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DIAGNOSTIC ROLE OF NT PRO BNP IN DIABETES TYPE 2 PATIENTS ASSOCIATED WITH CARDIOVASCULAR DISEASE RISK, A STUDY FROM CENTRAL INDIA

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Abstract

Cardiovascular disease is the most frequent cause of death in patient with diabetes. It is difficult to evaluate cardiovascular status of patients with diabetes because of complex symptomatology. NTproBNP, a split peptide from pro BNP molecule is a novel biomarker, released from cardiac myocytes in response to myocardial stretch, cardio vascular disease, endothelial dysfunction and heart failure. We aimed to test that is elevated NTproBNP levels associated with increased risk of cardiovascular disease in diabetes patients in comparison to matched control. Demographic, anthropometric measure, NT pro BNP, lipid profile, blood glucose were estimated and compared among angiographically proven cardiovascular disease patients with diabetes and healthy controls. Univariate and multivariate analysis were carried out to compare individual factor using t-Test, ANOVA and the inter group comparisons were done by using Bon ferroni Post Hoc test. Patients with type 2 diabetes were shown to have higher NTproBNP values (n=50, 1481.021±813.405) than control subjects (n=50, 23.562±23.395) (p <0.05). NTproBNP levels were independently related to diabetes after adjustment for age, sex, family history, smoking, obesity, blood pressure and lipid profile. Our data suggests that the secretion of NT pro BNP is increased in type II diabetes patients, suggesting association of diabetes and NTproBNP in cardio vascular disease with higher prevalence. Thus NTproBNP may serve as a screening tool to diagnose patients with type II diabetes with cardiovascular disease having complex symptomatology.

Keyword: *NTproBNP, Cardiovascular disease, Diabetes*

Introduction

With over 20 million diabetes subjects, India leads the world in the number of individual with diabetes.[2] Diabetic subjects have two or more fold higher risk of CVD compared to non diabetes population[3] but it is difficult to evaluate the CVD status of patients with diabetes because of complex symptomatology. The most evident cardiac complication is coronary atherosclerosis which appears clinically earlier and is more generalized in diabetic subjects.[4] Diabetes is also more prevalent among patients of heart failure.[5] Autopsy studies have suggested that heart from patients with diabetes have increased collagen content. Moreover patients with diabetes have a disproportional increase in left ventricular mass independent of blood pressure.[6- 8] All these factors may contribute to increased myocardial stiffness, higher cardiac morbidity and mortality in patients with diabetes.[9] Cardiovascular death accounts for ~ 70% of the deaths among subjects with diabetes. Evaluation of major coronary risk factors in Indian population undergoing angiography has shown that in about one third patients no major factors are detectable.

Brain natriuretic peptide (BNP) is a 32-amino acid peptide.[10] It is synthesized predominantly in the left ventricle of the heart as proBNP, a 108-amino acid

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peptide which is cleaved by a protease into its biologically active form BNP and NTproBNP, the 76-amino acid biologically inactive amino portion of proBNP.[11] Compared to BNP, NTproBNP has a longer half life than the active form BNP (60 to 120 min vs 15 to 20 min). The hormone is a potent vasodilator and a natriuretic factor regulating salt and water homeostasis. Its increased secretion occurs mainly with increased tension in the ventricular walls, cardiovascular disease including ischemia, arrhythmia, fibrosis, cardiac hypertrophy and coronary endothelial dysfunction. as several studies suggested that elevated NTproBNP levels represents a final common pathway for many cardiovascular pathologic states and it can be used as a biomarker of cardiac disease and associated pathologic states.[12,13] Keeping this aim, present study was conducted to evaluate role of NTproBNP in diabetes associated CVD risk in comparison to matched controls in population of central India.

Material and Methods

Patients included in the present study were all admitted to the intensive coronary care unit (ICCU) or attending the OPD of medicine medical college and hospital. Consecutive 100 patients undergoing coronary angiography at our hospital over a period of 1 year were included in the study. The diagnosis of CVD was made on the basis of clinical history and 12–lead standard electro diagram (ECG) before subjecting them to coronary angiography. The presence of any diameter stenosis ≥ 30% according to coronary angiography by visual assessment of coronary artery was included in the study. 100 matched subjects from medicine OPD and Blood Bank with no history of CVD or with normal electrocardiogram (ECG), were selected for the study. Previous histories of diabetes, smoking, HTN were noted. Informed consent was obtained from patients and controls of both groups. Subjects with kidney disorders, nephropathy and dyspnea were excluded.

