Determinants of C-reactive protein concentrations in pregnant women with type 1 diabetes

Paweł Gutaj¹, Patrycja Krzyżanowska², Jacek Brązert¹, Ewa Wender-Ożegowska¹

INTRODUCTION
Increased C-reactive protein (CRP) concentrations during pregnancy are associated with several perinatal complications.

OBJECTIVES
The aim of the study was to assess serum CRP concentrations and identify its determinants in pregnant women with type 1 diabetes.

PATIENTS AND METHODS
CRP concentrations were determined using a high-sensitivity assay (hs-CRP) in the first trimester (I, week <12 of gestation), in mid-pregnancy (II, weeks 20 to 24 of gestation), and in the late third trimester (III, weeks 34 to 39 of gestation) in a group of 73 patients with type 1 diabetes.

RESULTS
There was a significant increase in CRP concentrations between the first trimester and mid-pregnancy (median [interquartile range], 2.5 mg/l [1.3–4.5 mg/l] and 5.6 mg/l [2.5–11.6 mg/l]; P = 0.0001), which then stabilized with no further change between mid-pregnancy and the late third trimester (5.7 mg/l [2.5–9.6 mg/l]). CRP concentrations in all 3 trimesters were positively correlated with the waist-to-hip ratio (I, P <0.0001; II, P = 0.0004; III, P = 0.0369) and body mass index (I, P = 0.015; II, P = 0.0025; III, P = 0.0048), measured in the first trimester. CRP concentrations during pregnancy were positively correlated with a measure of insulin resistance, namely, the estimated glucose disposal rate, assessed in the first trimester (I, P = 0.01; II, P = 0.0165; III, P = 0.0062). There was a positive correlation between the levels of hs-CRP and total cholesterol (P = 0.001), low-density lipoprotein cholesterol (P = 0.013), and triglycerides (P = 0.0014) in the first trimester. There was no significant correlation between CRP and hemoglobin A1c, daily insulin requirement/kg, high-density lipoprotein cholesterol levels, maternal age, and diabetes duration.

CONCLUSIONS
Adiposity, abnormal body fat distribution, and insulin resistance are the major determinants of CRP concentrations in pregnant women with type 1 diabetes. Our results confirm the importance of weight control before pregnancy in women with type 1 diabetes.
women, higher levels of C-reactive protein (CRP) in the early pregnancy have been positively associated with perinatal complications including fetal growth restriction, preterm delivery, and gestational diabetes.\(^{10-12}\)

CRP is a clinically useful marker of systemic inflammation. Its synthesis takes place in the liver and is induced by infection and tissue injury. Previous work has shown that elevated levels of CRP are positively correlated with insulin requirements in nonpregnant women with type 1 diabetes.\(^{13}\) To date, there have been no studies analyzing the determinants of CRP levels in pregnant women with type 1 diabetes. Therefore, the aim of our study was to identify factors associated with CRP concentrations during pregnancy complicated by type 1 diabetes. We hypothesized that maternal metabolic control of diabetes may affect CRP levels in pregnancy.

**PATIENTS AND METHODS** This cross-sectional study was conducted in the Department of Obstetrics and Women’s Diseases, Poznań University of Medical Sciences, Poznań, Poland, in a population of pregnant Caucasian women with type 1 diabetes. The process of care delivered to pregnant diabetic women in our center is based on at least 3 planned short-stay hospital admissions during pregnancy: in the first trimester (week <12 of gestation), in mid-pregnancy (weeks 20 to 24 of gestation), and in the late third trimester (weeks 34 to 39 weeks of gestation). Patients who require more vigilant surveillance (diabetic complications, uncontrolled disease, obstetric comorbidities) are admitted more frequently. In-between hospital admissions, patients are referred biweekly for regular checkups in a hospital-based outpatient clinic.

For the purpose of this study, data were collected during the 3 main hospitalizations in the periods specified above. Patients received standard pregnancy care for type 1 diabetes, targeting a fasting glucose levels of 3.3 to 5.5 mmol/l, 1-hour postprandial glucose levels of more than 6.7 mmol/l, and hemoglobin A\(_1c\) (HbA\(_1c\)) levels of less than 6.1% (43 mmol/mol), as recommended by the Polish Diabetes Association and the Polish Gynecological Society.\(^{14}\) All women were treated with intensive insulin therapy using either multiple daily injections or continuous subcutaneous insulin infusion. Patients with multiple gestation, preterm rupture of membranes, preterm delivery, current infection, or acute illness of any kind were not included in the study.

