

Decreased Nitric Oxide in Women with Essential Hypertension in Prehypertensive Phase

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- Aim** To determine the concentrations of nitric oxide (NO) in plasma of women with essential hypertension in prehypertensive phase, its effect on blood pressure, and correlation with other vasoactive substances that regulate systemic and renal vascular tonus.
- Methods** The study performed at the Department of Nephrology, Hospital Center in Skopje, Macedonia, included 26 women with essential hypertension in prehypertensive phase and 11 normotensive women as healthy controls. Vasodilating factors NO and 6-keto-prostaglandin F1 alpha (6-keto-PGF1 α) were determined in plasma. Thromboxane B2 (TXB₂) as a vasoconstricting factor and electrolytes Na⁺, K⁺, and Ca²⁺ were determined in urine. Blood pressure was monitored over 24 hours. Systolic, diastolic, mean blood pressure were presented as average 24-hour values.
- Results** The concentrations of NO and 6-keto-PGF1 α were significantly lower in women with essential hypertension in prehypertensive phase than in their normotensive controls (NO: median 22, range 11-35 vs median 37.5, range 11-66; 6-keto-PGF1 α : 64.8 \pm 14.35 vs 98.21 \pm 43.45 μ mol/L; $P < 0.001$). The index of vascular reactivity (TXB₂/6-keto-PGF1 α ratio) was higher in women in prehypertensive phase than in normotensive women (1.3 vs 0.8, $P < 0.001$). Urinary calcium to creatinine ratio was significantly lower in the prehypertensive group (0.06 \pm 0.03 vs 0.24 \pm 0.13, $P < 0.001$). No direct correlations were found between NO, TXB₂, and 6-keto-PGF1 α , or between NO and electrolytes in the urine. Low NO and urinary Ca²⁺ were significant indicators of increased blood pressure ($P = 0.013$ and $P = 0.024$, respectively; backward stepwise multiple regression analysis).
- Conclusions** NO and 6-keto-PGF1 α were significantly lower in women in prehypertensive phase of essential hypertension. Lower NO correlated with increased systolic blood pressure, but not with on natriuresis and calciuresis. These findings, together with the higher vascular reactivity index, indicate that endothelial dysfunction precedes the establishment of essential hypertension.

Essential hypertension is characterized by endothelial dysfunction and increased vascular tone and resistance (1). Endothelial dysfunction is the result of a misbalance of the endothelium-derived relaxing and contracting factors. Still, little is known about the early, prehypertensive phase and about the interaction of constricting and dilating agents and their exact mechanism of action.

Vascular tone is preserved due to vasodilation that slightly prevails over the action of the vasoconstricting agents. Nitric oxide (NO), which is one of the many factors produced locally by the endothelium that intervene in endothelium-dependent vasodilation, is crucial for the maintenance of the vascular tone (2). It is unclear whether NO is a marker or a mediator of the endo-

thelial dysfunction in hypertension and whether changes in the NO concentration occur as primary or secondary events (3).

Increased release of NO may be stimulated by neurotransmitters, hormones, and substances derived from platelets and the coagulation system, as well as by forces of shear stress exerted on the blood vessel (2). NO regulates blood pressure by affecting not only systemic circulation, but also renal blood flow, renal autoregulation, tubulo-glomerular feedback, and renin release (4). Urinary excretion of 6-keto-prostaglandin F1- α (6-keto-PGF1 α) and thromboxane B₂ (TXB₂) is considered to reflect mainly the production of prostaglandin I₂ and thromboxane A₂ in the kidney, therefore giving information about the state of the renal and systemic vascular tone (5).

The aim of this study was to determine the concentration of NO in the prehypertensive phase and its association with blood pressure, TXB₂ as a vasoconstricting, and 6-keto-PGF1 α as a vasodilating factor.

Participants and Methods

Participants

The cross-sectional study conducted at the University St. Cyril and Methodius Hospital Center in Skopje, Macedonia, included 26 women with essential hypertension and 11 normotensive women as healthy controls. The study participants were all women to rule out bias that may occur due to the effect of estrogen to the blood vessels. The patients were referred to a clinician for a workup of hypertension. The mean age (\pm standard deviation) of the women with hypertension and their controls was 37.6 ± 4.6 and 35.0 ± 6.7 years, respectively ($P = 0.790$). All women with essential hypertension were in the prehypertensive phase and had a family history of hypertension. None of them were taking antihypertensive medications. Secondary hypertension was excluded by examination protocols according to the recommendations of the VII Joint National Committee (JNC VII) on prevention, detection, and treatment of high blood pressure (6). Basic laboratory tests of blood and urine, ultrasonography of the kidneys, electrocardiographic evaluation, ocular fundus examination, and 24-h ambulatory blood pressure measurements were performed in all women with hypertension. When indicated by medical history

or some other finding, thyroid hormones in plasma were determined to rule out thyroid dysfunction. Metanephrines were determined in urine and metaiodo-benzylguanidine scan was performed to rule out pheochromocytoma. Tc-99m diethylene triamine pentaacetate (DTPA) and captopril scans were done when renovascular hypertension was suspected. The women did not have any other comorbidities.

