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Are higher serum levels of myoglobin and creatine kinase enzymes indicative for progressive cardiovascular disease among patients with type 2 diabetes?

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ABSTRACT

Objective: The purpose of this study was to evaluate serum myoglobin as a cardiac muscle marker for the detection of early cardiovascular disorder and to assess total Creatine Kinase(CK) and creatinekinase-MB (CK-MB) levels among patients with Type 2 Diabetes mellitus (T2DM). *Methodology*: In this study, 300 study subjects were divided into two groups; 200 subjects diagnosed with T2DM (ascertained as cases) and 100 healthy individuals (ascertained as control group). *Results*: Significant increases in the serum levels of myoglobin, glucose, HbA1c, total creatine kinase and creatine kinase isoenzyme MB (CK-MB) were observed in diabetic patients compared to levels in healthy individuals. Besides, a significant increase in myoglobin, total CK and CK-MB levels was observed among diabetic patients with ischemic heart disease or hypertension. Moreover a strong positive correlation was observed between the duration of diabetes and the serum levels of myoglobin, total CK and CK-MB (r = 0.80, P = 0.00; r = 0.68, P = 0.030; and r=0.78, P= 0.000, respectively). Additionally, the present study showed significant positive correlations between the body mass index (BMI) of diabetic patients and the serum levels of myoglobin, total CK and CK-MB (r=0.77, P=0.000; r=0.65, P=0.028; and r=0.76, P=0.000. respectively). However, this study showed insignificant weak positive correlations between HbA_{1C} and the serum levels of myoglobin, total CK and CK-MB (r=0.28, P=0.068; r=0.27, P=0.075; and r=0.37, P=0.066, respectively). *Conclusion:* The higher levels of myoglobin, CK and CK-MB may be indicative of progressive cardiovascular disease among diabetic patients.

Keywords: Glucose, cardiovascular diseases, myoglobin, creatine kinase, diabetes mellitus, HbA1c

1. INTRODUCTION

The term diabetes mellitus (DM) describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances in carbohydrate, lipid, and protein metabolism resulting from defects in insulin secretion, insulin action or both (Guyton and Hall, 2015). DM includes type 1 (T1DM) and type 2 (T2DM), and T2DM constitute about 90-95% of the cases of diabetes. Chronic exposure to hyperglycemia can result in dysfunction and failure of various organs especially the eyes, kidneys, nerves, and heart and blood vessels. The long-term micro- and macro-vascular complications in T2DM include retinopathy, nephropathy, neuropathy, myocardial infarction, and stroke (American Diabetes Association, 2013; Donaghue et al., 2014). The basis of abnormalities in T2DM is deficient action of insulin on target tissues due to impairment of insulin secretion, defects in insulin action, or both. Insulin resistance, which represents a reduced physiological response of the peripheral tissues to the action of the normal levels of insulin, is a major finding in several metabolic disorders, including T2DM and metabolic syndrome (Adams-Huet et al., 2014; Rutter et al., 2014). Initially, insulin resistance is compensated by enhanced insulin secretion, but later, insulin secretion is impaired. In the progression from normal to impaired glucose tolerance and diabetes, insulin secretion deteriorates faster than insulin sensitivity (Nolan et al., 2015). DM assoicated complications are much loweramong patientswith controlled blood glucoselevels. Several factors has been implicated in the increased glucose levels comprising sustained elevated blood pressure, lack of physical activity, obeity,high chloestrol levels, and smoking (Nathan et al., 2005). According to the American Diabetes Association, cardiovascular disease (CVD) accounts for as much as 75-80% of mortality in patients with T2DM (Martín-Timón et al. 2014).

T2DM is present in 10-30% of patients presenting with acute myocardial infarction (AMI), and the incidence is expected to double in subsequent tow decatesrasing a major public health alarm. Hence, early AMI risk prediction provides an opportunity for appropriate intensive management. A raised myoglobin concentration and elevated total CK and CK-MB levels are now accepted as the standard biochemical markers for the diagnosis of AMI (Palanisamy et al., 2011). The HbA1c level is important not only for monitoring diabetes but also for assessing the risk of coronary heart disease in diabetic patients (Meisinger et al., 2005).

The above biomarkers may be used to develop patient demographics, which may be useful in developing health policies (Yue et al., 2011). Hence, this study aimed to evaluate serum myoglobin as a cardiac muscle marker for the detection of the early cardiovascular disorder among type 2 diabetic patients in addition to estimating total CK and CM-MB levels for those patients. Additionally, the assessment of glycemic control was performed by measuring HbA1c levels.

