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Original Article

Novel adipokines vaspin and irisin as risk biomarkers for cardiovascular diseases in type 2 diabetes mellitus

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ABSTRACT

Aims: Vaspin and irisin are novel cytokines proposed as potential new biomarkers of insulin resistance and endothelial dysfunction. This work is to investigate circulating levels of vaspin and irisin in patients with type 2 diabetes mellitus (T2DM) with and without cardiovascular disease (CVD) to study potential association with disease risk.

Materials and Methods: Circulating levels of vaspin and irisin were assayed in serum from 135 T2DM patients (with and without CVD) and 70 control subjects by ELISA.

Results: Vaspin levels were significantly higher in T2DM patients than in control subjects (6798 ± 3540 pg/ml vs. 3215 ± 3209 pg/ml, $p = 0.001$) and in CVD patients than in non-CVD patients (7417.3 ± 3507.6 pg/ml vs. 6017.3 ± 3606.4 pg/ml, $p = 0.001$), with significant positive correlations with BMI, FPG, serum insulin and HOMA-IR. Irisin levels were significantly lower in T2DM patients than in controls (71.15 ± 67.57 ng/ml vs.127 ± 71.57 ng/ml, $p = 0.004$), and in CVD patients than in non-CVD patients (55.77 ± 54.82 ng/ml vs. 115.5 ± 67 ng/ml, $p = 0.003$), with significant correlations with HbA1c, HOMA-IR and BMI in diabetic patients, and with HbA1c and TG in CVD patients. Elevated levels of vaspin was associated with 1.7 times increased CVD risk ($p = 0.001$, OR = 1.7, 95%CI = 1.21–2.39), while lower levels of irisin associated with 1.6 times increased CVD risk ($p = 0.007$, OR = 1.6, 95%CI = 1.45–2.28). ROC analysis indicated serum vaspin and irisin as independent CVD risk biomarkers with sensitivity, 94% and 73.7%, and specificity, 74% and 74.1%; respectively.

Conclusion: Our results indicate that circulating vaspin and irisin are potential new independent CVD risk biomarkers in T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common diseases with a rapidly increasing incidence worldwide [1]. Though, cardiovascular disease (CVD) is the major cause of morbidity and mortality in diabetes [2], yet, the underlying mechanisms linking T2DM with CVD still not fully elucidated. To date, several surrogate cytokines have been proposed as potential new markers of insulin resistance and endothelial dysfunction, such as vaspin and irisin.

Previous reports indicated that adipokines are related to insulin resistance, endothelial dysfunction, pro-inflammatory and pro-atherogenic states [3–5]. Elevated visceral adipose tissue mass has been accused for insulin resistance, T2DM, and risk of CVD [6,7]. Vaspin (visceral adipose tissue-derived serine protease inhibitor) is an adipokine first discovered in a rat model of abdominal obesity and T2DM [8], which inhibits proteases responsible for insulin resistance and plays an important role to protect against atherosclerosis and plaque development [9,10].

Previous studies indicated vaspin to be related to T2DM [11,12]. In mice, vaspin treatment improved glucose tolerance and enhanced insulin sensitivity [8], and associated with sustained glucose-lowering effects for at least 6 days after the injection [13]. In humans, vaspin mRNA was expressed in visceral and subcutaneous adipose tissue [14]. Both, gene expression and serum vaspin level associated with T2DM and obesity-associated disorders [15–18]. Also, vaspin has anti-atherogenic and anti-inflammatory activities by inhibiting the nuclear factor-kappa B (NF-kB) [19,20] and could prevent free fatty acid-induced endothelial apoptosis [21]. The relationship of serum vaspin with T2DM and cardiovascular complications is not fully clarified yet and results from previous studies are inconsistent.

Irisin is a novel exercise-regulated myokine inducing browning of white adipose tissue, energy expenditure, enhanced thermogenesis, weight loss and improved glucose tolerance [22]. Irisin is released by

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skeletal muscles (myokine) [23] and adipose tissues (adipokine) [24] in response to exercise [25]. It was postulated that mild increase in irisin serum level, either exercise-induced or injected, improves glucose homeostasis by increasing energy expenditure, thus irisin has gained interest as a potential promising treatment for metabolic diseases including T2DM [23]. However, later held out studies gave inconsistent results, while some studies reported lower levels of irisin in diabetic patients compared to healthy controls [26–31], other studies found T2D was associated with an increased irisin release in order to compensate the insulin resistance in the skeletal muscles and the poor glycaemic control in diabetic patients [32,33].

Both vaspin and irisin have been related to insulin resistance and T2DM [12], however, their real role in human insulin resistance is still unclear, besides the potential association with CVD in T2DM patients is still obscure. In this work, we aimed to assess circulating vaspin and irisin in patients with T2DM with and without CVD and investigate potential association with cardiovascular complications in order to evaluate their role as risk biomarkers for CVD in T2DM.

