Endothelin-1 and Metabolic Syndrome in Bangladeshi Rural Women

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Abstract

Background and purpose: Metabolic alterations and endothelial dysfunction in patients with type 2 diabetes and metabolic syndrome (Met S) often develop concurrently and measurement of circulating ET-1 levels is a well recognized marker of endothelial atherosclerotic and cardiovascular diseases. Here, we assess association between vasoactive peptide, endothelin-1 (ET-1), with Met S in rural Bangladeshi women.

Methods and subjects: Plasma levels of ET-1 were measured by ELISA and Met S was defined according to criteria of NCEP-ATP III. Multiple regressions were used to examine association between circulating ET-1 levels and Met S.

Results: A total of 1236 rural Bangladeshi women aged ≥15 years were studied using a population based cross sectional survey. The prevalence of Met S was 25.1%. Mean values of BMI, waist circumference, blood pressure (SBP, DBP), plasma fasting glucose, triglyceride, mean arterial pressure and fasting insulin were significantly higher, whereas levels of HDL cholesterol were significantly lower in Met S group compared to non Met S group. ET-1 levels significantly increased in Met S subjects [(Met S vs. non-Met S: 3.17±0.12 vs. 2.31±0.05, p=<0.001]. Based on univariate analyses, after adjusting for age, levels of ET-1 showed significant associations with waist circumference, SBP, DBP, mean arterial pressure, HDL cholesterol and fasting plasma glucose. However, in a stepwise multiple regression analysis, after adjusting for age and all other potential variables, only MAP, fasting plasma glucose and HDL cholesterol was found to be independently correlated with ET-1. We also found that mean plasma levels of ET-1 increased in direct proportion to levels of Met S components (p for trend=0.015).

Conclusions: Here, we provide the first evidence demonstrating an association between ET-1 plasma levels and Met S in Bangladeshi rural women. These data implicate ET-1 in Met S and indicate that plasma ET-1 could potentially be used as a surrogate biomarker for this disease and its associated complications. The present study is the first to assess relationship between levels of plasma ET-1 in Met S subjects from a South Asian country.

Introduction

Metabolic syndrome (Met S) is a constellation of metabolic disturbances, as well as a well established predictor of cardiovascular diseases (CVD), type 2 diabetes and their associated mortalities [1-3]. Although reports on the prevalence of Met S can be contradictory due to differences in the criteria used by various studies, it is obvious that there has been a dramatic increase in the number of cases reported not only in developed but also in developing countries [4-7]. Notably, among the developing nations, cardio metabolic risk factors are rapidly increasing in South Asians, starting at an early age [8]. Specifically, in Bangladesh, Met S is highly prevalent and more than 31% of rural women are affected [9]. It has become apparent that there is an urgent need to explore other novel preventative approaches besides the current methods, namely: dietary and lifestyle changes, such as weight loss, regular physical exercise, and adopting healthy diet to control Met S.

Endothelin (ET) is the most potent endogenous vasoconstricting factor known in the body and is derived from endothelial cells of the venous and arterial vessels. It (ET) functions as both a circulating hormone and a paracrine factor and is involved in regulating vascular tone, and, consequently, blood pressure [10,11]. Of all the members of the ET family of peptides, endothelin-1 (ET-1) is the most abundant and, functionally, most important peptide in the vasculature. An increase in levels of ET-1 has been described in a variety of pathological conditions,
including hypertension [12], cardiovascular disease [13] and diabetes mellitus [14]. For this reason, circulating levels of ET-1 are considered as potential markers of endothelial damage and atherosclerosis, as well as negative prognostic predictors of cardiovascular diseases [15].

Although the association between ET-1 and several metabolic abnormalities has been investigated [16-17], relatively limited and inconsistent evidence is available regarding the direct relationship between ET-1 (including big-ET-1, pro-ET-1) and Met S [17-20]. Moreover, most of these data are predominantly from developed countries, with very limited data from developing ones, particularly the South Asian region, where the lifestyle and genetics are substantially different. For instance, in an analysis of age standardized coronary heart disease (CHD) mortality in Canada conducted over a 15-year period, Canadians of South Asian heritage or residents had the highest CHD mortality compared with individuals of Chinese and European descent [21]. The prevalence and projections of diabetes mellitus is equally higher in South Asians compared to several other populations [22]. In fact, India alone is projected to experience the greatest global increase in type 2 diabetes mellitus by 2025 [22]. Further, South Asians were found to have a higher prevalence of subclinical atherosclerosis, and South Asian ethnicity was an independent predictor of cardiovascular diseases [23]. Unfortunately, to date no studies have been conducted among South Asian populations that were apparently healthy but were later diagnosed with Met S during routine screening for non communicable diseases. Because of these reasons, the present study is aimed at: 1) clarifying whether there is an association between circulating levels of ET-1 and Mets in rural Bangladeshi women; 2) elucidating the nature of the association between ET-1 and Mets cluster components, 3) determining independent factors likely to influence circulatory ET-1 levels in this population, and 4) lastly, analyzing the association of independent determinants of ET-1 with metabolic parameters. The present study is the first to assess the association of ET-1 with Met S and its parameter in a South Asian country, namely Bangladesh.

