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Osteopontin levels do not increase in gestational diabetes mellitus

Gestasyonel diabetes mellitusta osteopontin seviyeleri artmaz

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Abstract

Aim: Osteopontin (OPN) has recently been considered as a marker of coronary artery disease. The aim of this study is to evaluate OPN levels and investigate their correlation with the high sensitivity-CRP (hs-CRP) levels in patients with gestational diabetes mellitus (GDM), an increased state of insulin resistance.

Materials and Methods: Fifty-four GDM patients and 40 healthy pregnant patients were included in this study. Biochemical tests for lipid profile, fasting blood glucose, oral glucose tolerance test, OPN, HOMA-IR and hs-CRP were done at 24th gestational week. Serum levels of OPN were measured by enzyme-linked immunosorbent assays (ELISAs), serum hs-CRP levels were measured by particle association turbidometric assay.

Results: Gestational week, age, BMI of two groups were similar ($p > 0.05$). The GDM group had significantly higher fasting, and post-load (1st and 2nd hour) blood glucose, HbA1c, fasting insulin and HOMA-IR levels than those of the healthy group. Except triglyceride levels, the lipid profiles of two groups were not significantly different. The GDM group had higher triglyceride levels than the control group ($p<0.05$). The OPN levels were 3.6±2.2 ng/mL in the GDM and 3.4±2.6 ng/mL in the control groups ($p>0.05$). The hs-CRP levels were 0.94±0.8 mg/dL, 0.57±0.5 mg/dL in the GDM and control groups, respectively ($p<0.05$). There was no correlation between the OPN and hs-CRP levels ($r=0.080$; $p=0.442$). In GDM the patients are experiencing rapid metabolic changes, so these metabolic changes may not affect the level of OPN.

Conclusion: OPN levels were not significantly increased in GDM patients.

Keywords: Gestational diabetes mellitus, HOMA-IR, hs-CRP, osteopontin.

Öz

Amaç: *Osteopontin (OPN) son zamanlarda koroner arter hastalığının bir belirteci olarak düşünülmektedir. Bu çalışmada, insulin direncinin arttığı bir durum olan gestasyonel diabetes mellituslu (GDM) hastalarda,OPN seviyelerini ve bunun sensitif CRP (hs-CRP) seviyeleri ile korelasyonunu incelemek istedik.*

Gereç ve Yöntem: *Çalışmaya 54 GDM ve 40 sağlıklı hamile alındı. 24. Gebelik haftasında kan lipid düzeyleri, oral glukoz yükleme testi, OPN, HOMA-IR, hs-CRP düzeyleri için kan tahlilleri yapıldı. Serum OPN düzeyleri ELISA, hs-CRP düzeyleri parçacık ilişkili turbidimetrik test ile ölçüldü.*

Bulgular: *Her iki grubun gestasyonel haftası, yaş, VKI benzerdi ($p > 0.05$). GDM grubunda açlık glukoz, OGTT te 1 ve 2. saat kan glukoz, HbA1c, açlık insulin, HOMA-IR değerleri kontrol grubuna göre istatistiksel olarak anlamlı daha yüksek saptandı. Trigliserid seviyeleri dışında lipid profilerinde her iki grupta anlamlı fark saptanmadı. GDM grubunda kontrol grubuna göre trigliserid seviyeleri daha yüksek saptandı ($p<0.05$). GDM grubunda OPN seviyesi 3.6±2.2 ng/mL, kontrol grubunda 3.4±2.6 ng/mL saptandı ($p>0.05$). GDM ve kontrol gruplarında hs-CRP seviyeleri sırasıyla 0.94±0.8 mg/dL, 0.57±0.5 mg/dL saptandı ($p<0.05$). OPN ve hs-CRP seviyelerinde anlamlı korelasyon saptanmadı ($r=0.080$; $p=0.442$). Gestasyonel diyabette hastalar hızlı metabolik değişiklikler yaşadığı için metabolik değişiklikler OPN seviyesini etkilemeyebilir.*

Sonuç: *GDM'lu hastalarda OPN seviyelerinde anlamlı artış saptanmamıştır.*

Anahtar Sözcükler: Gestasyonel diabetes mellitus, HOMA-IR, hs-CRP, osteopontin.

