Chronic and gestational metabolic disorders have a different impact on late-pregnancy endothelial function in pregnant women

Reakcja śródbłonka naczyniowego na hiperglikemię i/lub nadciśnienie tętnicze w późnej ciąży jest zróżnicowana w zależności od przewlekłego lub indukowanego ciążą charakteru zaburzeń

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Abstract

Objectives: *We investigated how maternal endothelial function is affected by pregestational (Type 1) diabetes mellitus (PGDM) or gestational diabetes mellitus (GDM) and/or chronic hypertension (chHT) or gestational hypertension (PIH).*

Methods: *We conducted a prospective, observational study involving 78 participants with GDM, PGDM and/or hypertension (PIH-16, GDM + PIH-14, PGDM + chHT-8, PGDM-20, GDM-20) in the third trimester of a singleton viable pregnancy. Twenty healthy women with uncomplicated pregnancies matched for gestational age served as controls. We analysed maternal data, disease history and serum concentrations of E-selectin and Vascular cell adhesion molecule 1 (sVCAM-1).*

Results: *only the maternal serum concentration of sVCAM-1 differed significantly among the subgroups (p<0.0001), with the highest levels evident in women with PIH or GDM + PIH and the lowest in women with PGDM alone or PGDM + chHT.*

Conclusions: *pregestational or pregnancy associated disorders, although sharing similar clinical symptoms, have a different impact on endothelial function in pregnant women.*

Key words: **cell adhesion molecules** / **pregnancy** / **Type 1 diabetes** / **hypertension** / / **vascular endothelium** /

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Streszczenie

Śródbłonek naczyniowy jest uważany obecnie za narząd docelowy w rozwoju powikłań towarzyszących cukrzycy, jak również nadciśnieniu tętniczemu.

Celem badania była analiza wpływu cukrzycy ciążowej lub przedciążowej typu 1(GDM, PGDM) oraz nadciśnienia tętniczego przewlekłego lub indukowanego ciążą (chHT, PIH) na markery funkcji śródbłonka naczyniowego.

Materiał i metoda: *prospektywne badanie obserwacyjne na grupie 78 ciężarnych w III trymestrze pojedynczej ciąży (PIH-16, GDM+PIH-14, PGDM+chHT-8, PGDM-20, GDM-20). Grupę kontrolną stanowiło 20 zdrowych ciężarnych w pojedynczej, niepowikłanej ciąży dobranych pod względem wieku ciążowego. W grupie badanej analizowano dane antropometryczne i biochemiczne oraz stężenia rozpuszczalnych frakcji E-Selektyny (sE-Sel) i VCAM-1 (sVCAM-1) w surowicy krwi.*

Wyniki: *wykazano znamienną różnicę w stężeniach sVCAM-1 między analizowanymi podgrupami (p<0.0001) przy czym najwyższe stężenia zaobserwowano w podgrupach PIH oraz GDM+PIH, a najniższe stężenia w podgrupach z PGDM z lub bez chHT.*

Wnioski: *w ciążach powikłanych hiperglikemią i/lub nadciśnieniem tętniczym zróżnicowany wpływ chorób matczynych na śródbłonek naczyniowy ciężarnej zależy od przewlekłego lub indukowanego ciążą charakteru zaburzeń.*

Słowa kluczowe: **molekuły adhezyjne** / **cukrzyca typu 1** / **nadciśnienie tętnicze** / / **śródbłonek naczyniowy** /

Introduction

In the last decade, the vascular endothelium has gained attention as an autonomous endocrine organ that maintains homeostasis by altering its phenotype and function in response to changes in metabolic status. In disorders such as diabetes mellitus or hypertension, which are associated with prolonged metabolic stress, endothelial cells permanently adopt an abnormal phenotype, losing their normal responsiveness [1–3]. In non-pregnant individuals, these diseases, together with other components of metabolic syndromes, share similar mechanisms that have a negative impact on endothelial activity and function [4–6]. In summary, the vascular endothelium is a target organ for all components of metabolic syndromes, hence the generalized nature of these disorders.

