High temperature requirement A1 in placental tissues and serum from pre-eclamptic pregnancies with or without fetal growth restriction

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Abstract

Introduction: Pre-eclampsia (PE) is the most serious syndrome of human pregnancy and it is potentially life-threatening for both mother and fetus. The aim of the study was to identify the role of high temperature requirement A1 (HtrA1) in pre-eclampsia.

Material and methods: One hundred consecutive pregnancies complicated by PE and 100 normal controls were included in our study. The changes in serum HtrA1 and fetal growth restriction were recorded. The placentae after delivery was also obtained for laboratory analyses.

Results: High temperature requirement A1 expressed positively in all placenta tissues, but showed higher expression from control, PE with AGA (pre-eclamptic pregnancies with appropriate-for-gestational-age newborns) to PE with fetal growth restriction (FGR) groups. Early-onset PE happened more frequently while in PE with AGA, late-onset PE was more common. Additionally, we found that only during ~28-32 gestational weeks, sera HtrA1 level of PE with AGA and PE with FGR was increased significantly compared with the control group ($p < 0.05$). In contrast, there was no significant difference between groups in other gestational ages in the third trimester ($p > 0.05$).

Conclusions: HtrA1 could potentially affect trophoblast migration and invasion during placentation, resulting in the shallow invasion noted in pre-eclampsia. HtrA1 may play an important role in the etiology and severity of PE and FGR. But the actual mechanism still needs deep research.

Key words: pre-eclampsia, high temperature requirement A1, pregnancy, fetal growth restriction, etiology.

Introduction

Pre-eclampsia (PE) is the most serious syndrome of human pregnancy and it is potentially life-threatening for both mother and fetus. In developed countries, where the diagnosis and management of the disease is a major aim of prenatal care, maternal mortality attributable to PE has been reduced. However, perinatal and long-term morbidity and neurological sequelae, due to fetal growth restriction (FGR) and/or preterm delivery, are still critical problems [1, 2]. Nowadays, there are no effective interventions to prevent or cure PE except for a timed and often preterm delivery [3].

In many cases PE is associated with poor placentation in the beginning of pregnancy with a progressively hypoxic placenta [3], which contributes to the maternal signs of PE [1]. Recent studies show an ischemic placenta with a high-resistance vasculature, which cannot deliver an adequate blood supply to the feto-placental unit [2]. In addition, there is marked proliferation of villous cytotrophoblastic cells and the syncytiotrophoblast shows focal necrosis [4]. The cause of PE is a matter of debate, but recent studies in mice suggest that the primary feto-placental lesions are sufficient to initiate the disease [5]. The abnormal implantation may represent the unsuccessful interaction between the trophoblast of the placenta and the spiral arteries of the mother [6]. The pathology of shallow implantation is evident both in the placental extravillous trophoblast and in maternal spiral arteries. Extravillous trophoblast invasion into the spiral arteries is restricted to the decidua, and maternal spiral arteries fail to become low-resistance vessels [7, 8].

An increasing body of evidence shows that PE is associated with increased disordered proliferation and increased formation of syncytial knots [9-11]. In multicellular tissues such as placental tissues (trophoblastic layer, villous stroma, cell islands and cell columns), cell turnover is tightly regulated. Indeed, impaired proliferation and differentiation are associated with failure of normal placental development and pregnancy. When FGR is observed in PE, the surface area of placental villi is reduced and is associated with a decrease in umbilical blood flow [4, 12].

High temperature requirement A1 (HtrA1), a member of the family of HtrA proteins, is a secreted multidomain protein with serine protease activity. It is characterized by the presence of a trypsin-like serine protease domain and one PDZ domain. It also contains an IGFBP/mac25-like domain and a kazal-type inhibitor domain at the N-terminus [13]. Recently, it has been shown that HtrA1 can be expressed in a native protein of 50 kDa and a 30 kDa autocatalytic product [14]. Evidence is now accumulating to suggest that HtrA1 is involved in the physiological development of many organs [15-17] as well as in development and progression of several pathologies, including neoplastic and degenerative diseases [18-20]. High temperature requirement A1 is expressed during gestation, suggesting a role in placental development [17, 21].

