

# Influence of serum HMW adiponectin level in patients with pregnancy-induced hypertension syndrome on the occurrence of eclampsia in secondary pregnancy

QIAN XU, DONGMEI FAN, FAHONG LI and ZHANHONG ZHANG

Department of Obstetrics and Gynecology, Qingdao Women and Children Hospital,  
Qingdao, Shandong 266034, P.R. China

Received January 30, 2017; Accepted July 17, 2017

DOI: 10.3892/etm.2017.5112

**Abstract.** In the present study, we studied the influence of serum high-molecular weight adiponectin (HMWA) levels in patients with pregnancy-induced hypertension (PIH) on the occurrence of eclampsia in secondary pregnancy and its related mechanisms. In total, 130 patients who were diagnosed with PIH for the first time were selected for this study; the median interval of the secondary pregnancy was 28.5 months. The serum HMWA and leptin levels both times were detected, and the insulin resistance indexes (HOMA-IR) were calculated. The serum inflammatory indexes in the secondary pregnancy included interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels, and the oxidative stress indexes included methane dicarboxylic aldehyde (MDA) and oxidized low-density lipoprotein (ox-LDL) levels. The expression levels of adiponectin receptor 2 and cyclooxygenase-2 (COX-2) in placental tissue were detected. In secondary pregnancy, there were a total of 20 cases of eclampsia (15.38%), including 2 cases of mild PIH, 8 cases of moderate PIH and 10 cases of severe PIH; differences were statistically significant when compared to patients without eclampsia ( $p < 0.001$ ). The serum HMWA levels in patients with severe PIH in the first pregnancy were significantly lower than those in patients with mild and moderate PIH, and the serum levels in patients with mild PIH were the highest. The leptin levels in patients with severe PIH were significantly higher than those in patients with mild and moderate PIH, and the leptin levels in patients with mild PIH were the lowest ( $p < 0.05$ ). The HMWA levels in patients with eclampsia in the secondary pregnancy was significantly lower than those in patients without eclampsia, and the leptin levels in patients with eclampsia were significantly increased.

The HMWA levels in patients with eclampsia in the secondary pregnancy were lower than that in the first pregnancy, whereas the leptin levels were higher than that in the first pregnancy ( $p < 0.05$ ). HOMA-IR, IL-6, TNF- $\alpha$ , MDA and ox-LDL levels in patients with eclampsia were significantly higher than those in patients without eclampsia ( $p < 0.05$ ), and the adiponectin receptor 2 and COX-2 expression levels in the placental tissue were significantly higher than those in patients without eclampsia ( $p < 0.05$ ). Therefore, the serum HMWA levels are closely related to the occurrence of eclampsia in PIH patients in secondary pregnancy, and it influences insulin resistance, inflammatory response and oxidative stress response, which is correlated with increased adiponectin receptor 2 and COX-2 protein expression in placental tissue. Consequently, HMWA may be an important target for the intervention of preventing eclampsia for PIH patients in secondary pregnancy.

## Introduction

Pregnancy-induced hypertension (PIH) is an important risk factor that affects both the maternal and fetal health as well as the pregnancy outcome in the perinatal period. Under severe PIH, the failure rate of expectant treatment for patients with eclampsia is as high as 80-90%, and the fetal mortality is 5-20% (1). Studies have increasingly focused on the occurrence mechanism of PIH, which involves endothelial cell damage, immune response, oxidative stress, inflammatory reaction and abnormal blood lipid metabolism, thrombosis and unified theory (2). Adiponectin is an active cytokine that is secreted by fat cells and has been shown to have an important role in the formation of an atherosclerotic plaque, and in the occurrence and development of hypertension, insulin resistance, metabolic syndrome and diabetes (3). When PIH is aggravated, the adiponectin levels are gradually decreased, whereas the leptin levels are gradually increased. Under mutual coordination, adiponectin and leptin play important roles in endothelial inflammatory response and lipid metabolism (4). Currently, with the liberalization of the 'two-child policy', the proportion of pregnant women at an advanced age has been increased and the mortality of the second child has shown an increasing trend (5). There is less research on whether the occurrence of eclampsia in PIH patients in secondary pregnancy is related to

