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Original Article

Preeclampsia is associated with low placental transthyretin levels

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ABSTRACT

Objective: To investigate the relationship between placental transthyretin (TTR) level and preeclampsia.
Materials and Methods: Placental tissues from uncomplicated and preeclamptic pregnancies were analyzed using immunohistochemistry and image analysis. We measured the mean optical density (OD) of immunohistochemical staining of TTR across multiple sections using Image Pro Plus 6.0. To avoid bias, we used placental tissue array, which contained preeclamptic placentas ($n = 8$) and the control placentas ($n = 6$) on the same slide.
Results: The mean TTR OD of the syncytiotrophoblast layer of placentas (95% confidence interval) from the first trimester was higher than those from the second/third trimester, and term placentas [0.149 (0.014–0.285) for the 1st trimester, 0.037 (0.000–0.073) for the 2nd/3rd trimester, and 0.011 (0.035 –0.056) for term; $p < 0.01$]. Although the OD of the second/third trimester placentas appeared greater than that of term placentas, this was not statistically significant. The mean TTR OD of the syncytiotrophoblast layer of the severe preeclampsia group was lower than that of controls [0.010 (0.005–0.016) vs. 0.027 (0.013–0.041), $p < 0.05$].
Conclusion: The immunohistochemical expression of TTR in the syncytiotrophoblast layer of the placenta decreased significantly after 12 weeks of gestation, paralleling the changing demands of thyroid hormone uptake into the placenta. The reduced TTR expression in the syncytiotrophoblast layer of the preeclamptic placenta might impair thyroid hormone uptake and contribute to the pathophysiology of the disease.

Introduction

Preeclampsia (PE) is an idiopathic multisystem disorder characterized by new-onset hypertension and proteinuria after 20 weeks of gestation. It is the leading cause of maternal and perinatal morbidity and mortality [1]. The pathophysiology underlying PE is still incompletely understood, although it is widely accepted to involve a failure of appropriate placental development [2]. Differential gene and protein expression [3], or initial low oxygen environment [2] can cause abnormal growth of placenta and fetus [4]. Nevertheless, the precise cause of the pathological changes within the placenta prior to the onset of PE is still unclear.

The critical role of thyroid hormones (THs) in the development of many organs is well recognized. Hypothyroidism may cause infertility, increased risk of miscarriage, and other obstetric complications including PE [5,6]. Transthyretin (TTR), also known as prealbumin, is a TH carrier, and it has been suggested that TTR might influence the proliferation and differentiation of placental trophoblast through regulating exposure of placental cells to TH [7]. As a carrier protein, TTR transports multiple molecules, including THs and retinol. Elevated levels of TTR have been reported in the amniotic fluid of fetuses with abnormal karyotypes, including trisomy 21 and trisomy 18 [8]. In serum, TTR levels decrease after 12 weeks of gestation [9], similar to the changing demands of TH of fetus. In the placenta, TTR seems to be constant after 13 weeks of gestation [10]; however, the increased expression of TTR in the matrix and vessels within the placenta after 13 weeks suggests that

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expression in the syncytiotrophoblast (where the TTR transporter regulates TH uptake) should be reduced then.

Several studies compared total TTR levels between severe preeclampsia (sPE) and control placentas and found no correlation [11,12]. Recognizing TTR's role as a placental transporter, we aimed to study its spatial distribution. In this study, we measured TTR expression and distribution in normal placentas across gestation. We also compared TTR expression in sPE placentas and gestational matched controls to investigate the possible relationship between PE and placenta TTR.

Materials and methods

Patients

The diagnosis of sPE was based on the criteria of the National High Blood Pressure Education Program Working Group on high blood pressure in pregnancy. The criteria included the presence of high blood pressure of $\geq$ 160/110 mmHg on two occasions at least 6 hours apart that occurred after 20 weeks of gestation in women with previously normal blood pressure, accompanied with proteinuria ($\geq$ 5 g/24 hours) or proteinuria of $\geq$ 3+ on two random urine samples collected at least 4 hours apart. None of the patients had a history of hypertension or renal diseases. The control participants were age matched without hypertension or proteinuria or other pregnancy complications except preterm labor. This study was conducted in accordance with the provisions of the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the Research Centre for Women's and Infant's Health (RCWIH) Biobank at Mount Sinai Hospital (Mount Sinai Hospital Research Ethics Board, MSH REB# 04-0018-U). Written informed consent was obtained from all participants.