Particularly, in specimens from the first trimester of gestation, immunostaining for HtrA1 was generally found in both layers of the villous trophoblast, syncytiotrophoblast and cytotrophoblast [17, 21]. Cytoplasm of the extravillous trophoblast and extracellular matrix of cell islands and cell columns was labeled for HtrA1. Specimens from the third trimester of gestation showed a more intense positivity for HtrA1 in the syncytiotrophoblast than in the cytotrophoblast [17, 21]. The extravillous trophoblast and the decidual cells were positive for HtrA1 [21]. These data suggest a role of HtrA1 in placental formation and function, particularly in differentiation of the trophoblastic layer. Recently, two broad categories of PE, i.e. maternal and placental PE, have been described [22]. In placental pre-eclampsia, the problem arises from a placenta that is under hypoxic conditions and it is mainly associated with IUGR. In maternal PE the problem arises from the interaction between a normal placenta and a maternal constitution that suffers from microvascular disease [22, 23]. We hypothesized that HtrA1 expression could be altered in PE.

The purpose of this study was to investigate the expression pattern of HtrA1 in placentas and sera from PE with IUGR (placental PE) and without IUGR (maternal PE) to clarify the possible implication of HtrA1 in the pathogenesis of this disease.

Material and methods

Pre-eclamptic cases

One hundred consecutive pregnancies complicated by PE were included in our study. Pre-eclampsia was defined by presence of hypertension (systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg) accompanied by proteinuria (≥ 300 mg/24 h), after 20 weeks of gestational age in previously normotensive patients [24]. All patients were normotensive in the second trimester.

The diagnosis of FGR was made according to the following criteria:

1) Ultrasound measurement of the fetal abdominal circumference below the 10th centile [25] or fetal growth rate below 5 mm/week at serial measurements;
2) Abnormal Doppler flow velocity waveforms (Doppler-FVWs) of the uterine arteries [26] and abnormal Doppler-FVWs of the umbilical arteries [26];
3) Birth weight below the 10th centile according to our birth weight references values [27, 28].

Exclusion criteria were multiple pregnancies, pregnancies complicated by prenatal or postnatal diagnosis of structural and/or chromosomal anomalies, and pre-pregnancy diseases (chronic hypertension, diabetes, etc.). All PE pregnancies were delivered by caesarean section (upon requirement of the local patients, the population of which has a large proportion of caesarean section choice).

Control cases

Controls were 100 normotensive pregnancies with normal fetal growth and normal uterine and umbilical Doppler FVWs, delivered at term by cae-

sarean section, because of breech presentation or previous caesarean section.

In all cases and controls, pregnancies were dated by an ultrasound scan in the first trimester of pregnancy.

Clinical parameters

The following data were collected for both cases and controls: maternal age at delivery, parity, body mass index (body mass index – BMI = kg/m^2) at the beginning of pregnancy, BMI just before delivery, gestational age at birth, gestational age at onset of PE, mode of delivery, neonatal sex and weight at birth (neonatal weight was also expressed as Z-score, that is, the exact number of standard deviations from the mean for gestational age, using our birth-weight references [19]), placental weight, uterine and umbilical artery Doppler ultrasound velocimetry indexes, blood pressure, urinary protein levels, and exposure to drugs (such as antihypertensives, corticosteroids, antibiotics, aspirin).

Tissue samples

Immediately after delivery, normal and pathological placentae were transported from the delivery room to the laboratory. High temperature requirement A1, affinity-purified rabbit polyclonal antiserum raised against paraffin sections from placenta was analyzed for HtrA1 expression by immunohistochemistry (IHC). Anti-human HtrA1 monoclonal antibody (ABgent Company, USA), diluted 1 : 100 in TBS, was used. For each case, a negative control was obtained by using the antibody preadsorbed with the recombinant HtrA1 at the concentration of 20 µg/ml of diluted antibody. We counted 5 randomly chosen fields per section using a light microscope at high power (400 magnification; Q550CW Leica Corp., Germany) and the results were expressed as mean grey number (MGN). The higher the value of MGN, the greater was the expression of HtrA1 in placentas.

Blood samples

During the third trimester, every 4 weeks, peripheral venous blood samples were collected in vacutainer tubes without anticoagulant, from mothers with normal and pathological pregnancies. Serum was separated by centrifugation immediately after clotting and stored at –20°C until assayed. Concentration of HtrA1 in maternal serum samples was determined by a HtrA1 ELISA assay.

