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Estimation of insulin resistance and creatine kinase among Iraqi patients with type 2 diabetes mellitus

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Type 2 diabetes mellitus (T2DM) is a disorder in which the body's ability to control and profit glucose as fuel is impaired. Too much sugar flows into the circulation due to this chronic disease. Therefore, it can be defined as a group of metabolic disorders which can be characterized by different factors such as insulin resistance and followed by different complications. It has an influence on a variety of physiological bodily organs, making them less effective, including skeletal muscles. The aim of this study is to determine the activity of serum creatine kinase (CK) in Iraqi patients with T2DM. A total of 93 participants (68 T2DM divided into three groups: group I (19) patients with diabetic duration (<5 years), group II (19) patients with diabetic duration (≥5 to <10 years), and group III (30) patients with diabetic duration (≥10 years), as well as 25 healthy people as a control group gave their consent and participated in the study. All samples were collected from patients attending at Mustansiriya University, from National Diabetes Centre for Treatment and Research who were selected by the supervising specialist to be free of diabetic complications. Fasting serum glucose(FSG) was measured by the glucose oxidase method for patients and control group. Insulin was measured using ELISA kits. Also, Homeostatic model assessment of insulin resistance HOMA-IR (IR), Homeostatic model assessment of β (HOMA-β), and The activity of total CK were determined for the patient and control groups. In the present study, FSG, insulin, IR and HOMA-β were a significant increase (P≥0.0001) in T2DM patient group when compared with control group. During examination, the T2DM group II and III showed a significant increase in creatine kinase activity as compared with the control group, with p-values of (0.048, and 0.013) respectively. In T2DM patients, there was a positive correlation between CK activity and duration. In this study, There was also association between hyperglycemia and total CK activity.

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Introduction

Diabetes mellitus (DM) is one of the most important public health issues in the globe, bringing significant financial and socioeconomic implications to the public and private health systems. Although the number of diabetics has begun to fall in certain countries, diabetes rates have towering in most other developed and emerging nations in the past few decades [1-3].

Type 2 diabetes mellitus (T2DM) is a metabolic condition marked by hyperglycemia and attributed to both decreased insulin

action and insulin production in pancreatic beta cells. Homeostatic model assessment of insulin resistance HOMA-IR (IR) and insulin secretion may be caused by mitochondrial malfunction [4]. Risk factors for developing T2DM are, age over 45, metabolic syndrome, high fasting blood glucose, body mass index (BMI), and glycated haemoglobin (HbA1c>6%) [5-9]. T2DM affects about 1.4 million Iraqis, with prevalence ranging from 8.5% (IDF-age-adjusted) to 13.9 % [10,11].

When inherited and environmental variables like physical inactivity and obesity are concerned, insulin resistance develops before T2DM and leads to the failure of β-cell function and, as a result, a steady decrease in insulin production [12]. Insulin resistance is usually associated to a PI3K pathway that is suppressed, as well as IRS protein serine phosphorylation is elevated, but tyrosine phosphorylation is inhibited. In some circumstances, IRS protein degradation appears to occur [13]. In addition, despite lower muscle GLUT-4 levels, the insulin resistant in T2DM may result in inefficient GLUT-4 translocation. GLUT-4 is a transmembrane glucose transporter which allows skeletal muscle cells to absorb glucose [14]. Increased GLUT-4 content in the membrane could lead to enhanced insulin sensitivity and glucose tolerance [15].

