

# Endothelial Function among Patients with Hypertension Stage II with Varying Degrees of Arterial Hypertension

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## Abstract

**Aim of the study** was to determine the levels of endothelial cell biomarkers in patients with stage II of hypertension with varying degrees of arterial hypertension.

**Methods:** In order to carry out the study, a comprehensive examination of 224 patients with HT was conducted, including 126 patients with stage II HT of the 1-3 AH degree and 98 ones with stage I HT of the 1-3 AH degree, who were treated at PI "Zaporizhzhya city clinical hospital № 10" of Zaporizhzhya city Council. Practically healthy individuals were examined on an outpatient basis. To participate in the study, patients had to sign "Voluntary informed consent of the patient to participate in the study" form.

**Results:** The synthesis of endothelin-1 is triggered by vasoactive hormones, hypoxia, active oxygen forms, increased content of low-density lipoproteins, shear stress on the endothelium. Abnormalities of NO-dependent relaxation of arteries in patients with hypertension may be caused due to the following mechanisms: decrease in NO production, its accelerated degradation, the changes in cytoarchitectonics of blood vessels. But the greatest importance in the reduction of endothelium-dependent vasodilation is attached to intracellular oxidative stress: free radical oxidation significantly reduces the production of NO by endothelial cells

**Keywords:** endothelial dysfunction, hypertension

## Introduction

Arterial vessels are one of the earliest target organs which are affected in case of hypertension (HT). One of the early and key lesions in hypertension is a violation of the vascular endothelium, which leads to the formation of endothelial dysfunction (ED) [1].

A number of vasoactive mediators are formed in vascular endothelial cells of vessels, in particular, the vasodilator molecule of nitric oxide (NO), vasoconstrictor peptide - endothelin-1. Under physiological conditions, the endothelium produces and secretes a well-balanced number of mediators regulating the vasotonic vascular function [2].

Today it is proved that ED is not only a direct participant of arterial hypertension (AH), but also it determines its support and progression. The stigma of HT is increased risk of ED, the main manifestations of which include: reduced production of NO, increased synthesis of endothelin-1, violation of the integrity of the endothelial layer [3], [4].

Currently, biomarkers of endothelial cells are studied in patients at different stages of HT, since the development of coronary heart disease and cerebral strokes in most cases is realized through ED. From the above list, it becomes obvious that the determination of levels of ED markers in patients with stage II HT with varying degrees of hypertension is relevant and it determined the purpose of this study.

**Aim of the study** was to determine the levels of endothelial cell biomarkers in patients with stage II of hypertension with varying degrees of arterial hypertension.

**Material and methods:** In order to carry out the study, a comprehensive examination of 224 patients with HT was conducted, including 126 patients with stage II HT of the 1-3 AH degree and 98 ones with stage I HT of the 1-3 AH degree, who were treated at PI "Zaporizhzhya city clinical hospital № 10" of Zaporizhzhya city Council. Practically healthy individuals were examined on an outpatient basis. To participate in the study, patients had to sign "Voluntary informed consent of the patient to participate in the study" form.

*Criteria for inclusion in the study:* patients of both sexes aged 40 to 60 years; in the patient the presence of verified HT with a known duration of the disease of at least 1 year; informative written agreement of the patient to participate in the study.

*Criteria for exclusion from the study:* patients with high and very high cardiovascular risk; stage III HT; the presence in the patient of clinically significant comorbidities, primarily cardiovascular disease (heart failure more than II A stage and II FC (NYHA), heart rhythm disorders, diabetes, secondary hypertension); increased sensitivity to prescribed drugs and their components;

women of reproductive age a positive result of the pregnancy test, lactation, as well as the use of contraceptives during the study; acute disorders of cerebral circulation; cancer; the presence of contraindications for the appointment of prolonged calcium antagonists; pathological menopause in women; refusal of the patient to participate in the study.

*Distribution of patients into groups.* The distribution of patients into groups was carried out after the determination of the compliance with the inclusion/exclusion criteria in the study, after General clinical and instrumental examination and obtaining data of laboratory researches, depending on the stage of HT:

- the first (main) group included 126 patients with stage II HT, the median age was 52.0 [47.0-57.0] years;
- the second group included 98 patients with the first stage I HT, the median age was 52.0 [47.0-56.0] years;

*Determination of nitrate and nitrite ions.* The method of determining the final stable metabolites of nitric oxide in the blood is based on the reduction of nitrates to nitrites with the determination of the latter according to their reaction with the Gris reagent. The calculation of the amount of nitrite was carried out on a calibration graph, constructed via nitrite nitrogen. The study produced three results: the content of nitrite ions ( $\text{NO}_2$ ) ( $\mu\text{mol/l}$ ), the content of nitrate ions ( $\text{NO}_3$ ) ( $\mu\text{mol/l}$ ) and the total content of nitrite and nitrate ions ( $\text{NO}_x$ ) ( $\mu\text{mol/l}$ ).

