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C-Reactive Protein Levels in Non-Obese Pregnant Women with Gestational Diabetes

Simin Rota, Başak Yildirim,[1] Babür Kaleli,[1] Hülya Aybek, Koray Duman[1] and Bünyamin Kaptanoğlu

Departments of Biochemistry and [1]Obstetrics and Gynecology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

Rota, S., Yildirim, B., Kaleli, B., Aybek, H., Duman, K. and Kaptanoğlu, B. *C-Reactive Protein Levels in Non-Obese Pregnant Women with Gestational Diabetes*. Tohoku J. Exp. Med., 2005, **206** (4), 341-345 —— A low-grade systemic inflammation is concomitant in diabetes. There is a pathophysiological relation between gestational diabetes mellitus and type 2 diabetes mellitus, which was further supported by significantly elevated risk of type 2 diabetes in women with a history of previous gestational diabetes mellitus. We investigated the relation between low-grade systemic inflammation expressed as C-reactive protein and gestational diabetes in non-obese pregnant women. This study included 20 non-obese pregnant women with gestational diabetes mellitus and 30 non-obese pregnant women without gestational diabetes mellitus as a control group. The body mass index of all the subjects were < 25 kg/m^2. During 26-28 gestational weeks 100-g oral glucose tolerance test was performed and simultaneously fasting C-reactive protein levels were measured. Serum median C-reactive protein level was higher in patients with gestational diabetes mellitus ($p = 0.0001$). C-reactive protein was strongly associated with glycemic parameters and weight gain during pregnancy. A model consisting of glucose intolerance, age, parity, and weight gain during pregnancy accounted for 61% of the variance in log C-reactive protein. We demonstrated that serum C-reactive protein level was related with gestational diabetes mellitus and weight gain during pregnancy in late second and early third trimesters. ———— gestational diabetes; CRP; subclinical inflammation; pregnancy; BMI

C-reactive protein (CRP) is an acute-phase protein produced by hepatocytes in response to tissue injury, infection, and inflammation. CRP concentrations have been shown to be significantly associated with several cardiovascular risk factors such as obesity, diabetes mellitus (DM), smoking, and hypertension (Ridkel et al. 2000). Adiposity is strongly associated with circulating CRP level. The relationship between CRP and adiposity could be explained by the recent findings as adipose tissue being a source for chronic subclinical inflammation (Festa et al. 2001). Recently, independent relationship of obesity with plasma CRP levels has been shown (Fröhlich et al. 2000).

Studies regarding with CRP and diabetes demonstrated that women with elevated CRP levels had significantly increased risk of diabetes, in-

Received April 21, 2005; revision accepted for publication May 30, 2005.
Correspondence: Simin Rota, M.D., PK 259 Yenişehir, 06443 Ankara, Turkey.
e-mail: siminrota@yahoo.co.uk

dependent of adiposity and other risk factors (Pannacciulli et al. 2001). An independent relationship between insulin resistance and plasma CRP levels has also been reported (Fröhlich et al. 2000). Accordingly, chronic inflammation as manifested by acute-phase response has been proposed as a potential "common soil" underlying insulin resistance (Stern 1995). Gestational diabetes mellitus (GDM), which is an important risk factor for both fetus and the mother, is characterized by increased insulin resistance and impaired pancreatic insulin secretion like DM (Duncan et al. 2003). It was shown that though most women with GDM return to normal glucose tolerance after delivery, the presence of glucose intolerance during pregnancy is predictive of later maternal type 2 DM (Winzer et al. 2004). Though obesity is known to be often associated with GDM, the presence of lean pregnant women with GDM (Duncan et al. 2003) suggests that insulin resistance in GDM could not be explained only by the presence of obesity.

In this study, we investigated the association of GDM and CRP as a subclinic inflammation marker in non-obese pregnant women, thereby excluding the obesity factor which may affect serum CRP levels.