Of 73 women, 31 were covered with structured prepregnancy counseling delivered by a diabetologist or an obstetrician with a special interest in diabetic pregnancy. These data were self-reported by participants. The remaining women received standard care for nonpregnant patients with type 1 diabetes delivered by diabetologists as recommended by the Polish Diabetes Association. According to the Association, blood glucose levels and HbA\(_1c\) targets for women planning their pregnancies and being pregnant compared with those not planning pregnancy or nonpregnant were as follows: 3.3–5.0 mmol/l vs 3.9–6.1 mmol/l for fasting glucose levels, <7.5 mmol/l vs <7.8 mmol/l for postprandial glucose levels, and 6.1% (43 mmol/mol) vs 6.5% (48 mmol/mol) for HbA\(_1c\).\(^{15}\)

Anthropometric, clinical, and laboratory data were collected during the 3 planned hospitalizations. Blood samples for HbA\(_1c\) and lipid profiles were taken after overnight fasting and immediately transported to the central laboratory of the Gynecologic Obstetrical University Hospital in Poznań. After collection, blood samples were centrifuged immediately and sera were stored at −80°C for further analysis. All patients underwent a 24-hour urine collection to measure urinary protein excretion. Serum CRP concentrations were measured by an enzyme-linked immunosorbsent assay (high-sensitivity CRP [hs-CRP] test, EIA – 3954, DRG International, Inc., Springfield, New Jersey, United States). The HbA\(_1c\) percentage in whole blood was determined using a turbidometric inhibition immunoassay, Tina-quant\textsuperscript{®} HbA\(_1c\) test in a Cobas c311 analyzer (Roche Diagnostics, Basel, Switzerland). The normal range for this test is 4.8% to 6.0% (29–42 mmol/mol) for the nonpregnant population. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride (TG) levels were determined using Roche Diagnostics reagents (cholesterol CHOD-PAP, HDL-C plus, and triglycerides GPO-PAP, respectively) in a Cobas c501 analyzer. The following formula was used to calculate the level of low-density lipoprotein (LDL) cholesterol: LDLC = total cholesterol − HDL cholesterol − (TG/5).

IR was quantified using the estimated glucose disposal rate (eGDR) (milligrams/kilogram/minute), calculated with the following equation: eGDR = 24.31 − (12.22 × WHR) − (3.29 × HTN) − (0.57 × HbA\(_1c\)), where HTN is hypertensive status (0 = no; 1 = yes), WHR is waist-to-hip ratio, and HbA\(_1c\) is expressed in percentage. A decrease in eGDR reflects an increase in IR.\(^{16}\)

Anthropometric measurements (height, weight, and waist and hip circumference) and blood pressure measurements were performed at the onset of the study.

**Statistical analysis** Statistical analyses were performed using MedCalc for Windows, version 12.1.3.0 (MedCalc Software, Mariakerke, Belgium). Testing for normality of data distribution was performed using the D’Agostino–Pearson test. Nonparametric tests were used for the analysis of the data because CRP concentrations were not normally distributed. The Spearman rank correlation coefficient (rho) was used to test the relationship between CRP concentrations and clinical and laboratory data (continuous variables). The association between CRP and categorical variables (the presence of vascular complications, yes/no; smoking status, yes/no) was examined using linear regression. Comparisons of the CRP levels...
between the 3 trimesters were performed using the Kruskall–Wallis test. Those parameters found to be associated with CRP concentrations were included in multiple regression (stepwise) models built separately for each follow-up hospitalization. As CRP concentrations did not follow normal distribution, the values were log-transformed before inclusion in the regression analyses. According to the stepwise method, variables were entered into the model if their associated P values were lower than 0.05, and sequentially removed if their associated P values became greater than 0.2. Testing for goodness of fit of the logistic regression model was performed using the Hosmer–Lemeshow test. Statistical significance was defined as a P value of less than 0.05 (2-sided).

An institutional review board at the Poznan University of Medical Sciences (no. 673/12) approved the study protocol. Informed written consent was obtained from every patient before inclusion in the study.

