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ORIGINAL ARTICLE

Higher circulatory level of endothelin-1 in hypertensive subjects screened through a cross-sectional study of rural Bangladeshi women

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Endothelin-1 (ET-1) is a potential marker of the endothelial dysfunction, which has been shown to be elevated in hypertensive subjects. No previous study has investigated the circulatory level of ET-1 and hypertension in a South Asian country. The present study assessed the circulating levels of ET-1 in subjects with or without hypertension and further examined the association of ET-1 with clinical and metabolic parameters. A total of 2543 rural Bangladeshi women with a mean age of 44.5 years were studied using a cross-sectional survey. Multiple regressions were used to examine the association between the circulatory ET-1 levels and hypertension. The prevalence of hypertension was 29.3%. The ET-1 levels were significantly higher in the hypertensive (mean 3.08 pg ml^{-1}, s.e. (0.19)) than in the non-hypertensive subjects (mean 2.01 pg ml^{-1}, s.e. (0.03)) ($P=0.001$). After adjusting for age, the ET-1 level had significant positive associations with the diastolic blood pressure ($P=0.002$), systolic blood pressure ($P=0.001$), mean arterial pressure ($P=0.002$) and fasting blood glucose ($P=0.002$). In a tertile analysis, we found that hypertension in the subjects was significantly increased as the levels of ET-1 increased (P for the trend $=0.001$). In a stepwise multiple regression analysis, after adjusting for age and all other potential variables, we found that the mean arterial pressure and the fasting plasma levels have significant associations with the ET-1 level. The present study demonstrates that there is a higher concentration of ET-1 among the hypertensive subjects in an apparently healthy population of Bangladeshi rural women. The relationship between ET-1 and hypertension requires further investigation to define the clinical utility and predictive value of serum ET-1 levels for hypertension for a South Asian population.
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INTRODUCTION

High blood pressure is an important worldwide public health challenge because of its high frequency and its concurrent risks of stroke and cardiovascular and renal diseases.[1,2] Worldwide, 26% of the adult population were hypertensive in 2000, and 29% are projected to have this condition by 2025.[3] In Bangladesh, nearly 39% of the adults have hypertension, and this figure appears to be higher than among other South Asian populations.[4] The identification of risk factors and the control of hypertension are of the utmost importance in this region. The importance of blood pressure as a modifiable risk factor for cardiovascular disease is well recognized. Although many effective and inexpensive blood pressure-lowering treatments are available, other clinical approaches for controlling hypertension need to be explored.

Endothelin (ET) is a potent endothelial cell-derived venous and arterial vasoconstrictor peptide[5] that consists of a family of 21 amino acid peptides (ET-1, ET-2 and ET-3).[6] Among the family of peptides, ET-1 is the most abundant isoform and most potent vasoconstrictor in humans and contributes to the maintenance of the basal vascular tone.[7] In animal models of hypertension, ET-1 is overexpressed in the vascular wall,[8] and mice with an ET-1 gene that has been inactivated exhibit a slight elevation in blood pressure.[9] In addition, ET-1 is involved in a variety of other physiological processes in both animals and humans, including cell proliferation,[10] fibrosis,[11] endothelial dysfunction,[12] arterial stiffness[13] and cardiac hypertrophy;[14] all of which may contribute to the development and maintenance of hypertension. In this context, it is of interest to investigate the associations between ET-1 and blood pressure in humans.

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To date, the possible relationship of ET-1 with essential hypertension has been limited to experimental studies,[8,15] and few clinical studies in humans conducted in the developed countries have found elevated levels of ET-1 among hypertensive subjects,[16–20] whereas other studies have found no significant difference[21,22] compared with the healthy controls. To our knowledge, only one study has investigated the association between ET-1 and hypertension among an apparently healthy population.[23] Moreover, no previous studies on this topic have been conducted in South Asian populations or populations from low-income countries. As racial differences exist in the plasma levels of ET-1,[24] more research addressing the association between ET-1 and hypertension among other races requires investigation. As such, we aimed to examine the associations between the circulating ET-1 levels and hypertension among Bangladeshi rural women.

