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Original Article

Biochemistry Section

High Sensitivity Cardiac Troponin-T STAT in Type 2 Diabetes Mellitus Patients and Healthy Individuals: A Comparative Study

AK RASEEMA[1], PA GEETHA[2], NEETHI R KRISHNAN[3], ARUN MATHEW CHACKO[4], K MUHAMMED ASHRAF[5]

ABSTRACT

Introduction: Diabetes Mellitus (DM) is a metabolic disorder that shares the phenotype of hyperglycaemia, with several factors contributing to the disease, including decreased insulin secretion and glucose utilisation, as well as increased glucose production. There is a strong association between DM and Cardiovascular Disease (CVD). High-sensitivity cardiac troponin T (hs-cTnT), which is a marker of subclinical myocardial damage, is used in the risk stratification of asymptomatic individuals.

Aim: To estimate and compare hs-cTnT Short Turn Around Time (STAT) levels in diabetic patients without Acute Myocardial Infarction (AMI) with age and sex matched controls and also to investigate the correlation between hs-cTnT STAT and Glycated Haemoglobin (HbA1c) levels.

Materials and Methods: A comparative cross-sectional study was conducted in the Department of Biochemistry and Outpatient Clinic, Department of Medicine, Government Medical College, Kozhikode, Kerala, India, from April 2019 to April 2020. The study subjects were divided into two groups: Group 1 consisted of 58 patients with Type 2 Diabetes Mellitus (T2DM) without AMI, and Group 2 comprised 58 healthy individuals who were age and sex matched. No specific sampling technique was employed. After obtaining consent, T2DM patients who attended the outpatient clinic were evaluated with fasting blood glucose, HbA1c, Electrocardiogram (ECG), and hs-cTnT STAT estimation. Controls were selected and evaluated for the same from apparently healthy bystanders of other patients, medical and paramedical staff, and others willing to participate. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 22.0 software.

Results: The mean value of Fasting Blood Sugar (FBS) and HbA1c was higher in T2DM patients compared to healthy individuals. hs-cTnT showed a positive moderate correlation with HbA1c (rho=0.53), which was statistically significant (p-value <0.001). Simple linear regression analysis showed that in the case group, for a 1% increase in HbA1c levels, there was a 2.38 unit increase in hs-cTnT levels, which was statistically significant (p-value <0.001).

Conclusion: hs-cTnT levels are significantly elevated in T2DM patients without overt CVD compared to age and sex matched healthy individuals. T2DM is a risk factor for increased levels of biomarkers for atherosclerotic CVD, and proper glycaemic control reduces the levels of hs-cTnT in T2DM patients.

Keywords: Cardiovascular disease, Glycated haemoglobin, High sensitivity cardiac troponin T short turn around time

INTRODUCTION

The T2DM is characterised by variable degrees of insulin resistance, decreased insulin secretion, and increased glucose production. The common phenotype of hyperglycaemia in T2DM occurs due to specific genetic and metabolic defects in insulin action and/or secretion [1]. Cardiac troponin I (cTnI) and T (cTnT) form the contractile apparatus of myocardial cells and are expressed exclusively in the heart. High-sensitivity cTn assays are recommended for the routine detection of myocardial injury [2-4]. High-sensitivity cTn is a marker that indicates subclinical myocardial damage and has been used for risk stratification of asymptomatic individuals [5]. Diabetic patients have significantly higher hs-cTnT values than those without the disorder [6].

Since diabetes is an independent risk factor for MI, it is considered equivalent to Coronary Artery Disease (CAD) [7]. It has also been found that isolated diabetes and diabetes with certain other risk factors may be associated with MI, even in patients with a normal Body Mass Index (BMI) [8]. Previous studies have reported that patients with DM have increased atherosclerotic vascular disease, which may lead to subclinical myocardial damage in which endothelial dysfunction caused by hyperglycaemia plays an important role [9,10]. Previous studies have established a strong association between coronary atherosclerotic plaque burden and quantifiable circulating levels of troponin, as measured by hs-cTnT assay [11]. Therefore, the present study was undertaken to estimate and compare hs-cTnT STAT levels in diabetic patients without AMI with age and sex matched controls and to study the correlation between hs-cTnT STAT and HbA1c levels.

