ASSOCIATION OF IRISIN WITH THE OXIDANT-ANTI OXIDANT PARAMETERS IN TYPE 2 DIABETIC PATIENTS ACCORDING TO AGE IN THI-QAR PROVINCE/ IRAQ[1]

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ABSTRACT

The present study aimed to assessment of irisin level in patients with the newly onset type 2 "diabetes mellitus" (T2D M) and the scanning the association of irisin value with some physiological and oxidant-anti oxidant parameters. This study comprised 60 individuals diagnosed in newly onset T2DM and 40 healthy contributors (control group). Malondialdehyde (MDA), Ceruloplasmin and serum albumin concentration was calculated in patient who involved in this study. Serum irisin levels was evaluated by ELISA kit.

The present study showed a significant increasing ($P \leq 0.05$) of glycohemoglobin (HbA1C) and FBS level in patient with DM2 compared with the control groups (6.88 ± 1.02 vs 4.15±0.56; 7.85 ± 1.89 vs 4.94 ± 0.40) respectively. Also, the results explained a significant increased ($P \leq 0.05$) of T-Ch, Tg and LDL level in DM2 group compared with the control group (4.27±0.90 vs 4.23± 0.40; 2.14 ± 0.86 vs 1.89 ± 0.26; 2.84 ± 0.94 vs 2.23 ± 0.52) respectively. Whereas, the results showed a significant decrease ($P \leq 0.05$) of irisin, insulin, C-peptide and HLD in DM2 group compared with the control group (22.32 ± 4.55 vs 27.81 ± 2.93; 18.29 ± 3.66 vs 27.27 ± 6.90; 3.12 ± 0.99 vs 6.03 ± 0.48; 1.0 ± 0.17 vs 1.61±0.37) respectively.

In correlation analysis the results showed a negative association between irisin and (HbA1c) (r= -0.152), glucose (r= -0.331), insulin (r= -0.156), HDL(r= -0.114) and BMI (r= -0.219). Whereas, the results showed positive correlation between irisin and IL-6 (r=0. 0.115), CRP (r= 0.153), C-peptide (r=0.013), T-Ch (r= 0.057), Tg (r=0.209) and LDL(r=0.035).

Conclusion

1- The level of irisin in the type 2 patients reduced with high level of HOMA-IR and BMI.

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2- From the results of correlation analysis between irisin and oxidant factors we can concluded that irisin work as a anti agents of the oxidative condition.

3-The negative correlation between irisin and glucose refer to the important of it on the glucose homeostasis.

Keywords: *Irisin, T2DM, inflammatory parameters.*

1. INTRODUCTION

Diabetes mellitus (DM) is the continual disease common characterizes by hyperglycemia resulting from defects in secretion and/or its activity of insulin (WHO, 2016), its frequency elevate regularly every year. The universal commonness of diabetes, amongst adults were 6.4%, affecting 285 million patients in 2010, and is probable to augment to 7.7% (i.e., 439 million individuals) in 2030.

In humans, irisin is produced mainly by skeletal muscle in response to physical activity. It has been demonstrated that irisin plays a pivotal role in inducing fat browning and regulating energy expenditure. New findings from various studies conducted in both animals and humans suggest that irisin can affect bone and glucose metabolism. In particular, irisin is able to increase bone cortical mass by stimulating the osteoblast pathways, and irisin levels are inversely correlated with the incidence of fragility fractures among postmenopausal women affected by osteoporosis. Most available evidence shows that irisin significantly influences glucose and energy homeostasis (Endocrinologica, 2017).Bostrom *et al*., (2012) identified the irisin, an energetic metabolism-related myokine. Its secretion involves the increase of peroxisome proliferator- activated receptor-gamma coactivator 1 alpha (PGC1 alpha) in the muscle, inducted by exercise, promoting the expression and proteolysis cleavage of Fndc5, a type 1 membrane protein "fibronectin type III domain-containing protein 5", with release the irisin fragment for the blood flow, this hormone promotes a browning process on the white adipose tissue, a encoding for the thermo genesis in the tissue cells, through the increase of the mitochondrial uncoupling protein 1 (UCP1). So, the final effect of the hormonal signal promoted by the irisin is an enlarge on the physical energy spending, with the decrease of the obesity and development on the insulin resistance caused by diet (Bostrom *et al*., 2012).