2. MATERIALS AND METHODS

Study Design

In this study 300 study subjects were randomly selected at Alquayiyah General Hospital in the Riyadh region (Kingdom of Saudia Arabia) to evaluate the serum levels of myoglobin, total CK and CK isoenzyme MB in patients with T2DM. The 300 study subjects were divided into two groups; 200 subjects diagnosed with T2DM (ascertained as cases) and 100 healthy individuals (ascertained as control group). The control group was relatively matched for age and sex with the case group.

Ethical consideration

Informed verbal and written consent was obtained from each study participant.

Data and sample collection

An interview with a questionnaire was used to obtain the clinical data from each participant in this study. A physician took clinical history and examined the test and control groups. Venous blood samples were collected and then divided into three containers: fluoride oxalate anticoagulant container for glucose estimation, EDTA container for HbA1c (whole blood) and the last one in a plain container. After clotting, the samples were centrifuged at 1500 rpm for 5 min to obtain serum for myoglobin, total CK and CK-MB estimation. Serum or plasma was kept in clean dry containers at −20°C after separation until use.

Analytical procedure

Measurement of serum myoglobin (ELISA, Thermo Fisher Scientific, USA)

Serum myoglobin (ng/mL) was measured using an enzyme-linked immunosorbent assay technique (ELISA technique). ELISA is a method of specific antigen-antibody reaction in samples that use an enzyme reaction with its substrate to determine a target molecule concentration (Lequin, 2005).

Measurement of serum total creatine kinase (CK) (using the Optima UV/VIS Spectrophotometer -Japan)

Creatine kinase (CK) catalyzes the phosphorylation of ADP, in the existence of creatine phosphate, to form ATP and creatine. The catalytic concentration is determined from the rate of NADPH formation, measured at 340 nm, employing the hexokinase (HK) and glucose-6 phosphate dehydrogenase (G6p. DH) coupled reaction (Burtis et al., 2018) (Company: Human GMBH-Germany).

Measurement of serum creatine kinase isoenzyme MB (CK-MB) (using the Optima UV/VIS Spectrophotometer -Japan)

The M subunits of CK-MM (CK-3) and the soloM subunit of CK-MB (CK-2) were repressed by a specific antibody, consequently permitting assessment of the B subunit of CK-MB (supposing the nonexistence of CK-BB or CK-1). The CK-B catalytic concentration is determined from the rate of NADPH formation, measured at 340 nm using the hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) coupled reactions (Burtis et al., 2018) (Company: Human GMBH-Germany).

Estimation of glycated hemoglobin (using the chromatographic spectrophotometric ion exchange method).

This reaction depends on the elimination of the labile fraction of hemoglobin after preparing the hemolysate. Hemoglobin is reserved by a cationic exchange resin (HbA1c), which is precisely eluted after eliminating the hemoglobin A1a+b fraction (HbA1a+b) and is estimated by direct photometric reading at 415 nm (Burtis et al., 2018) (Company: Human GMBH-Germany).

Measurement of plasma glucose (using the Optima UV/VIS Spectrophotometer -Japan)

The glucose concentration is directly proportional to the color produced when the glucose oxidized by glucose oxidase yields H_2O_2, which oxidized to yield a color dye of quinonimine, which is measured photometrically at 505 nm (Burtis et al., 2018) (Company: Human GMBH-Germany).

Statistical analysis

Data from all patients are presented as percentages and (mean ± standard deviation). Differences between the means of patient and control groups were considered statistically significant with a P-value threshold <0.05 using the independent t-test. A significant correlation (r) was calculated using the Pearson correlation test, using SPSS version 21.

3. RESULTS

The test group was composed of 114 males (57%) and 86 females (43%), whereas the control group was composed of 56 males (56%), and 44 females (44%). A total of 32.5% (n = 65) of patients were hypertensive, and 15% (n = 30) had ischemic heart disease. A total of 35% (n = 70) of patients were described as obese based on body mass index calculation. The demographic characteristics of diabetic patients and their controls are shown in Table 1, while Table 2 shows the comparison of the means of plasma levels of myoglobin, total CK and CK-MB of the test group and the control group. Table 3 shows the comparison of the means of serum levels of myoglobin, total CK and CK-MB of the diabetic patients with ischemic heart disease or hypertension and those without ischemic heart disease or hypertension.

