Does brain natriuretic peptide have a significant diagnostic value in subclinical peripheral atrial disease type 2 diabetic patients?

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Background
Peripheral arterial disease (PAD) is a serious complication of diabetes mellitus (DM); it is also correlated with increase in the morbidity and mortality in diabetics owing to cardiovascular disease. Ankle–brachial index (ABI) is an established method to detect PAD.
Patients and methods
This is a cross-sectional study to detect brain natriuretic peptide (BNP) level in patient with type-2 DM with PAD using ABI.
Results
This study revealed 11 patients diagnosed with low ABI. Patients with low ABI showed statistical significance regarding mean age (P=0.038), duration of DM (P=0.004), concentration of glycated hemoglobin (P=0.044), BNP (P=0.013), and microalbuminurea (P=0.007). Moreover, patients with low ABI were significantly associated with nephropathy (P=0.001) and retinopathy (P=0.007). BNP at cutoff value of 360 pg/ml had sensitivity and specificity of 27.27 and 95.9, respectively. The BNP level was negatively correlated with the ABI (r=–0.183, P=0.162). BNP showed statistical significance with fasting blood sugar, postprandial glucose, nephropathy, retinopathy, and albumin/creatinine ratio in urine.
Conclusion
BNP is a potential and a promising biomarker for PAD screening in patients with type-2 DM.

Keywords:
brain natriuretic peptide, peripheral arterial disease, type-2 diabetes mellitus

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Introduction

Brain natriuretic peptide (BNP) is mainly secreted from cardiomyocytes after volume expansion or pressure overload. It has been linked to cardiac affection before appearance of symptoms. Lately, it has been linked to many noncardiac diseases such as rheumatic diseases, pulmonary diseases, and atherosclerosis [1,2]. It is found to be elevated in general population with peripheral arterial diseases (PAD) [3–5]. New data suggest its relation to diabetes mellitus (DM) in an inverse manner [6–8]. PAD is increased in diabetic than nondiabetic patients, with an increased risk of fourfolds of developing PAD in diabetic patients [9–11]. Being related to PAD and DM, this may suggest it having a role in developing DM with PAD. Ankle–brachial index (ABI) is a well-established noninvasive method of detecting PAD [12]. It depends on dividing the systolic pressure of the ankle by the brachial artery, which is supposed to be 1 in normal cases, as both systolic pressures will be equal. In case of atherosclerosis, ankle pressure will decrease, indicating PAD [13]. As atherosclerosis is one of the commonest complications of DM, we used ABI to diagnose its presence to be compared after that with BNP as a marker for complications of DM.

Patients

This is case–control study that was conducted at Helwan University hospitals. It included 60 patients with type 2 diabetes, with age between 35 and 65 years of (patient group), who were attending endocrine outpatient clinics, and another 40 age-matched and sex-matched participant, who were chosen as control group, with no past history of any chronic medical illness. Written consents were obtained from the patients and controls enrolled in this study.

Inclusion criteria

Inclusion criteria were a diagnosis of type-2 diabetes mellitus (T2DM) and PAD, defined as arterial

insufficiency with an ABI is less than or equal to 0.90 in either leg [14].

Exclusion criteria

T2DM and other endocrinal disorders, hypertension, renal failure, rheumatic heart disease, vascular disease (including coronary, cerebral, or PAD), chronic obstructive pulmonary disease, atrial fibrillation, and aortic disease, malignancy, and autoimmune disease were the exclusion criteria.

Methods

Each patient in this study was subjected to complete physical examination including full neurological examination. All the relevant examinations were completed, and the patients were categorized according to presence or absence of neuropathy.

Laboratory investigation

FPG and 2-h PG were measured. Lipid profiles including total serum cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and serum triglycerides were measured after a fasting period of 12–14 h. The concentration of total serum cholesterol, high-density lipoprotein cholesterol, and serum triglycerides were determined in fresh serum by conventional enzymatic assays. Serum creatinine level was measured. Random urine samples were collected for complete urine analysis and tested for pyuria, hematuria, and microalbuminuria. Plasma levels of glycosylated hemoglobin (HbA1c) were measured by ELISA. kit (Calbiotech Inc., USA, Catalog No.: IS098D).

