Serum Irisin In Individuals with Type 2 Diabetes Mellitus and Prediabetes in Duhok City

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Abstract
Type 2 diabetes mellitus is one of the most common chronic metabolic diseases characterized by hyperglycemia, with either lack of insulin secretion or decreased sensitivity to insulin metabolic effects. Prediabetes is an intermediate stage between normal glucose metabolism and diabetes mellitus with high prevalence rate of diabetes mellitus developments. We aimed in this study to evaluate serum irisin level among patients with type 2 diabetes mellitus and individuals with prediabetes. A cross-sectional study was done on 214 participants, who were attend to the Azadi Teaching Hospital, Diabetic unit at Duhok city between October 2021 and April 2022. The participants were classified to three groups: 94 patients with type 2 diabetes mellitus, 60 prediabetic individuals and 60 apparently healthy control. We collected blood samples in the morning after overnight fasting from all participants. All parameters were measured by cobas 6000 (Hitachi, Roche) and including glucose, insulin, total cholesterol, triglyceride, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol and blood HbA1c% except serum irisin that was measured by ELISA instrument. Serum irisin levels was lower in type 2 diabetic patients and prediabetic individuals compared to healthy control (P<0.001). There was a negative relation between serum irisin and total cholesterol, triglyceride, low-density lipoprotein-cholesterol, poor glycemic control and atherogenic indices, with positive relation of serum irisin with high-density lipoprotein-cholesterol. We found lower level of circulating serum irisin in type 2 diabetic patients and prediabetic individuals compared to healthy controls.

Keywords: Irisin, Type 2 Diabetes Mellitus, Prediabetes, HbA1c%, HOMA-IR.

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I. INTRODUCTION
Type 2 diabetes mellitus (T2DM) is one of the most common chronic metabolic diseases characterized by impaired carbohydrates metabolism (hyperglycemia), with either lack of insulin secretion (pancreatic β-cell dysfunction) or decreased sensitivity to insulin metabolic effects (insulin resistance) (Motahari-Tabari et al., 2014). T2DM prevalence is high worldwide; about 1 in 11 people are currently diagnosed with diabetes mellitus, and it is expected that diabetic patients will increase to 552 million by 2030 (Aune et al., 2015). Prediabetes indicates the earliest stage of impaired glucose metabolism characterized by plasma glucose levels between those of normal glucose tolerance and diabetes mellitus. Diabetes mellitus development is associated with prevalence rate of prediabetes (Tabá et al., 2012), therefore; modifiable risk factors in prediabetes, such as obesity, physical inactivity and diet high energy, can be targeted in prediabetes to delay or prevent T2DM development (Williamson, 2018).

Irisin is a peptide that has important roles in both human health and illness. It is a myokine; muscles and subcutaneous fat are responsible for its release, influenced by nutritional status and physical activity, and metabolic pathways influenced by it, like the metabolism of lipids and glucose. Human obesity and insulin resistance have both been linked to irisin (Boström et al., 2012), since it produces browning of white adipose tissue and enhances energy expenditure via rising uncoupling protein 1 levels that reduce body weight and improve insulin resistance (Huh et al., 2012).

Irisin's exact role is still unclear in T2DM and prediabetes, with controversial relation with glycemic indices and lipid profile (Mahmoodnia et al., 2017), therefore; in this study, we aimed to evaluate serum irisin levels among T2DM patients and prediabetic individuals, ascertain the association of serum irisin with lipid profile and atherogenic indices in
T2DM patients and prediabetic individuals, and in T2DM to estimate serum irisin relation with glycemic control.

II. MATERIALS AND METHODS

A. Population

We recruited 214 participants who attended to Azadi Teaching Hospital, Diabetic unit in Duhok city, between October 2021 and April 2022 in a cross-sectional study. The participants were classified into three groups; 94 T2DM patients, 60 prediabetic individuals and 60 apparently healthy as control.

The exclusion criteria include disorders affecting irisin level, including thyroid dysfunction, anaemia, cancer and renal diseases. We collected the clinical and demographic characteristics of the participants, including name, age, contact details, diabetes family history and gender. We obtained waist circumference, height and weight using a standard balance beam scale. The body mass index calculated as the body weight (kg)/height (m²), and categorized from 18.5 kg/m² to 24.9 kg/m² as normal weight, 25 kg/m² to 29.9 kg/m² overweight and 30 kg/m² and above for obese (Casadei et al., 2019). By using Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), we estimate Insulin resistance, HOMA-IR equation= fasting glucose (mg/dL) X fasting insulin (µU/mL) / 405 (Nuttall, 2015), and less than 3.0 was regarded as normal.