Plasma levels of myoglobin, total CK and CK-MB showed significant positive correlations with duration of diabetes (Figure 1, 2 and 3), BMI (figure 4, 5 and 6) and HbA_{1C} (Figures 7, 8 and 9), also there was a significant positive correlation between FBS and HbA_{1C} (Figure 10)

Table 1 Demographic characteristics of the study population (type 2 diabetic patients and control subjects)

Parameter	Diabetic patients	Control subjects
Number of subjects	200	100
Sex (male number)	114	56
Sex (female number)	86	44
Age (years)	60.72±9.18	59.75±12.36
Weight (kg)	74.30±14.85	69.89±14.33
Height (cm)	164.86±13.18	162.20±14.50
BMI (kg/m2)	28.92±5.29	25.86±4.91

BMI: Body mass index

Table 2 Comparison of the means of plasma levels of myoglobin, total CK and CK-MB of the test group and the control group.

Variable	Test group n=200	Control group n=100	P-value
S. Myoglobin(ng/mL)	48.89 ± 20.61	34.54 ± 15.43	0.019
S. Total CK (U/L)	110.72 ±36.3	99.18 ± 32.01	0.027
S. CK-MB(U/L)	21.20 ± 6.93	17.10 ± 4.05	0.033

Means ± SD and probability, CK: Creatine Kinase, CK-MB: Creatine Kinase isoenzyme MB SD: Standard deviation

Table 3 Comparison of the means of serum levels of myoglobin, total CK and CK-MB, of the diabetic patients with ischemic heart disease or hypertension and those without ischemic heart disease or hypertension.

Variable	Patients with ischemic heart disease n=30	Patients without ischemic heart disease n=170	P-value	Patients with hypertension n=65	Patients without hypertension n=135	P-value
S. Myoglobin(ng/mL)	71.00 ± 6.45	45.13 ± 19.82	0.002	70.30 ± 6.89	38.10 ± 16.32	0.000
S. Total CK (U/L)	138.48 ± 28.82	106.01 ± 35.42	0.013	138.72 ±23.16	96.62 ±33.52	0.032
S. CK-MB(U/L)	28.28 ± 1.79	20.00 ± 6.76	0.012	28.01 ± 1.75	17.77 ± 5.94	0.001

Means ± SD and probability, CK: Creatine Kinase, CK-MB: Creatine Kinase isoenzyme MB SD: Standard deviation

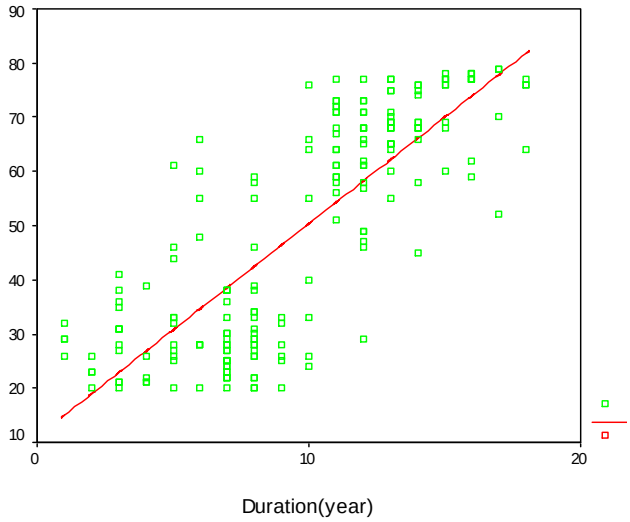

Figure 1 A scatter plot shows the relationship between the duration of diabetes (years) and the serum levels of myoglobin (ng/mL) (r=0.80, P=0.000). Squares indicating strength of association.

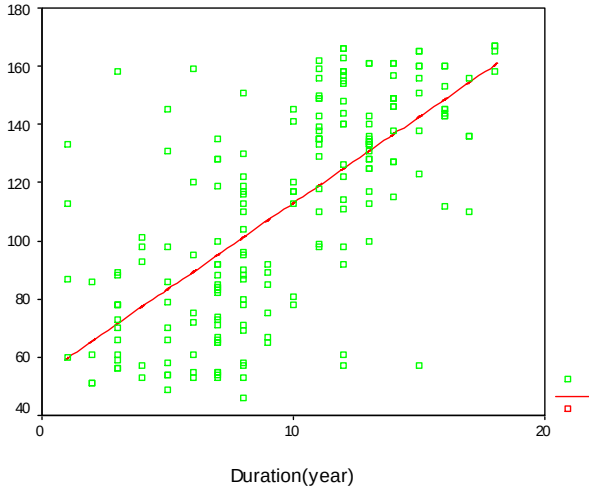

Figure 2 A scatter plot shows the relationship between the duration of diabetes (years) and the serum levels of total CK (U/L) (r=0.68, P=0.030). Squares indicating strength of association.

