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Study of NTproBNP, lipid profile and other conventional risk factors in cardio vascular disease patients

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ABSTRACT

There is lack of data on the relative importance of various conventional risk factors for cardio vascular disease among central Indians. However, cardio vascular disease risk factors have been shown to be more pronounced among diabetes subjects. The NTproBNP has been reported to be elevated in heart failure, ventricular stretch, and pressure overload. We conducted a prospective case-control study to determine the role of NTproBNP in cardio vascular disease among patients with or without diabetes in population of central India.We recruited 100 angio-graphically proven cardio vascular disease patients, 50 with diabetes and 50 without diabetes and 100 case matched controls from patients admitted to our hospital. Demographic, anthropometric measures, NTproBNP, lipids, blood glucose and other conventional risk factors were compared among cases and controls. Univariate and Multivariate analysis were carried out to compare individual factors using t-test, ANOVA and the inter group comparison were done by using Born ferroni Post Hoc tests. The levels of NTproBNP shows a significantly higher values in diabetes with cardio vascular disease patients (n=50, ±SD 1481.021±813.4059, 95% CI 1249.854-1712.188) as compared to patients with cardio vascular disease only (n=50, ±SD 704.062±359.269, 95% CI 601.959-806.165), diabetic control (n=50, ±SD 37.55±30.72, 95% CI 28.825-46.291) and control (n=50, ±SD 23.56±25.39, 95% CI 16.345-30.779). There is a significant association of NTproBNP with increased age, hyperglycemia, hyperlipidemia, obesity, smoking and hypertension. Conclusion:- This study evaluate that NTproBNP is an independent risk predictor of cardio vascular disease, acknowledging limitations resulting from study design, further larger studies are warranted to verify this conclusion in our population.

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INTRODUCTION

Cardio vascular disease (CVD) continues to be the major cause of death world wide, despite the use of new pharmacological strategies to lower blood glucose, lipids, more aggressive therapy of hypertension (HT) and change in life style [1]. Global burden of disease study estimates that Indian false the greatest burden due to CVD [2], reason could be epidemiological transformation in India [3,4]. Study from different parts of India including Chennai urban population study [5], the Jaipur watch -2 study [6] has reported an escalation in the prevalence rates of CVD among south and north Indians during passed 20 years [6], several other confirm the same [7-9].

Evaluation of major coronary risk factors in Indian patients undergoing angiography has shown that in about one third of the patients no major risk factors are detectable [10], yet they suffer from the dreaded disease. Therefore the primary challenge remains: the early and specific diagnosis of the acute coronary event so that prompt and appropriate therapy can be given. The increasing incidence of CVD in Indian patients especially in central India is possibly due to industrialization, stress of life, less exercise and increasing incidence of smoking and other factors [11].

An analysis on CVD related data from India describing the health impact due to acceleration of CVD among Indians emphasizes the increase in risk factor associated with CVD, particularly diabetes [12]. With over 20 million diabetes subjects, India leads the world in the number of individual with diabetes [13]. Diabetes subjects have two or more fold higher risk of CVD compared to non diabetes population [14].

Hyperlipidemia is a critical factor in 50% of patients with atherosclerotic CVD. Blood lipids level is having a positive correlation with CVD and its evolutions are used extensively in routine practice but their levels could be affected by various factors including physiological as well as analytical [15]. However, in recent years additional inciting factors (neurohormonal) have been identified. In CVD hormones are released from both cardiac and extra cardiac origins. NT pro BNP is a neurohormone, released by heart in both healthy individuals and patients with congestive heart failure [16], play a crucial role on body fluid homeostasis and vascular tone [17], has recently emerged as a potentially important risk factor in CVD and heart failure. Identification of this circulating biomarker may provide new windows into the pathophysiology and management of cardiovascular diseases [18].Therefore, the present study was conducted to find out role of NTproBNP in CVD in population of central India.

METHODS

Location

Patients included in the present study were all admitted to the intensive coronary care unit (ICCU) or attending the OPD of medicine of M.Y.Hospital attached to M.G.M. Medical College, Indore, Madhya Pradesh.

