Expression of IRS1 Gene in Pregnant Women with Gestational Diabetes Mellitus, in The Third Trimester

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Abstract
To investigate the genetic effect of gestational diabetes mellitus GDM by study expression of IRS1 gene was measured in women with GDM is characterized by high level of blood glucose, especially during 3rd trimester of the pregnancy period. The blood samples taken from one hundred twenty: 60 healthy pregnant and 60 pregnant with GDM in 3rd trimester of pregnancy, levels of fasting blood glucose (FBG) and HbA1c% was used to diagnose GDM. In addition to lipid profile (cholesterol, triglyceride, HDL, LDL, and VLDL). Molecular study was consisted of RNA extraction and qRT-PCR for IRS1gene expression determination. The fasting blood glucose mg/dl and HbA1c% level was highly significantly increase (P<0.01) between patients and control (healthy women) in 3rd trimester stage in addition lipid profile (serum cholesterol, serum triglyceride, LDL and VLDL) (mg/dl) but level of HDL (mg/dl) were decreased highly significantly (P<0.01) between patients and control. The result were showed significant increasing of IRS1 expression gene in control (1.00 ± 0.00) while decrease in patients (0.147 ± 0.02). It concluded that the low expression of IRS1 gene was connected with gestational diabetes mellitus comparison in control Iraqi women in third trimester of pregnancy.

Keywords: gestational diabetes mellitus, IRS1 gene expression, lipid profile, hyperglycemia

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Introduction

Gestational diabetes mellitus (GDM) is a medical condition when a woman normly didn’t have diabetes, showed an increase of blood sugar level throughout pregnancy in the 2nd and 3rd trimester of pregnancy, it is characterized by variable severity of carbohydrate intolerance [1]. Wahabi et al., (2013)[2] suggested that the occurrence of GDM, which influences 2–22% of the all pregnant, depend on ethnic groups of populations. In Caucasian women, gestational diabetes mellitus has been revealed to influence 4–7% of pregnant, whereas the rate is about 8–15% consistently, also increasing in Asian women rapidly [3,4]. Hunt and Schuller, (2007)[5] shown GDM is expected to affect 1 - 14% annually of pregnancies in the United States, while the popularity of GDM differs significantly between racial and ethnic groups. Insulin receptor substrate 1 (IRS1) is a connected of the insulin receptor tyrosine kinase and is essential to the insulin receptor sign transduction pathway, acting as an essential job in the insulin signaling pathway, in tissues of insulin sensitive [6]. Thrones, et al. (2006) [7] revealed that deregulation of IRS1 expression in diabetic patients, also low level in insulin-resistant of some conditions like obesity and diabetes type 2 as well as gestational diabetes mellitus. The aim of study that investigate the expression of IRS1 gene in women with GDM compared with health women.

Materials and methods

This study was achieved in University of Baghdad- Collage of Science. One hundred of study blood samples were collected from pregnant women at third trimester stage, collected from different hospital in Baghdad and were divided then two groups: 60 healthy pregnant (control group) and 60 pregnant with GDM. HbA1c% was calculated via using (Boditech kit, korea) and blood sugar test by using (Biosystem kit, Spain)[8]. Blood was taken from pregnant women after 10-14 hours fasting via vein puncture. The venous blood was put into tubes containing EDTA for RNA extraction and tubes without anti-coagulant for the biochemical tests. Serum was used to estimate (lipid profile consist of:Total cholesterol[9], triglyceride[10], high density lipoprotein (HDL)[11], low density lipoprotein (LDL)[12]and very low density lipoprotein (VLDL)[13]) by using enzymatic kits for (Spinreact, Spain). To study gene expression of IRS1, RNA extraction via the kit Direct-zol™ RNA, USA. In qRT-PCR technique was achieved by SYBR FAST one-step quantitative RT-PCR (KAPA kit, Canada) Figure-1.

The primers used in study IRS1 primer

5’GTGAACCTCAGTCCAAACCATAC3’(F)5’CCGGCACCTTGGCTCTGCT-3’(R) [14].

Housekeeping gene (a-tubulin) primer

5’AGAGTCGGCTGTAAGAACGC3’(F)5’TGGCTCTGTCGACTTGGCATC- 3’(R)[15]. Expression of IRS1 gene using (Smart cycler system)

Statistical Analysis

Statistical Analysis System- SAS program, version 2012, was used to analyzed the differences between means by using LSD test.

Results

The fasting blood glucose concentration (FBG) was estimated in patients with gestational diabetes and control. A highly significant increase in patients (186.01 ± 17.93 mg/dl) as compared with healthy control group (92.96 ± 4.40 mg/dl) . While HbA1C% test was highly significant increase in patients (9.10 ± 0.54) as compared with healthy control group (5.01 ± 0.27) in third trimester of pregnancy Table-1.
Table 1-Biochemical parameters in women GDM with and control group