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*Correspondence to:* Dr Zhanhong Zhang, Department of Obstetrics and Gynecology, Qingdao Women and Children Hospital, 6 Tong Fu Road, Qingdao, Shandong 266034, P.R. China  
E-mail: zhangzhanhong2016@163.com

*Key words:* pregnancy-induced hypertension, high-molecular weight adiponectin, eclampsia, insulin resistance, inflammatory response, oxidative stress, adiponectin receptor 2, cyclooxygenase-2

the serum high-molecular weight adiponectin (HMWA) levels, and whether the occurrence mechanism is different.

In this study, we aimed to provide a reasonable target for the intervention of reduction of the occurrence of eclampsia for PIH patients.

## Materials and methods

**Object data.** A total of 130 patients who were diagnosed with PIH for the first time at our hospital from January 2013 to June 2016 were selected. Inclusion criteria were as follows: i) Patients were aged 18-35 years, in the first pregnancy without a history of abortion, and primary and secondary hypertension; ii) patients with natural conception, single live birth, normal fetal development without intrauterine hypoxia and other symptoms; iii) patients at the gestational week of <20 weeks; iv) patients with an interval of secondary pregnancy of  $\geq 12.0$  months, natural conception without the history of abortion; v) patients with complete clinical data and informed consent rights were obtained. Exclusion criteria were as follows: Patients with obesity complicated with coronary heart disease, diabetes, liver and kidney dysfunction.

According to the PIH grading standard, there were 59 cases of mild PIH, 49 cases of moderate PIH and 22 cases of severe PIH. The average age was  $26.5 \pm 4.9$  years and the average pre-pregnancy BMI was  $20.8 \pm 3.4$  kg/m<sup>2</sup>. There were 89 cases of full-term birth and 41 cases of premature birth, 95 cases of spontaneous delivery and 35 cases of cesarean delivery, of which 121 fetuses survived. There were 2 cases of fetal death in patients with moderate PIH and 7 cases in patients with severe PIH; the average birth weight of fetuses was  $2.6 \pm 0.5$  kg and the interval of the pregnancy was 15.0-35.9 months with a median time of 28.5 months.

**Research methods and observation indexes.** The ELISA method was used to detect the serum HMWA and leptin levels of the two pregnancy types. Radioimmunoassay (RIA) was used to detect fasting insulin levels and full-automatic biochemical methods were employed to detect fasting blood glucose levels, and the insulin resistance index (HOMA-IR) was calculated. The serum inflammatory indexes in the secondary pregnancy was detected by ELISA, which included interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels. The oxidative stress indexes included methane dicarboxylic aldehyde (MDA) and oxidized low-density lipoprotein (ox-LDL) levels. Western blot analysis was used to detect the adiponectin receptor 2 and cyclooxygenase-2 (COX-2) protein expression levels in placental tissue. The incidence of preeclampsia and eclampsia in the secondary pregnancy were summarized.

Serum HMWA and leptin reagents were purchased from the Jiangsu Beyotime Biotechnology Co., Ltd. (Jiangsu, China) and the insulin reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA). The blood glucose test strip was purchased from Roche Diagnostics GmbH (Mannheim, Germany), and IL-6, TNF- $\alpha$ , MDA and ox-LDL reagents were all purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China). The procedures were conducted according to the instructions of the kit. The main procedures of the western blot analysis are as follows: We obtained placental tissue and added RIPA lysis buffer after tissue homogenate. Then, we

