

Maternal Serum C- Reactive Protein Concentration in Gestational Diabetes

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ABSTRACT

OBJECTIVE: Low-grade systemic inflammation is associated with an increased risk of type 2 diabetes. C-Reactive Protein (CRP), an acute phase protein produced by hepatocytes, may be associated with diabetes.

This study aimed to compare serum levels of CRP in women with gestational diabetes mellitus (GDM), impaired glucose tolerance test and control subjects.

MATERIALS AND METHODS: In this case control study, 30 women with GDM and 28 women with impaired glucose tolerance, (according to Carpenter and Coustan criteria) were compared with 31 normal pregnant women as control group. Groups were matched for gestational age, age and BMI before pregnancy. At 24-28 weeks of gestation, CRP levels were measured in three groups and compared with each other.

RESULTS: Our study showed serum CRP level was not significantly different among three groups. The median of serum CRP level in women with GDM, abnormal glucose tolerance test, and normal women was 8.8(7.5), 6.9(8.25) and 11.40(5.8) mg/dL respectively. In GDM patients, there was a significant correlation between CRP and BMI before pregnancy ($r=0.467$, $p=0.033$).

CONCLUSION: We didn't find a significant correlation between maternal serum CRP level and gestational diabetes but our study showed a significant correlation between pre-pregnancy BMI and CRP in gestational diabetic women.

KEY WORDS: C-reactive protein, gestational diabetes mellitus, body mass index, pregnancy, inflammation.

INTRODUCTION

Experimental and epidemiologic studies have shown an association between increased serum levels of acute-phase proteins and pathologic conditions, such as type 2 diabetes mellitus (DM), the metabolic syndrome X, obesity, and atherosclerotic cardiovascular diseases (1–2). A series of recent prospective studies have

linked such biomarkers as CRP, plasminogen activator inhibitor-1, and IL-6, the main upstream cytokine mediator of the acute-phase response with the prediction of type 2 DM in different populations (3-4). Gestational diabetes mellitus (GDM) is characterized by such metabolic defects as cell dysfunction and insulin resistance (5). There is a potential pathophysiological relationship between GDM

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and type 2 diabetes, hence these patients have a significantly elevated life-time risk for type 2 DM in the future (6). It has been hypothesized that GDM may represent the transient phase of a latent metabolic syndrome and may become clinically apparent later in life as type 2 DM (7).

Epidemiological studies have shown that CRP predicts the incidence of type 2 diabetes and cardiovascular diseases. In healthy middle-aged women, or young men for that matter, increased CRP levels were associated with a three to four fold increased risk of developing type 2 DM (5,6). Therefore, subclinical inflammation seems to be essentially involved in the pathogenesis of insulin resistance, metabolic syndrome and associated vascular diseases, although the exact pathophysiological mechanisms remain unclear (7,8)

Very limited attention has been paid to the role of inflammation as the etiology of gestational diabetes mellitus (GDM), a condition that is biochemically and epidemiologically similar to type 2 diabetes (6,8). Limited available data suggest that pro-inflammatory cytokines may be predictive of GDM (9,10). This hypothesis that GDM, in itself, may be a state of subclinical inflammation has not been directly evaluated, so this study compared serum levels of CRP in women with gestational diabetes, impaired glucose tolerance test and control subjects.

MATERIALS AND METHODS

A case control study was conducted at the pre-natal clinics of Mojibian and Shahid Sadoughi hospitals from 2007 till 2009 in Iran. Exclusion criteria included pre-gestational diabetes, chronic inflammatory diseases, hypertension, and preeclampsia or active infectious diseases. All subjects gave written informed consent for participation in the study, which was approved by the local ethics committee. In our study settings, all pregnant women were screened at 24–28 weeks of gestation using a 50-g glucose challenge test (GCT) (Carpenter and Coustan criteria). The

patients with post-challenge glucose concentrations of 130 mg/dL or higher were undertaken a standard 100-g, three-hour oral glucose tolerance test. GDM was diagnosed if at least 2 out of 4 diagnostic criteria were met (fasting plasma glucose \geq 95 mg/dl, 1, 2 and 3-hour plasma glucose \geq 180 mg/dL, \geq 155 mg/dL, and \geq 140 mg/dL, respectively). By consecutive patient selection, 29 women with GDM, 26 women with one abnormal OGTT value on the three-hour oral GTT (IGT), according to ADA criteria (ADA 2007) entered the study and 27 healthy pregnant women with normal GTT were considered as normal group. Normal pregnant women were matched with GDM and IGT groups for age, gestational age and BMI before pregnancy. We considered the ethical issues and described to recruited subjects for the study and obtained the consent to take blood samples. From their medical records, we got some information including maternal age, height, pre-pregnancy weight, reproductive and medical histories, and pre-pregnancy body mass index (BMI) (kg/m²) and entered them in analysis of the data as covariates. Maternal height and weight were measured by standard methods and body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters to the power of two. Pre-pregnancy weight was obtained by history.