Patient Selection

We have selected the patients as they are presented. Consecutive 100 patients undergoing coronary angiography at our hospital over a period of 1 year, April 2007 – May 2008 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.

Selection of controls

Age and Sex matched 50 Diabetes subjects and 50 Normal healthy subjects from medicine OPD and Blood Bank with no history of CVD or had normal electro diagram (ECG), were selected for the study. The name, age, sex, occupation and clinical history were taken on proforma. Previous histories of diabetes, smoking, HTN were noted.

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Fully informed consent was obtained from patients and controls of both groups. Subjects with kidney disorders, nephropathy and dyspnea were excluded.

Risk factors for Cardio vascular disease

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 [19], 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. Those patients whose body mass index is ≥25 kg/m^2 were considered as obese [20]. 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 [21, 22].

Collection of samples

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

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.

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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

The data was analyzed using SPSS 11.5 for windows (spss inc USA).Univariate and Multivariate analysis were carried out to compare individual factors using t-test, ANOVA and the inter group compression were done by using Bonferroni Post Hoc tests. Present work was approved by institutional research and ethical committee.

RESULTS

We enrolled 100 case and 100 case matched controls. Table I show the findings of various demographic parameters and clinical parameters of the 200 subjects. Patients were divided in to four groups 1) 50 had diabetes mellitus and cardiovascular disease, grouped as diabetics with cardiovascular disease or DwCVD. 2) 50 had cardiovascular disease without diabetes mellitus, grouped as non diabetics with cardiovascular disease or NDwCVD. 3) 50 had diabetes mellitus without cardiovascular disease, grouped as diabetic control or Dcontrol.4) 50 had no diabetes mellitus and cardiovascular disease were grouped as Control, of them DwCVD and NDwCVD are categorized as diseased group and others are categorized as non-diseased group. The univariate logistic regression analysis for risk factors versus CVD (as a dependent variable) was done to assess the relative risk of development

of CVD with each risk factor in the entire subject group that includes diseased and non diseased subjects. There was an association of more than one risk factor in most of the subjects, thus regression analysis was used to estimate the risk association of each risk factor. The results are shown in Table I. Both the diseased group and non diseased group is having 90% of male, diseased group is having age >40 years respectively. While the non diseased group with a respective mean age of 52.02 ± 9.47 for DwCVD and 38.56 ± 5.35 for Control. The mean age was found to be significantly higher for diseased group compared with non diseased group. Male sex was predominantly higher for each study group. In diseased group the female proportion was nearly one-fifth while non diseased group has a lesser number of female. Diseased group also show a considerably high smoker proportion, raised BMI, hypertension (raised diastolic blood pressure), diabetes mellitus, raised blood lipids and positive family history as shown in Table I. The lipid parameter total cholesterol (TC) was observed 287.58 ± 27.35 for DwCVD, 255.24 ± 26.04 for NDwCVD, 165.26 ± 22.84 for DControl and 148.64 ± 29.76 for control. The TC was observed higher in diseased group with rest other groups (Table I, $p<0.05$).The lipid parameter Triglyceride (TG) was observed 278.86 ± 46.87 for DwCVD, 173.46 ± 67.92 for NDwCVD, 183.10 ± 65.20 for DControl and 167.28 ± 68.32 for control. TG was observed higher in diseased group with rest of other group(Table I , $p<0.05$).The Lipid parameter high density lipoprotein (HDL) was observed 28.44 ± 9.022 for DwCVD, 29.48 ± 10.38 for NDwCVD, 43.28 ± 7.34 for DControl and 42.40 ±9.20 for control. HDL was observed lower in diseased group with rest of other group (Table I , $p<0.05$).The lipid parameter low density lipoprotein (LDL) was observed 166.36 ± 23.26 for DwCVD, 151.00 ± 34.35 for NDwCVD, 110.08 ± 29.94 for DControl and 92.08 ± 22.72 for control. LDL was observed higher in diseased group with rest of other group(Table I, $p<0.05$).The lipid parameter very low density lipoprotein (VLDL) was observed 45.54 ± 12.19 for DwCVD group ,43.08 ± 19.62 for NDwCVD ,34.74 ± 9.14 for DControl and 32.30 ± 13.25 for control. VLDL was observed higher in diseased group with rest of other group (Table I, $p<0.05$). Lipid parameters of control and Diabetic control were almost comparable having no significant differences. Age as a risk factor contributes significantly to diseased group ($p<0.05$), sex as a risk factor is non significant. Positive family history also contributes to the CVD; it was found to be statistically significant. We analyzed the role of conventional risk factors in affecting the pathogenesis of CVD in study population, among diseased group, prevalence of smoking was found to be significantly higher ($p<0.05$); diabetes mellitus has a direct relation with DwCVD as compared to controls and NDwCVD ($p<0.05$), hypertension (raised diastolic blood pressure) and body mass index were significantly higher in diseased group as compared to control ($p<0.05$). T-C, TG, LDL-C, HDL-C, VLDL were significantly associated with the CVD in patients ($p<0.05$). The findings of NT proBNP showed a remarkably high value associated with diseased groups, DwCVD group have a highest NT proBNP values 1481±813.4059.Whereas the NDwCVD group found with mean NT proBNP value 704.06±359.26, D control 37.55±30.72 and control with 23.56±25.39 as shown in Table II. The Inter group Comparisons using the Bonferroni Post Hoc test showed considerably higher NT-proBNP values in DwCVD group compared with each other groups as shown in Table III. The Multivariate regression analysis showed advanced age, raised BMI, hypertension (raised diastolic blood pressure), fasting glucose, TC, TG, decreased HDL, NT-proBNP and smoking all were significantly associated with diseased group subjects while sex and family history were insignificant as shown in Table IV.

