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Assessment of plasma B-type natriuretic peptide and highly sensitive C- reactive protein among diagnosed patients of diabetes mellitus with and without cardiovascular disease

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ABSTRACT

Background: Considering the burden of cardiovascular disease (CVD) complications in patients with diabetes and the production of BNP on the incidence of CVD. **Aims and Objectives:** We investigated the role of BNP and high sensitive C-reactive protein (hs-CRP) in predicting the presence of CVD in diabetes and determined if the concentration load of BNP and hs-CRP differed in diabetics participants with CVD and without CVD. **Materials and Methods:** Diabetic consenting participants fitting the study inclusion criteria were enrolled. Based on medical records, participants were grouped into diabetes with CVD and diabetes without CVD and tested for blood BNP, hs-CRP, Lipid Profile, and hemoglobin A1C (HbA1C). **Results:** Diabetes mellitus (DM) was relatively higher in the age group of 51–60 years with female preponderance. Ethnic distribution demonstrated a high percentage of DM among the Sikkimese Nepalese, followed by Bhutias, and Lepchas. On ascertaining the effects of BNP and hs-CRP on the likelihood that the diabetic participants have CVD, only BNP demonstrated statistical significance. However, unlike hs-CRP, BNP exhibited no association with HbA1C and lipid parameters. BNP and hs-CRP levels were higher among diabetics with CVD when compared to diabetics without CVD. **Conclusion:** Increasing BNP levels in diabetes is associated with an increased likelihood of exhibiting CVD but increasing hs-CRP level may not be associated with an increased likelihood of exhibiting CVD. Increase in BNP levels independent of influence by HbA1C and lipid profile should be investigated further and yet importantly diabetes with CVD exhibits extra inflammatory load (hs-CRP) compared to diabetes without CVDs.

Key words: Diabetes mellitus; Cardiovascular disease; Brain natriuretic peptide; High sensitive C-reactive protein; Sikkim

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INTRODUCTION

Cardiovascular disease (CVD) is a major complication associated with diabetes mellitus (DM). A robust number of epidemiological and pathological data have established the role of diabetes as an independent risk factor for the development of CVD.[1] It contributes to approximately 65% of deaths in persons with diabetes[2] and is therefore considered one of the major risk factor, an important cause of CVD.[3] This indicates an increased incidence of CVD in the Second Diabetes Capital of the World. India ranks second after China in the global diabetes epidemic, with

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77 million people with diabetes. Of these, 12.1 million are aged >65 years, which is estimated to increase to 27.5 million in the year 2045.[4]

Persistent high blood glucose level in diabetes induces changes in the micro and macro vessels. Microvasculature diseases manifest as retinopathy, nephropathy, and neuropathy, while the macrovasculature as CVDs. With rising fasting plasma glucose levels, the risk for CVD increases continuously even before reaching levels sufficient for a diabetes diagnosis.[5] These changes, in combination with increased oxidative stress, low-grade inflammation, and vasavasorum neovascularization, lead to macrovascular complications[6] making an assessment of the circulating metabolic biomarker as one of the most suitable ways of understanding the underlying pathology of the disease. One such cardiac marker we wish to explore and evaluate is the B –type natriuretic peptide considered the benchmark, against which other biomarkers are compared. B –type natriuretic peptide is also known as Brain natriuretic peptide(BNP) a cardiac neurohormone that is released from the ventricular myocytes in response to ventricular dilatation and volume overload.[7] Furthermore, the plasma BNP level is a stronger predictor of cardiovascular events than traditional risk factors, even in the absence of heart failure,[8] with an added capability of identifying high negative predictive value.[9] Yet another inflammatory marker that has been observed to rise in both diabetes and CVD is the C-reactive protein. It is an acute-phase protein released by the liver in response to inflammation. Many studies have shown that baseline levels of C-reactive protein (CRP) are useful in predicting cardiovascular events, even among healthy individuals.[10] However, there aren't many studies in India reporting high sensitive-CRP (hs-CRP) in patients with type-2 DM with accompanying cardiovascular events further, because diabetes is a risk factor for CVD and BNP is secreted on the incidence of CVD, we aimed to investigate the following.

Aims and objectives

The role of serum BNP and hs-CRP in predicting the presence of CVD in the diabetic study participants. Second, we evaluated the association of BNP and hs-CRP with the diabetic and cardiovascular risk profile, including hemoglobin A1C (HbA1c) and lipid profile. Finally, the last objective was to inspect if the concentration load of BNP and hs-CRP differed in diabetic participants with CVD and without CVD.

MATERIALS AND METHODS

This was a hospital-based, cross-sectional study conducted in the Department of Biochemistry and Medicine of Central Referral Hospital, Sikkim Manipal Institute of Medical Science. Before commencing the study, due approval from the Institutional Research Protocol Evaluation Committee and Institutional Ethics Committee [IEC/335/15-058/SMIMS] was obtained.

