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Circulating ficolin-2 and ficolin-3 in normal pregnancy and pre-eclampsia

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Summary

Ficolins are soluble molecules of the innate immune system that recognize carbohydrate molecules on microbial pathogens, apoptotic and necrotic cells. They act through two distinct routes: initiating the lectin pathway of complement activation and mediating a primitive opsonophagocytosis. In this study, we measured plasma levels of ficolin-2 and ficolin-3 in 60 pre-eclamptic patients, 60 healthy pregnant women and 59 healthy non-pregnant women by enzyme-linked immunosorbent assay (ELISA). Circulating levels of complement activation products (C4d, C3a, SC5b9), angiogenic factors (soluble fms-like tyrosine kinase-1, placental growth factor) and markers of endothelial activation (von Willebrand factor antigen), endothelial injury (fibronectin) and trophoblast debris (cell-free fetal DNA) were also determined. Plasma levels of ficolin-2 were significantly lower in healthy pregnant than in healthy non-pregnant women, while ficolin-3 levels did not differ significantly between the two groups. Furthermore, pre-eclamptic patients had significantly lower ficolin-2 and ficolin-3 concentrations than healthy non-pregnant and pregnant women. In the pre-eclamptic group, plasma ficolin-2 levels showed a significant positive correlation with serum placental growth factor (PlGF) concentrations and significant inverse correlations with serum levels of soluble fms-like tyrosine kinase-1 (sFlt-1), blood urea nitrogen and creatinine, serum lactate dehydrogenase activities, as well as with plasma VWF: antigen, fibronectin and cell-free fetal DNA concentrations. In conclusion, circulating levels of ficolin-2 are decreased in the third trimester of normal pregnancy. There is a further decrease in plasma ficolin-2 concentrations in pre-eclampsia, which might contribute to the development of the maternal syndrome of the disease through impaired removal of the trophoblast-derived material released into the maternal circulation by the hypoxic and oxidatively stressed pre-eclamptic placenta.

Keywords: complement, ficolin, pre-eclampsia, pregnancy, sFlt-1

Introduction

Pre-eclampsia, characterized by hypertension and proteinuria developing after mid-gestation, is a severe complication of human pregnancy, with a worldwide incidence of 2–10%. It is one of the leading causes of maternal, as well as perinatal morbidity and mortality, even in developed countries. Despite intensive research efforts, the aetiology and pathogenesis of pre-eclampsia are not understood completely. Increasing evidence suggests that an excessive maternal systemic inflammatory response to pregnancy with activation of both the innate and adaptive arms of the immune system is involved in the pathogenesis of the disease [1,2]. We have demonstrated previously that the complement system is activated with increased terminal complex formation in the third trimester of normal human pregnancy, and further in pre-eclampsia, as shown by the elevated amounts of activation markers in the systemic circulation [3]. However, in our recent study, the role of the mannose-binding lectin (MBL)-mediated lectin pathway has been ruled out in the pathological complement activation observed in pre-eclampsia [4].

Ficolins are pattern recognition molecules of the innate immune system that bind to carbohydrate moieties present

on the surface of microbial pathogens, apoptotic and necrotic cells. They act through two distinct routes: by initiating the lectin pathway of complement activation in concert with attached MBL-associated serine proteases (MASPs) and by a primitive opsonophagocytosis [5]. Ficolins are oligomeric proteins consisting of an N-terminal cysteine-rich region, a collagen-like domain and a C-terminal globular fibrinogen-like domain. The latter is responsible for carbohydrate binding [6]. Three types of ficolins have been identified in humans: ficolin-2 (L-ficolin), ficolin-3 (H-ficolin) and ficolin-1 (M-ficolin). The mRNA of ficolin-2 is expressed primarily in the liver and its protein product is secreted into the blood circulation. Ficolin-2 exhibits lectin activity toward N-acetyl-glucosamine (GlcNAc) and 1, 3-β-D-glucan. Ficolin-3 mRNA is expressed in the liver and lung. In the liver, ficolin-3 is produced by bile duct epithelial cells and hepatocytes, and is secreted into the bile and circulation. In the lung, ficolin-3 is produced by ciliated bronchial epithelial cells and type II alveolar epithelial cells, and is secreted into the bronchus and alveolus. Ficolin-3 binds to GlcNAc, N-acetyl-galactosamine (GalNAc) and fucose. Ficolin-1 mRNA is expressed in monocytes, the lung and spleen. Its protein product has been identified in secretory granules of neutrophils and monocytes, as well as in type II alveolar epithelial cells. Nevertheless, it is present in the circulation at very low levels compared to ficolin-2 and ficolin-3. Ficolin-1 exhibits binding activity towards GlcNAc, GalNAc and sialic acid [7].

