

Relationship of Total Antioxidant Capacity and Endothelin-1 levels in prehypertensive individuals among population attaining a sedentary lifestyle in central India

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Abstract

Objective: As known, hypertension has relation with increased peripheral vascular resistance. Vascular tone is regulated by multiple mediators; among them is Endothelin-1. Endothelin-1 (ET-1) is one of the most potent vasoconstrictors known to date. While its plasma /serum concentrations are elevated in some forms of hypertension. Prehypertension is one step towards hypertension, hence the same factor is involved in it. Oxidative stress is also found to be involved in pathogenesis of hypertension. Therefore our present study is designed to find the relationship between ET-1 levels and Total antioxidant capacity (TAC) in Prehypertension.

Methods: Total 100 prehypertensive cases and 100 sex matched controls were enrolled in this study. Inclusion criteria include patients with systolic blood pressure (SBP) in the range of 120 to 139 mmHg and diastolic blood pressure (DBP) in the range 80-90 mmHg, while patients with BP \geq 140/90 mmHg, diabetes mellitus (DM), Stroke, coronary artery disease (CAD) and myocardial infarction (MI) were excluded. Plasma ET -1 and plasma total antioxidant capacity were measured. We estimated ET -1 levels and TAC levels in Prehypertensive cases and Normotensive controls. Students T test were used for statistical analyses.

Results: Plasma ET -1 levels were found significantly ($p<0.001$) higher in prehypertensive cases as compared to normotensive controls. In contrary to this, Plasma Total Antioxidant capacity was found extremely significantly lower in prehypertensive cases as compared to normotensive controls($p<0.0001$).

Conclusion: The study demonstrated that in Prehypertensives, there is an inverse relationship between Endothelin -1 and Total antioxidant capacity.

Keywords: Endothelin-1, Total Antioxidant Capacity, DBP, SBP

Introduction

Recently, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (1) suggested a new classification for borderline blood pressure levels, the “prehypertension”. The new classification describes people with blood pressures between 120 and 139mmHg systolic or between 80 and 89mmHg diastolic blood pressures. This “new” category between normal blood pressure and established hypertension includes a population at high risk for developing hypertension and in which lifestyle modifications are needed (2).

Despite the high prevalence and intense efforts, understanding of the pathogenesis of essential hypertension is still limited (3). Among other mechanisms, hypertension has been associated with increased peripheral vascular resistance. Vascular tone is regulated by multiple mediators, among them endothelin-1(4).

Endothelin-1 is a 21-amino-acid peptide that is produced by vascular endothelial and smooth muscle cells. Endothelin-1 (ET-1) has potent vasopressor effects (5,6) and may therefore play a part in the regulation of blood pressure (BP) and in the pathogenesis of hypertension. An association between elevated circulating ET-1 levels and

Received 04-06-2018 Accepted 30-06-2018 Available Online 30-06-2018

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essential hypertension has been noted in several (7,8,9) but not in all studies (10).

The determination of total antioxidant capacity is now considered as a tool in medical diagnosis and treatment of several diseases, including cardiovascular disease, cancer, diabetes mellitus and aging [1]. Total antioxidant capacity (TAC) considers the cumulative action of all the antioxidants present in plasma and body fluids and provides an integrated parameter rather than the simple sum of measurable antioxidants. There is now a wide range of evidence indicating the importance of TAC in plasma and tissues and its modification during oxidative stress development, as well as its feasibility as a tool for investigating the association between diet and oxidative stress [2]. Therefore the aim of our present study was to determine the relationship between Endothelin 1 levels and total antioxidant capacity in prehypertensive subjects.

Materials and methods

This observational case control study was undertaken in the Department of Medical Biochemistry, Gandhi Medical College, Bhopal (M.P.) in association with Department of Medicine, Hamidia Hospital, Bhopal (M.P.), between January 2013 and December 2014 after approval from institutional ethical committee for biomedical research. The study included 100 prehypertensive patients diagnosed on the basis of clinical findings and having blood pressure in the range of 120 to 139 mmHg and DBP in the range 80-90 mmHg. The control group included 100 healthy normotensive individuals of both sexes. Fasting Blood samples were collected before giving any antioxidant drug/anti hypertensive treatment for the last 12 hrs for the estimation of TAC & Endothelin -1 levels. All the volunteers were well informed about the experimentation and their written consent was obtained, while patients with BP \geq 140/90 mmHg, DM, stroke, CAD and MI were excluded.

