Evaluation the Effect of 50 g Glucose Tolerance Test on Oxidative Stress and Interleukin-8 Parameters in Prediabetic Pregnancy

Prediabetic Gebelerde 50 gr Glukoz Yükleme Testinin Oksidatif Stres ve Interleukin-8 Parametreleri Üzerine Etkisinin Değerlendirilmesi

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Abstract

Background: Gestational diabetes mellitus (GDM) is a common medical complication of pregnancy, characterized by β-cell dysfunction and metabolic defects of insulin resistance in pregnancy. The aim of this study is to evaluate the effect of 50 g oral glucose tolerance test (OGTT) on oxidative stress and interleukin-8 (IL-8) parameters in prediabetic pregnant women.

Materials and Methods: Fasting and one hour blood samples were collected from 79 pregnant women who were administered 50 g OGTT. Patients with a one hour blood glucose level of 140–200 mg/dl were considered as the prediabetic group. Thereafter, routine biochemical parameters and the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA) and interleukin-8 (IL-8) parameters were measured from the serum samples taken during fasting and at one hour.

Results: The serum GPx and SOD levels of the prediabetic group (n=37) were remarkably lower than that of the control group (n=42) (p<0.05). Whereas the serum IL-8 levels of the prediabetic group were significantly higher than that of the control group (p<0.05). When the fasting and one hour levels of the parameters (SOD, GPx, IL-8 and MDA) were compared during OGTT, SOD levels were significantly decreased (p<0.001) and IL-8 levels were significantly higher (p<0.001). There was no significant difference in serum MDA levels of the prediabetic group compared to the control group (p=0.354).

Conclusions: In conclusion, it was found that serum GPx and SOD levels decreased, while serum IL-8 levels increased in prediabetic pregnant women; however, when 50 g OGTT was administered to these patients, SOD levels decreased and IL-8 levels increased. These results we obtained suggest that oxidative stress and systemic inflammation that are already present in prediabetic pregnant women may be triggered by 50 g OGTT, posing negative risk factors for pregnant women.

Key Words: inflammation, OGTT, oxidative stress, prediabetes

Oz


Materyal ve Metod: 50 gr OGTT yapılan 79 gebeden açlık ve birinci saat kan numuneleri alınması gerektir. Bununla birlikte, açlık ve birinci saat alınan serum numuneleri, rutin biyokimya parametrelerinin ve süperoksit dismutaz (SOD), glutatyon peroksidad (GPx), malondialdehit (MDA) ve interleukin-8 (IL-8) parametrelerinin düzeyleri ölçülmesi planlanmıştır.

Bulgular: SOD ve interleukin-8 parametreleri açlık ve birinci saat düzeyleri arasında anlamlı bir ilişki bulundu (p<0.05). Prediabetik grup serum IL-8 düzeyleri ise kontrol grubundan anlamlı olarak yüksek bulundu (p<0.05). OGTT sonrası SOD ve interleukin-8 parametreleri arasında anlamlı bir ilişki bulundu (p<0.001). Prediabetik grup serum MDA düzeyleri ise kontrol grubuna göre anlamlı fark saptanmadı (p=0.354).

Sonuç: Bu çalışmada, prediabetik gebelerde serum GPx ve SOD düzeyleri azaldı, ancak interleukin-8 düzeyleri arasında anlamlı bir ilişki bulundu (p<0.001). Prediabetik gebelerde sistemik inflamasyon 50 gr OGTT ile daha da tetiklenmek üzere olumsuz risk faktörleri oluşturulmuş oldu.

Anahtar Kelimeler: Enflamasyon, OGTT, oksidatif stres, prediabetik
Introduction
In general prediabetes is defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both. It has been reported to be associated with dyslipidemia, endothelial dysfunction, obesity, dysglycemia, procoagulant status, insulin resistance, hypertension and inflammation, which increase the risk of cardiovascular events (1). GDM is a common complication of pregnancy, characterized by β-cell dysfunction and defects of insulin resistance in pregnancy (2). Abnormal carbohydrate metabolism, such as impaired glucose tolerance in pregnancy and pregnancy diabetes, affects 1-14% of all pregnancies (3,4). Both the American College of Obstetricians and Gynecologists (ACOG) and the American Diabetes Association (ADA) advise screening all pregnant women for gestational diabetes (5).

