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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التعبير الجيني لجينIRS1 وصوره الدهون في النساء العراقيات المصابات بسكري الحمل في مرحله الثلث الثالث من الحمل

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الخلاصه

الكشف عن التاثير الجيني لسكري الحمل عن طريق دراسه التعبير الجيني لجين IRS1 في عينه من النساء العراقيات الحوامل المصابات بسكري الحمل ويزداد مستوى سكر في الدم ارتفاعا خصوصا في المرحله الثالثه من الحمل . اخذت مئه وعشرين عينه 60من النساء السليمات و60 من النساء مصابات بسكري الحمل في مرحله الحمل الثالثه. تم قياس سكر الدم الصائم وسكر الدم التراكمي لتحديد النساء المصابات بسكري الحمل وقياس الدهون(الكوليستيرول, الدهون الثلاثيه, الدهون عاليه الكثافه , دهون واطئه الكثافه, ودهون واطئه الكثافه جدا) لتحديد عوامل الخطورة. الدراسه الجزيئيه شملت استخلاص RNA واستخدام تقنيه qRT-PCR لدراسه التعبير الجيني لجينIRS1 في النساء الحوامل المصابات بسكري الحمل. ان مستوى سكر الدم الصائم والتراكمي ارتفع ارتفاع معنوي جدا (P<0.01) في النساء المصابات بسكري الحمل مقارنه

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بالسيطره كذلك ارتفاعا معنويا في نسبه الكوليسترول والدهون الثلاثيه والدهون الواطئه الكثافة وواطئه الكثافة

جدا على العكس انخفضت معنويا (0.01>P) الدهون العاليه الكثافة في النساء الحوامل المصابات بالسكري

الحمل مقارنه بالنساء السليمات. كشفت النتائج انخفاض التعبير الجيني لجينIRS1 في النساء الحوامل

المصابات بسكر الحمل. نستنتج من هذه الدراسه ان انخفاض التعبير الجيني لجين يرتبط مع الاصابه بسكري

الحمل في النساء الحوامل في مرحله الحمل الثالثه.

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 pregnants, depend on ethnic groups of populations. In Caucasian women, gestational diabetes mellitus has been revealed to influence 4–7% of pregnants, 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'GTGAACCTCAGTCCCAACCATAAC3'(F)5'CCGGCACCCTTGAGTGTCT-3'(R) [14].
Housekeeping gene (α-tubulin) primer
5'AGAGTCGCGCTGTAAGAAGC3'(F)5'TGGTCTTGTCACTTGGCATC- 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

Tests	Control (mean± SE)	Patient (mean± SE)	T-Test
Fasting blood glucose mg/dl	92.96 ± 4.40	186.01 ± 17.93	38.787 **
HbA1C%	5.01 ± 0.27	9.10 ± 0.54	1.264 **
cholesterol (mg/dl)	171.28 ± 9.01	278.44 ± 15.26	37.249 **
Triglyceride (mg/dl)	145.95 ± 11.48	246.22 ± 23.79	55.518 **
HDL (mg/dl)	64.57 ± 4.29	28.97 ± 2.34	10.284 **
LDL (mg/dl)	119.90 ± 11.21	200.20 ± 9.99	31.561 **
VLDL(mg/dl)	24.70 ± 1.96	45.00 ± 5.61	12.492 **
** ($P<0.01$)			

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)

