THE EVALUATION OF PARAOXONASE 1 ACTIVITY IN PATIENTS WITH GESTATIONAL DIABETES

AHMET ENGIN ATAY¹, MEHMET NAFI SAKAR², NURDAGÜL SERIFE NURANI CULCU¹, HAKKI SIMSEK¹, HALİT AKBAS¹, MURAT ACAR¹, BİRĞUL İŞIK¹, ALPASLAN KEMAL TÜZCU³
¹Department of Internal Medicine, Bagcılar Education and Research Hospital, Istanbul - ²Department of Obstetrics and Gynecology, Suleymaniya Education and Research Hospital, Istanbul - ³Department of Biochemistry, Medical School of Dicle University, Diyarbakır, Turkey - ⁴Department of Cardiology, Medical School of Yuzuncu Yıl University, Van, Turkey - ⁵Department of Medical Biology, Medical School of Harran University, Sanlıurfa, Turkey - ⁶Department of Internal Medicine, Genesis Hospital, Diyarbakır, Turkey - ⁷Department of Endocrinology, Medical School of Dicle University, Diyarbakır, Turkey

Introduction

Gestational diabetes mellitus (GDM) is the most frequent gestational metabolic disorder defined as glucose intolerance diagnosed during pregnancy for the first time⁴. Due to effects of placental hormones on maternal glucose metabolism, GDM usually occurs after 24th week of gestation, and affects approximately 1-14% of all pregnant⁵. Exact pathogenetic mechanism is not fully understood however GDM is believed to share some pathogenetic mechanisms with diabetes mellitus (DM)⁶. Pregnancies with or without GDM are associated with IR in different severity which resolves after delivery⁶.

Tissue responsiveness to insulin decreases as pregnancy advances; however insulin secretion and demand for insulin are balanced in significant proportion of pregnancies. In a small proportion, insulin supply is diminished or insulin resistance (IR) is enhanced, and GDM become evident⁶.

Oxidative stress (OS) is defined as excessive production of oxidants or diminished generation of antioxidants⁶. Oxidant/antioxidant imbalance leads to overproduction of reactive oxygen species (ROS). Paraoxonase 1 (PON1) is an antioxidant enzyme that has paraoxonase and arylesterase activity⁷. PON1 acts by inhibiting lipid peroxidation via hydrolyzing lipid peroxidase. The role of OS on GDM is still under debate. Few and contrasting data exist about the relation of PON1 activity and insulin resistance in patients with GDM⁸,⁹. The aim of the present study is to assess the relation of PON1 with IR in patients with GDM.

Patients and Methods

Sixty five patients with GDM and 66 healthy individuals were enrolled into the study. None of participants had been receiving any medication during the last 3 months. Patients with following disorders or conditions that may affect lipid profile or

ABSTRACT

Material and methods: Sixty five patients with GDM and 66 healthy pregnant were enrolled. Paraoxonase activity, insulin levels, HOMA-IR, demographic features and anthropometric measurements were evaluated.

Results: The mean paraoxonase activity was significantly diminished in patients with GDM (p:0.004). Insulin level and HOMA-IR were significantly higher in GDM patients (p:0.004 and p:0.001; respectively). The mean interval between present and previous pregnancy was significantly shorter in patients group (p:0.004). There was a significant correlation between PON1 activity and serum LDL, HDL levels and weight gained during pregnancy (p:0.001, p<0.001 and p:0.002; respectively) but not with weight and parity. However HOMA-IR was significantly correlated with weight and parity (p:0.001 and p:0.002; respectively).

Discussion: Diminished PON1 activity and enhanced oxidative stress accompany to insulin resistance in the pathogenesis of GDM. Oxidative stress is associated with weight gained during first 2 trimesters of pregnancy rather than obesity.

Key words: Paraoxonase activity, insulin resistance, gestational diabetes.

Received July 05, 2013; Accepted August 19, 2013
insulin resistance were excluded; liver or renal dysfunction, smoking, pre-pregnancy diabetes mellitus, hypertension, hyperlipidemia, hyperprolactinemia or thyroid disease. The control group was composed of age matched healthy pregnant with negative glucose challenge test. The study was approved by the ethical review committee of Van Yuzuncuyl University Turkey, and written informed consent was obtained from all participants. Pregnants with a history of GDM or glucose intolerance in their previous pregnancies were excluded. Also healthy pregnant with a family history of DM were not included.