In the pathogenesis of fetal and maternal complications in GDM, fetal hyperglycemia, maternal hyperlipidemia, hyperinsulinemia, and placental endothelial dysfunction have been reported to result in an increase in oxidative stress (6-8). It has been indicated that oxidative stress and IL-6 levels in pregnant women were significantly higher than that of the control group (p=0.05). The serum GPx and SOD levels of the prediabetic group were significantly higher than that of the control group (p=0.002). The median values age of the prediabetic group were significantly higher than that of the control group (p=0.354). Furthermore, the one hour levels of glucose and insulin were significantly higher in the prediabetic group than in the control group (p<0.05). There was no significant difference in serum MDA levels of the prediabetic group compared to the control group (p=0.000, p=0.023). There was no considerable difference between the groups in terms of other parameters (Table 1).

Materials and Methods
The study was approved by the ethical committee of our institute (Tokat Gaziosmanpasa University Clinical Research Ethical Committee; protocol number: 15-KAEK-034, 2015/04) and was planned and conducted according to the provisions of the Helsinki Declaration. The study included 79 pregnant women at 24–28 weeks’ gestation who were admitted to the General Secretariat of the Public Hospitals Union, Tokat State Hospital and GOU Health Research and Practice Center between 2015-2017, who were administered 50 g OGTT. Pregnant women with type 2 diabetes mellitus (T2DM) were excluded from the study. The patients did not need to be fasting for the screening test that can be run at any time of day. Fasting and one hour blood samples after OGTT were collected blood with serum separator tubes from the patients. Collected venous blood samples were centrifuged (4500 rpm for 10 minutes at +4°C) then divided into aliquots and stored at -80°C until required for laboratory analyses. Thereafter, in these two groups, routine biochemistry parameters and study parameters were measured from the serum samples taken during fasting and at one hour. Of these parameters, SOD (17), GPx (18) and MDA (19) levels were studied using the spectrophotometric method. IL-8 levels (DIAsource ImmunoAssay S.A., Belgium) were measured by enzyme linked immunosorbent assay (ELISA). Insulin, triglyceride, cholesterol, HDL, LDL levels of the pregnant women were spectrophotometrically evaluated by an autoanalyser (Cobas 501, Roche Diagnostic, Mannheim, Germany). Homeostatic model assessment for insulin resistance (HOMA-IR) value was calculated as HOMA-IR=Fasting Glucose (mg/dL) X Fasting Insulin (uIU/mL)/405, and the patients with a HOMA score of ≥2.5 were deemed positive for IR (20). Thirty-seven patients with a one hour blood glucose level of 140-200 mg/dl were considered as the prediabetic group. Whereas forty-two patients with one hour blood glucose below 140 mg/dl were considered as the control group.

Statistical analysis
All statistical analyses were performed using the SPSS 16.0 software. Descriptive statistics were given as arithmetic mean ± standard deviation. Non-normally distributed results were expressed as median (min-max) values. The Kolmogorov-Smirnov test was used for normality tests. The Student t-test was used for comparisons between subgroups for normally distributed tests. The Mann-Whitney U test was used for comparisons between subgroups for non-normally distributed tests and p values of less than 0.05 were considered as significant.

