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Original Research Paper

Biochemistry

STUDY OF SERUM IRISIN AND ITS ASSOCIATION WITH INSULIN RESISTANCE IN POLY CYSTIC OVARY SYNDROME

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ABSTRACT

OBJECTIVE: Poly cystic ovary syndrome (PCOS) is clinically heterogeneous endocrine disorders. Insulin resistance-related proteins play a role in the etiopathogenesis of PCOS. Irisin is a newly identified myokine which act like adipokines. Irisin has been shown to be associated with insulin-resistance and metabolic syndrome. The purpose of this study was to determine the serum levels of irisin in PCOS patients and evaluate its association with insulin resistance. **PATIENTS AND METHODS:** Eighty five PCOS patients and eighty five matched healthy controls were enrolled to study .Serum irisin levels , anthropometric and metabolic parameters including HOMA-IR were measured. Linear regression analysis was employed to study the relationship between irisin and metabolic parameters. **RESULTS:** Serum irisin level in PCOS patients (mean value ; 0.50±0.07 ng/ml) was significantly elevated when compared to control group (mean value; 0.203±0.043ng/ml)(p value <0.001).Linear regression analysis showed that serum irisin was positively associated with body mass index , fasting insulin and lipid profile in PCOS patients. **CONCLUSIONS:** Serum irisin level of PCOS patients was high compared to that of healthy control subjects . In patients with PCOS , this situation may be due to insulin resistance .

KEYWORDS : Poly cystic ovary syndrome, Metabolic syndrome, Insulin resistance , Irisin.

BACKGROUND

The first reports of polycystic ovary syndrome (PCOS) in modern medical literature were by **Stein and Leventhal** in **1935**, with descriptions of 7 women with the complaints of amenorrhea, hirsutism, and enlarged ovaries with multiple cysts[1].

PCOS is a common endocrine disorder, with a prevalence of approximately 7% in reproductive age women. It is now recognized as a common heterogeneous , inherited disorder characterized by hyperandrogenemia, menstrual irregularities, chronic anovulation, polycystic ovaries and reduced fertility[2,3] A joint ASRM/ESHRE publication gave the definition of this pathology, which is now accepted globally. For a confirmed diagnosis,2 of the following 3 clinical criteria are essential: oligomenorrhoea, hyperandrogenism or polycystic appearance of the ovaries on transvaginal ultrasonography [4].. There are numerous metabolic consequences of PCOS in addition to the effects on the reproductive system. These include a higher risk of obesity, insulin resistance (IR), type 2 diabetes mellitus (T2DM) and premature arteriosclerosis [5-7].

However,the aetiology of PCOS is underpinned by both insulin resistance and hyperandrogenism, with insulin resistance exacerbating hyperandrogenism.[6]

Insulin resistance occurs in approximately 80% of women with PCOS and occurs independently of obesity. Furthermore, women with PCOS are believed to be at and increased risk of developing type 2 diabetes mellitus (T2DM). A recent meta-analysis of 13 studies reported a 4-fold increased risk of T2DM in women with PCOS. Thus, PCOS is a well-defined clinical model of insulin resistance and the pre-diabetic state. [7,8] Visceral adipose tissue is a complex endocrine system that produces a large number of bioactive proteins, collectively termed adipokines . The deregulated production of adipokines in obese subjects appears to be implicated in the pathogenesis of IR , hyperandrogenism , and PCOS.[9]

Irisin , a newly identified peptide myokine is a novel Biomarker identified in patients with PCOS. Irisin was first reported by **Bostrom et al (2012)**;[10] secreted as a cleavage molecule from fibronectin type III domain containing 5 [FNDC 5] expressed on plasma membrane of Myocytes and to some extent ; adipocytes , stimulated by peroxisome proliferator activated receptor gamma coactivator-1-alpha[PGC-1alpha] in skeletal muscles[11]. Irisin was introduced to be an exercise-induced hormone secreted by muscle , assuming that exercise may increase irisin levels and so, result in benefit on metabolism and glucose tolerance then relationships with other myokines were determined and it has since been described as behaving in the same way as an adipokine expressed and secreted by white adipose tissue.[12]

Irisin has attracted great attention since its discovery as it is thought that this peptide could play an important role in both animal and human physiology and biology.[13]To date, however, the benefits to humans which could be attributable to irisin remain unclear, and the effects of exercise training on FNDC5 gene expression and irisin levels are as yet, undefined[14].

