SERUM LEVELS OF CARDIAC TROPONIN I, CREATINE KINASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND HYTPERTENSIVE CO-MOBIDITY IN UDUTH, SOKOTO STATE, NIGERIA

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ABSTRACT

Background: Diabetes mellitus (DM) and hypertension (HTN) are co-morbid conditions that may predispose to atherosclerotic cardiovascular diseases.

Aim: The aim of the study is to evaluate the serum levels of Cardiac troponin I and creatine kinase in patients with type 2 diabetes mellitus and Hypertension.

Materials and methods: A case -control study was conducted at Department of Medicine, endocrinology unit of Usmanu Danfodiyo University Teaching Hospital, from June 2015 to February 2016. A total of 180 subjects were recruited for the study and categorized into three groups. 60 patients diagnosed with diabetes and hypertension, 60 patients diagnosed with diabetes and 60 apparently healthy individuals were included in this study. Blood specimens were collected and processed from all group participants. Serum cardiac troponin I (CTnI) was analyzed using ELISA method, total Creatine Kinase (CK), creatine kinase-MB (CK-MB) was measured using IFCC reference procedure and plasma fasting plasma glucose levels were measured using enzymatic glucose oxidase method.

Results: Twenty three 23(19%) of the subjects with type 2 diabetes mellitus studied had increased CTnI levels above the 99th percentile (1.3ng/mL). Significant increase in CTnI was observed among participants with DM and HTN co-morbidity. In addition, the diabetic groups had significantly increase levels of total CK and CK-MB ($p=0.001$) compared to healthy controls. Furthermore, a strong positive correlation existed between CTnI, total CK and CK-MB (r-value 0.450, 0.569, $p<0.01$), and with CTnI and FPG (r-value 0.160, $p<0.05$). **Conclusion:** The study suggests increased levels of CTnI and CK-MB above safe values which may be a pointer to silent myocardial injury.

Key words: Cardiac troponin I, Creatine kinase, Diabetes mellitus, Hypertension.

INTRODUCTION

Diabetes Mellitus (DM) describes a group of metabolic disorders of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, action or both (ADA, 2005). The chronic hyperglycaemia of diabetes mellitus is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2005). Diabetes mellitus is one of the most common chronic diseases in nearly all countries, and continues to increase in numbers and significance, as changing lifestyles leads to reduced physical activity, and increased obesity (Shaw *et al.*, 2010).

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Diabetes mellitus poses a high risk for the development of cardiovascular diseases, because it heralds an accelerated process of atherosclerosis and an increased risk for atherothrombotic complications (Beckman *et al.*, 2002). Diabetes mellitus and hypertension are morbid conditions that strongly predispose to atherosclerotic cardiovascular disease than in those without diabetes and hypertension (Epstein and Sowers, 1992; Juutilainen *et al*., 2004).

Coronary heart disease (CHD) contributed to myocardial infarction (MI) and heart failure, attributed to most of the mortalities around the globe as reported by Ezekowitz *et al.* (2009) and Bui *et al*. (2011). Coronary heart disease (CHD) has been defined as impairment of heart function due to inadequate flow of blood to the heart compared to its need caused by obstructive changes in the coronary circulation to the heart (Hamshi and Woo, 1997). The occurrence of this co-morbid diseases represents a major public health concern making the assessment of the risk of coronary heart disease in diabetes patients important with acceptable standard biochemical biomarker which are troponin I and T (cTnI and cTnT) concentrations (Palanisamy *et al*., 2011). Heart contractility is evaluated by measuring the myocardial tissue specific protein Trop I, involved in cardiac contractility.

Previous studies indicated that cardiac troponin I is highly sensitive and specific marker of myocardial damage and therefore used as a diagnostic marker for Acute Myocardial Infarction (Tiwari, *et al.,* 2012). Similarly, Creatine kinase and CK-MB are two important indicators of myocardial necrosis (Alpert *et al.*, 2000). Previous studies shown that elevated high sensitivity troponin level suggested myocardial injury that was shown to be related with arterial stiffening in patients with type 2 diabetes mellitus (Yiu *et al.,* 2014).

