European Journal of Obstetrics & Gynecology and Reproductive Biology 225 (2018) 62–69

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

Endothelial dysfunction as a long-term effect of late onset hypertensive pregnancy disorders: High BMI is key

L.J. Alma[a,b], C.J.M. De Groot[a], R.X. De Menezes[c], W. Hermes[d], P.L. Hordijk[b], I. Kovačević[b,*]

[a] Department of Obstetrics and Gynecology, VU University Medical Center, Amsterdam, The Netherlands
[b] Department of Physiology, VU University Medical Center, Amsterdam, The Netherlands
[c] Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands
[d] Department of Obstetrics and Gynecology, Haaglanden Medical Center, The Hague, The Netherlands

ARTICLE INFO

Article history:
Received 11 March 2018
Received in revised form 4 April 2018
Accepted 5 April 2018
Available online xxx

Keywords:
Preeclampsia
Gestational hypertension
Cardiovascular risk factors and prevention
Endothelial activation
Biochemical markers
Obesity

ABSTRACT

Objective: Hypertensive disorders during pregnancy increase cardiovascular risk later in life by 2 to 9-fold. Endothelial activation is one of the underlying mechanisms of cardiovascular risk. Therefore, we decided to investigate endothelial activation in primiparous women, 2.5 years after a hypertensive pregnancy disorder.
Study design: Plasma samples were taken from women 2.5 years after gestational hypertension (GH) or late onset preeclampsia (cases) and from women 2.5 years after a normotensive pregnancy (controls). We studied the effects of patient plasma on the endothelial barrier function of primary human umbilical vein endothelial cells (HUVECs) using Electric Cell-Substrate Impedance Sensing (ECIS) and we measured levels of endothelial activation markers soluble intercellular adhesion molecule 1 (sICAM-1) and soluble endothelial selectin (sE-selectin) in the plasma samples of patients.
Results: Plasma from primiparous women with a history of late onset preeclampsia disrupted the endothelial barrier more than plasma from women with a history of GH. Endothelial resistance was reduced by 22% in samples taken after preeclampsia, 16% after normotensive pregnancy and 3% after GH ($p \leq 0.0001$ GH versus preeclampsia and $p = 0.0003$ versus normotensive pregnancy). We did not find differences in the levels of soluble endothelial activation markers (sICAM-1 $p = 0.326$ and sE-selectin $p = 0.978$). However, the BMI $\geq$25 showed a strong correlation with increased levels of sICAM-1 ($p = 0.046$) and sE-selectin ($p = 0.002$).
Conclusion: Our results indicate that GH and late onset preeclampsia are distinct disease entities with a different pathogenic mechanism underlying their cardiovascular risk. Furthermore, this study supports the hypothesis that these two diseases are early manifestations of cardiovascular vulnerability due to an unfavorable risk profile, and that obesity plays a main role. Our results suggest that this high-risk female population would be eligible for preventive care.

Introduction

Vascular dysfunction is evident in women with a former hypertensive pregnancy disorder, as has been shown via functional and structural imaging modalities [1]. Gestational hypertension (GH) and preeclampsia are two well-defined forms of hypertensive pregnancy disorders. GH is characterized by a new-onset of hypertension after 20 weeks of gestation and in preeclampsia, this is superimposed with proteinuria and/or maternal organ dysfunction [2]. Approximately 3–9% of all pregnancies worldwide are complicated by GH and 2–3% by preeclampsia [3].

The increased cardiovascular risk after hypertensive pregnancies ranges from 2-fold for all hypertensive pregnancy disorders to 9-fold for women who experienced early onset preeclampsia (before 34 weeks of gestation) [4]. It has been shown that hypertensive pregnancy disorders are associated with cardiovascular risk factors (obesity, insulin resistance and hypertension) and systemic activation of the maternal endothelium by soluble factors is crucial in the pathophysiology of hypertensive pregnancy disorders [5–10]. In the past, these circulating markers have been investigated extensively, however, inconsistency of the results did not allow identification of the underlying pathophysiologic cause of cardiovascular diseases (CVDs) after hypertensive pregnancy disorders [11].

Previous studies mainly focused on severe/early preeclampsia, although most hypertensive pregnancy disorders develop after 36 weeks of gestation. Therefore, we have chosen to set our focus on

* Corresponding author at: Department of Physiology, VU University Medical Center, De Boelelaan 1118, 1081 HV Amsterdam, The Netherlands.
E-mail address: i.kovacevic@vumc.nl (I. Kovačević).

https://doi.org/10.1016/j.ejogrb.2018.04.003
0301-2115/

the pathophysiology of cardiovascular disease after GH and late onset preeclampsia.

We hypothesized that endothelial activation/dysfunction plays a key role in the pathogenesis of CVDs after late onset preeclampsia and GH. To investigate this, we tested whether plasma from primiparous women, taken 2.5 years after a hypertensive pregnancy disorder, affects the endothelial barrier function of primary HUVECs. In addition, we measured the levels of endothelial activation/dysfunction markers sICAM-1 and sE-selectin in sera of these patients.

Materials and methods

Participants

Women with a history of GH or preeclampsia (cases), originate from a 2.5 year follow-up study [1] of the Hypertension and Preeclampsia Intervention Trial at Term (HYPITAT) study [12], a multi-center, parallel, open-labeled randomized controlled trial (study registration number ISRCTN08132825).