Brain natriuretic peptide

Enzyme-linked immunosorbent assay kit allows for the determination of BNP concentration in human serum, blood plasma, and other biological fluids.

Blood samples were collected from all patients from the venous line. The samples were collected in plastic tubes and were immediately centrifuged at 4°C. Serum was separated and stored at −20°C until assays were performed. The kit assay Human BNP level in the blood, using purified human BNP antibody to coat microtiter plate wells, make solid phase antibody, then add BNP to wells, combined BNP antibody which with enzyme labeled, become antibody–antigen–enzyme–antibody complex, after washing completely, add substrate, substrate becomes blue color at horseradish peroxidase enzyme-catalyzed reaction is terminated by the addition of a sulfuric acid solution and color change is measured spectrophotometrically at wavelength of 450 nm. The concentration of the samples is then determined by comparing the optical density (OD) of the samples to the standard curve.

Ankle–brachial index

The ABI is the ratio of the systolic pressure in the ankle to the systolic pressure in the arm. Device used: the ABI was determined using a bidirectional blood flow meter with wave form display of 8 MHz and a precalibrated mercury sphygmomanometer. Patient position: after the patient has been resting quietly for 5–10 min in the supine position, the systolic blood pressure was measured in both arms and in both ankles in the dorsalis pedis and posterior tibial arteries. The blood pressure cuff was placed ∼1 inch above the antecubital fossa for the brachial pressure and ∼2 inches above the medial malleolus for the ankle pressures.

A clear arterial pulse signal should be heard using the Doppler probe before inflating the blood pressure cuff. The cuff was inflated to at least 20 mmHg above the point where the arterial Doppler sounds disappear and then slowly deflated until the Doppler sounds reappear. The blood pressure at which the Doppler signal of the arterial pulse reappeared was the systolic pressure for that vessel.

The ABI was calculated by dividing the higher of the two ankle systolic blood pressures in each leg by the higher of the two brachial systolic blood pressures. The higher of the two brachial pressures was used as the denominator to account for the possibility of subclavian artery stenosis, which can decrease the blood pressure in the upper extremity.

The ABI was calculated for each leg, and the lower value was the patient's overall ABI. An abnormal value in either leg indicates PAD.

Interpreting the ankle–brachial index

Diagnostic criteria for the ABI were standardized. Most healthy adults have a value greater than 1.0. A value of less than 0.91 is consistent with significant PAD and a value lower than 0.40 at rest generally indicates severe disease. A value between 0.91 and 0.99 is borderline abnormal and does not rule out PAD. A value greater than 1.40 reflects no compressibility of the leg arteries and is not diagnostic.

Statistical analysis

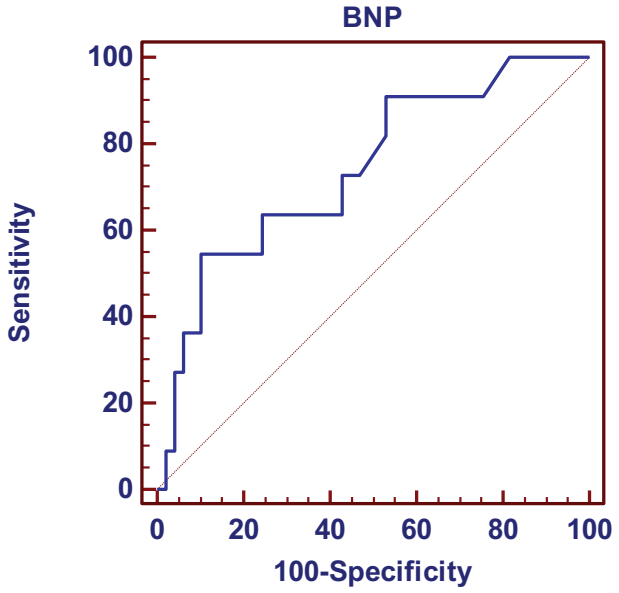
Data entry and statistical analysis were done by statistical package for the social sciences version 20 (SPSS; SPSS Inc., Chicago, Illinois, USA). Data for continuous variables were expressed as mean, SD, and SE.

