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Original article

# Serum irisin levels as a potential marker for diagnosis of gestational diabetes mellitus

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**Summary.** *Objectives:* The aim of this study was to compare serum irisin, trace elements (Zn, Cu, Mg) levels between the group of pregnant women with gestational diabetes mellitus (GDM) and healthy pregnant group. *Material and methods:* Sixty pregnant women with GDM and 30 healthy pregnant women. The two groups were matched for maternal age, gestational age. Maternal serum irisin levels were measured by enzyme-linked immunosorbent assay kit at 24-28 weeks of gestation. An association between maternal serum irisin levels and clinical and biochemical parameters was evaluated. Body mass index, serum levels of glucose, OGTT, insulin, HbA1C, HOMA IR, HOMAβ, Hb%, and irisin were investigated and analyzed in the study group and controls. *Results:* Pregnant women with GDM had significantly higher fasting blood glucose FBG (p = 0.004), first-houEr OGTT glucose (p = 0.001), second-hour OGTT glucose (p = 0.001), fasting insulin FI (p = 0.001) levels, HOMA IR (p = 0.001), HOMAβ (p = 0.001), HbA1C(p = 0.001), Hb% (p = 0.017), as compared to controls. serum irisin levels were significantly lower (p =0.001) in women subsequently developed GDM (mean ± SD =71.65±8.03) than healthy pregnant controls (mean ± SD 136.54±22.56). Correlation analysis between irisin levels and anthropometric and biochemical parameters in patients with gestational diabetes revealed that none of the investigated parameters correlated with serum irisin level. *Conclusions:* The present results suggest that serum irisin levels might presented as a novel marker for GDM, with decreased levels of irisin being symptomatic of GDM. (www.actabiomedica.it)

**Key words:** gestational diabetes mellitus, irisin, trace elements, glycemic indices

## Introduction

Gestational diabetes mellitus (GDM) is defined as a carbohydrate intolerance of varying severity, with onset or first recognition during pregnancy. GDM prevalence may range from 1% to 14% of all pregnancies, depending on the studied population and the diagnostic test employed (1). The pathogenesis of GDM is multifactorial and may include genetic and environmental factors, but the exact mechanism remains to be fully elucidated (2). Women with GDM are at increased risk of perinatal morbidity, impaired glucose tolerance, and type 2 diabetes in the years after pregnancy. Women with this condition may or may not have diabetes before. This condition goes away after delivery. When a woman is diagnosed with gestational diabetes mellitus, there is a risk of having it in the future pregnancies. Women who had this condition during pregnancy are more likely to develop Type 2 diabetes mellitus (3). Human gestation is characterized by weight gain and a progressive decrease in insulin sensitivity, which parallels the growth of the fetoplacental unit. Maternal insulin resistance in late gestation is an important mechanism to divert nutrients to the fetus to promote growth (4). Normal gestational insulin resistance is further enhanced in pregnancy complications, such as those resulting in abnormal fetal growth i.e. fetal macrosomia and intrauterine growth restriction (IUGR). Recent compelling evidence suggests that these pregnancy disorders are associated with

future development of maternal metabolic syndrome (5, 6). Insulin resistance plays an important role in the pathogenesis of GDM and despite extensive research, the mechanisms underlying insulin resistance are not fully understood (7). Insulin resistance in pregnancy is traditionally attributed to increased maternal adiposity and placental hormones with diabetogenic action (8, 9), although the underlying mechanisms are not fully understood. Recent investigations have focused on several new potential mediators of gestational insulin resistance (9). Since it is the largest organ in the body, skeletal muscle accounts for the majority of glucose uptake in response to insulin, and is quantitatively the most important site of insulin resistance. During the past decade, skeletal muscle has also been identified as a secretory organ and cytokines and other peptides produced and secreted by myocytes are classified as myokines (10). These myokines function as endocrine hormones and regulate the function of various distant organs.

