

Association between the presence of autoantibodies against adrenoreceptors and hypertensive disorders in pregnancy: a pilot study

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Introduction There are numerous factors considered to cause pregnancy-related hypertension, including genetic, immunological, environmental, and behavioral ones, as well as those related to endothelial dysfunction.

The immune system can identify foreign antigens and eliminate them from the human body. In the case of immune control mechanism failure, the immune system may be directed at a self-antigen. More recent data have shown characteristic molecular targets of autoantibodies common in patients with preeclampsia and essential hypertension, potentially explaining how elevated autoantibody titers might contribute to hypertension. Wallukat and Schimke¹ demonstrated the presence of autoantibodies against α_1 -, β_1 -, and β_2 -adrenoreceptors (anti- α_1 -ARs, anti- β_1 -ARs, and anti- β_2 -ARs, respectively) that bind to the second extracellular loop of receptors and are highly prevalent in the serum of hypertensive patients.

It is well known that neoangiogenesis is a multistep process closely associated with endothelial cell migration and proliferation.² Stimulation of α_1 -ARs localized in endothelial cells leads to negative regulation of angiogenesis, whereas β_2 -ARs stimulate neoangiogenesis. Moreover, in hypertension characterized by the impairment of angiogenesis, the α_1 -AR tone is higher than that of β_2 -ARs.³ Taking these facts into consideration, we hypothesized that abnormal stimulation of these receptors by autoantibodies might lead to abnormal angiogenesis, which is crucial for placental development. To verify this hypothesis, we evaluated the correlation between the titers of these autoantibodies and ultrasound Doppler flow measurements that reflect placental insufficiency. Moreover, the aim of our study was to investigate the differences in

anti- α_1 -AR, anti- β_1 -AR, and anti- β_2 -AR titers between pregnant patients with chronic hypertension (CH), gestational hypertension (GH), and preeclampsia and healthy pregnant women. Finally, we investigated the relationship between autoantibody titers and the severity of preeclampsia (based on systolic and diastolic blood pressure values and urinary protein excretion).

Patients and methods The study was performed with approval from the Bioethics Committee of the Poznan University of Medical Sciences, Poznań, Poland (No. 563/13). Each woman received detailed information about the project and provided informed consent before participating in the study.

A case control study was conducted in the Department of Perinatology and Gynecology of the Poznan University of Medical Sciences between November 2014 and March 2016. We analyzed the following groups of women—pregnant patients with preeclampsia ($n = 16$), those with CH ($n = 13$), and those with GH ($n = 17$). The control group consisted of 17 healthy, normotensive pregnant women. The compared groups were matched for the mother's age, parity, pre-pregnancy body mass index, and gestational age at the time of recruitment into the study and blood collection.

Hypertensive disorders were defined in accordance with the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy classification.⁴ The exclusion criteria for the control and study groups were systemic diseases related to endothelial dysfunction (eg, kidney diseases, diabetes mellitus, atherosclerotic diseases, inflammatory/infectious

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diseases, or cancer), multiple gestation, and autoimmune diseases.

Twenty-four-hour automatic blood pressure monitoring was carried out using the HolCARD CR-07 blood pressure recorder (Aspel, Zabierzów, Poland). Venous blood samples were collected from the antecubital vein in a 5-ml serum-separating vacuum tube. These samples were centrifuged at 2000 g for 10 minutes, and the plasma was collected in 1.5-ml Eppendorf tubes and stored at -80°C until batch processing. Enzyme-linked immunosorbent assays (ELISAs) were performed according to the manufacturer's instructions. Anti- β_1 -ARs, anti- β_2 -ARs, and anti- α_1 -ARs were assessed using the commercially available competitive ELISA kit (MBS733 845, MyBioSource, California, United States; CellTrend, Luckenwalde, Germany; and MBS705 594, MyBioSource, respectively). Simultaneously with blood collection, every woman underwent ultrasound Doppler flow measurements (Voluson E8; GE Healthcare, Little Chalfont, Great Britain) of the uterine arteries, umbilical vessels, and fetal middle cerebral artery. We calculated the uterine artery score for each patient based on the following scoring: 1 point for the "notch" sign in each uterine artery and 1 point for a pulsatility index exceeding 1.2. Additionally, we used the cerebral-umbilical ratio which is a quotient of the middle cerebral artery and umbilical artery pulsatility indices. Placental insufficiency was defined as a cerebral-umbilical ratio below the 5th percentile for gestational age and/or 3 to 4 points in the uterine artery score.^{5,6}

We used the Statistica v. 10 STATSOFT software (Statsoft Inc., Tulsa, Oklahoma, United States) for statistical analysis. The Shapiro-Wilk test was used to analyze the distribution. The *t* test, Mann-Whitney test, or Fisher exact test were used to assess the differences between the groups. Correlation analyses were performed by the Spearman rank correlation analysis. A *P* value of less than 0.05 was considered significant.