MATERIALS AND METHODS

Study procedure

The present study is a community-based cross-sectional study performed in women from rural Bangladesh between 2009 and 2010. A total of 2543 rural Bangladeshi women with a mean age of 44.5 years were studied using a cross-sectional survey. Females aged $\geqslant 15$ years were selected using the stratified multistage random sampling method. This sample size was sufficient to test all of our formulated research hypotheses at the 5% level of significance with a power of 80% ($\beta = 0.20$). We used the World Health Organization's STEPwise approach to surveillance (STEPS) approach (modified), which entails a stepwise collection of the risk factor data based on standardized questionnaires covering demographic characteristics, somatic illnesses, somatic and mental symptoms, medications, lifestyle and health-related behaviors (step 1), basic physical measures (step 2) and basic biochemical investigations, such as blood glucose and cholesterol (step 3). The women were recruited from the village communities of the Gaibandha district and Noagon district, covering two of the divisions of Bangladesh (Rajshahi and Rangpur). (A division is the largest administrative tier in Bangladesh, and there are a total of seven divisions in Bangladesh.). The respondents were selected randomly after selecting the division, district, Upazila and villages and were recruited through local announcements at the community level and by house-to-house visits. The details of the study area have been described before in our previous study.[25] The participants' data were obtained through interviews and clinical examinations at mobile examination centers, where blood samples were also collected. The study was approved by the Ethical Committee of the Health and Disease Research Center of Rural Peoples, Dhaka, Bangladesh, Shahid Ziaur Rahman Medical College, Bogra, Bangladesh and conforms to the principles outlined in the Helsinki Declaration. All of the participants gave their written informed consent prior to inclusion in the study.

Study subjects

From a total of 2720 recruited women, we excluded the subjects with chronic illnesses such as hypothyroidism, pregnant women, those on hormone replacement therapy, those with known illnesses such as ischemic heart disease and those with diabetes. After the exclusions, a total of 2543 subjects remained in this study.

Anthropometric and other variables

The anthropometric measurements of these individuals while they were wearing light clothing and no shoes were obtained by well-trained examiners as follows: height was measured to the nearest 0.1 cm using a portable stadiometer (Seca, Hamburg, Germany); weight was then measured in an upright position to the nearest 0.1 kg using a calibrated balance beam scale; body mass index was calculated as the body weight (kg) divided by the square of the body height (m^2); and waist circumference measurements were taken at the end of a normal expiration to the nearest 0.1 cm by measuring from the narrowest point between the lower borders of the rib cage and the iliac crest.

Blood pressure measurement

Blood pressure was measured twice in the right arm with the subject in a sitting position using a standard mercury manometer and cuff, to the nearest 2 mm Hg, with the initial reading taken at least 5 min after the subject was made comfortable, and again after an interval of 15 min. The average systolic and diastolic blood pressures were then estimated. Hypertension was defined as a systolic blood pressure $\geqslant 140$ mm Hg, a diastolic blood pressure $\geqslant 90$ mm Hg or the use of antihypertensive medication. The subjects who were determined to be hypertensive through the initial screening were followed up for 2 months, with four additional blood pressure measurements.

Biochemical analysis

Blood for the biochemical analysis was obtained from the participants after a 10–12 h overnight fast. The blood sample was collected using the standard blood sample collection procedure. Immediately after the collection of blood and labeling the blood vials, the samples were transported to the National Centre for Global Health and Medicine, Japan, for the biochemical assessment. For the analysis, the serum was immediately separated from the blood by centrifugation to evaluate the plasma concentration of the lipids. The triglycerides levels were measured using the lipoprotein lipase method (Wako Chemicals, Tokyo, Japan), high-density lipoprotein cholesterol levels were measured using the Determiner-L kit (Kyowa, Tokyo, Japan) and the fasting plasma glucose levels were measured using the Hexokinase G-6-PDH kit (Wako Pure Chemical Industries, Osaka, Japan).