MATERIALS AND METHODS

A comparative cross-sectional study was conducted in the Department of Biochemistry and Outpatient Clinic, Department of Medicine, Government Medical College, Kozhikode, Kerala, India, from April 2019 to April 2020. The necessary approval from the Institutional Ethics Committee (IEC) was obtained with the IEC number GMCKKD/RP2018/IEC/185.

Inclusion criteria:

- Patients with T2DM without clinical and electrocardiographic evidence of AMI, of both sexes, aged above 18 years, attending the medicine outpatient unit of Government Medical College, Kozhikode, and willing to give written informed consent to be a part of the study.
- For the control group: age and sex matched healthy non diabetic subjects selected from bystanders of other patients, medical and paramedical staff, and others willing to give written informed consent to be a part of the study.

Exclusion criteria:

- Patients with clinical and electrocardiographic evidence of MI, Type 1 DM, critical illness, pregnancy, malignancy, chronic liver disease, chronic renal disease, a history of cardiovascular disorders, and patients not willing to participate in the study.

- For the control group: subjects not matched to the age group and those who did not give written consent to be a part of the study were excluded from the study.

Sample size estimation: The sample size was calculated using the following formula:

$$n=\frac{(Z\alpha+z\beta)^2\times SD^2\times 2}{d^2}$$

Using an SD of 4.8 and d of 2.5, the total sample size obtained was 58. The study subjects were divided into two groups: Group 1 consisted of 58 patients with T2DM without acute MI, and Group 2 comprised 58 age- and sex-matched healthy individuals as controls. No specific sampling technique was employed.

Data collection: After obtaining written consent, T2DM patients who attended the medicine clinic were evaluated with fasting blood glucose, HbA1c, ECG, and hs-cTnT STAT estimation. Controls were evaluated using FBS, HbA1c, hs-cTnT, and ECG. Samples for hs-cTnT STAT and HbA1c were collected in EDTA tubes from both cases and controls for FBS blood was collected in sodium fluoride (NaF) containing tubes. 12-Lead Electrocardiogram recordings were obtained from the subjects.

Glucose estimation was done using the God-Pod Method [12], where glucose oxidase converts glucose to gluconic acid.

$$\beta\text{-D Glucose}+0_2+H_2O_2 \longrightarrow \text{Gluconic acid}+H_2O_2$$

$$H_2O_2+\text{4-aminoantipyrine}+\text{phenol} \longrightarrow \text{Red dye}+H_2O$$

The estimation of HbA1c [13] was done by determining HbA1c based on the Turbidimetric Inhibition Immunoassay (TINIA) for haemolysed whole blood, using the cobas c 501 analyser. All haemoglobin variants that were glycated at the β-chain N-terminus and had antibody recognisable regions identical to that of HbA1c were measured by this assay (TTAB=Tetradecyl trimethyl ammonium bromide). Liberated haemoglobin in the haemolysed sample was converted to a derivative with a characteristic absorption spectrum, which was measured bichromatically during the preincubation phase (sample+R1) of the above immunological reaction.

The ratio definition for the final result is expressed as mmol/mol HbA1c or % HbA1c and is calculated from the HbA1c/Hb ratio using different protocols:

- ✓ *Protocol 1* (% HbA1c according to International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine; not recommended for patient result reporting):
  - HbA1c (%)=(HbA1c/Hb)×100
- ✓ *Protocol 2* (% HbA1c according to Diabetes Control and Complications Trial/National Glycohaemoglobin Standardisation Program (DCCT/NGSP)):
  - HbA1c (%)=(HbA1c/Hb)×91.5+2.15
- ✓ *Protocol 3* (mmol/mol HbA1c according to IFCC):
  - HbA1c (mmol/mol)=(HbA1c/Hb)×1000

HbA1c levels above the established reference range indicated hyperglycaemia during the preceding 2 to 3 months or longer.