Some new studies have shown that the irisin values were lesser in patients with T2DM when compared with the non-diabetics (Arias-Loste *et al*., 2014), perhaps for a lacking expression of PGC1 alpha in the muscle (Liu*et al*., 2013). So, part of the diabetic subjects used a variety of medications. This variation also found on other forms of diabetes, like the type 1 diabetes mellitus (T1DM) (Espes and Arlsson, 2015), and gestational diabetes mellitus (GDM) (Eber*et al*., 2014). In addition, increases levels of irisin are also linked with other metabolic parameters such as body mass index (BMI), 2 h plasma glucose after OGTT "(oral glucose tolerance test)", HbA1c and triglycerides (Choi*et al*., 2013). Numerous studies have addressed the relationship between low of serum irisin levels and insulin resistance or diabetes. A lot of studies showed lower circulating irisin levels in type 2 diabetic patients (Zhang*et al*., 2016), and others explained a negative correlation with fasting glucose in blood and HbA1c (Yan *et al*., 2014).

This study aimed to measurement of irisin level in patients with newly onset- T2DM and to examine the association between irisin level and glycemic indices (BMI, fasting blood glucose, fasting insulin, C-peptide and lipid profile and some Oxidative Stress - antioxidant parameter).

2.MATERIAL AND METHODS

2.1 .Subjects

The aimed population of this study was 60 male persons who are already diagnosed as new onset of T2 DM., which referred to the Nasiriyah Endocrine and Diabetes Centre in Thi-Qar province, Iraq during February - August 2018. The patients are diagnosed as newly onset by the consultant medical staff, according to checked clinical examination and biochemical analysis. Another group of apparently healthy

individuals represented as the control group. The data was obtained from each patient including ages, BMI, medications, other disease, any other chronic disease and medical history. The patients were 60 males and the control grope involved 40 males individuals divided in to tow parts GI 35- 45, GII 46-55 years old matched with age in type2 group .

2.1.1. Blood collection

About (5 mL) of fasting venous sample of T2DM patients and controls divided to two parts the first part was (2ml) putting in tube with anti-conglutination (EDTA tube) this used to determination of HbA1C test, and the second part was (3ml) to obtain of serum.

2.2. Evaluation of Body Mass Index (BMI)

"Body mass index (BMI)" is a determine of someone´s weight in linked to their height, and then we put these measurements in the equation:

BMI= Weight (kg) ⁄ Height $(m)^2$ (Nuttall, 2015).

2.3. Biochemical parameters analysis

2.3.1. Hormones

The irisin, insulin and C-peptide hormone, concentration was calculated match up with to the ELIZA., based on the sandwich principle (Miyazawa*et al*.,1999).

2.3.2. Biochemical parameters

2.3.2.1. Evaluation of fasting blood sugar (F.B.S)

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase, with phenol and 4aminophenazone to form a red-violet quinonimine dye as indicator (Trinder, 1969).

2.3.2.2. HbA1C test

System reagents for the quantitative determination of HbA1c (Hemoglobin A1c), in human blood, on Beckman Coulter AU analyzers (Jeppsson*et al*., 2002).

2.3.2.3. Serum cholesterol and Triglyceride

Enzymatic method described by Allain *et al.*, (1974).

Cholesterol esters →cholesterol + free fatty acids

Cholesterol+ O_2→cholestero 4 one 3 +H_2O_2

$2H_2O_2$+phenol+PAP→Quinoneimine(pink)+$2H_2O$.

Evaluation of" High density lipoprotein" (HDL) 2.3.2.4.

The chemical substances only for healing of specimen previous to calculate of HDL.,- C add to reagent for sum cholesterol. "Low density lipoproteins (LDL) very low density (VLDL) " and "chylomicrons" from specimen are precipitate by "phosphotungstic acid (PTA) and magnesium chloride". HDL.,C obtained floating following of centrifuged, so then calculated add to sum cholesterol (Badimon *et al*., 1990).

2.3.2.5.Evaluation of" Low density lipid protein" (L D L)

By the following function (Peter and Kwiterovich, 2004).

$$L\ D\ L = Cholesterol\ con. - (Tg\backslash 5) - H\ D\ L\ con. = (mmol/L)$$

2.3.4.Determination. of oxidant-antioxidant. stress

2.3.4.1. Determination. of serum. malondialdehyde:

Lipid peroxidation items were one of the key pointers of oxidative pressure. Lipid peroxidation was dictated by utilizing the thiobarbituric corrosive technique (Fong *et al*., 1973).

