Myostatin and Myonectin Levels as a Potential Marker for Diabetes Mellitus with and without Cardiovascular Disease

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ABSTRACT

A prevalent metabolic condition known as type 2 diabetes mellitus puts people at risk for developing diabetic cardiomyopathy and atherosclerotic cardiovascular disease. This study's objective is to measure myokines levels (myostatin and myonectin) in relationship with other clinical varietals in Iraqi diabetic patients with and without CVD to assess their possible role as a potential marker for Diabetes mellitus with and without cardiovascular disease. The study included ninety participants divided into three groups as follows; 30 (G1) T2DM with CVD, 30 (G2) T2DM without CVD and control group 30 (G3) apparently healthy participants. There was a highly significant differences in the levels of myostatin and myonectin among studied groups. there was a positive correlation between myostatin and myonectin levels and BMI, SBP, HbA1C and VLDL and highly positive correlation with FBS, HOMA-IR, TC, TG and LDL-C in T2DM with CVD while, a negative correlation with HDL-C in T2DM with CVD. Conclusion: Changes in myokinase (myostatin and myonectin) levels have an influential relationship with the emergence of complications of diabetes, which is cardiovascular disease. Therefore, high levels of myokines in diabetic patients with and without cardiovascular disease, compared to healthy people, are considered an indicator of their numbness and can be considered markers for diagnosing diabetic patients with cardiovascular disease.

Keyword: Diabetes mellitus, CVD, Insulin resistance, Myostatin, Myonectin.

INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) and diabetic cardiomyopathy are common metabolic disorders that are leading to diabetes mellitus, one of the most common diseases in the world, affecting 8% of the population [1]. Type 2 diabetes also arises from insulin resistance, primarily in the liver, adipose tissue, and skeletal muscle, but diabetes mellitus is primarily defined by chronic hyperglycemia brought on by improper pancreatic function [2]. Excess body fat usually causes insulin resistance, which in turn causes hyperinsulinemia and a compensatory rise in pancreatic N cell production. But hyperglycemia occurs when t-cells are unable to counteract insulin resistance [3].

The search for proteins belonging to the transforming growth factor superfamily was focused on myostatin. In vitro myoblast development into myotubes is negatively regulated by myostatin, and obese women's myotubes secrete more myostatin than their thin counterparts [4]. Myostatin deletion animals have reduced inflammation and obesity-induced IR, according to studies conducted in mouse models. Moreover, myostatin-opposing antibodies shielded mice from age-related sarcopenia. Nonetheless, there appears to be conflicting data on myostatin's impact on insulin action and IR: in human patients, plasma myostatin levels fall as the number of metabolic syndrome criteria rises. It is mainly unknown how myostatin and insulin activity interact in humans [5]. As a novel myokine, myonectin serves as a link between the skeletal muscles and other tissues involved in metabolism, like the adipose tissues and liver [6]. It has been suggested that CTRP15 positively affects lipid metabolism and insulin sensitivity by increasing the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and compelling adipocytes and hepatocytes to absorb free plasma fatty acids, respectively [7]. In addition, CTRP15 reduces autophagy and has anti-inflammatory effects by suppressing inflammation cytokines like MCP-1, IL-6, and TNF-α. Furthermore, in individuals with metabolic syndrome and coronary artery disease, CTRP15 has been connected to insulin resistance.It has been demonstrated that myonectin increases women's plasma following aerobic exercise training and enhances the uptake of fatty acids (FA) by cultured hepatocytes [8]. Notably, myonectin was also the term given to another protein (CTRP5) in the early stages of study. However, CTRP15 was given the moniker myonectin because it is more selectively released by muscle tissue [9,10]. The effect of myonectin on human whole-body insulin action is mostly unknown. This study evaluated the levels of myostatin and myonectin in Iraqi diabetes mellitus patients with and without cardiovascular disease (CVD) in relation to other clinical factors. The goal of this research is to ascertain the myokine levels. (myostatin and myonectin) and their correlation with other clinical variables in Iraqi diabetic patients, both with and without cardiovascular disease.

