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Circulating monocyte chemoattractant protein-1 in women with gestational diabetes

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Abstract: Monocyte chemoattractant protein 1 (MCP-1) has been implicated as a key factor in the recruitment and activation of peripheral blood leukocytes in atherosclerotic lesions and adipose tissue. Elevated levels of circulating MCP-1 have been found in patients with type 1 and type 2 diabetes, as well as with coronary artery disease. In this study we compared serum MCP-1 concentrations between pregnant women with normal glucose tolerance (NGT), gestational diabetes mellitus (GDM) and non-pregnant healthy women. The studied group consisted of 62 patients with GDM (mean age 30.1 ± 5.0 years) at 29.0 ± 3.5 week of gestation, 64 pregnant women with NGT (mean age 30.0 ± 4.7 years) at 29.2 ± 2.9 week of gestation and 34 non-pregnant healthy women (mean age 29.8 ± 4.7 years). Serum MCP-1 concentration was measured using an enzyme - linked immunosorbent assay. Median MCP-1 concentrations did not differ significantly between women with GDM (median 342.3 [interquartile range 267.9-424.4] pg/ml) and NGT (338.0 [274.7-408.2] pg/ml), but were markedly lower than those found in non-pregnant women (485.2 [409.6-642.4] pg/ml, $p<0.0001$). After adjusting for glucose, the difference between pregnant and non-pregnant women remained highly significant ($p<0.0001$). In GDM patients MCP-1 levels correlated significantly with fasting glucose ($r=0.2665$, $p=0.0363$), insulin ($r=0.4330$, $p=0.0004$), HOMA-IR ($r=0.4402$, $p=0.0003$), ISQUICKI ($r=-0.4402$, $p=0.0003$), HbA1c ($r=0.2724$, $p=0.0322$), as well as with prepregnancy and current BMI ($r=0.3501$, $p=0.0057$ and $r=0.3250$, $p=0.0106$, respectively). Multiple regression analysis revealed that MCP-1 concentrations were significantly predicted only by plasma glucose ($\beta=0.3489$, $p=0.00004$). Our results suggest that MCP-1 levels are decreased in pregnant women, irrespective of their glucose tolerance status.

Key words: MCP-1 - Gestational diabetes - Insulin resistance - Pregnancy

Introduction

Chemokines (chemotactic cytokines) are small heparin-binding proteins that direct the migration of circulating leukocytes to sites of inflammation or injury [1-3]. The largest family is known as the CC chemokines, because of the fist two of the four conserved cysteine residues, and the most thoroughly characterized CC chemokine is MCP-1 (monocyte chemoattractant protein 1), also known as CCL2 - a potent agonist for monocytes, memory T cells and basophils [1]. MCP-1 has been implicated as a key player in the recruitment of monocytes from the blood into early atherosclerotic lesions, the development of intimal hyperplasia after angioplasty, as well as in vasculogenesis and thrombosis [1]. MCP-1 is also expressed and secreted by adipocytes and has been reported to be involved in the recruitment and activation of peripheral blood leukocytes in adipose tissue and in the induction of systemic insulin resistance [4-6]. Recently Zhou *et al.* [7] reported the identification of a novel protein, designated MCPIP (MCP-induced protein), which is expressed in human monocytes and cardiomyocytes after stimulation with MCP-1. Their results provided a novel molecular pathway, by which MCP-1 signal transduction is linked to transcriptional gene regulation leading to apoptosis [7].

Elevated levels of circulating MCP-1 have been found in patients with type 1 and type 2 diabetes [8-14], hypercholesterolemia [15], arterial hypertension [16], congestive heart failure [17] and coronary artery disease [18-20]. *In vitro* studies demonstrated that advanced glycation end-products, high glucose con-

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centration, glycated albumin and glycoxidized LDL enhanced MCP-1 expression in human endothelial cells [21].

Gestational diabetes mellitus (GDM), defined as a carbohydrate intolerance of varying severity, with onset or first recognition during pregnancy [22], is considered a prediabetic state or a transient unmasking of the metabolic syndrome, offering a unique opportunity to study abnormalities that may appear very early in the development of type 2 diabetes [23]. Di Benedetto *et al.* [24] demonstrated that women with and without a history of GDM had comparable serum levels of MCP-1, but to our best knowledge there are no reports regarding MCP-1 concentrations during pregnancy complicated by GDM. So, in the present study we evaluated MCP-1 serum levels in pregnant women with normal glucose tolerance (NGT) and GDM and established their relation to a range of anthropometric and metabolic factors conferring insulin resistance.