The skeletal muscle is one of the cells that is effect by insulin deficiency or resistant, resulting in decreased glucose utilization and prompting muscle damage, while increasing the size of free unsaturated fat due to fatty acid metabolism increase peripheral tissue resistance to insulin. The CK, a cytosolic substance which aids mitochondria in phosphate exchanging through cytoplasm as a source of elevated vitality, can be produced as a result of muscular deformity and is considered a muscle harm biomarker [16]. Likewise, CK is a muscle damage biomarker which is released into the bloodstream when muscle cells are damaged. In diabetic patients, the size of all out CK enzyme will increase due to an increase in this enzyme, and the source of this expansion is skeletal muscle devastation caused by a decrease in vitality [17]. All types of muscular dystrophy raise serum CK activity, but neurogenic muscle disease like poliomyelitis does not [18]. In patients with T2DM, skeletal muscles have been found to be out of balance in terms of glucose and lipid metabolism regulation. It was assumed that in this type of diabetes, changes in enzyme activity were more pronounced than changes in total activity of the enzyme creatine kinase [19].

Materials and methods

The current study included (68) patients of T2DM, were divided in to three groups, group I (19) patients with diabetic duration (<5 years), group II (19) patients with diabetic duration (≥5 to <10 years), and group III (30) patients with diabetic duration (≥10 years) (according to standard American Diabetes Association criteria) and (25) healthy individuals as control group. All samples were collected from patients attending at Mustansiriya University, from two places as National Diabetes Centre for Treatment and Research as well as AL-Yarmouk Teaching Hospital, who were selected by the supervising specialist to be free of diabetic complications. The Exclusion Criteria Patients who have type 1 diabetic mellitus (T1DM) and other diabetic complication such as patients on drugs which cause muscle injury or increase CK (e.g., Statins and Amiodarone), chronic renal failure, Cushing's disease, acromegaly, chronic pancreatitis, pancreatectomy, and malignancies as well as history of smoking and alcohol drinking were excluded from the study. Seven to ten millilitres of venous blood samples were collected from patients and controls groups. The samples were taken at the time between 8.00 and 11.00 a.m after 12-15 hours of fasting. serum collection by polyethylene tube the blood sample in a plain tube were allowed to clot for ten minutes at (37 ℃) in a water bath, then were centrifuged at (3000 x g) for 10 minutes. The collected serum was divided into several parts each part content (500 µL) serum was stored frozen at (-20 ℃) until being

used to estimate the different parameters included in the study. Hemolysed samples were discarded. The Ethics Committee of the College of Women Science/University of Baghdad authorized this study protocol. The activity of CK and glucose levels was measured in all of the samples. Serum glucose was determined by enzymatic colorimetric method using kit manufactured by Linear Chemicals based on Trinder reaction [20,21]. CK activity was measured using the CK NAC activated test kit (Bio systems, Inc., kinetic UV test). Following the general procedure, the enzyme activity was calculated using the following formula:

Creatin kinase activity (U/L) = Δ_{Abs}/min x Factor.

Factor = TV × 1000/6.3 × SV × P

Where:

TV= The volume of the overall reaction measuring in mL,

SV= The volume of the sample in mL,

6.3= The absorption coefficient in millimolar of NADH at wavelength 340 nm

P =The optical path length in cm

CK U/L = Δ_{Abs}/min x 3333

A kit provided by Mono Bind was used to assess serum insulin hormone using an Enzyme Linked Immunosorbent Assay. The following are the results of the HOMA-IR which is the homeostatic model assessment for insulin resistance and HOMA-β, which is the homeostatic model assessment for beta cell function [22].

HOMA-IR= [glucose(mg\dl) × fasting Insulin (μIU /mL)]/ 405.

HOMA-β = [fasting insulin (μIU/mL) × 360/ (fasting glucose (mg/d) [63]

Because the assessment of HOMA-IR and HOMA- is invalid when computed using fasting glucose <81 mmol/L or insulin <500 IU/L, the homeostatic model evaluations were statistically analysed exclusively in the subset of subjects with a fasting glucose >81 (mg\dl) and a fasting insulin >500 (IU/mL).