The aim of this study was to determine the serum levels of irisin in patients with poly cystic ovary syndrome (PCOS), to compare these levels and to understand its association with insulin resistance.

PATIENTS AND METHODS

The study has been conducted on two age & sex matched group of participants attending gynecology O.P.D. of J.L.N. Medical college and associated group of Hospitals, Ajmer (Rajasthan).

This study comprised 85 PCOS patients and 85 matched healthy controls. Diagnosis of poly cystic ovary syndrome (PCOS) will be based upon the diagnostic criteria of Rotterdam PCOS Consensus Criteria. Two of the following three criterias are required:1] Oligo/ anovulation , 2] Hyperandrogenism , 3] Polycystic ovaries on ultrasound.

PCOS was defined as the presence of 12 or more follicle in each ovary , each measuring 2-9mm in diameter , and/or increased ovarian volume > 10ml[4]. Patients suffering from Diabetes Mellitus, Hepatic and Renal dysfunction , Thyroid

dysfunction, Pregnant women and Smokers and Alcoholics were excluded.[16,17]

All patients had clinical and/ or biochemical hyperandrogenism and chronic anovulation and polycystic ovaries on ultrasound .The control group consisted of healthy women who had regular menstrual cycles without clinical or biochemical hyperandrogenism or poly cystic ovary and with no history of any drug intake .[18]

Anthropometric measurements(body mass and height) were measured.Body mass index was calculated according to the standard formula.[19]

In all the women serum level of irisin , fasting glucose and fasting insulin were measured.

Serum cholesterol , high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides levels were also measured. Biochemical parameters were studied by enzymatic colorimetric methods.[21,22] Irisin concentration in the blood samples were measured in the same experimental series using commercial ELISA kits (cat no: EH4702; Wuhan Fine Biotech co. ltd. China).HOMA-IR index , as calculated with the standard formula :

HOMA-IR= fasting concentration of insulin (mIU/ml) x fasting concentration of glucose(mmol/l)/22.5

Statistical Analysis

Unpaired t- test and Mann- Whitney U tests were used for variables with normal and not normal distribution , respectively . Values were given as mean $\pm$ SD . Pearson correlation analysis was employed to study the relationship between irisin and metabolic parameters. Multivariate linear regression models were used to study which variables were independently associated with irisin. A value of $p < 0.05$ was considered statistically significant.

RESULTS

BMI of the control group was 22.70 $\pm$ 1.82 . In the PCOS patients group BMI was 26.62 $\pm$ 2.62. The metabolic status of the PCOS and controls are shown in table 1 . In the control group the fasting glucose and fasting insulin values were determined as mean 93.09 $\pm$ 14.18 mg/dl and 4.14 $\pm$ 1.11 μIU/ml respectively and in the PCOS group , as 90.09 $\pm$ 6.87 mg/dl and 14.17 $\pm$ 3.15 μIU/ml respectively. The PCOS patients exhibited significantly higher levels of fasting insulin compared with those of the control group ($p < 0.001$).

HOMA-IR values were , then compared between the two groups for the assessment of insulin resistance . The HOMA-IR level was high in the PCOS group (5.02 $\pm$ 1.46) while in the control group values were low (2.72 $\pm$ 0.46)(p value < 0.001).

The mean serum irisin level was determined as 0.50 $\pm$ 0.07 μg/ml for PCOS patients and 0.203 $\pm$ 0.043 μg / ml for the control group . As a result of this , the PCOS patients had significantly elevated levels of fasting irisin compared to the control subjects.

Linear regression analysis showed that fasting irisin was positively associated with BMI, fasting insulin and lipid profile.

Table 1 : The metabolic status data of the control and PCOS patients

	Control	Case	p
BMI	22.70 + 1.82	26.60 + 2.62	0.001
Glucose(mg/dl)	93.09 + 14.18	90.09 + 6.87	0.08
Insulin (μIU/ml)	4.14 + 1.11	14.17 + 3.15	0.001
Irisin (μg/ml)	0.203 + 0.043	0.50 + 0.07	0.001
HOMA-IR	2.72 + 0.46	5.02 + 1.46	0.001
Cholesterol(mg/dl)	156.37 + 22.79	179.34 + 21.89	0.001
Triclyceride(mg/dl)	84.69 + 16.25	112.39 + 15.58	0.001
VLDL (mg/dl)	39.93 + 14.83	22.45 + 8.25	0.001
HDL (mg/dl)	24.14 + 12.66	45.87 + 6.44	0.001
LDL (mg/dl)	93.64 + 22.30	110.95 + 23.01	0.001

