Association of endothelin-1 with oxidative stress and inflammatory response in pre-hypertensives

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Endotelin-1
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Prehypertenze

SOUHRN

Úvod: Hypertenze je jedním z hlavních rizikových faktorů rozvoje kardiovaskulárních onemocnění. U osob s prehypertenzí existuje vyšší pravděpodobnost progrese k hypertenzi. Mechanismus progrese prehypertenze do hypertenze zůstává nejasný. Naše studie byla proto navržena s cílem prozkoumat vztah mezi endotelinem-1 na jedné straně a oxidačním stresem a zánětlivou reakcí na straně druhé u jedinců s prehypertenzí.
Metody: Do studie bylo zařazeno 30 osob s prehypertenzí a 30 normotenzních jedinců. Ve vzorcích krve odebraných nalačno se stanovoval lipidový profil a hodnoty kyseliny sialové vázané na lipidy (lipid-bound sialic acid, LBSA), malondialdehydu (MDA) a endotelinu-1 (ET-1).
Výsledky: Hodnoty celkového cholesterolu, triglyceridů (TG), cholesterolu lipoproteinů o velice nízké hustotě (very low-density lipoprotein cholesterol, VLDL-C) i cholesterolu lipoproteinů o nízké hustotě (low-density lipoprotein cholesterol, LDL-C) byly u osob s prehypertenzí statisticky významně zvýšené. U jedinců s prehypertenzí byly ve srovnání s normotoniky nalezeny i zvýšené hodnoty LBSA (39,58 ± 4,35), ET-1 (13,43 ± 5,1) a MDA (1,78 ± 3,5). U osob s prehypertenzí hodnoty ET-1 statisticky významně korelovaly s hodnotami LBSA a MDA. Analýza křivky ROC pro hodnoty ET-1, LBSA a MDA prokázala mezní hodnoty 7,0, resp. 31,5 a 1,23 pro stanovení diagnózy prehypertenze.
Závěr: Zvýšené hodnoty ET-1, LBSA a oxidačního stresu jsou spojeny se změnou krevního tlaku u osob s prehypertenzí, která může následně progredovat do esenciální hypertenze.

Keywords:
Endothelin-1
LDL-c
Lipid bound sialic acid
Malondialdehyde
Pre-hypertension

ABSTRACT

Introduction: Hypertension represents the major risk factor for cardiovascular diseases. Pre-hypertensives are more likely to progress to hypertension. The mechanism for the progression of pre-hypertension to hypertension remains unclear. Hence the present study was designed to investigate the association of endothelin-1 with oxidative stress and inflammatory response in pre-hypertensives.
Methods: The study was conducted in thirty subjects with pre-hypertension and thirty normotensive subjects. Lipid profile, lipid bound sialic acid (LBSA), malondialdehyde (MDA) and endothelin-1 (ET-1) levels were estimated in the fasting blood samples.
Results: Total cholesterol, triacylglycerol (TAG), very low density lipoprotein cholesterol (VLDL-c) and low density lipoprotein cholesterol (LDL-c) were significantly increased in pre-hypertensives. LBSA (39.58 ± 4.35), ET-1 (13.43 ± 5.1) and MDA (1.78 ± 3.5) were also significantly elevated in pre-hypertensives when compared to normotensive subjects. ET-1 was significantly correlated with LBSA and MDA in pre-hypertensives. Receiver operating curve analysis of ET-1, LBSA and MDA showed cut off values of 7.0, 31.5 and 1.23 respectively for the diagnosis of pre-hypertension.
Conclusion: Elevated ET-1, LBSA and oxidative stress are associated with altered blood pressure in pre-hypertensives and may progress to essential hypertension.