In our study, smoking was defined as regular smoking of cigarettes/beedies. Diabetes mellitus was diagnosed on the basis of fasting blood glucose concentration of ≥126mg/dl or a patient already on anti-diabetic medications. Systemic hypertension was considered to be present if the patient was taking anti-hypertensive treatment at the time of hospital admission or if blood pressure was recorded ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic[16], at least twice on examination during admission. A positive family history of CVD was defined as first degree relative that had documented CVD <55 years in males or <65 years in females. For lipid analysis, samples were obtained after an overnight fast. Patients whose body mass index is ≥25 kg/m^2 were considered as obese[17]. Patients who had serum concentration of total cholesterol (TC) ≥ 240 mg/dl, or triglyceride (TG) ≥300 mg/dl, or low-density lipoprotein cholesterol (LDL-C) ≥160 mg/dl or high-density lipoprotein cholesterol (HDL-C) ≤40.0mg/dl or very-low-density lipoprotein cholesterol (VLDL-C) ≥40.0 are considered as hyperlipidemics. NTproBNP levels ≥125.0 pg/ml were considered as higher or increased risk.[18, 19]

Venous blood was collected from all subjects after 12 hour overnight fasting. Serum was separated by low-speed centrifugation. The samples were stored at -20°C for prior analysis. Laboratory analysis was done in following ways—

1. Lipid profile done on fully automatic analyzer using a)Total cholesterol estimated by enzymatic, CHOD/PAP method Supplied by Roche Diagnostic Ltd. b)Triglyceride estimated by enzymatic, GPO/PAP method Supplied by Roche Diagnostic Ltd. c)High density lipoprotein estimated by enzymatic, CHOD/PAP method Supplied by Roche Diagnostic Ltd. d)Low density lipoprotein estimated by enzymatic, CHOD/PAP method Supplied by Roche Diagnostic Ltd. e)Very low density lipoprotein estimated by enzymatic, CHOD/PAP method Supplied by Roche Diagnostic Ltd.
2. Fasting blood sugar estimation done on fully automatic analyzer by using enzymatic assay kit.
3. NT-pro BNP was estimated on Elecsys 2010 fully automated immunoassays system by using pro BNP reagent kit, supplied by Roche Diagnostic Ltd.

Statistical analysis was performed in statistical software pack SPSS 11.5. Demographic data were initially described as mean ± SD value of groups. Significance of variance between groups was tested by student t -Test with $p < 0.05$ considered as Statistical significant. ANOVA and the inter compression were done by using Bon ferroni Post Hoc test. Present work was approved by institutional research and ethical committee.

Results

We enrolled a total of 200 subjects. Table I show the findings of various demographic parameters and clinical parameters of the subjects, of them 100 were diabetic, 100 were non-diabetic subjects. Patients with diabetes had a higher level of NTproBNP than population without diabetes (Figure-1).

Patients were divided in to four groups (1) 50 had diabetes mellitus and cardiovascular disease, grouped as diabetes with cardiovascular disease or DwCVD. (2) 50 had cardiovascular disease without diabetes mellitus, grouped as non diabetes with cardiovascular disease or NDwCVD. (3) 50 had diabetes mellitus without cardiovascular disease, grouped as diabetes control or Dcontrol. (4) 50 had no diabetes mellitus and cardiovascular disease were grouped as Control. Logistic regression analysis for risk factors versus CVD (DwCVD as a dependent variable) was done to assess the relative risk of development of CVD with each risk factor between DwCVD and control subjects (Table II).

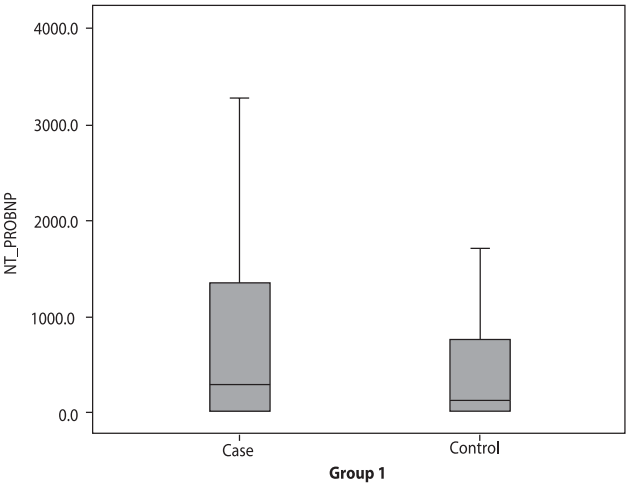

Fig.-1: *Distribution of NTproBNP values in the patient (n=100) and control (n=100) cohorts. P<0.001 for difference between groups.*