B. Blood collection

We collected blood samples in the morning after 12 hours of overnight fasting from all participants. EDTA tube were used to collect blood for HbA1c analysis and gel tube for analysis of serum irisin, fasting glucose sugar, cholesterol, triglyceride, LDL-c, and HDL-c. Tests kit used in this study were from Germany. All tests were done daily except irisin, triglyceride, LDL-c and HDL-c ratio. The biochemical analysis of the studied group was shown in Table 1.

C. Biochemical profile

All parameters were measured by Cobas 6000 (Hitachi, Roche) depending on different principles. HbA1c% based on the turbidimetric inhibition immunoassay for hemolyzed whole blood. Enzymatic, colorimetric method was used to measure concentration of serum glucose, serum cholesterol, triglyceride, low density lipoprotein-cholesterol and high-density lipoprotein-cholesterol. Plasma irisin was measured by using an enzyme-linked immunosorbent assay (ELISA) kit (Bioassay technology Laboratory, Zhejiang, China). Normal values of lipid profile depending on National Lipid Association (Jacobson et al., 2015). Electrochemiluminescence method was used for serum insulin measurement. Atherogenic indices have been calculated as follows: total cholesterol/HDL-c ratio (less than 4.5 as normal range), triglyceride/HDL-c ratio less than 3.0 as normal range, less than 2.5 for LDL-c/HDL-c ratio as normal range and less than 130 mg/dl as normal range for non-HDL-c = total cholesterol- HDL-c (Hussain et al., 2020). The study was approved by the Research Ethics Committee of the General Directorate of Health at Duhok City (18 August 2021) and registered by reference number: 18082021-8-23.

D. Statistics

Statistical analyses were done using the SPSS Version 25.0 program (IBM). To compare baseline differences between groups continuous variables analysis of variance and participant’s t-tests were used. For categorical variables, the chi-squared (χ²) test was used. Multiple linear regression analysis was used to look at the relationship between cognitive function and the concentration of irisin in circulation, as well as demographic and clinical factors. Statistical significance at p<0.05.

III. RESULTS

In this study, 214 individuals were recruited, 94 type 2 diabetic patients, 60 prediabetic individuals and 60 were apparently healthy controls. Mean age of T2DM patients was significantly higher than prediabetic individuals and healthy controls individuals. Majority of T2DM patients (87, 92.6%) and prediabetic individual (52, 86.7%) were equal to or more than 40 years old.

T2DM patients’ females were predominant (53, 56.4%). Anthropometric measurements such as body mass index and waist circumference showed statistically significant difference among studied groups, with higher mean ±SD among patients with T2DM (30.22±4.32) and prediabetic individuals (27.76±3.18) as shown in Table 1.

The biochemical analysis of the studied group was shown in Table 2. Serum glucose, Insulin, HbA1c% and HOMA-IR in T2DM patients and prediabetic individuals were significantly higher than healthy controls p<0.001. Moreover, in T2DM
patients and prediabetic individual’s, serum cholesterol, triglyceride and LDL-c were higher than in healthy control, while HDL-c had lower values in patients of T2DM and prediabetic individuals compared to healthy controls. Mean ±SD of irisin in T2DM patients was (7.67±1.57 ng/ml) and in prediabetic individuals (8.5±1.74 ng/ml) that were lower than the mean level of controls (9.01±2.36 ng/ml) and the difference was statistically significant (P <0.001).

