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Predictors of cardiomyopathy in patients with type-2 diabetes mellitus with and without cardiovascular complications: A cross-sectional study

Aamir Bashir [a], *Md Azharuddin* [b], *Ishfaq Rashid* [c], *Krishna Murti* [a,*], *Krishna Pandey* [d,**]

[a] *Department of Pharmacy Practice, National Institute of Pharmaceutical Education and Research, Hajipur 844102, Bihar, India*
[b] *Department of Pharmaceutical Medicine (Division of Pharmacology), School of Pharmaceutical Education and Research, Jamia Hamdard, 110062 New Delhi, India*
[c] *Department of Pharmacy Practice, National Institute of Pharmaceutical Education and Research, SAS Nagar, 160062 Punjab, India*
[d] *Division of Clinical Medicine, Rajendra Memorial Research Institute of Medical Sciences (Indian Council of Medical Research), Agamkuan, Patna, 800007 Bihar, India*

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ABSTRACT

Aim: This study was aimed to evaluate the cardiomyopathy in patients with type 2 diabetes mellitus (T2DM) who live with or without cardiovascular complications by estimating different cardiac biomarkers.

Methods: This cross-sectional study enrolled 125 participants including 25 healthy volunteers and 100 T2DM patients. After meeting all inclusion criteria, the participants were categorized into five groups (N = 25 in each) as; healthy volunteers (I), T2DM (II), T2DM with hypertension (III), T2DM with dyslipidemia (IV), T2DM with hypertension and dyslipidemia (V). Pearson's correlation analysis was performed to assess the significant association between cardiac biomarkers other biochemical parameters. P-values <0.05 were considered statistically significant.

Results: The average age of the participants was found to be 55.04 ± 7.51 years. The positive correlation was found between HbA1c and calcium or BNP levels however, a negative association was observed with zinc level. Group V showed higher mean of BNP (pg/mL) as 86.73 ± 64.49 followed by Group III (61.02 ± 53.69), IV (33.88 ± 33.71), II (13.49 ± 11.67) and I (5.54 ± 1.49) which predicts the subclinical cardiomyopathies in the respective groups. Serum zinc (μg/dL) level were significantly lower in Group V (52.72 ± 12.16) followed by III (56.15 ± 9.64), IV (58.10 ± 10.05), II (59.49 ± 11.33) and I (73.96 ± 21.91).

Conclusions: In summary, BNP and calcium levels were significantly elevated while zinc was significantly reduced in T2DM patients with cardiovascular complication. Results from the study also shown positive correlation between BNP, calcium, Troponin-I levels and blood pressure. However, further longitudinal studies required to confirm these findings.

* Corresponding author at: Department of Pharmacy Practice, National Institute of Pharmaceutical Education and Research, Hajipur 804102, India.
** Corresponding author.
E-mail addresses: krishnamurti74@gmail.com (K. Murti), drkrishnapandey@yahoo.com (K. Pandey).

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1. Introduction

India leads the world with the largest number of diabetes patients, thereby known as "Diabetic Capital of the World", having more than 62 million diabetic individuals diagnosed with the disease [1,2]. The rapid epidemiological transition associated with unhealthy dietary patterns, physical inactivity are considered as main drivers to give a higher prevalence of diabetes in the urban population [3]. Type 2 diabetes mellitus (T2DM) is the commonest form of diabetes with a prevalence of 2.4% in the rural population and 11.6% in the urban population [4].

Diabetes mellitus is a significant risk factor for developing cardiac complications, which specifically contribute to subclinical cardiomyopathy [5]. T2DM have two-four fold increased risk of cardiovascular morbidity and mortality, however, the exact mechanism is undetermined [6]. Diabetes-associated cardiovascular complications including hypertension and dyslipidemia are also believed to have a critical deteriorating impact on myocardium [7]. Several studies have reported that diabetes and related cardiovascular abnormalities lead to chronic myocyte injury [5,7].

Diabetic cardiomyopathy is believed to have an extensive preclinical course. The pathophysiological alterations are stimulated by the metabolic changes in hyperglycemic status [8]. There are various underlying mechanisms to relate diabetes with myocardial injury as; hyperglycemia-induced structural abnormalities, microvascular dysfunction, cardiac autonomic neuropathy and neurohormonal abnormalities [9,10]. Initially, it involves myocardial fibrosis, dysfunctional remodelling of the myocardium and associated diastolic dysfunction and later on systolic dysfunction and clinical heart failure is observed [11].

There are several micro and macronutrients which are possibly involved in cardiomyopathy among diabetes patients such as zinc deficiency, abnormal calcium levels. Systemic zinc deficiency is associated with the high incidence of diabetes and its related complications [12]. Zinc as an important microelement plays a significant antioxidant role in cell structure and function by inhibiting damage associated with lipid peroxidation and also initiates the clearance of free radicals [13,14].

In addition, cytosolic calcium plays a crucial role in insulin secretion from pancreatic cells [15]. It has been reported that the calcium homeostasis gets disturbed during the metabolic abnormalities like in diabetes and potentiate its damaging effect on myocardium [16].Previous literature shows varying results for the association between calcium and incident diabetes [17,18]. However, there is lack of evidence related to the estimation of these nutrients for their diagnostic utility in cardiovascular disorder prediction.