Results
Of the 79 pregnant women involved in the study, 46.8% constituted the prediabetic group (n=37) and 53.2% constituted the non-prediabetic control group (n=42) (p=0.05). The median values age of the prediabetic group were significantly higher than that of the control group (p=0.002). The serum GPx and SOD levels of the prediabetic group were significantly lower than that of the control group (p=0.05). Whereas, the serum IL-8 levels of the prediabetic group were significantly higher than that of the control group (p=0.05). There was no significant difference in serum MDA levels of the prediabetic group compared to the control group (p=0.354). Furthermore, the one hour levels of glucose and insulin were significantly higher in the prediabetic group than in the control group (p=0.000, p=0.023). There was no considerable difference between the groups in terms of other parameters (Table 1).
The levels of the serum study parameters after glucose intake during OGTT in the prediabetic and control groups are shown in the table (Table 2). When the prediabetic and non-prediabetic groups were compared during fasting, a decrease in GPx and SOD levels and an increase in IL-8 levels were found (p<0.003, p=0.017 and p=0.042). A decrease in SOD levels and an increase in IL-8 levels were observed between the one hour (p=0.005 and p=0.005). When the fasting and one hour levels of the prediabetic group were compared during the OGTT, a significant decrease in SOD levels (p<0.001) and a significant increase in IL-8 levels (p<0.001) were found. However, when the fasting and one hour levels of serum MDA and GPx levels were compared, there was no significant difference (p=0.118 and p=0.576). When fasting and one hour levels were compared in the non-prediabetic group, a significant decrease in SOD levels (p<0.001) and a significant increase in IL-8 levels (p<0.001) were found. When the fasting and one hour levels of serum MDA and GPx levels were compared, there was no significant difference (p=0.153 and p=0.914).

Table 1. Comparison of study parameters before OGTT in prediabetic and nonprediabetic groups

<table>
<thead>
<tr>
<th></th>
<th>Prediabetic group (n=37)</th>
<th>Nonprediabetic group (n=42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30 (19-42)</td>
<td>25 (17-39)</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI</td>
<td>25.91±3.07</td>
<td>25.26±2.97</td>
<td>0.338</td>
</tr>
<tr>
<td>Glucose fasting (mg/dL)</td>
<td>79.78 ± 12.9</td>
<td>79.24 (9.8)</td>
<td>0.832</td>
</tr>
<tr>
<td>Glucose one hour (mg/dL)</td>
<td>155.3±15.3</td>
<td>103.86±16.21</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Insulin fasting (µU/mL)</td>
<td>7.15 (2-94)</td>
<td>7.83 (3-83)</td>
<td>0.295</td>
</tr>
<tr>
<td>Insulin one hour (µU/mL)</td>
<td>64.74 (9-248)</td>
<td>44.71 (19-102)</td>
<td>0.023 *</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>2.62 ± 4.54</td>
<td>3.03 ± 4.07</td>
<td>0.679</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>47.84±17.56</td>
<td>38.49±13.29</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>110.08±36.19</td>
<td>96.78±41.3</td>
<td>0.140</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>203.7±62.07</td>
<td>213.0±78.6</td>
<td>0.572</td>
</tr>
<tr>
<td>GPx (µL)</td>
<td>682.6±232.51</td>
<td>847.52±236.12</td>
<td>0.003 **</td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>13.98±4.57</td>
<td>11.07±8.16</td>
<td>0.354</td>
</tr>
<tr>
<td>SOD (µL/mL)</td>
<td>11.42 (2.22-18.50)</td>
<td>13.82 (2.53-17.47)</td>
<td>0.017 *</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>5.16 (1.22-89.69)</td>
<td>3.85 (1.22-90.22)</td>
<td>0.042 *</td>
</tr>
</tbody>
</table>

* Mann-Whitney U, p<0.05
** Student t, p<0.01
BMI: Body mass index, HOMAIR: Homeostatic model assessment for insulin resistance, GPx: Glutathione peroxidase, MDA: Malondialdehyde, SOD: Superoxide dismutase, IL-8: Interleukin-8

Table 2. Comparison of study parameters prediabetic and nonprediabetic groups during fasting and OGTT at the one hour.