Categorical variables were expressed as absolute numbers and percentages. Comparison between two groups was analyzed by nonparametric test Mann–Whitney test for continuous variables and χ^2 for qualitative data. *P* value of less than 0.05 was statistically significant (Tables 1 and 2, and Fig. 1).

Results

In receiver operator characteristic curve, BNP yielded an area under the curve of 0.74 (95% confidence interval, 0.61–0.84; *P*=0.005) for detection of PAD. BNP level of greater than 360 pg/ml was determined as the cutoff value that gave the best combination of sensitivity and specificity (27.27 and 95.9, respectively) for detecting the presence or absence of PAD (Fig. 2, and Tables 3 and 4).

Fig. 1

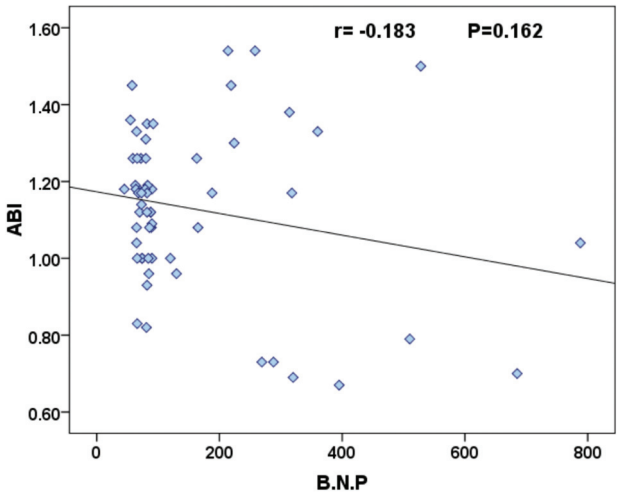


Brain natriuretic peptide receiver operator characteristic curve. BNP, brain natriuretic peptide.

Discussion

DM affects the levels of natriuretic peptides regardless of cardiovascular disease [15,16].

Fig. 2



The brain natriuretic peptide level was negatively correlated with the ankle–brachial index (*r*=–0.183, *P*=0.162). ABI, ankle–brachial index; BNP, brain natriuretic peptide.

Table 1 Demographic data

Variables	Diabetic group (*n*=60) [*n* (%)]	Control group (*n*=40) [*n* (%)]	*P* value
Age			
<50 years	20 (33.3)	16 (40.0)	0.737
50–60 years	22 (36.7)	12 (30.0)	
>60 years	18 (30.0)	12 (30.0)	
Mean±SD	53.87±7.98	52.55±8.38	0.430
Sex			
Male	23 (38.3)	16 (40.0)	0.867
Female	37 (61.7)	24 (60.0)	
BMI			
Normal	3 (5.0)	29 (72.5)	0.000
Overweight	34 (56.7)	11 (27.5)	
Obese	23 (38.3)	0 (0.0)	
Mean±SD	29.84±4.74	24.14±2.00	0.000
Smoking habit			
Smoker	18 (30.0)	15 (37.5)	0.435
Nonsmoker	42 (70.0)	25 (62.5)	
Nephropathy	19 (31.7)	0 (0.0)	0.000
Retinopathy	14 (23.3)	0 (0.0)	0.001
Neuropathy	10 (16.7)	0 (0.0)	0.017
Ankle-brachial index	1.05±0.05	1.13±0.21	0.025