Result and discussion

Among the control subjects 52% were males and 48% were female. Besides, the group I patients 68% were males and 32% were female and in (group II and group III) 73%, 83% were male and 27%, 17% were female respectively. The base line characteristics of study subjects are shown in (Table-1). The mean age of control was (45.96±10.71) years when compared to (49.47±9.63) years in group I patients, (52.26±8.37) years in group II patients and (57.40±8.33) years in group III patients. The mean duration of diabetes in groups I, II and III were (2.05±1.08), (6.53±1.68) and (14.60±4.28) years respectively. Statistically a significant increase (p =0.0001) was observed when compared between them.

TABLE 1 Demographic details of studied groups

Characteristics		Control	Patients I	Patients II	Patients III	p-value
Number		25	19	19	30	-
Age (year)	Mean±SD	45.96±10.71	49.47±9.63	52.26±8.37	57.40±8.33	0.0001*
	Min-Max	25-70	32-62	30-67	40-75	
Sex	M/N (%)	13 (52)	13 (68)	14 (73)	25 (83)	0.089
	FM/N (%)	12 (48)	6 (32)	5 (27)	5 (17)	
Duration	Mean±SD	-	2.05±1.08	6.53±1.68	14.60±4.28	0.0001*
	Min-Max	-	1.0-4.0	5.0-9.0	10.0-30.0	

According to biochemical parameter, FSG level was determined in all studied groups. The FSG distribution (mean±SD) of T2DM patients' groups (I, II, and III) and control group expressed in (mg/dL) as indicated in (Table 2). The results reveal that there is a significant increase (p <0.0001) in the level of glucose. Our results are in agreement with this study [23]. In comparison between the results of (group I and II) with (group III) were obtained a significant increase in this parameter, while no significant differences

were observed in comparison between the results of group I and group II patients.

In the group of patients with longer illness durations, glucose levels increased significantly. The findings of this study, which found a link between FSG and diabetes length, are consistent with the findings from a prior study, which illustrated that the risk of severe hyperglycemia increased with duration and age.

The insulin resistance increased (or decreased insulin sensitivity) and impaired insulin secretion play a main role in the pathogenesis of DM. Assessing insulin resistance/sensitivity and pancreatic β-cell activity to diagnose the type of diabetes and predict an effective treatment and protection plan for diabetes is highly fascinating. Multiple approaches and indices have been established so far to measure insulin resistance/sensitivity and β-cell function using variables models such as dynamic and static tests as well as calculations. A number of approaches, indices, and measurements were used to assess pancreatic β-cell function and insulin resistance/sensitivity. Moreover, Table 2 depicts the values of insulin, HOMA-IR and HOMA-β as a measurement of pancreatic β-cell function and insulin resistance/sensitivity with DM. It is obvious from the results in (Table-2), that there was a high significant increase in the level of HOMA-IR value in T2DM patient group III (p =0.0001) when compared with control group. On comparing patient groups with each other, a significant increase is further evident between (group I and II), and group III (p <0.01). There was no significant difference between the observed groups (I and II) patient with control group. Similarly, there was no statistically significant difference between the T2DM and control groups for values of the HOMβ (p >0.05).

High glucose levels caused insulin excretion in normal physiology. With regard to IR, the increased levels in T2DM patients were associated with elevated insulin levels. Our results are in agreement with these studies [25,26] that reported elevations in IR and insulin levels in DM patients than in the control group. This finding can be clarified on the ground that IR is likely to be the initial metabolic abnormality in T2DM. As a result, IR induced elevated serum glucose, which caused insulin hyper-excretion by the pancreas. The pancreatic beta-cells are impaired when hyperglycemia is chronic and persistent and they stop functioning [26].

After analysis, the T2DM groups revealed a substantial increase in CK activity when compared to the control group, As indicated in Table 3 and Figure 1, with p-values of (0.832, 0.048, and 0.013) respectively. There was an insignificant correlation in the activity of total CK p-value (0.440) between the group I (with duration <5 years) and group II (with duration ≥5 to <10 years), but the significant correlation increased between the group I (with duration<5 years) and group III (with duration ≥10 years, and P value = 0.033). No significant difference (p-value = 0.107) was observed between group II (with duration ≥5 to < 10 years) and group III (with duration ≥10 years). T2DM patients exhibit significant differences at the level of CK activity.