Measurement of blood pressure: Each subject was seated in a quiet and comfortable position for five minutes and BP was measured, five minutes apart with a mercury sphygmomanometer (cuff size 12.5X40 cm) with auscultator method of BP measurement.

Sample collection: Fasting blood samples were obtained by vein puncture of anticubital vein .5 ml of blood was taken in plain vials. The blood samples were centrifuged at 3000 RPM for 10 min .After which the serum was separated for the estimation of Total antioxidant capacity and Endothelin-1 level.

Determination of Total Antioxidant Capacity (D. Koracevic et al method, 2001)(13).

A standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton type reaction, leading to the formation of hydroxyl radicals (OH^{*}). These ROS degrade benzoate, resulting in the release of Thiobarbituric Acid Reactive Substances .

Antioxidants from the added sample of human fluid cause suppression of production of TBARS .This reaction can be measured spectrophotometrically at 530 nm and the inhibition of color development defined as AOA.

Reagents- Sodium phosphate buffer :100 mmol/l, pH7.4, Sodium benzoate:10 mmol/l, Na OH ; 50 mmol/l, EDTA (Solution), Fe (NH₄)₂SO₄:2mmol/l, Fe-EDTA complex (prepared freshly H₂O₂: 10mmol/l, Acetic acid:20%, Thiobarbituric acid (TBA):0.8% (w/v) in 50 mmol/l NaOH, Uric acid :1 mmol/l in 5 mmol/l NaOH. (all the above chemicals are supplied by thermofisher scientific).

Incubate for 10 min at 100°C (in boiling water bath) then cooled in an ice bath, measure absorbance at 532 against deionized water.

Calculation: TAC (mmol)= [CUA x (K-A)]/(K-UA)

Where, K=absorbance of control (K₁-K₀), A=absorbance of sample (A₁-A₀), UA=absorbance of uric acid solution (UA₁-UA₀), CUA=concentration of uric acid (in mmol/l)

Pipette into tubes (in milliliters) as follows:

	A ₁	A ₀	K ₁	K ₀	UA ₁	UA ₀
Serum	0.01	0.01	-	-	-	-
Uric acid	-	-	-	-	0.01	0.01
Buffer	0.49	0.49	0.50	0.50	0.49	0.49
Na benzoate	0.50	0.50	0.50	0.50	0.50	0.50
Acetic acid	-	1.00	-	1.00	-	1.00
Fe EDTA	0.20	0.20	0.20	0.20	0.20	0.20
H ₂ O ₂	0.20	0.20	0.20	0.20	0.20	0.20
Incubate for 10 minutes at 100°C (in boiling water bath) then cooled						
Acetic acid	1.00	-	1.00	-	1.00	-
TBA	1.00	1.00	1.00	1.00	1.00	1.00

Estimation of Endothelin -1 levels by ELISA Method

The Endothelin-1 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of endothelin-1 in serum, EDTA and heparin plasma. Endothelin-1 (ET-1) is a pleiotropic molecule of 21 amino acid residues involved in cardiac and vascular function, and inflammatory responses. It is highly expressed in the vascular endothelium, and also produced by leukocytes, smooth muscle cells, mesangial cells, cardiac myocytes, and astrocytes.

Statistical analysis: Statistical Analysis was carried out by using student’s unpaired ‘t’ test .The p<0.0001 was extremely considered significant.

Limitation of the study: Test samples were collected from patients who attended the OPD of Medicine and diagnosed as prehypertensives. This study was subjected to 100 prehypertensive cases within 30-60 years of age. The laboratory of biochemistry department is well equipped with ELISA, Semiautoanalyser, colorimeter and spectrophotometer. All investigation methods used in this study are standardized in our laboratory.

Results

Table 1: shows the classification of hypertension, which is classified into Prehypertension and Hypertension. According to the JNC VII hypertension is classified as Prehypertension, stage 1 and stage 2.

	Systolic (mm Hg)	Diastolic (mm Hg)
Normal	<120	<80
Prehypertension	120-139	80-89
Stage 1 hypertension	140-159	90-99
Stage 2 hypertension	≥160	≥100

Table 2: Shows demographic and anthropometric parameters of study population

Variables	Normotensives (n=100)	Prehypertensives (n=100)	P value
	32.96±6.12	42.52±8.74	<0.0001**
Sex (M/F)	55/45	53/47	---
Weight (kg)	67.57±7.39	71.23±5.95	<0.001*
Height (cm)	166.70±6.23	162.47±4.51	<0.0001**
BMI (kg/m ²)	24.20±1.47	25.43±1.62	<0.0001**
Waist-Hip ratio	0.91±0.02	0.92±0.02	<0.001*
Obesity (%)	61%	65%	---
SBP	118.7±4.89	128.46±6.04	<0.0001**
DBP	77.67±5.94	83.26±3.06	<0.0001**