extracted total cell protein, followed by rough quantification using a Coomassie brilliant blue method. Before detecting the protein, we used  $\beta$ -actin antibody to detect the protein content in each sample and then took 30  $\mu$ g of total protein, which was followed by separation via 8% SDS-PAGE. The protein was transferred onto the PVDF membrane, the rat anti-human adiponectin receptor 2 and COX-2 monoclonal antibody was added (1:2,000, cat. no. 187562; Invitrogen, Carlsbad, CA, USA) overnight at 4°C. Then, we added the rabbit anti-rat polyclonal secondary antibody (1:500, cat. no. BR16503; Bio-Rad Laboratories, Inc., Hercules, CA, USA) for incubation at room temperature for 4 h and washed the protein using phosphate-buffered saline (PBS), which was followed by color development using ECL. The results were scanned and saved, and semi-quantitatively analysed using Lab Works 4.5 gel imaging software (Applied Biosystems, Foster City, CA, USA), which was presented as integral optical density (IOD).

**Statistical analysis.** SPSS 20.0 statistical software (Chicago, IL, USA) was used for statistical analysis and the measurement data was presented as mean  $\pm$  standard deviation (SD). The independent sample t-test was used for intergroup comparison, and the paired t-test was used for intragroup comparison. The enumeration data are presented as case or percentage. The Chi-square test was used for intergroup comparison, and the rank sum test was used for comparison of ranked data.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Analysis of incidence of preeclampsia and eclampsia in secondary pregnancy.** In secondary pregnancy, there were a total of 20 cases of eclampsia (15.38%), including 2 cases of mild PIH, 8 cases of moderate PIH and 10 cases of severe PIH; differences were statistically significant in comparison to patients without eclampsia ( $Z = -9.309$ ,  $p < 0.001$ ).

**Comparisons of serum HMWA and leptin levels in the first and second pregnancy.** The serum HMWA levels in patients with severe PIH in the first pregnancy were significantly lower than those in patients with mild and moderate PIH, and the serum levels in patients with mild PIH were the highest. The leptin levels in patients with severe PIH were significantly higher than those in patients with mild and moderate PIH, and the leptin levels in patients with mild PIH were the lowest; differences were statistically significant ( $p < 0.05$ ). The HMWA levels in patients with eclampsia in secondary pregnancy were significantly lower than those in patients without eclampsia, and the leptin levels in patients with eclampsia were increased significantly. The HMWA level in patients with eclampsia in secondary pregnancy was lower than that in the first pregnancy, whereas the leptin level was higher than that in the first pregnancy; differences were statistically significant ( $p < 0.05$ ) (Fig. 1 and Table I).

**Comparisons of HOMA-IR, IL-6, TNF- $\alpha$ , MDA and ox-LDL levels.** The HOMA-IR, IL-6, TNF- $\alpha$ , MDA and ox-LDL levels in patients with eclampsia were significantly higher than those in patients without eclampsia; differences were statistically significant ( $p < 0.05$ ) (Table II).

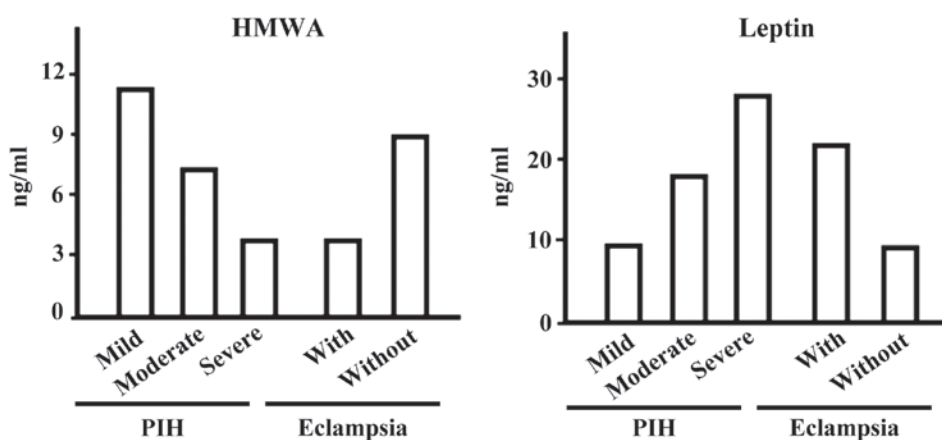


Figure 1. Comparisons of serum HMWA and leptin levels in the two pregnancy types. HMWA, high-molecular weight adiponectin.