2.3.4.2. Determination. of ceruloplasmin concentrations

Ceruloplasmin focus in serum was estimated by Menden *et al*., 1977.

2.5. Statistical Analysis

All statistical analysis was performed by using the Statistical Package Social Sciences version 20 software (SPSS v.20) for Windows, due to sample T-test.

3. RESULTS

3.1. BMI

The present study showed a significant increase (P ≤ 0.05) of BMI level in patient with DM2 compared with the control group (25.83 ±2.13 *vs* 21.82 ±1.65) **(**table 2.1).

3.2. Biochemical parameters

The present study showed a significant increase (P ≤ 0.05) of glycohemoglobin (HbA1C) and FBS level in patient with DM2 compared with the control group (6.88 ± 1.02 *vs* 4.15±0.56; 7.85 ± 1.89 *vs* 4.94 ± 0.40) respectively (table 2.1). Also, the results explained a significant increased (P ≤ 0.05) of T-Ch, Tg and LDL level in DM2 group compared with the control group (4.27±0.90 *vs* 4.23± 0.40; 2.14 ± 0.86 *vs* 1.89 ± 0.26; 2.84 ± 0.94 *vs* 2.23 ± 0.52) respectively. Whereas, the results showed a significant decrease (P ≤ 0.05) of irisin, insulin, C-peptide and HLD in DM2 group compared with the control group (22.32 ± 4.55 *vs* 27.81 ± 2.93; 18.29 ± 3.66 *vs* 27.27 ± 6.90; 3.12 ± 0.99 *vs* 6.03 ± 0.48; 1.0 ± 0.17 *vs* 1.61±0.37) respectively (table 2.2).

3.3.Oxidative Stress - antioxidant parameter

4.3.1 Lipid Peroxidation Status (Malondialdehyde)

The present study showed a significant. increase in the concentration of serum MDA in patients (p≤.0.05)) compared. with group of control (Table 2.1).

4.3.2. Ceruloplasmin Concentrations

The present study showing a significant elevation in Cp levels in patients compared with control group (P≤.0.05).

3.4. Correlation analysis

In correlation analysis the results showed a negative association between irisin and (HbA1c) (r= -0.152), hyperglycemia (r= -0.331), insulin (r= -0.156), HDL(r= -0.114) and BMI (r= -0.219). Whereas, the results showed positive correlation between irisin and IL-6 (r=0. 0.115), CRP (r= 0.153), C-peptide (r=0.013), T-Ch (r= 0.057), Tg (r=0.209) and LDL(r=0.035) (Table 2.3).

Table (2.1): Level of hormonal, physiological and oxidative stress - antioxidant parameter parameters in type 2 DM.

Parameters	GI N= 60	GII N=60	Control I N=20	Control II N=20	LSD
BMI	22.67 ±1.458	23.41±0.62	21.48± 1.29	22.11± 1.456	0.51
FBG (mmol/L)	7.87± 1.93	12.97± 0.90	5.00± 036	4.88±0.44	0.53
HbA1C (mmol/L)	6.92±1.02	10.72± 1.11	4.143± 0.52	4.149± 0.60	0.39
Irisin ng/dl	22.09± 3.69	20.87±2.48	26.23± 1.72	28.10± 2.57	1.21

Insulin ng/dl	18.05± 3.77	16.63 ±2.11	26.91± 8.65	28.19± 5.81	2.08
C-peptide	3.22±1.15	2.59±0.93	6.15± 0.45	5.94± 0.49	0.38
MDA	2.74±0.45	3.30±0.40	1.84±0.35	1.70±0.30	0.17
Ceruloplasmin	7.66± 1.57	6.01 ±0.82	2.78± 0.89	2.50± 0.52	0.47

Table (2.2): Level of lipid profile parameters in Type 2 DM.

Parameters	**GI N= 60**	**GII N=60**	**Control I N=20**	**Control II N=20**	**LSD**
TG (mmmol/L)	1.40 ± 0.20	1.54 ± 0.21	1.42 ± 0.29	1.61± 0.38	0.11
T-Ch (mmol/L)	3.72 ± 0.486	3.73 ± 0.479	3.95 ± 0.474	3.97± 0.471	0.20
HDL-Ch(mmol/L)	1.06 ± 0.16	1.04 ± 0.18	1.51 ± 0.38	1.54 ± 0.36	0.10
LDL (mmol/L)	2.37 ± 0.58	2.38 ± 0.60	2.15 ± 0.43	2.11 ± 0.45[b]	0.23

Table(2.3): Correlation analysis between irisin and other parameters.