<table>
<thead>
<tr>
<th></th>
<th>Prediabetic group (n=37)</th>
<th>Nonprediabetic group (n=42)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx fasting</td>
<td>682.59±232.51</td>
<td>847.52±236.12</td>
<td>0.003</td>
</tr>
<tr>
<td>GPx one hour</td>
<td>655.26±298.90</td>
<td>834.32±764.56</td>
<td>0.914 b</td>
</tr>
<tr>
<td>MDA fasting</td>
<td>13.98±4.57</td>
<td>11.07±8.16</td>
<td>0.454</td>
</tr>
<tr>
<td>MDA one hour</td>
<td>14.76±6.41</td>
<td>13.34±4.78</td>
<td>0.153 b</td>
</tr>
<tr>
<td>SOD fasting</td>
<td>11.42 (2.22-18.50)</td>
<td>13.82 (2.53-17.42)</td>
<td>0.017 b</td>
</tr>
<tr>
<td>SOD one hour</td>
<td>7.8 (1.24-13.07)</td>
<td>10.28 (3.62-16.33)</td>
<td>0.005 c</td>
</tr>
<tr>
<td>IL-8 fasting</td>
<td>5.16 (1.22-89.69)</td>
<td>3.85 (1.22-90.22)</td>
<td>0.042 c</td>
</tr>
<tr>
<td>IL-8 one hour</td>
<td>18.07 (3.85-428.86)</td>
<td>10.17 (1.74-143.41)</td>
<td>0.005 c</td>
</tr>
</tbody>
</table>

a: Comparison of study parameters prediabetic groups during fasting and OGTT at the one hour.
b: Comparison of study parameters nonprediabetic groups during fasting and OGTT at the one hour.

Discussion

In this study, the effect of 50 g OGTT on serum oxidative stress and IL-8 parameters in prediabetic and non-prediabetic pregnant women were evaluated. For this purpose, pre- and post-OGTT serum glucose, insulin, HOMA-IR, HDL, LDL, triglyceride, GPx, MDA, SOD, and IL-8 levels were compared in pregnant women. It was found that the serum GPx and SOD levels of the prediabetic group were lower, while their IL-8 levels were higher than that of the non-prediabetic group. In the prediabetic group, one hour serum SOD levels were decreased and insulin and IL-8 levels were increased during OGTT compared to the fasting values. There was no significant difference in serum MDA levels of the prediabetic group compared to the control group.

