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Comparative Study of New Biomarkers in Iraqi DM2 with and without Complications

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Abstract

Background: Recent research indicates that persistent inflammatory responses may contribute to the rise of diabetic nephropathy (DN) and diabetic cardiovascular disease (DCVD) in type 2 diabetes mellitus patients (DM2). Numerous molecules associated with inflammation and angiogenesis have been implicated in the development and progression of DN and DCVD, respectively.
Methods: The subjects were separated into five groups: healthy controls (n= 25), type 2 diabetes mellitus patients (n= 30), type 2 diabetes mellitus patients with nephropathy DN (n= 30), and type 2 diabetes mellitus patients with cardiovascular disease DCVD (n= 30). The blood levels of irisin, IL-8, HbA1C, urea, and creatinine were determined.
Results: In current study there was high significant increased irisin levels ($p< 0.001$) in DN patients than other groups and a high significant decreased IL-8 level in DCVD.
Conclusions: Serum IL-8 and irisin levels may serve as early indicators of DM2 problems (DN, DCVD).

Keywords: DM2, DN, DCVD, HbA1C, IL-8, Irisin.

Introduction

Diabetes type 2 (DM2) is a common disease and is a complex and heterogenous group of chronic metabolic diseases that are characterized by hyperglycemia. Type 2 diabetes is a gradual metabolic disorder marked by insulin resistance and eventually pancreatic cell dysfunction.

Diabetic nephropathy (DN) is a chronic microvascular consequence of DM2 that affects approximately 20% to 30% of patients. It is often regarded as the most common cause of end-stage renal failure necessitating renal replacement. Numerous studies have suggested that cardiovascular problems are already common in DM2 patients. It has been known so many years ago the role of hyperglycemia in the development of cardiovascular problems.

Irisin is a novel myokine that was discovered to be released into the bloodstream by skeletal muscles during exercise. Inteleukin-8 (IL-8) is a chemokine that draws neutrophils to areas of inflammation and is involved in the host's defense against bacterial infections. It is generated by fetal thymic epithelial cells and IL-1 stimulates its *in vitro* synthesis. Glycated hemoglobin (HbA1C) was initially identified as an abnormal hemoglobin in people with DM2 over the age of 40. The blood test for HbA1C determines the average plasma glucose level during the preceding eight to twelve weeks and is used to assess glycemic control in people who have been diagnosed with diabetes. The WHO recommends a HbA1C level of 6.5 percent as the cut-off point for diabetes diagnosis (1). Blood urea, a breakdown product of a significant nitrogenous end product of protein and amino acid catabolism and creatinine, a byproduct of creatine phosphate hydrolysis in

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muscle which is eliminated by kidneys. Blood urea nitrogen is an indirect and imprecise indicator of renal function since it quantifies the amount of urea nitrogen in the blood. Urea and creatinine levels are strong indications of a healthy kidney, while serum levels indicate renal impairment.

In this study, these factors were assessed in DM2 male patients and their correlation with DN and DCVD were investigated.

Materials and Methods

The samples of blood were collected from 115 Iraqi male with age ranged between (32-55) years were enrolled in this study in Al-Kadhimiya hospital in Baghdad city from September 2021 to January 2022 which are divided into four groups as following:

G1: 25 healthy controls,

G2: 30 diabetic type 2 patients,

G3: 30 diabetic type 2 with nephropathy (DN) patients.

G4: 30 diabetic type 2 with cardiovascular disease (DCVD) patients.

Irisin, IL-8 and HbA1C were estimated by ELISA kits. Urea and creatinine levels were determined by colorimetric methods.

Statistical analysis

The t data present study was expressed as mean±SD in addition, The t-test was performed to compare the patient and control groups, also, p value≤ 0.05, 0.001 were considered significant.

Results

Irisin, IL-8, HbA1C, urea and creatinine in four study groups: control group (G1), DM2 (G2), DN (G3) and DCVD (G4) in male population. The data showed high significant decreased in patients of DN (G3) compared with other groups and high significant decreased in DCVD (G4) compared with G1 and G2 and there was a high significant decreased in G2 than G1 (Table 1).

The correlations of irisin and Il-8 with DN and DCVM in DM2 patients were found (Tables 2 and 3).

Table 1. Comparative study of some biochemical parameters in Iraqi Type 2 diabetes mellitus (DM2) with and without complication and healthy controls.

Parameters	Groups				p value					
	G1 no.25 mean±SD	G2 no.30 mean±SD	G3 no.30 mean±SD	G4 no.30 mean±SD	G1&G2	G1&G3	G1&G4	G2&G3	G2&G4	G3&G4
IRISIN (ng/Ml)	151±5.9	39.1±6.15	19.67±3.3	23.44±2.35	HS	HS	HS	HS	HS	HS
IL-8 (ng/Ml)	4.79±0,38	6.54±0.25	7.89±0.3	8.7±0.23	HS	HS	HS	HS	HS	HS
HbA1C (mmoL/moL)	4.33±0.73	6.26±0.2	8.55±0.3	8.23±0.45	HS	HS	HS	HS	HS	NS
Urea (mg/Ml)	21.6±1.43	38.69±3.2	47±3.22	27±2	HS	HS	HS	HS	HS	HS
Creatinine (mg/Ml)	5±0.274	0.8±0.01	0.99±0.01	0.76±0.03	HS	HS	HS	HS	HS	HS

HS: high significant **p** value≤ 0.001.