In addition to metabolic disorders, pregnancy also modulates endothelial function. High circulating concentrations of sex steroid hormones (oestrogens, progesterone) are leading modulators of endothelial activity. Moreover, the placenta is a unique organ whose function depends on appropriate endothelial activity. Increased insulin resistance, an altered lipid profile, modified clotting homeostasis – all characteristic in pregnancy – combined with changes in maternal body weight and composition contribute to chronic inflammation triggered by angiogenesis. Both increased maternal adiposity and placenta formation stimulate the production of angiogenic factors necessary to regulate the formation of new vessels and maintain their function.

Gestational hypertension and gestational diabetes mellitus are frequent and severe complications of pregnancy that increase neonatal mortality and morbidity. These conditions also have a negative impact on the remote prognosis of women who develop them during pregnancy. In addition, maternal Type 1 or 2 diabetes are commonly acknowledged as serious risk factors for foetomaternal complications. Hypertensive disorders often coexist with different forms of maternal hyperglycaemia, leading to a further deterioration in perinatal outcomes in this high-risk population of pregnant women. Recent studies explain this coincidence, showing a clear association between insulin resistance and pre-eclampsia [7–12]. Also, persistent alterations in the concentrations of cell adhesion molecules are evident in women with a remote history of pre-eclampsia [13]. The authors suggest that this mechanism may mediate an increased risk of cardiovascular disorders in this population.

Endothelial dysfunction in women with hypertensive disorders of pregnancy has been described by many researchers [14–18]. In these conditions, endothelial dysfunction is mainly driven by sustained inflammation and changes in angiogenic factors. However, data on endothelial function in pregnant women with diabetes are limited and some derive from small populations [19, 20]. To date, no studies have investigated markers of endothelial function in combined disorders affecting maternal metabolism, e.g. maternal hypertension with hyperglycaemia. This gap in our knowledge must be filled, because the vascular endothelium has recently been identified as a target organ for both hypertension and hyperglycaemia.

We investigated endothelial function, as indicated by the concentrations of cell adhesion molecules, in pregnant women with hypertension and/or diabetes. We hypothesized that maternal disorders such as hypertension or diabetes are associated with altered endothelial function, and are manifest in changes in the serum levels of the cell adhesion molecules sE-selectin (sE-sel) and Vascular cell adhesion molecule 1 (sVCAM-1). We also hypothesized that each of these disorders would have a unique profile of markers of endothelial function.

Materials and methods

To test our hypothesis, we designed a prospective, observational study with a control group. Our study group consisted of 78 women with singleton pregnancies complicated by at least one of the following: chronic or gestational hypertension; gestational diabetes mellitus; and pregestational (Type 1) diabetes mellitus. Participants were recruited from a population receiving antenatal care at the Department of Obstetrics and Women's

Diseases, a tertiary, academic unit serving women with high-risk pregnancies. All patients gave informed consent to participate in this study and the study protocol was approved by the Bioethics Committee of the University of Medical Sciences in Poznan, Poland. Data on the history of concomitant disorders, gestational age/maternal age at onset, concomitant medications and vascular complications were collected from patients upon admission to our centre. Data on maternal glycaemia, glycated haemoglobin (HbA1c) levels and blood pressure values were retrieved from maternal records. We also reviewed neonatal records to obtain data on the gestational age at delivery and birth weight of the newborns. Neonates with a birth weight below the 10th percentile for their gestational age at delivery and sex were defined as small for gestational age. Those with a birth weight above the 90th percentile for their gestational age at delivery and sex were classified as large for gestational age. To assess foetal growth, we used local growth charts customized for gestational age at birth and sex.

Maternal proteinuria was defined as a daily protein loss of more than 0.3g/24 h, calculated from a 24-h urine collection, after the exclusion of maternal urinary tract infection.

The control group was recruited from healthy pregnant women with otherwise uncomplicated pregnancies admitted for short-term antenatal surveillance in the third trimester because of reduced foetal movements. The control group had no symptoms of foetal distress on cardiotocography (CTG) monitoring or mild premature contractions and did not require tocolytics or glucocorticoids to improve foetal lung maturity.