Sample size

The sample size for the difference in between means was calculated ($N= 2\sigma^2[Z_{1-\alpha/2}+Z_{1-\beta}]^2/d^2$). Assuming a 5% level of significance ($\alpha=0.05$), power 80% ($\beta=0.84$), and taking Sahu et al.,[11] 2010 as reference, a sample size (n) of 11 was found in each group. However, a total of 36 DM with CVD and 38 DM without CVD consenting participants during the span of 1 year were enrolled for the study.

Study sample

All diabetic consenting participants visiting the Medicine outpatient department, Central Referral Hospital, Sikkim, were enrolled for the study. Participants with a history of liver disease, acute illness, thyroid disease, anemia, hemochromatosis, malignancy, acute infections, and acute inflammatory conditions who were likely to have an added acute phase response were excluded from the study. Likewise, women with gestational diabetes and women on hormone replacement therapy were also excluded from the study. Based on their medical records, general information on age, gender, anthropometric measurements, ethnicity, and history of past/present diseases were noted down. 8 mL of fasting venous blood was then collected from all qualifying participants under aseptic conditions to estimate fasting blood sugar, lipid profile, and HbA1c using ERBA Kits for ERBA Manheim EM 200 full auto analyzer. BNP (Elabscience Biotechnology Co., Ltd) and hs-CRP levels (Biomerica Inc.) were measured by ELISA technique in the Lab Life ER 2007, Microplate Reader (India). Thereafter, the participants were further put into two groups; those with diabetes and accompanying cardiovascular disorder and those without cardiovascular disorders. From the 74 diabetic study participants qualifying the study criteria, 36 had DM with CVD like coronary artery disease, congestive cardiac failure, and left ventricular dysfunction, while 38 participants had only DM.

Statistical analysis

All statistical analysis was performed using the IBM SPSS 20 software package (SPSS Inc, Chicago, IL, USA). The proportion of adults with DM and all other categorical variables are presented in frequency percentages. Data were inspected for normal distribution and appropriate statistical tests were applied –a logistic regression was conducted to ascertain the effects of the independent variable-BNP and hs-CRP on the likelihood that the study participants (diabetics) have cardiovascular diseases. Thereafter, a Mann-Whitney U-test/Wilcoxon-Mann–Whitney test was calculated to determine if there were differences between hs-CRP and BNP levels between diabetes with CVD and

diabetes without CVD. Finally, a Pearson's r correlation was calculated to measure the strength and direction of association between BNP and CRP levels with the diabetic and lipid risk parameters.

RESULTS

Of the 74 consenting participants, diabetes was frequently found in the age group of 51–60 years (37.8%) and the least common in the age group of 71–80 and above (9.4% and 1.3%, respectively). Female preponderance was more (55%) than males (44%) among the diabetic participants. Ethnical observation of diabetes was the highest for the Sikkimese Nepalese participants (72.9%), followed by the Bhutias (16.2%), Lepchas (06.7%), and others (04.0%) (Table 1).

Table 2 represents the baseline diabetic and cardiometabolic parameters of the case-diabetes with CVD and the Control-diabetes without CVD. Body Mass Index of the two study groups were similar, exhibiting no significant difference at P=0.27. Likewise, glycated Hb (HbA1c) was also not significantly different at P=0.09. However, their levels were higher in the cases (diabetes with CVD=7.7%) than in the control (diabetes without CVD=6.8%). Of the lipid profile parameters, only total cholesterol differed significantly between patients having diabetes with CVD (185.mg/dL) and diabetes without CVD (162 mg/dL), at P=0.04.

Thereafter, a logistic regression was performed to ascertain the effects of BNP and hs-CRP levels on the likelihood that the participants have CVD (Table 3). The logistic regression model was statistically significant, $\chi^2(4) = 67.62$, P=0.001. The model explained 78.0% (Nagelkerke R2) of the variance in heart disease and correctly classified 90.5% of cases. Sensitivity was 88.9%, specificity was 92.1%, positive predictive value was 91.4% and negative predictive value was 89.7%. Of BNP and hs-CRP, only BNP (P=0.001) demonstrated statistical significance. Increasing BNP levels in the diabetic participants was associated with an increased likelihood of exhibiting CVDs, but increasing hs-CRP level may not be associated with an increased likelihood of exhibiting CVDs.