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Introduction

Systolic blood pressure greater than 120 mmHg and less than 139 and diastolic blood pressure (DBP) greater than 80 mmHg and less than 89 are defined as pre-hypertension.[1] Pre-hypertension is most prevalent in developed as well as in developing countries. Pre-hypertensives increases the risk of developing essential hypertension twice when compared to normotensive individuals. Nearly one billion individuals are affected with essential hypertension and expected to reach 1.56 billion by 2025.[2] Hypertension is associated with cardiovascular disease and accounts for 24% coronary heart diseases in India.[3] Dyslipidemia, oxidative stress and inflammatory response play an important role in the pathogenesis of hypertension and predictive factors for cardiovascular diseases.[4] The enhanced production of free radicals is associated with a decline in the antioxidant status and this imbalance leads to oxidative stress. Increased oxidative stress further augments the activation of inflammatory proteins and exacerbates the inflammatory status. Many clinical and experimental studies have shown that hypertension is associated with oxidative stress.[5–7] Chrysohoou et al. have documented the association of pre-hypertension with oxidative stress and dyslipidemia independently of other co-existing factors.[8]

Endothelial dysfunction is a characteristic of hypertension associated with atherosclerosis and is considered as an early feature in atherogenesis.[9] Systemic inflammation induces oxidative stress and endothelial dysfunction that play a significant role in the clinical manifestation of cardiovascular disease. Factors contributing to hypertension along with oxidative stress and inflammation may be the production of endothelin-1. Endothelin-1 is a peptide produced by vascular endothelium and has very potent systemic vasoconstricting properties and attributes to elevated blood pressure.[10] Further epidemiological, experimental and clinical studies indicated the presence of endothelial dysfunction in hypertensive subjects.[11] Saito et al. have documented the relationship between ET-1 levels and essential hypertension suggesting the causative role for ET-1 in the pathogenesis of the disease.[12,13] But the association between endothelial dysfunction, oxidation stress and systemic inflammation in the development of pre-hypertension remains clear. Hence the present study was designed to investigate the association of endothelin-1 with oxidative stress and inflammatory response in pre-hypertensives.

Materials and methods

This study was conducted in the Department of Biochemistry in collaboration with Department of General Medicine, Mahatma Gandhi Medical College and Research Institute, SBV, Pillaiyarkuppam, Puducherry. The study was conducted after obtaining the permission from institutional research council and institute human ethics committee (IHEC). Blood pressure was measured using a mercury sphygmomanometer and average of the three values was recorded as the actual blood pressure of the volunteer's.

The study comprises of two groups, normotensive (healthy control) and pre-hypertensive groups with thirty subjects in each. Thirty subjects having systolic blood pressure greater than 120 mmHg and less than 139 and diastolic blood pressure (DBP) greater than 80 mmHg and less than 89 were included in pre-hypertensive group. Systolic blood pressure <120 mm Hg and diastolic blood pressure < 80 mm Hg were included in normotensive group. Subjects with history of hypertension, diabetes mellitus, coronary artery disease, known organ dysfunction, present smokers and chronic alcoholics and individuals suffering from any other endocrine disorders were excluded from the study.

Sample collection

Three millilitre of venous blood samples were collected in EDTA vial after an overnight fasting of 12 hours. The blood samples were centrifuged at 3000 g for 10 min and the plasma was separated. Lipid profile such as total cholesterol (TC), triacylgycerol (TAG) and high-density lipoprotein (HDL) cholesterol were estimated immediately using kits adapted to clinical chemistry autoanalyser. Very low-density lipoprotein (VLDL) cholesterol was calculated using the formula (TAG/5). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [TC-(HDL+VLDL)].[14] Endothelin-1 was estimated using ELISA method and procured kit from UBI Magiwell, California, USA. Plasma Lipid bound sialic acid (LBSA) and malondialdehyde Aminoff's method[15] and method of Yagi et al.[16] respectively.