The diagnosis of diabetes-related cardiomyopathy and its other complications have been done with different novel biomarkers. The principle behind the predictive value of biomarker is that, it measures the progression of damage in the myocardium. Till date, several established diagnostic biomarkers are available including, B-type natriuretic peptide (BNP), N-terminal pro b-type natriuretic peptide (NT-proBNP), atrial natriuretic peptide (ANP), troponin T and I (Trop-T, Trop-I), creatine kinase-muscle/brain (CPK-MB) etc. [19]. Their use in conjunction with other clinical variables identifies the patients with a high risk of future cardiovascular events and may benefit in the clinical management of these particular illnesses [20].

BNP is a clinically useful and cost-effective diagnostic tool to measure the early risk of cardiovascular disease in patients with diabetes mellitus, hypertension, impaired renal function [21,22]. Troponins are also considered as key cardiac biomarkers to diagnose the myocardial damage. Several studies evident that, elevated levels of BNP and troponin I are observed in T2DM patients without any early clinical evidence of active cardiovascular disease [22–24]. The cumulative evidence through these biomarkers can provide an important prognostic value in patients who have an ongoing subtle myocardial injury.

Data from several pre-clinical and clinical studies suggest that hypertension and lipid-induced cardiomyopathies are characterized by contractile dysfunction and ventricular hypertrophy [25–27]. However, there is limited number of studies evaluating the cardiomyopathic status with co-existent diabetes. Thus, the unmet need for early detection and diagnosis of cardiovascular (CV) risk in T2DM patients is required to ensure adequate prevention of diabetic complications and to determine specific health targets [28].

In this backdrop, the present study aimed to evaluate the cardiomyopathy status in T2DM who live with or without different cardiovascular complications like hypertension and dyslipidemia by estimating different diagnostic cardiac biomarkers.

2. Material and methods

2.1. Study design

This cross-sectional study was conducted at the outpatient department (OPD) of Rajendra Memorial Research Institute of Medical Sciences-Indian Council of Medical Research (RMRIMS-ICMR) Patna, Bihar. The study protocol was approved by the Institutional Ethics Committee of Rajendra Memorial Research Institute of Medical Sciences (RMRIMS), Patna (Reference No.46/RMRI/EC/2017). The study was carried out in accordance with the declaration of Helsinki and its amendments [29]. From all study participants, written informed consent was obtained and were assured for their confidentiality and anonymity. The manuscript was written as per the STROBE guidelines for strengthening the reporting of observational studies in epidemiology [30].

2.2. Subject selection

A total of 125 participants were enrolled in this study including 25 healthy volunteers and 100 T2DM. On the basis of inclusion criteria, subjects were categorized into five groups such as, healthy volunteers (I), type 2 diabetes mellitus(II), T2DM + hypertension (III), T2DM + dyslipidemia (IV), T2DM + hypertension and dyslipidemia (V). Each group consists of 25 participants (Fig. 1).

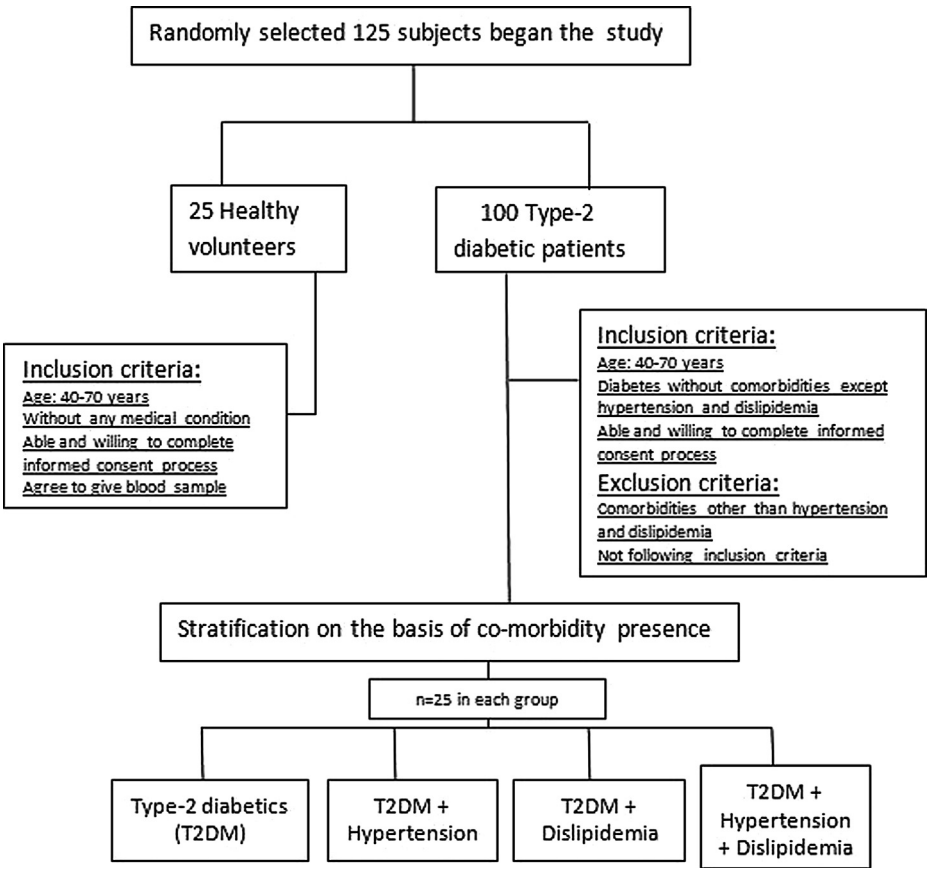

Fig. 1 – Flowchart of study population.