All participants gave their informed consent to participate in the study.

Measurements

The following parameters were compared between women with essential hypertension in prehypertensive phase and normotensive healthy controls: 1) concentration of NO; 2) concentrations of TXB₂ and 6-keto-PGF1 α and the TXB₂/6-keto-PGF1 α ratio as an index of vascular reactivity; 3) Na⁺, K⁺, and Ca²⁺ in urine; and 4) average values of 24-hour systolic, diastolic, and mean blood pressures.

Blood pressure was measured by a 24-h blood pressure monitoring device (SpaceLab, Redmond, WA, USA). Nitric oxide was determined by an indirect method. After being released from endothelial cells, NO autoxidizes within several seconds into NO₂, which interacts with hemoglobin and turns into nitrates. Total nitrates and nitrites in plasma are relatively stable and considered an indicator of the endogenous formation of NO. We measured nitrites in plasma by the OXIS non-enzymatic assay (BIOXYTECH Nitric Oxide Non-Enzymatic Assay; OXIS, Portland, OR, USA), using the method modified by Conrad (7). This method provides determination of nitrites as metabolites of NO through enzyme conversion and a spectrophotometrical reading. The method was established by the Institute of Physiology in Skopje, Macedonia, to determine only NO in plasma. Correlations with creatinine clearance were investigated as indirect indicators of NO in urine.

We used radioimmunoassay method (Amersham, Buckinghamshire, UK) to determine TXB₂ and 6-keto-PGF1 α in urine, because these values reflect not only their systemic, but also their intrarenal production (5). We also determined Ca²⁺ in urine with a chloranilate method, and Na⁺ and K⁺ in urine, because of the possible effect of the measured vasoactive factors on electrolytes in urine. Differential examinations were performed

at the beginning of the study to rule out secondary hypertension.

Statistical Analysis

Differences were compared with either Mann-Whitney U test or unpaired Student t test, when applicable. Possible associations were tested with correlations and multiple regression analysis. NO and 24-hour blood pressure were dependent variables, and TXB₂, PgF₆, Na⁺, K⁺, and Ca²⁺ in urine were independent variables. Electrolytes in the urine were presented as a ratio of electrolytes and creatinine to obtain a corrected value. Statistical software Statistica for Windows (Version 6.0, StatSoft, Tulsa, OK, USA) was used for the analysis. *P* value of <0.05 was considered statistically significant.

Results

Women with essential hypertension in the prehypertensive phase had significantly higher 24-hour systolic, diastolic, and mean blood pressure than their normotensive controls (Table 1).

The concentration of nitrites was significantly lower in women with essential hypertension in the prehypertensive phase than in normotensive controls (*P*=0.024; Table 1). The index of vascular reactivity, TXB₂/6-keto-PGF1α, was significantly higher in women with essential hypertension than in normotensive controls. Thromboxane B₂, as a metabolite of thromboxane A₂, was not significantly different between the two groups, but the concentration of 6-keto-PGF1α, as a metabolite of PGI₂, was lower in the women with hypertension in the prehypertensive phase. Thus,

TXB₂ as a vasoconstrictor was in a relative excess (Table 1).

Multiple regression analysis (backward stepwise analysis) with NO as a dependent variable did not show that TXB₂, 6-keto-PGF1α, Na⁺, K⁺, and Ca²⁺ in urine and average 24-hour systolic, diastolic, and mean arterial pressure were predictors of values of NO in the group of women with essential hypertension (data not shown).

The ratios of Na⁺/creatinine and K⁺/creatinine in urine were similar in both groups. The ratio of urinary calcium and creatinine in urine was significantly lower in the group of hypertensive patients (Table 1). Hyperfiltration was present in most women in prehypertensive phase (calculated creatinine clearance 142.0±42.7, reference values up to 120 mL/min). No direct relationship was found between 24-hour blood pressure and NO in the women in prehypertensive phase. The model of multiple regression analysis, with the average 24-hour systolic blood pressure as a dependent predictor and NO, 6-keto-PGF1α, TXB₂, Na⁺, and Ca²⁺ in urine as independent predictors, showed that NO and calciuria were predictors of increased systolic blood pressure (*P*=0.013 and *P*=0.024, respectively).