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Introduction

Gestational diabetes mellitus (GDM) is a transient carbohydrate metabolism disturbance in pregnancy. GDM is observed in 1-10% of pregnancies (1). Up to 24 weeks of pregnancy, various hormonal changes affecting insulin resistance lead to the emergence of GDM. GDM may be defined as early-onset type 2 DM in insulin resistance (1). GDM leads to a variety of risks for the fetus and the mother during pregnancy (2). Pregnants with GDM have an 18-50% risk of developing type 2 DM within 5 years after parturition (3). Cardiovascular disorders, hypertension, dyslipidemia and metabolic syndrome can also develop years after onset of GDM (4).

According to experimental and epidemiological studies, the association between serum acute phase proteins with type 2 DM, metabolic syndrome, obesity and coronary heart disease has been proven (5). Chronic inflammatory conditions developing in these diseases may cause acute-phase response (6). Studies performed in healthy population have indicated that the inflammatory biomarkers like CRP and plasminogen activator inhibitor-1 are independent predictors of coronary artery diseases (CAD) (7). In the pathophysiology of GDM, insulin resistance and chronic subclinical inflammation may lead to acute phase response (8). In this study, the GDM mothers were expected to have higher levels of hs-CRP than the control mothers.

Osteopontin (OPN) is a protein compound which is secreted by many cells (9). The role of OPN has been revealed in many cellular, humoral and metabolic mechanisms (9,10). Therefore, OPN also has an important role in atherosclerosis, vascular calcification and remodeling (11). However in a recently published study, it was reported that in a young population without any CAD symptoms, OPN levels were not associated with the vascular markers of subclinical atherosclerosis (12). In another report, OPN levels were correlated with fasting blood glucose levels and increased in obese/overweight individuals (13). Stenczer et al. (14) reported that preeclampsia patients who had serious endothelial injury had demonstrated higher fibronectin, and osteopontin levels. Pregnant patients who develop insulin resistance in the first trimester have a higher risk of becoming preeclamptic later in pregnancy (15). Recently, Winhofer et al. (16) have reported that the serum OPN levels were lower in GDM group than the control group, but the correlation between serum OPN levels and the insulin sensitivity/secretion was not statistically significant. After all, we could not find adequate number of literature studies about the significance of OPN levels in GDM.

Materials and Methods

This prospective study included 54 GDM patients and also 40 healthy pregnant as control group (overall age range, 18-34 years) at their 24 - 28 weeks of gestation who presented to our endocrinology and obstetrics clinics between April 2012 and March 2015. Patients with any of the following criteria were excluded from the study: History of smoking; abnormal blood pressure, previously diagnosed thyroid or pituitary disease; CAD; diabetes mellitus; chronic renal failure; rheumatic disease; and recent history of psychiatric disorders. Blood samples were drawn once for the measurement of OPN, hs-CRP, lipid profile, HOMA-IR levels, after the patients with established diagnosis of GDM, gave their informed consent for the study.

A 2-hour oral glucose tolerance test (OGTT) was performed using 75 g glucose after 8 hours of fasting. OGTT equal or above reference values (fasting: 92 mg/dL, 1-h: 180 mg/dL, and 2-h: 153 mg/dL) indicated presence of GDM (17).

Medical ethics committee of our university approved the study. The researchers declared that they had complied with the World Medical Association *Declaration of Helsinki*: Ethical principles for medical *research* involving *human* subjects. The written and informed consent forms were provided for all patients.

Biochemical parameters

Serum glucose, triglyceride, total and HDL-cholesterol levels were determined using enzymatic procedures, and serum insulin was measured with chemiluminescent methods. Serum LDL levels were calculated using the Friedwald formula. Serum levels of OPN were measured by enzyme-linked immunosorbent assays (ELISAs) (Human Osteopontin Platinum ELISA BMS2066 / BMS2066TEN eBioscience Kit; normal range 2.30-75.24 ng/mL). High- sensitivity CRP (hs-CRP) levels were measured by particle-enhanced turbidometric immuno-assay (normal range 0.03-2.76 mg/dL) (Cobas Integra C-Reactive Protein Latex, Roche Diagnostics, Indianapolis, USA). HbA1c was measured from blood samples by the turbidometric inhibition immunoassay test method (Roche COBAS Autoanalysis 6000, USA) (18).

Firstly, serum insulin and fasting glucose levels were measured, then the insulin resistance was calculated using the homeostasis model assessment (HOMA) based on the following formula (19).