Table I. Comparisons of serum HMWA and leptin levels in PIH patients in different degrees in the two pregnancy types (ng/ml).

Parameters	Mild PIH (n=59)		Moderate PIH (n=49)		Severe PIH (n=22)	
	Eclampsia (n=2)	No eclampsia (n=57)	Eclampsia (n=8)	No eclampsia (n=41)	Eclampsia (n=10)	No eclampsia (n=12)
<b>HMWA</b>						
First pregnancy	10.8±3.6	11.2±3.9	7.3±2.5	7.5±2.4	4.2±1.5	4.5±1.4
Secondary pregnancy	8.5±3.2	10.9±3.7	6.2±2.2	7.6±2.3	3.6±1.3	4.4±1.2
<b>Leptin</b>						
First pregnancy	9.5±2.7	9.3±2.8	17.8±3.1	17.5±3.2	28.9±3.6	28.2±3.5
Secondary pregnancy	11.2±2.9	9.2±2.8	20.1±3.5	17.6±3.3	33.6±3.9	28.4±3.6

HMWA, high-molecular weight adiponectin; PIH, pregnancy-induced hypertension.

Table II. Comparisons of HOMA-IR, IL-6, TNF- $\alpha$ , MDA and ox-LDL levels.

Groups	HOMA-IR	IL-6 ( $\mu\text{mol/l}$ )	TNF- $\alpha$ ( $\mu\text{mol/l}$ )	MDA ( $\mu\text{mol/l}$ )	ox-LDL ( $\mu\text{g/l}$ )
Eclampsia	2.33±0.54	66.95±25.62	32.56±10.32	4.65±1.02	659.73±123.54
No eclampsia	1.98±0.49	45.62±15.64	15.93±7.58	2.64±0.68	352.89±102.52
t-test	3.659	4.527	4.869	5.027	5.329
P-value	0.037	0.032	0.026	0.023	0.018

IL-6, interleukin-6; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; MDA, methane dicarboxylic aldehyde; ox-LDL, oxidized low-density lipoprotein.

Table III. Comparisons of adiponectin receptor 2 and COX-2 protein expression levels in placental tissue.

Groups	Adiponectin receptor 2	COX-2
Eclampsia	0.65±0.17	0.58±0.19
No eclampsia	0.34±0.11	0.26±0.08
t-test	6.235	6.127
P-value	0.006	0.010

COX-2, cyclooxygenase-2.

*Comparisons of adiponectin receptor 2 and COX-2 protein expression levels in placental tissue.* Adiponectin receptor 2 and COX-2 protein expression levels in placental tissue in patients with eclampsia were significantly higher than those in patients without eclampsia; differences were statistically significant ( $p < 0.05$ ) (Table III).

## Discussion

The results of the present study showed that the serum HMWA levels in the first pregnancy were decreased, whereas the leptin levels were increased with an increase of PIH grading,

