Assessment of Circulatory Endothelin-1 Level among Pre-and Post-Menopausal Rural Women in Bangladesh: Result from a Population-Based Study

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Abstract
Aims: Prevalence of non-communicable diseases is a challenging problem among menopausal women especially in a least developing country like Bangladesh. Recently, plasma levels of endothelin-1 or related molecules are reported to be associated with metabolic syndrome and its components. So we determined the circulatory level of endothelin-1 in Bangladeshi rural pre- and post-menopausal women and its association with various cardio-metabolic risk factors.

Main methods: This study is based on a community based cross-sectional survey among 1678 rural women over the age of 15. We measured plasma level of endothelin-1 by ELISAand used muliplergressions to estimate the association between circulatory endothelin-1 level and cardio-metabolic risk factors.

Key findings: Endothelin-1 levels were significantly higher in post-menopausal subjects than in pre-menopausal (post-menopausal vs. pre-menopausal: 3.13 ± 0.09 vs. 2.29 ± 0.05pg/ml, p=0.001). Mean values of age, systolic blood pressure, diastolic blood pressure, plasma triglyceride level, fasting plasma glucose level, and HDL cholesterol were significantly higher in post-menopause compared to pre-menopausal group (P<0.05). In both univariate and multiple regression analysis, we found that ET-1 had significant positive association with age only. Metabolic syndrome was present in 25.6% respondents and it was more prevalent among post-menopausal (39.3%) as compared to pre-menopausal (16.8%) women. Prevalence of hypertension was also greater in post-menopausal women than in pre-menopausal women (30.7% vs. 9.8%).

Significance: The higher level of circulatory endothelin-1 level may be associated with various medical problems and events/syndromes of post-menopausal women in rural Bangladesh. Proper intervention strategies should be warranted among these high-risk subjects.

INTRODUCTION
In Bangladesh, the prevalence of chronic non-communicable diseases such as cardiovascular diseases (CVD) and type 2 diabetes is increasing significantly [1]. This increase is observed not only in the urban area but also in the rural population. Especially in Bangladesh, rural women who are mostly poor and physically active seem to be more vulnerable [2].

The risk of CVD increases sharply after middle age in women,
especially after menopause [3,4]. The transition from pre- to post-menopause is a remarkable change in the endogenous sex hormone milieu in women. It is characterized by markedly decreased production of 17β-estradiol (estradiol), although androgen production from the adrenal cortex and ovarian stromal cells remains fairly steady [3,4]. Because estrogen reduces LDL-cholesterol and triglyceride, and has a function of increasing HDL-cholesterol, plasma levels of LDL-cholesterol and triglyceride will increase in post-menopausal women with estrogen deficiency.

Endothelin-1 (ET-1) is a peptide with potent and characteristically sustained vasoconstrictor, is implicated in the proliferation of vascular smooth muscle cells, and involved in the pathogenesis of hypertension [5], diabetes mellitus [6,7]. So ET-1 has been considered as a bio-marker of endothelial damage and a negative prognostic predictor of CVD [8]. Evidence suggests that sex hormones may modulate plasma ET-1 levels. Webb et al [9] found that 17β-estradiol decreased ET-1 levels in the coronary circulation of postmenopausal women. More recently, Silvestri et al [10] also showed a reduction of ET-1 levels in postmenopausal women under oral hormone therapy.

Thus, the aim of this study was to determine the circulatory level of ET-1 in pre- and post-menopausal rural women in Bangladesh, and also investigated its association with cardiometabolic risk factors.

METHODS

Participants

A total of 1678 apparently healthy rural Bangladesh women were enrolled in this study. Eligible people were all 15 years old or older. We performed this research using the population-based cross-sectional survey according to the World Health Organization’s 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 behavior (step 1), basic physical measures (step 2) and basic biochemical investigations, such as blood glucose and cholesterol (step 3). The study was carried out in fifteen villages from three districts in Bangladesh (Rajshahi, Rangpur and Bogra) under Rajshahi and Rangpur divisions in Bangladesh. Briefly, women were recruited through local public announcements using a loudspeaker and door-to-door neighborhood visits. Data from participants 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 (HDRCRP), Dhaka, Bangladesh, and conforms to the principles outlined in the Helsinki Declaration. All participating subjects gave their written informed consent before they were included in the study.