RESULTS Data from 73 pregnant women with type 1 diabetes were included in the analysis. Clinical and laboratory characteristics of the study group are shown in TABLES 1 and 2. Longitudinal changes in hs-CRP concentrations are shown in TABLE 2. Hs-CRP concentrations were the lowest in the first trimester, with a significant increase observed between the first trimester and mid-pregnancy. There was no change in hs-CRP concentrations between mid-pregnancy and the late third trimester, with a significant increase observed between the first trimester and mid-pregnancy (β = 0.076; P = 0.001; β = 0.052; P = 0.05; respectively) after adjustment for WHR. A negative correlation between hs-CRP and eGDR was observed (ρ = –0.301; P = 0.01). There was no correlation between hs-CRP and HDL cholesterol levels (β = 0.019; P = 0.19; β = 0.52), daily urinary protein excretion (ρ = –0.145; P = 0.23), and creatinine clearance (ρ = 0.019, P = 0.88). There were also no correlations between hs-CRP levels and maternal age (ρ = –0.084; P = 0.48), diabetes duration (ρ = –0.008; P = 0.94), and daily insulin requirement/kg (ρ = 0.073; P = 0.54). Moreover, hs-CRP was not associated with smoking status (β = 0.032; P = 0.81), the presence of vascular complications (β = 0.093; P = 0.45), and prepregnancy counseling (β = –1.67; P = 0.189).

Second trimester (weeks 20 to 24 of gestation) In the second trimester, hs-CRP levels were positively correlated with first-trimester WHR (ρ = 0.52, P = 0.0004) and BMI (ρ = 0.459, P = 0.0025). There was a negative correlation between mid-pregnancy hs-CRP levels and first-trimester eGDR (ρ = –0.372; P = 0.0165). Mid-pregnancy hs-CRP levels were not correlated with mid-pregnancy levels of total cholesterol (ρ = 0.025; P = 0.88), HDL cholesterol (ρ = –0.287; P = 0.073), LDL cholesterol (ρ = 0.231; P = 0.146), TG (ρ = 0.138; P = 0.389), and HbA1c (ρ = 0.092; P = 0.57), or with daily urinary protein excretion (ρ = 0.256; P = 0.121) and creatinine clearance (ρ = 0.256; P = 0.121). In addition, they were not correlated with maternal age (ρ = 0.163; P = 0.308), diabetes duration (ρ = –0.041; P = 0.8), and daily insulin requirement/kg (ρ = 0.157; P = 0.33). Finally, mid-pregnancy hs-CRP levels were not associated with smoking status (β = 0.20; P = 0.242), the presence of vascular complications (β = 0.219; P = 0.107), and prepregnancy counseling (β = –1.57; P = 0.512).

Third trimester (weeks 34 to 39 of gestation) In the late third trimester, hs-CRP levels were positively correlated with first-trimester WHR (ρ = 0.331; P = 0.0369) and BMI (ρ = 0.437; P = 0.0048). However, there was a negative correlation between hs-CRP levels and first-trimester eGDR (ρ = –0.426; P = 0.0062). In addition, hs-CRP levels were not correlated with the levels of TG (ρ = 0.256; P = 0.115), total cholesterol (ρ = 0.227; P = 0.176), HDL cholesterol (ρ = –0.157; P = 0.347), LDL cholesterol (ρ = 0.142; P = 0.402), and HbA1c (ρ = 0.236; P = 0.149), or with daily urinary protein excretion (ρ = 0.251; P = 0.189) and creatinine clearance (ρ = 0.0599;
TABLE 2  Laboratory parameters of the study group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>&lt;12 weeks</th>
<th>20–24 weeks</th>
<th>34–39 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP, mg/l</td>
<td>2.5 (1.3–4.5)</td>
<td>5.6 (2.5–11.6)</td>
<td>5.7 (2.5–9.6)</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>6.5 (5.9–7.5)</td>
<td>5.6 (5.3–5.9)</td>
<td>5.9 (5.6–6.3)</td>
</tr>
<tr>
<td>mmol/mol</td>
<td>48 (41–58)</td>
<td>38 (34–41)</td>
<td>41 (38–45)</td>
</tr>
<tr>
<td>eGDR, mg/kg/min</td>
<td>10.8 (9.5–11.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>serum total cholesterol, mmol/l</td>
<td>165.9 ±24.1</td>
<td>233.0 ±41.0</td>
<td>269.4 ±59.7</td>
</tr>
<tr>
<td>serum HDL cholesterol, mmol/l</td>
<td>72.6 (63.2–81.2)</td>
<td>87.9 (81.4–99.8)</td>
<td>77.1 (64.1–91.8)</td>
</tr>
<tr>
<td>serum LDL cholesterol, mmol/l</td>
<td>78.4 (67.1–93.8)</td>
<td>119.7 (99.1–134.5)</td>
<td>138.1 (115.3–169.9)</td>
</tr>
<tr>
<td>serum triglycerides, mmol/l</td>
<td>57.9 (45.1–75.5)</td>
<td>127.9 (103.8–159.5)</td>
<td>228.8 (180.9–281.4)</td>
</tr>
<tr>
<td>daily urinary protein excretion, g/24 h</td>
<td>0.13 (0.1–0.2)</td>
<td>0.16 (0.09–0.2)</td>
<td>0.19 (0.14–0.35)</td>
</tr>
<tr>
<td>creatinine clearance, ml/min</td>
<td>127.5 (106.5–144.0)</td>
<td>125.9 (108.9–145.5)</td>
<td>124.7 (108.5–140.6)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation or median (interquartile range).

