Effects of Obesity on Vascular Endothelial Growth Factor and Erythropoietin in Patients with Arterial Hypertension

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Abstract

Objective: to compare level of the main cytokines induced by ischemia (vascular endothelial growth factor and erythropoietin) in patients with first grade arterial hypertension with and without obesity and in normotensive persons.

Methods. 79 patients with first grade arterial hypertension (50 with accompany obesity) were examined. 54 individuals with optimal or normal blood pressure were included into the control group. Vascular endothelial growth factor, erythropoietin, soluble vascular endothelial growth factor receptor-1 and serum placental growth factor were determined by the enzyme immunoassay. Vascular endothelial growth factor -634 G/C and -2578 C/A gene polymorphisms were investigated by polymerase chain reaction.
**Results.** An average concentration of vascular endothelial growth factor (312.8±25.6 pg/ml) and erythropoietin (9.1±0.7 mU/ml) in patients with arterial hypertension was higher (p<0.01) than in individuals without blood pressure elevation (206.1±18.6 pg/ml and 6.4±0.6 mU/ml respectively). Concomitant obesity significantly affects these results: level of vascular endothelial growth factor and erythropoietin was elevated only in patients with combination of arterial hypertension and obesity when it became higher than in patients with isolated arterial hypertension (p<0.01) or in control group (p<0.001).

There are no intergroup differences in soluble vascular endothelial growth factor receptor-1 and placental growth factor concentration as well as in the detection rate of alleles -634 G/C and -2578 C/A and genotypes -634 GG, GC, CC and -2578 CC, CA, AA of the promoter region of vascular endothelial growth factor gene.

**Conclusion.** Increased serum vascular endothelial growth factor and erythropoietin concentration is typical for patients with combination of first grade arterial hypertension and obesity. Level of these cytokines doesn’t differ in patients with isolated arterial hypertension and normal blood pressure.

**Keywords:** arterial hypertension; obesity; vascular endothelial growth factor; erythropoietin; soluble vascular endothelial growth factor receptor-1; placental growth factor; vascular endothelial growth factor gene polymorphism

The main inductor for production of vascular endothelial growth factor (VEGF) and erythropoietin (EPO) is hypoxia which lead to stabilisation of HIF-1α (hypoxia-inducible factor-1α) usually quickly destroyed in sufficient oxygen concentration. In hypoxia, HIF-1α and HIF-1β co-operate; and this complex is bound with specific hypoxia-sensitive regions of VEGF [4, 31] and EPO [11, 20] genes that increase expression of cytokines and their receptors. Such reaction of the organism is aimed to preserve oxygen concentration sufficient for metabolic needs both due to greater number of erythrocytes carrying oxygen and as a result of microvessels growth which promote its transport to organs and tissues.

In obesity vascularization becomes insufficient for adequate oxygen supply in adipose tissue [17, 24, 27] which leads to local hypoxia and stimulates VEGF and EPO expression for metabolic processes improvement.

It is proved that arterial hypertension (HTN) is accompanied by elevation of VEGF and EPO level in serum [3, 5, 12, 19, 21]. Evidences about blood pressure (BP) changes after administration of drugs that affect VEGF or EPO system confirm important role of these cytokines in BP control. HTN is the most frequent side reaction in treatment with both VEGF/VEGFR axis blockers [23, 26, 28, 37] and recombinant EPO [6, 18].

Acute BP elevation as a result of treatment with VEGV inhibitors occurs due to decreased nitric oxide (NO) concentration and reduced prostaglandin level,
Effects of obesity on vascular endothelial growth factor

as well as because of increased thromboxane and endothelin-1 concentration [7, 10, 33]. HTN becomes chronic due to imbalance between renal perfusion pressure and potassium excretion [15] caused by reduced NO formation in renal endothelium [14].

As far as EPO-induced mechanisms of BP elevation are concerned, one can single out higher blood viscosity, greater vascular reactivity as a result of increased sensitivity to catecholamines, activation of the renin-angiotensin-aldosterone system owing to a greater number of angiotensin receptors in smooth muscle cells of the vessels, elevation of intracellular calcium level, smooth muscle spasm, arterial remodeling due to stimulation of the growth of vessel wall cells, higher endothelin-1 and serotonin concentration. And though EPO can stimulate NO-dependent vasodilatation, the ultimate effect of interaction of all the mentioned mechanisms will lead to BP elevation [18, 32].