The purpose of this study was to determine whether circulating levels of ficolin-2 and ficolin-3 are altered in normal pregnancy and pre-eclampsia and related to the clinical features and laboratory parameters of the patients, including complement activation products (C4d, C3a, SC5b9), angiogenic factors (soluble fms-like tyrosine kinase-1, placental growth factor) and markers of endothelial activation (von Willebrand factor antigen), endothelial injury (fibronectin) or trophoblast debris (cell-free fetal DNA).

Materials and methods

Study patients

Our study was designed using a case–control approach. Sixty pre-eclamptic patients, 60 healthy pregnant women with uncomplicated pregnancies and 59 healthy non-pregnant women were involved in the study. The study participants were enrolled from the First Department of Obstetrics and Gynecology and from the Department of Obstetrics and Gynecology of Kútvölgyi Clinical Center, at the Semmelweis University, Budapest, Hungary. All women were Caucasian and resided in the same geographic area in Hungary. Exclusion criteria were multi-fetal gestation, chronic hypertension, diabetes mellitus, autoimmune disease, angiopathy, renal disorder, maternal or fetal infection and fetal congenital anomaly. The women were fasting; none of the pregnant women were in active labour, and none had rupture of membranes. The healthy non-pregnant women were in the early follicular phase of the menstrual cycle (between cycle days 3 and 5), and none of them received hormonal contraception.

Pre-eclampsia was defined by increased blood pressure (≥140 mmHg systolic or ≥90 mmHg diastolic on ≥2 occasions at least 6 h apart) that occurred after 20 weeks of gestation in women with previously normal blood pressure, accompanied by proteinuria (≥0·3 g/24 h or ≥1 + on dipstick in the absence of urinary tract infection). Blood pressure returned to normal by 12 weeks postpartum in each pre-eclamptic study patient. Pre-eclampsia was regarded as severe if any of the following criteria was present: blood pressure ≥ 160 mmHg systolic or ≥110 mmHg diastolic, or proteinuria ≥ 5 g/24 h (or ≥3 + on dipstick). Pregnant women with eclampsia or HELLP (haemolysis, elevated liver enzymes and low platelet count) syndrome were not enrolled into this study. Early onset of pre-eclampsia was defined as onset of the disease before 34 weeks of gestation (between 20 and 33 completed gestational weeks). Fetal growth restriction was diagnosed if the fetal birth weight was below the 10th percentile for gestational age and gender, based on Hungarian birth weight percentiles.

The study protocol was approved by the Regional and Institutional Committee of Science and Research Ethics of the Semmelweis University, and written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki.

Biological samples

Blood samples were taken from an antecubital vein into plain tubes, as well as ethylenediamine tetraacetic acid (EDTA) or sodium citrate anti-coagulated tubes, and then centrifuged at room temperature with a relative centrifugal force of 3000 ***g*** for 10 min. The aliquots of serum and plasma were stored at −80°C until the measurements.