MATERIAL AND METHODS

There are 90 participants in the study. Based on the lineage of the original mutant cell, three groups were established for this investigation: 30 (G1) T2DM with CVD, 30 (G2) T2DM without CVD, with ages ranging from 42 to 67 years, and 30 (G3) seemingly healthy with matched sex and age were included in the study. The body mass index of each individual was determined by dividing their height (m2) by their weight (kg). BMI is computed as follows: BMI = mass (kg) / height (m)2. Using an auto analyzer Cobas, the serum fasting glucose, HbA1c, and lipid profile (TC, TG, HDL-C, and LDLC) were

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estimated. The DRG insulin ELISA kit was used to measure serum insulin concentrations. The formula ((fasting glucose (mmol/l)] × [fasting insulin (μU/ml)) represents the homeostasis model of IR.5. The ELISA kit was used to measure the amounts of myonectin and myostatin in serum.

Statistical Analysis

A Microsoft Office Excel 2010 work sheet was used for all statistical analysis and data registration. The (means ± SD) of the data were reported. differences that the t-test at P<0.05 deems to be significant.

Table 1. Demographic measures and biochemical values.

Parameters	T2DM with CVD Mean ± SD (G1) N=30	T2DM without CVD Mean ± SD (G2) N=30	Control Mean ± SD (G3) N=30	(G1) Vs (G2)	(G1) Vs (G3)	(G2) Vs (G3)
Sex (M/F)	(12 /18)	(17 /13)	(19 /11)	/	/	/
Age (Years)	40.23±3.21	38.25±4.44	32.21± 6.23	NS	NS	NS
Weight (kg)	95.56±10.23	84.77±8.36	75.85±8.95	0.05	0.05	0.05
High (cm)	178.22±8.10	172.31±5.25	173.21±7.85	NS	NS	NS
BMI (Kg/m2)	29.62±3.21	25.10±2.33	23.25±3.15	0.05	0.05	0.05
SBP (mmHg)	170.0±5.00	145.0±5.00	12.00±5.00	0.05	0.05	0.05
DBP (mmHg)	145.0±10.00	135±5.00	12.00±5.0	0.05	0.05	0.05
S.FBG (mg/dl)	178.66±14.10	105.12±6,54	88.11±5.63	0.01	0.01	0.05
HbA1c (%)	9.23±2.13	7.01±1.25	4.81±1.19	0.05	0.05	0.05
Insulin (ng/dl)	16.21±2.72	10.20±1.32	9.13±2.10	0.05	0.05	NS
HOMA-IR%	5.22±2.10	3.00±0.82	2.10±0.93	0.05	0.05	NS
TC (mg/dl)	250.50±12.32	205.14±10.25	185.77±8.66	0.05	0.05	0.05
TG (mg/dl)	188.13±11.02	158.22±8.23	90.30±15.12	0.05	0.01	0.05
LDL-C (mg/dl)	145.41±10.10	128.22±8.12	88.02±8.12	0.05	0.05	0.05
HDL-C (mg/dl)	38.12±4.23	42.25±3.21	48.51±3.23	0.05	0.05	0.05

Information is presented as mean ± SD, NS= non-significant,
* Significance: P-Value ≤ 0.05, ** High significance: P-Value ≤ 0.01

Table 2. Myostatin and myonectin level among groups.

Parameters	T2DM with CVD Mean ± SD (G1) N=30	T2DM without CVD Mean ± SD (G2) N=30	Control Mean ± SD (G3) N=30	(G1) Vs (G2)	(G1) Vs (G3)	(G2) Vs (G3)
Myostatin (ng/ml)	26.22±5.21	17.44±5.36	10.65 ±4.22	0.001	0.001	0.001
Myonectin (ng/dl)	11.74±2.11	8.13±1.05	5.81±1.26	0.01	0.01	0.01

Information is presented as mean ± SD, * Significance: P-Value ≤ 0.05,

** High significance: P-Value ≤ 0.01

As shown in table (3): there was a positive correlation between myostatin level and BMI, SBP,HbA1Cand VLDL in and highly positive correlation with FBS,HOMA-IR, TC, TG and LDL-C in T2DM with CVD while, a negative correlarton with HDL-C in T2DM with CVD. Also, there was a positive correlation between myostatin level and SBP, FBS, HbA1c, FBS TC,TG LDL-C in T2DM without CVD.

