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Effect of Pro-inflammatory Markers on Gestational Diabetes

Gebelik Diyabetinde Pro-enflamatuvar Belirteçlerin Etkisi

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Abstract

Introduction: Gestational diabetes, which is a subtype of hyperglycemia; can cause fetal mortality and also may cause diabetes mellitus after pregnancy. Even though there are studies indicating that inflammation may cause the development of diabetes during gestation, roles of osteoprotegerin and resistin remain unknown. In this study, we aimed to evaluate in a broad perspective the pro-inflammatory cytokines which may be linked to gestational diabetes in relation to biochemical parameters.

Method: This study was performed with 49 patients who had gestational diabetes mellitus (GDM) diagnosis and 28 healthy pregnant women. Serum levels of leptin, resistin, CD40L, TNF-R (Tumor Necrotizing Factor Receptor), OPG (Osteoprotegerin), MCP-1 (Monocyte Chemoattractant Protein), MPO (Myeloperoxidase), ICAM-1 (Intercellular Adhesion Molecule) were analysed and compared to blood glucose levels, HbA1c, total cholesterol, triglycerid, LDL and HDL cholesterol levels.

Results: Serum OPG (p=0.043) and sTNF-R (p=0.001) levels were found significantly higher in GDM patients compared to healthy controls, whereas resistin levels were found lower. Even though a statistically meaningful difference was not observed, inflammatory markers MPO, ICAM-1, CD40L, and leptin were also found higher compared to control group. A positive correlation between MPO and triglyceride and negative correlation between MPO and HDL was observed (p=0.017; p<0.05).

Discussion: The role of pro-inflammatory markers in diabetes is not clear. High levels of inflammatory markers in GDM patients may be helpful for clarifying the GDM pathogenesis. Multicenter studies with larger patient populations could enlighten the role of pro-inflammatory cytokines in GDM.

Key words: Gestational diabetes, pro-inflammatory markers, cytokine, osteoprotegerin

Öz

Giriş: Hipergliseminin bir alt tipi olan Gebelik Diyabeti fetal ölüme ve gebelik sonrasında diyabet mellitusa neden olabilmektedir. İnflamasyonun gebelik sırasında diyabete yol açtığını bildiren araştırmalar olmasına rağmen, osteoprotegerin ve resistinin rolü henüz bilinmemektedir. Bu araştırmada, gebelik diyabeti ile i-lişkili olabilecek pro-enflamatuvar sitokinleri geniş bir perspektifle inceleyerek biyokimyasal parametrelerle birlikte değerlendirdik.

Yöntem: Bu çalışma Gebelik Diyabeti tanısı almış 49 gebe ve 28 sağlıklı gebe kadın ile gerçekleştirilmiştir. Leptin, resistin, CD40L, TNF-R (Tümör Nekrotizan Faktör Reseptörü), OPG (Osteoprotegerin), MCP-1 (Monosit Kemoatraktan Protein-1), MPO (Miyeloperoksidaz), ICAM-1 (Hücreler arası adhezyon Molekülü) düzeyleri ölçülüp kan glukoz, HbA1c, total kolesterol, trigliserid, LDL ve HDL kolesterol düzeyleri ile birlikte değerlendirilmiştir.

Sonuçlar: Resistin düzeyleri düşük düzeyde saptanırken, serum OPG (p=0,043) ve sTNF-R (p=0,001) düzeyleri sağlıklı kontrollere göre gebelik diyabeti olanlarda belirgin olarak artmış düzeydedir. Bu hasta grubunda, istatistiksel olarak anlamlı olmasa da, MPO, ICAM-1, CD40L ve leptin düzeyleri sağlıklı gebelerden daha yüksek düzeyde saptanmıştır. MPO ve trigliserid düzeyleri arasında pozitif yönde bir korelasyon varken, MPO ve HDL düzeyleri arasında negatif korelasyon olduğu saptanmıştır (p=0,017; p0,05).