Laboratory methods

Standard laboratory parameters (clinical chemistry) were determined by an autoanalyser (Cobas Integra 800; Roche, Mannheim, Germany) using the manufacturer's kits. Plasma levels of ficolin-2 and ficolin-3 were measured by enzyme-linked immunosorbent assay (ELISA) (Hycult Biotech, Uden, the Netherlands; cat. no. HK336 and HK340, respectively) on an automated ELISA analyser (Elisys UNO; Human GmBH, Wiesbaden, Germany), according to the manufacturer's instructions. Levels of C4d, C3a and SC5b9 in maternal plasma were assessed with Quidel ELISA kits (San Diego, CA, USA; cat. no. A008, A015 and A029, respectively). Serum total soluble fms-like tyrosine kinase-1 (sFlt-1) and biologically active placental growth factor

(PlGF) levels were measured by electrochemiluminescence immunoassay (Elecsys; Roche; cat. no. 05109523 and 05144671, respectively) on a Cobas e 411 analyser (Roche). Plasma von Willebrand factor antigen (VWF:antigen) levels were quantified by ELISA (Dakopatts, Glostrup, Denmark), while plasma fibronectin concentration was measured by nephelometry (Dade Behring, Marburg, Germany), according to the manufacturer's protocol. After extracting DNA with the silica adsorption method, the amount of cell-free fetal DNA in maternal plasma was determined in patients with male newborns by quantitative real-time polymerase chain reaction (PCR) analysis of the sex-determining region Y (*SRY*) gene, as we have described previously [8].

Statistical analysis

The normality of continuous variables was assessed using the Shapiro–Wilk's *W*-test. As the continuous variables were not distributed normally, non-parametric statistical methods were used. To compare continuous variables between two groups, the Mann–Whitney *U*-test was applied; to compare them among multiple groups, the Kruskal–Wallis analysis of variance by rank test was performed. Multiple comparisons of mean ranks for all groups were carried out as *post-hoc* tests. Fisher's exact and Pearson's χ^2 tests were used to compare categorical variables between groups. Spearman's rank order correlation was applied to calculate correlation coefficients. Multiple linear regression analyses were undertaken, as a non-parametric method, with logarithmically transformed values of the dependent variable. Odds ratios (OR) with 95% confidence intervals (CI) were calculated by logistic regression analyses.

Statistical analyses were performed using the following software: STATISTICA (version 8·0; StatSoft, Inc., Tulsa, OK, USA) and SPSS (version 18·0 for Windows; SPSS, Inc., Chicago, IL, USA). For all statistical analyses, $P < 0{\cdot}05$ was considered statistically significant.

In this paper, data are reported as median (25–75 percentile) for continuous variables and as number (percentage) for categorical variables.

Results

Patient characteristics

The clinical characteristics of the study participants are described in Table 1. There was no statistically significant difference in terms of age among the study groups. Additionally, no significant differences were observed in gestational age at blood collection and the percentage of primiparas or parity between pre-eclamptic patients and healthy pregnant women. However, all the other clinical features presented in Table 1 differed significantly among our study groups. Fetal growth restriction was absent in healthy pregnant women, whereas the frequency of this condition was 18·3% in the pre-eclamptic group. Twenty-one women had severe pre-eclampsia and five patients experienced early onset of the disease. In our pre-eclamptic group, multiparous women had significantly higher age [32 (29–35) *versus* 28 (25–31) years, $P < 0{\cdot}001$] and pre-pregnancy body mass index (BMI) [27·2 (25·5–29·0) *versus* 23·1 (19·8–26·1) kg/m^2, $P < 0{\cdot}05$] than primiparous women.

Laboratory parameters

The laboratory parameters of the study subjects are displayed in Table 2. As can be seen in the table, there were significant differences in most of the measured laboratory parameters among the three study groups except for serum aspartate aminotransferase (AST) activity. As shown in

Table 1. Clinical characteristics of healthy non-pregnant and pregnant women and pre-eclamptic patients.