Table 3. Correlation coefficient of myostatin level with biochemical values

Parameters	T2DM with CVD (r value)	T2DM without CVD (r value)
BMI (Kg/m^2)	0.271*	0.162
SBP (mmHg)	0.243*	0.286*
DBP (mmHg)	0.037	0.08
S.FBG(mg/dl)	0.435**	0.471**
HbA1c (%)	0.320*	0.231*
HOMA-IR%	0.551**	0.452**
TC (mg/dl)	0.572**	0.352*
TG (mg/dl)	0.339**	0.313*
LDL (mg/dl)	0.515**	0.248*
HDL (mg/dl)	-0.367*	0.09
VLDL (mg/dl)	0.390*	0.313

*Correlation is significant at 0.05 level, ** Correlation is significant at 0.01 level.

As shown in table (4): there was a positive correlation between myonectin level and BMI, SBP, HbA1C TC, TG and LDL-C in and highly positive correlation with FBS and HOMA-IR, in T2DM with CVD while, a negative correlarton with HDL-C in T2DM with CVD. Also, there was a positive correlation between myonectin level and BMI, FBS and HOMA-IR in T2DM without CVD.

RESULTS

As shown in table (1) there was a significant increased appears of weight, BMI,SBS,DBS, FBS,HbA1c,Insulin level, HOMA-IR, TC TG and LDL-C among study groups(T2DM with CVD, T2DM without CVD ,and control groups). While, a significant decreased of HDL-C among study groups(T2DM with CVD, T2DM without CVD ,and control groups). And there was no significant appears of age and high among study groups(T2DM with CVD, T2DM without CVD ,and control groups).

In table (2): There was a highly significant study of myostatin and myonectin levels among study groups (T2DM with CVD, T2DM without CVD ,and control groups).

Table 4. Correlation coefficient of myonectin level with biochemical values

Parameters	T2DM with CVD	T2DM without CVD
	(r value)	(r value)
BMI (Kg/m^2)	0.271*	0.241*
SBP (mmHg)	0.223*	0.184
DBP (mmHg)	0.117	0.012
S.FBG (mg/dl)	0.635**	0.375*
HbA1c (%)	0.425*	-0.113
HOMA-IR%	0.320*	0.304*
TC (mg/dl)	0.502*	0.018
TG (mg/dl)	0.450	-0.045
LDL (mg/dl)	0.305**	0.022
HDL (mg/dl)	-0.215*	0.211
VLDL (mg/dl)	-0.024	-0.023

*Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

DISCUSSION

A common and dangerous microvascular consequence of diabetes that results in blindness and visual impairment is diabetes mellitus [11]. Apart from its detrimental effects on eyesight, it could also be linked to increased risks of systemic vascular problems, such as heart failure, stroke, and coronary heart disease, as well as death in those with type 2 diabetes mellitus. The main cause of the pathophysiology of diabetes mellitus is persistent hyperglycemia. Intensive glycemic management, however, cannot totally eradicate the risk of diabetes mellitus [12].

Therefore, the pathophysiology of such a disorder may entail other aspects. a correlation that is positive between the risk of diabetes and cardiovascular disease and serum myostatin levels This discovery suggests that myostatin may play more functions besides controlling the growth of muscles. Additionally, myostatin may have an impact on T2DM associated with CVD, according to earlier experimental research [13]. Patients with T2Dm and CVD had the greatest circulating serum myostatin levels, while participants without CVD had lower levels. Additionally, it was demonstrated that myostatin positively correlated with glucose level and HOMA-IR. A decline in glucose tolerance is correlated with elevated serum myostatin levels. Moreover, there is a positive correlation between circulating myostatin and conventional biochemical measures of poor metabolic health. In line with previous research, our data provide more proof that this myokine plays a role in the pathophysiology of type 2 diabetes [14, 15]. A portion of the connection between MSTN and diabetes mellitus has also been disclosed. The incidence and progression of diabetes or insulin resistance are strongly correlated with the concentration of MSTN [16]. According to Dial et al., type 1 diabetes patients' serum myostatin levels were considerably greater than those of the control group [17]. Similar results have been reported in type 2 diabetes, where it was discovered that T2D patients' muscles had higher levels of MSTN mRNA than the control group. Furthermore, there was a correlation found between MSTN mRNA and the amount of plasma IL-6 as well as the homeostasis model assessment of insulin resistance (HOMA2-IR). Similar findings have been noted: in diabetic rats' muscular and subcutaneous adipose tissue, mRNA expression of MSTN was up relative to the control group, and in their brown adipose tissue, mRNA expression of MSTN receptor was elevated. Furthermore, Hittel et al. discovered that insulin sensitivity and plasma MSTN levels were highly correlated, and that myostatin injection caused insulin resistance in mice [18]. The prevalence of metabolic syndrome, atherogenic dyslipidemia, and type 2 diabetes is rising globally, especially in southern Asia and the Middle East. In these high-risk patients, statins can lower low-density lipoprotein cholesterol and lower the risk of CVD; nevertheless, residual risk of CVD is linked to additional lipid abnormalities, such as low levels of high-density lipoprotein cholesterol and high levels of triglycerides.