Discussion

Our study showed that NO has an impact as a vasodilator on the regulation of blood pressure in co-action with other factors and that it is decreased even in patients in prehypertensive phase. The other vasodilating factor, prostaglandin metabolite 6-keto-PGF1α, was also decreased, and the ratio TXB₂/6-keto-PGF1α, as an index of

Table 1. Values of 24-hour blood pressures and vasoactive factors in urine in women with essential hypertension and their normotensive controls*

Parameter (mean±SD)	Women with			Reference value range
	essential hypertension (n=26)	normotension (n=11)	<i>P</i>	
24-hour blood pressure (mm Hg):				
systolic	133.9±11.6	113.0±14.7	0.002 [†]	
diastolic	89.04±9.8	70.6±8.3	0.001 [†]	
mean	104.1±10.1	87.8±7.2	0.005 [†]	
Nitrites (μmol/L, median, range)	22 (11-35)	38 (11-66)	0.024 [‡]	10-50
Vasoactive factors:				
TXB ₂ (pg/mL)	87.07±16.08	79.76±32.6	0.370	49-65
6-keto-PGF1α (pg/mL)	64.80±14.35	98.21±43.45	0.002 [†]	128-338
TXB ₂ /6-keto PGF 1α ratio	1.34±0.30	0.80±0.10	<0.001 [†]	0.14-0.51
24-hour urinary electrolyte/creatinine ratio (mmol/L/μmol/L):				
Na ⁺	15.90±7.01	12.94±6.19	0.200	100-220
K ⁺	4.60±2.05	3.87±1.58	0.300	35-80
Ca ²⁺	0.06±0.03	0.24±0.13	<0.001 [‡]	1.3-10.0

*Abbreviations: SD – standard deviation; TXB₂ – thromboxane B₂; PGF – prostaglandin.

[†]Unpaired Student t-test, *P*<0.05 was considered significant.

[‡]Mann-Whitney U test, *P*<0.05 was considered significant.

vascular reactivity, was higher. Thromboxane B₂, 6-keto-PGF1 α , and electrolytes in urine did not correlate directly with NO and none of them was a predictor of decreased concentration of NO. Calcium in urine was lower in women in prehypertensive phase and, together with NO, was a significant indicator of increased blood pressure.

Prehypertensive phase was recently recognized by the JNC-VII as a separate phase of hypertension and a comprehensive clinical study investigated patients in this phase (8). Many authors reported that lack of NO and relative excess of vasoconstrictors existed in already established hypertension (2,5,7,9,10), but it is not yet clear whether these are primary or secondary events in endothelial dysfunction. Altered production of NO was proven in hypertensive rats and in human form of essential hypertension (13). Our study did not establish if the lack of NO was due to the decreased synthesis of NO or low substrate availability, or due to degradation by superoxide anions or inhibition by endogenous inhibitors (3). Sex differences in the susceptibility of vascular walls to vasoactive factors determined by estrogen and testosterone were avoided by including only women.

Several vasodilating (NO and prostaglandin derivatives) and vasoconstricting (endothelin and TXB₂) factors are known as mediators and markers of endothelial dysfunction in essential hypertension (3). They exert their action directly on the vascular wall or may act indirectly through a change of clearance of electrolytes (3). Significant decrease in vasodilating factors, NO and prostaglandin metabolite, in our study was a marker of endothelial dysfunction, occurring in a prehypertensive phase. Thus, women in prehypertensive phase might benefit from an early start of antihypertensive therapy, especially of medications that have an impact on NO (enhance its production or slow down its degradation) and improve the endothelial dysfunction (inhibitors of angiotensin-converting enzyme or Ca-antagonists). This could be even more useful in patients with a family history of hypertension.

Changes in the renal vascular tone, indicated by decreased prostaglandin metabolite, occur concomitantly. Increased vascular reactivity index shows that blood vessels are more susceptible to the action of other vasoconstrictors, which were not investigated in our study. Our findings showed that hyperfiltration was present despite

the lack of prostaglandin metabolite in urine and NO in plasma. This might be due to intrarenal production of NO, which has the effect opposite to that of vasoconstrictors; this was also shown in animal studies (4). Excretion of Na⁺ in urine was not reduced in patients in prehypertensive phase, which also speaks in favor of the above presumption.

Decreased Ca²⁺ in urine was not a result of direct action of the NO, as no direct correlation was found between the two parameters. Together with NO, this was a significant indicator of increased blood pressure. Hypocalciuria in essential hypertension has also been reported by other authors (11,12).

Our study had several limitations. First, it included a small number of patients who should be followed to determine the transition of prehypertensive to hypertensive phase. Second, NO was determined on a single occasion.

Within these limitations, we can conclude that low plasma NO in prehypertensive women is a marker of endothelial dysfunction and is related to increased blood pressure, but probably in the setting where intrarenal production of NO is still high and manages to counteract vasoconstrictors. Therefore, further studies of these interactions in prehypertensive phase may elucidate the early changes in vascular tone and contribute to decisions for an early start of antihypertensive therapy.

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