	Healthy non-pregnant women ($n = 59$)	Healthy pregnant women ($n = 60$)	Pre-eclamptic patients ($n = 60$)
Age (years)	28 (23–35)	30 (28–32)	29 (26–32)
BMI at blood draw (kg/m^2)	20·8 (19·6–22·9)	25·8 (24·3–27·9)[b]	29·9 (26·9–33·3)[b,d]
Pre-pregnancy BMI (kg/m^2)	n.a.	21·0 (19·5–22·6)	25·5 (21·6–28·1)[d]
Smokers	14 (23·7%)	0 (0%)[b]	3 (5·0%)[a]
Primiparas	n.a.	37 (61·7%)	38 (63·3%)
Parity	n.a.	1 (1–2)	1 (1–2)
Systolic blood pressure at blood draw (mmHg)	115 (110–120)	110 (107–120)	162 (155–180)[b,d]
Diastolic blood pressure at blood draw (mmHg)	80 (70–80)	70 (60–80)[b]	100 (97–110)[b,d]
Gestational age at blood draw (weeks)	n.a.	36 (36–37)	37 (36–39)
Gestational age at delivery (weeks)	n.a.	39 (38–40)	38 (37–39)[d]
Fetal birth weight (grams)	n.a.	3450 (3150–3700)	3125 (2450–3475)[d]
Fetal growth restriction	n.a.	0 (0%)	11 (18·3%)[d]

[a]$P < 0{\cdot}05$ *versus* healthy non-pregnant women. [b]$P < 0{\cdot}001$ *versus* healthy non-pregnant women. [c]$P < 0{\cdot}05$ pre-eclamptic patients *versus* healthy pregnant women. [d]$P < 0{\cdot}001$ pre-eclamptic patients *versus* healthy pregnant women. Data are presented as median (25–75 percentile) for continuous variables and as number (percentage) for categorical variables. BMI: body mass index; n.a.: not applicable.

Table 2. Laboratory parameters of healthy non-pregnant and pregnant women and pre-eclamptic patients.

	Healthy non-pregnant women ($n = 59$)	Healthy pregnant women ($n = 60$)	Pre-eclamptic patients ($n = 60$)
Serum BUN level (mmol/l)	4·1 (3·5–4·8)	2·8 (2·0–3·3)[b]	3·5 (2·7–4·2)[a,c]
Serum creatinine level (μmol/l)	66 (61–72)	49 (42–56)[b]	63 (55–71)[d]
Serum bilirubin level (μmol/l)	9·3 (6·6–12·4)	5·4 (4·0–6·8)[b]	7·3 (5·7–8·9)[a,c]
Serum AST activity (U/l)	17 (15–20)	19 (17–21)	19 (15–25)
Serum ALT activity (U/l)	14 (12–17)	12 (10–15)[a]	16 (11–23)[c]
Serum LDH activity (U/l)	154 (128–170)	158 (138–169)	192 (153–225)[b,d]
Plasma C4d level (μg/ml)	0·04 (0·02–0·06)	0·11 (0·08–0·15)[b]	0·16 (0·10–0·21)[b,c]
Plasma C3a level (ng/ml)	85·5 (29·7–173·8)	751·6 (194·6–1660)[b]	1358 (854·8–2142)[b,c]
Plasma SC5b9 level (ng/ml)	32·5 (20·5–52·8)	59·9 (42·1–86·6)[b]	75·9 (50·8–116·3)[b,c]
Serum sFlt-1 level (pg/ml)	76·3 (67·1–83·6)*	3252 (2509–4751)[†,b]	6814 (3736–12720)[‡,b,d]
Serum PlGF level (pg/ml)	16·2 (14·0–18·0)*	183 (126–307)[†,b]	98·0 (63·7–146)[‡,b,d]
Plasma VWF:antigen level (%)	70·0 (60·2–87·3)	152·6 (112·7–199·0)[b]	184·8 (139·9–243·1)[b,c]
Plasma fibronectin level (g/l)	n.m.	0·37 (0·31–0·47)	0·58 (0·41–0·82)[d]
Plasma cell-free fetal DNA level (pg/μl)	n.m.	0·002 (0·0–0·172)[§]	0·082 (0·033–0·292)[¶,c]

[a]$P < 0{\cdot}05$ *versus* healthy non-pregnant women. [b]$P < 0{\cdot}001$ *versus* healthy non-pregnant women. [c]$P < 0{\cdot}05$ pre-eclamptic patients *versus* healthy pregnant women. [d]$P < 0{\cdot}001$ pre-eclamptic patients *versus* healthy pregnant women. *$n = 52$; [†]$n = 58$; [‡]$n = 54$; [§]$n = 19$; [¶]$n = 33$. Data are presented as median (25–75 percentile). ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; DNA: deoxyribonucleic acid; LDH: lactate dehydrogenase; n.m.: not measured; PlGF: placental growth factor; sFlt: soluble fms-like tyrosine kinase; VWF:antigen: von Willebrand factor antigen.

