Original Article

# METABOLIC BLOOD CHANGES IN HYPERTENSIVE WOMEN OF REPRODUCTIVE AGE

Oksana G. Ishonina, Elena V. Olempieva<sup>1</sup>, Tatyana D. Kovalenko

State Educational Enterprise of Higher Professional, Rostov-on-Don State Medical University, Federal Agency of Health Protection and Social Development Department of Common and Clinical Biochemistry №1 <sup>2</sup> Laboratory №02035 Rostov-on-Don Russia

#### **Corresponding Author:**

Kovalenko Tatyana Dmytryevna 29, Nachichevansky str. Rostov-on-Don, 344022 Russia e-mail: <u>friends 81@mail.ru</u>

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#### Summary

Our investigation established that early stage of hypertension disease in women of reproductive age was complicated by disbalance in the action of enzymatic antioxidants that lead to intensification of lipid peroxidation processes. Such changes lead to oxidative modification of proteins and lipid components of circulating lipoproteins, activation of the process of connective tissue enzymatic degradation and, as a result, to endothelial dysfunction and angiopathy.

**Key words**: antioxidant status, hypertension disease, lipoproteins, lipid peroxidation.

## Introduction

Hypertension presents as a major risk factor, associated with cardiovascular pathology cardiac insufficiency. According to National Health and Nutrition Examination Survey (NHANES) results, hypertension is diagnosed in 31% of people over 20 years of age. To increase the efficiency of hypertension treatment, federal and regional programs were designed such as "Prophylaxis and treatment of arterial hypertension in the Russian Federation" (July 23, 2001, Decree of Russian Federation Government №1171, October 7, 1996 [1]. These programs help to identify the key factors for hypertension disease. Many researchers have attempted to find a correlation between disturbance of haemodynamics, molecular mechanisms of oxygen transport and the state of intracellular metabolism. The results of these investigations indicate that hypertension is one of the so-called "diseases of dysadaptation". That is why it is very important to compare the history of onset of hypertension, the physiological state of the patient and features of metabolism in various functional conditions of the hypertensive patient for the purpose of monitoring and designing programs for prevention and treatment of hypertension.

The aim of our investigation was to study criteria

for adaptation to stage 1, 1<sup>st</sup> degree of hypertension disease in women of reproductive age as such as antioxidant state, estimation of free radical processes, lipoprotein metabolism and parameters of connective tissue metabolism.

## **Materials and Methods**

Two groups were studied, matched by data from physical examination, medical history, and results from ultrasound and electrophysiological examinations.

The control group included 33 healthy women of reproductive age (mean age  $22.3\pm1.2$  years), without any signs of cardiovascular pathology.

The clinical group included 35 women of reproductive age with stage1,  $1^{st}$  degree hypertension (mean age 25.6±2.4 years).

Erythrocytes, blood serum and blood plasma were used in the investigation. The activity of superoxide dismutase (SOD) [2], catalase [3], glutathione peroxidase (GPO) [4] and concentration of reduced form of glutathione [5] was established in erythrocytes. The activity of myeloperoxidase (MPO) [6] and concentration of non-erythrocyte hemoglobin [7] was studied in blood plasma. The level of middle mass molecules (MMM)[8], concentration of malonic dialdehyde (MDA) [9], level of  $\beta$ -lipoproteins [10], oxidative form of lipoproteins [11] and resistance to oxidation lipoproteins [12], activity of kallikrein [13], oxyproline concentration [14] were found in the blood plasma.

Statistical programs "Statistica 6.0" for Windows XP with paired and unpaired statistic methods were used for statistical analyses of results. Mean values and standard error of mean (m $\pm$ SEM) were estimated for results from analyses. Reliable data was assessed at significance level of p<0.05.