Participants with the age group of 40–70 years, who have confirmed T2DM i.e. with fasting blood sugar (FBS) level of ≥126 mg/dl and are already on anti-diabetic therapy from more than 1 year, were included. Blood pressure (BP) was measured in a sitting position after at least 5 min rest by experienced staff using an automatic digital device. Patients were considered hypertensive when systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg or using antihypertensive drugs [31]. Hyperlipidemia was defined by total cholesterol concentration of ≥200 mg/dL or using antilipidemic drugs. The participants with co-morbidities other than hypertension and dyslipidemia were excluded from the study. Patients who have not given their consent were also excluded.

2.3. Socio-demographic and anthropometric characteristics

All the relevant data were collected from the enrolled participants who attended the general outpatient department (OPD) via personal interview. The data includes socio-demographic details, such as gender, age, level of education, monthly income, family history of diabetes, addictions and other cardiovascular complications. Anthropometric characters were obtained by using standardized procedures at the time of the interview. A wall-mounted height measurement scale was used to measure height, nearest to 0.5 cm. Waist circumference (WC) was measured by standard tape nearest to inches in the horizontal plane just above the umbilicus. Body weight was measured to an accuracy of 0.1 kg and body mass index (BMI) computed as BMI = body mass (kg)/[height (m)]^2.

2.4. Clinical and biochemical assessment

After overnight fasting, venous blood (2 mL) was collected in serum and EDTA-Vacutainers (BD, USA) for biochemical measurements. American Diabetes Association (ADA) recommendations were followed to diagnose diabetes mellitus. Fasting blood glucose (FBG) level of ≥126 mg/dL after minimum 12-hour fasting and two-hour after meal glucose level (OGTT) of ≥200 mg/dL were considered as diabetic [32]. Blood glucose was estimated by using the commercially available kit (Meril, India) following manufacturer's instruction. BNP and Troponin-I were estimated by Alere Triage® cardio2 panel (Fluorescence immunoassay) using Alere Triage®MeterPro. Glycosylated haemoglobin (HbA1c) and lipid profile were estimated by using the Alere Afinion diagnostic kit with Afinion™ AS100 Analyser (Alere, USA). Other parameters like urea (GLDH-method), creatinine (Jaffe's kinetic method), zinc (Colorimetric method), calcium (Arsenazo III method) and albumin (BCG Dye method) were analysed by using commercially available kits by Microlab 300 clinical chemistry analyzer (Merck, Netherlands).

2.5. Sampling technique

Selection of study subjects was done by simple random sampling technique. The standardized statistical formulas were used to determine the sample size for quantitative variables [33]. The sample size was calculated on the basis of an assumed prevalence of 8.5% for T2DM in a general hospital setting of Bihar. On precision of 5% at a 95% confidence level the sample size of T2DM cases was found to be 117.

2.6. Assessment of other covariates

Covariates were analyzed at baseline of study for socio-demographic variables, anthropometric parameters, family history of diabetes, duration of diabetes and other complications (hypertension or dyslipidemia) [7]. The occupation was categorized into 3 categories as not working, labourer/farmer and businessman/employee. Educational qualification was grouped into primary, matriculate, intermediate, graduate and postgraduate. Monthly income was assessed by 4 groups as less than 5000, 5000–10,000, 10,000–20,000 and more than 20,000[INR]. The associated complications were divided into four groups as no complications, hypertension, dyslipidemia, and both hypertension and dyslipidemia.

2.7. Statistical analysis

Data were processed in Excel-sheet and analyzed by using the SPSS software (Version 21; CDC Atlanta, USA). Socio-demographic variables were analyzed by using descriptive statistics. Frequencies and percentages were compared for categorical variables using Pearson's chi-square (χ^2) test. The continuous variables were shown as mean ± Standard Deviation (SD) and compared by using the independent Student's *t*-test or one-way analysis of variance as appropriate. Furthermore, Pearson's correlation analysis was performed between BNP, troponin-I, calcium, zinc and continuous variables in order to assess the significant associations. All tests were two-tailed and differences were accepted as significant when the *P*-value was <0.05 [34].

3. Results

The results of the 125 participants are presented. Table 1 represents the socio-demographic characteristics of the study participants. The average age of the patients was found to be 55.04 ± 7.51 years. Male participants outnumbered the females in the diabetic (60%) and diabetic hypertensive (72%) groups respectively. Most of the patients were unemployed across all study groups. The non-vegetarian dietary habit was also dominating as high as 92% in diabetic group while a low percentage of 8% of the patients in the same group were vegetarians. A non-significant number of patients were addicted to alcohol whereas a countable number was addicted to tobacco chewing (36%) among both diabetic and diabetic hypertensive's. Education up to primary level was seen in the majority of participants (56%) in diabetic hypertensive with dyslipidemia. Duration of diabetes among all groups with diabetes had shown a similar median of 3 i.e. 3 to 6 years of diabetes history. The monthly income was predominantly less than 5000 INR in majority of the participants. Among participants, 44% of diabetic dyslipidemics and diabetic hypertensive with dyslipidemia had family history of diabetes.