DISCUSSION

Studies in the past have shown that high rates of CVD in Asian Indians are accompanied by paradoxically low prevalence of conventional risk factors [23]. Our study showed that high NTproBNP levels are independently associated with CVD and diabetes along with raised lipids level, blood pressure (diastolic), smoking, age, sex, family history, BMI. In this study the mean age was 60.99±10.34 year for diseased group, the sex distribution is 81% male and 19% females. The study was predominately male oriented as CVD affects males more severely commonly than females; this can be attributed to the protective effect of estrogens in females. Our current findings shows increased NTproBNP values in diseased group as compare to control, which is having NTproBNP values below 125pg/ml. Several different explanation for over findings are possible of them patients with diabetes have a higher prevalence of diastolic dysfunction or have more peripheral and distal atherosclerotic changes in the coronary tree. Previous study from outside of India have convincingly demonstrated that circulating NTproBNP are increased in CVD patients, Charlotte shows NTproBNP as a screening marker and increased risk marker in diabetes [24-26].

Our study shows increased value of NTproBNP with age in diseased and non diseased group (P 0.001). The effect of age is more complicated although data are limited there is evidence that alteration in the degradation, clearance or production of NTproBNP occurs with aging, in normal individuals. Clark et al [27] showed that with increasing age and in the absence of renal dysfunction, there is decrease in renal clearance of BNP and an increase in plasma BNP levels [28, 29]. Another key function is with aging there is a decrease in the ratio of cGMP to BNP which indicate decrease response to BNP therefore dictate increased endogenous BNP production [30] as well as platelet associated clearance receptor decrease as individuals age increase [31]. Same for NTproBNP because it is an inactive part of BNP.

This study shows raised diastolic blood pressure or hypertension for diseased group as compared to controls ($p<0.05$). However BNP values cannot be used for differentiate between systolic and diastolic heart failure, reason is undiagnosed diastolic dysfunction is common as shown by Redfilled MM [32]. Thomas et al stated that arterial volume does not increases with healthy aging but is related to left ventricular systolic and diastolic dysfunction, it is clear that age and arterial volume are independent predictors of NTproBNP levels [33].

This study shows positive association of increased BMI with CVD, many studies that concluded there is inverse correlation between BMI and plasma NTproBNP levels [34-36] but as per our results we may state that obese patients associated with diabetes mellitus, hyperlipidemia could have increased NTproBNP levels as compared to non diseased group.