HOMA-IR = [fasting plasma insulin (μU/mL)] x [fasting plasma glucose (mmol/L)] / 22.5

Statistical analyses

Statistical analyses were performed using the Rstudio software (version 0.98.501). The Kolmogorov-Smirnov test was done to analyze the normality of data. All

numerical variables with a normal distribution were expressed as mean±standard deviation. For comparison between the parameters of GDM and control groups the independent sample t-test was used. Nonhomogeneous data were analyzed using the Mann-Whitney U test. The Pearson correlation test was used to assign the association between OPN and hs-CRP levels.

Results

The characteristics of the groups are shown in Table-1. There was no significant difference in age, BMI and gestational week between the groups.

Table-1. Demographic Characteristics of Patients (n=94).

	GDM Group (n=54)	Control Group (n=40)	*p*
Age (years)	32.6 ± 5.3	29.2±4.7	NS
BMI (kg/m^2)	28.6 ± 4.3	25.9 ± 3.8	NS
Gestational week	24.9 ± 3.2	25.5 ± 2.3	NS

GDM: Gestational diabetes mellitus, NS: Non-significant. Values are given as mean ± SD (standard deviation)

Table- 2. Results of the Study

	GDM Group (n=50)	Control Group (n=40)	*p*
Fasting blood glucose*	94.8±16.7	80.2±6.1	<0.001
Fasting insulin (µU/mL)	12.8 ± 5.4	7.2 ± 3.1	<0.001
HOMA-IR	3.06 ± 1.4	1.5 ± 0.7	<0.001
Post load glucose* (1st hour)	200.4±49.6	143.6±29.6	<0.001
Post load glucose* (2nd hour)	161.7±42.0	114.8±22.6	<0.001
HbA1c (%)	5.6±0.6	5.0±0.4	<0.001
Total cholesterol*	238.9 ± 54.8	220.1 ± 36.6	0.062
Triglyceride*	197.3 ± 84.6	145.7 ± 56.5	≤0.001
LDL-cholesterol*	138.6 ± 40.2	131.7 ± 31.4	0.363
HDL-cholesterol*	64.2 ± 16.4	63.1 ± 18.3	0.769
Osteopontin (ng/mL)	3.6±2.2	3.4±2.6	0.666
hs-CRP*	0.94±0.8	0.57±0.5	0.004

GDM: Gestational diabetes mellitus, SD: Standard deviation, hs-CRP: High-sensitivity C-reactive protein, *: (mg/dL)

As shown in Table-2, women with GDM had statistically significantly higher fasting glucose, 1st and 2nd post load glucose, and HbA1c levels than women in the healthy group.

Significant intergroup differences were also found as for fasting insulin, HOMA-IR and triglyceride levels ($p<0.05$). Total, LDL, HDL-cholesterol levels were not significantly different between groups. There was no statistically significant difference in serum OPN levels between the groups. The hs-CRP levels were significantly higher in the GDM group compared with the control group. There was no statistically significant correlation between the OPN and hs-CRP levels ($r=0.080$; $p=0.442$).

Discussion

The fasting and post load glucose, HbA1c, fasting insulin, and HOMA-IR levels were statistically significantly higher in the GDM group. Because GDM occurs as a result of the progressive advancement of insulin resistance, blood glucose, and insulin levels were expected to be higher than those of the healthy group. Significant increases in fasting insulin levels and HOMA-IR were determined. Metabolic parameters of women with GDM that were comparable to those observed in obesity and type 2 DM (20).

Lipid profiles of the two groups were not significantly different except triglyceride levels. In pregnancy, total cholesterol and triglyceride levels are expected to increase, but the ranges are variable according to various studies performed (21-23). High levels of triglyceride and low levels of HDL-cholesterol are typical findings of metabolic syndrome and diabetes mellitus. Insulin resistance and GDM, may lead to the development of higher triglyceride levels in the GDM group.