The anthropometric measurements, biochemical and cardiac parameters of study participants are presented in Table 2. The BNP and calcium levels were significantly higher in diabetic hypertensive with dyslipidemia (Group V = 86.73 ± 64.49) than diabetic hypertensive's (Group III = 61.02 ± 53.69), followed by diabetic dyslipidemic (Group IV = 33.88 ± 33.71) and diabetic patients (Group II = 13.49 ± 11.67) (Fig. 2, Fig. 3), compared to healthy volunteers (Group I). Whereas, the serum zinc levels were found vice versa to the above order of concentrations (Fig. 4). Meanwhile, there was no statistically significant difference ($p = 0.269$) between troponin-I levels in all the study groups. A significant difference of HbA1c levels was observed in Group 5 ($p < 0.001$) and Group IV ($p = 0.033$), when compared to diabetic group, indicating that cardiovascular comorbidities with diabetes mellitus negatively impact glycemic status.. CHO/HDL-ratio shown a significant difference when diabetic group was compared with Group V ($p = 0.016$) and then with Group IV ($p = 0.028$) (see Fig. 5).

Furthermore, Pearson correlation was performed to established the association between HbA1c, blood pressure and lipid profile with different cardiac biomarkers depicted in Table 3. HbA1c showed a positive association with calcium and BNP levels while a negative association was observed with zinc levels. There was, no association observed between HbA1c and Troponin-I levels. Blood pressure also showed a positive correlation with BNP, calcium and Troponin-I whereas a negative correlation was found with zinc. Total cholesterol and triglyceride levels were not significantly correlated with any of these cardiac markers however, the CHO/HDL-ratio showed a positive correlation with serum calcium levels.

4. Discussion

The incidence of diabetes is rising at a rapid pace and the prevalence of heart failure among diabetic patients is also escalating worldwide. The present findings highlight the importance of research in this particular area to develop potential strategies and to overcome such pandemic conditions. To the best of our knowledge, this is the first study carried out to demonstrate the myocardial injury status by estimating different cardiac markers in patients with T2DM with and without CVD comorbidities. Although the estimated biomarkers are usually targeted for suspected cardiac patients, there is a growing body of evidence showing that the elevated levels of these may predict the ongoing myocardial damage in diabetes patients with or without cardiovascular comorbidities [21,23,35]. Our results revealed that periodic estimation of the circulating markers like BNP, troponins, calcium and zinc could be an important tool to predict the risk of developing cardiovascular complications. In patients with T2DM, cardiovascular complications are the main determinants of morbidity and

Table 1 – Socio-demographic characteristics of different study population groups.

Variables n (%)	Group I (n = 25)	Group II (n = 25)	Group III (n = 25)	Group IV (n = 25)	Group V (n = 25)
Gender					
Male	12(48)	15(60)	18(72)	10(40)	11(52.8)
Female	13(52)	10(40)	7(28)	15(60)	14(47.2)
Occupation					
Unemployed	12(48)	11(44)	11(44)	16(64)	13(52)
Labourer/farmer	3(12)	4(16)	3(12%)	1(4)	3(12)
Businessman/employee	10(40)	10(40)	11(44)	8(32)	9(36)
Diet					
Vegetarian	4(16)	2(8)	6(24)	5(20)	9(36)
Non-vegetarian	21(84)	23(92)	19(76)	20(80)	16(64)
Religious belief					
Hindu	21(84)	22(88)	23(92)	25(1 0 0)	23(92)
Muslim	4(16)	3(12)	1(4)	0	1(4)
Other	0	0	1(4)	0	1(4)
Addiction					
No addiction	20(80)	15(60)	14(56)	20(80)	17(68)
Tobacco chewing	4(16)	9(36)	9(36)	5(20)	6(24)
Alcohol	0	0	1(4)	0	0
Multiple	1(4)	1(4)	1(4)	0	2(8)
Education					
Primary	12(48)	12(48)	9(36)	8(32)	14(56)
Matriculation	5(20)	4(16)	6(24)	10(40)	8(32)
Intermediate	1(4)	4(16)	3(12)	2(8)	1(4)
Graduation	3(12)	3(12)	7(28)	3(12)	2(8)
Post graduation	4(16)	2(8)	0	2(8)	0
Duration of diabetes*					
Mean ± SD	0	2.72 ± 1.13	3.28 ± 1.48	2.84 ± 1.21	3.32 ± 1.46
(Median)	(0)	(3)	(3)	(3)	(3)
Income (monthly in INR)					
<5000	8(32)	13(52)	12(48)	9(36)	16(64)
5000–10,000	9(36)	4(16)	8(32)	4(16)	3(12)
10,000–20,000	0	3(12)	3(12)	9(36)	1(4)
>20,000	8(32)	5(20)	2(8)	3(12)	5(20)
Family history of diabetes					
No history	21(84)	16(64)	17(68)	14(56)	14(56)
Primary relatives	4(16)	9(36)	8(32)	11(44)	11(44)

Abbreviations: SD = standard deviation, Group I = Healthy volunteers, Group II = Diabetic patients, Group III = Diabetic hypertensive patients, Group IV = Diabetic dislipidemic patients, Group V = Diabetic hypertensive with dislipidemia patients.