Fig. 1a,b, plasma levels of ficolin-2 were significantly lower in healthy pregnant than in healthy non-pregnant women, while ficolin-3 levels did not differ significantly between the two groups. Furthermore, pre-eclamptic patients had significantly lower ficolin-2 and ficolin-3 concentrations than healthy non-pregnant and pregnant women.

Using the receiver operating characteristic (ROC) curve analysis, we determined cut-off values for plasma levels of ficolin-2 (<2·84 μg/ml; sensitivity: 70·2%, specificity: 66·1%) and ficolin-3 (<24·0 μg/ml; sensitivity: 68·3%, specificity: 54·2%) to discriminate pre-eclamptic patients from healthy pregnant women. Both low ficolin-2 and ficolin-3 levels were associated significantly with pre-eclampsia [OR (95% CI) for ficolin-2: 4·58 (2·07–10·1), $P < 0{\cdot}001$; for ficolin-3: 2·56 (1·21–5·40), $P < 0{\cdot}05$], even after adjustment for maternal age, BMI and gestational age at blood draw in multiple logistic regression analysis [adjusted OR with 95% CI for ficolin-2: 8·74 (2·90–26·4), $P < 0{\cdot}001$; for ficolin-3: 3·30 (1·24–8·77), $P < 0{\cdot}05$].

In the group of pre-eclamptic patients, no statistically significant differences were found in plasma levels of ficolin-2 and ficolin-3 between patients with mild and severe