The hs-cTnT Stat assay [14] employs two monoclonal antibodies specifically directed against human cardiac troponin T. The antibodies recognise two epitopes (amino acid positions 125-131 and 136-147) located in the central part of the cardiac troponin T protein, which consists of 288 amino acids. After fermentation, the cells are disrupted by sonication, and Recombinant human cardiac troponin T (rec. hcTnT) is purified by ion exchange chromatography. Purified rec. hcTnT is further characterised by Sodium Dodecyl-sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), western blotting, immunological activity, and protein content. Results were determined using a calibration curve generated specifically for the instrument by a 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS for Windows version 22.0. Chi-square test was used for comparison for categorical data and for non normally data Mann-Whitney U test was used. Linear correlation between two variables was explored using correlation coefficient. A p-value <0.05 was considered as statistically significant.

RESULTS

The mean age for cases was 54.09±8.99 years and for controls, the mean age was 51.53±8.60 years. The p-value was 0.12, indicating that the age distribution among the controls and cases was equal [Table/Fig-1].

Variable	Category	Mean±SD	Range	p-value
Age (years)	Cases	54.09±8.99	38-76	0.12*
	Controls	51.53±8.60	36-68	

[Table/Fig-1]: Age distribution among the study groups.
*Mann-Whitney U Test

[Table/Fig-2] shows gender distribution. The majority of both cases and controls were females. The p-value was 0.22, indicating that the gender distribution among the controls and cases was equal.

Variable	Category	Cases n (%)	Control n (%)	p-value
Gender	Males	14 (24.1)	20 (34.5)	0.22*
	Females	44 (75.9)	38 (65.5)	

[Table/Fig-2]: Gender distribution among the study groups.
*Chi-square test

[Table/Fig-3] depicts FBS in cases and controls. The p-value was <0.001, indicating a highly significant difference in FBS levels between cases and controls.

Variable	Groups	N	Mean±SD	Mean Diff	p-value
FBS (mg/dL)	Cases	58	208.74±67.84	123.98	<0.001*
	Controls	58	84.76±8.44		

[Table/Fig-3]: Comparison of mean values of FBS between two groups using Mann-whitney U Test.

[Table/Fig-4] depicts HbA1c levels between cases and controls. The p-value was <0.001, indicating a highly significant difference in HbA1c levels between cases and controls.

Variable	Groups	N	Mean±SD	Mean difference	p-value
HbA1c (%)	Cases	58	9.45±1.60	4.23	<0.001*
	Controls	58	5.22±0.35		

[Table/Fig-4]: Comparison of mean values of HbA1cvariable between two groups using Mann-Whitney U Test.

[Table/Fig-5] shows hs Trop T levels between cases and controls. The p-value was <0.001, indicating a highly significant difference in hs Trop T levels between cases and controls.

Variable	Groups	N	Mean±SD	Mean difference	p-value
hs Trop T (ng/dL)	Cases	58	10.54±6.10	7.44	<0.001*
	Controls	58	3.10±0.23		

[Table/Fig-5]: Comparison of mean values of hs Trop T between two groups using Mann-Whitney U Test.

[Table/Fig-6] shows the comparison of FBS status between cases and control groups using the Chi-square Test, with a statistically significant value (p-value <0.001).

[Table/Fig-7] shows the comparison of HbA1c status between cases and control groups using the Chi-square test, with a statistically significant value (p-value <0.001).

Variables	Category	Cases n (%)	Controls n (%)	p-value
FBS	Normal	0	58 (100.0)	<0.001*
	Diabetic	58 (100.0)	0	

[Table/Fig-6]: Comparison of FBS status between cases and control groups using Chi-square test.

Variables	Category	Cases n (%)	Controls n (%)	p-value
HbA1c	Normal	0	58 (100.0)	<0.001*
	Diabetic	58 (100.0)	0	

[Table/Fig-7]: Comparison of HbA1c status between cases and control groups using Chi-square test.

[Table/Fig-8] shows that among the 58 control group individuals, the hs Trop T level was below the detection level. Among cases, 81% showed hs Trop T levels in the detectable range, and 19% showed elevated levels. The p-value was <0.001, indicating a statistically significant difference in hs Trop T levels between cases and controls.