Table 2. biochemical parameters of T2DM patients, prediabetic individuals and healthy controls.

| Parameters          | T2DM Mean±SD  | Pre-DM Mean±SD  | Control Mean±SD | P-value |
|---------------------|---------------|-----------------|-----------------|---------|
| Glucose (mg/dl)     | 189.0±47.88   | 196.3±30.11     | 167.7±18.71     |<0.001  |
| Cholesterol (mg/dl) | 191.6±39.86   | 169.9±34.51     | 167.7±18.71     |<0.001  |
| Triglyceride (mg/dl)| 163.3±41.33   | 133.5±38.27     | 128.4±22.74     |<0.001  |
| HDL-c (mg/dl)       | 43.9±9.04     | 45.1±7.91       | 47.4±7.3        | 0.037   |
| LDL-c (mg/dl)       | 111.4±30.84   | 98.4±27.42      | 93.4±16.34      |<0.001  |
| HbA1c (%)           | 0.9±2.15      | 5.9±0.13        | 5.2±0.59        |<0.001  |
| Insulin (IU/ml)     | 18.5±16.93    | 14.6±10.31      | 11.2±4.19       |<0.001  |
| Irisin (ng/ml)      | 7.6±1.56      | 8.5±1.74        | 9.0±2.36        |<0.001  |
| HOMA-IR             | 8.5±7.43      | 3.8±2.78        | 2.6±1.05        |<0.001  |

HDL-c, high density lipoprotein-cholesterol; LDL-c, Low-density lipoprotein-cholesterol; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

Atherogenic indices association between studied groups were shown in table 3. Differences found between mean ±SD of atherogenic indices among studied groups were statistically significant; patients of T2DM and prediabetic individuals had higher mean ±SD of atherogenic indices than healthy control.

Table 3. Atherogenic indices of T2DM patients, prediabetic individuals and healthy controls.

| Atherogenic indices | T2DM    | Prediabetic individuals | Healthy controls | P-value |
|---------------------|---------|--------------------------|------------------|---------|
| CHO/HDL-c           | 4.49±1.15| 3.89±1.12                | 3.61±0.69        |<0.001  |
| TG/HDL-c            | 3.91±1.38| 3.11±1.26                | 2.79±0.71        |<0.001  |
| LDL-c/HDL-c         | 2.63±0.913| 2.28±0.85                | 2.03±0.55        |<0.001  |
| non-HDL-c (mg/dl)   | 147.6±37.84| 124.7±35.11              | 120.3±20.7       |<0.001  |

The mean level of irisin with different levels of biochemical parameters was shown in table 4. In patients of T2DM, there were statistically significant lower levels of mean irisin with poor glycemic controls. Moreover, there was an insignificant low level of irisin among patients with T2DM and prediabetic individuals with abnormally high cholesterol, triglyceride and HOMA-IR.

Table 4. Association of serum irisin mean level with abnormal biochemical parameters of T2DM patients, prediabetic individuals and healthy controls.

| Parameters          | Irisin in T2DM No=94 | Irisin in Pre-DM No=60 | Irisin in Control No=60 | Mean±SD | P-value | Mean±SD | P-value | Mean±SD | P-value |
|---------------------|----------------------|------------------------|-------------------------|---------|---------|---------|---------|---------|---------|
| Glucose (mg/dl) <100 | 7.90±1.79            | 8.67±1.55              | 8.57±0.61               |<0.001  |<0.001  | 9.01±2.36 | NA       |
| Cholesterol (mg/dl) <200 | 7.80±1.58            | 8.61±1.86              | 8.75±0.59               |<0.001  |<0.001  | 9.01±2.36 | NA       |
| Triglyceride (mg/dl) <150 | 7.69±1.12            | 8.63±1.98              | 8.28±1.26               | 0.467   | 0.90±2.29 | 9.00±2.84 | 0.990    |
| HDL-c (mg/dl) >130 Male | 7.80±1.51            | 9.03±2.48              | 8.21±1.89               | 0.178   | 0.187   | 11.67±1.16 | 0.020    |
| | Female >50 | 7.75±1.63            | 8.21±1.14              | 8.18±1.53               | 0.956   | 0.030   | 9.36±2.82 | 0.561    |
| LDL-c (mg/dl) >130 Male | 7.80±1.53            | 8.63±1.80              | 7.49±0.58               | 1.030   |<0.001  | 10.9±2.43 | 0.365    |
| | Female >50 | 7.68±1.64            | 8.60±1.83              | 8.11±1.32               | 0.391   | 0.021   | 9.11±2.43 | 0.273    |
| HOMA-IR >3.0 | 7.91±1.81            | 8.68±1.76              | 8.31±1.73               | 0.426   |<0.001  | 9.01±2.38 | 0.893    |

NA, no association

In relation to all abnormal levels of atherogenic indices, the mean level of irisin was lower in patients of T2DM and prediabetic individuals than apparently healthy controls, and they were statistically insignificant, as shown in table 5.