A Mann–Whitney U-test was then run to determine if there were differences in serum BNP and hs-CRP concentration between diabetes with CVD and diabetes without CVD. The median BNP value was significantly higher in diabetes with CVD group (979 pg/mL) and lower in diabetes without CVD (182 pg/mL), at P=0.001. A significant difference was also observed for hs-CRP levels between the two groups at P=0.001. Plasma hs-CRP levels were

Table 1: Percentage distribution of diabetes in terms of age, gender, and ethnicity

Age group distribution of diabetes mellitus in the ethnic population of Sikkim						
Age group (years)	**30–40**	**41–50**	**51–60**	**61–70**	**71–80**	**>80**
Total no. (n)	05	21	28	12	07	01
Percentage	6.7	28.3	37.8	16.2	9.4	1.3
Gender-wise distribution of diabetes mellitus in the ethnic population of Sikkim						
Gender	**Male**	**Female**				
Total no.(n)	33	41				
Percentage	44.5	55.4				
Ethnic distribution of diabetes mellitus in the study participants						
Ethnic groups	**Total no. (n=74)**	**Percentage**				
Bhutia	12	16.2				
Lepcha	05	06.7				
Nepali	54	72.9				
Others	03	04.0				

Table 2: Diabetic risk parameters of the study group

Parameter	DM without CVD (n=38)	DM with CVD (n=36)	P-value
Age (in years)	54.9±8.1	57.5±11.9	0.02*
BMI (kg/m^2)	26.5±2.6	27.2±3.8	0.27
HbA1C (%)	6.8±1.0	7.7±1.6	0.09
Lipid profile			
Total cholesterol (mg/dl)	162.31±38.3	185±56.4	0.04*
Triglyceride (mg/dl)	158±73.8	169±86.9	0.74
LDL-cholesterol (mg/dl)	94.3±31.6	106.7±43.3	0.12
HDL-cholesterol (mg/dl)	48.7±10.5	51.8±14.3	0.23

Values are Mean±SD; P-value significant at <0.05. DM: Diabetes mellitus, CVD: Cardiovascular disease, BMI: Body mass index, HbA1C: Hemoglobin A1C, LDL: Low-density lipoprotein, HDL: High-density lipoprotein

higher (3.8 mg/dL) for diabetes with CVD than for diabetes without CVD (0.85 mg/dL) (Table 4).

Finally, a Pearson's correlation was run to assess the relationship between serum BNP, and hs-CRP concentration with the lipid profile parameters and HbA1C of the diabetic participants. Preliminary analysis showed the relationship to be linear, demonstrated by a scatter plot. There was no significant correlation between the BNP levels and HbA1c and the lipid profile of the diabetic participants. However, there was a moderate positive correlation between serum hs-CRP and HbA1c (r=0.270, P=0.020), including total choslesteral (r=0.334, P=0.004) and low-density lipoprotein (LDL) (r=0.303, P=0.009) of the lipid parameters (Table 5).

DISCUSSION

Diabetes is an ever-growing public health problem worldwide and the disease burden is rapidly increasing in our Indian subcontinent. Sikkim, a small state in the north-eastern region of India, has a population of only 610,577 people, and as per the data retrieved from the National Programme for Prevention and Control of Cancer, Diabetes, CVDs and Stroke (NPCDCS) 2011, the prevalence of DM is relatively high as 13.6% across the states and till date, studies demonstrate to support the growing trend of diabetes in Sikkim.[12] Our study included 74 diagnosed patients with type 2 diabetes of which 36 had associated cardiovascular events. These diabetic patients were in the age group of 37–85 years and the majority fell in the age group category of 51–60 (37.8%). There were more female diabetics (55%) than male diabetics (44%). The distribution of the ethnic population of Sikkim is broadly divided into three predominant groups as Lepcha (8%),Bhutia (13%) and the Nepalis (57.76%).[13] Our study demonstrated the ethnic distribution of DM as Lepcha −6.7%, Bhutias −16%, and Nepali −73% (Table 1), which corresponded to the ethnic distribution of the Sikkimese population, placing Sikkimese Nepali under high-risk ethnic group for diabetes. Ethnicity, genetic, and environmental factors that influence individuals lifestyle are known to affect the risk related to the pathogenesis of type 2DM. In addition, there are also studies reporting that Asian Indians progress more rapidly through the pre-diabetes stage as compared to people of other racial groups.[14]

As the first objective, BNP and hs-CRP were evaluated for its capability in prediction the presence of CVD in the diabetic study participants. Our study demonstrated that increasing BNP levels in diabetic participants is associated with an increased likelihood of exhibiting CVDs, but increasing hs-CRP level may not be associated with an increased likelihood of exhibiting CVD (Table 3). To support the findings, we had BNP and hs-CRP again evaluated; however, this time, categorizing the diabetic study participants into those that had accompanying CVDs and those without. The median values of BNP in the two diabetic study group- those with CVDs and those without CVDs was found to be 978±356 pg/mL and 182±129 pg/mL, respectively, which was statistically significant. Median values of hs-CRP were also found to be significantly higher in diabetics (3.8 mg/dL) with CVDs than diabetics without CVDs (0.85 mg/dL) (Table 4). The findings are in concordance with a study demonstrated by Miller et al., where they report CRPs little benefit over BNP in considering them as a biomarker in cardio vascular diseases.[15] Natriuretic peptides, BNP and NT-pro BNP, provides much predictive information about myocardial infarction, heart failure, stroke, and death in patients