Enzyme-linked immunosorbent assay for plasma ET-1 level

The concentration of the ET-1 in the plasma was determined using a Quantikine ET-1 Enzyme Immuno Assay Kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocol. A 4.5 h solid-phase enzyme-linked immunosorbent assay was used, which contained synthetic ET-1 and antibodies raised against synthetic ET-1. This immunoassay has been shown to accurately quantitate synthetic and naturally occurring ET-1. The standards and samples were pipetted into the wells, and if present, the ET-1 antigen was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific to ET-1 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells, and the color developed in proportion to the amount of ET-1 bound in the initial step. The color development was then stopped, and its intensity was measured. The ET-1 concentration of each sample was calculated with a standard curve constructed by plotting the absorbance of each standard solution.

Statistical analysis

The differences in the clinical characteristics between the hypertensive and non-hypertensive subjects were assessed by the *t*-test and the Mann–Whitney test for normal and skewed continuous variables, respectively. The mean $\pm$ s.e. and the median (interquartile range) are presented, where appropriate. A linear regression analysis was used to evaluate the association between the ET-1 plasma levels and the clinical/metabolic parameters for the unadjusted and age-adjusted models. To identify the independent determinants of ET-1 and hypertension, a stepwise multiple regression analysis was performed in forward direction with the significance level for additions to the model set to 0.20. The trend association between the tertiles of the ET-1 levels and the percentage of subjects with hypertension was tested using linear regression analysis. Receiver operator characteristic curves and the corresponding area under the curves were used to evaluate the diagnostic values of the tertiles of the ET-1 for the hypertensive status. The optimal cut-off point was selected based on the receiver operator characteristic curves providing the maximum diagnostic efficiency (the maximum value of specificity % plus sensitivity %). Two-sided P values <0.05 were considered statistically significant. All of the analyses were performed using Stata version 12.0 (Stata Corp, College Station, TX, USA).

RESULTS

The individuals screened in this study as hypertensive were unaware that they had hypertension. This screening was conducted as part of a non-communicable disease screening of apparently healthy women in rural Bangladesh.

Table 1 shows the clinical characteristics of the subjects according to their hypertensive and non-hypertensive status. The hypertensive subjects were relatively older, and many of their cardiometabolic factors, including systolic blood pressure, diastolic blood pressure, fasting blood glucose, triglycerides levels and waist circumference were significantly higher than their non-hypertensive counterparts. However, the high-density lipoprotein cholesterol levels were significantly lower in the hypertensive subjects than in the non-hypertensive subjects ($P<0.05$ for all).

The plasma ET-1 levels were significantly higher in the hypertensive subjects than in the non-hypertensive subjects ($P=0.001$) (Figure 1).

Table 2 shows the association between the ET-1 levels and the metabolic or other clinical parameters. After adjusting for age, the ET-1 levels were significantly associated with the systolic blood pressure ($P=0.001$), diastolic blood pressure ($P=0.002$), mean arterial pressure (MAP) ($P=0.001$), fasting plasma glucose level ($P=0.002$) and plasma high-density lipoprotein levels ($P=0.03$). Other variables, including the body mass index, waist circumference, triglycerides and fasting insulin levels, were not associated with the ET-1 levels. A stepwise multiple regression analysis considering all of the variables presented in Table 1 revealed that the mean arterial pressure and fasting plasma glucose levels were independent determinants of the plasma ET-1 levels (Table 3). In addition, when we performed multiple logistic regressions for the hypertension status and the other predictors, we found that an elevated plasma ET-1 level had a strong association with hypertension (Table 4).

Figure 2 shows the association between the tertiles of the ET-1 levels and the percentage of hypertension. As shown in the figure, hypertension was significantly increased in subjects as the levels of ET-1 increased (P for trend $=0.001$).