Materials and methods

Patients. The studied group consisted of 62 patients with GDM and 64 pregnant women with NGT, attending the gynaecological out - patient clinic of the Medical University of Bialystok: The control group consisted of 34 non-pregnant healthy women, eight of whom had one or more pregnancies without a history of GDM and 26 had no previous pregnancy. All subjects were non-smokers and had not taken any anti-inflammatory drugs or drugs known to affect carbohydrate metabolism in the previous 3 months. Patients with pregnancy induced hypertension (PIH), preeclampsia and other pregnancy complications (except GDM) were not included. All pregnancies were singletons. The estimation of pregnancy duration was based on routine ultrasonographic examination preformed between 10 and 12 weeks of gestation. GDM was diagnosed according to the Polish Diabetological Association criteria (fasting plasma glucose 100 mg/dl [5.55 mmol/L] or/and plasma glucose 2-h after 75g glucose load 140 mg/dl [7.8 mmol/L]). Written informed consent was obtained from all participants, and the protocol was approved by the local ethics committee (Medical University of Bialystok).

For each patient body mass index (BMI) was calculated at the time of blood collection as weight in kilograms divided by height in meters squared. Medical records were also revised to collect information concerning subject's weight shortly before conception, and prepregnancy BMI was calculated as described above. Fasting plasma glucose concentration was measured using oxidase method (CORMAY, Poland) and fasting serum insulin level was assayed by immunoradiometric method (Biosource Europe SA, Belgium). MCP-1 serum concentration was measured using human ultrasensitive ELISA kit (Bender MedSystems GmbH, Austria) with the detection limit of less than 2.31 pg/ml. The intraassay and interassay coefficients of variation (CVs) were less than 4.7% and 8.7%, respectively.

Insulin resistance. Insulin resistance was estimated by HOMA (Homeostasis Model Assessment) (25) and QUICKI (Quantitative Insulin Sensitivity Check Index) (26). An insulin resistance score (HOMA-IR) was computed with the formula: fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5 (25). Insulin sensitivity index (IS_{QUICKI}) was calculated as the inverse log sum of fasting insulin (I0 [mU/l]) and fasting glucose (G_0 [mg/dl]): $IS_{QUICKI}=1/[\log(I_0) + \log(G_0)]$ (26).

Statistical analysis. Statistical analysis was performed using the STATISTICA 7.0 for Windows software package (StatSoft.Inc, Tulsa, USA). The variables the distribution of which was substantially skewed, were expressed as medians and interquartile ranges (IR). Other results were showed as means ± SD. The differences between the groups were compared by Mann - Whitney U test. Multiple least - square regression procedure was used to estimate mean case - control differences in serum MCP-1 concentration after adjusting for potential confounders. Relationships between variables were tested by Spearman's rank correlations. Moreover, multivariate linear regression analysis was performed to establish which factors were significantly and independently associated with the variance in MCP-1 serum concentration. p value less than 0.05 was regarded as statistically significant.

Results

Clinical and biochemical characteristics of the groups studied are summarized in Table 1. Patients with GDM were significantly more obese before pregnancy, had higher fasting insulin levels and HOMA-IR and lower IS_{QUICKI} values than the control group, as well as significantly higher plasma glucose, HOMA-IR and lower IS_{QUICKI} than the pregnant women with NGT. The subjects with NGT had markedly lower fasting glucose and higher insulin levels in comparison with the non-pregnant women. MCP-1 concentrations were comparable in the NGT and GDM groups, but significantly lower than in the non-pregnant group (Table 1).

In a pooled analysis MCP-1 correlated significantly with fasting glucose ($r=0.3330$, $p<0.0001$), HOMA-IR ($r=0.1726$, $p=0.0296$) and ISQUICKI ($r=-0.1726$, $p=0.0296$). In the GDM group MCP-1 correlated significantly with fasting glucose ($r=0.2665$, $p=0.0363$), insulin ($r=0.4330$, $p=0.0004$), HOMA-IR ($r=0.4402$, $p=0.0003$), ISQUICKI ($r=-0.4402$, $p=0.0003$), HbA1c ($r=0.2724$, $p=0.0322$), as well as with prepregnancy and current BMI ($r=0.3501$, $p=0.0057$ and $r=0.3250$, $p=0.0106$, respectively). In the non-pregnant women there was only a correlation between MCP-1 and fasting glucose ($r=0.3580$, $p=0.0376$). In the pregnant subjects with NGT MCP-1 values were not associated with any of the clinical or metabolic parameters studied.

Because MCP-1 levels were most strongly associated with fasting glucose, an adjustment for this parameter was made, and the differences in MCP-1 concentrations between the control group and the women with NGT and GDM remained highly significant ($p<0.0001$). Multiple regression analysis with MCP-1 as a dependent value revealed that in the whole population plasma glucose was the only significant predictor ($\beta=0.3489$, $p=0.00004$), explaining about 9% of the variance in MCP-1 concentration. In the subgroup of pregnant women MCP-1 levels were significantly predicted only by fasting insulin values ($\beta=0.2715$, $p=0.0323$).