## **Results and Discussion**

Our investigation revealed that activity of SOD in the study group was 14.5% higher (p<0.05) than that in the control group (Table 1). The activity of catalase (Table 1) showed a tendency to rise – it was 9.5% higher than that in the control group (0.05 ). These changes testify to thepresence of hyperproduction of superoxyde anionradical and hydrogen peroxide accumulation inthe early stage of hypertensive disease. It isknown that hyperproduction of superoxyde anionradical leads to elevation of blood pressure owing to the nitroxilic radical inactivation and hyperproduction of endotheline I. But accumulation of hydrogen peroxide, which is endothelium independent factor of relaxation can be considered as an adaptation mechanism which is required for lowering blood pressure.

GPO, like catalase, is able to destroy the  $H_2O_2$ . Our results showed that activity of GPO was the same as in the control group (Table 1). The coefficient of protective efficiency of the antioxidant enzymes SOD/GPO was 18.1% (p<0.05) higher than in control group (Table 1). This testifies that first-line antioxidant enzymes deactivate free radicals more intensively than other antioxidant enzymes.

The concentration of reduced form of glutathione was 43.3% (p<0.05) lower than in the control group (Table 1). This suggests the presence of endogenous intoxication at an early stage of hypertensive disease.

The activity of MPO in the study group was significantly higher (61.5%, p<0.05) as compared to the control group (Table 1), thus leading to hyperproduction of hypohaloids. This could be attributed to the fact that both hydroxylic radicals and singlet oxygen contribute to the initiation of lipid peroxidation.

Taking into account the results obtained, we assume that discrepancy between first- and second-line antioxidant system and activation of lipid peroxidation characterizes the early stage of hypertensive disease. That is why hypertension disease could be considered as free radical pathology.

There are special markers of lipid peroxidation activation. MDA, MMM and nonerythrocyte hemoglobin are some of these markers. The results we obtained showed that MDA concentration was 94.3% higher (p<0.05) in the clinical group, as compared with the control group (Table 2). It is known that MDA is a powerful vasoconstrictor, responsible for smooth muscle cells construction and blood pressure elevation.

The concentration of MMM increased significantly (to 53.5%, p<0.05) in women with hypertensive disease (Table 2), thus testifying about endogenous intoxication owing to activation of lipid peroxidation of both lipid and proteins components in blood serum.

It is known that singlet oxygen [15] breaks down proteins and nucleic acids of the cell. That is why we have come to a conclusion that the increase of MMM concentration is a result from singlet oxygen interaction with polypeptide

Parameters	Control group , n=33	Clinical group, n=35
Superoxidedismutase,	4.68±0.23	5.34±0.107
conditional units/g Hb		P<0.05
Catalase,	2.43±0.097	2.66±0.106
mcat/g Hb		0.05 <p<0.1< td=""></p<0.1<>
SOD/GPO coefficient	2.32±0.225	2.74±0.251
		P<0.05
Glutathioneperoxidase,	2.02±0.081	1.97±0.177
conditional units/g Hb		P>0.05
Reduced glutathione, mcmol/g	5.82±0.349	3.30±0.29
Hb		P<0.05
Myeloperoxidase, mcmol/mg of	11.63±0.461	18.78±1.27
protein per min		P<0.05
Kallikrein,	15.36±6.38	45.49±1.36
mcmol /min		P<0.005

 Table 1. Activity of first and second line antioxidant enzymes, myeloperoxidase and kallikrein in women with hypertensive disease

chains of the proteins.

The activity of kallikrein in the blood serum in women with early stages of hypertensive disease was 196.2% (p<0.05) higher, as compared with the control group (Table 1). On the one hand, this testifies to an adaptational reaction to hypertension development due to activating the formation of a strong vasodilator - bradykinine. On the other hand, kallikrein activates tissue collagenase, which breaks the peptide links in the helical domains of collagen molecules. Collagen is the main component of connective tissue, as well as of vascular walls. It is known that singlet oxygen increases the gene expression of mRNA to collagenase, and it could be assumed that at an early stage of hypertensive disease formation this significantly affects the connective tissue of the vascular wall. This could explain why we observed such a significant elevation of free oxyproline – to 53.7% (p<0.05) in the clinical group, as compared with the control group. (Table 2). Oxyproline is a biochemical marker of the connective tissue structure damage.