* (1 = 6 months to 1 year, 2 = 1 to 3 years, 3 = 3 to 6 years, 4 = 6 to 9 years, 5 = >9 years).

mortality and simultaneously counted as main contributors to the treatment costs. There are several factors such as abdominal obesity, hypertension, hyperlipidemia etc. which promotes cardiovascular complications [36].

BNP and troponins are released from the myocardium in response to myocardial injury, increased pressure, cardiac pump dysfunction, ventricular hypertrophy due to hypertension [37]. In our study, we observed that BNP is significantly elevated in those diabetic individuals who have coexistent cardiovascular complications including hypertension and dyslipidemia when compared to diabetic control. Our findings are in line with several previous studies who have reported a significant association between plasma BNP and increased CV risk events [38,39]. In addition, our results are also supporting the evidence from the study conducted by Masayuki Onodera and colleagues suggested that BNP (50 pg/mL) could be a signal of increased cardiovascular risk among diabetic patients [40]. Another study conducted by Clerico et al. has proposed that under physiological conditions, the normal BNP level should be considered as 30 pg/mL [41]. The TnI, levels were found non-significant across all the study groups In contrast to our study, data from the previous study reported that TnI levels were significantly higher in patients with diabetes having coexisted cardiovascular complications resulting from cardiac injury [42,43]. Prospective studies have reported that, diabetic patients often exhibit electrolytic imbalance for characteristic ions like potassium, magnesium, zinc, phosphate and calcium [18]. The current study found a predominant deficiency of serum zinc levels when comorbidities started to coexist. Our findings support the results from Ye Song

Table 2 – Anthropometric, biochemical and cardiac markers of different study population groups.

Variables Mean ± SD p-value	Group I (n = 25)	Group II (n = 25)	Group III (n = 25)	Group IV (n = 25)	Group V (n = 25)	All Participants (n = 125)	P
Age (years)	55.12 ± 5.04	55.20 ± 7.26 0.964[a]	54.32 ± 7.14 0.650[b] 0.668[e]	53.24 ± 8.91 0.363[c] 0.398[f]	57.32 ± 8.55 0.272[d] 0.350 [g]	55.04 ± 7.51	0.413
Weight (kg)	61.32 ± 8.85	65.00 ± 10.82 0.195[a]	63.36 ± 10.28 0.456[b] 0.585[e]	60.96 ± 8.21 0.182[c] 0.144[f]	64.24 ± 16.79 0.446[d] 0.850 [g]	62.98 ± 11.33	0.658
Height (cms)	154.80 ± 7.69	157.04 ± 6.52 0.272[a]	155.28 ± 9.14 0.842[b] 0.437[e]	154.16 ± 6.97 0.759[c] 0.138[f]	153.32 ± 8.28 0.516[d] 0.084 [g]	154.92 ± 7.75	0.526
BMI (kg/m^2)	25.56 ± 2.18	26.48 ± 4.51 0.364[a]	26.32 ± 3.88 0.401[b] 0.891[e]	25.62 ± 2.79 0.942[c] 0.418[f]	27.08 ± 5.35 0.196[d] 0.672 [g]	26.21 ± 3.89	0.623
W.C (inches)	35.28 ± 2.15	34.40 ± 2.38 0.177[a]	35.60 ± 2.70 0.646[b] 0.103[e]	34.96 ± 2.31 0.615[c] 0.404[f]	36.32 ± 3.72 0.233[d] 0.035 [g,*]	35.31 ± 2.74	0.145
Systolic pressure (mmHg)	127.60 ± 9.25	123.20 ± 5.56 0.047[a*]	145.20 ± 12.28 <0.001[b,*] <0.001[e,*]	125.20 ± 5.09 0.262[c] 0.192[f]	146.40 ± 10.36 <0.001[d,*] <0.001 [g,*]	133.52 ± 13.45	<0.001
Diastolic pressure (mmHg)	82.40 ± 6.63	80.80 ± 4.00 0.307[a]	91.20 ± 5.26 <0.001[b,*] <0.001[e,*]	82.80 ± 5.41 0.816[c] 0.144[f]	90.40 ± 4.54 <0.001[d,*] <0.001 [g,*]	85.52 ± 6.77	<0.001
HbA1c (%)	5.56 ± 0.46	7.76 ± 1.76 <0.001[a,*]	8.01 ± 2.09 <0.001[b,*] 0.653[e]	9.08 ± 2.41 <0.001[c,*] 0.033[f,*]	9.97 ± 2.34 <0.001[d,*] <0.001 [g,*]	8.07 ± 2.43	<0.001
FBG (mg/dL)	88.76 ± 13.30	156.24 ± 54.10 <0.001[a,*]	155.00 ± 71.35 <0.001[b,*] 0.945[e]	207.00 ± 89.71 <0.001[c,*] 0.019[f,*]	229.48 ± 113.61 <0.001[d,*] 0.005 [g,*]	167.30 ± 89.65	<0.001
2 h AMG (mg/dL)	116.48 ± 22.89	227.92 ± 98.27 <0.001[a,*]	258.20 ± 100.36 <0.001[b,*] 0.287[e]	292.52 ± 129.01 <0.001[c,*] 0.050[f,*]	321.60 ± 136.16 <0.001[d,*] 0.008 [g,*]	243.34 ± 125.66	<0.001
T. Cho. (mg/dL)	167.56 ± 31.08	154.92 ± 32.23 0.165[a]	153.04 ± 25.83 0.079[b] 0.821[e]	204.52 ± 53.57 0.004[c,*] <0.001[f,*]	200.88 ± 45.09 0.004[d,*] <0.001 [g,*]	176.18 ± 44.33	<0.001
Triglyceride (mg/dL)	148.08 ± 74.20	110.04 ± 44.97 0.033 [a,*]	105.60 ± 35.68 0.014[b,*] 0.701[e]	217.80 ± 109.21 0.011[c,*] <0.001[f,*]	196.00 ± 94.17 0.050[d,*] <0.001 [g,*]	155.50 ± 88.15	<0.001
HDL (mg/dL)	44.76 ± 14.19	45.28 ± 15.95 0.904[a]	45.40 ± 22.58 0.905[b] 0.983[e]	53.32 ± 25.84 0.153[c] 0.192[f]	50.20 ± 22.84 0.317[d] 0.382 [g]	47.79 ± 20.70	0.506
LDL (mg/dL)	101.68 ± 26.06	96.92 ± 27.40 0.532[a]	95.12 ± 24.30 0.362[b] 0.807[e]	114.72 ± 34.29 0.137[c] 0.048[f,*]	112.92 ± 43.24 0.271[d] 0.125 [g]	104.27 ± 32.35	0.095