*Definition of endothelin-1.* The quantitative content of endothelin-1 was determined by immunoassay method using a set of reagents "Endothelin-1 ELISA kit" (Biomedica, Austria). A standard curve was constructed to determine the content of endothelin-1 with reference values from 0.5 to 10 fmol/ml. Optical density was estimated by spectrophotometric method at a wavelength of 540 Nm. The extent is determined using a semi-automatic tablet analyzer "SUNRISE TS" (Austria). The content of endothelin-1 in blood plasma was presented in fmol/ml.

**Statistical Analysis.** Statistical processing of the obtained data was carried out on a personal electronic computer using the PSPP application software package (version 0.10.2, GNU Project, 1998-2016). The analysis of distribution for each studied criterion was carried out. The obtained data are presented in the form of the median and interquartile range Me [Q25-Q75]. When testing statistical hypotheses, the null hypothesis was rejected at the level of statistical significance (p) below 0.05.

**Results and Discussion.** The levels of Endothelin-1 and metabolites' NO in the examined persons are presented in Table 1.

Table 1. The levels of Endothelin-1 and metabolites' NO in patients examined (Me [25-75], n = 224)

Variable	The group surveyed persons	
	HT patients with stage II (n = 126)	HT patients with stage I (n = 98)
	1	2
Endothelin-1, fmol/l	1,63 [1,13-2,18]	0,86 [0,72-0,99]
P-value	p = 0,0001	
NO <sub>3</sub> , μmol/l	13,00 [11,00-15,00]	15,50 [14,00-16,00]
P-value	p = 0,0001	
NO <sub>2</sub> , μmol/l	6,00 [5,00-7,00]	7,00 [6,00-9,00]
P-value	p = 0,0001	
NO <sub>3</sub> +NO <sub>2</sub> , μmol/l	18,50 [16,00-21,00]	24,00 [20,00-24,00]
P-value	p = 0,0001	

The level of endothelin-1 between groups of patients with hypertension was significantly higher in patients with stage II HT than in patients with stage I HT - it was 1.63 [1.13-2.18] fmol/l versus 0.86 [0.72-0.99] fmol/l, respectively ( $p < 0.05$ ). The level of NO<sub>3</sub> in patients with stage II HT was 13.00 [11.00-15.00] μmol/l, which was significantly lower by 19.2 % compared to the group of patients with stage I HT ( $p < 0.05$ ).

NO<sub>2</sub> index had the lowest value in the group of patients with stage II HT - it was 6.00 [5.00-7.00] μmol/l and was significantly lower by 16.7 % compared with the group of patients with stage I HT - it was 7.00 [6.00-9.00] μmol/l ( $p < 0.05$ ). The median sum of NO<sub>3</sub>+NO<sub>2</sub> metabolites in the group of patients with stage II HT was 18.50 [16.00-21.00] μmol/l and was significantly lower by 29.7 % of the median of this indicator in the group of patients with stage I HT - it was 24.00 [20.00-24.00] μmol/l ( $p < 0.05$ ). Depending on the degree of increase of blood pressure, we divided into 3 subgroups the patients with stage II HT and analyzed the indicators of endothelial dysfunction. The results are presented in table 2.

Table 2. Indicators of endothelial dysfunction in HT patients with stage II with varying degrees of AH

Variable	1 degree of AH (n = 41)	2 degree of AH (n = 43)	3 degree of AH (n = 42)	P-value
	1	2	3	
Endothelin-1, fmol/l	1,10 [1,07- 1,12]	1,62 [1,52- 1,79]	2,28 [2,10- 3,20]	p <sub>1-2</sub> = 0,001 p <sub>2-3</sub> = 0,001 p <sub>1-3</sub> = 0,001
NO <sub>3</sub> , µmol/l	15,00 [14,00- 16,00]	13,00 [10,00- 14,00]	11,00 [10,00- 13,00]	p <sub>1-2</sub> = 0,001 p <sub>2-3</sub> = 0,014 p <sub>1-3</sub> = 0,001
NO <sub>2</sub> , µmol/l	7,00 [6,00- 7,00]	6,00 [5,00- 7,00]	5,00 [4,00- 6,00]	p <sub>1-2</sub> = 0,09 p <sub>2-3</sub> = 0,04 p <sub>1-3</sub> = 0,001
NO <sub>3</sub> +NO <sub>2</sub> , µmol/l	22,00 [20,00- 23,00]	18,00 [16,00- 20,00]	16,00 [15,00- 18,00]	p <sub>1-2</sub> = 0,001 p <sub>2-3</sub> = 0,001 p <sub>1-3</sub> = 0,001