In our study, the hs-CRP level in the GDM group was significantly higher than that of the healthy group. The hs-CRP is accepted as a risk factor for atherosclerosis, myocardial infarction and stroke (24). C-reactive protein secreted from liver has an important role in immune response. The hs-CRP activates many inflammatory processes. In human endothelial cells, hs-CRP stimulates adhesion proteins like vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), E-selectin and monocyte chemo-attractant protein-1 (MCP-1) (5). Also in human monocytes hs-CRP stimulates the production of tissue factor that has a role in extrinsic coagulation pathway (24). Retnakaran et al. (8) reported that the obesity is the factor that influences the CRP levels in GDM patients. In another report, the patients who previously had GDM also had higher levels of CRP (25). Even after GDM, chronic inflammation and thus the CAD risk probably continue. Bo et al. (26) reported that post-GDM women had higher levels of E-selectin, intracellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6), hs-CRP and IMT (intima media thickness) than the controls. Thus, these studies have led to the conclusion that GDM patients will have an increased risk of cardiovascular disease in the future (26).

OPN is a glycoprotein and acts as a multifunctional pro-inflammatory cytokine. It is secreted from activated T cells, NK cells, dendritic cells and monocytes/macrophages. OPN plays an important role in the physiologic and pathologic events including angiogenesis, apoptosis, inflammation, wound healing and tumor metastasis (27, 28). OPN levels

increase in chronic inflammation, and it may have a function in the pathogenesis of atherosclerosis (29). Nakamachi et al. (30) reported that PPRα agonists suppress OPN expression in macrophage cell cultures and decrease OPN levels in type 2 diabetic patients. In conclusion, all these information suggest the possible role of OPN in macrophage-derived inflammatory process. In another paper, the authors reported that in pre-eclamptic patients OPN concentrations increased in association with extensive endothelial injury (14). Yan et al. (31) reported higher OPN levels in type 2 diabetic patients and demonstrated the existence of an independent association with the presence and severity of nephropathy and CAD. In another article, Ahmad et al. (13) reported that IL-8 plays a significant role in the secretion of plasma OPN levels. They also noticed that IL-8 and OPN levels increased in correlation with insulin resistance in obese patients (13). In a large-scale study conducted in young adults, OPN was shown not to be of interest regarding vascular markers of subclinical atherosclerosis (12). In another study, OPN was suggested to be the first indicator of coronary artery calcification in asymptomatic CAD in patients with type 2 DM (32). Despite contradictory publications, in an article involvement of OPN in insulin resistance, obesity, chronic inflammation, such as CAD has been demonstrated (33). As indicated in this paper, in spite of favourable changes in insulin resistance, levels of OPN increased in bariatric surgery (33). In this study, increased OPN levels were correlated with the increased osteocalcin and C-telopeptide levels (33). The association of OPN with insulin/glucose levels and atherosclerosis has been reported in an experimental study (34). As demonstrated in that study, an increase in blood glucose levels activates NFAT (Nuclear Factor of Activated T-cell) which leads to an increase in OPN mRNA (34). A similar study was done previously by Winhofer et al (16). In that study, lower OPN levels were detected in patients with GDM compared with the control group, and any correlation could not be found between OPN and insulin levels/secretion (16). The authors also reported that OPN levels had increased during postpartum period (16). The authors concluded that OPN might have local importance and it is not an underlying factor in the development of insulin resistance in GDM patients. Correlation between OPN, and CRP/hs-CRP levels was also reported. In our study we reported that OPN levels were not different in GDM group than the healthy group and there was no correlation with the hs-CRP levels. We did not study the postpartum OPN levels, so we do not know any postpartum effect on OPN levels. The important role of OPN in vascular calcification has been shown in insulin resistance, obesity, and chronic inflammation in atherosclerosis. In GDM or bariatric surgery, patients are experiencing rapid metabolic changes. So these metabolic changes may not affect the level of OPN. However, in chronic diseases such as type 2 Diabetes or CAD, blood OPN levels may be correlated with the disease duration.

The limitations of our study were as follows: More significant results could be obtained if greater number of patients could be investigated in a longer study period. And also if a group of diabetic patients who were not pregnant could be enrolled in the study; then more meaningful results could be reported. If the weight gain during pregnancy had been considered, there might have been statistically significant correlation with the OPN. We could have obtained highly significant results, if we could evaluate OPN levels during postpartum period.

Conclusion

OPN has recently been considered as a marker for the risk of CAD. We found a significant increase in serum hs-CRP levels but no significant increase in OPN levels in the GDM which increase insulin resistance and where metabolic changes are rapid. There is a need for studies involving more patients and postpartum period. OPN levels do not significantly increase in GDM patients.

The authors declare that they have not any conflicts of interest.

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