2. Materials and methods

2.1. Study participants

We retrospectively studied 135 patients with T2DM and 70 matched healthy volunteers serving as a control group, all participants were recruited from the outpatients'clinic of the National Research Center in Cairo, Egypt. Data of medical and family history was obtained by questionnaire. Physical activity was defined as exercise for 2–3 days/week for at least 30 min. Clinical examination including systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements was applied. Anthropometric measurements (weight and height) were collected and used for BMI calculation according to the standard formula BMI = weight (kg)/[height (m)]^2. Hypertension was defined as blood pressure above 140/90 mmHg or under antihypertensive drugs. Diagnosis of diabetes was based on the criteria of American Diabetes Association [34]. Our subjects were classified into 3 groups:

T2DM patients without CVD: included 67 subjects fulfilling the diagnostic criteria of diabetes or were under oral antidiabetic treatment with no history or signs of any CVD.

T2DM complicated with CVD: included 68 diabetic subjects complicated with any form of CVD e.g. ischemic heart disease (IHD), macroangiopathy and/or cerebrovascular disease. IHD included myocardial infarction, ischemic electrocardiographic (ECG) changes and angina pectoris. Macroangiopathy included peripheral arterial diseases (ankle brachial index ≤ 0.9) and cerebrovascular disease (history of transient ischemic attack, reversible ischemic neurological deficit or stroke caused by cerebral infarction).

Control group: included 70 healthy subjects with a normal glucose tolerance test, with no history of hypertension, hyperlipidemia, CVD or family history of any form of CVD, renal disease, hepatic disease, endocrine disease, other metabolic disorders, and autoimmune diseases or under any long-term medication.

Exclusion criteria for diabetic patients included insulin treatment (for the need to measure serum insulin level), renal disease, hepatic disease, endocrine disease, other metabolic disorders, and autoimmune diseases.

Informed consent was obtained from all participants and the study protocol was approved by the Ethics Committee of the National Research Center.

2.2. Methods

2.2.1. Assay of biochemical markers

Venous blood samples were collected from all subjects after 12 h of overnight fast, centrifuged within 2 h and assayed for biochemical markers. For ELISA assays, aliquots were frozen at −80 °C till time of assay to avoid erroneous results from repeated freeze/thaw cycles.

Fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were assayed on c311 clinical chemistry auto analyser (Roche Diagnostics, Germany). Glycosylated hemoglobin (HbA1c) was assayed by ion-exchange high-performance liquid chromatographic (HPLC) method using Agilent 1200 series HPLC system (Agilent Technologies, USA) equipped with UV/VIS-Detector 415 nm using the commercially available HbA1c test kit (RECIPE Chemicals and Instruments GmbH, Germany). Serum insulin was quantitatively assayed by commercially available enzyme immunoassay test kit (IMMUNOSPEC, USA), with assay sensitivity of 2.0 μU/ml. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from fasting insulin and fasting plasma glucose (FPG) by the following equation: HOMA-IR = fasting insulin (μU/ml) × FPG (mg/dl)/405.

2.2.2. Assay of circulating vaspin and irisin in serum

Serum aliquots were thawed and centrifuged just prior to assay. Vaspin was assayed using Human vaspin enzyme-linked immunosorbent assay (ELISA) kit *MBS2506005* (MyBioSource, Inc., USA) with detection range from 62.5 pg/ml to 4000 pg/ml and sensitivity of 37.5 pg/ml, with no significant cross-reactivity or interference with other analogues. Irisin was assayed using a commercially available human irisin ELISA kit MBS706887 (MyBioSource, Inc., USA). The detection range is from 3.12 ng/ml to 200 ng/ml, with an estimated sensitivity of less than 0.78 ng/ml. Intra-assay and inter-assay precision coefficients of variation (CV%) were <8% and <10%, respectively.

2.3. Statistical analysis

Analysis of data was performed using the IBM SPSS version 20.0 software. Data were expressed as mean ± standard deviation (SD) for continuous variables and as frequency (%) for categorical variables. Normally distributed data were compared using Student's t test for two groups and ANOVA test for more than two groups followed by posthoc Bonferroni multiple comparison test. For categorical clinical variables, differences between groups were evaluated by chi-square test. The correlations between serum vaspin and irisin with other variables were tested using Pearson correlation coefficient (r) and correlations were analyzed using Spearman's correlation coefficient. Logistic regression analyses were used to assess association of serum vaspin and irisin with the incidence of CVD in T2DM patients with unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (CIs). A receiver operating characteristics (ROC) analysis was used to calculate the area under the curve (AUC) to find the best cutoff value of serum vaspin and irisin providing the highest diagnostic specificity followed by the best sensitivity to differentiate between T2DM patients with CVD from those without CVD. P value <0.05 was considered significant.

3. Results

3.1. Characteristics of the study participants

The study included 205 subjects classified into 3 groups: T2DM (n = 68), T2DM complicated with CVD (n = 67) and control group (n = 70). Age ranged from 45 to 64 years. The frequencies of CVD in our patients were: 65% IHD, 15% cerebrovascular disease, 10% macroangiopathy, 10%combined IHD and cerebrovascular disease.

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