We examined TTR expression in normal placentas across gestation. First trimester placentas (6–12 gestational weeks) were obtained from elective terminations of pregnancies by dilatation and curettage. Second trimester and preterm placentas were from elective termination and from preterm labor patients without other pregnancy complications. Term placentas were from spontaneous labor or cesarean delivery.

Placenta immunohistochemistry

All samples were subjected to the same experimental methods. Briefly, paraffin blocks were cut into 4-μm-thick sections. After dewaxing in xylene and rehydration in graded alcohol, the sections were heated in 0.1 mol/L sodium citrate buffer (pH 6.0) in a microwave oven for 10 minutes for antigen retrieval. The slides were then incubated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Nonspecific binding was blocked by incubation in blocking solution (protein block, serum-free solution; Dako, Carpinteria, CA, USA) for 10 minutes. Subsequently, the slides were incubated for 1 hour at room temperature with specific Anti-Human-Transthyretin antibody (Dako) diluted 1:200; in the case of the negative controls, the primary antibody was replaced with phosphate-buffered saline. Slides were then incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (Dako; 1:300 dilution) for 30 minutes at room temperature. Substrate-chromogen DAB was added to each slice for 2 minutes, and the reaction was stopped by washing in excess tap water. Hematoxylin was used as a counterstain.

Western blotting

Placental tissue from 10 sPE patients and six gestational age-matched controls was homogenized in 250mM sucrose, 10mM Hepes/Tris, pH 7.4 buffer containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Next, 40 μg total protein was separated in NuPAGE Novex 4–12% Bis/Tris gradient gels (Invitrogen, Grand Island, NY, USA) and transferred to a nitrocellulose membrane (Millipore, Billerica, MA, USA) for 30 minutes. Blotting was performed by incubation of the membranes with Rabbit anti-TTR (Dako) antibody (1:1000 dilution) overnight at 4°C. Membranes were incubated with the secondary antibody (antirabbit immunoglobulin G-HRP; 1:3000 in Tris-buffered saline/Tween 20; Dako) for 30 minutes. Detection of bound antibody was performed with Pierce chemiluminescent (ECL) kit (Thermo, Waltham, MA, USA). Levels of proteins were then quantified using Quantity-One program (Bio-Rad Laboratories). TTR protein levels were normalized to β-actin.

Placental tissue array

Immunohistochemical (IHC) technique is a qualitative examination, and its quantitative analysis might be influenced by many procedures including tissue selection, paraffin blocks section, and staining. To avoid these biases as much as possible, we used the method of placental tissue array. Tissue array slides were obtained from the RCWIH BioBank program of Mount Sinai Hospital, Toronto, Ontario, Canada, in accordance with the policies of the Mount Sinai Hospital Research Ethics Board (MSH, 10-0128-E). Each of the tissue array slides contains sections from eight placentas from sPE patients and six gestational matched preterm labor controls (Table 1). The placentas used for tissue array were chosen by an experienced pathologist, and each placenta was sampled from four separate locations. The tissue array makes it possible to test TTR in all the placentas at the same time using exactly the same IHC procedures. All tissues are located randomly on the slides and coded until the quantitative analysis was completed.

There was no significant difference in gestational age, gravidity, and parity between the sPE group and the control group. For all the placentas, no fetal growth restriction was diagnosed.

Quantitative analysis

TTR is expressed in both the syncytiotrophoblast layer and the stroma of the placenta. We used Image Pro Plus version 6.0 (Media Cybernetics, Bethesda, Maryland, USA) to analyze the digital images. This method of IHC quantification has been used by several other groups [13,14]. To avoid possible bias in light intensity during image capture and to ensure the accuracy of measurements, image brightness was normalized to background levels. All images are taken at exactly the same conditions using the same microscope and camera (Olympus DP72, ISO 200, Olympus, Tokyo, Japan). We selected syncytiotrophoblast layer and stroma separately as area of interest (AOI) for measurements. Using Image Pro Plus, we changed the images to grayscale digital images to test total optical density (OD) and calculate the area of AOI. We then calculated the mean OD using total OD/area of AOI. The mean OD represents the density of dye staining and reflects the content of TTR.

Table 1
Characteristics of the severe PE and control groups.

	sPE	Controls
n	8	6
Gestational age (wk)	29 ± 1.7	31 ± 2.4
Gravidity	1.4 ± 0.5	2.0 ± 1.1
Parity	1.0 ± 0.0	1.5 ± 0.8
Placental weight (g)	281 ± 60	385 ± 91
Birth weight (g)	1046 ± 238	1822 ± 328

sPE = severe preeclampsia.