Tartışma: Pro-enflamatuvar belirteçlerin diyabette rolü henüz açıklığa kavuşmamış durumdadır. Gebelik diyabeti olanlarda inflamasyon belirteçlerinin yüksek oranda olması Gebelik Diyabeti patogenezinin anlaşılmasında yararlı olabilir. Daha büyük hasta grupları ile yapılacak çok merkezli araştırmalar pro-enflamatuvar belirteçlerin gebelik diyabetine etkisini açıklığa kavuşturucu olacaktır.

Anahtar Kelimeler: Gebelik diyabeti, pro-enflamatuvar belirteçler, sitokinler, osteoprotegerin

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Introduction

Gestational diabetes which may occur as a pregnancy complication is characterized by disrupted glucose tolerance, and seen in women who have relatively disrupted insulin secretion and resistance. These women carry the risk of diabetes for their entire lives.[1]

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During pregnancy, insulin sensitivity decreases due to elevated glucose requirement for fetus.

Placental hormones contribute to this situation. Even though development rate of gestational diabetes among pregnant women is 1–14%, some patients have normal glucose tolerance after gestation but some of them remain to have disrupted glucose tolerance or become diabetic patients.[2]

Placenta has pleotropic role during fetal development, and also has relationship with maternal and fetal circulation while having contact with different surfaces. In this situation, placenta is exposed to regulatory effects of hormones, cytokines, and growth factors. Placenta expresses leptin, resistin and TNF-α which are also expressed by adipose tissue.[3]

During gestation-induced insulin resistance, the unique relationship between placenta and adipose tissue points out that adipokines play role in the regulation of insulin action.

Maternal diabetic environment causes alterations in adipose tissue and placental cells. Cytokine levels which are secreted at minimum levels increases during pregnancy. Compared to women who are not pregnant, insulin resistance was reported to be 3 times higher in pregnant women.[2] Increase in insulin resistance causes alterations of circulating TNF-α, leptin, and resistin levels. Insulin, leptin and other cytokines also increase at fetal environment. Insulin and cytokines contribute to the regulation of placental function.[3]

Maternal insulin is regulated by placenta which is in contact with syncytiotrophoblasts. This process causes alterations of cytokine synthesis and secretion. Number of insulin receptors change in placental resistin, leptin, and insulin regulation. Fetal insulin is controlled by mother and it has effect on the placenta of healthy pregnants, and female patients with gestational diabetes.[3]

It is known that cytokines produced by adipose tissue such as resistin and IL-6, adhesion molecules such as ICAM-1, and adipokines play role in type 2 diabetes and atherosclerosis pathogenesis.[4] In this study, we aimed to evaluate the pro-inflammatory cytokine levels in GDM patients, and their relation to resistin and osteoprotegerin.

Material and Methods

Patients and Healthy Controls

This study was performed with 49 patients who have GDM diagnosis (age 18–42) and 28 healthy pregnant women (age 18–40). Ethical approval was obtained from Yeditepe University Hospital on 18.09.2016 with decision number 238. Study Consent Forms were signed by the patients before they enrolled to the study. Samples were collected from patients admitted to Endocrinology Department of Bağcılar Research Hospital İstanbul, Turkey. All experiments were carried out in accordance with World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subject.[5]

Method

Blood samples of healthy and GDM patients are collected into biochemical analysis tubes (red top, with no additives) and centrifuged to obtain serum. One of the aliquoted serum samples is stored at -80°C until cytokine measurements.

Glucose, glycosylated hemoglobin (HbA1c), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and trigylceride evaluations are analysed with an automated enzyme immuno-assay (EIA) system (PRISM, Abbott, USA) at the institution where the samples were collected. Cytokine analyses are performed at Yeditepe University. For evaluation of leptin, resistin, CD40L, TNF-R, OPG, MCP-1, MPO, and ICAM-1 levels; a bead array analysis (Flow Cytomix Human Obesity 9 plex Kit-BMS816FF, BenderMed Systems, USA) is performed with flow cytometry (FC500, Beckman Coulter, USA) according to manufacturer instructions. The results are analysed with FlowCytomics Pro 1.0 software (BenderMed Systems, USA).