Irisin is a novel myokine (1), adipokine (2) and neurokine (3) consisting of 112 amino acid residues, with a molecular weight of 12 587 kDa (11,12). Proteolytically processed from the product of fibronectin type III domain containing 5 (FNDC5) gene in response to the activation of peroxisome proliferatoractivated receptor g (PPARg) co-activator-1a (PGC-1a) (11) and is an anti-diabetic hormone that regulates the glucose metabolism and energy consumption via converting white to brown adipose tissue (13).

Recently has been identified as an exercise-induced hormone secreted by skeletal muscle and has been proposed to mediate the beneficial effects of exercise on metabolism (14). Sedentary lifestyle is a major risk factor for type 2 diabetes mellitus. Randomized controlled trials have demonstrated that physical activity improves glucose tolerance and reduces the risk of type 2 diabetes mellitus (15). Therefore, it has been speculated that physical exercise may exert its beneficial effects on energy metabolism through secreted factors from myocytes such as irisin (16). Recent studies have shown that circulating irisin levels were significantly lower in patients with type 2 diabetes compared to people without diabetes (17, 18).

Studies in mice have shown that FNDC5 which directly stimulates the conversion of white adipose tissue (WAT) to brown-like adipose tissue (BAT), leading to increased total energy expenditure and, subsequently, to weight loss, improved glucose tolerance and insulin sensitization (19). Due to its metabolic properties, irisin has recently attracted a lot of interest as a potential new target for the treatment of obesity and its associated disorders. In clinical settings, circulating irisin levels are reportedly lower in patients with obesity and type 2 diabetes mellitus (DM) (20, 21), indicating that irisin may play an essential role in glucose intolerance. However, circulating irisin is reported to be paradoxically higher in adults with the metabolic syndrome (22), suggesting that states of irisin resistance or tolerance may exist (23). Data regarding irisin in human pregnancy are scarce. Irisin precursor is expressed in human placenta during gestation and its serum levels are higher during the entire pregnancy, when compared with nonpregnant women. After adjusting for body mass index (BMI), maternal irisin levels were associated with the homeostasis model assessment of estimated insulin resistance, suggesting that irisin may contribute to thedevelopment of normal gestational insulin resistance (24).

## Objective

The aim of the study was to compare Serum irisin concentrations between pregnant women with GDM and healthy pregnant women. irisin levels may have a potential as a novel marker for diagnosis and follow-up of gestational diabetes mellitus.and also to evaluate the correlations between Zn2+, Cu2+and Mg2 and alteration in serum irisin concentrations between pregnant women with GDM and healthy pregnant women.

## Materials and methods

The case-control study was conducted at Pegnant care center, AL-Najaf province, Iraq, between June 2017 and March 2018. The Ethics Committee of the institution approved the study, and all participants provided informed consent. The study group comprised 60 women diagnosed with GDM and 30 healthy pregnant controls with normal oral glucose tolerance test

(OGTT) results. All participants were recruited at the time of screening for GDM using a 75 g, 2-h OGTT between 24 and 28 weeks of pregnancy. GDM was diagnosed when one or more abnormal plasma glucose values (fasting_92 mg/dL, 1h_180 mg/dL, 2 h_153 mg/dL) were obtained using the criteria of The International Association of Diabetes and Pregnancy Study Groups (25).

The GDM and control groups were matched for maternal age, gestational age and current body mass index (BMI). Gestational age was determined by the last menstrual period and confirmed by ultrasonographic examination performed during the first trimester of pregnancy. BMIs measured during OGTT screening using the following formula: weight (kg)/height (m2). No patients received medications that interfered with glucose or lipid metabolism before blood sampling. Patients with multiple pregnancy, pre-existing glucose intolerance, pregnancy-induced hypertension, preeclampsia, acute or chronic inflammation, as well as active smokers were not included. An overnight fasting venous blood sample was obtained from all participants to assess Iris in levels and other biochemical parameters on the day of OGTT screening. All samples were stored at room temperature for at least 30 min to allow the blood to clot, followed by centrifugation (3000 rpm) for 15 min to separate serum. Serum specimens were aliquoted and stored at _80 _C until Iris in levels were analyzed. Glucose levels during OGTT were measured with the hexokinase method using a commercially available kit (Beckman AU5800; Beckman Coulter Diagnostics, Brea, CA). Insulin levels were determined using a chemiluminescent assay (AccessDxI800; Beckman Coulter Inc., Brea, CA), and glycosylated hemoglobin (HbA1c) levels were determined using commercially available kits and high-performance liquid chromatography (Tosoh HLC 723 G8, Tosoh Bioscience, Tokyo, Japan). Serum triglyceride, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterollevels were determined using an autoanalyzer (Beckman AU5800; Beckman Coulter Diagnostics, Brea, CA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: fasting glucose (mmol/L)_fasting insulin (IU/mL)/22.5 (26). Serum concentrations of magnesium, zinc, and copper were measured by colorimetric method using Randox kit (Randox, UK).