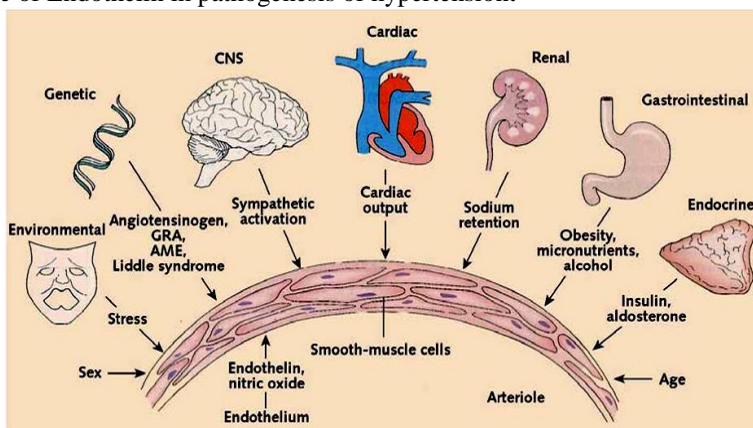
(** depicts extremely statistically significant, *depicts statistically significant)

Table 3: shows Endothelin -1 levels and Total Antioxidant Capacity in study population

Variables	Normotensives(n=100)	Prehypertensives (n=100)	P value
	3.50 ± 1.78	4.52 ± 2.29	<0.001*
	1.94±1.35	1.32±0.51	<0.0001**

(** depicts extremely statistically significant, *depicts statistically significant)

Figure 1: shows the role of Endothelin in pathogenesis of hypertension.



ET-1 a peptide isolated from the media of cultured vascular endothelial cells exerts marked vasomodulatory, with mainly vasoconstrictive effects (5) and may represent a major factor in the regulation of BP. The presence in plasma of ET-1 raises the possibility that an excess in production of this substance may play a role in the development or maintenance of hypertension (14).

The sex-specific distribution of several demographic and anthropometric parameters is presented in Table II. We observed that normotensive subjects were at extremely statistically significantly younger age as compared to prehypertensives and hypertensives ($p < 0.0001$). Among 100 prehypertensive subjects 53 were men and 47 were women. Moreover pre-hypertensive participants were more frequently obese as compared to normotensive. Systolic blood pressure of normotensive individuals was found 118.7 ± 4.89 mmHg while in prehypertensives it was 128.46 ± 6.04 mmHg which was extremely statistically significantly ($p < 0.0001$) higher. Similarly, Mean Diastolic blood pressure of normotensive individuals was found 77.67 ± 5.94 mmHg while in prehypertensives it was mean 83.26 ± 3.06 mmHg which was extremely statistically significantly ($p < 0.0001$) higher.

Comparison of Total Antioxidant Capacity and Endothelin-1 levels were shown in Table 3. It was also cleared by Figure 2 that ET-1 levels in prehypertensives (4.52 ± 2.29) were statistically significantly higher than normotensives (3.50 ± 1.78). In contrary to this, TAC was found lower in prehypertensives

(1.32 ± 0.51) as compared to normotensives (1.94 ± 1.35). Difference found was extremely statistically significant ($p < 0.001$). Therefore, it can be illustrated that there is an inverse relationship between Endothelin 1 levels and Total antioxidant capacity. An association was found between hypertension status, total antioxidant capacity and Endothelin 1 levels.

Discussion

Since the discovery of endothelium –dependent relaxation of vascular smooth muscle (15) vascular endothelium has been recognized as an important functional unit involved in the regulation of vascular smooth-muscle tone. Relaxation results from release of a labile endothelium derived relaxing factor probably identical to nitric oxide (16,17). In addition to endothelium derived vasoconstriction factors, with a characteristically slow onset and long duration of action have also recently been demonstrated (17,18,19,20).

In this work we revealed the differences in the levels of Endothelin-1 which has potent vasopressor effect and oxidative stress markers between prehypertensive subjects and normotensive subjects, without any clinical evidence of cardiovascular and metabolic disorders. The data that emerged from our study demonstrated that in prehypertensives the serum Endothelin levels were significantly increased respect to normotensives. While Total antioxidant capacity was found extremely significantly decreased in prehypertensives compared to normotensives. Moreover we found a negative correlation between ET-1 levels and TAC in patients with prehypertension.