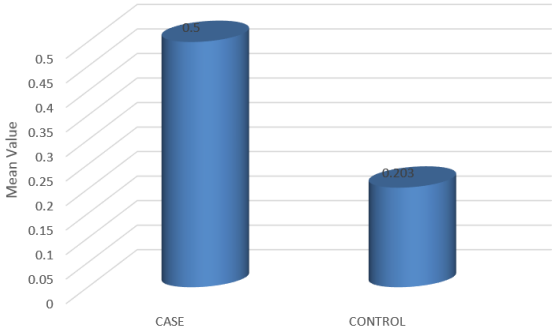
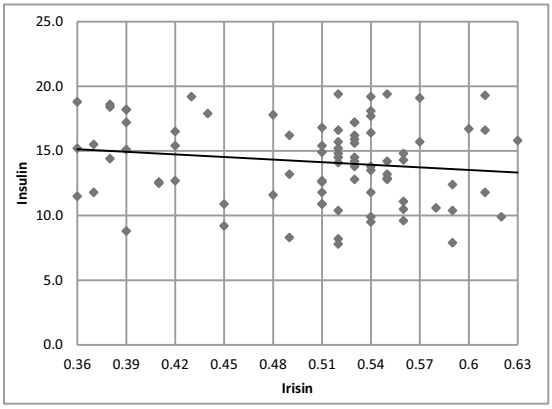


Figure 1 : The mean serum irisin level for PCOS and control group

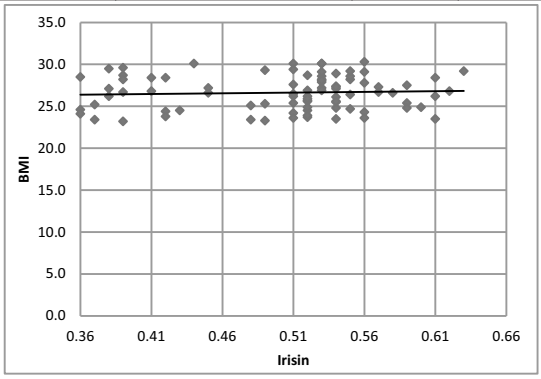
Correlation between Irisin and Insulin

Correlations			
		Irisin	Insulin
Irisin	Pearson Correlation	1	-.150
	Sig. (2-tailed)		.172
	N	85	85
Insulin	Pearson Correlation	-.150	1
	Sig. (2-tailed)	.172	
	N	85	85



Correlation between Irisin and BMI

Correlations			
		Irisin	BMI
Irisin	Pearson Correlation	1	.058
	Sig. (2-tailed)		.597
	N	85	85
BMI	Pearson Correlation	.058	1
	Sig. (2-tailed)	.597	
	N	85	85