Table 2 Ankle–brachial index with demographic data in diabetic patients

Variables	ABI		*P* value
	Low (*n*=11) (18.3%) [*n* (%)]	Normal (*n*=49) (81.6%) [*n* (%)]	
Age			
<50 years	2 (10.0)	18 (90.0)	0.138
50–60 years	3 (13.6)	19 (86.4)	
>60 years	6 (33.3)	12 (66.7)	
Mean±SD	58.36±6.70	52.86±7.96	0.038
Sex			
Male	7 (30.4)	16 (69.6)	0.056
Female	4 (10.8)	33 (89.2)	
BMI			
Normal	1 (33.3)	2 (66.7)	0.789
Overweight	6 (17.6)	28 (82.4)	
Obese	4 (17.4)	19 (82.6)	
Mean±SD	29.77±5.25	29.86±4.68	0.959
Smoking habit			
Smoker	5 (27.8)	13 (72.2)	0.216
Nonsmoker	6 (14.3)	36 (85.7)	
Diabetic duration			
<5 years	2 (5.9)	32 (94.1)	0.004
≥5 years	9 (34.6)	17 (65.4)	
Type of treatment			
Oral antidiabetic agents	8 (20.0)	32 (80.0)	0.637
Insulin treatment	3 (15.0)	17 (85.0)	
Waist circumference	110.36±15.64	107.49±14.95	0.569
Total cholesterol (mg/dl)	200.45±36.12	191.88±35.83	0.228
HDL (mg/dl)	37.18±11.59	39.40±10.23	0.379
LDL (mg/dl)	136.95±24.88	123.14±28.24	0.075
Total triglyceride (mg/dl)	144.64±61.51	141.57±81.73	0.462
HbA1c	9.97±1.93	8.84±1.79	0.044
Creatinine (μmol/l)	72.43±15.83	70.97±25.28	0.534
Microalbuminurea	134.17±111.53	42.29±72.94	0.007
B-natriuretic peptide	264.64±203.58	133.32±135.05	0.013
Nephropathy	8 (72.7)	11 (22.4)	0.001
Retinopathy	6 (54.5)	8 (16.3)	0.007
Neuropathy	3 (27.3)	7 (14.3)	0.296

ABI, ankle–brachial index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

The relation between BNP and PAD is not fully understood, but there is a postulation that antimigration factors for vascular smooth muscle cells are affected by the natriuretic peptide family [17].

Added to this, they have a protective effect as they promote angiogenesis and decrease cardiac load by their diuretic effect [18–20].

This led to the postulation of presence of a strong relationship between PAD and BNP in diabetic population, and in the past few years, this point was under investigation.

In our study, we divided the work into three stages. In the first stage, we examined different well-known confounders related to diabetes and calculated their statistical significance in diabetic and control groups. BMI, ABI, nephropathy, neuropathy, and retinopathy showed statistical significance.

In the second stage, we used ABI as a noninvasive method to determine PAD in noncomplaining diabetic individuals. Mean age, sex, duration of diabetes, glycated hemoglobin, creatinine, nephropathy, retinopathy, microalbuminuria, and BNP showed statistical significance when comparing between having low and normal ABI. These results were similar to that concluded by Jin *et al.* [21]. Renal affection in the form of creatinine level, microalbuminuria, and nephropathy can be explained by the settled information about the correlation between diabetes and presence of cardiac and renal affection [22]. Moreover, microalbuminuria is one of the risk factors for cardiovascular complications in diabetic patients [23].

Table 3 Brain natriuretic peptide cutoff value in relation to different variables

Variables	<360 (*n*=11)		>360 (*n*=49)		*P* value
	Mean	SD	Mean	SD	
Age	56.18±5.363		53.35±2.418		0.315
SBP	124.55±10.113		123.67±9.724		0.782
DBP	76.82±5.600		77.55±5.874		0.610
BMI	31.727±6.1492		29.416±4.333		0.276
WC	115.545±17.0784		106.327±14.06		0.095
FBG	11.864±5.0532		8.6±3.26		0.055
PPG	14.800±5.1361		11.16±3.62		0.025
HbA1C	9.97±1.929		8.84±1.79		0.084
Urea	5.536±1.929		5.05±1.79		0.250
Creatinine	72.427±15.8263		70.96±25.28		0.534
Serum albumin	3.782±0.5056		3.76±0.417		0.921
TC	200.45±36.123		191.88±35.8		0.228
LDL	136.945±24.8820		123.141±28.24		0.141
HDL	37.182±11.5915		39.4±10.22		0.527
TG	144.64±61.505		141.57±81.73		0.462
UACR	134.173±111.5266		42.28±72.93		0.007
GFR	111.862±31.2118		117.4±29.67		0.580
EF	64.27±6.035		66.98±5.23		0.137
Sex (male/female)	6/5		17/32		0.221
Smoking (yes/no)	4/7		14/35		0.610
Retinopathy (yes/no)	6/5		8/41		0.007
Nephropathy (yes/no)	8/3		11/38		0.001
Neuropathy (yes/no)	3/8		7/42		0.296
Therapy (oral/insulin)	8/3		42/17		0.637