Variables	Category	Cases n (%)	Controls n (%)	p-value
hs Trop T	Blank	0	58 (100.0)	<0.001*
	Detection	(77.6)	0	
	Quantitation	2 (3.4)	0	
	Elevated	11 (19.0)	0	

[Table/Fig-8]: Comparison of hs Trop T status between cases and control groups using Chi-square test.

[Table/Fig-9] shows the Spearman's correlation for the relationship between hs Trop T and HbA1c in Case and Control groups, with statistically significant p-values of <0.001 and 0.44 for cases and controls, respectively.

Groups	Variable	Values	HbA1c
Cases	hs Trop T	rho	0.53
		p-value	<0.001*
		N	58
Controls	hs Trop T	rho	0.10
		p-value	0.44
		N	58

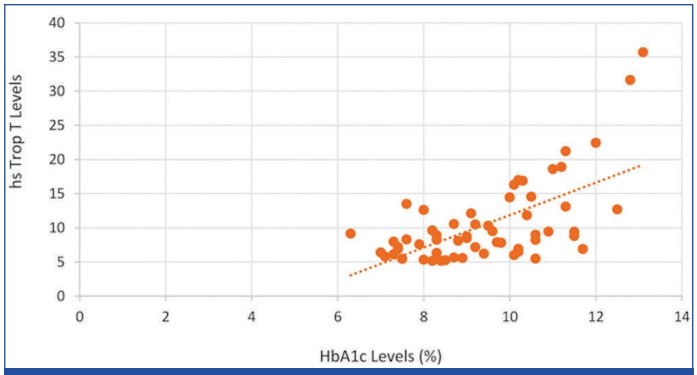
[Table/Fig-9]: Spearman's correlation to assess the relationship between hs Trop T and HbA1c in case and control groups.

In case group, there was a positive (rho=0.53) moderate correlation between hs Trop T and HbA1c (p-value <0.001), with a statistically significant result. An increase of 1% in HbA1c levels is associated with a 2.38 units increase in hs Trop T levels, which was statistically significant (p-value <0.001). The hs Trop T levels prediction equation is (2.38×HbA1c)-11.92. Up to 39% of variations in the hs Trop T values can be attributed to variations in HbA1c [Table/Fig-10].

Group	Independent variables	β coefficients β	β coefficients Std. Error	t	p-value	R^2
Case	Constant	-11.92	3.82	-3.123	0.003*	0.39
	HbA1c	2.38	0.40	5.967	<0.001*	

[Table/Fig-10]: Simple linear regression analysis to predict the hs Trop T levels by HbA1c in case group.

[Table/Fig-11] shows that the trend line in the scatter plot indicates an upward trend, depicting a positive moderate correlation between hs Trop T and HbA1c levels.



[Table/Fig-11]: Scatter plot depicting the relationship between hs Trop T and HbA1c levels in cases.

DISCUSSION

The present comparative cross-sectional study aimed to estimate and compare the levels of hs-cTnT STAT (high-sensitivity cardiac troponin T) in diabetic patients without AMI in 116 individuals, including both cases and controls. The mean serum hs-cTnT levels were found to be 10.54±6.10 ng/L in cases and 3.10±0.23 ng/L in controls. The difference in levels between cases and controls was found to be statistically significant (p-value <0.001). The 99th percentile upper reference limit of the hs-cTnT test was determined to be 14 ng/L, at which the sensitivity for diagnosing MI was found to be 100%. In the present study, controls reported hs-cTnT levels below the detection level (<5 ng/L), while none of the cases were below the detection level. Among cases, 81% (n=47) had hs-cTnT levels in the detectable range (<14 ng/L but ≥5 ng/L), and 19% (n=11) showed elevated values (≥14 ng/L). Previous studies have shown varying prevalence rates of detectable and elevated levels of cTnT in different populations [15-17].