Diabetes mellitus is not a predisposing factor for cardiovascular diseases, but once patients with diabetes mellitus develop coronary artery disease, they have significantly worse outcomes when compared to non diabetic patients. The goal of the study was to assess cardiac troponin I, and creatine kinase in diabetic patients which may aid the earlier detection of silent myocardial injury as there is paucity of data for the assessment of such cardiac markers in these patients in this environment.

MATERIALS AND METHODS

Sokoto State was created on 3rd February 1976 with an area of 25,973 square kilometres and an estimated population of 4,244,399. It has 23 local government areas and it is bordered north by Niger Republic, west by Kebbi State and Benin Republic and east by Zamfara State.

Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, a 700 bed tertiary health center, serves Sokoto, Kebbi, and Zamfara States in Nigeria and also receives patients from the neighboring Niger Republic. It is among the crop of Teaching Hospitals that was established by the federal government in May 1980 as second generation Teaching Hospitals. The population served is mainly the Hausa/Fulani ethnic group of both Islamic and Christian faith. The main occupation of the men is subsistence farming and animal husbandry. The study was based in the Department of Medicine, Endocrinology Unit, UDUTH, Sokoto.

Ethical Consideration: This study was approved by the ethical committee at the Usmanu Danfodiyo University Teaching Hospital, Sokoto (UDUTH/HREC/2014/311).

A total of 180 subjects were recruited into this study. This consisted of 120 subjects with type 2 diabetes mellitus attending the diabetic clinic of Usmanu Danfodiyo University Teaching Hospital Sokoto; the patients with type 2 diabetes were divided into two groups. Sixty subjects with diabetes mellitus without hypertension and sixty subjects with type 2 diabetes mellitus and hypertension, and 60 apparently healthy individuals who were aged and sex matched with the subjects were used as control subjects. Diabetic hypertensive participants were selected based on their record history.

The controls were recruited from the general staff and students of UDUTH Sokoto.

Participants with type 2 diabetes mellitus with or without hypertension were included into the study. Apparently normal individuals without diabetes mellitus, hypertension and any chronic illness were recruited as controls. Patients with type 1 diabetes, chronic diseases apart from diabetes mellitus and hypertension were excluded from this study.

Five milliliters (5ml) of fasting venous blood was collected using standard technique from the ante-cubital vein. Two milliliters (2ml) was dispensed into fluoride oxalate bottle for glucose analysis and three milliliters (3mL) into sterile plain blood specimen bottle which was allowed to clot at room temperature after which it was centrifuged at 3000 rpm for 5 minutes. Serum was aspirated, aliquoted and stored at -20^{o}C for analysis. The serum levels of CTnI using Accubind ELISA method described by (Bodor *et al*., 1992), Creatine Kinase Total, and CK-MB by Schumann *et al.* (2002). IFCC, Reference procedure for the measurement of catalytic concentration of creatine kinase and fasting plasma glucose levels using the enzymatic method of Trinder (1969) were measured.

Statistical Analysis

Statistical analysis was performed using Statistical package for social science (SPSS) windows version 20 was employed for the analysis. The results were presented as mean ±standard deviation. One-way ANOVA was used to compare quantitative variables between three groups and a post hoc test was used to confirm where the differences occurred between groups. The Pearson correlation coefficient was used to correlate the studied variables.

RESULTS

Table 1. Shows the frequency in percentage of elevated CTnI, total CK, CK- MB and lipid profile in subjects with type 2 diabetes mellitus. Of the 120 subjects with type 2 diabetes mellitus recruited into the study, twenty three 23(19.2%) had increased troponin levels above the 99th percentile (1.3ng/mL), with fifteen 15(12.5%) in DM with hypertension and eight 8(6.7%) in DM without hypertension. Thirty six 36 (30.0%) had elevated total creatine kinase above the reference range (171U/L), with 23 (19.2%) in DM with hypertension and 13(10.8%) in DM without hypertension. Twenty nine 29 (24.1%) had elevated CK-MB above the reference range 25U/L with 19 (15.8%) in DM with hypertension and 10(8.3%) in DM without hypertension.