It is known that singlet oxygen inhibits  $Ca^{2+}$ -ATPase [15]. It could be assumed that the function of  $Ca^{2+}$ -pump is impaired at an early stage of hypertensive disease. Such events lead to elevation of the intracellular  $Ca^{2+}$  concentration, and  $Ca^{2+}$  is responsible for vasoconstriction and blood pressure elevation.

Enzymatic degradation of connective tissue components in women with early stages of hypertensive disease correlates with dyslipidemia. Our results showed that concentration of  $\beta$ -lipoproteins (78.2%;p<0.05) was higher, and oxidative forms of lipoproteins lower (85% p<0.05) in the clinical group (Table 2). It could be assumed that hyperproduction of hypohaloids against the background of MPO activation leads to oxidative modification of lipoproteins. Decreased resistance to oxidation forms of lipoproteins is a bad prognostic sign and indicates lowering of reserves of LPO substrates.

These findings could be considered as a molecular base of hypertensive disease development, attributable to oxidative forms of lipoproteins. The latter damage the vascular wall due tothe rise of cytosol  $Ca^{2+}$  in the endothelial cells, and inhibit endothelial factors of relaxation caused by inhibition of nitroxilic radical formation. They also increase the expression of endothelin I. Oxidative forms of lipoproteins affect the L-arginine transport into endothelial cells due to increase the NO-synthesis and leads to endothelial dysfunction.

The concentration of non-erythrocyte hemoglobin (NEH) was 51.7% (p<0.05) higher in the clinical group than in the controls (Table 2). This is because oxidative modification of blood lipoproteins involves both active form of oxygen and metals with changeable valence.

It could be assumed that the rise of MDA and MMM concentration affect lipid and protein layers of lipoprotein, leading to endogenous intoxication in early stages of hypertension (as was mentioned above). This is in support of the peptide theory of vasoconstriction.

**Table 2.** Level of lipoproteins, middle mass molecules, malonic dialdehyde, nonerythrocyte hemoglobin and free oxyproline at women with hypertensive disease

Parameters	Control group , n=33	Clinical group, n=35
$\beta$ -LP, g/l	3.76±0.312	6.7±0.453
		P<0.05
Oxidative LP,	5.35±0.221	8.81±0.47
nmol MDA/g protein		P<0.05
Resistant to oxidation LP, nmol	4.21±0.336	0.63±0.05
MDA/g protein		P<0.01
Middle mass molecules, g/l	2.1±0.126	3.22±0.3
		P<0.05
Malonic dialdehyde,	14.02±0.56	27.24±0.827
nmol		P<0.05
Nonerythrocyte haemoglobin,	29.4±1.13	44.6±1.56
mmol/l		P<0.05
Free oxyproline,	9.59±0.83	14.74±0.74
Mcg/ml		P<0.05

## Conclusions

Our investigation has revealed that early stage of hypertension is characterized by molecular mechanisms of vasoconstriction:

• Hypertensive disease in women of reproductive age at reproductive age women is accompanied by severe dyslipidemia and LPO activation, that lead to increased concentrations of MDA, MMM and NEH;

• Decrease of reduced form of glutathione concentration against the background of increased concentration of MMM indicate formation of endogenous intoxication in women with hypertension;

• Hyperproduction of superoxide anion radical, hypohaloids and singlet oxygen leads to destructive changes in early stages of hypertension;

• Elevation of hydrogen peroxide concentration and kallikrein activation could be considered as a mechanism of adaptation to increased blood pressure, activation of lipid peroxidation and accumulation of molecular products of lipids and proteins degradation;

• The metabolic changes revealed lead to endothelial dysfunction and affects hemodynamics.

The metabolic changes we observed in hypertensive women warrants the assumption

that these multiple metabolic disorders could be responsible for the onset and development of development of hypertension. This is why we find it appropriate that treatment of hypertension should include both angiotensin converting enzyme inhibitors and  $Ca^{2+}$ -ATPase inhibitors and antioxidants for the purpose of decreasing the level of endogenous intoxication and activating the glutathione-dependent line of enzymatic antioxidant system.

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