There are enough evidences of pro-angiogenic anti-ischaemic effects of VEGF and EPO and its impact on BP level. Wide spread combination of obesity which characterises by local hypoxia with HTN make it possible to suppose that adipose tissue takes part in augmentation of VEGF and EPO production and thus in BP control. In previ ous scientific reports we didn’t find information about effects of obesity on VEGF and EPO level in patients with first grade HTN. Analysis of these correlations became the main aim of our study.

Methods

79 patients with first degree HTN were examined; 54 individuals with optimal or normal BP composed the control group (Table 1). Common entry criteria were age from 40 to 60 and absence of severe accompanies diseases. Untreated patients with office BP level in the range 140 to 159 mmHg for systolic and 90 to 99 mmHg for diastolic BP were enrolled in the main group. In control group were included individuals without signs of HTN. An average level of both systolic and diastolic office BP was higher in the main group than in the control one (p<0.001).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=54)</th>
<th>HTN (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (years)</td>
<td>50.0±0.9</td>
<td>51.5±0.8</td>
</tr>
<tr>
<td>Office systolic BP (mmHg.)</td>
<td>120.7±2.1</td>
<td>147.6±3.5***</td>
</tr>
<tr>
<td>Office diastolic BP (mmHg)</td>
<td>77.5±1.1</td>
<td>90.7±1.2***</td>
</tr>
<tr>
<td>Number of men</td>
<td>16 (29.6%)</td>
<td>33 (41.7%)</td>
</tr>
<tr>
<td>Number of smokers</td>
<td>10 (18.5%)</td>
<td>14 (17.7%)</td>
</tr>
<tr>
<td>HTN in relatives</td>
<td>24 (44.4%)</td>
<td>59 (74.7%)***</td>
</tr>
<tr>
<td>Angina pectoris I-III functional classes</td>
<td>21 (38.8%)</td>
<td>31 (39.2%)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.9±0.6</td>
<td>31.3±0.5***</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>79.2±2.5</td>
<td>82.6±2.1</td>
</tr>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>79.8±2.3</td>
<td>78.5±2.2</td>
</tr>
</tbody>
</table>
Note. BMI – body mass index; GFR – glomerular filtration rate. Differences in the parameters are reliable in comparison with those in the control group; *** p<0.001. Data are presented as mean ± se

The groups were matched by the age, sex, number of smokers and angina pectoris detection rate. There were no differences between the groups in blood creatinine level and glomerular filtration rate (GFR) calculated using the MDRD formula:

\[
GFR = 32788 \times \left[\text{plasma creatinine (μmol/L)}\right]^{-1.154} \times \text{age}^{-0.203} \times 0.742 \quad \text{(if female)}
\]

Close relatives (predominantly female: 69%) had HTN much more often in the main group than in the control one (p<0.001). Body mass index (BMI) determined using the Quetelet formula:

\[
\text{BMI} = \frac{\text{body weight (kg)}}{(\text{height (m)})^2}
\]

in patients with HTN was higher than in those with normal BP (p<0.001). Third degree obesity was revealed in 4 patients, second degree obesity in 12 patients and first degree obesity in 34 patients of the main group, 25 individuals in this group were overweight. In the control group 2 persons had second degree obesity; 4 subjects had first degree obesity; 24 persons were overweight.

The level of free VEGF, sVEGFR1 (soluble VEGF receptor-1), PlGF (placental growth factor) and EPO were determined in blood serum using the enzyme immunoassay (capture/sandwich immunoassays) on the Stat Fax 2100 laboratory instrument (USA). VEGF and sVEGFR1 concentrations were measured by eBioscience tests (Austria), PlGF and EPO using DRG Diagnostics tests (USA). Detection limit for VEGF was 7.9 pg/ml; sVEGFR1 – 0.03 ng/ml, PlGF – 1.06 pg/ml, Epo – 1.2 mU/ml. Intra-Assay coefficient of variation for VEGF – 3.7%, sVEGFR1 – 6.5%, PlGF – 2.8%, Epo – 5.1%. Blood was taken from 8 to 9 o’clock a.m. Treatment (if prescribed) was interrupted three days before blood sample collection.