Tobacco smoking is an important modifiable risk factor of CVD. A recent study from southern India estimates that 700 000 deaths per year in India are a result of smoking also smokers live about 20 year less than an average Indian. As per results of this study smokers have increased levels of NTproBNP. Smoking is established risk factor for CVD. It affects the vascular endothelium, lipid peroxidation and associated complications causes increased stress on vessels and heart, causes increase in NTproBNP. Smoking impairs sympathovagal balance and decreases the heart rate, variability in healthy subjects, even a single citrate smoking leads to overt sympathetic excitation. Furthermore, smoking results in an increased in NT pro BNP levels, the change in adrenergic nervous system and NT pro BNP levels are well correlates [37].

Lipid abnormalities are well known to increased risk of CVD. Studies in Asian communities in the UK have suggested that obesity, type 2 diabetes mellitus, lower HDL cholesterol and triglyceride concentrations are important risk factors for CVD in this racial group [38, 39]. In our study, hyperlipidemia was observed in 68% of subjects increased serum cholesterol is casually associated with increased risk of CVD. Specifically a 10% increase in serum cholesterol is associated with 20% to 30% increase risk of CVD and elevation earlier in life may associated with higher risk of CVD as given by Lo Rosa [40]. Similar Case–control studies from different parts of India have shown that TC, TG levels are more in CVD patient. Hughes et al [41] in his study showed that relative risk of MI correlates directly with TG and inversely with HDL-C levels in Asian Indians, as well as low levels of HDL cholesterol have been shown to be a power full risk factor of CVD [42]. The association of serum triglyceride concentration with risk of CVD is not as strong, and is subject to confounding by serum LDL and HDL cholesterol, diabetes and other factors. In our study the logistic regression analysis showed that after adjusting for other risk factors, raised triglycerides levels were associated with higher risk of CVD. But lipid profile level could be affected by various factors including physiological as well as analytical. Therefore here needs to be evaluation of a risk factor without variations and more specifications. We believe that changes in life style and urbanization over the past two decades might have led to a higher prevalence of hyperglycemia, hyperlipidemia and CVD in our population.

Increasing plasma brain natriuretic peptide concentrations are correlated with the development of cardiac arrhythmias and the degree of homodynamic compromise [43], and high concentrations predict poor long-term survival. The volume-contracting and vasodilative properties of brain natriuretic peptide reduce systemic vascular resistance, decreases intra cardiac filling pressure, and improve myocardial performance. As shown in vitro studies, brain natriuretic peptide inhibits the growth of cardiac fibroblasts, potentially limiting the proliferative remodeling of the heart by retarding collagen deposition and can also induce cardiac myocyte apoptosis [44]. Thus, through direct actions and indirect actions, the natriuretic peptides potentially limit the myocardial proliferative or hypertrophic response to injury or ischemia. NTproBNP has a multiplicity of cardiac

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Table I. Univariate analysis of confounding factors

		Diseased		Non Disesased			
Factor	Particulars	DwCVD[a] (n=50)	NDwCVD[b] (n=50)	DControl[c] (n=50)	Control[d] (n=50)	p-value	95% CI
Age	(Mean±SD)	61.32±10.19	60.36±10.52	52.02±9.47	38.56±5.35	a/d, a/c <0.05	17.89-27.63, 4.43-14.17
Sex	M	41	40	47	47	-	-
FH	Yes	20	9	12	5	a/b, a/d <0.05	-
Smoking	Yes	22	20	5	0	a/c, a/d, b/c, b/d, c/d <0.05	-
BMI	≥25	6	12	10	7	a/d, a/c <0.05	4.319-0.958, 5.247-1.886
BPS	≥140mm	13	8	1	0	>0.05	
BPD	≥90mm	21	14	0	0	a/d, a/c <0.05	2.12-8.96, 3.68-10.52
FS	≥126mg/dl	50	0	50	0	a/d, a/b <0.05	84.02-102.70, 80.82-99.50
TC	≥229.0 mg/dl	49	47	0	0	a/d, a/b, a/c <0.05	124.75-153.13, 18.15-46.53, 108.13-136.51
TG	≥160.0 mg/dl	50	13	12	9	a/d, a/b, a/c <0.05	78.15-145.01, 71.97-138.83, 62.33-129.19
HDL	≤40.0 mg/dl	27	25	1	4	a/d, a/c <0.05	18.79-9.13, 19.67-10.01
LDL	≤138.0 mg/dl	37	33	7	0	a/d, a/b, a/c <0.05	59.36-89.20, 0.44-30.28, 41.36-71.20
VLDL	≥40.0 mg/dl	32	24	17	13	a/d, a/c <0.05	5.73-20.75, 3.29-18.31
NT-proBNP	≥125.0 pg/ml	50	50	0	0	a/d, a/b, a/c <0.05	1220.218-1694.700,539.718-1014.200, 1206.222-1680.704