Figure 3 shows the receiver operator characteristic curve for hypertension and the tertiles of ET-1. The area under the curves values were moderately higher (0.758; 95% CI = 0.687–0.829; s.e. = 0.036; $P<0.001$), demonstrating the sensitivity and 1-specificity of the prediction of the hypertension risk for the different levels of ET-1. The cut-off point for the tertiles of ET-1 was found to be T3, as the ET-1 levels of T3 were 2.38 or more. The sensitivities and specificities of ET-1 were 0.563 and 0.866, respectively.

Table 1 Clinical characteristics of subject according to hypertensive status

Characteristics	*Non-hypertensive*	*Hypertensive*	P-*value*[a]
Subjects (prevalence)	1798 (70.7)	745 (29.3)	
Age (years)	40.52 ± 1.34[b]	48.85 ± 1.21	<0.001
BMI (kg m^{-2})[c]	22.67 (20.00–25.00)	22.89 (19.74–25.73)	0.799
Waist circumference (cm)	75.48 ± 0.75	81.49 ± 1.03	<0.001
Triglycerides (mg dl^{-1})[c]	159.65 (100.35–176.96)	179.78 (128.44–232.01)	<0.001
HDL cholesterol (mg dl^{-1})	42.06 ± 1.36	36.50 ± 1.45	0.006
Systolic blood pressure (mm Hg)	106.95 ± 1.49	151.57 ± 1.96	<0.001
Diastolic blood pressure (mm Hg)	71.26 ± 0.79	89.56 ± 1.01	<0.001
MAP (mm Hg)	83.18 ± 0.98	110.24 ± 1.21	<0.001
Fasting blood glucose (mmol l^{-1})[c]	6.20 (5.20–6.80)	6.60 (5.60–7.95)	<0.001
Insulin (μU ml^{-1})[c]	6.55 (2.66–9.74)	6.70 (3.78–13.64)	0.359

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein, MAP, mean arterial pressure.
[a]Based on *t*-test for normal continuous variables, Mann–Whitney test for non-normal continuous variables.
[b]Mean ± s.e. for normal variables (all such values.
[c]Median (interquartile range) for non-normal variables (all such values).

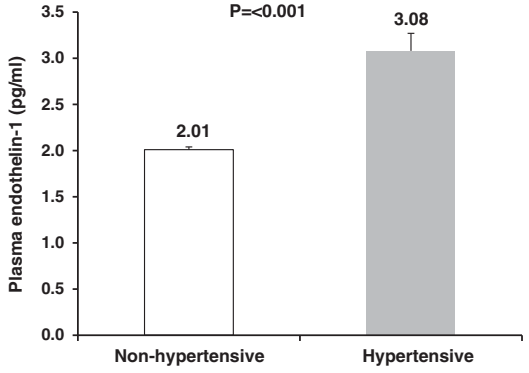

Figure 1 Mean levels of plasma endothelin-1 according to hypertensive and non-hypertensive status.

DISCUSSION

In this cross-sectional study of Bangladeshi women, the plasma ET-1 levels were significantly higher in the subjects with hypertension than in the non-hypertensive subjects, and both the systolic and diastolic blood pressures were positively associated with ET-1 after adjusting for age. To our knowledge, this is the first study in an apparently healthy South Asian population to address the association between the circulating levels of ET-1 and hypertension.

The elevated ET-1 levels among the subjects with hypertension and the significant positive association between the ET-1 and the blood pressure in our study is consistent with some of the previous clinical studies.[16–19] According to these previous studies, the plasma ET-1 levels were significantly higher in the patients with essential hypertension compared with the healthy controls. Moreover, in line with our study, Seissler *et al.*[26] found a significant positive association between proET-1 and high blood pressure. However, the findings of our study were not consistent with a number of other studies. In an apparently healthy population, Hirai *et al.*[23] found a significant positive association between ET-1 and the systolic and diastolic blood pressures in a univariate analysis; however, in a multiple stepwise regression model, both the systolic and diastolic blood pressures were not associated with ET-1. Similarly, Piatti *et al.*[21] found no significant positive association of ET-1 with the systolic and diastolic blood pressures in a multiple regression analysis. Differences in the background characteristics of the study population may be a potential reason for the inconsistent findings across the studies. The present findings, together with a majority of clinical studies, suggest that hypertension may be associated with elevated ET-1 levels, but more studies from South Asian countries may clarify the relationship between ET and hypertension that we observed in the present study.