Statistical analysis

All data are expressed as mean ± standard deviation. Independent 't' test was used to find the significant difference among the two groups. Pearson's correlation analysis was done to find the association between the biochemical parameters. Receiver operating curves (ROC) were done to find the sensitivity and specificity of biochemical parameters for predicting pre-hypertension in normotensive individuals. Statistical analysis was proved using Statistical Package of Social Service (SPSS), Version 19.0.

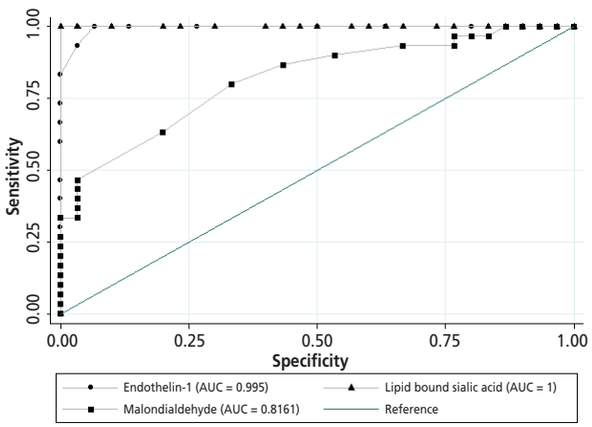
Results

Table 1 shows the baseline characteristics of the subjects. There was no significant difference between the age, BMI and waist to hip ratio. SBP (125 ± 5.4) and DBP (85 ± 6.2)

Table 1 – Basic characteristic of subjects

Parameter	Control (n = 30)	Pre-hypertensive (n = 30)
Age (years)	36.4 ± 7.0	38.2 ± 10.0
BMI (kg/m^2)	22.5 ± 4.4	24.9 ± 3.5
Waist to hip ratio	0.89 ± 0.03	0.92 ± 0.04
SBP (mmHg)	112 ± 3.0	125 ± 5.4*
DBP (mmHg)	70 ± 5.5	85 ± 6.2*

BMI – body mass index; DBP – diastolic blood pressure; SBP – systolic blood pressure.
Data are expressed as mean ± SD.
The parametric data were compared by using unpaired student's t test.
* Statistically significant compared to other group, with p value < 0.05.



Parameters	Cut off value	Sensitivity (%)	Specificity (%)	AUC
ET-1	7.0	100	93	0.99
LBSA	31.5	100	100	1.0
MDA	1.23	80	67	0.81

Fig. 1 – ROC of endothelin-1, lipid bound sialic acid and malondialdehyde for the diagnosis of pre-hypertension.

were significantly increased in pre-hypertensives when compared to control.

Comparison of mean ± SD of the lipid bound sialic acid, malondialdehyde, endothelin-1 and lipid profile of normotensive and pre-hypertensive are shown in Table 1. Lipid bound sialic acid (LBSA) and malondialdehyde (MDA) levels were significantly elevated in pre-hypertensives when compared to normotensive subjects. Similarly endothelin-1 was also significantly elevated in pre-hypertensive when compared to control. Total cholesterol (246.5 ± 6.61), triacylglycerols (129 ± 13.8). Very low-density lipoprotein (25.8±2.74) and low-density lipoprotein (181.8±9.24) were significantly increased in pre-hypertensives when compared to control. No significant change was found in HDL-c among the two groups.

Receiver operating characteristic curve for ET-1, LBSA and MDA are shown in Figure 1. Area under the curve of the Receiver operative curve (ROC) of ET-1, LBSA and MDA was found to be 0.99, 1.0 and 0.81 respectively. The optimal cut off values of ET-1, LBSA and MDA were 7.0 (100% sensitivity and 93% specificity), 31.5 (100% sensitivity and 100% specificity) and 1.23 (80% sensitivity and 67% specificity) respectively.

Figure 2 shows the association of circulating ET-1 levels with lipid bound sialic acid and malondialdehyde levels. ET-1 was positively correlated with lipid bound sialic acid ($r = 0.79$, $p < 0.001$) and MDA ($r = 0.75$, $p<0.001$) in Pre-hypertensives.