Table II. Study of Mean NT pro BNP

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CONTROL	50	23.562	25.3955	3.5915	16.345	30.779	5.0	91.0
NDwCVD	50	704.062	359.2697	50.8084	601.959	806.165	145.8	1695.0
DwCVD	50	1481.021	813.4059	115.0330	1249.854	1712.188	452.3	3256.0
DCONTROL	50	37.558	30.7279	4.3456	28.825	46.291	8.0	123.8

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Table III. Post Hoc Tests;Bonferroni

					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
	NDwCVD	776.959(*)	89.0108	.000	539.718	1014.200
	DCONTROL	1443.463(*)	89.0108	.000	1206.222	1680.704

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

Table IV. Multivariate regression analysis; Beta-Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	-.793	.216		-3.668	.000	-1.219	-.366
AGE	.007	.001	.186	6.019	.000	.005	.010
BMI	-.014	.004	-.098	-3.684	.000	-.022	-.007
BPS	.000	.000	.034	1.373	.171	.000	.001
BPD	.005	.002	.067	2.554	.011	.001	.009
FS	-.002	.000	-.218	-8.123	.000	-.003	-.002
TC	.004	.000	.567	11.277	.000	.004	.005
TG	.000	.000	.059	2.039	.043	.000	.001
HDL	-.003	.001	-.064	-2.107	.037	-.005	.000
LDL	.000	.001	.038	.931	.353	-.001	.001
VLDL	-.001	.001	-.022	-.809	.419	-.002	.001
NT_PROBNP	.000	.000	.152	4.358	.000	.000	.000
Family H	-.033	.029	-.028	-1.135	.258	-.091	.024
SMOKER	.075	.032	.064	2.319	.022	.011	.139
SEX1	.075	.040	.049	1.846	.066	-.005	.154

functions and is released as a counter-regulatory hormone in response to a variety of cardiac stress but most particularly cardiac stretch. It is significantly affected by changes in volume and in cardiac performance, and among its effect are volume reduction and vasodilatation. Thus this hormone is a sensitive marker for changes in ventricular physiology and could be used as a routine marker better than lipid profile which is affected by daily life style. Observations of this study increase speculation that NTproBNP can serve as a biomarker for non-HF mechanisms, preclinical disease, and other pathologic states of myocardial disease including coronary endothelial dysfunction, myocardial ischemia, and arrhythmias. Wang et al from the Framingham Heart Study reported in a prospective investigation in the general population without heart failure that with each SD increase in log BNP

levels, there were significant increases in the risk of death, HF, AF, stroke or transient ischemic attack, and first cardiovascular event. Thus, these findings render BNP as more of a global marker for myocardial injury and suggest that NTproBNP production is the final common pathway for a host of cardiovascular diseases.

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 choose case matched controls and performed multivariate analysis to adjust for other potential confounders. We avoided misclassification of disease status by identifying case according to established criteria. We increased the efficiency of our design by recruiting diabetic control and healthy controls.
Our study has certain limitations, First, our sample size is small it needs to be increased. Second we measured glucose, lipids, NTproBNP only once. Third, CVD may be related to non traditional risk factors such as C-reactive protein, fibrinogen, lipoprotein (a) and homocystien. However, the current evidences are insufficient to conclusively support the additive value of these specific risk factors over conventional risk factors.

In conclusion our results suggest that raised levels of NTproBNP it self is an independent major risk factor of CVD. However our result shows hyperglycemia, hyperlipidemia, hypertension, raised BMI and smoking is also important risk factors of CVD. Diabetes and CVD are major socioeconomic burden for us. Evaluation of earlier and specific risk predictor of CVD like NTproBNP as our results suggests will help in reduction and treatment of CVD in our population.

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