Methods

Study procedure and subjects

The present study is a community-based cross-sectional study performed on women from rural Bangladesh. A total of 1236 participants aged ≥15 years were selected using the stratified multistage random sampling. We used the World Health Organization’s (WHO) 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, life style and health related behaviours (step 1), basic physical measures (step 2) and basic biochemical investigations, such as blood glucose and cholesterol (step 3). Following these steps, advanced biomarker assessments were then taken, i.e., circulatory levels of ET-1 and nitric oxide (NO).

The study was carried out in rural villages of three districts in Bangladesh, namely Rajshahi, Bogra and Naogoan (two villages for each district). Briefly, women were recruited through local public announcements using a loud speaker and door-to-door neighbourhood visits. Data from participants were obtained through interviews and clinical examinations at mobile examination centers, in addition to collection of blood samples. The study was approved by the Ethical Committee of the Health and Disease Research Center of Rural Peoples (HDRCRP), Dhaka, Bangladesh, and Bogra Medical College, Bogra, Bangladesh, and conformed to the principles outlined in the Helsinki Declaration. Also, the nature of the study was fully explained to all participating subjects in the language they understood well and only those (participants) that gave written informed consent were included in the study.

Finally, subjects with the following conditions were excluded from the study: participants with/on: a) chronic illness, such as hypothyroidism; b) pregnant women, c) hormone replacement therapy, as well as women with known illness, such as ischaemic heart disease or diabetes or hypertension. After exclusion a total of 1236 subjects remained in the study and in the final analysis.

Anthropometric measurements

Anthropometric measurements on individuals wearing light clothing and without shoes were conducted by well-trained examiners, as described here: a) height was measured to the nearest 0.1 cm using the portable stadiometer; b) weight was measured in an upright position, to the nearest 0.1 kg, using a calibrated balance beam scale; c) body mass index (BMI) was determined by dividing weight (kg) by height squared (m²); and d) waist circumference measurements were taken at the end of 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. In addition, blood pressure was measured twice in the right arm in a sitting position using the standard mercury manometer and cuff, to the nearest 2 mmHg, with the initial reading taken at least 5 minutes after the subject was made comfortable, and again after an interval of 15 minutes. The average systolic and diastolic blood pressures were then estimated.

Biochemical analysis

Blood for biochemical analysis was obtained from the participants after a 10-12 hour overnight fast. For analysis, both serum and plasma samples from processed from collected blood. Following parameters were measured in plasma triglycerides [lipoprotein lipase method; Wako Chemicals, Tokyo, Japan], total cholesterol [Cholesterol E, Wako Pure Chemical Industries, Ltd. Osaka, Japan], as well as levels of the fraction high-density lipoprotein cholesterol [HDL-C] [HDL-C with the Determiner-L kit (Kyowa Co Ltd, Tokyo, Japan)] and low-density lipoprotein cholesterol [(LDL-C)] with the Determiner-L kit (Kyowa Co Ltd, Tokyo, Japan). Fasting plasma glucose (FPG) [glucose with the Hexokinase G-6-PDH kit (Wako Pure Chemical Industries Ltd, Osaka, Japan)] were also measured.

Enzyme-Linked Immunosorbent Assay for plasma and cardiac ET-1 levels

Concentration of ET-1 in plasma was determined using a Quantikine ET-1 Enzyme Immuno Assay Kit (R&D Systems, Minneapolis, MN), according to the manufacturer’s protocol. A 4.5 h solid phase ELISA was used, and 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. Standards and samples were pipetted into the wells and if present, 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 and enzyme reagent, a substrate solution was added to the wells and a colour developed in proportion to the amount of ET-1 bound in the initial step. Development of the colour was then stopped and its intensity measured. The ET-1 concentration of each sample was calculated with a standard curve constructed by plotting the absorbance of each standard solution.