VEGF genetic polymorphism was determined in 41 patients with elevated and 23 people with optimal (normal) BP included into the research sequentially. To study polymorphism of the VEGF gene, DNA was isolated from 1.0 mL of the citrate blood using the phenol-chloroform method. Single nucleotide polymorphism was detected via polymerase chain reaction using the primers and two hydrolysis TaqMan probes by the method of allele discrimination on the registering amplifier DT-322 in the volume of 25 mcL by Sintol kits (Russia). The following primers were used:

-2578 forward 5′ TCAGTCCATGCCTCCACAGA 3′
-2578 reverse 5′ GGAACAAAGTTGGGGCTCTGA 3′
-2578 A probe R6G-TATCCACCCAGATCtTGCCAGGGTC-TAMRA
-2578 C probe FAM-CCACCCAGATCgTGCCAGGGT-TAMRA
-634 forward 5′ TCCAGAGAGAAAGTGCCAGGAGAGA 3′
-634 reverse 5’ CCCAAAAAGCAGGACTCCTCA 3’
-634 C probe R6G-TGCCCTCGTCgCTTTCTGCTG-TAMRA
-634 G probe FAM-TTCGCCCTGTCcCTTTCTGCTG-TAMRA
Exclusion criteria: secondary HTN; complicated HTN including stroke, transient ischaemic attack, myocardial infarction, coronary revascularization; diabetes mellitus; kidney diseases; peripheral artery disease; severe retinopathy; arrhythmias and conduction disorders; as well as heart failure of the third and fourth functional classes according to NYHA classification and signs of renal failure.

Statistical analysis was performed using Statistica7 program. The comparison of the average values was made using the Student’s t-test for the data with normal distribution and the nonparametric Mann-Whitney test for the findings with distribution deviating from normal. The Pearson’s chi-squared ($\chi^2$) method was used to test the hypothesis of normal distribution. Data are presented as mean ± se (standard error of the mean). Stepwise multiple linear regression was used to identify effects of categorical factors on the VEGF and EPO levels. Correlation analysis was performed by the Spearman’s method. The genotype frequencies of VEGF were checked for the Hardy–Weinberg equilibrium using a chi-square test. The chi-square test was also used to analyze the distribution of genotypes and alleles. P-value <0.05 was considered statistically significant.

Results

The average concentration of free VEGF in serum of the patients with first grade HTN was 312.8±25.6 pg/ml which was considerably higher than in the control group – 206.1±18.6 pg/ml (Fig. 1). Such differences were observed in both male (314.9±48.6 pg/ml versus 207.1±36.3 pg/ml, p<0.05) and female (310.4±35.7 pg/ml versus 205.3±23.8 pg/ml, p<0.01) patients.

The EPO concentration in the patients of the main group irrespective of their sex was higher than in the control group (p<0.01) being equal to 9.1±0.7 mU/ml and 6.4±0.6 mU/ml respectively (9.0±0.8 mU/ml and 6.2±0.6 mU/ml, p<0.05 for men and 9.2±0.9 mU/ml and 6.5±0.8 mU/ml, p<0.01 for women). The haematocrit level in patients with HTN (45.8±0.6%) was also higher (p<0.05) than in the participants with normal BP (43.0±0.7%). However, no significant differences in the haemoglobin content between these groups (143.2±1.9 g/l versus 139.8±2.3g/l) were detected.
As the VEGF-induced effects are influenced considerably by its soluble receptors (sVEGFR1) and placental growth factor (PIGF), their concentrations were measured concurrently. The sVEGFR1 level in blood did not vary in the individuals of the main and control groups being equal to 8.3±5.9 ng/ml and 30.5±17.0 ng/ml respectively though there were considerable individual variations of its level. No differences in the PIGF concentration between the groups amounting to 40.1±5.2 pg/ml in patients with elevated BP and 47.2±9.4 pg/ml in patients with normal BP were revealed (Fig. 1).