which was consistent with results of previous studies (6-8). The physiological function of adiponectin includes adjusting glucolipid metabolism, increasing insulin sensitivity, anti-inflammation and anti-atherosclerosis (6). Adiponectin is a type of important cardiovascular protective adipokine, and hypoadiponectinemia is associated with abnormal endothelium-dependent vasodilatation (7). The placenta is an important organ that secretes the leptin, and the serum leptin levels in eclampsia patients is increased significantly, which is consistent with the degree of proteinuria, although it drops rapidly after treatment. The increased leptin inhibits mRNA expression in placental neuropeptide Y gene via the paracrine effect, thus reducing the placental blood flow and placental hypoxia, involved in the occurrence of eclampsia (8). The incidence of eclampsia in secondary pregnancy is 15.38%. The HMWA levels in patients with eclampsia is significantly lower than that in patients without eclampsia, but the leptin levels in patients with eclampsia is increased significantly. The HMWA levels in patients with eclampsia in secondary pregnancy is lower than that in the first pregnancy, but the leptin levels are higher than those in the first pregnancy. The HMWA and leptin levels in patients without eclampsia were not changed in the two pregnancy types. It indicates that both decreased HMWA levels and increased serum leptin levels participate in the occurrence of eclampsia in secondary pregnancy. The PIH grading and change in HMWA and leptin levels in secondary pregnancy may have an important application value in predicting the occurrence of eclampsia.

Our study also determined that HOMA-IR, IL-6, TNF- $\alpha$ , MDA and ox-LDL levels in patients with eclampsia were significantly higher than those in patients without eclampsia, and the adiponectin receptor 2 and COX-2 protein expression levels in placental tissue were significantly higher than those in patients without eclampsia. Insulin resistance is an independent risk factor for the occurrence and poor prognosis of PIH, which is closely associated with the inflammatory response and oxidative stress (9). The secretion of adiponectin is closely related to the IL-6, TNF- $\alpha$ , MDA and ox-LDL expression levels (10). The inflammatory response plays an important role in angiogenesis, immune rejection and hypoxia-ischemia in placental tissue. Moderate immuno-inflammatory response exists in normal pregnancy, and the extensive inflammatory response in blood vessels is involved in the occurrence of eclampsia (11). IL-6 is a type of glycoprotein, which is mainly secreted by lymphocytes and mononuclear macrophages, an important proinflammatory factor with extensive biological effects. In addition, IL-6 can induce the B-cell differentiation, produce immunoglobulins, promote T-cell proliferation. TNF- $\alpha$  is a type of polypeptide material mainly produced by mononuclear macrophages, which is an important media involved in a variety of physiological and immune responses in the body. IL-6 and TNF- $\alpha$  levels play an important role in pregnancy, childbirth and fetal growth and development (12,13).

Enhanced oxidative stress is also an important mechanism for the occurrence of eclampsia. Harma *et al* (14) found that supplementing antioxidants may reduce the incidence of preeclampsia in high-risk pregnant women by two-thirds. The clinical trials of antioxidant therapy are carried out step by step, which is expected to be an important method of intervention

in the occurrence of eclampsia. ox-LDL is an oxidized product after the lipoprotein is attacked by oxygen free radicals, as well as the potential inducer in the inflammatory process, which can directly damage vascular endothelial cells, and induce mononuclear macrophages, adhesion molecule of endothelial cell, cytokines and pro-inflammatory factors (15). Three types of adiponectin receptors have been found currently, namely R1, R2 and T-calcium adhesion protein. R2 is mainly expressed in human placental tissues, and it is mainly expressed in placental villus syncytiotrophoblast and cytotrophoblast during early pregnancy, and only expressed in syncytiotrophoblast in the full term (16). IL-6 and TNF- $\alpha$  levels can inhibit R2 expression. After adiponectin and receptor are combined, activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK) and peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) signaling transduction pathway are activated in order to increase insulin sensitivity and improve insulin resistance and glucolipid metabolism (17).

COX-2 is the important rate-limiting enzyme that is synthesized by prostaglandin (PG), which is associated with inflammation and atherosclerosis (18). PG is a type of important tissue-derived medium, and the vasoactive PG products, such as angiotensin and thromboxane, play key roles in vascular endothelial injury and thrombosis (19). The main action target of aspirin is COX-2, and there remains a controversy about whether a large-scale, double-blind, randomized, placebo-controlled trial of early application of aspirin can significantly decrease the occurrence of eclampsia (20).

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