Table 3: Logistic regression predicting the likelihood of cardiovascular disease based on serum BNP and hs-CRP levels

Study Variable	Walds	Sig	Exp (B)	95% CI for EXP (B)	
				Lower	Upper
BNP (pg/mL)	13.216	0.001*	1.007	1.003	1.010
hs-CRP (mg/dL)	1.654	0.198	1.297	0.873	1.929

Statistical significance at P<0.05*. hs-CRP: High sensitive C-reactive protein

Table 4: BNP and hs-CRP levels in Diabetes with CVD and Diabetes without CVD

Study Variable	DM without CVD (n=38)	DM with CVD (n=36)	P-value
BNP (pg/mL)	182±129	979±356	0.001*
hs-CRP (mg/dL)	0.85±0.58	3.8±1.2	0.001*

Values are median±SD. hs-CRP: High-sensitive C-reactive protein with statistical significance at P<0.05*

Table 5: Pearson's correlation for BNP and hs-CRP with HbA1c and lipid profile parameters

Study Variable	HbA1c	Total cholesterol	Triglyceride	HDL	LDL
BNP	r=0.072	r=0.127	r=0.046	r=0.071	r=0.079
	P=0.542	P=0.280	P=0.695	P=0.550	P=0.501
hs-CRP	r=0.270	r=0.334	r=0.064	r=0.081	r=0.303
	P=0.020*	P=0.004*	P=0.588	P=0.491	P=0.009*

r=pearsons correlation, *Statistically significant at P<0.05. LDH: LOW-density lipoprotein, HDL: High-density lipoprotein

with acute coronary syndrome as compared to all other conventional variables put together.[16] A recent 2020 study by Yan et al., also supports the study findings, as it reports high circulating levels of BNP in patients with diabetes.[17]

Although BNP levels increase in diabetes, its significant blood marker, glycated hemoglobin (HbA1C) used in the diagnosis and management of diabetes did not show a significant association with the BNP levels (P=0.542) and neither did the lipid profile parameters, including TC, TG, high-density lipoprotein and LDL (P>0.05) (Table 5) suggesting BNP levels in diabetics may not be under the influence of HbA1C and Lipid profile parameters or vice versa. Partially contradicting this finding, a study reports poor glycemic control may cause an increase in BNP levels, while there was no association established between the decrease in BNP levels and the lipid parameters.[18]

Low-grade systemic inflammation is thought to be responsible for the development of DM[19], and hs-CRP is an acute phase reactant that is produced in the liver during inflammatory conditions. American Heart Association recommends hs-CRP levels below 1 mg/l as low risk, between 1 and 3 mg/l as average risk, and above 3 mg/l as high risk for CHD.[20] CRP was found to be a likely independent predictor for cardiovascular death in patients of DM with acute coronary syndrome.[21] Although Asian Indians are considered a very high-risk group for DM, there were fewer studies of hs-CRP among Asian Indians.[22] In our hospital-based observational study, hs-CRP failed to predict CVDs in the diabetic participants (P=0.198) (Table 3). Nevertheless, hs-CRP concentration was much higher in the diabetic participants with CVDs (3.8 mg/mL) than those without CVDs (0.85 mg/mL) and exhibited a moderate positive correlation with HbA1c (r=0.270) and the lipid parameters including TC (r=0.334) and LDL (r=0.303) (Table 5).

Limitations of the study

It was a hospital based study and selection bias may have been introduced. Short study period and financial constraints failed to demonstrate a representation of the population . Nevertheless the facts reported in the study can be used to generate health data in terms of BNPs potential role in Diabetes with CVDs.

CONCLUSION

BNP concentration increases in diabetes. Increasing BNP levels in diabetes is associated with an increased likelihood of exhibiting CVDs, but increasing hs-CRP level may not be associated with an increased likelihood of exhibiting CVDs. Increase in BNP levels independent of influence by HbA1C and lipid profile parameters can be investigated further and finally, yet importantly, Diabetes with CVDs exhibit extra inflammatory load (hs-CRP) than Diabetes without CVDs.

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SR- Performed work, **MLS-** Designed and generated idea; **BK-** Supervised study sample recruitment and other related activities performed in the department of medicine; SMIMS, **SB-** Provided technical guidance while performing work in the department of biochemistry/SMIMS, **RDB-** Prepared manuscript and helped designing the study.

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