The HYPITAT included cases with GH or preeclampsia at a gestational age between 36^{+0} and 41^{+0} weeks. Gestational hypertension was defined as diastolic blood pressure ≥95 mm Hg on two occasions at least six hours apart without proteinuria. Preeclampsia was defined as diastolic blood pressure ≥90 mm Hg on two occasions at least six hours apart in combination with proteinuria (measured two or more times through protein on a dipstick, >0.3 g/24 h or a protein to creatinine ratio >30 mg/mmol) [13–15]. All women had a singleton pregnancy. Exclusion criteria: anti-hypertensive drug use for pre-existing hypertension, diabetes mellitus, insulin dependent gestational diabetes, renal disease, heart disease, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), oliguria <500 mL/24 h, pulmonary edema or cyanosis, human immunodeficiency virus seropositivity (HIV), use of intravenous anti-hypertensive drugs, suspected intrauterine growth restriction, fetal anomalies or abnormal fetal heartrate monitoring.

Between June 2008 and November 2010, patients from the HYPITAT trial were asked to join a longitudinal follow-up study in hospitals throughout the Netherlands. The study was approved by the institutional review board of all participating hospitals. Recruitment of the control patients is described in the follow-up studies of the HYPITAT trial [1,16]. In brief, control patients experienced a healthy pregnancy approximately 2.5 years earlier, and were either friends of women from the HYPITAT study, or women from regional midwifery practices. Exclusion criteria for controls were HELLP syndrome, GH, preeclampsia, pre-existing hypertension, diabetes mellitus, gestational diabetes, premature delivery, delivery of a neonate with intrauterine growth restriction, renal disease, heart disease, HIV or pregnancy/lactation within the last three months. Clinical characteristics of the control participants were recruited from medical records. Each participant provided written informed consent. All participants went through clinical assessment and filled out a questionnaire regarding their medical history.

In the present study, we used frozen plasma samples from all women who were not using anti-histamines, antibiotics, non-steroidal anti-inflammatory drugs, glucocorticoids or biopharmaceuticals at the time of investigation. Additionally, all multiparous women, cases and controls, were excluded from the present study.

For part of the analyses, we studied the effect of body weight after hypertensive pregnancy on endothelial dysfunction. According to the guidelines of European Association for the Study of Obesity (EASO) we defined two categories of patients: normal body weight (BMI 18.5–25) and overweight (BMI ≥ 25). Additionally, we investigated the correlation between metabolic syndrome after hypertensive pregnancy disorder and endothelial (dys)function. We defined metabolic syndrome as the presence of ≥3 of the following criteria: waist circumference ≥88 cm (35 inches), plasma triglycerides ≥1.7 mmol/l, plasma high-density lipid cholesterol (HDLc) <1.3 mg/dl, fasting plasma glucose ≥ 5.6 mmol/l, blood pressure ≥90 mm Hg diastolic and/or ≥140 mmHg systolic [17,18].

Plasma samples

Fasting venous heparin blood samples were drawn during assessment approximately 2.5 years after pregnancy. All samples were immediately centrifuged after collection and kept on ice throughout the entire process. Within 36 h, the plasma samples were sent to one central laboratory (Medical Center Haaglanden, The Hague, the Netherlands) and analyzed for levels of glucose, total cholesterol, high-density lipoprotein cholesterol (HDLc) and triglycerides. Subsequently, the samples were stored at −80 °C until further use. For the present study, heparin plasma samples were slowly thawed on ice and centrifuged (5 min, 15000 RPM at 4 °C) before use.

Isolation and culture of endothelial cells

We isolated primary human umbilical vein endothelial cells (HUVECs) from umbilical cords which were obtained from the Department of Obstetrics of the Amstelland Ziekenhuis (Amstelveen, the Netherlands). In brief, HUVECs were isolated freshly from umbilical veins which were cannulated and infused with 134 U/ml Collagenase type II solution (Worthington). After 30 min incubation with Collagenase type II at 37 °C, detached endothelial cells were collected and cultured in M199 endothelial cell medium for a maximum of 3 passages. The procedure for HUVEC isolation and culturing has been previously described [19].

Endothelial barrier analysis

Endothelial barrier function upon exposure to patient plasma samples was evaluated through Electrical Cell-Substrate Impedance Sensing (ECIS) (Applied Biophysics) as described previously [20]. Confluent, second passage HUVECs were harvested with trypsin/EDTA and seeded into a 1% gelatin pre-coated 96w10idfPET ECIS array from Applied Biophysics. Once cells had grown to confluence, the growth medium was replaced by a 1:5 dilution of patient plasma in M199 and subsequent changes in barrier resistance were measured continuously. 24 h later, the response of the pre-incubated cells to a strong inflammatory agonist (10 ng/ml TNF-α) was measured for another 24 h.

Endothelial barrier resistance was assessed at a low frequency (4000 Hz), which best reflects the intercellular contact and thus monolayer integrity and barrier function. For interpretation of the ECIS results, all changes in resistance were normalized to resistance measured shortly before addition of plasma or TNF-α.

ELISA measurement of soluble biomarkers

The levels of two endothelial activation markers in patient plasma were measured using ELISA-kits purchased from R&D Systems: sE-selectin and sICAM-1, catalogue numbers DY724 and DY720 respectively. Plasma samples were diluted 1:10 for sE-selectin, 1:1000 for sICAM-1.

Statistical analysis

Throughout all experiments we compared the different pregnancy groups (normotensive pregnancy, preeclampsia and GH) and we analyzed for potential confounding properties of cardiovascular risk