Results The clinical characteristics of the control and study groups are shown in [TABLE 1](#). There were no significant differences between these groups in terms of patient age, parity, prepregnancy body mass index, and gestational age at the time of recruitment to the study and blood collection. The mean (SD) urinary protein excretion in patients with preeclampsia was 2.6 (2.15) g/24 h.

The median of anti- α_1 -AR, anti- β_1 -AR, and anti- β_2 -AR titers in each group of women are presented in [TABLE 1](#). Interestingly, there were only 2 patients who were positive for anti- α_1 -ARs. The first was a 28-year-old patient in the 39th week of an uncomplicated pregnancy and the other was a 22-year-old woman with CH in the 26th week of gestation. There were no significant differences in anti- α_1 -AR, anti- β_1 -AR, and anti- β_2 -AR titers between the study and control groups ([TABLE 1](#)). Moreover, in the CH group, we found a negative correlation between anti- β_2 -AR titers

and maximum systolic blood pressure ($r = -0.62$; $P = 0.02$). There were no significant correlations between autoantibody titers and the severity of preeclampsia. We did not observe a correlation between gestational age at sampling and autoantibodies titers in any of the groups.

In 73% of patients with preeclampsia, placental insufficiency was detected by ultrasound examination, whereas it was detected in only 33% and 8% of women with CH and GH, respectively. Furthermore, our analysis did not reveal any correlations between anti- α_1 -AR and anti- β_2 -AR titers and ultrasonographic features of placental insufficiency in any of the groups.

Discussion Based on our hypothesis, we expected that the titers of adrenoreceptor autoantibodies would be significantly different in the serum of pregnant women with hypertensive disorders than in that of healthy pregnant controls. The exact pathophysiology and etiology of hypertensive disorders in pregnant women remain elusive, but there is growing evidence for the involvement of the immune system in these processes. Dysfunction in immunological mechanisms may impair spiral artery invasion, leading to placental insufficiency and elevated blood pressure.⁷

The α_1 -ARs are primarily expressed on vascular smooth muscle cells and proximal renal tubules, and their activation causing elevated blood pressure.³ In 1994, anti- α_1 -ARs were detected for the first time in patients with malignant hypertension and soon after were reported in patients with primary hypertension.^{8,9} The β_1 -ARs are localized mainly in cardiac tissue, and their stimulation is followed by an increase in cardiac output, which is the main blood pressure regulator. It is widely accepted that β_2 -ARs are localized on endothelial cells and regulate vasomotor tone and cause endothelial nitric oxide activation, which is followed by vasorelaxation.³ Therefore, we hypothesized that there is a relationship between the presence of these autoantibodies and the development of different types of hypertensive disorders in pregnancy.

Only one published study examined the association between the presence of autoantibodies against adrenoreceptors and severe preeclampsia.¹⁰ In contrast to our results, the authors showed that anti- α_1 -ARs, anti- β_1 -ARs, and anti- β_2 -ARs titers were significantly increased in patients with severe preeclampsia and concluded that these autoantibodies may be involved in the pathogenesis of this disease. Interestingly, in our study, there were only 2 patients with positive anti- α_1 -AR titers, and these patients differed in health status. This finding may suggest that these autoantibodies occur incidentally, independently from any underlying disease.