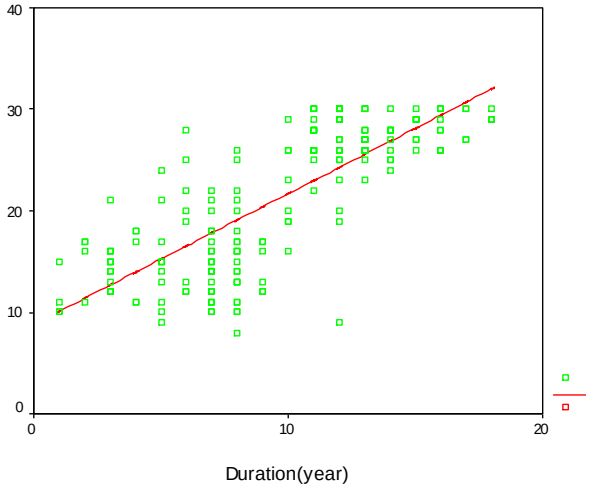

Figure 3 A scatter plot shows the relationship between the duration of diabetes (years) and the serum levels of CK-MB (U/L) (r=0.78, P=0.000). Squares indicating strength of association.

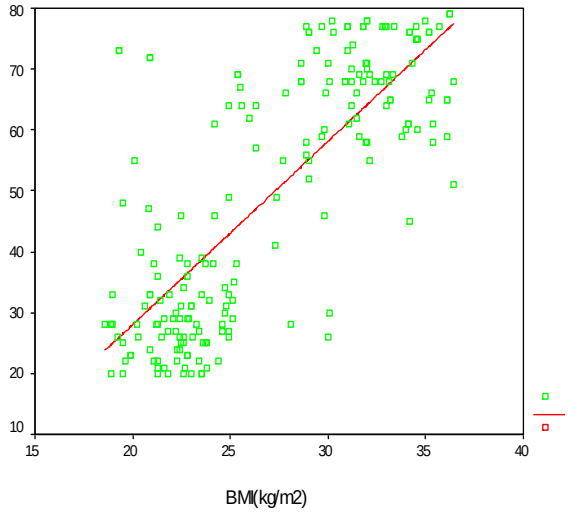

Figure 4 A scatter plot shows the relationship between BMI (kg/m^2) and the serum levels of myoglobin (ng/mL) (r=0.77, P=0.000). Squares indicating strength of association.

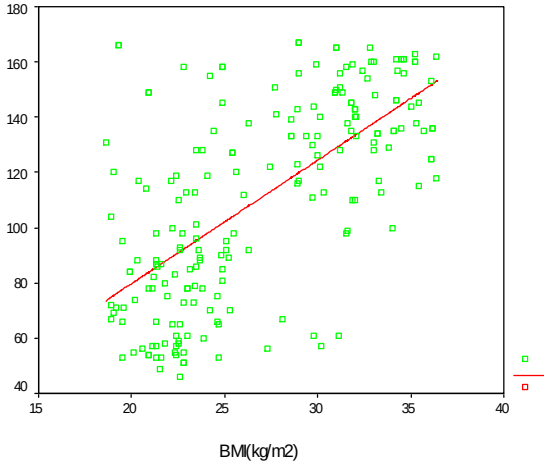

Figure 5 A scatter plot shows the relationship between BMI (kg/m^2) and the serum levels of total CK (U/L) (r=0.65, P=0.028). Squares indicating strength of association.

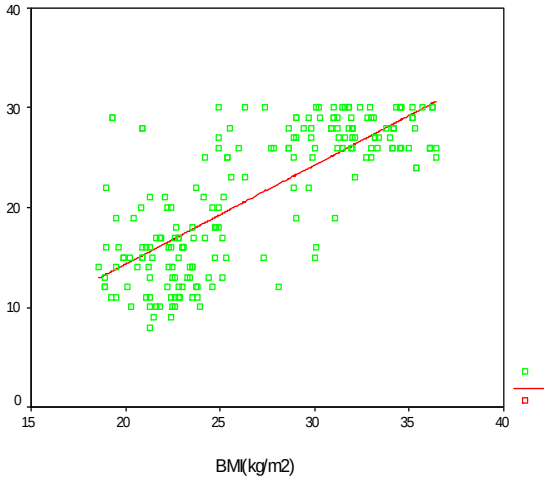

Figure 6 A scatter plot shows the relationship between BMI (kg/m^2) and the serum levels of CK-MB (U/L) (r=0.76, P=0.000). Squares indicating strength of association.