Our protocol included the collection of maternal blood once in the third trimester (between 32 and 36 gestational weeks). The samples were centrifuged, aliquoted and stored at -80°C until assayed. Concentrations of sE-sel and sVCAM-1 were examined using commercially available enzyme-linked immunosorbent assay kits: the Human sE-Selectin Quantikine and Human sVCAM-1 Quantikine Kits (R&D Systems, Inc., Minneapolis, MN, USA).

Statistical analysis was performed using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA). Because our data violated the assumption of normality, we used the non-parametric Kruskal-Wallis test to evaluate differences in the cell adhesion molecules among subgroups, with a Bonferroni correction to allow for multiple comparisons. The data are given as medians and interquartile ranges unless stated otherwise.

Results

The characteristics of the study group are summarized in Table I. Eleven of the 28 participants with Type 1 diabetes mellitus exhibited vascular complications (mainly retinopathy). Eight of the 37 women with hypertensive disorders (either alone or with coexistent diabetes) developed proteinuria and therefore met the criteria for pre-eclampsia. Seven of these eight women were in the subgroup with gestational hypertension.

The perinatal outcomes in our cohort are listed in Table II. In nineteen of 78 individuals, delivery was performed via caesarean section. There were no cases of foetal malformation or early neonatal death (defined as neonatal death within the first week of life).

For further analysis, patients were grouped on the basis of whether they suffered from gestational disorders (gestational diabetes and/or gestational hypertension) or chronic conditions (Type 1 diabetes mellitus, either isolated or with coexisting chronic hypertension).

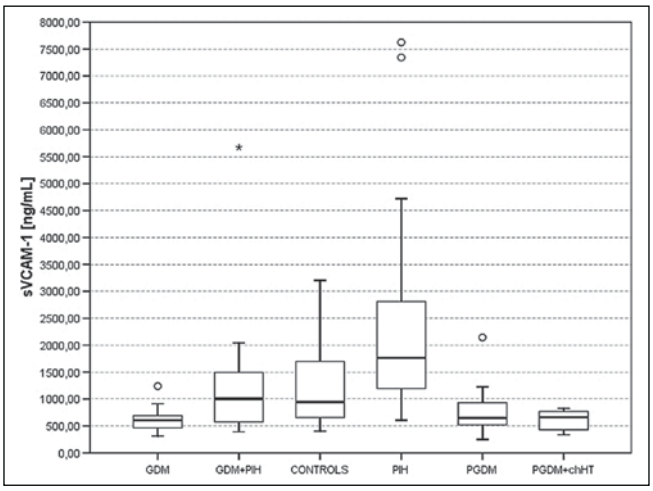

Figure 1. Serum concentrations of sE-selectin in the study group (p = 0.04)
GDM – gestational diabetes mellitus; PGDM – pregestational diabetes mellitus; PIH – gestational hypertension; chHT – chronic hypertension.

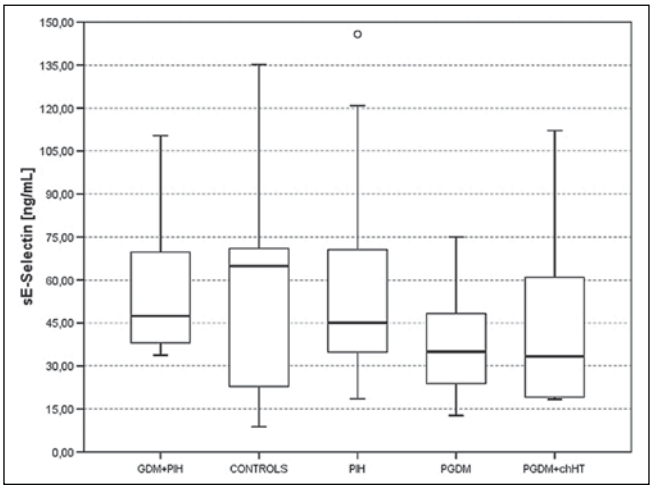

Figure 2. Serum concentrations of Vascular cell adhesion molecule 1 in the study group (p < 0.0001)
GDM – gestational diabetes mellitus; PGDM – pregestational diabetes mellitus; PIH – gestational hypertension; chHT – chronic hypertension

Comparison of the concentrations of sE-sel and sVCAM-1 across the subgroups revealed an insignificant trend for reduced levels of sE-sel in women suffering from any form of either hypertension or diabetes, or both, when compared with controls (see Figure 1). Comparison of sVCAM-1 levels across the subgroups revealed a highly significant difference among concentrations, with the highest levels in the subgroups with gestational hypertension alone or with coexisting gestational diabetes and the lowest in women with Type 1 diabetes with or without chronic hypertension (see Figure 2).