TABLE 2 Biochemical parameters of studied groups

Parameters	Groups	Mean±SD	Min-Max.	*p*-value		
				I	II	III
Insulin	Control	12.77±5.09	7.0-22.50	0.105	0.120	0.0001*
	Patients I	23.51±15.76	4.0-52.60	-	0.949	0.025*
	Patients II	23.06±15.66	7.0-63.66	0.949	-	0.021*
	Patients III	37.90±33.12	6.50-31.80	0.025*	0.021*	-
Insulin Resistant	Control	2.82±1.18	1.31-5.02	0.057	0.133	0.0001*
	Patients I	10.59±9.25	1.29-33.70	-	0.701	0.013*
	Patients II	8.94±5.85	3.80-26.0	0.701	-	0.004*
	Patients III	20.49±21.55	2.46-94.0	0.013*	0.004*	-

HOMβ	Control	163.86±132.60	25.65-76.0	0.015*	0.072	0.098
	Patients I	78.68±72.15	21.10-348.40	-	0.540	0.307
	Patients II	101.23±86.65	14.0-298.0	0.540	-	0.730
	Patients III	112.70±129.37	11.52-640.20	0.307	0.730	-
FSG(mg/dl)	Control	100.52±16.82	78.0-137.0	0.001*	0.001*	0.0001*
	Patients I	168.0±45.50	90.0-271.0	-	0.923	0.008*
	Patients II	166.04±35.65	100.0-243.0	0.923	-	0.006*
	Patients III	217.95±97.82	91.0-526.0	0.008*	0.006*	-

TABLE 3 Serum CK (Mean±SD) of studied groups

Groups	Mean±SD Activity in U/L	Min-Max.	*p*-value I	II	III
Control	49.92±10.12	34.50-66.60	0.832	0.048*	0.013*
Patients I	52.29±19.46	31.50-98.90	-	0.440	0.033*
Patients II	69.83±37.03	22.80-178.80	0.440	-	0.107
Patients III	78.18±62.82	21.40-295.40	0.033*	0.107	-