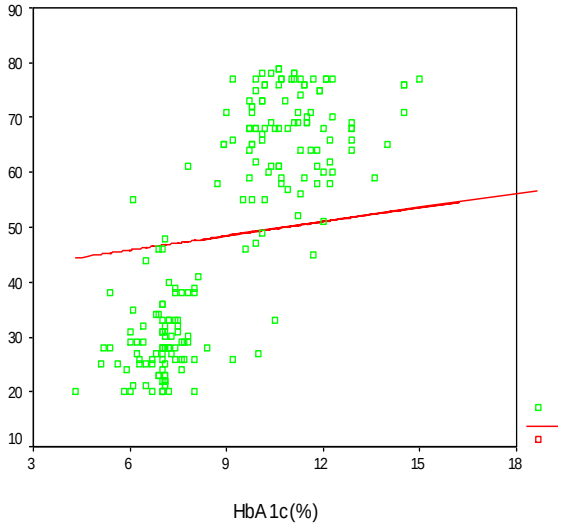

Figure 7 A scatter plot shows the relationship between HbA_{1C} (%) and the serum levels of myoglobin (ng/mL) (r=0.28, P=0.068). Squares indicating strength of association.

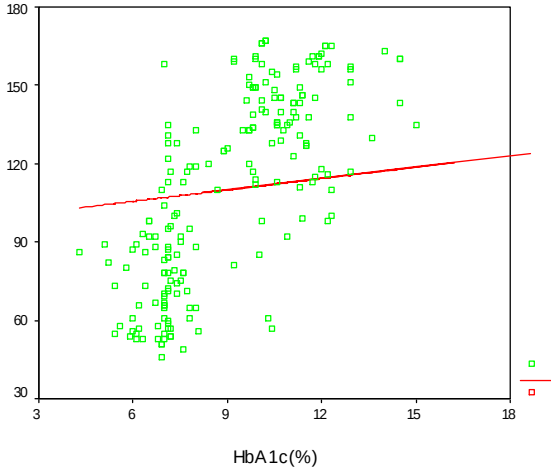

Figure 8 A scatter plot shows the relationship between HbA_{1C} (%) and the serum levels of total CK (U/L) (r=0.27, P=0.075). Squares indicating strength of association.

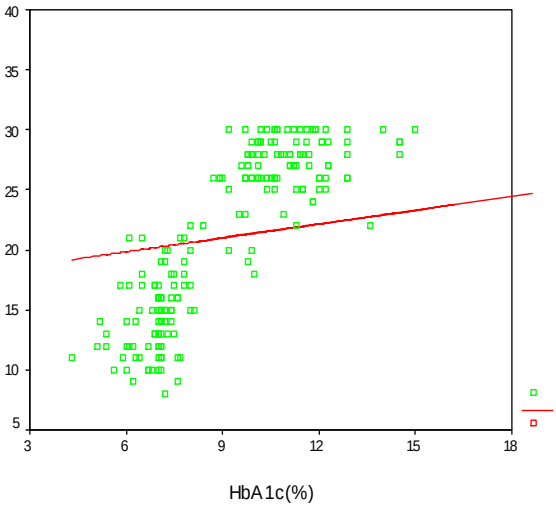

Figure 9 A scatter plot shows the relationship between HbA_{1C} (%) and the serum levels of CK-MB (U/L) (r=0.37, P=0.066). Squares indicating strength of association.

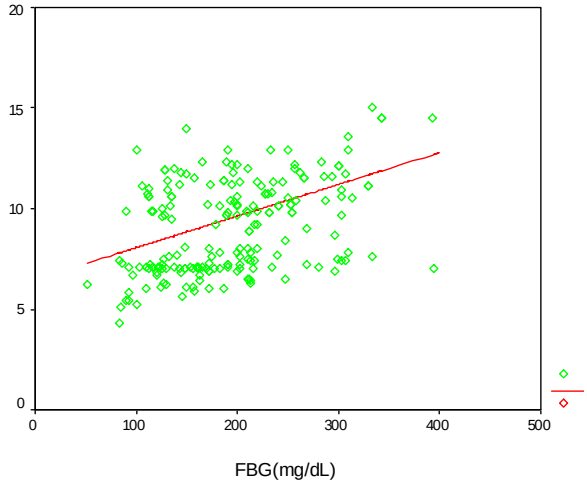

Figure 10 A scatter plot shows the relationship between FPG (mg/dL) and HbA_{1C} (%) (r=0.69, P=0.002). Squares indicating strength of association.