Abbreviations: HbA1c, hemoglobin A1c; hs-CRP, high-sensitivity C-reactive protein; eGDR, estimated glucose disposal rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein

In another study involving young patients with type 1 diabetes, Ladeia et al. demonstrated a positive association between CRP levels and diabetes duration. We did not find such an association; however, this may be because our study group consisted of older patients (29 ±4 years vs 14 ±4 years) who had a longer duration of diabetes (12 ±7 years vs 5 ±4 years). Our results are in line with the results of Kilpatrick et al., who analyzed an older cohort (median age, 30 years) and showed that diabetes duration was unrelated to CRP levels.

We also tested whether CRP levels were correlated with body fat distribution, and found the correlation between CRP and WHR to be stronger than that for BMI, especially in the first trimester and mid-pregnancy. This provides additional evidence for an association between central adiposity and elevated CRP levels. Our results also suggest that central, rather than total, adiposity might be the major determinant of inflammatory status, which was previously demonstrated by Hermsdorff et al. in healthy young adults. Important data regarding the association between CRP levels in pregnancy and maternal obesity come from a cross-sectional study of Retnakaran et al. carried out in healthy women and those with gestational diabetes. The authors demonstrated that maternal prepregnancy BMI, but not maternal glycemic tolerance status, was a significant determinant of CRP levels during glucose tolerance testing in the late second or early third trimester. Another study confirmed these results at the time of screening for gestational diabetes. However, the authors repeated CRP measurements in the late third trimester (weeks 37 to 38 of gestation) and found that impaired glucose metabolism was a major predictor of CRP concentrations during this period. The authors hypothesized that subclinical inflammation in the early stages of pregnancy is driven by maternal prepregnancy obesity and a subsequent increase in IR and that resultant impaired...
In this study, which is consistent with earlier reports, 23-28 to date, there have been no studies linking CRP levels with IR and lipids in pregnancy; nonetheless, both elevated IR and dyslipidemia have been linked to chronic low-grade inflammation in adipose tissue. 29-31 In this study, CRP levels were correlated with total cholesterol, LDL cholesterol, and TG levels. To test whether these associations were secondary to adiposity, we performed the multiple regression analysis and found that the association remained significant for total cholesterol and LDL cholesterol, but was no longer observed for TGs after adjustment for WHR. It is interesting to note that these associations were found only in the first trimester. Pregnancy is associated with physiological changes in lipids induced by increasing levels of steroids and placental hormones; however, we found concentrations of lipids in early pregnancy to be relatively unaffected by these changes. Thus, it seems that lipids in early pregnancy reflect the prepregnancy status. Although total cholesterol, LDL, and CRP levels have all been associated with increased cardiovascular risk, the causal relationship between lipids and CRP levels was not established in our study.

To evaluate the possible association between IR and CRP levels, we calculated eGDR, which is a noninvasive measure of IR in patients with type 1 diabetes. The eGDR has been standardized for a nonpregnant population and has been found to correlate closely with a clamp technique, which is the gold standard for the estimation of insulin sensitivity in patients with absent β-cell function. We measured eGDR early in the pregnancy when WHR has not yet been affected by the enlarging uterus. Therefore, we assumed that the calculation of eGDR in our study group was not significantly biased by the pregnant state. 3 Negative correlation between first-trimester eGDR and CRP levels suggests that chronic low-grade inflammation may be linked with IR in pregnant women with type 1 diabetes. In addition, we did not find any association between smoking status and elevated CRP levels, 32,33 which is consistent with earlier reports. 34 The discrepancies between the studies might be explained by different methodological and population characteristics.