VEGF expression may be affected by polymorphism of the VEGF gene promoter region, -2578 C/A and -634 G/C in particular. Neither allele -2578 C nor allele -2578 A frequencies varied in the patients with first grade HTN and those from the control group. There were no differences in -634 G and -634 C allele frequencies in the people with elevated and normal BP (Table 2). No differences in frequencies of allele combinations -634 G/C and -2578 C/A were revealed in the main and control groups (Table. 3), which corresponds to the Hardy-Weinberg law of random distribution.

**Fig1.** VEGF, sVEGFR1 and PIGF levels in patients with first grade hypertension and individuals without BP elevation.
Table 2
Frequencies of VEGF gene allelic variations -634 G/C and -2578 C/A in the main and control groups

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Control (n = 23)</th>
<th>HTN (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-634 G/C polymorphism</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>34 (74%)</td>
<td>61 (74%)</td>
</tr>
<tr>
<td>C</td>
<td>12 (26%)</td>
<td>21 (26%)</td>
</tr>
<tr>
<td></td>
<td>-2578 C/A polymorphism</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>22 (48%)</td>
<td>33 (40%)</td>
</tr>
<tr>
<td>A</td>
<td>24 (52%)</td>
<td>49 (60%)</td>
</tr>
</tbody>
</table>

Table 3
Frequencies of VEGF gene allelic combinations -634 G/C and -2578 C/A in the main and control groups

<table>
<thead>
<tr>
<th>Allelic combinations</th>
<th>Control (n = 23)</th>
<th>HTN (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allele frequency</td>
<td>Expected frequency</td>
</tr>
<tr>
<td>-634 G/C polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>11 (48%)</td>
<td>55%</td>
</tr>
<tr>
<td>GC</td>
<td>12 (52%)</td>
<td>38%</td>
</tr>
<tr>
<td>CC</td>
<td>0 (0%)</td>
<td>7%</td>
</tr>
<tr>
<td>-2578 C/A polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>3 (13%)</td>
<td>23%</td>
</tr>
<tr>
<td>CA</td>
<td>16 (70%)</td>
<td>50%</td>
</tr>
<tr>
<td>AA</td>
<td>4 (17%)</td>
<td>27%</td>
</tr>
</tbody>
</table>

In patients with first grade HTN having -634 GG genotype the average VEGF concentration was 306.9±42.1 pg/ml which was higher (p<0.05) than in the patients with -634 GC genotype (173.4±61.2 pg/ml). In individuals without BP elevation, the plasma VEGF content amounted to 215.2±49.7 pg/ml if they had -634 GG genotype and 144.7±33.2 pg/mL if they had -634 GC genotype (p>0.05). -634 CC genotype was found out only in two patients from the main group which did not give an opportunity to estimate the extent of its effect on the level of VEGF secretion. -2678 C/A genotype did not affect level of VEGF expression in either hyper- or normotensive patients.

Influence of the age, sex, BMI, level of systolic and diastolic BP and GFR on VEGF and EPO concentration in serum was estimated by the method of the step-by-step linear regression analysis. GFR (β = 0.39; p < 0.05) proved to be an independent factor affecting VEGF concentration in the patients with first grade HTN whereas EPO level was influenced by the BMI (β = 0.47; p < 0.05). No reliable effect of these categorical predictors on the VEGF and EPO concentrations in serum in the control group was revealed.
In the main group there were positive correlations between EPO content, level of systolic BP (r = 0.33; p < 0.05) and BMI (r = 0.38; p < 0.05) and a negative correlation between VEGF and PlGF (r = -0.34; p < 0.05). No significant correlations were detected in the control group.

As far as concomitant obesity may affect VEGF and EPO expression we compared concentrations of these growth factors in hypertensive patients without obesity with their concentrations in the control group. After BMI normalization there were no differences in the serum level of VEGF and EPO in patients with and without HTN (table 4). VEGF (p<0.01) and EPO (p<0.05) concentrations were higher in cases of combination of obesity and HTN then in HTN alone. At the same time there were no intergroup differences in the serum level of sVEGFR1 and PlGF. Most important differences in VEGF and EPO levels were revealed between subgroup of patients with combination of HTN and obesity and control group (p<0.001). These findings suggest that obesity may affects VEGF and EPO expression with greater force then BP elevation.