Subjects and Methods

Subjects

Non-obese 20 pregnant women with GDM as study group and 30 non-obese pregnant women without GDM as control group were included to the study. The pre-pregnancy body mass index (BMI) of the subjects in both group were < 25 kg/m^2. The demographic characteristics of the subjects were given in Table 1. The exclusion criteria included pregnant women with known cardiovascular diseases, chronic inflammatory diseases and acute infections which may affect acute phase proteins. Multiple pregnant women, type 1 and 2 DM, smokers and corticosteroid using individuals were not included to the study. An oral informed consent was obtained from all of the participants. Demographic, obstetrical and personal information as infections, medications, pregnancy weight, weight gain during pregnancy, smoking were obtained by a questionnaire. Height (m) and weight (kg) were measured by using a standard scale. Weight gain was calculated with the difference between the pre-pregnancy weight and the weight measured while performing the OGTT.

Laboratory tests

All the 50 patients underwent to a 100-g oral glucose tolerance test (OGTT) interpreted by the World Health Organization criteria (Wolf et al. 2003) during the 26-28 gestational weeks. The 100-g OGTT was performed in the morning after an overnight fasting. Venous blood samples were drawn at baseline, 60, 120 and 180 min following ingestion of a standard 100-g glucose load. GDM diagnose was based on an abnormal OGTT as recommended by the American Diabetes Association (Wolf et al. 2003). Serum glucose levels were measured by an enzymatic method (IL test glucose) in ILAB900 (Instrumentation Laboratory, Lexington, MA, USA) autoanalyzer. Fasting CRP was measured by using a latex immunoturbidimetric assay (Scil Diagnostics GmbH, Martinsried, Germany).

Statistical analyses

Statistical analyses were performed using a commercial computer package programme (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL, USA). For non-parametric variables chi-square and for parametric variables *t*-test were used to assess the univariate differences between the variables. The distribution of CRP was skewed, thus median value was used for the presentation of this variable. The natural logarithmic transformation of CRP (logCRP) was used in multivariate analyses which were presented in tables. Bivariate associations of logCRP with continuous measures of weight gain in pregnancy, prepregnancy BMI, fasting blood glucose, 2-hr blood glucose were assessed by Pearson correlation analysis. Multiple linear regression analyses were used to determine which factors were significantly and independently associated with variation in logCRP.

Results

As shown in Table 1 the mean prepregnancy BMI of the subjects were less than 25 kg/m^2 and there was no significant difference among the groups. There were proportionally more nulliparas in the patients with GDM. There were no significant differences among the groups in age, gestational week, family history of GDM or type 2 DM, previous GDM and macrosomic infant. In GDM group weight gain during pregnancy was significantly higher compared to control group.

TABLE 1. *Demographic characteristics of study subjects*

Characteristics	GDM (Mean ± S.D.)	Control (Mean ± S.D.)	*p*
Age (yr)	25.40 ± 4.04	27.37 ± 5.20	0.16
Gestational age (week)	26.8 ± 1.2	27.1 ± 2.1	0.12
Prepregnancy BMI (kg/m^2)	23.34 ± 1.22	23.28 ± 0.98	0.848
Weight gain in pregnancy (kg)	7.25 ± 2.23	5.73 ± 1.78	0.01
Previous hx of GDM/DM 2	20%	13.3%	0.697
Previous GDM/macrosomic infant	5%	6.7%	1.00
Parity Nulliparous	75.0%	33.3%	0.036
One	15.0%	40.0%	
Greater than one	10.0%	26.6%	

Previous hx of GDM/DM 2; Previous history of GDM/DM 2.

In GDM group fasting and 2 h blood glucose levels were significantly higher than the control group. Serum median CRP level was higher in the GDM group compared to the control group (Table 2).

The reference range of CRP in our laboratory is 0-1 mg/100 ml. In our study, interestingly all the pregnant women with GDM had CRP levels above 1 mg/100 ml. In pregnant women without GDM only 4 subjects had CRP levels above 1 mg/100 ml. As in our control group there are only a few subjects with CRP levels above 1 mg/100 ml, and all the subjects with GDM had serum CRP levels above 1 mg/100 ml, the statistical analyses were done on the basis of CRP levels.

As shown in Table 3, in Pearson correlation analysis CRP was found to be strongly associated with weight gain during pregnancy and glycemic parameters. In multivariate linear regression analysis (Table 4), a model consisting of glucose intolerance, age, parity, weight gain during pregnancy accounted for 61% ($r^2 = 0.618$) of the vari-

TABLE 2. *Metabolic characteristics of study subjects*

Characteristics	GDM	Control	*p*
2 h blood glucose (mg/100 ml)[a]	206.6 ± 5.08	95.1 ± 14.22	0.0001*
Fasting blood glucose (mg/100 ml)[a]	91.60 ± 9.71	80.86 ± 8.03	0.0001*
CRP (mg/100 ml)[b]	2.8 (2.0 – 4.8)	0.95 (0.20 – 4.8)	0.0001*

[a] Mean ± S.D.
[b] Median (interquartile range).
* Statistically significant.