## Statistical Analysis

Statistical analysis was performed using two statistical software, the Statistical Package of Social Science (SPSS ver. 21) and Graphpad Prism ver.5. Continuous variables were expressed as mean ± standard deviation (SD). Significant differences were assessed using Paired t-test and independent t-test for variables with equal and unequal frequencies respectively. Bivariate correlations were assessed using standardized Pearson coefficients. The *p* values obtained of less than 0.05 and 0.01 were considered as statistically and highly statistically significant respectively.

## Results and discussion

The demographic characteristics of all participants are shown in Table 1, The total study population was 60 gestational diabetes mellitus, 30 normal pregnant in each group. The mean of maternal ages, and gestational ages of the two groups were not significantly (NS) different. In addition, BMI at the time of sample collection were differ in both groups.

Comparisons of clinical data between the two groups are presented in Table 2. In the fasting glucose, OGTT, insulin, and HOMA-IR values (p = 0.001), also Hb% (p = 0.017) were significantly higher, except HOMAβ (p = 0.001) were significantly lower in the GDM group in comparison to control at the time of GDM screening serum irisin levels were significantly

**Table 1.** The demographics characterizes of the study population

| Variables | GDM | Control | P value |
|---|---|---|---|
| Mother's age (Years) | 26.33±3.21 | 26.07±3.58 | 0.772 NS |
| BMI Kg/m² at sampling | 34.39±2.00 | 31.04±1.82 | 0.000** |
| Gestational age (weeks) at sampling | 28.47±0.96 | 28.07±0.98 | 0.069 NS |

BMI: body mass index

**Table 2.** Clinical characteristics of the healthy pregnant controls and women diagnosed with GDM

| Parameters | GDM Mean ± SD | Control Mean±SD | P value |
|---|---|---|---|
| Glucose(mg/dl) | 115.35±11.82 | 99.00±15.37 | 0.000** |
| OGTT(mg/dl) 1h | 182.04±4.23 | 133.23±5.22 | 0.000** |
| OGTT(mg/dl) 2h | 149.38±8.19 | 103.11±2.15 | 0.000** |
| Isulin(µlu/ml) | 15.32±2.70 | 8.63±1.20 | 0.000** |
| HOMA IR | 2.51±0.22 | 1.97±0.21 | 0.000** |
| HOMO β | 103.00±23.86 | 84.70±6.92 | 0.000** |
| HbA1C% | 5.08±0.23 | 4.47±0.19 | 0.000** |
| Hb % | 11.70±0.77 | 11.27±0.81 | 0.017* |
| Irisin(ng/ml) | 71.65±8.03 | 136.54±22.56 | 0.000** |

OGTT: oral glucose tolerance test, HbA1c: Glycated heamoglobin A1c, Hb: Hemoglobin

lower ($p < 0.001$) in women subsequently developed GDM (mean ± SD =71.65±8.03) than in controls (mean ± SD 136.54±22.56).

Relationships between serum irisin levels and other variables analyzed independently at the time of GDM screening are presented in Table 3. In the GDM group, no significant correlations were observed between serum irisin levels and other clinical or biochemical parameters.

As shown in Table 4, serum Zn levels were significantly lower in GDM women as compared to normal pregnancy (p=0.001) However, a significantly serum Cu lower level was observed in the healthy pregnant compared to GDM group (p=0.001).Conversely, serum Mg levels significantly lower was observed in GDM group were compared to healthy pregnant women (p=0.001).