Table 1. Clinical and biochemical parameters of the studied population.

Parameter	Control group n=34	NGT n=64	GDM n=62
Age (years)	29.8 ± 4.7	30.0 ± 4.7	30.7 ± 5.0
Parity	1.8 ± 0.95	1.9 ± 1.04	2.0 ± 1.31
Gestational age (week)	-	29.2 ± 2.9	29.0 ± 3.3
Prepregnancy BMI (kg/m^2)	22.0 (20.4-23.8)	23.1 (20.4-26.7)	23.6 (20.8-27.2) p=0.0115*
Current BMI (kg/m^2)	-	27.9 (24.6-30.9)	28.3 (25.1-31.3)
Fasting glucose (mmol/l)	4.82 (4.33-5.22)	4.14 (3.94-4.42) p<0.0001*	4.44 (4.28-4.94) p<0.0001†
Fasting insulin (pmol/l)	77.1 (59.5-102.6)	98.3 (65.3-138.5) p=0.0355*	114.4 (79.6-142.8) p=0.0004*
HOMA-IR	2.29 (1.79-3.08)	2.54 (1.71-3.81)	3.21 (2.14-3.99) p=0.0066* p=0.0203†
QUICKI	0.146 (0.140-0.152)	0.144 (0.136-0.153)	0.139 (0.135-0.148) p=0.0066* p=0.0203†
HbA1c (%)	4.9 (4.7-5.2)	5.0 (4.6-5.3)	5.1 (4.8-5.3)
MCP-1 (pg/ml)	485.2 (409.8-642.4)	338.0 (274.7-408.2) p<0.0001*	342.3 (287.9-424.4) p<0.0001*

Data are shown as means ± SD or medians (interquartile range)
*p *vs* control; †p *vs* NGT group

Discussion

In the present study we showed that patients with GDM were significantly more obese before pregnancy, had higher fasting insulin levels and higher indices of insulin resistance, whereas the subjects with NGT demonstrated markedly lower fasting glucose and higher insulin concentrations in comparison with the non-pregnant group. These findings correspond to the well known metabolic changes that occur in normal and diabetic pregnancy. Non-diabetic pregnancy produces an "accelerated starvation" in the fasting state, with an earlier and more profound hypoglycemia and an increased fasting insulin level, whereas women with GDM first of all demonstrate elevated fasting insulin concentrations [27].

In this study we also showed that serum MCP-1 levels did not differ between women with NGT and GDM, but were significantly lower than those observed in the non-pregnant subjects. These findings are in part consistent with the results obtained by Di Benedetto *et al.* [24], who found similar MCP-1 serum concentrations in women with and without a recent history of GDM. Conversely, Denison *et al.* [28] observed significantly higher release of MCP-1 from peripheral blood cultures from pregnant women at term as compared with non-pregnant women. The explanation of this dicrepancy is quite a problem. In our study MCP-1 levels were most strongly associated with glucose concentrations, but after an adjustment for this confounder, the difference in MCP-1 levels between the control group and the women with NTG and GDM remained highly significant. Moreover, in contrast to the NGT group - demonstrating comparatively low fasting glucose values - glycemic levels in GDM patients were comparable to those found in the non-pregnant women, whereas MCP-1 concentrations differed significantly between the two groups. These findings suggest that factors other than glucose are probably responsible for the observed difference in serum MCP-1 levels between pregnant and non-pregnant women. One of such factors may be transforming growth factor - beta1 (TGF-beta1) - a multifunctional cytokine that exhibits potent immunoregulatory and anti-inflammatory properties and prolongs graft survival [29,30]. Recent reports showed that TGF-beta1 levels were elevated in healthy pregnant women in comparison with the non-pregnant controls and suggested that TGF-beta1 may function as a regulatory factor in fetal allograft survival during pregnancy [29,30]. On the other hand, TGF-beta has been shown to have immunosuppressive effects on endothelial cells (EC). Ayatollahi *et al.* [31] demonstrated that EC expressed MCP-1 mRNA and protein in response to tumor necrosis factor-alpha (TNF alpha), interferon-gamma (IFN-gamma) or interleukin-1beta (IL-1beta), but not TGF-beta1. TGF-beta1 in co-treatment with either TNF alpha or IL-1beta, significantly decreased MCP-1 mRNA and protein expression, as compared to TNF alpha or IL-1beta treatment alone, indicating that TGF-beta down-modulates cytokine-induced MCP-1 expression in human EC [31]. Of course, we can only hypothesize that TGF-beta may be a key factor responsible for the alterations in circulating MCP-1 levels, but the secretion and action of this chemokine in pregnant women need further investigations.

In conclusion, our results suggest that serum MCP-1 concentrations are decreased in pregnant women, irrespective of their glucose tolerance status.

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