**Nitric Oxide Colorimetric Assay**

We placed 300μl serum and 300μl of potassium phosphate buffer in an ultra filter (e.g., 10 000 MWCO from SARTORIUS, VIVASCIENCE Cat. No. 13 239-E) and centrifuged at 20°C for 45 min (1250×g resp. 4000 rpm, r=7 cm). Then we collected the ultra filtrate and used in the nitric oxide colorimetric assay. Nitric oxide (NO) was indirectly detected in serum as nitrite using a NO Colorimetric Assay kit (Roche Diagnostics, Mannheim, Germany). In this method, the nitrate present in the sample was reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate in the presence of the enzyme nitrate reductase. The nitrate formed reacted with sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride to give a red violet diazo dye. The diazo dye was measured at 550 nm, on the basis of its absorbance within the visible range.

**Definition of metabolic syndrome and risk factors**

Met S was defined using the standard National Cholesterol Education Program Adult Treatment Panel III definition [24], with participants having three or more of the following five criteria: a) high blood pressure (SBP≥130 or DBP≥85mmHg) or subjects diagnosed with hypertension; b) elevated fasting plasma glucose (FPG≥110 mg/dl or FPG≥6.1 mmol/L) or patients diagnosed with diabetes; c) elevated triglycerides (≥150 mg/dl or ≥1.7 mmol/L); d) high density lipoprotein (HDL) cholesterol (<50 mg/dl or <1.29 mmol/L); and e) abdominal obesity, as measured by a waist circumference of ≥88 cm for women. In the present study, we included apparently healthy study subjects.

**Statistical analysis**

Differences in clinical characteristic between subjects with and without Met S were assessed by t-test and the Mann-Whitney test for normal and skewed continuous variables, respectively. Mean ± S.E. and median (interquartile range) are presented, where appropriate. Multiple linear regression analysis was used to evaluate the association between plasma ET-1 levels and clinical/metabolic parameters for unadjusted and age adjusted model. Trend association between mean ET-1 levels stratified according to the number of components of Met S were tested using linear regression analysis. Two sided P values of less than 0.05 were considered statistically significant. All analyses were performed using Stata version 11.0 (Lake way Drive, College Station, Texas USA).

**Results**

Table 1 shows clinical characteristics of the target population, based on subjects with or without Met S. The prevalence of Met S in the current study was 25.08%. Subjects with Met S were found to be older and had higher BMI and waist circumferences.

All cardio metabolic features, including triglycerides, waist circumference, SBP, DBP, mean arterial pressure, fasting plasma glucose (FPG), and fasting insulin levels were significantly higher in subjects with Met S, except HDL-C levels, which were found to be significantly lower in Met S.

Plasma ET-1 levels were significantly higher in Met S group compared to the non Met S group, i.e., without Met S (P<0.05) (Figure 1A). On the other hand, there was no significant difference in serum nitrite/nitrate levels between Met S and non Met S group.

Table 2 shows a dear association between ET-1 and other parameters of Met S. ET-1 levels were significantly and positively associated with waist circumference, SBP, DBP, mean arterial pressure, fasting plasma glucose levels and inversely associated with HDL cholesterol in age-adjusted model (P<0.05).

As stated earlier, multiple regression analysis was utilized to assess the independent effects of various clinical parameters on levels of circulatory ET-1. Multiple stepwise regression analysis of ET-1, including data on BMI, waist circumference, systolic blood pressure, diastolic blood pressure, mean arterial pressure, fasting insulin, fasting plasma glucose, and HDL cholesterol, after adjustment for age was performed in (Table 3). The results showed that only mean arterial pressure, fasting plasma glucose and HDL cholesterol were significantly associated with ET-1 levels.

Table 1: Clinical characteristics of subject with and without metabolic syndrome according to the NCEP-ATP III criteria.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Subject without MetS</th>
<th>Subject with MetS</th>
<th>P values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject and prevalence</td>
<td>926/74.92</td>
<td>310/25.08</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.18 ± 1.50</td>
<td>47.18 ± 0.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.74 ± 0.39</td>
<td>23.49 ± 0.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>74.02 ± 1.03</td>
<td>80.79 ± 0.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>98.49 (62.46 - 117.07)</td>
<td>185.58 (156.41 - 257.63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>45.25 ± 2.57</td>
<td>36.76 ± 1.86</td>
<td>0.019</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>104.43 ± 2.35</td>
<td>132.49 ± 1.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70.33 ± 1.24</td>
<td>81.98 ± 0.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>81.69 ± 1.56</td>
<td>98.82 ± 1.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.10 (4.70 - 5.50)</td>
<td>6.90 (6.20 - 8.80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>2.97 (1.51 - 5.21)</td>
<td>7.19 (3.78-13.64)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Based on t-test for normal continuous variables, Mann-Whitney test for non-normal continuous variables