The level of endothelin-1 was significantly lower in the subgroup of 1 degree of AH - it was 1.10 [1,07-1,12] fmol/l than in group of 2 degrees of AH - it was 1,62 [1,52-1,79] fmol/l and in the subgroup of degree 3 AH - it was 2,28 [2,10-3,20] fmol/l ( $p < 0.05$ ). The increase in this index in the subgroup of degree 3 hypertension was reliable at 28.9 % vs the subgroup 2 degree of hypertension and in 2.1 times compared to subgroup of degree 1 AH ( $p < 0.05$ ).

The value of NO<sub>3</sub> was significantly higher in the subgroup of degree 1 AH and was 15.00 [14.00-16.00] µmol/l versus 13.00 [10.00-14.00] µmol/l of the subgroup of degree 2 AH, and against 11.00 [10.00-13.00] µmol/l of the subgroup of degree 3 AH ( $p < 0.05$ ). There was a significant gradual decrease in this indicator with an increase in the degree of AH, the level in subgroup of the degree 3 of AH was lower by 18.2 % against subgroup of the degree 2 of AH and by 36.4 % against subgroup of the degree 1 of AH ( $p < 0.05$ ).

NO<sub>2</sub> was significantly more than 28.6 % in the subgroup of degree 1 AH than in the subgroup of degree 3 AH where the level was 5.00 [4.00-6.00] µmol/l ( $p < 0.05$ ). This indicator had no significant difference between subgroup of degree 2 AH and subgroup of degree 1 AH ( $p > 0.05$ ).

The median amounts of metabolites of  $\text{NO}_3 + \text{NO}_2$  was also significantly higher in the subgroup of degree 1 AH and worked out 22.00 [20,00-23,00]  $\mu\text{mol/l}$  vs 18,00 [16,00-20,00]  $\text{mmol/l}$  of subgroup of degree 2 of hypertension, and against 16,00 [15,00-18,00]  $\text{mmol/l}$  of subgroup of degree 3 AH ( $p < 0.05$ ). There was a significant gradual decrease in this indicator with an increase in the degree of AH, the level in subgroup of the degree 3 AH was lower by 11.1 % against the subgroup of the degree 2 of AH and by 27.3 % against the subgroup of the degree 1 AH ( $p < 0.05$ ).

The synthesis of endothelin-1 is triggered by vasoactive hormones, hypoxia, active oxygen forms, increased content of low-density lipoproteins, shear stress on the endothelium. Abnormalities of NO-dependent relaxation of arteries in patients with hypertension may be caused due to the following mechanisms: decrease in NO production, its accelerated degradation, the changes in cytoarchitectonics of blood vessels. But the greatest importance in the reduction of endothelium-dependent vasodilation is attached to intracellular oxidative stress: free radical oxidation significantly reduces the production of NO by endothelial cells [5].

The endothelial cells's reaction to the stimuli is the vasoconstriction and proliferation, and the reduction of endothelium-dependent vascular relaxation is considered to be one of the diagnostic criteria of endothelial dysfunction. According to modern concepts, endothelial dysfunction is an imbalance between the Pressor and depressor mediators synthesized by endothelial cells of the vascular wall providing normally the optimal course of all endothelial processes, in which there is an abnormality of dilatation vessel ability [6].

Today it is proved that endothelial dysfunction is not only a direct participant of hypertension, but also it determines its support and progression. It requires the further investigation of the role of endothelial dysfunction, the main manifestations of which include: reduced NO production, increased synthesis of endothelin-1, in increasing the risk of adverse course of HT [7].

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**Ethical Declaration.** The study was approved by the local ethics committee of *State Institute «Zaporizhzhia Medical Academy of Postgraduate Education of Ministry of Health of Ukraine»*. The study was carried out in conformity with the Declaration of Helsinki.

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