Table 5. Association of irisin with abnormal atherogenic indices of T2DM patients, prediabetic individuals and healthy controls.

| Parameters          | Irisin in T2DM No=94 | Irisin in Pre-DM No=60 | Irisin in Control No=60 | Mean±SD | P-value | Mean±SD | P-value | Mean±SD | P-value |
|---------------------|----------------------|------------------------|-------------------------|---------|---------|---------|---------|---------|---------|
| LDL-c/HDL-c <2.5 | 7.61±1.68            | 8.46±1.92              | 8.56±1.47               | 0.825   |<0.001  | 8.92±2.30 | 9.40±2.69 | 0.551    |
| >2.5 | 7.74±1.48            | 8.65±1.74              | 9.01±2.36               | NA      |<0.001  | 9.01±2.36 | 9.40±2.69 |<0.001   |
| CHD/HDL-c <4.5 | 7.62±1.65            | 8.57±1.90              | 8.26±1.12               |<0.001  |<0.001  | 8.90±2.24 | 9.84±2.22 | 0.326    |
| >4.5 | 7.74±1.47            | 8.69±1.22              | 9.13±2.45               | 0.387   | 0.030   | 8.84±2.28 | 0.637    |
| TG/HDL-c >3.0 | 7.71±1.63            | 8.28±1.34              | 8.84±2.28               |<0.001  |<0.001  | 9.07±2.50 | NA       | 0.306    |

IV. DISCUSSION

The prevalence of diabetes mellitus is growing globally, and over 90% of patients with diabetes have T2DM (Holman et al., 2015). T2DM is associated with numerous complications,
including cardiovascular disease, which significantly contributes to the high mortality, morbidity, and socioeconomic burden associated with diabetes (Shah et al., 2018). Prediabetic individuals’ risk for acquiring T2DM is high, as most T2DM patients have hyperglycemia for several years prior to its diagnosis, and it is associated with complications like cardiovascular disease (Aroda et al., 2008). Depending on American Diabetes Association, the following are the diagnostic criteria for type 2 diabetes mellitus and prediabetes (American Diabetes Association (ADA), 2021): For type 2 diabetes mellitus, HbA1c% level of 6.5% and higher, fasting plasma glucose level of 126 mg/dL (7.0 mmol/L) or higher, 2-hour plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher during 75-gram oral glucose tolerance test and a random plasma glucose of 200 mg/dL (11.1 mmol/L) or higher. For prediabetes: HbA1c% between 5.7%-6.4%, fasting plasma glucose level of 100-125 mg/dL (5.6-6.9 mmol/L) and 2-hour plasma glucose level of 144-199 mg/dL (7.8-11 mmol/L) during a 75-gram oral glucose tolerance test.

Irisin is a new myokine that plays a crucial function in energy balance, homeostasis and metabolism (Lv et al., 2015). Moreover, circulating irisin levels gradually decline as glucose tolerance deteriorates (Zhang et al., 2016). Our study showed a higher mean of waist circumference and body mass index in patients with T2DM and prediabetic individuals than healthy controls with statistically significant (P<0.001). We found consistency with another study done in China that found T2DM patients and prediabetic individuals had higher waist circumference and body mass index (Sun et al., 2022). Obesity causes both insulin resistance and β-cell dysfunction, which are significant risk factors for acquiring T2DM and prediabetes.

In constituent and conflicting data were reported by different studies in a different area about the relation between the mean level of irisin among patients of T2DM and prediabetic individuals, and evaluate the involvement of irisin in the initiation as well as the evolution of diabetes mellitus and prediabetes (Park et al., 2020; Akour et al., 2017). We found that in patients with T2DM and prediabetes, the mean circulating levels of irisin was lower compared to healthy controls (7.67±1.57 ng/ml, 8.5±1.74 ng/ml and 9.01±2.36 ng/ml). Peroxisome proliferator-activated receptor gamma coactivator 1-alpha activity and expression were significantly downregulated in the skeletal muscle of T2DM patients and individuals with prediabetes, which plays an important role in glucose metabolism, insulin sensitivity and insulin secretion in muscle and liver, and this may explain the lower serum irisin levels as irisin is an exercise-induced myokine activated via the peroxisome proliferator-activated receptor gamma coactivator-1-α pathway (Soyal et al., 2006).