The prevalence of detectable levels of cTnT in healthy individuals was found to be much lower in previous studies [18]. The Dallas Heart Study observed that cTnT elevation is rare in subjects without Diabetes Mellitus (DM) and other cardiovascular risk factors in the general population [19]. Another study classified subjects based on cTnT values into undetectable, minimally increased, and increased categories and found that 44% of people with DM had increased cTnT levels compared to 11% with undetectable levels (p-value <0.001) [18]. A study by Zheng J et al., concluded that hs-cTnT exhibited diverse distribution in a community-based population with various blood glucose levels, and the prevalence of detectable and elevated hs-cTnT was higher in the diabetic population, possibly due to multiple risk factors for CVD and the independent interpretation of blood glucose levels [20].

Jonas Hallén et al., reported that circulating troponin T was measurable in 90% of individuals using novel high-sensitivity assay techniques, and a considerable proportion had levels above the 99th percentile of a reference population. This study suggested that troponin T release reflects underlying chronic pathophysiological progressions and can serve as a useful surrogate marker for outlining risk factors in patients with T2DM [21].

In the Atherosclerosis Risk in Communities (ARIC) study, the association between baseline HbA1c and hs-cTnT was examined, and it was observed that higher baseline values of HbA1c were related to elevated hs-cTnT levels in a graded manner [22]. Another ohort study by Everett BM et al., examined the risk of incident cardiovascular disease in women with and without diabetes mellitus and found that high-sensitivity cardiac troponin T was detectable in a higher proportion of diabetic women compared to non-diabetic women. Even though the majority of women had troponin T levels within the normal range, the presence of very low but detectable levels was associated with a >3-fold increase in the adjusted risk of cardiovascular death [23].

In the present study, a moderate correlation (rho=0.53) was observed between hs-cTnT levels and HbA1c, which was statistically significant (p-value <0.001). Simple linear regression analysis showed that in the case group, a 1% increase in HbA1c levels was associated with a 2.38 unit increase in hs-cTnT levels, which was statistically significant (p-value <0.001).

Selvin et al., observed that people with diabetes had a higher proportion of individuals in the higher range of hs-cTnT values compared to pre-diabetics and non-diabetics [6]. The present study also found significantly elevated hs-cTnT levels in diabetic patients without overt CVD compared to age and gender-matched healthy individuals. Additionally, hs-cTnT levels were found to positively correlate with HbA1c levels.

Limitation(s)

However, there are certain limitations to consider. The limited sample size may not fully represent the general population. The cost and availability of hs-cTnT kits constrained the final sample size. The cross-sectional design of the study prevents determining causal relationships. The lack of cardiac imaging data could have influenced the results, as occult CVD may be present. Insulin resistance measurements were not included, and there is a scarcity of data on the Indian population.

CONCLUSION(S)

Based on the present study, it can be concluded that hs-cTnT levels are significantly elevated in T2DM patients without overt CVD compared to otherwise healthy individuals. hs-cTnT levels are associated with HbA1c levels in people with T2DM, which is a risk factor for increased levels of biomarkers for atherosclerotic cardiovascular disease. Thus, proper glycaemic control reduces these levels in T2DM patients. Further evaluation of hs-cTnT STAT in T2DM patients without overt cardiovascular morbidities is recommended, as it is more sensitive than Troponin I. Conducting a large-scale study with a bigger sample size may provide better correlation and outcomes, aiding clinicians in utilising hs-cTnT STAT as a parameter to predict the risk of developing CVD in diabetic patients.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, KMCT Medical College, Kozhikode, Kerala, India.
2. Professor, Department of Biochemistry, Government Medical College, Kozhikode, Kerala, India.
3. Assistant Professor, Department of Biochemistry, KMCT Medical College, Kozhikode, Kerala, India.
4. Associate Professor, Department of Biochemistry, KMCT Medical College, Kozhikode, Kerala, India.
5. Professor, Department of Biochemistry, Government Medical College, Wayanad, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Arun Mathew Chacko,
Associate Professor, Department of Biochemistry, KMCT Medical College,
Kozhikode-673602, Kerala, India.
E-mail: dr_arunmathew@yahoo.com

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