Statistical analysis

Patient age, BMI, gestational age, neonatal and placental weight, blood pressure readings, and HtrA1 expression on the placental tissues and HtrA1 concentrations in sera were reported as mean and standard deviations (SDs). Means among groups were compared using a one-way analysis of variance (ANOVA). Tamhane *post hoc* tests, chosen to account for unequal variances, were performed to identify significant differences between the dependent variables at $\alpha < 0.05$. Categorical and nominal values (urinary protein levels, exposure to pharmaceuticals) were analyzed by the χ^2 test. A value of $p \leq 0.05$ was considered significant. Statistical evaluation was performed using SPSS 11.0 for Windows.

Results

Patient characteristics

The three study groups, controls, PE-AGA, and PE-FGR, were comparable for maternal age and prepregnancy BMI (Table I).

All PE pregnancies differed from controls for neonatal birth weight, placental weight, blood pressure, urinary protein values and percentage of patients receiving corticosteroids or antihypertensives (Table I). For PE-AGA, the gestational age of delivery and the S/D ratio did not differ from controls, while for PE-FGR, those two values were significantly different from controls ($p < 0.001$, 0.038) (Table I).

The two subgroups of pregnancies complicated by PE did not significantly differ only for diastolic pressure. In the PE-FGR group, gestational age at delivery and onset of PE, and neonatal and placental weight, were lower than in PE-AGA, while the systolic pressure and S/D ratio were higher than in PE-AGA; moreover, there were differences between the two groups in urinary protein levels (Table I).

High temperature requirement A1 expression in placental tissues and in sera

We observed that HtrA1 was dispersed in the cytosol of placental cells. Moreover, we observed clear positivity for HtrA1 close to the microvilli that characterize the plasma membrane of syncytiotrophoblast and cytotrophoblast cells, in accordance with the fact that HtrA1 can be a secreted protein. In addition, we observed a peculiar localization of HtrA1 protein in extracellular matrix of decidual tissue, consistent with a secreted protein (Figures 1-3). But for PE-AGA, the MGN was less than the control ($p = 0.043$). The MGN of PE-FGR was less than both the control and PE-AGA ($p = 0.017$, 0.027, respectively) (Table II).

Only when the gestational week was within ~28–32 weeks was the mean serum concentration of HtrA1 in PE-AGA higher than the control, and serum HtrA1 of PE-FGR was higher than the control and the PE-AGA. In other gestation weeks in the third

Table I. Clinical characteristics of control and pathological pregnancies

Parameter	Control	PE-AGA	PE-FGR	Value of *p*
Number of patients	100	83	17	
Maternal age of delivery, mean ± SD [years]	28.6 ±4.9	27.3 ±7.5	29.1 ±5.2	NS
Pre-pregnancy BMI, mean ± SD [kg/m^2]	21.2 ±3.8*#	23.3 ±2.8*&	24.6 ±3.2#&	NS
Gestational age at delivery, mean ± SD [weeks]	39.2 ±2.5*#	37.3 ±3.2*&	31.6 ±2.1#&	*NS #&< 0.001
Neonatal birth weight, mean ± SD [g]	3317.9 ±408.6*#	2902.5 ±734.6*&	2011.6 ±506.4#&	*0.013 #< 0.001 &0.027
Placental weight, mean ± SD [g]	522.2 ±67.0*#	408.2 ±82.2*&	254.2 ±43.2#&	*0.043 #&< 0.001
Gestational age at onset of PE, mean ± SD [weeks]	NA	37.2 ±7.2&	30.2 ±3.5&	&< 0.001
Blood pressure, mean ± SD [mm Hg]				
Systolic	116.2 ±30.0*#	155.8 ±24.6*&	168.5 ±37.2#&	*#<0.001 &0.042
Diastolic	79.3 ±13.6*#	96.2 ±17.2*&	95.4 ±21.2#&	*#< 0.001 &NS
Proteinuria, number and %				
< 1 g/24 h	0 (0)*#	22 (26.5)*&	0 (0)#&	*#&< 0.001
< 5 g/24 h	0 (0)*#	60 (72.3)*&	8 (47.1)#&	*#&< 0.001
≥ 5 g/24 h	0 (0)*#	1 (0.02)*&	9 (52.9)#&	*#&< 0.001
S/D ratio, mean mean ± SD	2.6 ±0.6*#	2.9 ±1.1*&	3.7 ±0.7#&	*NS #0.038 &0.03
Patients receiving, number and %				
Corticosteroids	0 (0)*#	36 (43.4)*&	15 (88.2)#&	*#&< 0.001
Antihypertensives	0 (0)*#	56 (67.5)*&	17 (100)#&	*#&< 0.001
Complication and %				
HELLP syndrome	0 (0)*#	0 (0)*&	1 (5. 9)#&	*NS #&< 0.001
Placental abruption	0 (0)*#	2 (2.4)*&	1 (5. 9)#&	*#&< 0.001