The Group	Mean ± SE				
	Ct	Ct (HKG)	△ ct	△△ ct	Fold change
Control	23.50 ± 0.64	31.54 ± 0.51	-8.04 ± 1.03	0.00 ± 0.00	1.00 ± 0.00
Patient GDM	29.13 ± 0.85	31.69 ± 0.58	-4.88 ± 1.15	-4.03 ± 1.26	0.147 ± 0.02
T-Test	3.594 **	2.108 NS	2.673 **	2.631 **	0.361 **
P-value	0.0103	0.783	0.0001	0.0087	0.0062
** ($P<0.01$).					
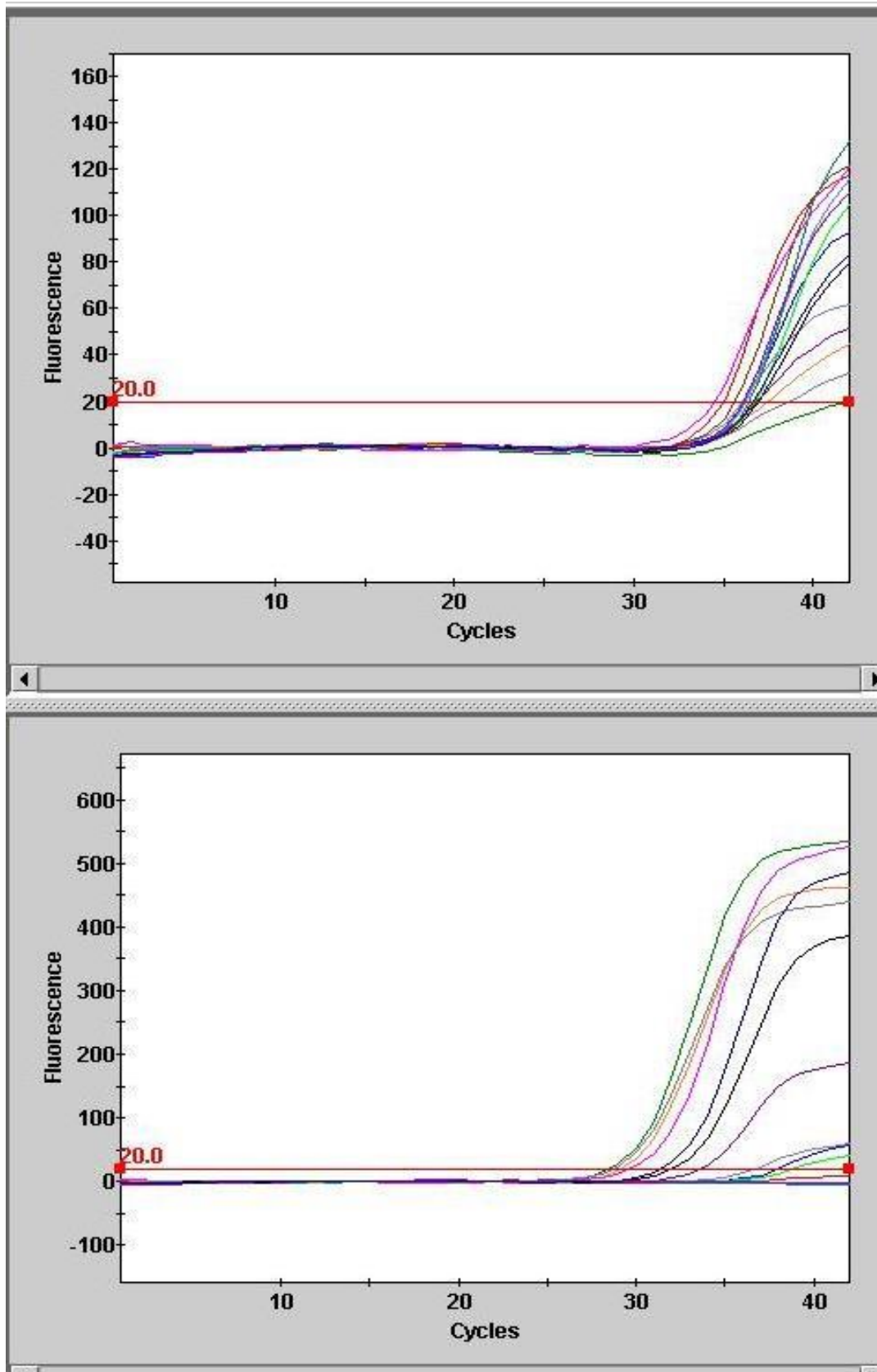
Figure 1-Expression of IRS1 gene (Real time PCR assay by SYBR FAST one-step) Smart cycler®
2.0 software

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 A1c (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
1. Zhang, C., Bao, W. and Rong, Y. **2013**. "Genetic variants and the risk of gestational diabetes mellitus: a systematic review," *Human Reproduction Update*, **19**: 376–390.
2. Wahabi, H., Esmaeil, S., Fayed, A. and Alzeidan, R. **2013.** "Gestational diabetes mellitus: maternal and perinatal outcomes inKing Khalid University Hospital, Saudi Arabia," *The Journal of the Egyptian Public Health Association*, **88**: 104–108.
3. Rosenberg, T.J., Garbers, S., Lipkind, H. and Chiasson, M.A. **2005**. Materal obesity and diabetes as risk factors for adverse pregnancy outcomes: differences among4 racial/ethnic groups. *Am.J.PublicHealth* **95**: 1545–1551.
4. Hunsberger, M., Rosenberg, K.D. and Donatelle, R.J. **2010**. Racial/ethnic disparities in gestational diabetes mellitus: findings from a population-based survey. *Womens Health Issues,* **20**: 323–328.
5. Hunt, K.J. and Schuller, K.L. **2007**. The increasing prevalence of diabetes in pregnancy. *Obstet Gynecol Clin,* **34**(2): 173–99.
6. Zhande, R. John J. Mitchell, Jiong Wu, and Xiao Jian Sun. **2002**. Molecular Mechanism of Insulin-Induced Degradation of Insulin Receptor Substrate 1, *Mol Cell Biol.* 2002 Feb; **22**(4): 1016–1026.
7. Thrones AC., Huang C. and Klip A. **2006**. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. *Trends Endocrinol Metab*.; **17**: 72–8.
8. Biadgo, B., Melku, M., Mekonnen, S. and Abebe, M. **2016**. Hematological indices and their correlation with fasting blood glucose level and anthropometric measurements in type 2 diabetes mellitus patients in Gondar, Northwest Ethiopia. Diabetes Metab. Syndr. Obes., **9**: 91–99.
9. Griffin, J., Alice, M. and Lichtenstein, D. **2014**. Dietary Cholesterol and Plasma Lipoprotein Profiles: Randomized-Controlled Trials. *CurrNutr Rep*.; **2**(4): 274–282.
10. Thomas, A**. 2002**. "Fats and Fatty Oils". Ullmann's Encyclopedia of Industrial Chemistry. Ullmann's Encyclopedia of Industrial Chemistry: Wiley-VCH.:10_173.