Table 2 – (continued).

Variables Mean ± SD p-value	Group I (n = 25)	Group II (n = 25)	Group III (n = 25)	Group IV (n = 25)	Group V (n = 25)	All Participants (n = 125)	P
Non HDL (mg/dL)	120.16 ± 24.82	114.84 ± 31.17 0.508[a]	112.56 ± 24.58 0.282[b] 0.775[e]	145.84 ± 36.46 0.005[c,*] 0.002[f,*]	150.24 ± 45.58 0.006[d,*] 0.002 [g,*]	128.73 ± 36.64	<0.001
CHO/HDL-ratio	3.82 ± 0.89	3.74 ± 1.04 0.671[a]	3.79 ± 0.95 0.903[b] 0.855[e]	4.36 ± 0.88 0.039[c,*] 0.028[f,*]	4.64 ± 1.46 0.021[d,*] 0.016 [g,*]	4.07 ± 1.11	0.080
Zinc (μg/dL)	73.96 ± 21.91	59.49 ± 11.33 0.005[a,*]	56.15 ± 9.64 0.001[b,*] 0.267[e]	58.10 ± 10.05 0.002[c,*] 0.647[f]	52.72 ± 12.16 <0.001[d,*] 0.047 [g,*]	60.08 ± 15.41	<0.001
BNP (pg/mL)	5.54 ± 1.49	13.49 ± 11.67 0.001[a,*]	61.02 ± 53.69 <0.001[b,*] <0.001[e,*]	33.88 ± 33.71 <0.001[c,*] 0.006[f,*]	86.73 ± 64.49 <0.001[d,*] <0.001 [g,*]	40.13 ± 50.27	<0.001
Calcium (mg/dL)	9.48 ± 1.10	9.86 ± 1.43 0.293[a]	10.92 ± 1.63 0.001[b,*] 0.019[e,*]	10.63 ± 1.87 0.011[c,*] 0.112[f]	11.36 ± 1.93 <0.001[d] 0.003 [g]	11.20 ± 8.63	<0.001
Troponin-I (ng/mL)	0.01 ± 0.00	0.01 ± 0.00 1.000[a]	0.01 ± 0.04 0.322[b] 0.322[e]	0.01 ± 0.00 1.000[c] 1.000[f]	0.02 ± 0.06 0.157[d] 0.157 [g]	0.01 ± 0.03	0.269

Abbreviations: BMI = body mass index, W.C = waist circumference, HbA1c = glycated haemoglobin, FBG = fasting blood glucose, 2 h AMG = 2 h after meal glucose T. Cho = total cholesterol, HDL = high density lipoprotein, LDL = low density lipoprotein, CHO/HDL-ratio = total cholesterol high density lipoprotein ratio, BNP = brain natriuretic peptide, $p < 0.05$ is taken statistically significant.

[a] Healthy subjects versus diabetic patients.
[b] Healthy subjects versus diabetic hypertensive patients.
[c] Healthy subjects versus diabetic dislipidemic patients.
[d] Healthy subjects versus diabetic hypertensive dislipidemic patients.
[e] Diabetic versus diabetic hypertensive.
[f] Diabetic versus diabetic dislipidemic patients.
[g] Diabetic versus diabetic hypertensive dislipidemic patients.
[*] Indicates statistically significant differences.