Maternal fasting plasma samples were collected in 10-mL tubes, and frozen at -80°C. Samples were thawed at room temperature. Plasma glucose concentrations were measured in certified clinical laboratories using photometric method. The intra-assay coefficient of variation for the glucose was 1.74% and inter-assay coefficient of variation was 1.19%. This assay had a sensitivity margin of 5mg/dl. Serum CRP concentrations were measured using a sensitive DRG kit (USA). The intra-assay coefficient of variation for CRP was 7.5% and the inter-assay coefficient of variation for CRP was 4.1%. Subjects were divided into CRP tertiles using cut-points defined by the distribution of CRP (tertile 1, <1.3; tertile2, 1.3-4; tertile3, >4)

Statistical Analysis: All statistical analysis was performed using SPSS for Windows, version 11.50. Data of continuous variables were expressed as mean \pm standard deviation and median (interquartile range). For comparison of median of CRP between three groups Kruskal-Wallis test was used and for comparison of mean of fasting plasma glucose one way ANOVA was used. For assessment of correlation between variables on three groups Pearson's correlation was used.

RESULTS

Eighty nine pregnant women participated in this study, 30 and 28 subjects with GDM and IGT and 31 with normal GTT were considered as control group. Control group was matched for age, gestational age, and BMI before pregnancy with IGT and GDM groups. The mean age of pregnant women was 27.9 ± 6.1 years, mean of parity was 2 ± 1.1 and mean of pre-pregnancy BMI was $26.7 \pm 3.7 \text{ kg/m}^2$. Serum CRP level was not

significantly difference among three groups. The median of serum CRP level in women with gestational diabetes, abnormal glucose tolerance test group and control group was [median (interquartile range)] $8.8(7.5) \text{ mg/l}$, $6.9(8.25) \text{ mg/l}$ and $11.40(5.8) \text{ mg/l}$, respectively (Table1). This study showed a significant correlation between CRP level and BMI before pregnancy in GDM groups ($r=0.467$, $p=0.033$) and was not correlated with other groups. At 24th-28th weeks of gestation, CRP was not related to BMI at the time of OGTT in women with gestational diabetes. In all participants there was a positive correlation between CRP level and BMI before pregnancy, but the correlation was not significant ($r=0.23$, $p=0.08$) and the level was not correlated with age, parity and fasting blood glucose ($r=-0.114$, $p=0.385$) (Table2).

Table 3 shows the odds ratio of GDM within the CRP tertile relative to the lowest tertile. We didn't find any significant association between gestational diabetes and different CRP tertiles.

Table 1- Characteristic of study subjects.

	GDM n=30	IGT n=28	Control n=31	pvalue
CRP(mg/l)*	8.8(7.5)	6.9(8.25)	11.40(5.8)	0.1
BMI at OGTT (kg/m2)•	30.44 \pm 3.87	30.36 \pm 3.5	27.82 \pm 5.62	0.10
Fasting value at OGTT(mg/dl)•	98.79 \pm 14.78	97.07 \pm 7.29	88.20 \pm 11.18	0.18
1-h value at OGTT(mg/dl)•	201.37 \pm 29.9	160.15 \pm 31.36	151.33 \pm 14.97	0.01
2 h-value at OGTT(mg/dl)•	176.06 \pm 29.24	128.65 \pm 17.75	121.33 \pm 35.92	0.01
3-h value at OGTT(mg/dl)•	131.58 \pm 28.43	103.42 \pm 27.37	103.33 \pm 27.93	0.001

Median \pm interquartile range*
Mean \pm SD•

Table 2- Correlation (r) analysis of maternal C-reactive protein of women with gestational diabetes mellitus and other groups with different parameters.