Although we studied many possible determinants of CRP concentrations, and our study population was relatively homogeneous, we cannot exclude the possibility of residual confounding effects of unmeasured variables, such as lifestyle factors.

In summary, we determined hs-CRP levels in pregnancy complicated by type 1 diabetes and demonstrated an association between hs-CRP and maternal adiposity, lipid profile, and IR in the first trimester. As CRP levels were positively associated with an increased risk of several perinatal complications, our results support the important role of weight control before pregnancy in women with type 1 diabetes.

**Contribution statement** PG participated in the study design; collected, analyzed, and interpreted the data; and participated in the preparation of the manuscript. PK performed a laboratory analysis and reviewed the manuscript. JB reviewed and accepted the final version of the manuscript. EW-O designed the study and interpreted and participated in the preparation of the manuscript.

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**REFERENCES**

Determinants of C-reactive protein concentrations in pregnant women with type 1 diabetes


Determinanty stężenia białka C-reaktywnego u kobiet ciężarnych z cukrzycą typu 1

Paweł Gutaj¹, Patrycja Krzyżanowska², Jacek Brązert¹, Ewa Wender-Ożegowska¹

¹ Klinika Położnictwa i chorób kobiecych, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu, Poznań
² Klinika Gastroenterologii Dziecięcej i chorób metabolicznych, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu, Poznań

SŁOWA KLUCZOWE
białko C-reaktywne, ciąża, cukrzyca typu 1, zapalenie

STRESZCZENIE

WPROWADZENIE
Podwyższone stężenie białka C-reaktywnego (C-reactive protein – CRP) w ciąży wiąże się z wieloma powikłaniami położniczymi.

CELE
Celem badania była analiza stężeń CRP w surowicy oraz wpływających na nie czynników u pacjentek ciężarnych z cukrzycą typu 1.

PACJENTI I METODY
Stężenia CRP oznaczono metodą o dużej czułości (high-sensitivity CRP – hsCRP) w I trymestrze ciąży (I: <12. tydzień), w połowie ciąży (II: 20.–24. tydzień ciąży) i pod koniec III trymestru (III: 34.–39. tydzień ciąży) w grupie 73 pacjentek z cukrzycą typu 1.

WYNIKI
Zaobserwowano istotny wzrost stężenia CRP między I trymestrą a połową ciąży (mediana [przedział międzykwartylowy]: 2,5 mg/l [1,3–4,5 mg/l] vs 5,6 mg/l [2,5–11,6 mg/l]; p = 0,0001), nie odnotowując dalszych zmian między połową ciąży a końcem III trymestru (5,7 mg/l [2,5–9,6 mg/l]). Stężenia CRP we wszystkich 3 trymestrach były dodatnio skorelowane ze wskaźnikiem talia–biodro (I: p <0,0001; II: p = 0,0004; III: p = 0,0369) i wskaźnikiem masy ciała (I: p = 0,015; II: p = 0,0025; III: p = 0,0048) zmierzonymi w I trymestrze ciąży. Stężenia CRP w trakcie ciąży były dodatnio skorelowane z wyliczonym w I trymestrze wskaźnikiem insulinooporności, tzw. estimated glucose disposal rate (I: p = 0,01; II: p = 0,0165; III: p = 0,0062). Stwierdzono dodatnią korelację między stężeniem CRP a stężeniem cholesterolu całkowitego (p = 0,001), cholesterolu LDL (p = 0,013) oraz triglicerydów (p = 0,0014) w I trymestrze ciąży. Nie stwierdzono korelacji między stężeniem CRP a stężeniami hemoglobinii A₁c, dobowym zapotrzebowaniem na insulinę/kg, stężeniem cholesterolu HDL, wiekiem matki oraz czasem trwania ciąży.

WNIOSKI
Nadmierna masa ciała, nieprawidłowa dystrybucja tkanki tłuszczowej oraz insulinooporność są głównymi czynnikami wpływającymi na stężenie CRP u ciężarnych z cukrzycą typu 1. Wyniki pracy potwierdzają istotną rolę kontroli masy ciała w okresie przedciążowym u pacjentek z cukrzycą typu 1.