4. DISCUSSION

DM is commonly associated with both microvascular and macrovascular complications, and specifically, T2DM contributes to subclinical myocardial injury. T2DM is also associated with increased arterial stiffness as a consequence of increased oxidative stress and accelerated endothelial cell apoptosis, endothelial dysfunction, and depletion of endothelial progenitor cells which may predispose individuals to increased cardiovascular risk (Schram et al., 2004). Early prediction of this risk will be very valuable in the clinical management of the disease. Consequently, several studies have focused on the prediction of cardiovascular diseases among diabetic patients (Jenkins et al., 2007; Bruno et al., 2012; Thygesen et al., 2007).

The present study showed a significant elevation of the mean serum levels of myoglobin (P=0.019), total CK (P =0.027), and CK-MB (P =0.033) in diabetic patients compared with levels in the control group, hence indicating that myoglobin, total CK and CK-MB estimation may be a valuable biomarker to assess or predict the progression of adverse cardiovascular events in patients with T2DM. Furthermore, hypertensive diabetic patients are at a much higher risk of developing cardiac pathology (Petitti et al., 2007). This study showed a significant increase in the mean serum levels of myoglobin (P = 0.000), total CK (P =0.033) and CK-MB (P =0.001) in diabetic hypertensive patients compared to normotensive diabetic patients, which may be indicated by the synergistic effects of hypertension and diabetes on myocardial damage (Idonije et al., 2011). Besides, the current study shows a significant elevation of the mean of the serum levels of myoglobin, total CK and CK-MB among the diabetic patients with ischemic heart disease when compared to the mean of those without ischemic heart disease (P =0.002; P =0.013; and P =0.012, respectively). The myoglobin, total CK and CK-MB are extremely sensitive and considered specific biomarkers of myocardial necrosis and crucial biomarkers for the diagnosis of AMI (Jaffe, 2002).

In the current study, there was a significant strong positive correlation between the duration of the disease and the serum levels of myoglobin (r=0.80, P=0.000). Also, there was a significant strong positive correlation between BMI and the serum levels of myoglobin (r=0.77, P=0.000). This finding is consistent with that reported by (Lavie et al., 2013), who found a correlation between BMI and the severity of atherosclerosis, AMI and heart failure (HF). The present study shows an insignificant weak positive correlation between HbA1C and the serum levels of myoglobin (r=0.28, P=0.068), which could be because HbA1C is greatly affected by glycemic control and not by the severity of atherosclerosis.

In the current study, there was a significant moderate positive correlation between the duration of diabetes and the serum levels of total CK (r=0.69, P=0.031). Also, there was a significantly strong, positive correlation between the duration of diabetes and the serum levels of CK-MB (r=0.79, P=0.000), this corresponds to Odumand Young (2018); who reported that all the cardiac markers were significantly higher in diabetic patients than non-diabetic one, also they noted that the patients with long term DM had increased levels of total CK, CK-MB, and myoglobin (Odum and Young, 2018). Likewise, the present study shows a significant moderate positive correlation between BMI and serum levels of total CK (r=0.65, P=0.028) and a significant strong positive correlation between BMI and serum levels of CK-MB (r=0.76, P=0.000). This finding corresponds to Alberti et al. 2009, who showed that obesity is associated with an increased risk for AMI and HF (Alberti et al., 2009). In this study, there was an insignificant weak positive correlation between HbA1C and the serum levels of total CK and CK-MB (r=0.27, P=0.075, and r=0.37, P=0.066, respectively). The current study also indicates a significant strong positive correlation between FPG and HbA1C (r=0.69), (P=0.002).

5. CONCLUSION

The higher levels of myoglobin, CK, and CK-MB may be indicative of progressive cardiovascular disease (CVD) among diabetic patients. The risk of CVD may increase among patients with hypertension and ischemic heart disease, consequently, cardiac markers should be measured regularly among these patients.

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Conflicts of Interest:

The authors declare no conflict of interest.

Ethical approval

Ethical committee approval code number: EC 00015/CM-UOH.8/17.

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