In the present study, we clearly demonstrated through a tertile analysis that hypertension was significantly increased in subjects as the levels of ET-1 increased (P for trend $=0.001$). Among the hypertensive subjects who belonged to the highest tertile of the ET-1 level, 50% of them had a fasting blood glucose level $\geqslant 7$ mmol l^{-1}, 10% of them were overweight (body mass index $\geqslant 25$) and 100% of them had

Table 2 Association between endothelin-1 and others parameters

	Unadjusted			*Age-adjusted*		
Variables	*Coefficient (β)*	*s.e.*	P-*value*[a]	*Coefficient (β)*	*s.e.*	P-*value*[a]
Age (years)	0.0170	0.0077	0.029	—	—	—
BMI (kg m^{-2})	0.0055	0.0225	0.808	0.0090	0.0223	0.689
Waist circumference (cm)	0.0202	0.0112	0.074	0.0188	0.0112	0.095
Triglycerides (mg dl^{-1})	0.0015	0.0016	0.338	0.0012	0.0015	0.436
HDL cholesterol (mg dl^{-1})	−0.0213	0.0088	0.017	−0.0193	0.0088	0.030
Systolic blood pressure (mm Hg)	0.0144	0.0035	<0.001	0.0132	0.0037	<0.001
Diastolic blood pressure (mm Hg)	0.0294	0.0078	<0.001	0.0265	0.0083	0.002
MAP (mm Hg)	0.0232	0.0056	<0.001	0.0213	0.0061	0.001
Fasting blood glucose (mmol l^{-1})	0.0180	0.0540	0.001	0.0168	0.0542	0.002
Insulin (μU ml^{-1})	−0.0030	0.0060	0.615	−0.0020	0.0059	0.740

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; MAP, mean arterial pressure.
[a]Based on the regression analysis.

Table 3 Multiple linear regression of plasma levels of endothelin-1 (pg ml^{-1}) and others predictors

Characteristics	*Coefficient of (β)*	*s.e.*	P-*value*
MAP (mm Hg)	0.0251	0.0078	0.054
Fasting blood glucose (mmol l^{-1})	0.0157	0.0617	0.012

Abbreviation: MAP, mean arterial pressure.

Table 4 Multiple logistic regression of hypertension status and others predictors

Characteristics	*Odds ratio*	*s.e.*	P-*value*
Age (years)	1.058	0.019	0.004
Waist circumference (cm)	1.147	0.037	<0.001
Triglycerides (mg dl^{-1})	1.015	0.004	0.001
Endothelin-1 (pg ml^{-1})	17.319	0.829	0.001

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; MAP, mean arterial pressure.
nn-Whitney test for non-normal continuous variables.
Mean ± s.e. for normal variables (all such values).

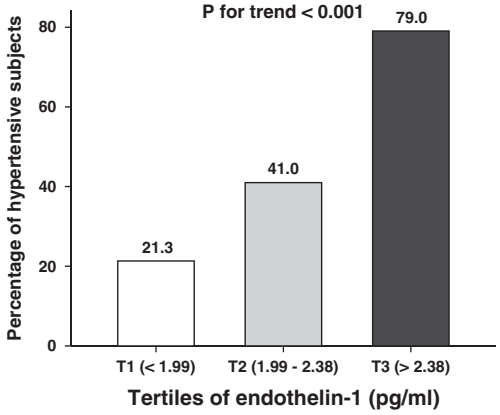

Figure 2 Percentage of subjects with hypertension according to the tertiles of endothelin-1.