Subjects with the following conditions were excluded from the study: those with chronic illness, such as hypothyroidism; pregnant women, those on hormone replacement therapy, as well as women with known illness, such as ischemic heart disease or diabetes. After exclusion, a total of 1802 subjects remained for the present study analysis.

Menopausal status

Women were defined as post-menopausal, if they had reported their last menses to be at least 12 months previously, and pre-menopausal, if they had an unchanged and regular menstrual pattern during the last five years, without typical climacteric complaints. Women with peri-menopausal status, such as those whose last menses were within 12 months, but not regular, were not included in the study. A standard questionnaire on health and menopausal status written in the local language was used to identify the pre- and post-menopausal subjects.

Anthropometric measurements

Anthropometric measurements on individuals wearing light clothing and without shoes were conducted by well-trained examiners: height was measured to the nearest 0.1 cm using the portable stadiometer; weight was measured in an upright position, to the nearest 0.1 kg, using a calibrated balance beam scale; body mass index (BMI) was determined by dividing weight (kg) by height squared (m²); and 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. 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. The blood sample was collected using the standard blood sample collection procedure. For analysis, the serum was immediately separated from the blood by centrifugation, for evaluating plasma concentration of lipids including triglycerides (TG) [lipoprotein lipase method; Wako Chemicals, Tokyo, Japan], total cholesterol (TC) [Cholesterol E, Wako Pure Chemical Industries, Ltd. Osaka, Japan], its fraction high-density lipoprotein cholesterol (HDL-C) [HDL-C with the Determiner-L kit (Kyowa Co Ltd, Tokyo, Japan)]. Fasting plasma glucose [glucose with the Hexokinase G-6-PDH kit (Wako Pure Chemical Industries Ltd, Osaka, Japan)] were also measured. Circulatory levels of ET-1 were measured using specific commercial enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer’s directions (Quantikine, R & D systems, Minneapolis, MN, USA).

Definition of metabolic syndrome and risk factors

Metabolic syndrome (MS) was defined using the standard National Cholesterol Education Program Adult Treatment Panel III definition [11], with participants having three or more of the following five criteria: a) high blood pressure (≥130/≥85 mmHg) or subjects diagnosed with hypertension; b) elevated fasting blood glucose (≥110 mg/dl or ≥6.1 mmol/L) or patients diagnosed with diabetes; c) elevated triglycerides (≥150 mg/dl or ≥1.7 mmol/L); d) high density lipoprotein cholesterol (HDL-C <50 mg/dl or <1.29 mmol/L); and e) abdominal obesity, as measured by a waist circumference of ≥88 cm for women.
Hypertension was defined as a systolic blood pressure ≥140 mm Hg and a diastolic blood pressure ≥90 mm Hg. Also, participants who took some medical treatments at the time of the study, for example, anti-hypertensive or anti-diabetic medications (insulin or oral agents), were considered as having high blood pressure or elevated fasting blood glucose, respectively.

**Statistical analysis**

Differences in clinical characteristics of subjects between pre- and post-menopausal women were assessed by t-test, and all values were presented as mean ± S.E. Additionally, multiple regression analysis was performed to assess the association between circulatory ET-1 levels and menopausal status and cardio-metabolic risk factors. 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 subjects according to menopausal status. Mean values of age, systolic blood pressure, diastolic blood pressure, plasma level of triglyceride, fasting plasma glucose and plasma HDL levels were significantly higher in post-menopausal women compared to pre-menopausal women.

Plasma levels of ET-1 were significantly higher in post-menopausal subjects (post-menopausal vs. pre-menopausal: 3.13 ± 0.09 vs. 2.29 ± 0.05 pg/ml, P=0.04) (Figure 1).

Table 2 shows the association between circulatory levels of ET-1 and other predictors through univariate analysis. We found that ET-1 had significant positive associations with age (beta=0.02, P=0.001) and fasting plasma glucose level (beta=0.08, P=0.016). When we performed multivariate linear regression analysis, we found only age shows significant positive association with ET-1 (Table 3).

When we performed multiple logistic regressions considering menopausal status as dependent variable, we found plasma ET-1 level had strong association with menopause (Table 4).

Prevalence of MS according to menopausal status was presented in Figure 2. MS was present in 25.6% respondents and it was more prevalent among post-menopausal (39.3%) compared to pre-menopausal (16.8%) women.