GDM is defined as a carbohydrate intolerance including insulin resistance that begins or is identified during pregnancy due to hormonal changes (9). Abnormal glucose tolerance is a risk factor for the development of T2DM (21). Unless treated, this condition creates a serious clinical picture that may lead to diabetes and may cause micro and macrovascular complications (22). Chronic hyperglycemia leads to oxidative...
stress, which is believed to cause the development of diabetic complications. However, it has been reported that acute hyperglycemia also causes oxidative stress and increases inflammatory cytokine concentration in non-diabetic individuals (12,14). Oxidative stress is indicated to be a pathogenic factor that causes insulin resistance, β-cell dysfunction, glucose intolerance, and consequently the development of T2DM. Although hyperglycemia is considered as the major factor responsible for the complications of T2DM, it is known that oxidative stress has an essential role not only in the pathogenesis of T2DM, but also in the development of GDM and maternal-fetal complications (9). The studies have shown a correlation between GDM and oxidative stress markers. Moreover, antioxidant defense systems have been reported to reduce in GDM (11). Peuchant et al. observed that malondialdehyde MDA levels increased and Gpx levels decreased in GDM patients (23). Chaudhari et al. found that MDA levels were high and SOD levels were low in GDM patients (24). Rajdl et al. observed that GSH levels decreased in diabetic pregnant women (25). López-Tinoco et al. found SOD and Gpx levels to be lower in GDM patients in comparison with the control group. Therefore, it has been stated that oxidative stress may be effective in the progression and/or pathogenesis of GDM and that the reduction in antioxidant defense may be a response to increased oxidative stress (9). Furthermore, oxidative stress may result in fetal stress by inducing vascular dysfunction in the placenta (26). In this study, the serum Gpx and SOD levels of the prediabetic group were found to be lower than that of the non-prediabetic group. It was also found that there was a decrease in the one hour serum SOD levels of the prediabetic group during OGTT compared to the fasting values. Although the serum MDA levels of the prediabetic group were higher than the non-prediabetic group, no significant difference was observed. The pathophysiology of GDM has not been clearly understood; however, chronic subclinical inflammation caused by hormonal change in pregnancy has been reported to have an important role. Some oxidative stress markers and inflammatory and anti-inflammatory cytokines have been shown to increase in T2DM patients and GDM (27,28). Although there is controversy regarding cytokine levels, T2DM is considered as a chronic inflammatory disease. Owing to the similarity between T2DM and GDM and the correlation between T2DM and inflammation, a relationship between inflammation and pathophysiology of GDM has been assumed (11). Circulating IL-8 levels have been reported to be high in type 1 diabetes mellitus (T1DM) and T2DM, assuming that this cytokine may play a role in the pathogenesis of diabetic macroangiopathy. IL-8 is one of the proinflammatory cytokines that may have atherogenic properties, and may therefore promote intimal thickening and atherosclerosis (29). In a study of endothelial cell culture, it was reported that in increasing IL-8 production from endothelial cells (EC) as a result of high glucose concentration, protein kinase C is activated by hyperglycemia in EC, IL-8 gene expression is regulated by protein kinase C (PKC) activity, and therefore, activation of PKC by high glucose concentration may lead to specific increase in IL-8 release from EC (30). It has been reported that 75 g glucose load increases the monocyte nuclear factor (NF) –κB, the cardinal cellular signal of inflammation, also it induces proinflammatory cytokines and enzymes transcription that produce ROS (13). Esposito et al. showed that acute hyperglycemia caused an increase in inflammatory cytokine concentrations in non-diabetic subjects (14). It has been shown that C-reactive protein (CRP) and IL-6 plasma concentrations increase during OGTT in patients with T2DM, circulating IL-8 increases after OGTT in the IGT group in obese patients (29), and high levels of glucose induce IL-8 production and secretion in cultured EC (26). In a study, glucose, insulin, IL-6 and IL-8 parameters were studied during 75 g glucose OGTT load. As a result, an increase in IL-6 and IL-8 concentrations was found throughout OGTT (31). Acute hyperglycemia has been shown to induce plasma cytokines in IGT patients who were administered glucose infusion. It has been shown that short-term hyperglycemic increase may influence cytokine concentrations more than continuous hyperglycemia and an oxidative mechanism may mediate the effect of hyperglycemia (14). In addition, Straczkowski et al. found high levels of IL-8 after OGTT in the IGT group in obese patients and reported that acute hyperglycemia upregulated IL-8 levels (29). In the present study, we found that the serum IL-8 levels of the prediabetic group were higher than that of the non-prediabetic group, and there was an increase in the one hour serum IL-8 levels of the prediabetic group during OGTT compared to the fasting values. It has been stated that besides playing a physiological role in the fetoplacental part during pregnancy, cytokines may have a pathophysiological role, when expressed in abnormal amounts (11). In conclusion, it was found that serum Gpx and SOD levels decreased, while serum IL-8 levels increased in prediabetic pregnant women; however, when 50 g OGTT was administered to these patients, SOD levels decreased and IL-8 levels increased. These results we obtained suggest that oxidative stress and systemic inflammation that are already present in prediabetic pregnant women may be triggered by 50 g OGTT, posing negative risk factors for pregnant women.

**Ethical Approval:** The study was approved by the ethical committee of our institute (Tokat Gaziosmanpasa University Clinical Research Ethics Committee; protocol number: 15-KAEK-034, 2015/04) and was planned and conducted according to the provisions of the Helsinki Declaration.

**Author Contributions:**

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Literature Review: L.A.

Design: Z.C.O, C.M.


Analysis and interpretation: K.D., M.K.

Writing manuscript: Z.C.O, K.D.

Critical revision of manuscript: K.D., M.K.

**Conflict of Interest:** The authors have no conflicts of interest to declare.
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