In the absence of obvious hyperglycemia, The American Diabetes Association (ADA) criterion for GDM were used for the diagnosis of GDM\(^{(15)}\). Screening with the 2-hour 75 g oral glucose tolerance test (OGTT) was performed. The diagnosis of GDM was confirmed if two or more values equal or exceed upper limit of following; fasting 95 mg/dl, 1-hour 180 mg/dl, 2-hour 155 mg/dl. The pregnant with one or less positive values were considered as healthy controls.

Obstetric history, demographic features, alcohol consumption, smoking status, medications and medical family history were questioned in detail for each patient. Anthropometric measurements like weight, height, BMI, pregestational weight were documented. All participants were examined by the same physician. BMI was determined as the ratio of weight in kilograms to heightxheight in meters. Weight gained during pregnancy was estimated by subtracting the weight before pregnancy from the weight at the time of blood sample collection.

Blood samples were obtained after 12-hour fasting period. Serum levels of total cholesterol, triglycerides, HDL and LDL were determined using an Aeroset autoanalyser (Abbott Laboratories Inc., Abbott Park, IL, USA). Paraoxonase activity was measured using commercially available kits (Relassay,Turkey). The activity of PON1 was measured as described by Furlong et al\(^{(11)}\). The rate of hydrolysis of paraoxon was assessed by monitoring the increase in absorbance at 405 nm and 25° C. Plasma glucose levels were measured using the glucose oxidase method. Plasma insulin concentrations were analyzed by Beckman Coulter chemiluminescent immunoassay (Beckman Instruments, Brea, California, USA). The Homeostasis Model Assessment (HOMA) of insulin resistance (HOMA-IR) formula (HOMA-IR= fasting glucose (mm0/L)xfasting insulin (µU/ml)/22,5) was used to determine insulin resistance\(^{(12)}\).

Statistical Analysis:Statistical analysis were performed using SPSS for Windows (release 15.0; SPSS Inc., Chicago, IL, USA). All data were expressed as the mean ± standard deviation (SD). Comparison of variables with a normal distribution between the study groups were analyzed using Student’s t-test. Correlations were determined by Pearson’s simple linear regression analysis. A P-value < 0.05 was considered to be statistically significant.

Results

Clinical and demographical data were shown in table 1. The differences of parity and interval between present and the previous pregnancy were significant (p:0,002 and p:0,004; respectively). When the anthropometric measurements were compared, patients with GDM were obese and had higher BMI (p:0,005 and p<0,001; respectively). Also the weight gained during pregnancy was significantly higher in patients group (9,2 kilograms(kg) vs 6,7 kg, p<0,001).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients (n:65)</th>
<th>Control subjects (n:66)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28,1±3,1</td>
<td>27,3±3,7</td>
<td>NS</td>
</tr>
<tr>
<td>Parity (n)</td>
<td>3,14 ±1,5</td>
<td>2,1±1,6</td>
<td>p:0,002</td>
</tr>
<tr>
<td>Interval between pregnancies (years)</td>
<td>2,0±0,23</td>
<td>2,5±0,27</td>
<td>p:0,004</td>
</tr>
<tr>
<td>Weight (kilograms(kg))</td>
<td>76,4±17</td>
<td>67,2±10</td>
<td>p=0,009</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>32,2±4,8</td>
<td>27,3±4,2</td>
<td>p:0,001</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>9,2±2,5</td>
<td>6,7±2,1</td>
<td>p&lt;0,001</td>
</tr>
<tr>
<td>Glukoz (mg/dl)</td>
<td>153±23</td>
<td>88±14</td>
<td>p&lt;0,001</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>13,1±16,6</td>
<td>7,1±9,3</td>
<td>p:0,004</td>
</tr>
<tr>
<td>HOMA-I (mg/dl x µU/ml)</td>
<td>5,54±2,41</td>
<td>1,51±0,76</td>
<td>p&lt;0,001</td>
</tr>
<tr>
<td>PON 1 activity (U/L)</td>
<td>82,1±52,9</td>
<td>89,8±43,3</td>
<td>p:0,004</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>221±32</td>
<td>158±25</td>
<td>p:0,001</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>142±28</td>
<td>78±24</td>
<td>p&lt;0,001</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>43±5</td>
<td>49±5</td>
<td>p&lt;0,001</td>
</tr>
<tr>
<td>Trygliceride (mg/dl)</td>
<td>205±35</td>
<td>150±59</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 1: The comparison of serum PON1 activity, HOMA-IR, biochemical variables and demographical data between patients and control subjects.
There was no significant difference between groups in terms of triglycerides levels. However patients had significantly higher LDL and lower HDL levels (p<0.001 and p=0.02; respectively). The patients group had diminished PON1 activity when compared to the control subjects (p=0.004). Serum insulin levels and HOMA-IR were significantly higher in patients group (p=0.004 and p=0.001; respectively).