Table I

Demographic data (Mean ± SD) for the study population

Factor	DwCVD (n=50)	NDwCVD (n=50)	DControl(n=50)	Control (n=50)
Age	61.32±10.19	60.36±10.52	52.02±9.47	38.56±5.35
BMI	21.86±2.83	22.56±3.06	25.43±3.45	24.50±3.56
BPS	132.22±8.90	129.42±7.73	124.72±3.35	122.76±2.96
BPD	85.90±5.78	84.08±6.80	78.80±8.13	80.36±4.36
FS	175.66±25.76	85.50±11.49	176.32±16.51	82.30±12.60
TC	287.86±27.35	255.24±26.04	165.26±22.84	148.64±29.76
TG	278.86±46.87	173.46±67.92	183.10±65.20	167.28±68.32
HDL	28.44±9.02	29.48±10.38	43.28±7.34	42.40±9.20
LDL	166.36±23.26	151.00±34.35	110.08±29.29	92.08±22.72
VLDL	45.54±12.19	43.08±19.62	34.74±9.19	32.30±13.25
NTproBNP	1481.021±813.405	704.062±359.269	37.558±30.727	23.562±23.395

Table II

Study of selected risk factor in studied groups.

Factor	Particular**s**	DwCVD[a] (n=50)	Control[d] (n=50)	p-value	95% CI
FS	≥ 126mg/dl	50	0	<0.05	84.02-102.70
TC	≥ 240.0 mg/dl	49 (98%)	0	<0.05	124.75-153.13
TG	≥ 300.0 mg/dl	50	09 (18%)	<0.05	78.15-145.01
HDL	≤ 40.0 mg/dl	27 (54%)	04 (08%)	<0.05	18.79-9.13
LDL	≤ 160.0 mg/dl	37 (76%)	0	<0.05	59.36-89.20
VLDL	≥ 40.0 mg/dl	32 (64%)	13 (26%)	<0.05	5.73-20.75
NTproBNP	≥ 125.0 pg/ml	50	0	<0.05	1220.218-1694.700

Table III

Post Hoc Tests: Bonferroni (NTproBNP)

					95% CI	
	Group	Mean Difference	Std. Error	p<0.05	Lower Bound	Upper Bound
DwCVD	CONTROL	1457.459(*)	89.0108	.000	1220.218	1694.700

*The mean difference is significant at the .05 level.

DwCVD Patients had considerable higher mean value of NTproBNP (1481.021±813.405) than control subjects NTproBNP (23.562±23.395) ($p < 0.05$).

Each group was consisting higher proportion of males. DwCVD group is having mean age 61.32 ± 10.19 years respectively as compared to control group having mean age of 38.56 ± 5.35 years. The mean age was found to be significantly higher for DwCVD group compared with control. DwCVD group also showed a considerably high proportion of smoker, hypertension (raised diastolic blood pressure), fasting glucose, raised blood lipids and positive family history (Table II).

Blood lipids of DwCVD observed significantly higher values compared to their counterparts, diabetes has a direct relation with DwCVD as compared to control. The Inter group Comparisons using the Bonferroni Post Hoc test showed considerably higher NTproBNP values in DwCVD group compared with control (Table III).

A multivariate regression analysis considering age, sex, BMI, BPS, BPD, fasting glucose, TC, TG, HDL, LDL, VLDL, FH, smoking, NTproBNP as independent variable was applied. The finding showed advanced age, raised BMI, hypertension, fasting glucose, TC, TG, decreased HDL, NTproBNP and smoking had significant association while sex and family history were insignificant.

An attempt was also made to see any linear relationship in age and NTproBNP using the regression method but no such linearity was found. Thus there is no positive linear trend of increase in age and raised values of NTproBNP.

Discussion

Increased secretion of BNP and NTproBNP occurs mainly with increased tension in the ventricular walls, decreased oxygen supply, acute myocardial infarction, chronic cardiac heart failure and hypertrophy of the heart.[12, 13] NTproBNP is a split product from the BNP and it is more stable, circulating concentration is not dependent on the receptor population and it is solely eliminated through glomurular filtration.