Those with metabolic syndrome and type 2 diabetes are frequently reported to have these anomalies. There are more lipid-modifying treatments that focus on these anomalies [19,20]. Furthermore, in mice models of high-fat diet-induced obesity susceptibility, high-fat feeding may cause a large rise in body weight and MSTN expression in the muscle; but, in mice models of high fat diet induced obesity resistance, MSTN expression does not alter considerably in the muscle. These findings suggest that MSTN may be a major factor in obesity, and other research has shown that modifying MSTN expression can impact the onset of obesity [21]. Because myonectin and blood glucose, insulin, and HbA1c are positively correlated, it is plausible that either blood glucose or insulin regulates the amount of circulating myonectin in the blood. This makes it possible to evaluate how quickly rising glucose and insulin levels affect circulating myonectin in specific people. Circulating myonectin levels, however, did not alter in response to the oral glucose challenge-induced hyperglycemia and hyperinsulinemia. This finding suggested that myonectin release might not be impacted by an abrupt rise in blood glucose or insulin levels brought on by OGTT [22]. The recent findings showed that the diabetic individuals with and without CVD had significantly different mynoectin levels and insulin resistance. Skeletal muscle secretes a new myokine agent called myonectin, which modifies fat metabolism and lowers blood lipid levels [23]. Moreover, Myonectin promotes increased glucose uptake and the oxidation of fatty acids., and the phosphorylation of adenosine monophosphate kinase. According to this study, diabetic patients' circulation levels of myonectin were considerably raised by endurance training [24]. Myonectin's main biological function is to increase the uptake of free fatty acids by skeletal muscles and aid in the metabolism of fat and glucose levels in adipose tissue. However, it has several pleiotropic properties that protect against ischemia-reperfusion injury, promote endothelial function, and decrease the inflammatory response. These benefits are mediated by the S1P/cAMP/Akt signaling pathway [25]. Since lipids play crucial roles in the development of CVDs, it is imperative to create a new classification of lipids based on whether they enhance CVDs, have an influence on CVDs conditionally, or have no effect at all. The risk of CVDs and all-cause mortality was found to be strongly correlated with the variabilities of TC, HDL-C, and LDL-C in our meta-analysis. The pathophysiological processes behind these relationships are still unknown, despite an increasing amount of evidence from epidemiological studies; numerous reasonable interpretations have been put out to corroborate our findings [26]. Dyslipidemia may cause repetitive cholesterol crystallization and dissolution inside the restricted area of plaques, reducing plaque stability and ultimately raising the risk of CVD-related events. This instability of atherosclerotic plaque components may also raise the likelihood of plaque rupture.

CONCLUSION

Changes in myokinase (myostatin and myonectin) levels have an influential relationship with the emergence of complications of diabetes, which is cardiovascular disease. Therefore, high levels of myokines in diabetic patients with and without cardiovascular disease, compared to healthy people, are considered an indicator of their numbness and can be considered markers for diagnosing diabetic patients with cardiovascular disease.

RECOMMENDATION: It is recommended to enlarge the sample size with the addition of a fourth group which include patients with CVD only to elucidate the exact role of the studied markers in the diagnosis of diabetic patients with or without CVD.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published.

Limitations: We face difficulty in collecting samples from more patients and healthy volunteers since it is difficult to convince patients and explain the goal of the study to all subjects. We were also faced with difficulty in choosing healthy subjects with a BMI non-significantly differ from those of patients.

Potential Conflict of Interest: None

Competing Interest: None

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