Prevalence of hypertension in our current study subjects based on menopausal status has been shown in (Figure 3A) (pre-menopause vs. post-menopause: 9.8% vs. 30.7%). An age-dependent increase of hypertension prevalence has been observed in our current study subjects (Figure 3B).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient (β)</th>
<th>Standard error</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.0274</td>
<td>0.0040</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.0318</td>
<td>0.0146</td>
<td>0.032</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.0055</td>
<td>0.0076</td>
<td>0.467</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.0010</td>
<td>0.0011</td>
<td>0.380</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>0.00001</td>
<td>0.0028</td>
<td>0.996</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.0043</td>
<td>0.00210</td>
<td>0.151</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.0024</td>
<td>0.0058</td>
<td>0.673</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>0.0045</td>
<td>0.0048</td>
<td>0.343</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>0.0885</td>
<td>0.0363</td>
<td>0.016</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>0.0006</td>
<td>0.0008</td>
<td>0.450</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>-0.0055</td>
<td>0.0033</td>
<td>0.094</td>
</tr>
</tbody>
</table>

**Table 1**: Clinical characteristics of subject according to menopausal status.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pre-menopause</th>
<th>Menopause</th>
<th>P valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (prevalence)</td>
<td>750</td>
<td>928</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.28 ± 0.97</td>
<td>53.44 ± 0.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.98 ± 0.36</td>
<td>22.39 ± 0.46</td>
<td>0.468</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>77.68 ± 0.82</td>
<td>76.91 ± 0.68</td>
<td>0.500</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>115.03 ± 6.08</td>
<td>154.27 ± 6.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>37.90 ± 1.40</td>
<td>45.91 ± 3.34</td>
<td>0.013</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110.78 ± 1.72</td>
<td>127.96 ± 2.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73.82 ± 0.90</td>
<td>79.92 ± 1.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>85.41 ± 1.13</td>
<td>95.51 ± 1.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.89 ± 0.10</td>
<td>6.98 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>12.21 ± 2.89</td>
<td>9.92 ± 1.34</td>
<td>0.488</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>176.22 ± 5.49</td>
<td>190.02 ± 8.80</td>
<td>0.174</td>
</tr>
</tbody>
</table>

**Table 2**: Association between endothelin-1 and others parameters.

*Based on t-test for normal continuous variables, *Mean ± S.E for normal variables (all such values)

BMI: Body Mass Index; HDL: High Density Lipoprotein.

Figure 1 Mean plasma levels of endothelin-1 according to pre-menopausal and post-menopausal status in rural Bangladesh.
Numerous evidences indicate that the increased bioactive ET-1, a potent vasoconstrictor, contributes to vascular dysfunction and progress of CVD via multiple pathways. ET-1 has been implicated in the pathogenesis of vascular oxidative stress, inflammatory activity, mitogenic stimulation of the vascular smooth muscle cells, and fibrosis. Regardless of gender, advancing age itself is the major risk factor for the development of CVD [17-19]. Aging is associated with elevation of ET-1 levels (mRNA and protein) in plasma and vascular tissues [20-22], which was found to be correlated well with vascular tone or endothelium-dependent vascular dilation [23,24]. ET-1-mediated vasoconstriction is augmented in older adults [25], and synthesis of ET-1 is elevated in cultured aortic endothelial cells from older compared with young donors [26]. As for mechanism stimulating ET-1 system in aging, some studies have shown that preproET-1 mRNA is up-regulated by shear stress, cytokines, and oxidative stress [27], all of which are age-related stimulation. In our current study both in univariate and multivariate regression analysis, we found ET-1 has a significant positive association with age. Thus ET-1 may be involved in ageing-related pathophysiology and pathogenesis in rural women in Bangladesh warranting extensive investigations in near future.

Healthy pre-menopausal women are protected against CVD, compared with age-matched men, this protection is impaired or lost in post-menopause [28], which is characterized by depletion of estrogen. Estrogen has been shown to have anti-inflammatory,

**DISCUSSION**

This is the first report showing that circulatory ET-1 levels are significantly higher in post-menopausal women than in pre-menopausal women using a population-based cross-sectional study in rural Bangladesh.