*PE – preeclampsia, PE-AGA – pre-eclamptic pregnancies with appropriate-for-gestational-age newborns, PE-FGR – pre-eclamptic pregnancies with fetal growth restriction, BMI – body mass index, NS – not significant, NA – not available, values of p were calculated by ANOVA test, followed by Tamhane test for pairwise comparison, or by χ^2 test; *Comparison between controls and PE-AGA group, #comparison between controls and PE-FGR group, &comparison between PE-AGA and PE-FGR groups*

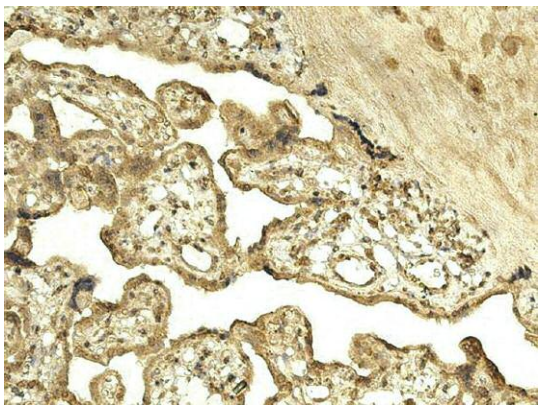

Figure 1. Expression of HtrA1 in control group (200×)

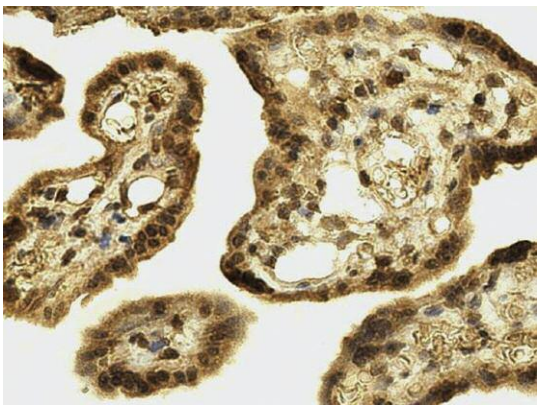

Figure 2. Expression of HtrA1 in PE-AGA (400×)

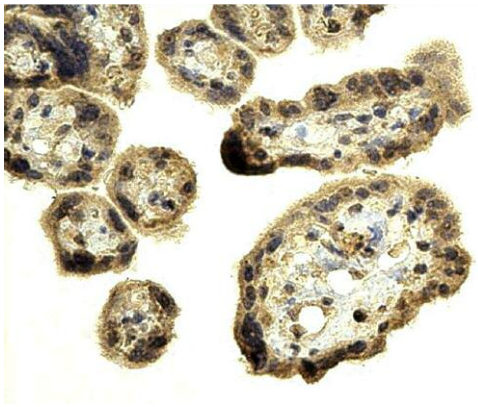

Figure 3. Expression of HtrA1 in PE-FGR (400×)

trimester, there was no significant difference between any groups (Table II).

Discussion

Pre-eclampsia occurs in approximately 5% to 10% of nulliparous pregnancies. It is among the top three causes of maternal mortality in both developed and developing countries and is one of the leading causes of maternal and fetal morbidity. We found that women who experience pre-eclampsia are at increased risk for adverse pregnancy outcomes, with its prognosis depending on the severity of the disease and the gestational age at the time of disease onset and at delivery. Pre-eclampsia-fetal growth restriction occurred more frequently in early-onset PE, which has been associated with worse perinatal outcomes such as small-for-gestational age infants, compared to pre-eclampsia that has onset at term. Pre-eclampsia-fetal growth restriction is also characterized by increased severity, including HELLP syndrome and placental abruption. All these conclusions were the same as those of Jen Jen Chang *et al*. [29].