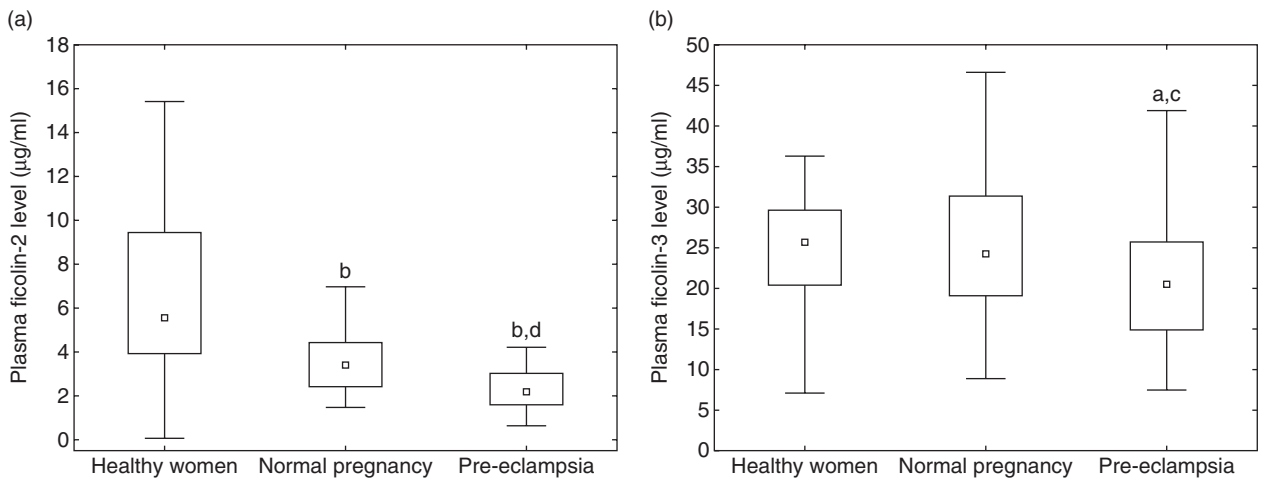

Fig. 1. Plasma ficolin-2 (a) and ficolin-3 levels (b) of healthy non-pregnant and pregnant women and pre-eclamptic patients. Middle point: median; box: interquartile range (25–75 percentile); whisker: range (excluding outliers); [a]$P < 0{\cdot}05$ *versus* healthy non-pregnant women; [b]$P < 0{\cdot}001$ *versus* healthy non-pregnant women; [c]$P < 0{\cdot}05$ pre-eclamptic patients *versus* healthy pregnant women; [d]$P < 0{\cdot}001$ pre-eclamptic patients *versus* healthy pregnant women.

pre-eclampsia, between patients with late and early onset of the disease or between pre-eclamptic patients with and without fetal growth restriction (data not shown).

Relationship of plasma ficolin-2 and ficolin-3 levels of the study subjects to their clinical characteristics and laboratory parameters

We also investigated whether plasma ficolin-2 and ficolin-3 concentrations of the study participants were related to their clinical features and laboratory parameters by calculating the Spearman's rank order correlation coefficients (continuous variables) or by Mann–Whitney *U*-test (categorical variables). In healthy pregnant women, there was a statistically significant positive correlation between plasma ficolin-2 and serum PlGF concentrations (Spearman's $R = 0{\cdot}33$, $P < 0{\cdot}05$), while a significant inverse correlation was observed between their ficolin-2 and sFlt-1 levels ($R = -0{\cdot}59$, $P < 0{\cdot}001$; Fig. 2a). In the pre-eclamptic group, plasma ficolin-2 levels showed a significant positive correlation with serum PlGF concentrations ($R = 0{\cdot}34$, $P < 0{\cdot}05$) and significant inverse correlations with serum levels of sFlt-1 ($R = -0{\cdot}72$, $P < 0{\cdot}001$; Fig. 2b), blood urea nitrogen ($R = -0{\cdot}36$, $P < 0{\cdot}05$) and creatinine ($R = -0{\cdot}38$, $P < 0{\cdot}05$), serum lactate dehydrogenase activities ($R = -0{\cdot}32$, $P < 0{\cdot}05$), as well as with plasma VWF:antigen ($R = -0{\cdot}34$, $P < 0{\cdot}05$), fibronectin ($R = -0{\cdot}50$, $P < 0{\cdot}001$) and cell-free fetal DNA ($R = -0{\cdot}41$, $P < 0{\cdot}05$) concentrations. However, after adjustment for serum sFlt-1 levels in multiple linear regression analyses, only the association between ficolin-2 and creatinine concentrations remained significant [standardized regression coefficient (β) $= -0{\cdot}41$, $P < 0{\cdot}05$]. There was no other relationship between plasma ficolin-2 or ficolin-3 levels of the study subjects and their clinical features and measured laboratory parameters – including complement activation products – in either study group.

Discussion

In this study, we determined plasma levels of ficolin-2 and ficolin-3 in healthy non-pregnant and pregnant women and pre-eclamptic patients. Simultaneous measurement of complement activation products, angiogenic factors and markers of endothelial activation, endothelial injury and trophoblast debris enabled us to investigate their relationship, which can help in understanding the role of circulating ficolins in normal pregnancy and pre-eclampsia.

A major function of circulating ficolins is activation of the complement system through the lectin pathway by association with effector MASPs [6]. However, in this study, circulating levels of ficolins did not correlate with those of complement activation products, suggesting that the ficolin-mediated lectin pathway does not play a remarkable role in systemic complement activation during normal pregnancy and pre-eclampsia. Instead, circulating immune complexes and C-reactive protein have been implicated to activate complement through the classical pathway both in normal pregnancy and further in pre-eclampsia [3,9,10]. The MBL-mediated lectin pathway has also been shown to be activated in normal pregnancy [11]. Circulating mannose-binding lectin (MBL) concentration was elevated in patients with pre-eclampsia, and MBL genotypes were found to be associated with the disease [12–14]. Nevertheless, contradictory data also exist [15,16] and functional activity of the MBL-MASP2 complex is unchanged in pre-eclampsia, according to our previous results [4]. Recently, elevated levels of the complement activation fragment Bb in early pregnancy have been demonstrated to associate with the development of pre-eclampsia later in gestation, indicating the role of the