Table 1: Frequency of cardiac biomarkers among study population

Analytes	n	DM+ HTN (%)	DM without HTN (%)	Percentage
CTnI	120	15(12.5%)	8(6.7%)	23(19.2%)
Total CK	120	23(19.2%)	13(10.8%)	36(30.0%)
CK-MB	120	19(15.8%)	10(8.3%)	29(24.1%)

n= total number of patients with diabetes mellitus enrolled, DM without HTN=Diabetes mellitus without hypertension, DM+HTN= Diabetes mellitus and hypertension, CTnI=cardiac troponin I, Total CK= Total Creatine Kinase, CK-MB= Creatine kinase MB isoenzyme

Table 2. shows the comparison of cardiac troponin I, total creatine kinase, and CK-MB across the three groups. The cardiac troponin I of patients with type 2 diabetes mellitus without hypertension and that of control group showed a significant statistical difference when compared to the patients with diabetes mellitus and hypertension group ($p<0.05$) .Total creatine kinase of patients with type 2 diabetes mellitus without hypertension and diabetes mellitus with hypertension showed a significant difference when compared to the control group $p<0.05$). CK-MB differed significantly across the three groups.

Table 2: Cardiac troponin I, Total Creatine Kinase and CK-MB Isoenzyme of diabetics without hypertension, diabetics with hypertension and controls

Variable	n	CTnI (ng/mL)	Total CK (IU)	CK- MB (IU)
DM	60	0.90±1.94[a]	161.29±180.59[b]	26.67±35.99[b]
DM + HTN	60	1.53±2.68[b]	188.76±203.54[b]	37.70±37.25[c]
Controls	60	0.36±0.48[a]	53.57±35.48[a]	14.03±8.05[a]
f- value	-	5.29	11.58	8.71

n= number of subjects, Values are expressed as mean±SD, values with different superscript are statistically significant across column, DM=Diabetes mellitus, DM+HTN= Diabetes mellitus and hypertension, CTnI=Cardiac troponin I, Total CK= Total Creatine Kinase, CK-MB= Creatine kinase MB isoenzyme

Table 3. Shows Pearson correlation between cardiac troponin I, total CK and CK-MB in subjects with diabetes mellitus. There was a significant positive correlations between cardiac troponin I and total CK, CK- MB, ($p<0.01$) and cardiac troponin I and fasting plasma glucose ($p<0.05$).

Table 3: Correlation between Cardiac troponin I, Total CK and CK-MB in diabetics

Variables	r- value	p- value
CTnI vs Total CK	0.450	0.001**
CTnI vs CK- MB	0.569	0.001**
CTnI vs FPG	0.160	0.032*

CTnI=cardiac troponin I, Total CK= Total Creatine Kinase, CK-MB= Creatine kinase MB isoenzyme, FPG fasting plasma glucose, r = correlation coefficient, **Significant correlations at ($p<0.01$) * (significant correlations at ($p< 0.05$)

DISCUSSION

The study shows that nineteen percent (19%) of the patients with diabetes mellitus studied had increased cardiac troponin I levels above the 99th percentile (1.3ng/mL). This is in line with the study by Yiu *et al.* (2014), which demonstrates that up to 25% of patients with type 2 diabetes mellitus without clinical evidence of active cardiovascular disease have ongoing subtle myocardial injury/necrosis as detected by an elevated serum level of high sensitive troponin I. It agrees also with the study where 20–50% of asymptomatic patients with diabetes mellitus may have silent coronary heart disease (Prasad *et al.*, 2014). The study also is in line with studied by Hernandez *et al.* (2011) where he noted that 21% of diabetics have silent ischemia and Zheng *et al.* (2012) where he suggested that patients with diabetes mellitus have a chronic level of myocardial damage injury as identified by a correlation with blood glucose and elevated levels of serum high sensitive cardiac troponin. Cardiac troponin I showed a significant increase in DM with HTN when compared to DM without HTN and control. When DM with HTN was compared to DM without HTN in our study, higher significant levels ($p< 0.05$) was observed in diabetes mellitus with hypertension. This is line with the observation reported by Karar *et al.* (2015), where cardiac troponin I levels were significantly ($p = 0.004$) higher among diabetic hypertensive patients compared to normotensive diabetic patients. This may be indicative of synergistic effects of hypertension and diabetes on myocardial damage.