High temperature requirement A1 is a member of the HtrA serine protease family, and is widely present in microorganisms, plants and animals. High temperature requirement A1 can be secreted by various tissue cells, including the mature epithelial tissue layer of cells, mammary epithelial cells, liver bile duct cells, and ovarian granulosa cells; during pregnancy the HtrA1 is often sourced from placental cells. Previous studies showed that HtrA1 was upregulated in secretory endometrial glands and decidual cells during pregnancy, suggesting potential function in fecundity implantation processes [17]. Placental development is a rather complex process involving the degradation of extracellular matrix (ECM), and HtrA1 is involved in degradation of ECM proteins such as fibronectin [30] and aggrecan [31]. During the early pregnancy period the decidua decorin was accumulated, which could inhibit the migration and invasion of trophoblast cells. High temperature requirement A1 is a secreted protease believed to be involved in degradation of ECM [13]. Its specific expression in the trophoblast during the period of active invasion strongly suggests that HtrA1 is a previously unidentified protease involved in trophoblast invasion. Our study showed that HtrA1 expressed positively in all placental tissues, but exhibited higher expression from control, PE with AGA to PE with FGR groups. It was in accordance with our previous study [32]. For PE with FGR, we also found that early-onset PE happened more frequently while in PE with AGA, late-onset PE was more common. Thus, we assumed that HtrA1 could potentially affect trophoblast migration and invasion during placentation, resulting in the shallow invasion noted in PE.

We found that only during gestational weeks ~28–32, sera HtrA1 level of PE with AGA and PE with FGR was increased significantly compared with the control group. In contrast, there was no significant difference between any groups in other gestational ages in the third trimester. The placenta has a dynamic and continuous capacity for self-renewal. Throughout gestation the placenta shows a high cell proliferation rate and lack of cell contact inhibition. Most of the continuous remodeling of this tissue is the result of apoptotic events. Moreover, the stepwise progression from the villous cytotrophoblast to the invasive extravillous cytotrophoblast is characterized by dramatic change in expression of cell adhesion molecules and proteinases that degrade the ECM. Abnormalities in either apoptotic or invasive function of the tissue may cause some

Table II. Mean expression of HtrA1 in placental tissues and in sera

	Controls	PE-AGA	PE-FGR	Value of *p*
MGN in placental tissues, mean ± SD	165.82 ±3.20$^{*\#}$	156.03 ±5.07$^{*\&}$	152.69 ±4.11$^{\#\&}$	*0.043 $^{\#}$0.017 $^{\&}$0.027
Concentration in sera in different gestational weeks, mean ± SD				
~28–32 [week]	28.18 ±2.40$^{*\#}$	42.66 ±2.24$^{*\&}$	47.32 ±3.22$^{\#\&}$	$^{*\#}$ < 0.001 $^{\&}$NS
~32–36 [week]	44.68 ±2.40$^{*\#}$	46.77 ±2.57$^{*\&}$	46.43 ±2.70$^{\#\&}$	$^{*\#\&}$NS
~36–40 [week]	46.77 ±2.45$^{*\#}$	44.68 ±2.24$^{*\&}$	47.64 ±2.94$^{\#\&}$	$^{*\#\&}$NS

Abbreviations – see Table I

diseases, such as miscarriage, PE, etc. A recent study showed that HtrA1 expressed differently in different gestational ages according to different trophoblast cells [16, 17]. From our research, it seemed that HtrA1 changed continuously accompanied with placentation. During ~28–32 weeks, changes of serum HtrA1 may have a relationship with onset of PE. After this time, the placenta may enter a comparatively stable situation, so that it causes stable serum HtrA1.

This is the first study to analyze the placental and sera expression of HtrA1 protein in pregnancies complicated by PE with and without FGR. Interestingly, Ajayi *et al*. [33] found that the ectopic expression of HtrA1 in cultured extravillous trophoblastic cell line HTR-8/SVneo attenuated chemotactic migration and invasion of these cells. All these data suggest that HtrA1 could potentially affect trophoblast migration and invasion during placentation, resulting in the shallow invasion noted in preeclampsia. High temperature requirement A1 may play an important role in the etiology and severity of PE and FGR. But the actual mechanism still needs deep research.

Acknowledgments

Lu Zong and Lijuan Wang contributed equally to this work.

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