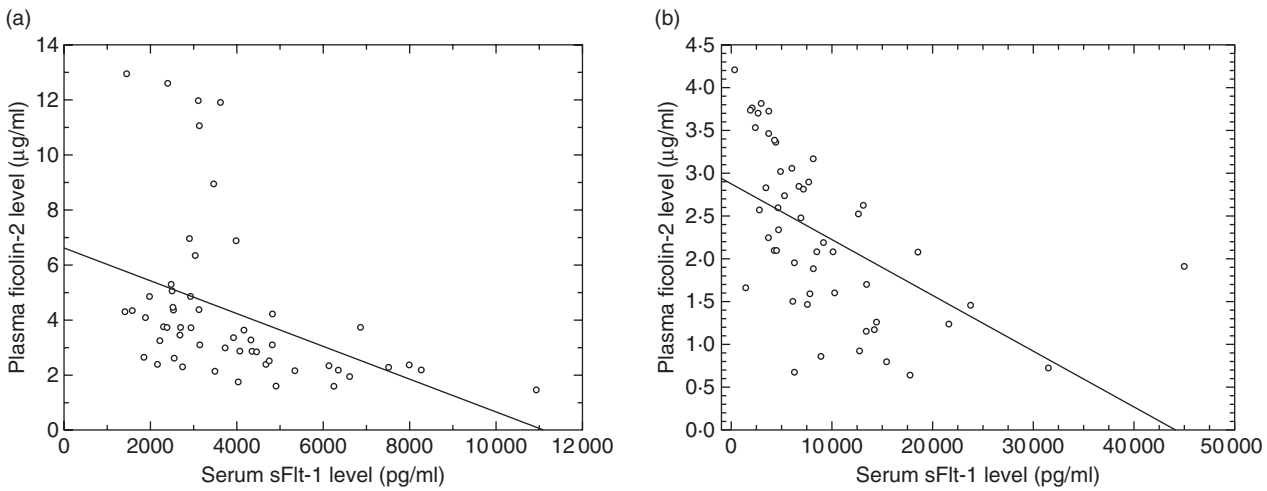

Fig. 2. Scatterplots and the regression line of plasma ficolin-2 levels *versus* serum soluble fms-like tyrosine kinase-1 (sFlt-1) concentrations in healthy pregnant women (a) and pre-eclamptic patients (b).

alternative pathway in the pathogenesis of this disorder [17,18].

In addition to their ability to activate the complement system, ficolins can also act as direct opsonins and mediate the clearance of microorganisms, apoptotic and necrotic cells through phagocytosis [19–23]. Trophoblast apoptosis is a feature of normal pregnancy with an increment in pre-eclampsia, complicated further by necrosis [24–27]. Indeed, ficolins have been reported to bind to the trophoblast cells undergoing apoptosis in the pre-eclamptic placenta [15]. Additionally, the placenta sheds apoptotic and even living cellular and subcellular material (also called as trophoblast debris), containing cell-free fetal DNA and sFlt-1, into the maternal circulation both in normal pregnancy and with elevated amounts in pre-eclampsia [28–33]. Given the significant inverse correlation of circulating levels of ficolin-2 with those of cell-free fetal DNA and sFlt-1 in our healthy pregnant and pre-eclamptic groups, it is tempting to speculate that ficolin-2 may be involved in the direct removal of trophoblast-derived material from the maternal circulation. In pre-eclampsia, consumption (or primary deficiency) of circulating ficolin-2, as suggested by its diminished plasma concentration, might impair the clearance of shed apoptotic and necrotic placental material leading to the maternal syndrome of the disease. Although plasma ficolin-3 concentration was also decreased in our pre-eclamptic women, circulating levels of ficolin-3 did not correlate with those of cell-free fetal DNA or sFlt-1 in our pregnant study groups. This discrepancy might be explained by the differences in ligand specificity of ficolin-2 and ficolin-3, i.e. ficolin-2 can recognize DNA [22]. It is possible that low plasma concentration of ficolin-3 in pre-eclampsia is simply a consequence of its sequestration in the apoptotic placenta [15].