metabolic syndrome (as determined by modified National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III criteria). In addition, in the current analysis we found that the fasting blood glucose level had a positive association with the plasma ET-1 level, as demonstrated through both the univariate and multivariate regression analyses. Moreover, the plasma high-density lipoprotein level had a negative significant association with the plasma ET-1 level only in the univariate regression analysis. Thus, in addition to the blood pressure, the fasting blood glucose level was also a determinant for the plasma ET-1 levels in our subjects. However, when we performed a multiple logistic regression analysis for the hypertensive status and the other predictors, there was no significant association between hypertension and the fasting blood glucose level; conversely, there was a strong association between hypertension and the plasma ET-1 level in the logistic regression analysis.

Although the mechanisms involved in the induction of ET-1 in hypertensive subjects remains unclear, several possible explanations have been suggested. The intravenous infusion of ET causes a rapid and transient vasodilation followed by a profound and long-lasting increase in blood pressure.[5,27] *In vitro* experiments have demonstrated that this increase in blood pressure is related to the profound vasoconstriction of the resistance arteries of the different vascular beds of the circulation.[28,29] ET-1 not only exerts direct vasoconstrictor effects but is also able to potentiate other vasoconstrictor substances such as norepinephrine and serotonin,[30] which may be involved in the development of hypertension.[31] Another mechanism may be the profound renal effects of ET-1.[17,23] ET decreases the renal plasma flow and glomerular filtration rate at a level of the range at which no generalized alterations in hemodynamics occur.[29] As the kidney plays a central role in the regulation of the chronic hemostasis of the pressure–volume regulation, these effects may have important roles for the development of hypertension.

The major strengths of the present study include the use of a large community-based survey with a relatively large sample size and the exclusion of subjects with chronic diseases such as heart diseases and diabetes that may interact with the possible relationship between ET-1 and hypertension. However, the present study also has several limitations that need to be mentioned. First, the existence of an association derived from a cross-sectional study does not necessarily indicate causality. Thus, the present study cannot rule out whether the higher plasma ET-1 level observed in the present study in hypertensive subjects is the result of high blood pressure. A prospective cohort

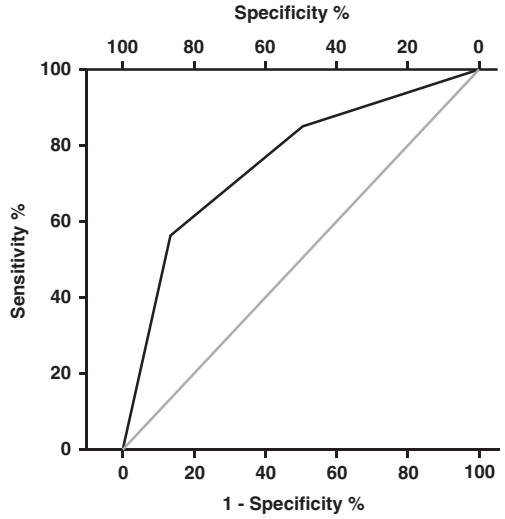

Figure 3 Receiver operating characteristic for the hypertensive subjects and the tertiles of endothelin-1.

study design and serial plasma ET-1 measurements are essential to explore the relationship between the hypertensive status and plasma ET-1 levels. Second, in assessing the association between ET-1 and blood pressure, we were unable to adjust for important lifestyle risk factors, including smoking, alcohol consumption and physical activity. However, alcohol consumption and smoking are very uncommon among Bangladeshi women and thus are unlikely to alter the results after the additional adjustment for these variables.

In conclusion, the findings of our study suggest that the plasma ET-1 level was elevated among the hypertensive subjects and was significantly and positively associated with the blood pressure among the rural women in Bangladesh. Prospective studies are needed to confirm the present cross-sectional findings among South Asian subjects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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