Discussion

Hypertension is becoming a serious threat to human health and positively associated with cardiovascular disease. The Seventh Report of the Joint National Commit-

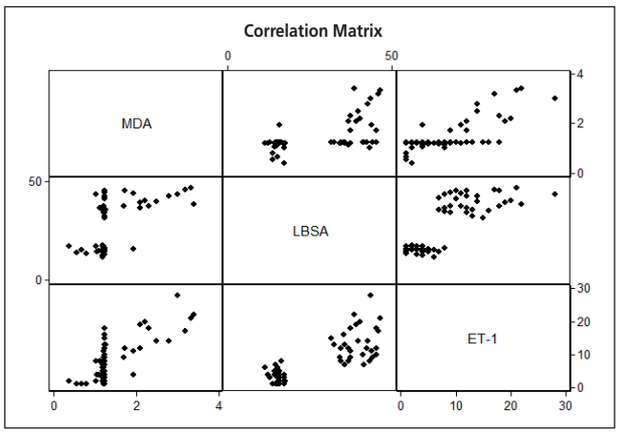


Fig. 2 – Correlation between endothelin-1, LBSA and MDA

Table 2 – Comparison of serum lipids, MDA, LBSA and ET-1 levels between the normotensives and subjects with pre-hypertension

Parameters	Normotensives (n = 30)		Pre-hypertensive subjects (n = 30)	
	Mean ± SD	95% CI	Mean ± SD	95% CI
LBSA (µg/mg of protein)	15.26 ± 1.49	14–15	39.58 ± 4.35*	38–41
ET-1 (pg/mL)	3.44 ± 1.90	3–4	13.43 ± 5.10*	11–15
MDA (µmol/L)	1.14 ± 0.26	1–2	1.78 ± 3.5*	1–2
TC (mg/dL)	163.7 ± 21.28	156–172	246.5 ± 6.61*	244–249
TAG (mg/dL)	116 ± 14.18	111–122	129 ± 13.8*	124–134
VLDL (mg/dL)	23.3 ± 2.83	22–24	25.8 ± 2.74*	24–26
LDL-c (mg/dL)	79.90 ± 25.19	70–89	181.8 ± 9.24*	178–185
HDL-c (mg/dL)	43.34 ± 10.7*	39–47	41.4 ± 3.54	40–43

CI – confidence interval; ET-1 – endothelin-1; HDL-c – high density lipoprotein-cholesterol; LBSA – lipid bound sialic acid; LDL-c – low density lipoprotein-cholesterol; MDA – malondialdehyde; SD – standard deviation; SE – standard error of mean; TAG – triacylglycerols; TC – total cholesterol; VLDL – very low density lipoprotein.
The parametric data were compared by using unpaired student's t test.
* Statistically significant compared to other group, with p value < 0.001.

tee (JNC 7) describes prehypertension as an independent category of blood pressure.[17] Studies have demonstrated that pre-hypertension precedes the development of hypertension in 90% of people and also increase the risk of cardiovascular disease.[18–20] It has been reported that patients with elevated blood pressure are more susceptible for the advancement of overt hypertension, cardiovascular disease and its associated complications when compared to normotensive subjects. Hence the identification of the risk factors or the markers for the early detection of pre-hypertension before the development of essential hypertension would be of great significance in preventing the cardiovascular risk.

There are many reports showing increased pro-atherogenic lipid profile, resulting in increased cardiovascular events. Abnormalities in lipid metabolism may be another important contributory factor in the pathogenesis of hypertension. Consistent with this, total cholesterol (246.5 ± 6.61), triacylglycerol (129 ± 13.8), very low-density lipoprotein (25.8 ± 2.74) and low-density lipoprotein (181.8 ± 9.24) were significantly increased in pre-hypertensives when compared to control. No significant change was found in HDL-c among the two groups. These findings were in concordance with the results shown by Sathiyapriya et al.[21] Similarly another study has shown the lipid derangements in pre-hypertensives compared to healthy subjects.[22] It has been reported that higher TC, TAG, LDL-c and non HDL-c have been found to be associated with an increased risk of hypertension which supports the findings of our study.