		Irisin	**C_peptide**	**Insulin**	**MDA**	**CP**	**FBS**	**HbA1c**	**Chol**	**TGs**	**HDL**	**LDL**	**BMI**
Irisin	**Correlation**	**1**	**0.013**	**-0.156**	**0.063**	**0.428**	**-0.331****	**-0.152**	**0.057**	**0.209**	**-0.114**	**0.035**	**- 0.219**
	Sig.		**0.922**	**0.234**	**0.428**	**0.634**	**0.010**	**0.246**	**0.667**	**0.108**	**0.384**	**0.789**	**0.093**
	N	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**

(**= significant).

DISCUSSION

Biochemical parameters

Glycohemoglobin (HbA1c)

The results explain an important elevated in levels of HbA1c in DM2 compared with the control group, that is might be most exactly reflects the preceding 2-3 months of glycemic control, thus the patient with 2-3 months period of DM., and bad organize to the disease so this situation lead to highly level of HbA1c in blood (Harris, 1998). The high level of HbA1C in this study was coordinated with other study by Kamran (2010) who reported the bad control to the long period as 2-3 months to the DM disease lead to higher HbA1c levels and diabetic difficulty (Kamran, 2010). This study showed a negative association among irisin with hemoglobin A1C (HbA1c). Thus, level of irisin might reveal the metabolic condition of patients suffer as of metabolic disorders. In adding to glycemic or HbA1c, "irisinemia" can also grow to be a new gifted idea to observe disorders of metabolism like obesity or T 2DM in future might be appear for a useful means in organization of metabolic diseases (Sanchis *et al.*, 2012).

A negative association has been shown in this study to the irisin values with insulin and HOMA-IR, this might be of all individuals in this study were health with BMI., (At the time indicated by the results of BMI in this study, which observing that it is within the standard range due to the World Health Organization. Association among irisin with insulin resistances confirming by the hypothesized participation of the "p-38-PGC1a- betatrophin pathway of irisin " (Sanchis-Gomar and Perez-Quilis 2014).

Blood glucose

The results showed a significant raise of blood sugar in DM2 compared with the control group. The confusion of beta cells in pancreas organ lead to reduce production of insulin hormone, if beta cells don't make sufficient insulin, glucose accumulation in the blood in its place when absorbing by cells of the body, pre-diabetes or diabetes might be take place in this condition. The cells of body are hungry of energy in spite of high blood glucose levels in diabetes condition (Forouhi and Wareham, 2014).

Irisin

Decreased of irisin level were observed in the DM2 compared with the control group, this might be because of the information that irisin was progressively reduced with decrease tolerance of glucose in quantity to insulin resistance or due to a high of fat at the expenditure of muscle mass for require of activity in patients with type2 DM, this explanation matched with the study which done by (Yan *et al*., 2014; Assyov *et al*., 2016).

So the irisin and myonectin, ruling by insulin resistance. Irisin and myonectin, are possible involved, in lipid and glucose metabolism, and thus possibly will be stop the development, of insulin resistance. on the other hand, their secretion could also be influence by the enlargement of muscle insulin resistance.

Since irisin and myonectin showing to act in the adipose tissue, their deregulation might have an effect on the crosstalk between the tissues and further has a say to insulin resistance and impair glucose and lipid metabolism. Numerous studies found lesser circulating irisin levels in type 2 diabetic patients (Moreno-Navarrete
et al.,2013; Zhang *et al.*, 2014; Zhang *et al.*, 2016).

Lipid profile

The results showed a significant increase in (triglyceride and Low density lipoprotein) of new onset patients. Typically, the dyslipidemia is reflected largely in enlarged serum levels of triglycerides and low levels of HDL, cholesterol levels may be very high in proteinuria patients (Schofield *et al*., 2016). These results are corresponding with the result of Vaziri, (2003).

The model of dyslipidemia, in diabetes is different, from that in non-diabetic people. This explain the significance of lipid and lipoprotein

examination in diabetic patients and recommend a different lipid lowering agents from that used in non-diabetic population (Rustemeijer *et al*., 1997). Accordingly, this study showed a negative association between irisin and cholesterol, might be that irisin possibly will inhibit the production of hepatic cholesterol through "AMPK-dependent inhibition of sterol regulatory element-binding proteins (SREBP2) and downstream of its genes target. Obstruction of irisin-induced adenosine monophosphate-activated protein kinase (AMPK) activation by complex C., or knockdown of "AMPKα1" (Xiong et al., 2015).