TABLE 3. *Pearson correlation analysis of CRP with adiposity and metabolic parameters*

	CRP	*p*
Pre-pregnancy BMI	–0.037	0.80
Weight gain in pregnancy	0.536	< 0.0001*
Fasting blood glucose	0.398	0.004*
2 h blood glucose	0.683	< 0.0001*

* Statistically significant.

TABLE 4. *Multiple linear regression analysis with dependent variable logCRP*

Variable	Parameter estimate	SE	*t*	*p*
Presence of GDM	0.445	0.079	5.664	0.0001*
Age	–0.004	0.007	–0.606	0.548
Parity	0.031	0.043	0.715	0.478
Weight gain in pregnancy	0.052	0.017	3.037	0.004*

* Statistically significant.

ance in logCRP. While glucose intolerance was found to be the most important factor ($p = 0.0001$) affecting CRP levels, weight gain was also shown to be another important factor ($p = 0.004$).

DISCUSSION

In the present study, we demonstrated that maternal serum CRP level was related with GDM and weight gain during pregnancy in late second and early third trimester. Although lean pregnant women were chosen for the study, CRP level was found to be associated with weight gain during pregnancy. This may raise a question whether also in pregnant women an increase in adipose tissue may associate with serum CRP levels. Adipose tissue is believed to be a source of subclinical inflammation (Festa et al. 2001). CRP as an inflammatory marker is produced and released by the liver under the stimulation of cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (Retnakaran et al. 2003). Cytokines promote *de novo* synthesis of hepatic fatty acid and interfere with lipoprotein lipase activity (Fröhlich et al. 2000). Hepatic uptake of insulin is inhibited by elevated portal free fatty acids. The elevated production of free fatty acids might hypothetically increase hepatic secretion of glucose as well as contribute to elevated peripheral insulin concentration (Duncan et al. 2003). Cytokines also may impede insulin stimulated glucose uptake (Fröhlich et al. 2000).

The correlation between CRP and glucose intolerance was also confirmed in our study. In a study, TNF-α another subclinical inflammation marker was found to be the strongest independent predictor of insulin resistance throughout pregnancy (Kirwan et al. 2002). Radaelli et al. (2003) suggested that in gestational diabetes, placenta showed increased expression of genes for chronic stress and inflammatory pathways. It was also reported that total sialic acid, an acute phase marker, was found to be elevated in women with a history of previous GDM, and was suggested as a potentially implicating factor in the link between previous GDM and type 2 DM (Retnakaran et al. 2003). Taken together, these data suggest a model of placental-driven systemic inflammation, leading to insulin resistance.

In our data, we showed a correlation between CRP levels and weight gain during pregnancy. According to our multiple regression analysis, weight gain difference between the two groups contribute to the difference in CRP levels among the groups. Several studies have established an association between prepregnancy and pregnancy BMIs and elevated inflammatory markers in first, late second, and early third trimesters. Wolf et al. (2003) suggested that the increased risk of GDM was attenuated when adjusted for BMI, which was highly correlated with CRP in the first trimester. It was also stated that despite the correlation of CRP with insulin resistance, an independent contribution of CRP to the risk of GDM could not be confirmed (Retnakaran et al. 2003). In our study a correlation was detected between CRP and glucose intolerance in GDM. Retnakaran et al. (2003) suggested that hormonal and metabolic factors specific to pregnancy may affect the relationship between inflammation and diabetogenesis.

In conclusion, we demonstrated that serum CRP levels were higher in lean pregnant women

with GDM compared to lean pregnant women without GDM and the elevated CRP levels did not correlate with prepregnancy BMI. The higher CRP level was found to be associated with glucose intolerance and weight gain during pregnancy. We propose a model wherein weight gain and GDM mediate a systemic inflammatory response resulting an increase in CRP levels. To our opinion future prospective studies on lean pregnant women are required to investigate the etiology of higher CRP levels in pregnant women with GDM.

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