In our study NTproBNP levels were shown to be significantly elevated in cohort of patients with diabetes particularly. However, patients with diabetes had a higher BMI, systolic, diastolic blood pressure, increased total cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein than the control subjects, which might have confounded our results. The results of multivariate regression analysis suggest NTproBNP as an independent variable associated with CVD, even when the above mentioned possible confounders were taken into account. Studies in Asian communities conducted in UK have shown that obesity, type II diabetes, lower HDL and increased TG concentration were important risk factors for CVD in this racial group.[20, 21] Several Indian studies had shown strong association of CVD with diabetes compared to non diabetes population.[22, 23] Study of Bibbins-Domingo K and Omland T had convincingly demonstrated that circulating NTproBNP levels were increased in CVD, Charoltte shows similar findings and suggested NTproBNP a screening marker and increased risk predictor of CVD in diabetes giving a strong base to our study. [24-26]

There are several possible explanations for elevated NTproBNP levels in patients with diabetes as they have a higher prevalence of diastolic dysfunction as our result shows or have more peripheral and distal atherosclerotic changes in the coronary tree due to increased blood lipids. Hearts from patients with diabetes have increased collagen content, as have been verified in autopsy studies and it is proposed that natriuretic peptide synthesis increases by the same mechanism that transform cardiac fibroblast into a collagen-secreting cell. [27] Another possible mechanism working from the very start of diabetes could be decreased relaxation of the myocardium

because of ATP deficiency. The intracellular glucose deficiency among patients with diabetes leads to a higher use of free fatty acids through beta oxidation in the myocardium. A sufficient amount of carbohydrate break down is of great importance for assuming an adequate function of the ion pumps, meaning Na^+/K^+- ATPase and Ca^{2+} - ATPase , which maintains the right cardiomyocytes membrane potential and intracellular Ca+ transport, that triggers relaxation. In the diabetes heart, this balance is disturbed, proposing a functional explanation to the impaired relaxation in the myocardium.[28, 29, 30, 31] These effects could be so strong that it would over run the age related effect of NTproBNP in diabetes with CVD patients as it has been suggested that NTproBNP is more age sensitive, but in our study there is no such relation was found. Thus, NTproBNP might be especially useful for screening CVD risk in diabetes subjects independent of age effect. [32]

Studies had shown increased intimal media thickness (IMT), endothelial dysfunction (ED) and arterial stiffness in diabetic subjects compared to non diabetic subjects.[33, 34] Increased IMT, ED and arterial stiffness leads to increase in myocardial stretch resulting increased NTproBNP secretion in circulation as a compensatory mechanism.

Increased blood lipids level are well known to associate with CVD; association of diabetes makes it more complicated and increases the risk of having coronary atheromatosis, increased myocardial stretch and increased secretion of NTproBNP.

Our study shows increased levels of NTproBNP in smokers. Smoking is established risk factor for CVD and it affects the vascular endothelium, lipid peroxidation, decreased antioxidant level and other associated complications causes increased stress on vessels and heart, resulting increase in concentration of NTproBNP. Smoking impairs sympathovagal balance and decreases the heart rate variability in a normal human being, even a single cigarette smoking leads to overt sympathetic excitation, change in adrenergic nervous system and results in an increased in NT pro BNP seceration from myocytes.[35]

This study has its inherent disadvantage of being only a one time observation of each individual. Still the study was able to detect a difference in levels of NTproBNP between two groups and it could be used as a screening tool to separate patients with diabetes eligible for an angiographical examination. Our study has certain strengths, both cases and controls were drawn from the same catchment area representing a fairly homogeneous population with minimal migration. The hospital based design was optimal for our study, because cases and controls were similarly sensitized towards recalling exposure information. We performed multivariate analysis to adjust for other potential confounders. We avoided misclassification of disease status by identifying case according to established criteria. Diabetes and CVD are major socioeconomic burden for the presenting geographical location, evaluation of earlier and specific risk predictor of CVD like NTproBNP will help in reduction and treatment of CVD in diabetes population as our results suggests. In conclusion, we may state that secretion of NTproBNP is increased in patients with type 2 diabetes with CVD as compared to control subjects. Therefore measurement of NTproBNP might be a simple screening tool to identify patients with diabetes at risk of CVD and requiring further examination and treatment. However this is a very small study that needs confirmation in larger-scale studies.

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