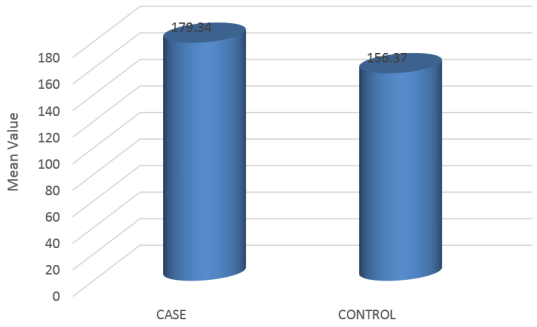


Figure 2 : The mean serum cholesterol level for PCOS and Control group

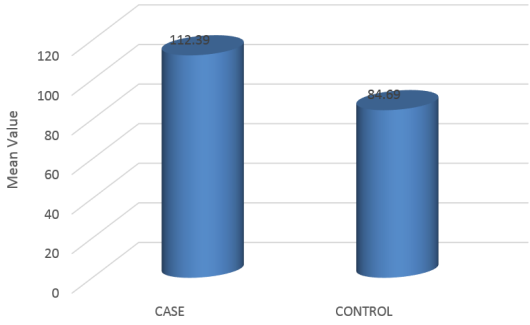


Figure 3 : The mean serum Triglyceride level for PCOS and Control group

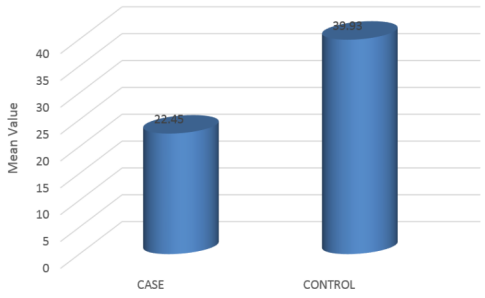


Figure 4 : The mean serum VLDL level for PCOS and Control group

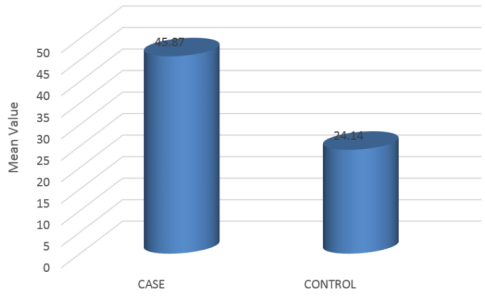


Figure 5 : The mean serum HDL level for PCOS and Control group

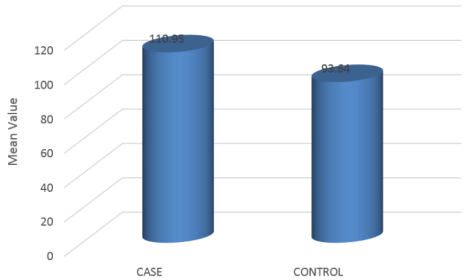


Figure 6 : The mean serum LDL level for PCOS and Control group

DISCUSSION

According to the ASRM/ESHRE criteria definition , PCOS is frequently associated with insulin resistance and metabolic syndrome. Insulin has a key role in carbohydrate metabolism and insulin resistance is associated with PCOS at a reported prevalence of 50%-70% occurringindependently of obesity[22] . Increased serine phosphorylation and reduced tyrosine phosphorylation, which is a specific abnormal pattern of insulin receptor phosphorylation seems to be responsible for the insulin resis tance observed in PCOS[23] .

It has been widely accepted that insulin signal Transduction might be diminished in PCOS. PCOS has been linked to a higher prevalence of impaired glucose tolerance and T2DM, independent of obesity levels. Although the majority of PCOS patients retain sufficient beta-cell function to prevent deterioration in glucose tolerance, a considerable number, especially those with first-degree relatives with type 2 diabetes, produce an abnormal beta-cell response in response to glucose challenges[24] . Impaired glucose tolerance has been reported in approximately 30%- 40% of PCOS patients and diabetes mellitus type 2 in 7.5%-10%[25]. In a meta-analysis[26] , a subgroup analysis of BMI-matched studies reported the OR for impaired glucose tolerance to be 2.54 (95% CI 1.54-4.47) for women with PCOS.

From the ongoing discussion we come to know that obesity is a worldwide health burden , accompanied by a number of comorbidities including glucose intolerance, insulin resistance, type 2 diabetes and pcos. Myokine Irisin , which is a cleavage product of the type 1 membrane protein fibronectin type III domain – containing 5 , has been associated with adiposity and body weight in humans.[27]

In healthy controls our study shows that as BMI increases value of Irisin increases this means obese non-pcos persons have high level of Irisin. Irisin level is greater in men than women this may be because of greater muscle mass in men than women . Circulating Irisin has been found to be directly associated with muscle mass.[28,29]

In our study we found higher level of Irisin in pcos patients. As Irisin is a myokine secreted in response to PGC-1α is activation . Studies suggest that PGC-1α is important for mitochondrial homeostasis for it regulates mitochondrial biogenesis and oxidative metabolism , and mitochondrial function also plays a role in Insulin resistance. [30]

This can be because of obesity associated lower brown or beige adipocyte in human adipose tissue. Furthermore, expression and activity of PGC-1α are higher in patients with pcos .[31,32]

A moderate increase in circulating Irisin levels augmented energy expenditure , not only by reducing weight gain due to a high –fat diet , but also by improving diet induced insulin resistance.[33]

Overall , the blood concentration of Irisin may reflect the metabolic status of patients suffering from metabolism disorders. Although optimism should be guarded [34], the identification of Irisin opens new possibilities because the application of Irisin may prove beneficial not only in monitoring and/or treatment of obesity and pcos but also for a wide range of pathological conditions that are characterized by a variable imbalance of energy demand and expenditure.[35,36]

CONCLUSION

The result of this study showed the serum irisin level of PCOS patients was found high compared to that of healthy control subjects.In patients with PCOS , this

situation may be due to insulin resistance.

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