Statistical Analysis

All data is evaluated by IBM SPSS Statistics 22 (IBM SPSS, Turkey) software. Parameters' convenience to normal distribution is evaluated by Shapiro Wilks test. For intragroup comparisons; Student t test is performed for parameters showing normal distribution, whereas Mann-Whitney U test is performed for parameters not showing normal distribution. Pearson correlation analysis is used for evaluating relevance between parameters showing normal distribution. For parameters not showing

normal distribution, Spearman's rho correlation analysis is performed. Significance is evaluated at p<0.05 level.

Results

Intergroup Comparison

Mean levels of blood glucose (p=0.006), HbA1c (p=0.029), total cholesterol (p=0.019) and triglyceride (p=0.001) were higher in GDM patients, whereas HDL (p=0.001) and resistin (p<0.001) levels were found decreased compared to healthy controls (Table 1). OPG (p=0.043), TNF-R (p=0.001), and MPO levels were found elevated at the patient group.

Intragroup Comparison

Patient group: Triglyceride (p=0.001), total cholesterol (p=0.019), glucose (p=0.006) and HBA1c (p=0.029) mean levels are found higher compared to control group, and were statistically significant. While there was a correlation between MPO and triglyceride (p=0.011) levels, an inverse relation between MPO and HDL (p=0.017) is detected. No significant correlations between MPO and biochemical parameters; total cholesterol, glucose, HDL, LDL, and HA1c were observed. No significant correlations between leptin, resistin, CD40L, OPG, TNF-R, MCP1, ICAM-1 levels and biochemical parameters; total cholesterol, triglyceride, glucose, HDL, LDL and HbA1c were detected (Table 2); and also, no relationship between postprandial blood sugar (PBS) and cytokines was observed (Table 2).

Control group: No significant correlation between MPO and biochemical parameters; total cholesterol, triglyceride, HDL, LDL and HbA1c was observed. Likewise, no significant correlation between CD40L, OPG, TNF-R, MCP-1, ICAM-1 and biochemical parameters parameters; total cholesterol, triglyceride, glucose, HDL, LDL and HbA1c was observed (Table 2). Even though inverse correlation between leptin and glucose was detected (p: 0.043), no correlation between leptin and biochemical parameters; total cholesterol, triglyceride, HDL, LDL and HbA1c was observed. No significant correlations between resistin and HbA1c, total cholesterol, triglyceride, HDL, LDL and glucose were detected.

Discussion

In our study, markers except the resistin; OPG, CD40L, TNF-R, MPO, MCP-1, ICAM-1, were shown to be higher in patient group compared to control group. The increase in levels of TNF-R and OPG were found statistically significant.

Table 1. Distribution of biochemical parameters according to patient groups

	Patient (n=49)	Control (n=28)	p
	Mean±SD (Median)	Mean±SD (Median)	
Leptin[1]	18.02±6.87	14.78±8.9	0.079
Resistin[2]	619.35±556.73 (471.54)	4802.5±2527.01 (4020)	0.001
CD40[1]	1704.99±1143.99	1668.79±790.12	0.883
TNF[1]	0.88±0.27	0.66±0.25	0.001
OPG[2]	11.90±11.23 (9.69)	8.25±5.72 (6.46)	0.043
MCP1[2]	1183.38±1148.72 (891.22)	1011.27±739.62 (874.94)	0.346
MPO[1]	219.28±86.43	199.24±54.94	0.273
ICAM[1]	465.77±245.76	409.39±175.95	0.290
Triglyceride[1]	197.92±83.89	132±63.95	0.001
Cholesterol[1]	205.04±34	186.32±31.42	0.019
HDL[1]	46.67±11.85	58.02±11.02	0.001
LDL[1]	115.73±24.33	104.18±26.82	0.057
Glucose[1]	105.82±34.99	81.06±10.73	0.006
HbA1c[1]	5.80±0.44	5.33±0.55	0.029