Sialic acid is an acute-phase protein which is a component of oligosaccharide and abundant in vascular endothelial cells. Elevated sialic acid is associated with cardiovascular morbidity and mortality. In the present study, lipid bound sialic acid (LBSA) was significantly elevated in pre-hypertensives. This suggested that LBSA has been increased before the development of hypertension which could be used as early marker for the prediction of hypertension and risk of cardiovascular complications. The ROC cut off value for LBSA was 31.5 with sensitivity 100% and specificity 100% for the diagnosis of pre-hypertension in the present study. Increased levels of LBSA indirectly reflect the inflammatory response observed in this study. Elevated sialic acid also accelerates atherosclerosis and enhances the clot formation in vascular endothelium perhaps due to inflammatory response.[22]

In the present study lipid peroxidation product, malondialdehyde is significantly elevated in subjects with pre-hypertension when compared to control. It was concordance with previous study which shows the increased MDA level in pre-hypertensive subject.[23] The optimum cut off value for MDA was found to be 1.23 with 80% sensitivity and 67% specificity in this study. Increased inflammatory response due to elevation of sialic acid leads to oxidative stress that would have resulted in the elevation of lipid peroxidation products, malondialdehyde. Oxidative stress is reflected as one of the risk factors for the onset of hypertension and its associated complications. Mounting evidence indicates that oxidative stress is directly related to endothelial dysfunction. Oxidative stress may cause endothelial dysfunction, vascular damage and associated with pre-hypertension or essential hypertension.

Endothelin-1 (ET-1) is a vasoconstrictor peptide, abundant in vascular endothelium and smooth muscle cells. Many experimental and clinical studies have shown the association between endothelin levels and hypertension.[4,24] In accordance with this we also found elevated levels of ET-1 in pre-hypertensive subjects when compared to normotensives which show that elevation of ET-1 occurs in the circulation well before the development of overt hypertension. This finding was consistent with the previous literature.[25] The optimal cut off values of ET-1 was 7.0 with 100% sensitivity and 93% specificity which is a better marker for the prediction of pre-hypertension when compared to MDA which has 80% sensitivity and 67% specificity when compared to ET-1. In the present study we have shown that ET-1 was positively correlated with lipid bound sialic acid and MDA. Elevated ET-1 reveals the endothelial dysfunction in pre-hypertension in this study and concordance with the previous study.[26] Increased circulating levels of sialic acid indirectly reflect the inflammatory response in pre-hypertension which is also associated with oxidative stress. The inflammatory response and oxidative stress observed in this study may promote the oxidation of LDL via endothelial dysfunction, cause the vascular wall damage and ultimately culminate in atherosclerosis in future. This may be partially attributed to variations in blood pressure, plasma cholesterol, TAG and lipoproteins in pre-hypertensives. Indeed pre-hypertensive subjects have increased risk for the development of overt hypertension and cardiovascular diseases in future. Hence ET-1, LBSA and MDA levels could be used as adjunct markers for the early diagnosis of pre-hypertension and cardiovascular risk.

Conclusion

Our findings confirm the presence of inflammation and oxidative stress in pre-hypertensives as evidenced by elevation of lipid bound sialic acid and malondialdehyde levels. In addition endothelial dysfunction marker, endothelin-1 and atherogenic lipid profile are significantly elevated in pre-hypertensives which may contribute to elevation of blood pressure in a patient with pre-hypertension. These could be used as early marker for the assessment of endothelial dysfunction and cardiovascular risk in pre-hypertension and also helps in the prevention of progression to essential hypertension and early management of the same.

Conflicts of interest

There are no conflicts of interest.

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