetal compartment.

sFas ligand we krwi matczynej i pępowinowej w ciąży powikłanej preeklampsją

Celem badania była analiza rozpuszczalnego ligandu Fas (sFasL) we krwi matczynej i pępowinowej w ciążach powikłanych preeklampsją z prawidłowym wewnątrzmacicznym wzrostem płodu. Badaniami objęto 17 kobiet rodzących z ciążą powikłaną ciężką preeklampsją. Grupę kontrolną stanowiło 12 zdrowych kobiet z fizjologicznym przebiegiem ciąży i prawidłowym ciśnieniem tętniczym krwi. Rozpuszczalny Fas ligand (sFasL) w surowicy krwi matczynej i pępowinowej oceniano metodą ELISA. Otrzymane wartości sFasL w surowicy krwi matczynej były podobne w obu grupach badanych kobiet. Średnie wartości sFasL we krwi pępowinowej były wyższe w grupie kobiet z ciążą powikłaną stanem przedrzucawkowym. Uzyskane wartości sFasL we krwi matczynej i pępowinowej były podobne w grupie zdrowych kobiet rodzących, natomiast zaobserwowano wyższe wartości sFasL we krwi pępowinowej niż we krwi matczynej w grupie kobiet z ciążą powikłaną preeklampsją. Wyniki naszych badań mogą sugerować zaburzenia równowagi programowanej śmierci komórkowej (apoptozy) pośredniczonej przez system Fas/FasL w ciąży powikłanej preeklampsją, zwłaszcza w kompartmencie płodowym.