Table 2 shows the distribution of intervals between present and previous pregnancy in the patient and control groups. Thirty three patients and seventeen controls had shorter duration (≤ 2 years) between present and previous pregnancy. Seven patients and 16 controls had a duration of more than 3 years since the last pregnancy.

<table>
<thead>
<tr>
<th>Interval between present and previous pregnancy</th>
<th>≤2 years</th>
<th>2-3 years</th>
<th>&gt;3 years</th>
<th>First pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (65)</td>
<td>33</td>
<td>18</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Controls (66)</td>
<td>17</td>
<td>22</td>
<td>16</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2: The distribution of intervals between present and previous pregnancies in patient and control group.

Table 3: Distribution of parities between patient and control groups.

<table>
<thead>
<tr>
<th>Number of Parities</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>&gt;5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n=65)</td>
<td>6</td>
<td>12</td>
<td>21</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Controls (n=66)</td>
<td>13</td>
<td>22</td>
<td>21</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3: Distribution of parities between patient and control groups.

Table 4: Correlation of PON1 activity and HOMA-IR with biochemical and demographical variables of patients with gestational diabetes.

<table>
<thead>
<tr>
<th>Variables</th>
<th>PON1</th>
<th>HOMA-IR</th>
<th>Parity</th>
<th>Weight</th>
<th>HDL</th>
<th>LDL</th>
<th>Weight-gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>PON1 activity</td>
<td>0.318</td>
<td>0.003</td>
<td>0.114</td>
<td>NS</td>
<td>0.128</td>
<td>NS</td>
<td>0.497</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.318</td>
<td>0.003</td>
<td>0.339</td>
<td>0.002</td>
<td>0.369</td>
<td>0.001</td>
<td>0.136</td>
</tr>
</tbody>
</table>

However no significant correlation exist between PON1 activity and parity or BMI. In contrast, HOMA-IR was significantly correlated with parity and BMI (p=0.002 and p=0.001; respectively). Also correlation of HOMA-IR and weight gained during pregnancy were significant (p<0.001, r=0.498). Although HOMA-IR and LDL were significantly correlated (p=0.03, r=0.232), there was no significant correlation between HOMA-IR and HDL.

Discussion

GDM is a useful model to establish the preventive strategies against the development of type II DM and cardiovascular disease. Approximately half of patients with GDM experience the development of type II DM after delivery (13). The incidence of postpartum type II DM among pregnant with GDM ranges from 3 to 65% depending on ethnicity, lack of uniformity in diagnostic criteria and follow-up procedures and differences in statistical analysis (14).

Impaired B-cell function and IR is regarded as the strongest predictors of postpartum diabetes in patients with GDM (15). As expected, insulin and HOMA-IR levels were significantly higher in patients group in the present study. Insulin resistance defines a decreased ability of insulin to promote uptake, storage and inhibit the release of glucose (16). Hyperglycemia impairs B-cell response to glucose (17). A possible mechanism of decreased PON1 activity is inactivation of PON1 as a result of increased superoxide radicals and hydrogen peroxide.

The mechanism that OS trigger GDM remain an active area of research. It is not definitely clear whether OS precedes IR or is a result of IR. In the present study, glucose level and PON1 activity were inversely and significantly correlated, and GDM patients with better glucose control had higher PON1 activity than GDM patients with poorer glycemic control. Placental hormones like human placental lactogen and increased fatty tissue in pregnant, precede the onset of IR (18).