Some studies found a probable role of irisin in the homeostasis of fat metabolism or lipid abnormalities but still, the relation of the mean level of irisin with lipid profile was controversial (de la Iglesia et al., 2014). In our study, a statistically insignificant negative relation was found between mean level of serum irisin and serum cholesterol, triglyceride and LDL-c among patients with T2DM and prediabetic individuals, as a lower level of irisin was found with hypercholesterolemia and hypertriglyceridemia among T2DM patients and individuals with prediabetes. Moreover, we found a positive correlation between serum irisin level and HDL-c levels. Other studies discovered that circulating irisin levels were negatively associated to total cholesterol, triglyceride and LDL-c levels (Ebert et al., 2015; Duran et al., 2015) and positively with HDL-c (Akour et al., 2017; Wen et al., 2013). At the same time, we found lower circulating irisin levels in patients with T2DM and prediabetic individuals with highly abnormal atherogenic indices than apparently healthy controls. A study done in Iran showed no significant association between irisin and atherogenic indices (Ommati et al., 2019).

It is well known that glycemic control is the cornerstone of managing T2DM and is essential for long-term diabetes complications prevention (Afroz et al., 2019). However, irisin and glycemic indices relationship in diabetes remain contradictory. As serum irisin levels were lower in patients of T2DM with poor glycemic control and higher glucose levels, as we established a negative correlation between irisin and glycemic control in these patients. Liu et al. found pancreatic β cell proliferation was promoted by irisin to protect cells from apoptosis as well as ameliorate glucose metabolism in skeletal muscle and liver cells (Liu et al., 2017; So et al., 2016). In other research’s, irisin and serum glucose levels, insulin and HbA1c% were found to be negatively correlated in T2DM patients (Mehrabian et al., 2016). Fibronectin type III domain-containing 5 is the protein precursor whose proteolytic cleavage generates irisin (Boström et al., 2012). Increased serum fibronectin type III domain containing 5 in T2DM is related to increased age and poor glycemic control (Mágero et al., 2021).

In this study, we found lipid profile in patients of T2DM and prediabetes were abnormal compared to healthy controls. Patients of T2DM and prediabetic individual’s had higher serum cholesterol, triglyceride, and LDL-c mean levels than healthy controls, while HDL-c means level was lower in patients of T2DM and prediabetic groups than healthy controls, and the results were statistically significant. Studies done in Jordan and Bangladesh have shown the same results as ours (Bhowmik et al., 2018; Akour et al., 2017). An increase in blood glucose level will enhance acetyl CoA formation by activating of the Krebs cycle pathway that end ups with cholesterol synthesis (Krivoruchko et al., 2015). A decrease in the activity of insulin in patients of T2DM and prediabetic individuals’ results in decrease in lipoprotein lipase and hepatic lipase activity, which lead to an increase in serum triglyceride concentration (ADA, 2003). Since the apoprotein B lysine residues become glycated as glycemia rises, the LDL receptor cannot identify it, which results in decreased LDL-c catabolism (Kim-Dorner et al., 2010).
Reduced blood HDL-c levels are caused by increased VLDL acted upon by cholesterol ester transfer protein to create small HDL-c, which is quickly removed from circulation (McLaughlin et al., 2005). Diabetes related dyslipidemia is caused by a number of causes, including insulin’s effects on the formation of apoproteins in the liver, and regulation of lipoprotein lipase, cholesteryl ester transfer protein actions, and the peripheral effects of insulin on muscle and adipose tissue (Goldberg, 2001). In patients with T2DM and prediabetic individuals, statistically significant results were found in our study for atherogenic indices compared to healthy controls with higher levels in patients of T2DM and prediabetic individuals.

V. CONCLUSION

In T2DM and prediabetes low mean level of serum irisin was found. Collectively low mean level of irisin, abnormal lipid profile and abnormally high atherogenic indices among patients with T2DM and prediabetes will enhance risks development for cardiovascular disease, therefore; early diagnosis and management of both are crucial as they associated with increased levels of irisin and normalization of lipid profile, thus decreasing the risk of development of cardiovascular diseases.

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