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Original Article

Serum Irisin levels as a marker in some phenotypes of PCOS

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ABSTRACT

Study objectives: The aim of the study was to assay the serum levels of Irisin in women with PCOS.
Study design: Serum Irisin levels were investigated in obese and normal weight cases with PCOS and in control cases.
Study setting: The study was done in the Obstetrics and Gynecology Department, Mansoura University, Egypt.
Material and methods: The study included 80 cases with PCOS and 80 control cases. The patients were stratified by BMI as obese and normal weight.
Statistical analysis was performed by using Statistical Package for Social scientists (SPSS) for Windows 7 (SPSS Inc., Chicago, IL, USA).
Main outcome measure was to measure the serum Irisin levels in obese and in normal weight cases. Also, some metabolic indices including lipid profile and HOMA-IR were measured in the cases of study.
Results: Fasting Irisin levels were significantly elevated in obese and in normal weight PCOS patients as compared to the levels in obese and normal weight control women ($P = 0.000$). The serum Irisin levels in obese and in normal weight PCOS cases were found to be significantly positively correlated with BMI, insulin levels, HOMA-IR, triglycerides levels, and triglycerides/HDL ratio ($P < 0.05$). The serum Irisin levels were found to be significantly negatively correlated with HDL levels ($P < 0.05$).
Major conclusion: Changes in Irisin levels may be considered as a biomarker for the detection polycystic ovarian syndrome. In the future, it may be used as a marker to follow the disease under various modalities of treatments.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive-aged women. Studies in which ultrasound examination for presence of polycystic ovaries were conducted reveal a prevalence rate of approximately 20–30% in Caucasian women in the reproductive age [1]. It is associated with reproductive problems, and with metabolic disorders including insulin resistance and dyslipidemia. It is believed that dyslipidemia plays an important role in the pathogenesis of PCOS-associated metabolic dysfunction [2].

It was suggested that impaired regulation of some adipomyokine hormones may play a role in the development of PCOS because most PCOS patients have a tendency to become overweight or obese and insulin resistant.

Irisin is a newly identified muscle-derived myokine that acts as a messenger from skeletal muscle to other parts of the body. Irisin is named for Iris, the Greek goddess who served as a messenger between the Gods [3]. The circulating factor Irisin, cleaved from fibronectin type III domain containing 5 (FNDC5) activates thermogenic programs (browning) in white adipose tissue (WAT). The browning of WAT is associated with augmented mitochondrial density and oxygen consumption, producing heat leading to weight loss [3].

Skeletal muscle is not the main source of irisin. Irisin is produced by many tissues as adipose tissue, liver, the cardiovascular

Abbreviations: BMI, body mass index; HOMA-IR, Homeostasis Model of Assessment-Insulin Resistance index; IR, insulin resistance; MS, metabolic syndrome; PCOS, polycystic ovary syndrome; WAT, white adipose tissue; ELISA, Enzyme Linked immune-Sorbent Assay.

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system, the brain, the bone, the pancreas, the kidney, the immune system, the ovary, the peripheral myelin sheath, the intestinal L cells and pancreatic islets, the testis, the spleen, the stomach, the cardiac tissues, and the fetal skeletal muscle cells as evidenced by Irisin staining [4].

Irisin may have a role in the pathophysiology of a number of diseases characterized by insulin resistance such as type-2 diabetes and metabolic syndromes [5]. Higher Irisin levels are reported to be associated with body mass index (BMI), muscle mass and adipose tissue mass [6].

It was reported that Irisin plasma concentration in PCOS cases and in healthy subjects is related to body adipose content [7].

Exercise has a beneficial effect on metabolism through the activation of insulin action, due to increased Irisin secretion [8]. Irisin discovery may raise hopes regarding the hypothesis that it may provide a new modality of treatment for obesity and diabetes [9].

1.1. Objectives

The cause of PCOS is still unknown, many patients with PCOS are obese and present with dyslipidemia and insulin resistance. It was suggested that Irisin may play a role in the development of insulin resistance.

The aim of this study was to determine the serum levels of irisin in obese, normal weight and normal weight cases with PCOS, and to correlate the levels with insulin resistance (IR) parameters.

2. Materials and methods

2.1. Patients

The present study was a case-control study carried on 80 cases with PCOS divided into two groups, and 80 control cases divided into 2 groups, matched for age, BMI, and WHR). The cases were selected from infertile cases attending the outpatient clinic at the Department of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University, during the period between September 2015 and January 2017, in accordance with the following inclusion/exclusion criteria.