C-Peptide

The necessary role of C-peptide is a helpful and broadly use method of assess pancreatic beta cell purpose (Jones and Hattersley, 2013; Leighton *et al*., 2017), not as good as C-peptide levels have been linked with lesser glycemic organize and for this reason elevated HbA1c values (Lachin *et al*., 2014; Kuhtreiber *et al*., 2015). Decreases value of C-peptide and decrease beta cell function has been related to bigger levels of glucose change capability (Kramer *et al*., 2014; Hope *et al*., 2016).

Insulin

In study by Fukushima *et al*., 2016 in obese patients create the positive correlation between irisin and insulin resistance (Fukushima *et al*., 2016), Though others reported either no association (Liu *et al*., 2013; Choi *et al*., 2013) or even a negative relationship
(Yan *et al*.,2014) among serum irisin with homeostatic model assessment of insulin resistance (HOMA-IR) score. Level of irisin was negative associated with BMI and insulin in our study individuals, this could be showed by the fact that all participants in our study were metabolically in good physical shape with BMI (At the time indicated by the results of BMI in our study, which showed that it is within the normal range according to the World Health Organization), table 2.

Oxidant- antioxidant system

Oxidant system

MDA

From our results we observed elevated MDA level in DM2 compared with the control grope, Because of oxidative stress results from an inequity between radical- formation and radical-scavenging systems, i.e. amplified free radical making or lowering activity of antioxidant defenses, or both. Hyperglycemia-activate oxidative stress has also been linked with enlarged endothelial cell apoptosis in vitro and in vivo (Bajaj and Khan, 2012). Several studies have shown that DM(types 1 and 2) is accompanied by elevate structuring of free radicals and looser antioxidant capacity, leading to oxidative injure of cell components (Bashan *et al*.,2009).

There are numerous sources of "reactive oxygen species (ROS)" manufacture in diabetes including those of mitochondrial and non-mitochondrial origins; ROS accelerate the four important molecular mechanisms involved in "hyperglycemia-induced oxidative tissue harm., these four pathways are activation of "protein kinase C (PKC)", "enlarged hexosamine pathway flux, increased advanced glycation end-product (AGE), and increased polyol pathway flux (Roloand Palmeira, 2006).

Ceruloplasmin

Ceruloplasmin was elevated in diabetic group than non-diabetic group. like result has found, in different studies manage by (Lee *et al*., 2015, Sharma *et al*., 2018).The possible clarification could be that highest level of ceruloplasmin in pre-diabetic group but does not fluctuate significantly in the diabetic range. Similarly, considering both groups ceruloplasmin linked with "age, fasting glucose, post prandial glucose, glycated hemoglobin, triglycerides and TG/HDL-C ratio. Thus, it implies, that serum triglycerides, and TG/HDL-C rang which are substitute, indicator of insulin resistance might, be reflect by ceruloplasmin. Ceruloplasmin could be used as substitute to mark, the insulin resistance (Sharma *et al*., 2018). Cells have evolved highly

complex enzymatic and non-enzymatic antioxidant systems, which work synergistically, and in combination with each other, to protect the body against free radical-induced damage (Bajaj and Khan, 2012). In patients with T2DM, the content of oxidized fatty acids is increased, and the anti-inflammatory and antioxidant activities of HDLs are impaired (Morgantini *et al*., 2011). In other study suggested of The increased, glucose for the long period of time without controlled is one of the motivation factors, for malignancy, the insulin resistance and the unbalanced lipid profile additionally increase the risks, thus, enlarged ceruloplasmin level in diabetic patients can be show the risk of malignancy (Ryu and Park, Scherer, 2014).

So, level of ceruloplasmin, which is considered as a very, important inflammatory marker, enlarged ceruloplasmin was clearly in type2 newly diagnosed patients, our result were in supporting with various authors who researched on acute phase proteins in type 2 diabetes (McMillan ,1989; De Feo *et al*.,1993). The significance of chronic low grade, inflammation with, activate innate immune system in the pathogenesis of type 2 diabetes seems possible out of doubt, ceruloplasmin is also an acute, phase reactant with a response, of intermediate magnitude and is known to have antioxidant action (Goldstein *et al*.,1979).

Insulin resistance might also be correlated with irisin secretion, because an increase in irisin promotes energy consumption, which contributes to weight loss, fat reduction and improved insulin resistance (3)

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