NS: non-significant **p** value≥ 0.05.

Table 2. The correlations of Irisin level in Type 2 diabetes mellitus (DM2) with diabetic nephropathy (DN) and diabetic cardiovascular disease (DCVD).

Parameters		DM	DN
DN	R	- 0.007	
	P	0.986	
DCVD	R	0.664	- 0.001
	P	0.05*	0.997

***Significant.**

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Table 3. The correlations in IL-8 level in diabetic nephropathy (DN) and diabetic cardiovascular disease (DCVD).

Parameters		DM2	DN
DN	R	0.387	
	P	0.270	
DCVD	R	0.535	0.133
	P	0.05*	0.715

***Significant.**

Discussion

Irisin is a new myokine and exercise hormone that has been demonstrated to play a role in energy balance and insulin sensitivity control. Due to its relationship with insulin resistance, irisin has been a subject of study in diabetes mellitus. This study indicated decreased serum irisin concentrations in patients with chronic renal disease with or without DM2 and low irisin levels in patients with diabetes nephropathy (DN) (2, 3). Genetic factors are risk factors for DN, in general, poor glycemic management, hypertension, advanced age, hyperfiltration, male gender, and pathological alterations in renal tissue contributed to the development of diffuse glomerulosclerosis. Although irisin levels were significantly lower in all groups (G2, G3, and G4) than in healthy control participants, it is unclear if irisin levels differ between diabetic nephropathic patients with and without diabetic cardiovascular disease. Thus, the DN produced by protein urea is a critical state that can result in a variety of problems and decrease in irisin concentration (4). Because irisin is a protein hormone produced by skeletal muscles in response to the activation of peroxisome proliferator-activated receptor c during exercise (5). Furthermore, multiple investigations have established that skeletal muscle has the ability to release this hormone, which regulates metabolism (6) and exercise is a preventive factor against a variety of diseases, most notably metabolic and cardiovascular disorders. Additionally, irisin levels increased as HbA1C levels dropped, suggesting that the lower levels of irisin in DN, DCVD, and DM2 may be attributed to a rise in HbA1C (7). Thus, the levels of irisin have been recommended as a biomarker for a variety of diseases, including metabolic disorders (8).

In this study, there was a substantial increase in IL-8 levels in the DCVD, DN, and DM2 patient groups, respectively. Interleukin 8, which is expressed in vascular endothelial cells, fibroblasts, monocytes, and epithelial cells, can produce a variety of triggered reactions, including white blood cell movement, neutrophil peroxide generation, activation of lysosome release, and chemotaxis. As a result, it is hypothesized that IL-8 has a role in the development and progression of diabetic nephropathy, cardiovascular disease, and infection.

IL-8 stimulates neutrophils (9), resulting in a considerable increase in the proliferation of human renal mesangial cells. Additionally, it has been demonstrated that IL-8 induces oxidative stress, changes in vascular permeability, enhanced endothelial coagulation ability, and decreased diastolic function, resulting in aberrant blood flow regulation and leading to the development of DN and DCVD (10). The fact that IL-8 expression was lower in DN than in DCVD may be attributable to the innate and acquired immune responses sharing cytokine activities concurrently (11). So the increasing levels of IL-8 in DM2 patients with and without complication may related to proinflammatory profiles (12). We showed high significant levels of HbA1C in G4 and G3 than other groups (G1&G2). HbA1C is recommended as a standards of care for testing and monitoring diabetes (12). Hyperglycemia is intimately linked to the consequences of diabetes, which include retinopathy,

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nephropathy and cardiovascular disease. HbA1C variability was found to be independently linked with the development of vascular consequences of HbA1C and nephropathy in the current meta-analysis. This is because HbA1C variability reflects long-term changes in blood glucose, which coincides with variations in HbA1C levels between visits (13). Also, here was showed high significant increased levels of urea in DM2 and DM2 complications.

Urea is a result of the breakdown of proteins (14) whereas creatinine is a byproduct of creatine that is predominantly found in skeletal muscles. Because the compounds are not eliminated regularly in renal illness, they accumulate in the body, resulting in an increase in urea levels in the blood (14). Additionally, that data indicated that creatinine levels were not significantly elevated in DM2 problems. Reduced serum creatinine levels are associated with an increased risk of DM2, as skeletal muscle volume is decreased. Skeletal muscle is a significant insulin target tissue, and a reduction in skeletal muscle results in fewer insulin target sites, which results in insulin resistance and DM2 development (15). The results of this study showed a significant positive correlation in irisin with DM2 and irisin in DCVD and non-significant negative correlation in irisin for DM2 with irisin in DN and non-significant negative correlation in irisin with DN with irisin in DCVD. In addition, there was a significant positive correlation in IL-8 in DM2 with IL-8 in DCVD and there was non-significant negative correlation between IL-8 in DN and IL-8 in DCVD and IL-8 in DM2 with IL-8 in DN.

In conclusion, DM2 is a highly complex and multifaceted inflammatory disease. Serum irisin may be used as a biomarker for the etiology of DM2, nephropathy and cardiovascular disease, and increasing levels of IL-8 contribute significantly to the progression of complications such as diabetes mellitus or interstitial inflammation in DN and DCVD.

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