The study was approved by the IRB committee of Faculty of Medicine, Mansoura University, Egypt [code number; R/15.08.61, date: 1/9/2015]. Informed written consents were taken from the cases.

Sample size calculation was done by using creative research systems, the survey software-system.

The incidence of PCOS in anovulatory infertility is from 35% up to 75% [10]

By using the mean incidence of PCOS in infertility as 55% and the confidence level of 95%: the confidence interval is calculated to be 15.42. The sample size is calculated by using the confidence level of 95% & confidence interval of 15.42: it was found to be 40 cases in each group.

The power of the study was 95%

Inclusion criteria:

Women with PCOS were diagnosed according to the revised Rotterdam criteria [11], by the presence of two of the following three manifestations: (1) oligo-ovulation/or anovulation, (2) clinical and/or biochemical hyperandrogenism, and (3) polycystic ovaries on ultrasound scanning. The cases were not under treatment for at least 3 months.

The control cases had regular menstrual cycles and normal ultrasonography findings on pelvic ultrasound scanning, without clinical or biochemical hyperandrogenism. The cases were not receiving any treatment for at least 3 months.

Exclusion criteria for all cases of the study included age >40 years, known cardiovascular disease, thyroid disease, other endocrinopathies, neoplasms, endometriosis, current smoking, diabetes, renal impairment, hypertension, oral contraceptives users, insulin sensitizer drugs, hormonal or anti-hypertensive medication for at least 3 months before the study.

The final clinical study involved five groups of cases:

Group 1: consisted of 40 obese PCOS cases (with BMI >30).
Group 2: consisted of 40 normal weight PCOS cases (with BMI <25).
Group 3: consisted of 40 control cases with obesity (BMI >30).
Group 4: consisted 40 normal weight control cases (BMI <25).

BMI (kg/m^2) was calculated by dividing weight (kg) by height squared (m^2). Obesity is considered if BMI $\geq$ 30 kg/m^2 (according to the WHO criteria).

2.1.1. Sampling

All samples were obtained in the morning after an overnight fast (10–12 h) during the early follicular phase (days 2–4 of the spontaneous cycle). Five ml of maternal venous blood was withdrawn from anti-cubital vein under complete aseptic conditions.

All samples were collected in a vacutainer tube with gel, and the serum was separated by centrifugation and divided into three parts: one part was used for assessment of fasting blood glucose and insulin levels, the second part is used to perform total lipid profile (total cholesterol, triglycerides, HDL-C, and LDL-C), and the third part is stored at −20 °C until the time of assay of irisn.

2.1.2. Methods of assay

All biochemical tests were done on Cobas Integra 400 plus autoanalyzer by using Boehringer Mannheim reagents.

- Serum levels of glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) were measured by the enzyme-calorimetric methods. Low-density lipoprotein (LDL) levels were calculated by using Friedwald et al., equation [12]. LDL (mg/dl) = total cholesterol − [HDL + (triglycerides/5)].
- Insulin: was measured by electro-chemiluminescence immunoassay method.
- Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR) index for the assessment of insulin resistance was measured by calculation using Matthews et al., equation [13].
  HOMA-IR = [glucose (mg/dl) × insulin (IU/ml)]/405.
  Insulin resistance (IR) was accepted as HOMA-IR $\geq$ 2.5 [13].
- *Serum Irisin* concentrations were measured with enzyme-linked immunosorbent assay (ELISA) by using commercial Human Irisin ELISA Kit, supplied by BioVendor with Cat No RAG018R, Lot No X14-369, according to the methods recommended by the manufacturer.

This assay is a specific competitive Enzyme Linked-Immuno-Sorbent Assay (ELISA) for quantitative determination of irisin in human biological fluids. A polyclonal antibody recognizing native irisin reacts with a series of predetermined recombinant irisin standard proteins n the irisin coated plate. Their relative reactivity is plotted with that of the standard proteins. The color was measured at OD 450 in an ELISA reader within 30 minutes. Generate the standard curve by plotting the average absorbance obtained by each standard concentration on vertical (Y) axis vs the corresponding irisin concentration on the horizontal axis. Calculate the irisin concentrations by interpolation of the regression curve formula. If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of irisin in the sample.

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