**Table 3.** Correlations between irisin levels with other biochemical parameters in control subjects and in women diagnosed with GDM

| Parameters | R | P-value |
|---|---|---|
| Glucose(mg/dl) | -0.240 NS | 0.065 |
| OGTT(mg/dl)1hr. | -0.232 NS | 0.074 |
| Insulin(µlu/ml) | -0.038NS | 0.774 |
| OMMA IR | -0.154 NS | 0.241 |
| HOMMA β | 0.044 NS | 0.740 |
| HbA1C(%) | -0.077 NS | 0.558 |
| Hb(%) | 0.038 NS | 0.773 |

**Table 4.** Comparisons of trace Elements in patients with gestational diabetes mellitus and control group

| Parameters | Groups | Mean±SD | P value |
|---|---|---|---|
| Cu (µg/dl) | GDM | 109.00±14.62 | 0.001** |
| | Control | 85.43±5.06 | |
| Zn (µg/dl) | GDM | 79.27±6.87 | 0.001** |
| | Control | 101.30±7.20 | |
| Mg (mg/dl) | GDM | 1.99±0.07 | 0.001** |
| | Control | 2.35±0.07 | |

Gestational diabetes mellitus (GDM) is a metabolic disorder during pregnancy leading to acute and chronic complications in both mother and newborn. Thus, GDM patients have an increased risk of co-morbidities during pregnancy, e.g. preeclampsia, pregnancy-induced hypertension, and shoulder dystocia with impeded delivery (27). Furthermore, chronic complications might occur after delivery including type 2 diabetes mellitus (T2DM) and cardiovascular disease (28, 29).

Therefore, early diagnosis and appropriate treatment of GDM is helpful in reducing the adverse maternal and fetal outcomes and in protecting mothers and infants from long-term complications. Thus, previous studies have tried to determine the predictive value of maternal or placental biomarkers before the development of GDM, and these identified in many biological process involving insulin resistance, carbo-

hydrate metabolism, oxidative stress, and inflammation (30). To the best of present knowledge, this result are the first to use a case- control study to measure irisin in the serum of GDM patients and healthy controls in Iraqi population. Furthermore in the present conducted this analysis to evaluate the circulating irisin between GDM patients and healthy pregnant. Consistent with these findings, this study confirmed that pregnant with GDM have lower circulating irisin. implicated in the maternal metabolic disturbances associated with abnormal fetal growth. Pregnancy is associated with substantial changes in maternal metabolism, which provide sufficient energy and nutrients to the fetus (31, 32). In this context, mothers develop a state of insulin resistance during midpregnancy, which progresses throughout the third trimester, leading to reduced consumption of glucose by maternal tissues and increased gluconeogenesis (31). However, in a substantial proportion of pregnancies, the insulin-resistant condition is greatly increased, resulting to adverse maternal metabolic state and fetal growth abnormalities (33-34).

Irisin is a novel myokine and adipokine which induces an increase in total body energy expenditure, improving insulin sensitivity and glucose tolerance in experimental animals. In the present study showed that serum irisin levels were significantly lower in the patients with GDM than in the healthy pregnant women, the present results are agreement with the findings of Yuksel et al. (35), who also reported a decrease in circulating irisin in women with GDM; however, Kuzmicki et al. found that serum irisin increased significantly in pregnant women, but this increase was significantly lower in subjects with GDM (36). The concentration of irisin increased significantly from colostrum to transitional and mature milk, and plasma irisin also increased in lactating women with and without GDM compared to non-lactating women (37). In contrast, Ebert et al. (38) In GDM, there is enhanced ability of glucose to cross the placenta, with resultant fetal hyperglycaemia, hyperinsulinaemia and macrosomia. This may lead to a variety of fetal pathologies postpartum and pregnancyassociated morbidity, such as preeclampsia (39-40) and susceptibility to development of GD in subsequent pregnancies. Up to 90% of GDMafflicted women develop type 2 diabetes (41). GDM may therefore, serve to unmask women who are predisposed and destined to develop type 2 diabetes later in life (42) - found no difference in circulating irisin between pregnant women with and without GDM, although 4 years after childbirth irisin levels were significantly higher in patients with previous gestational diabetes mellitus than in women with normal glucose tolerance. Conversely, Aydin et al. and other studies (37, 43, 44) showed lower serum irisin in lactating women with GDM in comparison with healthy lactating women. No significant differences in serum irisin between non-obese, obese and GDM subjects at term were recently reported by Piya et al. (45). However, further studies revealed that after adjusting for BMI, lipids and glucose, irisin levels were significantly lower in non-obese pregnant women as compared with obese and GDM groups. Our results showed that irisin levels were markedly lower than healthy pregnant, disagreement with other studies which may suggest a compensation for a physiologic increase in insulin resistance or a stimulating effect of high estrogens levels (46). or possibly its additional secretion by the placenta, although the influence of placental tissue to circulating irisin appears insignificant (46, 47). The authors suggested that these findings may reflect irisin resistance developing together with insulin resistance.