After calculating the concentrations of the molecules under investigation as multiples of the median, we found that the levels of sVCAM-1 underwent a different pattern of changes depending on whether the maternal disease was of pregestational or pregnancy associated origin (see Table III).

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Table I. Characteristics of the study group.

	N=80
Maternal age [years]	29.3 (24.0-35.0)
Prepregnancy BMI [kg/m^2] *)	23.9 (21.0-30.7)
History of pregestational diabetes [years]	11.0 (5.5-16.5)
Gestational age at the GDM diagnosis	26.0 (24.0-28.0)
History of chronic hypertension [years]	6.0 (3.0-11.0)
Gestational age at the diagnosis of gestational hypertension [weeks]	29.5 (26.0-34.0)
HbA_{1c} at sampling (GDM/PGDM patients) [%]	6.0 (5.5-6.5)
Proportion of patients with prepregnancy obesity [BMI ≥30 kg/m^2]	25.0%

*) for patients with GDM, only BMI at the moment of the diagnosis was available: 31.3 (29.2-34.4) kg/m^2, data presented as a median and interquartile range (in brackets).

Table II. Perinatal outcomes in the study group.

	N=78
Gestational age at delivery [weeks]	37.5 (34.0-39.0)
Proportion of premature deliveries [%]	23.4
Proportion of deliveries before 34 gestational weeks completed [%]	14.3
Birth weight [g]	3210 (2830-3720)
Proportion of SGA newborns [%]	11.0
Proportion of LGA newborns [%]	18.7

SGA – small for gestational age; LGA – large for gestational age; data presented as a median and interquartile range (in brackets).

In a regression analysis, performed separately for the subgroup with chronic conditions and the subgroup with gestational complications, we found no association between the concentrations of the cell adhesion molecules investigated and maternal characteristics such as age, prepregnancy body weight, history of diabetes or chronic hypertension, HbA1c level at sampling (in participants with diabetes), maximum diastolic or systolic blood pressure in pregnancy (in patients with hypertension) and gestational age at sampling. In addition, pre-eclampsia had no impact on the concentrations of sE-sel and sVCAM-1, except for an insignificant trend for lower sVCAM-1 levels in participants with pre-eclampsia.

Comparison of the concentrations of sE-sel and sVCAM-1 with respect to foetal growth revealed no correlation between the levels of these molecules and birth weight in the study group.

Discussion

In this study, we confirmed that maternal disorders affecting insulin resistance and vascular function have a measurable and differing impact on the circulating levels of cell adhesion molecules. However, we did not observe a dose-dependent pattern, suggesting an additive influence of concomitant conditions.

Limited clinical data are available on the changes in cell adhesion molecules that occur during pregnancy in humans. Moreover, these studies compared small cohorts of women with hypertensive pregnancies with healthy individuals and yielded inconclusive results. Some evidence on changes in the concentrations of cell adhesion molecules was provided by our previous studies on Type 1 diabetes in pregnant women. In this population, we found an association between microvascular complications and low levels of circulating sE-sel and sVCAM-1 and a relationship between the levels of these molecules and foetal growth [19, 21].