	GDM n=30		IGT n=28		Control n=31		All of the women n=89	
	r	p	r	p	r	p	r	p
BMI before pregnancy (kg/m2)	0.467	0.03	0.19	0.4	0.14	0.5	0.23	0.08
BMI at OGTT (kg/m2)	0.2	0.3	0.17	0.4	0.15	0.4	0.04	0.7
Fasting value at OGTT(mg/dl)	-0.104	0.5	-0.06	0.7	0.08	0.8	-0.1	0.38
1-h value at OGTT(mg/dl)	-0.36	0.05	0.15	0.4	-0.7	0.5	-0.2	0.87
2 h-value at OGTT(mg/dl)	-0.35	0.05	0.21	0.2	-0.62	0.5	-0.01	0.9
3-h value at OGTT(mg/dl)	0.04	0.81	0.03	0.9	-0.34	0.7	0.08	0.52

Table 3- Odds ratio of GDM according to CRP tertile with tertile 1 serving as the reference group

	Tertile 1	Tertile 2	Tertile 3	P.value
CRP Range	<1.3	1.3-4	>4	
Odds Ratio(95%CI)	1	1.29(0.38-4.3)	0.95(0.76-1.1)	0.47

DISCUSSION

The second and third trimesters of pregnancy represent a physiological type of insulin resistance (11). Insulin resistance is associated with dysfunction of endothelial and inflammation as well as increased production of cytokines by adipose tissue (12-13). CRP, a sensitive marker of inflammation, is associated with a high prevalence of atherosclerosis (3, 14, 15). There is some evidence to support the theory that chronic inflammation might be a risk factor for type 2 diabetes (3). Gestational diabetes is a biochemical and epidemiological condition similar to type 2 diabetes (16), so pro-inflammatory cytokines such as CRP may be associated with GDM. Some studies have measured CRP at various gestational ages in pregnant women and found inconsistent results regarding the association between inflammatory markers and the incidence of GDM and the interdependence with the degree of adiposity (17). The interpretation of the results was influenced by coexistence of hypertension, preeclampsia, and different racial groups or small sample size in some studies. In our study, there was no relationship between level of CRP and GDM at the 24th - 28th weeks of gestation. This finding is similar to reports of the Leipold et al (18) that showed GDM was not related to increased CRP at the 24th -28th weeks of gestation but was related to CRP at the 37th -38th weeks of pregnancy. Rentankaran et al (17) also demonstrated that maternal serum levels of CRP are not related to GDM at the time of oral glucose tolerance testing in late second or early third trimester. But our result is in part contradictory to reports of the Rota et al (19) who demonstrated that CRP protein level was higher in patients with gestational diabetes. Wolf et al (20) found that CRP concentrations in the first trimester predicted the development of GDM in the ongoing pregnancy. Also, in our study CRP concentrations correlated with BMI before pregnancy in women with gestational diabetes. This result is consistent with the study of Rentankaran et al (17) and Leipold et al (18) that showed a significant

correlation between pre-pregnancy BMI and CRP level. In a prospective study conducted by Qiu et al to examine the association between CRP and GDM risk, women were recruited before 16 weeks of gestation and were followed until delivery. This study demonstrated that elevated CRP was associated with GDM risk. After adjustment of maternal pre-pregnancy BMI, family history of diabetes and nulliparity, women with CRP in the highest tertile experienced a 3.5-fold increased risk of GDM (95% CI 1.2-9.8) as compared with those in the lowest tertile. The association between CRP and GDM was obvious when analyses were restricted to lean women (BMI<25kg/m²). Lean women with CRP \geq 5.3mg/l had a 3.7-fold increased risk of GDM (95%CI 1.6-8.7) as compared with women with CRP < 5.3mg/l. This study concluded that systemic inflammation is associated with an increased risk of GDM and this association is independent of maternal pre-pregnancy adiposity (21). Li et al evaluated serum CRP in gestational diabetes and pregnant control women and showed that CRP was positively related with fructose amine hemoglobin A1c, triglyceride and BMI. This study concluded that CRP plays a role in pathogenesis of GDM (22). Smirnakis et al assessed the association of sex hormone-binding globulin, high-sensitive C-reactive protein and fasting blood glucose and insulin in the late first trimester and early second trimester of pregnancy with the diagnosis of gestational diabetes. In this study sex hormone-binding globulin level was lower and high-sensitive CRP level was higher among women who subsequently developed gestational diabetes. Multivariate analysis suggested that sex hormone-binding globulin measurement was the best predictor of GDM (23). Inconsistent results regarding the association between inflammatory markers and GDM are related to different racial groups or small sample size and different methods of researches. The limitations of our study included: small sample size, cross-sectional design of our research (cross-sectional), measuring of CRP level at one juncture in

pregnancy .We need a longitudinal study to measure CRP in the first trimester and follow the patients to show the effect of elevated CRP in the pathogenesis of GDM.

In conclusion we didn't find a significant correlation between maternal serum CRP level and gestational diabetes but our study showed a significant correlation between pre-pregnancy BMI and CRP in gestational

diabetic women.

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