The concept of irisin resistance with compensatory hyperirisinemia was also proposed by Hee Park et al. (48), who showed that high irisin levels were associated with an increased risk of the metabolic syndrome and cardiovascular disease. However, an association between irisin and insulin resistance, in particular during pregnancy, seems still unclear. Piya et al. (49) confirmed that in pregnant women serum irisin was positively correlated with fasting blood glucose, insulin and HOMA-IR. Ebert et al. (47) In contrast, Yuksel et al. (43). reported that serum irisin level was negatively correlated with HOMA-IR in individuals with various degree of obesity. Additionally, we observed that in the whole group of pregnant women serum irisin concentration correlated negatively with glucose level at 120 min of the OGTT, which is consistent with the results of Choi et al. (50), who found that 2 h plasma glucose was an independent negative predictor of irisin concentration in the patients with newly-diagnosed type 2 diabetes.. All these discrepancies may

result from differences in clinical characteristics of the subjects studied and various diagnostic criteria; however, the potential effect of BMI or weight gain during pregnancy and gestational week at sampling appears controversial since a positive correlation between irisin level and body mass index at the last weeks in third trimester of gestation (47) and a negative one at term (49), were reported by different authors. In the present study, no associations between circulating irisin and BMI were observed. Moreover, controversial results, i.e. higher irisin concentration in pregnant than in non-pregnant women (47) or no significant differences during and after pregnancy (49), have been found in different studies.

## Results of trace elements

In the present study suggest that this element also contributes at some level to the pathogenesis of GD and pregnancy in diabetes. This is consistent with the role of this metal as a regulator of carbohydrate metabolism in pregnancy (51) The effect of diabetes in pregnancy may arise through two related mechanisms, namely, the direct effect of trace elements and oxidative stress on immune regulation (52). A significant decrease in Zn concentration was shown in the diet-treated diabetic group relative to healthy pregnancy which supports the hypothesis that Zn and Cu may play a role in the mechanisms regulating the immune response (53, 54).

Another study found that deficiency of Mg++ is associated with immunosuppression in athletes, suggesting that Mg++ has a role in immunoregulation (55, 56).

Magri et al. did not find a relationship between the serum levels of calcium, magnesium, and zinc and gestational hypertension, therefore, they proposed that these elements might not clinically participate in the pathogenesis of the gestational hypertension (57). The mean serum levels of magnesium, copper and zinc between the two groups were significantly different. For defining, the role of serum electrolytes in GDM more research is necessary. The results of the present study showed that these elements did play a prominent role in the pathogenesis of GDM.

## Conclusions

Maternal serum irisin levels of patients with GDM are significantly lower compared with healthy pregnant as controls. However, The present results suggest that serum irisin levels might presented as a novel marker for GDM, with decreased levels of irisin being symptomatic of GDM, and revealed that these trace elements Cu,Zn,Mg did play a conspicuous role in the pathogenesis of GDM.

The important issues of the associations between maternal insulin resistance during pregnancy, and future risk of the metabolic syndrome in mother need to be further addressed in future prospective studies.

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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