The elevated serum high sensitive troponin I level in patients with type 2 diabetes mellitus, was found to be associated with a more than 2-fold increase in the adjusted risk of major adverse cardiovascular events, and that a normal serum high sensitive troponin I level had an excellent negative predictive value for future adverse cardiovascular outcome in patients with type 2 diabetes mellitus after up to 4 years of follow-up. Elevated high sensitivity troponin level suggested myocardial injury that was shown to be related with arterial stiffening in patients with type 2 diabetes mellitus (Yiu *et al.,* 2014). The increased levels of the cardiac troponins may be associated with increased arterial stiffness, consequence to increased oxidative stress, endothelial dysfunction and accelerated cell apoptosis seen in subjects with type 2diabetes mellitus. Total creatine kinase of the entire two diabetic groups differs significantly compared to control. Hesham *et al.* (2012), reported a significant higher levels of serum total troponin I, total CK (total and MB) in patients with diabetes mellitus with myocardial infarction than in myocardial infarction patients without diabetes mellitus. Elevated concentrations of troponin I are significantly and independently associated with both prior and the incidence of subsequent acute myocardial infarction (Omland *et al.* 2013), Elfaki (2011), in his study also reported a significantly raised level of these cardiac markers in patients with type 2 diabetes mellitus with and without ischemic heart disease when compared with the control group, and in a study by Awais *et al.* (2016), a strong association of cardiac enzymes i.e. CK-MB and troponin was seen in diabetic and non diabetic patients with myocardial infarction. The increased levels of creatine kinase could be as a result of hyperglycaemia recorded in patients with diabetes mellitus. This may lead to shortage of ATP which decreases the synthesis of creatine phosphate thereby leading to the activation of creatine kinase enzyme. When the ratio of creatine phosphate to creatine falls, it leads to the inhibition and leaking of the enzyme into the blood circulation (Adlija *et al.,* 2006).

CONCLUSION AND RECOMMENDATION

The study showed that a significant number of patients with diabetes mellitus with or without hypertension had increased levels of cardiac troponin I and CK-MB above safe values, which may be a pointer to silent myocardial injury. Patients with type 2 diabetes mellitus and hypertension may have a higher risk of having silent myocardial ischemia than those with only diabetes mellitus as demonstrated by the increased levels of Cardiac troponin I and CK-MB in the study.

Limitations

The patients were not followed up to detect any major adverse cardiac events.

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APPENDIX 1

USMANU DANFODIYO UNIVERSITY TEACHING HOSPITAL, SOKOTO.

PRIVATE MAIL BAG 2370 SOKOTO, NIGERIA

Chairman Board
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Our Ref: UDUTH/HREC/2014/311 Your Ref: Date: 23rd April, 2015

Rabiatu Ibrahim Mahmud (PG Adm. No. 12211226004)
Department of Chemical Pathology,
Faculty of Medical Laboratory Sciences,
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RE: APPLICATION FOR ETHICAL CLEARANCE

With reference to application on the above subject dated 31st December, 2014 on a research proposal titled: ***"Evaluation of some cardiac marker and Lipid Profile in Patients Type 2 Diabetes Mellitus attending Usmanu Danfodiyo University Teaching Hospital, Sokoto"***, I write to acknowledge its receipt and to convey Ethical Committee's approval to you. The approval is given with the understanding that the data obtained would be used to substantiate the above topic.

Please ensure that the study is guided by the methodology presented in the proposal.

Thank you.

Prof. Nma M. Jiya, ***FWACP***
Chairman HREC

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