[1]Student t Test
[2]Mann-Whitney U Test

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Table 2. Correlation between cytokines and biochemical parameters

Group			Leptin	Resistin	CD40	TNF	OPG	MCP1	MPO	ICAM
Patient	Triglyceride	r	-0.192	0.166	0.182	0.056	0.035	0.151	0.359	0.209
		p	0.186	0.254	0.210	0.702	0.813	0.301	0.011	0.150
	Cholesterol	r	0.002	0.089	0.022	0.141	0.092	-0.039	-0.092	0.137
		p	0.987	0.543	0.880	0.335	0.531	0.789	0.529	0.348
	HDL	r	0.026	-0.132	-0.115	0.166	0.101	-0.087	-0.339	0.022
		p	0.861	0.365	0.431	0.253	0.488	0.550	0.017	0.882
	LDL	r	0.047	0.003	-0.006	0.128	0.016	-0.090	-0.120	0.125
		p	0.746	0.981	0.966	0.382	0.914	0.538	0.413	0.394
	Glucose	r	0.125	0.059	0.236	-0.151	-0.024	0.128	-0.054	0.076
		p	0.413	0.702	0.119	0.322	0.875	0.403	0.725	0.618
	HbA1c	r	0.107	-0.105	0.153	-0.024	-0.125	-0.117	0.066	0.460
		p	0.693	0.698	0.572	0.930	0.644	0.667	0.807	0.073
	PBS	r	-0.022	0.103	-0.229	0.269	0.188	0.074	0.012	0.026
		p	0.912	0.602	0.240	0.166	0.338	0.707	0.951	0.894
Control	Triglyceride	r	0.133	0.144	-0.256	-0.138	-0.065	-0.017	0.051	-0.019
		p	0.500	0.464	0.188	0.485	0.741	0.930	0.797	0.925
	Cholesterol	r	0.209	-0.023	-0.357	-0.104	-0.115	0.011	0.290	-0.086
		p	0.285	0.909	0.062	0.600	0.561	0.955	0.134	0.663
	HDL	r	0.218	-0.215	-0.210	-0.008	-0.069	0.156	-0.048	0.059
		p	0.264	0.273	0.284	0.969	0.728	0.427	0.809	0.767
	LDL	r	0.061	0.058	-0.112	-0.131	-0.082	-0.138	0.315	-0.182
		p	0.758	0.768	0.572	0.506	0.677	0.484	0.102	0.354
	Glucose	r	-0.477	-0.124	0.058	-0.121	-0.005	0.008	-0.525	0.111
		p	0.043	0.636	0.824	0.643	0.985	0.976	0.031*	0.671
	HbA1c	r	-0.112	0.373	0.123	-0.044	-0.231	-0.247	-0.054	0.595
		p	0.774	0.323	0.752	0.911	0.550	0.522	0.891	0.091

Pearson correlation analysis and Spearman's rho correlation analysis was performed

ICAM-1 is an important intercellular adhesion molecule. It facilitates the interaction between leukocytes and endothelial cells at the site of inflammation.[6] Studies report that hyperglycaemia induces the serum ICAM-1 levels. Our study supports previous studies that report the predisposition of diabetes to inflammation with high level of serum ICAM-1 in patients with gestational diabetes.

In this study, low level of serum resistin is observed in contrast with many previous studies that were designed similarly. The contrasting result may be explained with variations in study models, individual intrinsic or extrinsic differences, different methods application at different times; and also, hormonal and physical factors with respect to patient groups may have influenced the results. The level of serum OPG is found to be elevated in patients when compared to the control group; a result which is in concert with previously reported animal studies. This finding also coincides with the studies which demonstrate increased OPG levels due to the effect of estrogen, even though the similar effect suppresses the resistin mRNA serum levels.[7]

The roles of OPG and resistin in signaling pathways of glucose metabolism will be explored by further studies.