Due to our results, normotensive controls and in prehypertensive cases were similar to those reported by previous investigators (9,10,20). In our study, TAC was found 1.32 ± 0.51 mmol/l among prehypertensive cases. It was hypothesized that high blood pressure which is the clinical manifestation of hypertensive, is associated with loss of balance between per oxidation and various antioxidant factors which are reactive oxygen species (Krouf et al., 2003) (23). The difference was statically highly significant when compared to controls S.C. Onuoha et al (2012) reported it as 1.70 ± 0.05 mmol/l (25).

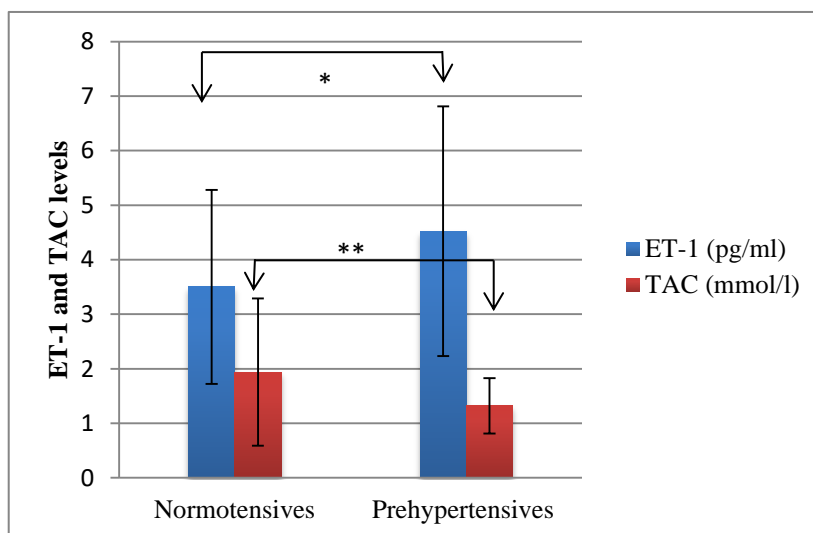


Figure 2: The comparison of Endothelin -1 levels and Total Antioxidant Capacity in Normotensives and Prehypertensives.

In hypertensive patients, the relative increase of forearm blood flow in response to nonselective endothelin-1 antagonists appears to be larger when compared with prehypertensive patients. Previous studies have shown an increase in forearm blood flow of 65% to 80% from baseline after endothelin-1 antagonism in hypertensive patients, whereas in prehypertensive subjects, increases of about 30% were demonstrated in the study presented by Weil et al. (7,8,16). These results suggest that prehypertension might be a less pronounced, intermediate stage of endothelial dysfunction. In our study, ET-1 levels were found to be 4.52 ± 2.29 pg/ml in prehypertensives where as Letizia et al (1997) reported it as 10.4 ± 3.4 pg/ml in hypertensives (25).

Conclusion

We revealed a relationship between pre-hypertension status, Endothelin-1 levels

and oxidative markers among cardiovascular disease free adults, independently of other coexisting risk factors or sedentary lifestyle behaviors. This evidence may suggest that excessive production of oxidative markers could be an early event in the pathogenesis of hypertension, preceding excess rise in blood pressure levels and could also be an element that contributes to vascular injury. However, the opposite relationship of Endothelin levels and total antioxidant capacity also be supported. Since endothelial dysfunction is a well-known predictor of cardiovascular events, the confirmation of its existence in prehypertensive subjects emphasizes that prehypertension is not a benign condition. Furthermore, increased levels of endothelin-1 can lead to increased arterial stiffness and vascular remodeling via induction of vascular smooth muscle proliferation (21,22). Thus, enhanced activity of endothelin-1 in prehypertensive patients not only may result in increased vascular tone but also may be involved in the structural changes. Therefore in this present study it is conceivable that in prehypertensives with low antioxidant capacity increased Endothelin levels may induce hypertension.

In conclusion, the result of this work demonstrates an inverse relationship of Endothelin-1 levels and Total antioxidant capacity which may play a role in the pathogenesis of hypertension.

Acknowledgement

Authors are thankful to the staff of Biochemistry department of Gandhi Medical College Bhopal(M.P.) for their technical support and involvement during this research.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: SN, TS: Protocol or project development, Data collection or management SN: Data analysis Manuscript editing or writing, Revisions. All authors approved the final version of the manuscript.

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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