11. Renjith, R. and Jayakumari, N**. 2011**. A Simple Economical Method for Assay of Atherogenic Small Dense Low-Density Lipoprotein-Cholesterol (sdLDL-C). Indian *Journal of Clinical Biochemistry*, **26**(4): 385–388.

12. Martin, S., Blaha, M., Elshazly, M., Toth, P., Kwiterovich, P., Blumenthal, R., Jones, S. **2013**. Comparison of a Novel Method vs the Friedewald Equation for Estimating Low-Density Lipoprotein Cholesterol Levels From the Standard Lipid Profile. *JAMA*.; 310(19): 2061-2068.

13. Vujovic, A., Kotur-Stevuljevic, J., Spasic, S.; Bujisic, N., Martinovic, J., Vujovic, M.,;and Pajic, D. **2010**. Evaluation of different formulas for LDL-C calculation. *Lipids in Health and Disease*, **9**(27).

14. Jennifer, J., Thierry, G., Mireille, C., Yannick Le, M. and Brustel, T. **2007**. Interleukin-1β-Induced Insulin Resistance in Adipocytes through Down-Regulation of Insulin Receptor Substrate-1 Expression, *Endocrinology*; **148**(1): 241-251.

15. Shu, L., Sauter, N., Schulthess, F., Matveyenko, A., Oberholzer, J. and Maedler, K. **2008**.Transcription Factor 7-Like 2 Regulates β-Cell Survival and Function in Human Pancreatic Islets,*Diabetes*; **57**(3): 645-653

16. Wei, J., Gao, J. and Cheng, J. **2014**. Gestational diabetes mellitus and impaired glucose tolerance pregnant women.; *Pak J Med Sci*.; **30**(6): 1203–1208.

17. Artal, R., Mosley, G.M. and Dorey, F.J. **1984**. Glycohemoglobin as a screening test for gestational diabetes. *Am J Obstet Gynecol*.; **148**(4): 412–4.

18. Hughes, R.C., Rowan, J. and Florkowski, C.M. **2016**. Is There a Role for HbA1c in Pregnancy? *Curr Diab Rep*.; **16**(1): 5.

19. Leiva, A., Pardo, F., Ramirez, M.A., Farias, M., Casanello, P. and Sobrevia, L. **2011**. Fetoplacental vascular endothelial dysfunction as an early phenomenon in the programming of human adult diseases in subjects born from gestational diabetes mellitus or obesity in pregnancy. *Experimental diabetes research*: **34**: 9286.

20. Herrera, E. and Ortega-Senovilla, H. **2010**. Disturbances in lipid metabolism in diabetic pregnancy—are these the cause of the problem? *Best Pract Res Clin Endocrinol Metab*; **24**: 515–25.

21. Aziz, R. and Mahboob, T. **2008**. Lipid profile and serum insulin levels in Gestational Diabetes. Journal of the Dow University of Health Sciences; **2**(3): 102-106.

22. Rossner, S. and Ohlin, A.**1995**. Pregnancy as a risk factor for obesity.Lessons from the Stockholm Pregnancy and Weight Development Study. *Obes Res,* **3**(suppl 2): 267-275.

23. Sparks, J.D., Sparks, C.E. and Adeli, K. **2012**. Selective hepatic insulin resistance, VLDL overproduction, and hypertriglyceridemia. *Arterioscler Thromb Vasc Bio*l; **32**: 2104–12.

24. Colomiere, M., Permezel, M. and Lappas, M. **2010**. Diabetes and Obesity during Pregnancy Alter Insulin Signalling and Glucose Transporter Expression in Maternal Skeletal Muscle and Subcutaneous Adipose Tissue. *Journal of Molecular Endocrinology*, **44**: 213-223.

25. Zainab k. Hussain*, Jabbar H. Yenzeel, Hayfa H.Hassani. **2018**. Genetic variation of IRS1 gene in women with gestational diabetes mellitus in third trimester stage in Iraq. *Iraqi Journal of Science*, **59**(3A): 1176-1182.

26. Wadood1 S.A., Al- Shawk R.S. and Sabir S.F. **2016**. The Correlation of Lipocalin-2 and Retinol Binding Protein-4 with the Inflammatory State in Iraqi Patients with T2DM, *Iraqi Journal of Science,* **57**(2A): 802-807