Importantly, in our study, we included women with preeclampsia as well as those with CH and GH, which is a novel aspect of our investigation. There are few similar risk factors that contribute to the development of GH and preeclampsia,

TABLE 1 Clinical characteristics and autoantibody titers of the study and control groups

Parameter	Gestational hypertension (n = 17)		Preeclampsia (n = 16)		Chronic hypertension (n = 13)		Control group (n = 17)
Age, y, mean (SD)	29 (3)	<i>P</i> = 0.1	30 (7)	<i>P</i> = 0.7	32 (5)	<i>P</i> = 0.6	32 (4)
Gestational age at recruitment, wk, median (min–max)	38 (20–41)	<i>P</i> = 0.8	32 (26–40)	<i>P</i> = 0.06	34 (22–40)	<i>P</i> = 0.09	38 (21–41)
Parity, median (min–max)	1 (1–3)	<i>P</i> = 0.3	1 (1–3)	<i>P</i> = 0.1	2 (1–8)	<i>P</i> = 0.8	2 (1–5)
Prepregnancy BMI, kg/m ² , mean (SD)	28.3 (4.6)	<i>P</i> = 0.06	28.0 (4.3)	<i>P</i> = 0.07	28.4 (9.6)	<i>P</i> = 0.25	25.1 (4.6)
Mean SBP, mm Hg, mean (SD)	134 (15)	<i>P</i> < 0.01	139 (12)	<i>P</i> < 0.01	131 (11)	<i>P</i> < 0.01	111 (9)
Mean DBP, mm Hg, mean (SD)	84 (10)	<i>P</i> < 0.01	90 (8)	<i>P</i> < 0.01	83 (9)	<i>P</i> < 0.01	70 (7)
Maximum SBP, mm Hg, mean (SD)	160 (23)	<i>P</i> < 0.01	169 (20)	<i>P</i> < 0.01	164 (23)	<i>P</i> < 0.01	119 (11)
Maximum DBP, mm Hg, mean (SD)	106 (17)	<i>P</i> < 0.01	109 (11)	<i>P</i> < 0.01	113 (19)	<i>P</i> < 0.01	78 (12)
Anti- α_1 -ARs, n (%)	0	<i>P</i> = 1.0	0	<i>P</i> = 1.0	1 (7.7)	<i>P</i> = 1.0	1 (5.9)
Anti- β_1 -ARs, ng/ml, median (IQR)	1.4 (1.07–1.68)	<i>P</i> = 0.5	1.13 (0.74–3.45)	<i>P</i> = 0.6	1.51 (1.07–1.70)	<i>P</i> = 0.4	1.27 (0.88–1.53)
Anti- β_2 -ARs, U/ml, median (IQR)	4.39 (2.94–6.65)	<i>P</i> = 0.6	3.6 (2.83–4.75)	<i>P</i> = 0.2	3.36 (1.99–4.54)	<i>P</i> = 0.06	5.25 (3.16–6.57)

Abbreviations: anti- α_1 -ARs, autoantibodies against α_1 -adrenoreceptors; anti- β_1 -ARs, autoantibodies against β_1 -adrenoreceptors; anti- β_2 -ARs, autoantibodies against β_2 -adrenoreceptors; BMI, body mass index; DBP, diastolic blood pressure; IQR, interquartile range; SBP, systolic blood pressure

which may suggest that they are congenital diseases.¹¹ Furthermore, patients with severe GH have an independently increased perinatal risk and even worse outcomes than those with mild preeclampsia.^{11,12} These factors suggest that patients with CH and GH should be included in studies examining hypertensive disorders in pregnancy.

We found a significant negative correlation between anti- β_2 -AR titers and maximum systolic blood pressure in the CH group. It is well known that agonist binding of these autoantibodies to β_2 -ARs causes receptor activation followed by vasorelaxation.³ Our results suggest that these autoantibodies may be protective in patients with CH; moreover, in patients with higher levels of these autoantibodies blood pressure may be lower.

Our analysis did not reveal any correlation between anti- α_1 -AR or anti- β_2 -AR titers and ultrasonographic features of placental insufficiency. Unfortunately, our hypothesis was not confirmed, which may indicate that the abnormal stimulation of these receptors by autoantibodies is not related to the pathogenesis of hypertensive disorders in pregnancy and with placental insufficiency.

Based on our findings, we assume that anti- α_1 -ARs, anti- β_1 -ARs, and anti- β_2 -ARs are unlikely to be involved in the pathogenesis of hypertensive disorders in pregnant women. In pregnant patients with CH, anti- β_2 -ARs may play a protective role. However, to date, there have been few studies on this topic, so further research is needed to investigate these findings and to

expand the knowledge about the etiopathogenesis of hypertension in pregnancy.

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