DBP, diastolic blood pressure; EF, ejection fraction; FBG, fasting blood glucose; GFR, glomerular filtration rate; HbA1C, glycated hemoglobin; HDL, high-density lipoproteins; LDL, low-density lipoproteins; PPG, postprandial glucose; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; UACR, urinary albumin creatinine ratio.

Table 4 Regression analysis for predictors of patients with brain natriuretic peptide greater than 360

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value reference	OR	95% CI	*P* value reference
Retinopathy	6.15	1.505–25.139	0.011	0.668	0.049–9.012	0.761
Nephropathy	9.212	2.083–40.75	0.003	26.98	1.383–526.265	0.03
PPG	1.248	1.034–1.505	0.021	1.291	1.022–1.630	0.032
UACR	1.014	1.005–1.023	0.003	1.014	1.003–1.026	0.013

CI, confidence interval; OR, odds ratio; PPG, postprandial glucose; UACR, urinary albumin creatinine ratio.

BNP less than 100 pg/ml does not indicate heart failure, whereas BNP greater than 400 pg/ml is a strong indication of heart failure. BNP of 100–400 pg/ml is left for clinical correlation [24,25]. Moreover, BNP could be related to many factors other than cardiovascular ones [26]. Accordingly, in the last stage, we examined specific confounders to BNP with calculating a cutoff value for detecting PAD. In receiver operator characteristic curve, BNP yielded an area under the curve of 0.74 (95% confidence interval, 0.61–0.84; *P*=0.005) for detection of PAD. After calculating the cutoff value, more than 360 pg/dl had the best specificity (95.9%) but with low sensitivity (27.27%). This cutoff value was different from a study carried by Jin *et al.* [21] which had 72.8 as a cutoff value with sensitivity and specificity of 71 and 68%, respectively. We compared below and above this cutoff value, resulting in high statistical significance with fasting and postprandial blood glucose levels, urinary albumin to creatinine ratio, nephropathy, and retinopathy. This was assured by regression analysis to examine the previously mentioned confounders and their relation to BNP greater than 360. We had statistical significance with retinopathy on univariate analysis only but it was not in the case with nephropathy, postprandial glucose, and urinary albumin/creatinine ratio, where all of them showed high statistical significance on univariate and multivariate analyses. The finding of vascular affection in the form of retinopathy and nephropathy can be explained by the point of having high BNP is associated with lower vascular functional capacity [27]. Moreover, pretreatment of Wistar rats with BNP was found to attenuate production of radical

oxygen species [28]. A study carried out by Seki *et al.* [29] mentioned that BNP at baseline is an independent predictor of annual rate of decline in estimated glomerular filtration rate, and its monitoring can be used as a tool in management of diabetic nephropathy. All of the previous data point in the direction of investigating diabetic patients without obvious cardiovascular complications for BNP levels for early prediction and management of such complications at early stages.Studying the relation between glycemic control and BNP level was carried by Dal *et al.* [30] where a poor glycemic control was concluded to raise BNP which may lead to overdiagnosis of congestive heart failure. This was emphasized by our study with the high statistical significance between postprandial glucose and BNP.

Then, the correlation between the noninvasive used tool (ABI) and the investigation under examination (BNP) was calculated. The BNP level was negatively correlated with the ABI (r=−0.183, P=0.162). This negative correlation was mentioned by Jin *et al.* [21]. This assures the investigated point of the importance of measuring the BNP in diabetic patients even in absence of any obvious vascular complications, as low ABI indicates presence of PAD, and this was negatively correlated to BNP levels.

Our points of weakness are the small sample size in a widely distributed disease. We also used ABI to identify PAD, which is not the best method in case of atherosclerosis.

Conclusion

BNP is a potential marker for detecting occult PAD in diabetic patients.

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Conflicts of interest

There are no conflicts of interest.

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