<table>
<thead>
<tr>
<th>Tests</th>
<th>Control (mean± SE)</th>
<th>Patient (mean± SE)</th>
<th>T-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose mg/dl</td>
<td>92.96 ± 4.40</td>
<td>186.01 ± 17.93</td>
<td>38.787 **</td>
</tr>
<tr>
<td>HbA1C%</td>
<td>5.01 ± 0.27</td>
<td>9.10 ± 0.54</td>
<td>1.264 **</td>
</tr>
<tr>
<td>cholesterol (mg/dl)</td>
<td>171.28 ± 9.01</td>
<td>278.44 ± 15.26</td>
<td>37.249 **</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>145.95 ± 11.48</td>
<td>246.22 ± 23.79</td>
<td>55.518 **</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>64.57 ± 4.29</td>
<td>28.97 ± 2.34</td>
<td>10.284 **</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>119.90 ± 11.21</td>
<td>200.20 ± 9.99</td>
<td>31.561 **</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>24.70 ± 1.96</td>
<td>45.00 ± 5.61</td>
<td>12.492 **</td>
</tr>
</tbody>
</table>

** (P<0.01)

The result above showed highly significant increase of the cholesterol concentration in patients (278.44 ± 15.26 mg/dl) as compared with control group (171.28 ± 9.01 mg/dl), also the study revealed the concentration of triglyceride mg/dl was highly significantly increase (P<0.01) (246.22 ± 23.79 mg/dl) in patient while in control (145.95 ± 11.48 mg/dl). the concentration HDL mg/dl result was low significant (P<0.01) in patient (28.97 ± 2.34 mg/dl) and (64.57 ± 4.29 mg/dl) in control. Further, this study demonstrated the concentration of LDL mg/dl was highly significantly increase (P<0.01) (200.20 ± 9.99 mg/dl) in patient while (119.90 ± 11.21 mg/dl) in control and highly significantly increase (P<0.01) of VLDL concentration mg/dl in patient (45.00 ± 5.61 mg/dl) and in control (24.70 ± 1.96 mg/dl). The results demonstrated high significant in expression of IRS1 gene in control (1.00 ± 0.00) while patient with gestational diabetes (0.147 ± 0.02) Table-2.

Table 2-Gene expression of IRS1 (gestational diabetes patient and control)

<table>
<thead>
<tr>
<th>The Group</th>
<th>Mean ± SE</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ct</td>
<td>Ct (HKG)</td>
<td>Δ ct</td>
<td>ΔΔ ct</td>
</tr>
<tr>
<td>Control</td>
<td>23.50 ± 0.64</td>
<td>31.54 ± 0.51</td>
<td>-8.04 ± 1.03</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Patient GDM</td>
<td>29.13 ± 0.85</td>
<td>31.69 ± 0.58</td>
<td>-4.88 ± 1.15</td>
<td>-4.03 ± 1.26</td>
</tr>
<tr>
<td>T-Test</td>
<td>3.594 **</td>
<td>2.108 NS</td>
<td>2.673 **</td>
<td>2.631 **</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0103</td>
<td>0.783</td>
<td>0.0001</td>
<td>0.0087</td>
</tr>
</tbody>
</table>

** (P<0.01).
Figure 1-Expression of IRS1 gene (Real time PCR assay by SYBR FAST one-step) Smart cycler® 2.0 software
Discussion

The gestational diabetes pathogenesis is a desert of function of beta cells and insulin resistance. It submits to a decline in the physiological response to insulin, consequential in more insulin secretion to balance for the transport of glucose to skeletal muscles and adipose tissue and reserve production of liver glycogen [16]. Previous studies discovered that Alc (HbA1c) test as diagnostic test of diabetic through pregnancy [17]. Other research has indicated that the beginning pregnancy 1st trimester which HbA1c level increasing may characterize as indicator to women with GDM in the 2nd - and 3rd trimester [18]. GDM is broadly connected with dysfunction of placenta tissue essentially prompted hyperinsulinemia, hyperglycemia, also dyslipidemia linked with this pathology since dyslipidemia is a danger factor to expand endothelial dysfunction and atherosclerosis [19]. This result is agree with Herrera and Ortega-Senovilla, 2010 which indicate in addition to numerous studies that triglyceride are elevated in third trimester of pregnancy with GDM [20] have been claim that serum lipid models in GDM against normal pregnancy have been widely studied, with the majority studies observing most elevated levels of triglyceride in each of trimesters of pregnancy in women with diabetes. The results of this study, are agree with a details by Aziz and Mahboob (2008)[21] that significantly lower levels of HDL in women with GDM compared with normal pregnant. The results in this study compared with other reports of Rossner and Ohlin, 1995[22] that showed that LDL cholesterol concentration raised significantly through pregnancy as complicated by gestational diabetes, also insulin resistance raises very low-density lipoprotein cholesterol beside with LDL and intermediate-density lipoprotein[23]. This result exposed the low expression of IRS1 in women with GDM compared in control through third trimester of pregnancy. Colomiere et al., (2010) [24] has been demonstrated that insulin-signaling intermediates reduced in levels of IRS-1 proteins, reduced GLUT4 translocation and following glucose uptake in fat cells of overweight women with gestational diabetes, and type2 diabetes in comparison to health women. In addition, the genetic variation of IRS1 gene was linked with gestational diabetes mellitus comparison in healthy Iraqi women in third trimester of pregnancy [25]. Also recent study showed that a dipokines are associated with insulin resistance and obesity-related metabolic disorders in many diseases[26].

References