Comparing patient and control groups, OPG and TNF-R levels are found to be significantly higher in patient group; however, the level of resistin are found lower in patient group. These data suggest a signaling pathway in which OPG and resistin play different roles. OPG and RANKL

(RANK ligand) are expressed in osteoblasts. Osteoclasts contain RANK receptor for the RANKL. OPG represses the RANK-RANKL interaction by binding to RANKL. This interaction is triggered by resistin.[8]

OPG and resistin might have opposite roles in this pathway by the effect of estrogen. The increase in the levels of OPG by the effect of estrogen was shown in previous studies.[9] It is also known that estogen level increases during pregnancy.[2]

OPG level was found to be higher in GDM patients when compared to normal pregnants.[10] This evidence might be related with low resistin levels in gestational diabetes. Furthermore, estrogen regulates the effect of insulin in heathy individuals; however, this regulatory effect of estrogen on insulin decreases in diabetes.[11]

The correlation between glucose level and OPG production and secretion has been shown in DM patients, especially those who were diagnosed in last two years.[12] To this end, it is of importance to address how the level of serum OPG is altered. Evaluation of this alteration requires the consideration of the opposite functions of OPG and resistin.[9] The relationship between OPG and diabetes, and between diabetes and estrogen are also considerable in this concept. Estrogen might lose the regulatory effect on insulin secretion, and might trigger the elevation of OPG level in diabetes.

TNF is the most studied cytokine in studies related to diabetes.[13] High level of TNF-R in diabetic patient group might be related to the inflammatory effect of diabetes. TNF-α related to free TNF receptor (TNF-R1, TNR2) and receptor-ligand complex is called as TNF pathway marker. The role of TNF pathway in progression of diabetic nephropathy was studied. Soluble markers of TNF pathway has been associated with abnormal excretion of albumin and disrupted renal function in diabetes.[14]

In addition to TNF-R level, high level of pro-inflammatory cytokines such as MCP-1, MPO, ICAM-1 in patient group supports the hypothesis suggesting that gestational diabetes has also predisposition to inflammation like diabetes mellitus. Besides, high leptin level which is the marker of triggered inflammation supports the correlation between diabetes and inflammation, all together with high level of other inflammation markers such as MPO and TNF-R. MPO has been found to be negatively correlated with glucose levels in the control group. MPO might be measured at elevated levels in a healthy pregnant because of the elevated number of granulocytes. On the other hand, in our study, positive correlation between MPO and triglyceride reveals a correlation between inflammatory markers and alterations in adipose tissue. Triglycerides are stored in adipose tissue, and when energy is needed, fatty acid and glycerol release from the triglyceride in adipose tissue.[15] In a study, mice on a high fat diet showed that increased MPO levels which were released by neutrophils lead to insulin resistance.[16]

Decreasing sensitivity to leptin production in obesity induced diabetes has been reported previously.[17] In our study, leptin and glucose levels are found to be higher when compared to control group, but not significantly. This does not explain the negative correlation between leptin and glucose in control group.

There are some studies indicating high levels of resistin in diabetes. It is thought that this situation is the result of the direct effect of pro-inflammatory cytokines on resistin. Our study could not explain the correlation between leptin and resistin; however, studies that report the correlation between resistin and inflammation have been published.[18]

Eventually, variations in studies and discussion about resistin indicate the requirement for further studies that include the factors influencing the pathway, especially the RANKL-RANK.

GDM is a process related with inflammation in which pro-inflammatory cytokines are secreted. However, further studies are required because of inconsistent results about resistin. The roles of pro-inflammatory markers in diabetes remain to be elusive. For the risk of gestational diabetes to progress into diabetes after gestation, new inflammation markers should be evaluated with more sensitive methods by considering the parameters such as pregnancy age, duration and hormonal alterations.

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