There is a complex interaction between hyperglycemia, IR and OS in GDM. A possible association between IR and OS is explained by enhanced glycosylation leading to an increase in reactive oxygen species (19). Increased adipose tissue is a source of subclinical inflam-
mation that decreases insulin sensitivity and increases OS\(^{10}\). Suzuki et al stated that increased glucose levels in diabetes react non-enzymatically with proteins and become advanced glycation end products that increase endothelial production of ROS\(^{19}\). Even in non-diabetic subjects, hyperglycemia may trigger glucose oxidation and impair endothelium which regresses with normoglycemia\(^{4}\).

According to current data, the recurrence rate of GDM is 35.6%, and it varies depending on the interval between pregnancies. An interval of less shorter than 24 months apparently increases the risk of recurrence\(^{10}\). In our study, pregnant women with history of GDM were excluded. However interval between present and previous pregnancy was significantly shorter in patients group. Mcneill et al determined that approximately one-third of subjects had overt DM before subsequent pregnancy\(^{20}\). Patients with GDM exhibit features of metabolic syndrome like hyperlipidemia, hyperglycemia and obesity. A transient occurrence of MS that is observed during pregnancy become permanent when pregnancies become sooner and increases in number\(^{10}\). The correlation of PON1 activity and parity or interval between pregnancies were no significant. However a significant correlation exist between HOMA-IR and parity or interval between pregnancies.

Maternal obesity and amount of weight gained during pregnancy are other risk factors associated with GDM\(^{21}\). Women with GDM are more likely to be obese when compared to normoglycemic counterparts\(^{20}\). Obese individuals have diminished ability to suppress plasma concentrations of FFA which can induce a insulin resistant state. Adipocytes produce some specific molecules that may trigger the development of IR\(^{22}\). Obesity is a strong predictor of development of overt DM for pregnant with GDM later in life\(^{23}\).

In our study, correlation of PON1 and BMI was nonsignificant. However weight gained during pregnancy and PON1 activity was significantly correlated. Excessive fat accumulation in a short period may stimulate production of cytokines which are also involved in the pathogenesis of OS\(^{24}\). Beside well documented association between IR and obesity, obese individuals have higher TG and LDL levels\(^{29}\).

Enhanced lipid levels have adverse effect on glucose metabolism like accelerated hepatic gluconeogenesis and diminished muscle glucose metabolism\(^{29}\). Results of some recent studies revealed that IR accompany to high cholesterol levels in GDM\(^{27}\).

Zavaroni et al stated that IR accompanies to dyslipidemia and HT\(^{29}\). Patients with IR and OS experience enhanced LDL oxidation which is inhibited by PON1 activity\(^{29}\). Higher triglyceride and lower HDL levels are strongly correlated with decreased insulin sensitivity; especially in obese individuals\(^{30}\). Bo et al stated that elevated triglyceride level leads to impaired insulin action via enhanced OS\(^{31}\). PON1 activity and HDL levels were significantly and positively correlated. Li et al concluded that PON1 is considered to play key role in the antiatherosclerotic activity of HDL\(^{32}\).

The present study have some limitations. First, the number of participants were relatively low. Patients and control subjects with history of GDM in their previous pregnancies or impaired glucose tolerance in the present pregnancy were excluded to eliminate the cumulative effect of hyperglycemic state. Second, repetitive measurements of these parameters may increase the significance of the results.

**Conclusion**

Preventive measures to decrease excessive weight gain during pregnancy may reduce OS and IR for patients with GDM. Women at high risk of GDM such as obesity, excessive weight gain and high number of parity should undergo screening as soon as possible after conception. Patients should be informed about the increased risk of GDM when interval between pregnancies decrease and number of parities increase. Efforts for better understanding the pathophysiology of GDM would also help in determining novel therapeutic approach for both GDM and DM. Further studies with large number of participants are required to establish the exact role of OS and IR on the development of GDM.

**References**

The evaluation of paraoxonase 1 activity in patients with gestational diabetes


HALIT AKBAS
Department of Medical Biology
Medical School of Harran University
Sanliurfa
(Turkey)