There is an increasing body of evidence that an imbalance between circulating angiogenic factors and their antagonists plays a crucial role in the pathogenesis of pre-eclampsia [34,35]. We have reported previously that increased serum sFlt-1 and decreased PlGF levels are associated with blood pressure, renal and endothelial dysfunction, trophoblast deportation, as well as with a shorter duration of pregnancy, fetal growth restriction and the severity and preterm onset of the disease in pre-eclampsia [36]. In the present study, plasma ficolin-2 levels showed significant inverse correlations with renal and liver function parameters, as well as with markers of endothelial activation and injury in women with pre-eclampsia. However, after adjustment for serum sFlt-1 levels, these associations disappeared except for that with serum creatinine concentrations. These results suggest that low levels of circulating ficolin-2 due to its consumption or primary deficiency (e.g. genetically determined) might contribute to the development of generalized endothelial dysfunction and the maternal syndrome of the disease indirectly through impaired elimination from the circulation of the placentally derived material containing sFlt-1. Nevertheless, the independent inverse linear relationship of ficolin-2 levels with creatinine concentrations observed in our pre-eclamptic group might also imply a direct role of ficolin-2 in renal dysfunction. Interestingly, in IgA nephropathy, glomerular deposition of ficolin-2 with local lectin pathway activation was associated with more severe renal disease [37].

According to our findings, pregnant women with low circulating levels of ficolin-2 or ficolin-3 have an increased risk for pre-eclampsia. Low ficolin-2 and ficolin-3 levels have already been linked to various pathological conditions, such as combined allergic and infectious respiratory disease in children [38,39], bronchiectasis [40], prematurity, low birth weight and perinatal infections [41], sarcoidosis [42], susceptibility to fever and neutropenia in pediatric cancer patients [43] and to neonatal sepsis [44]. Moreover, our research group has demonstrated recently that low ficolin-3 levels in early follow-up serum samples are related to the severity and unfavourable outcome of acute ischaemic stroke [45]. Genetic variations were shown to affect ligand binding or circulating levels of ficolins [46–48] and to associate with several disorders, including rheumatic fever and chronic rheumatic heart disease [49], bacterial and cytomegalovirus infections after orthotopic liver transplantation [50,51], and even immunodeficiency [52]. As pre-eclampsia is a multifactorial disease with genetic components, the role of ficolin gene polymorphisms should be examined in the future in the risk of this pregnancy-specific disorder.

In our study, the similar plasma ficolin-2 and ficolin-3 levels of pre-eclamptic patients regardless of the severity, the time of onset of the disease or the presence of fetal growth restriction might be explained by the complex aetiology of pre-eclampsia. Several genetic, behavioural and environmental factors need to interact to produce the complete picture of this pregnancy-specific disorder. We reported various genetic and soluble factors that were associated with the severity or complications of pre-eclampsia, including HELLP syndrome and fetal growth restriction [53–56]. Nevertheless, it is also possible that the relatively small sample size of this study prevented the detection of an effect in the subgroup analyses. Although pre-eclampsia is predominantly a disease of primiparas, multiparous women, especially with advanced age or over weight, can also be affected, as in our cases.

In conclusion, circulating levels of ficolin-2 are decreased in the third trimester of normal pregnancy. There is a further decrease in plasma ficolin-2 concentrations in pre-eclampsia, which might contribute to the development of the maternal syndrome of the disease through impaired removal of the trophoblast-derived material released into the maternal circulation by the hypoxic and oxidatively stressed pre-eclamptic placenta.

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Disclosure

The authors have no conflicts of interest to disclose.

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