*Mean ± S.E for normal variables (all such values)

Median (interquartile range) for non-normal variables (all such values)

BMI: body mass index; HDL: high density lipoprotein, MAP: mean arterial pressure.
cholesterol were significantly associated with ET-1 (Table 3) and these three variables were independent determinants of plasma ET-1 levels. In addition, when we performed multiple logistic regressions for the Met S status and the other predictors, we found that an elevated plasma ET-1 level had a strong association with Met S (Table 4).

Figure 2 shows mean plasma levels of ET-1, according to the number of Met S components. The data shows that mean ET-1 levels were significantly increased with increased number of Met S components ($P$ for trend =0.015). In addition, (Figure 3) shows the association between the tertiles of the ET-1 levels and the percentage of elevated fasting plasma glucose, hypertension, low HDL and Met S. As shown in (Figure 3), percentage of subjects of elevated fasting plasma glucose level, hypertension, low HDL and Met S increased as the levels of ET-1 increased.

**Discussion**

In this cross sectional study of Bangladesh, we evaluated the relationship between circulating levels of ET-1 and cardio metabolic risk factors among apparently healthy rural women who were later diagnosed with Mets during a health screening for non communicable diseases. We found that levels of plasma ET-1 were significantly higher among subjects with Met S than the subjects without Met S. We also found that mean plasma levels of ET-1 increased in direct proportion to the presence of Met S components, whereas, in contrast, levels of NO metabolites (nitrite/nitrate) were unchanged, irrespective of the Met S status. Among the cardio metabolic risk factors, waist circumference, SBP, DBP, mean arterial pressure, fasting plasma glucose displayed significant positive associations with levels of plasma ET-1 whereas HDL cholesterol had significant negative association with ET-1 levels. Assessment by multiple stepwise regression analysis revealed that after adjusting for age and all other potential variables only mean arterial pressure, fasting plasma glucose and HDL cholesterol were found to be independently correlated with ET-1. To our knowledge, this is

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient (β)</th>
<th>S.E</th>
<th>P value*</th>
<th>Coefficient (β)</th>
<th>S.E</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.0130</td>
<td>0.0081</td>
<td>0.108</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.0227</td>
<td>0.0210</td>
<td>0.280</td>
<td>0.0247</td>
<td>0.0209</td>
<td>0.239</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.0219</td>
<td>0.0098</td>
<td>0.026</td>
<td>0.0219</td>
<td>0.0097</td>
<td>0.025</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.0013</td>
<td>0.0006</td>
<td>0.041</td>
<td>0.0011</td>
<td>0.0007</td>
<td>0.083</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>-0.0101</td>
<td>0.0040</td>
<td>0.012</td>
<td>-0.0103</td>
<td>0.0040</td>
<td>0.010</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.0103</td>
<td>0.0038</td>
<td>0.007</td>
<td>0.0093</td>
<td>0.0040</td>
<td>0.022</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.0195</td>
<td>0.0084</td>
<td>0.020</td>
<td>0.0171</td>
<td>0.0087</td>
<td>0.050</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>0.0160</td>
<td>0.0061</td>
<td>0.009</td>
<td>0.0143</td>
<td>0.0065</td>
<td>0.028</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>0.1022</td>
<td>0.0238</td>
<td>&lt;0.001</td>
<td>0.0983</td>
<td>0.0241</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>0.0014</td>
<td>0.0097</td>
<td>0.882</td>
<td>0.0026</td>
<td>0.0097</td>
<td>0.786</td>
</tr>
</tbody>
</table>

BMI: body mass index; HDL: high-density lipoprotein; MAP: mean arterial pressure.

*Based on regression analysis.
Interestingly, our target population reported here was comprised entirely of physically active individuals who were isolated from urbanization and had limited access to adequate health care services due to poverty. Bangladesh is one of the poorest South Asian countries, with a GDP per capita income of $588 USD, an average life expectancy at birth of 68 years and an adult literacy rate of about 55% (World Bank, 2012). Besides, in Bangladesh more than one third of the population lives below the poverty line and are unable to even meet the barest of their basic needs (World Fact Book). It should be noted in this context that in Bangladeshi rural women, of the components of metabolic syndrome studied here, the most prevalent was low HDL levels, followed by diabetes, hypertension, high triglyceride level and the lastly elevated waist circumference [25].