In the current study, we did not find any significant association between ET-1 and blood pressure both in univariate and multivariate analysis. The significant positive association between ET-1 and blood pressures has been shown in previous clinical studies [12-15]. According to these previous studies, plasma ET-1 levels were significantly higher in patients with essential hypertension as compared with healthy controls. In addition, Seissler et al. found a significant positive association between proET-1 and high blood pressure [16]. As demonstrated in (Figure 3), Bangladeshi rural women demonstrate an age dependent prevalence of hypertension and the prevalence of hypertension is significantly higher in post-menopausal women than in pre-menopausal women (Figure 3). But in these populations, circulatory ET-1 did not show any significant positive association with blood pressure. Thus, increased blood pressure may not be a contributory factor for the elevated ET-1 level in post-menopausal women in rural Bangladesh.

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Healthy pre-menopausal women are protected against CVD, compared with age-matched men, this protection is impaired or lost in post-menopause [28], which is characterized by depletion of estrogen. Estrogen has been shown to have anti-inflammatory,
anti-oxidant, anti-hypertensive effects on the vasculature [29]. Besides, experimental studies have demonstrated that estrogen can inhibit the expression of ET-1 at mRNA and/or protein levels [30-32]. Ovariectomized female animals show an elevation of ET-1 levels in plasma and vascular tissues, whereas this elevation of ET-1 was attenuated significantly by administration of estrogen [33,34]. Especially in clinical studies, plasma ET-1 levels were shown to be suppressed on hormone replacement therapy with estradiol or estrone in post-menopausal women [35-37]. All these studies suggest the inhibitory effects of estrogen on ET-1 levels. Thus the above findings that depletion of estrogen causes up regulation of ET-1 can be also evident from the present findings conducted in a rural situation of a South Asian country.

MS was presented in 25.6% participants and it was more prevalent among post-menopausal (39.3%) as compared to pre-menopausal women in current study. This finding is consistent with many of previous studies [38-40]. The emergence of metabolic risk factors in post-menopausal phase may be a direct result of ovarian failure represented as estrogen deficiency. Decrease in estrogen production is thought to be responsible for substantial proportion of increased cardio-metabolic risk factors in post-menopausal women. The production and plasma levels of ET-1 are elevated in patients with type 2 diabetes, obese, and hypertensive patients [41,42], and a positive correlation between plasma ET-1 levels and diabetic microangiopathy has been reported [43,44]. [45] showed that dual ET receptor antagonist attenuated the chronic type 2 diabetes complications in animal experimental study. Besides, ET-1 was found to induce insulin resistance [46], mainly through the impairment of insulin receptor and glucose transport signaling and also dysregulation of adipose tissue signaling [47,48], which may take part in the development of the MS [49]. ET-1 augments endothelial uptake of oxidized LDL, which in turn stimulate ET-1 production [50]. Statin therapy, which is a key medicine with lipid-lowering effect, inhibits production of preproET-1 mRNA in endothelial cells [51]. ET receptors antagonism improves endothelial function in hyperlipidemic animals [52]. All of these findings suggest a potential role of the endothelin system in the pathophysiology of diabetes [53], MS and its vascular complications. In the current study, we tried to find an association between plasma ET-1 level and the metabolic syndrome components, but we did not find any significant association between them. Thus the elevated ET-1 level in post-menopause may not have role in the development of metabolic disorders in Bangladeshi rural women as evident from the present study.

The major strengths of the present study include use of a large community-based survey with a relatively large sample size. However, the study also has some limitations that warrants mentioned. Firstly, existence of an association from cross-sectional study does not necessarily indicate causality. Secondly, each biomarker was sampled and evaluated only once in each participant. Ideally, there should be more than one sampling and evaluation per participant at different time-points. Thirdly, this study is based on only rural women from a lower socioeconomic class, thus limiting generalization of the data obtained to the whole adult women in Bangladesh. Future studies should also focus on ET-1 levels using urban population. To date, a comparison study using urban and rural population separately in this research field is lacking [54,55].

CONCLUSION

The findings of our study suggest that circulating ET-1 levels are higher in post-menopausal women compared to pre-menopausal women in rural Bangladesh. Longitudinal studies are needed to determine if post-menopause represents a risk factor for CVD and to establish the clinical relevance of the association between ET-1, and menopausal status.

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