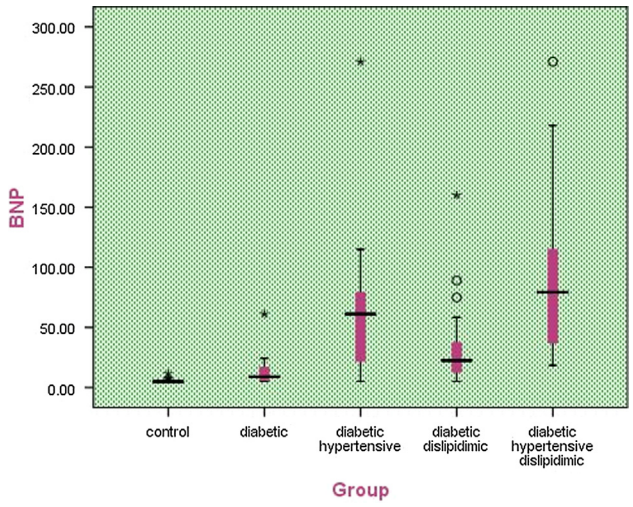

Fig. 2 – Plasma B-type natriuretic peptide (BNP) levels of study groups.

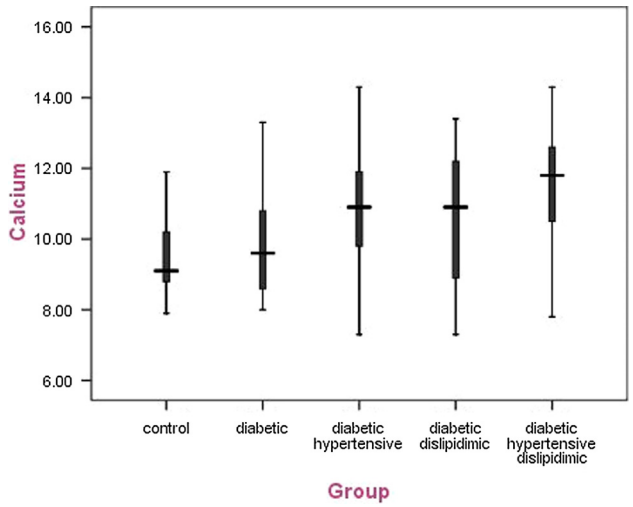

Fig. 3 – Calcium concentration of study groups.

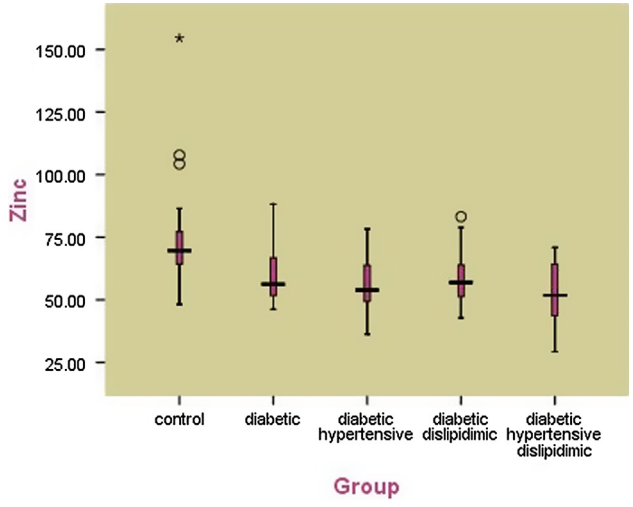

Fig. 4 – Zinc concentration of study groups.

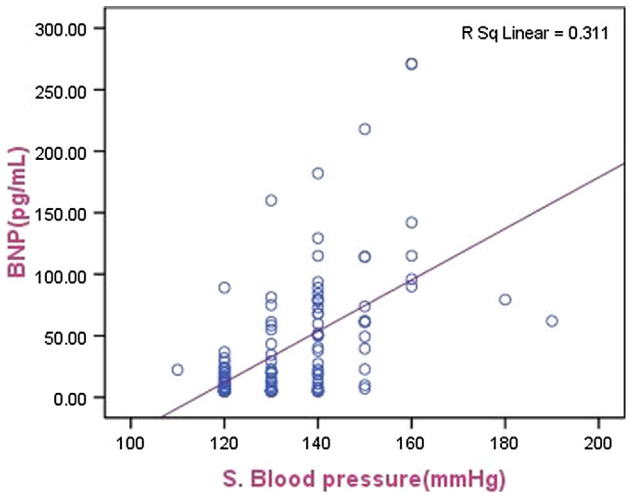

Fig. 5 – Corelation between BNP and Systolic Blood pressure.

et al. and Soinio et al. who have reported that zinc deficiency increases the susceptibility of oxidative damage to the heart and may turn to cardiac events [44,45]. Other epidemiological studies have also shown that Zn supplementation has a preventive role and delays the diabetic cardiomyopathy in diabetic patients [46]. The disturbed calcium metabolism in diabetes strongly affect tissues like heart, skeletal muscle and pancreas so complicate the on-going cardiovascular disorder status. In our study, we found an elevated serum calcium level resulting into low intracellular calcium. This elevation in serum calcium levels have also been linked to hypertension in earlier studies [47]. Anastassios G. Pittas et al. have performed a systematic review and *meta*-analysis and the results were not surprising, they have also reported the significant association between calcium and the risk of diabetes [48].