Among the components of Met S, SBP, DBP, waist circumference, fasting plasma glucose and plasma HDL levels showed significant association with plasma levels of ET-1. In stepwise regression analysis, mean arterial pressure, fasting plasma glucose and plasma HDL levels were found to be significantly associated with ET-1 plasma levels, after adjusting all other significant cofactors. In many of the previous studies, plasma ET-1 levels were significantly higher in hypertensive than normotensive subjects [26-28]. However, the relationship between ET-1 and blood pressures were not entirely consistent. In some studies, plasma ET-1 levels were not related to SBP or DBP [17, 29]. In our current study, we also did find significant association between plasma ET-1 levels and those of fasting plasma glucose levels and plasma HDL levels. To date, only a few studies have examined the relationship between these Met S bio markers and plasma ET-1 levels. In an epidemiologic study, Hirai et al. found a positive association between levels of total cholesterol and plasma ET-1 [29]. However, among patients with type 2 diabetes, Hermans et al. found no significant association between plasma ET-1 levels and Met S [18]. These apparent inconsistencies between the studies could be attributed to differences in the background of the target populations. It is interesting to note that our target population reported here were apparently healthy at the onset of the study, whereas in the first population based study that assessed the relationship between plasma ET-1 levels and cardio metabolic risk factors in a South Asian country.

In the present study we found a significantly higher plasma concentration of ET-1 among subjects with Met S than the control group. Also mean plasma ET-1 levels were found to significantly increase with a number of Mets components. The present finding is consistent with several previous studies conducted in this field [17-20]. For instance, previously, plasma ET-1 levels were found to be significantly higher among impaired glucose tolerance (IGT) or non insulin dependent diabetes mellitus (NIDDM) subjects with Met S compared with IGT or NIDDM subjects without Met S [17]. Similarly, circulating ET-1 levels were found to be significantly higher in obese men (BMI nearly 31kg/m²) with Met S than normal controls [19]. In another previous population based KORA F4 study, pro-ET-1 was found to be significantly associated with Met S [20]. Among patients with type 2 diabetes mellitus, Hermans et al. found that Big ET-1 levels were significantly higher in subjects with Met S than control [18]. Taken together with these previous studies, the findings of our current study suggest that ET-1 levels are significantly elevated in subjects with Met S than controls even in apparently healthy populations. This positive association between Met S and circulatory levels of ET-1 is likely to be independent of ethnicity, socio economic demographics, environmental and genetic factors.

Table 3: Multiple linear regression of plasma levels of endothelin-1 (pg/ml) and others predictors.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Coefficient of (β)</th>
<th>S.E</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>0.152</td>
<td>0.006</td>
<td>0.017</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>0.248</td>
<td>0.024</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>-0.136</td>
<td>0.004</td>
<td>0.033</td>
</tr>
</tbody>
</table>

MAP: Mean Arterial Pressure; HDL: High Density Lipoprotein.

Figure 2 Mean plasma levels of endothelin-1 stratified according to the number of components of metabolic syndrome.

Figure 3 Percentage of subjects with elevated fasting plasma glucose levels, hypertension, low HDL levels and metabolic syndrome according to the tertiles of plasma endothelin-1 levels.

Table 4: Multiple logistic regression of metabolic syndrome status and others predictors.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Odds ratio</th>
<th>S.E</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>1.065</td>
<td>0.018</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>1.143</td>
<td>0.037</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>24.794</td>
<td>0.904</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endothelin-1 (pg/ml)</td>
<td>41.680</td>
<td>1.844</td>
<td>0.043</td>
</tr>
</tbody>
</table>

The study subjects of the current investigation were from rural Bangladesh, where, although the majority of the population are physically active, they are isolated from urbanization and have limited access to adequate health care services due to poverty. Bangladesh is one of the poorest South Asian countries, with a GDP per capita income of $588 USD, an average life expectancy at birth of 67 years and an adult literacy rate of about 55% (World Bank, 2012). Besides, in Bangladesh more than one third of the population lives below the poverty line and are unable to even meet the barest of their basic needs (World Fact Book). It should be noted in this context that in Bangladeshi rural women, of the components of metabolic syndrome studied here, the most prevalent was low HDL levels, followed by diabetes, hypertension, high triglyceride level and the lastly elevated waist circumference [25].
majority of previous reports, their target populations were either diabetic, obese, or patients with heart diseases.