In this study, we observed low levels of sVCAM-1 in patients with chronic disorders primarily affecting vascular function, i.e. Type 1 diabetes alone or with chronic hypertension. Notably, the subgroup with gestational hypertension alone or with gestational hyperglycaemia had much higher sVCAM-1 concentrations than participants with pregestational diabetes with/without chronic hypertension. Based on these results, we conclude that maternal complications are associated with both abnormally elevated and reduced levels of sVCAM-1. We suspect that transient maternal hyperglycaemia, probably combined with hyperinsulinaemia, could be behind the increased sVCAM-1 concentrations evident in our cohort. Also, the fact that insulin resistance is a known pathomechanism for gestational hypertension explains the increased sVCAM-1 levels in the subgroup with gestational diabetes and hypertensive disorders. Interestingly, maternal hypertension differs from hyperglycaemia in terms of its impact on the vascular endothelium: the latter is associated with reduced levels of sVCAM-1, whereas in women with gestational hypertension, we observed substantially elevated levels of this protein.

In contrast, altered vascular function, which is commonly present in patients with long-term Type 1 diabetes, may cause a severe reduction in the level of sVCAM-1. Moreover, placental tissue is a separate source of sVCAM-1 that contributes to the total amount of this molecule present in the maternal circulation. Given that gestational diabetes is frequently associated with accelerated foetal growth and large placental size, this may contribute to elevated concentrations of sVCAM-1. However, in a pregnancy complicated with Type 1 diabetes, one would expect impaired placentation and poor placental development, which ultimately translate into intrauterine growth restriction (IUGR). In our early observations on a small cohort of women with pregnancies complicated by Type 1 diabetes, we observed no association between placental mass and the concentration of sVCAM-1, either in early or late gestation. It should be emphasized that placental mass is not necessarily directly associated with placental function. Furthermore, foetal growth restriction due to impaired placental function in pregnant women with diabetes is masked by the stimulation of relative foetal overgrowth by maternal hyperglycaemia.

Nevertheless, these results must be considered cautiously, because of our small sample size and the risk of the differences being driven by outliers. Some data are available from older studies involving small cohorts of pregnant women with pre-eclampsia. The authors of these studies consistently report elevated sVCAM-1 levels relative to normotensive controls in this population [14–16]. Similar to other studies involving women with high-risk pregnancies, we also found a substantial variation in the levels of sVCAM-1.

Currently, little is known about the relationship between foetal growth and markers of maternal endothelial function. However, altered endothelial function is a marker of several maternal disorders known for their negative impact on foetal

Table III. Concentrations of sE-Selectin and sVCAM-1 in the study group as MoM (multiple of median).

molecules	controls	GDM+PIH	PGDM+chHT	PGDM	PIH	GDM
sVCAM-1	1.00	1.06	0.70	0.62	1.86	0.64
sE-Selectin	1.00	0.73	0.51	0.54	0.70	--

GDM – gestational diabetes mellitus; PGDM – pregestational diabetes mellitus, PIH – gestational hypertension; data presented as a multiple of median versus the control group

growth. As foetal growth and development strongly depend on the uteroplacental vascular interface, an association between foetal growth and measurable markers of impaired endothelial function may indicate an inadequate supply of nutrients and oxygen to the foetus. In a study comparing maternal concentrations of sP-selectin (sP-sel) between normotensive and hypertensive pregnant women with or without IUGR, Laskowska *et al*. [22] found that concentrations of the molecule were elevated in patients with pre-eclampsia, irrespective of foetal growth, whereas in normotensive patients, isolated IUGR was associated with reduced concentrations of sP-sel relative to controls.

Research involving a larger cohort is necessary in future to identify the maternal and foetoplacental contributions to the specific profiles of markers of endothelial function present in the maternal circulation.

In summary, based on our findings, we conclude that maternal hyperglycaemia and/or hypertension in pregnancy is associated with reduced levels of sE-sel, irrespective of the chronic or gestational nature of the disorder. With regard to the concentrations of sVCAM-1, we propose that maternal disorders associated with insulin resistance manifest themselves in an elevated sVCAM-1 level and that these disorders tend to be pregnancy induced manifestations of severe maternal metabolic syndrome. In contrast, chronic maternal conditions in which vascular dysfunction develops are more likely to present with significantly lower concentrations of this molecule relative to healthy controls.

Conclusions

Pregestational or pregnancy associated disorders, although sharing similar clinical symptoms, have different impacts on endothelial function in pregnant women.

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