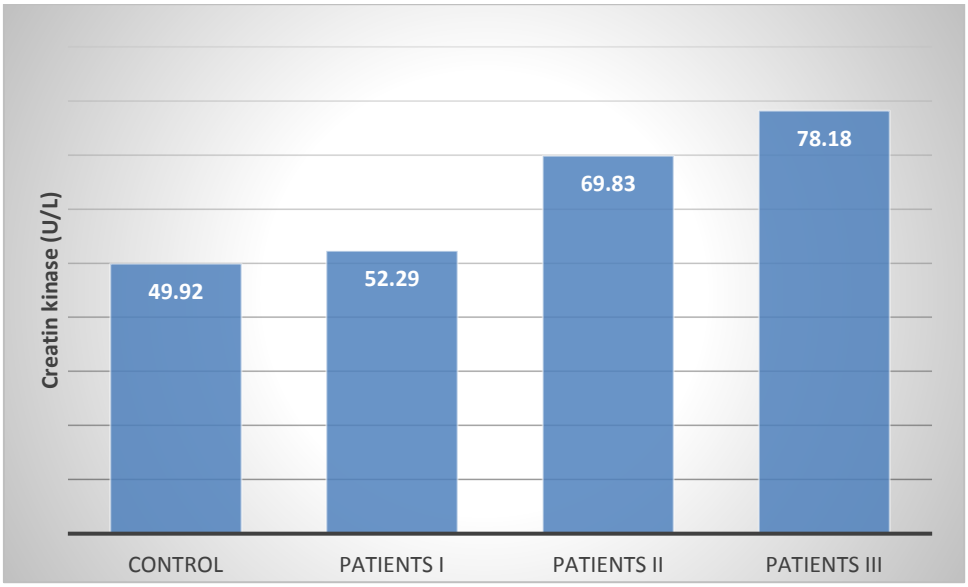

FIGURE 1 Mean values of serum CK activity for all the studied groups

Total CK activity was higher in T2DM groups than in control groups (p-values of 0.832, 0.048, and 0.013, respectively). This finding was consistent with the other research, which found that patients with T2DM had increased total CK activity. Total CK activity is increased owing to a variety of reasons, including Krebs cycle dysfunction, which is caused by an increase in proteolysis in diabetics [27]. The morphological appearances of skeletal muscle cells are affected by disorders in the Krebs cycle and respiratory chain, which reduce mitochondrial activity and ATP generation. T2DM patients' skeletal muscles have smaller mitochondria than normal, making energy production difficult. Low ATP can cause a drop in creatine phosphate, which can activate the (AMP activated protein kinase) as a late consequence. Diabetic individuals are at a higher risk of muscular atrophy and alteration [28].

Another result with previous study was observed that a mild link between CK elevation and the degree of glycemia, but the CK level became normal with carbohydrate metabolism improvement [29].

TABLE 4 Correlation between variables in patients group I

Variable	Insulin		IR		HOMAB	
	R	P	R	P	R	P
IR	0.904**	0.0001	-	-	0.377	0.112
HOMAB	0.964**	0.001	0.377	0.112	-	-
Age	-0.112	0.649	0.093	0.706	-0.429	0.067
Duration	-0.340	0.155	-0.278	0.249	-0.247	0.307
Glucose	0.478*	0.039	0.774**	0.0001	-0.107	0.664
CK	0.527*	0.020	0.495*	0.031	0.347	0.145

* The significant difference means the p-value (**P** ≤0.05) based on the LSD post-hoc test.

TABLE 5 Correlation between variables in Patients group II

Variable	Insulin		IR		HOMAB	
	R	P	R	P	R	P
IR	0.947**	0.0001	-	-	0.628**	0.004
HOMAB	0.838**	0.0001	0.628**	0.004	-	-
Age	0.198	0.417	0.183	0.453	0.100	0.683
Duration	0.311	0.196	0.328	0.170	0.177	0.469
Glucose	-0.385	0.104	-0.117	0.634	-0.730**	0.0001
CK	0.226	0.351	0.104	0.671	0.333	0.164

* The significant difference means the p- value (**P** ≤0.05) based on the LSD post-hoc test.

TABLE 6 Correlation between variables in Patients group III

Variable	Insulin		IR		HOMAB	
	R	*P*	R	P	R	*P*
IR	0.864**	0.0001	-	-	0.371*	0.044
HOMAB	0.747**	0.0001	0.371*	0.044	-	-
Age	0.040	0.833	-0.019	0.922	0.039	0.839
Duration	-0.112	0.555	0.005	0.978	-0.222	0.239
Glucose	0.009	0.963	0.413*	0.023	-0.359	0.051
CK	-0.176	0.352	-0.194	0.304	-0.127	0.504

* The significant difference means the p- value (**P** ≤0.05) based on LSD post-Hoc test.

The correlation among all the studied groups and parameters in serum was conducted as illustrated in Tables 4, 5, and 6. According to the results, in patients group I, the results revealed a substantial positive association between insulin and (IR, HOMAB, Glucose, CK) as well as a strong association between IR and (Glucose, CK). In patients group II, the results showed a significant positive correlation between insulin and (IR, HOMAB), while the results demonstrated a significant negative correlation between HOMAB and Glucose. Whereas in patients group III, a significant positive correlation between insulin and (IR, HOMAB) as well as between IR and (HOMAB, Glucose).

Conclusion

The activity of total CK among T2DM groups was elevated when compared with the control group. There was a strong association between hyperglycemia and total CK activity.

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