In the present study we found, ET-1 has significant positive association with fasting plasma glucose. Indeed, hyperglycaemia, the primary metabolic disturbance of diabetes, has been shown to increase [30] the release of ET-1 from endothelial cells in culture. Importantly, it was shown that altered plasma ET-1 in diabetes can be restored to normal by restoration of metabolic control [31]. Thus, elevated plasma glucose level might be one of the contributing factors for the increased ET-1 level in the Met S subjects in the current study population. In addition, ET-1 level has a negative significant association with plasma HDL level in current study. In fact, in vitro studies demonstrated HDLs exert anti-atherogenic effects by inhibiting ET-1 release in a polar manner [32] in human endothelial cells. In our most recent study, we found that the prevalence of low HDL-cholesterol is as nearly as 85% among rural Bangladeshi women [24]. Therefore low HDL cholesterol is a potential Met S component for Bangladeshi rural women and ET-1 has negative association with plasma HDL level in this study population. In addition, when we performed a multiple logistic regression analysis for the Met S status and the other predictors, there was significant association with Met S and mean arterial pressure and the fasting blood glucose level; there was also a strong association between Met S and the plasma ET-1 level in the logistic regression analysis.

Endothelial dysfunction, the widely recognized first precursor to atherosclerotic disease, is evident in patients with obesity-related Met S. This observation is not only seen as a failure to vasodilate adequately due to impaired NO bioavailability [33], but also as chronic and excess vasoconstrictor tone via increased ET-1-mediated vasoconstriction [34,35]. Indeed, endothelial-dependent vasodilatation is impaired in Met S [36]. This condition is most likely mediated by reduced expression of vasodilators (nitric oxide and prostacyclin), with a concomitant increase of vasoconstrictors (endothelin-1, angiotensin II and thromboxane A2) [36]. For this reason, here, we wanted to check also the expression levels of nitric oxide (as NO metabolites) in subjects with elevated plasma ET-1 levels. Although circulating ET-1 levels were found to be significantly higher in Met S compared to control group, in the present study, it was surprising to observe that there was no significant change in NO metabolites (nitrite/nitrate) levels between the two groups studied. Thus, it seems that there might be impairment of vascular homeostasis in our Met S subjects of the current study, due to an imbalance between the protective effects of the nitric oxide and the dysfunctional ET-1 system.

The major strengths of the present study include use of a large community based survey with a relatively large sample size, coupled by a comprehensive analysis of plasma ET-1 levels and the associated cardio metabolic risk factors in an apparently healthy population that was later diagnosed with Met S. However, the study also has some limitations: a) firstly, existence of an association from cross sectional study does not necessarily indicate causality; b) in assessing the association between ET-1 and cardio metabolic risk factors, we were not able to adjust for important lifestyle risk factor, including smoking and alcohol drinking. However, it is less likely to adjust for these variables because alcohol drinking and smoking are very uncommon among women in Bangladesh. In addition, in the present study, we did not use modified NCEP ATP III criteria or IDF criteria for Met S definition, future analysis should be done in current research design using those criteria. Moreover, future studies are needed to clarify the data obtained on NO in the current study. Indeed, plasma concentration of NO metabolites is affected not only by NO production but also by alimentary intake of nitrates as well as renal function (urinary nitrate excretion) and these factors were not controlled in this study.

In conclusion, the present study is the first comprehensive approach using a large number of sample sizes to establish the association between circulatory levels of ET-1 and Met S in a South Asian context. We show that circulatory levels of ET-1 are significantly higher in Met S subjects compared to non-Met S. Among the cardio metabolic risk factors waist circumference, SBP, DBP, fasting plasma glucose level and plasma HDL levels showed significant association with plasma ET-1 levels. The current cross sectional study indicates that only mean arterial pressure, fasting plasma glucose and HDL levels are independently correlated with plasma ET-1 levels in subjects with Met S. Further prospective studies are needed to elucidate the role of plasma ET-1 levels in subjects with MetS and its associated disorders.

Acknowledgments

This work was supported by Grant-in-Aid for Scientific Research (overseas academic) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (23406037, 23406016, 23406029, 24406026, 25305034), and Japan Society for the Promotion of Science. Current project (WDF11-610) on gestational diabetes from World Diabetes Foundation (WDF), Denmark to HDRCRP has also supported a part of this work.

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