HbA1c was significantly higher in diabetic patients comorbid with both hypertension and dyslipidemia compared to diabetic hypertensive subjects, thereby extending the literature that, diabetic status worsens with cardiovascular complications [7]. Similarly, the previous studies confirmed our results by showing elevation of plasma BNP and serum calcium levels, whereas reducing the serum zinc concentration in diabetics with coexistent cardiovascular complications [12,21,48]. In contrary to the previous reports, this study reported a non-significant plasma Tn-I levels in all the study groups [23], which supports the fact that TnI as a diagnostic marker may actually predict the acute cardiac injury rather than diagnose the on-going subclinical cardiomyopathy. This study also attempted to assess the relationship between different clinical characteristics and cardiac biomarker. Results from the study found that, HbA1c was positively associated with serum calcium ($r = 0.473$; $p < 0.001$) and BNP ($r = 0.306$; $p < 0.001$). These findings are similar with previous research demonstrating that hyperglycemia is associated with intracellular calcium deficiency and increased level of BNP [48,20]. In addition, this study observed a significant negative association ($r = -0.480$; $p < 0.001$) between HbA1c and zinc, which confirms earlier results reported by Soinio et al. [45]. In that study report, they have predicted high cardiac events

Table 3 – Pearson correlation coefficient between HbA1c, blood pressure and lipid profile with cardiac biomarkers of different study population groups.

	BNP	Troponin-I	Calcium	Zinc
HbA1c (%)	r = 0.306**	r = 0.055	r = 0.473**	r = −0.480**
	p < 0.001	p = 0.544	p < 0.001	p < 0.001
Systolic BP (mmHg)	r = 0.558**	r = 0.240**	r = 0.278**	r = −0.225*
	p < 0.001	p = 0.007	p = 0.002	p = 0.012
Diastolic BP (mmHg)	r = 0.530**	r = 0.267**	r = 0.331**	r = −0.249**
	p < 0.001	p = 0.003	p < 0.001	p = 0.005
Total cholesterol (mg/dL)	r = 0.163	r = 0.017	r = −0.084	r = −0.055
	p = 0.069	p = 0.852	p = 0.354	p = 0.540
Triglyceride (mg/dL)	r = 0.012	r = −0.087	r = 0.076	r = −0.055
	p = 0.895	p = 0.333	p = 0.401	p = 0.545
CHO/HDL-ratio	r = 0.114	r = 0.011	r = 0.267**	r = −0.092
	p = 0.204	p = 0.900	p = 0.003	p = 0.308

Abbreviations: r = Pearson correlation coefficient, p < 0.05 is taken statistically significant.
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

in diabetics who have relatively low plasma Zn than normal. Meanwhile, we found a non-significant association between HbA1c and Troponin-I, instead of accepting the fact that sub-clinical elevations of TnI occur in patients with type 2 diabetes mellitus reported by Ehimen Phyllis Odum et al. [42]. Therefore for a confirmed diagnosis, other highly sensitive cardiac predictors need to be considered. Blood pressure measurements have also shown a significant positive correlation with BNP, calcium and Troponin-I whereas a negative relationship with zinc. The results are supporting the fact that, hypertension deteriorates the subclinical cardiomyopathy in diabetics reported by Francisco Arrieta et al. who have recently updated the recommendations for diabetes and cardiovascular disease in Spain. Francisco Arrieta et al. documented that, the total cholesterol and triglyceride were not significantly related to any of these cardiac markers ($p > 0.05$). However, in difference with the previous literature, they have recommended that hyperlipidemia is a key pathogenic factor to develop cardiovascular injury [49]. To summarize, our findings suggest that discussed biomarkers should be considered for the early diagnosis of cardiomyopathy, especially in the non-acute cases to predict an expected cardiovascular disorder [50].

4.1. *Strengths and limitations*

There are several strengths and limitations in our study that are worth to mention. Strengths included; first, this study has been carried out in an economically less developed region of India where the healthcare facilities are not meeting the necessary medical needs of the population. Second, the study groups involved may represent the real-world situation as the random sample selection procedure was followed. Third, the present study has estimated multiple biomarkers simultaneously to strengthen the predictive value of diagnostic approach. Our study has several limitations; first, due to the small sample size in each class of diabetic co-morbidity, we couldn't have performed the sub group analysis on the basis of age to determine the impact of age on cardiomyopathy status. Second, this study involves one-point measurement of biomarkers for risk stratification which may add some bias in these findings. Therefore more number of longitudinal cohorts is needed to provide additional information.

5. Conclusion

Early detection and diagnosis of cardiovascular (CV) risk in T2DM patients is necessary to ensure adequate prevention of diabetic complications. BNP and calcium levels were significantly elevated in patients with T2DM having coexistent cardiovascular complication however, zinc levels were significantly reduced. The study also shown positive correlation between blood pressure and BNP, calcium, Troponin-I levels. Lipid profile have not shown any significant correlation with BNP, Troponin-I and zinc. Therefore, further longitudinal studies are required to confirm these findings.

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Declaration of Competing Interest

There is no known conflict of interest associated with this publication.

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Authors Contribution

Conceived and designed the experiments: AB, KM, KP.
Performed the experiments: AB.
Analyzed the data: AB.
Wrote the paper: AB, MA, IR, KM, KP

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.06.012.

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