Table 4
Comparison of observed dates in patients with combination of HTN and obesity, isolated HTN, and control group

<table>
<thead>
<tr>
<th>Index</th>
<th>Control (n=24)</th>
<th>HTN (n=29)</th>
<th>HTN + obesity (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mmHg</td>
<td>120,8±2,3</td>
<td>145,6±2,2***</td>
<td>148,5±2,3***</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>77,5±1,3</td>
<td>91,0±1,4***</td>
<td>90,5±1,5***</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52,0±1,2</td>
<td>52,1±1,2</td>
<td>50,8±1,1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27,0±0,7</td>
<td>27,9±0,6</td>
<td>34,1±0,5***/000</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>203,3±36,4</td>
<td>232,3±33,6</td>
<td>352,0±36,4***/000</td>
</tr>
<tr>
<td>sVEGFR1 (pg/ml)</td>
<td>20,7±13,0</td>
<td>0</td>
<td>6,7±7,8</td>
</tr>
<tr>
<td>PlGF (pg/ml)</td>
<td>45,0±9,0</td>
<td>46,9±6,9</td>
<td>35,1±7,5</td>
</tr>
<tr>
<td>Epo (mU/ml)</td>
<td>6,4±1,3</td>
<td>7,4±1,1</td>
<td>9,9±0,9***/0</td>
</tr>
<tr>
<td>Ht, %</td>
<td>43,8±1,1</td>
<td>45,7±1,2</td>
<td>45,8±0,9</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>92,3±4,3</td>
<td>114,9±4,4***</td>
<td>119,5±4,3***</td>
</tr>
<tr>
<td>GFR (ml/min/1,73/m²)</td>
<td>79,3±3,1</td>
<td>76,8±4,4</td>
<td>79,6±2,6</td>
</tr>
</tbody>
</table>

Note. BP - blood pressure, BMI – body mass index, GFR – glomerular filtration rate, LVMI – left ventricular mass index.

Differences in the parameters are reliable in comparison with those in the control group: *** p<0.001. Data are presented as mean ± se.

Differences in the parameters are reliable in comparison of patients with combination of HTN and obesity and in subjects with isolated HTN: o - p<0.05, oo - p<0.01, oo0 - p<0.001.
Discussion

It was shown in a series of observations that serum VEGF concentration in patients with HTN is higher than in subjects with normal BP [3, 12]. In hypertensive patients VEGF level in circulation is greater in certain conditions which include microalbuminuria [1], retinopathy [36], myocardial infarction, angina pectoris, insult or chronic renal failure [21]. The increased level of VEGF in serum in people with high BP also revealed in our research seems to be paradoxical because VEGF stimulates NO-dependent vasodilatation [39], growth of the microvessels and, consequently, have to reduce BP. The main known causes of the fact that VEGF has no effects are increased sVEGFR1 expression and decreased VEGFR2 and PlGF formation or their combination. The genetically conditioned growth of VEGF expression and its reduced elimination by the kidneys may in turn lead to the higher levels of cytokines in blood and tissues [25].

In this work concentration of both sVEGFR1 and PlGF did not vary in the patients with first grade HTN and the individuals from the control group so it is doubtful that they influence the level of free VEGF and development of its effects significantly. Average GFR was also the same in the main and control groups.

Neither alleles nor allelic combinations of the promoter region of the VEGF gene specific for patients with HTN were detected. However, average VEGF concentration in serum was higher in the patients with first grade HTN if they have -634 GG genotype than if they have -634 GC genotype. According to the data obtained from the literature, -634 G/C VEGF polymorphism has an influence on the level of ligand expression and severity of sunitinib-induced HTN [9, 30]. -634 GG genotype increases release of the large poorly soluble VEGF isoforms [29] most of which are bound with heparan sulfate proteoglycan of the intracellular matrix [38]. Elevated pressure on the vessel wall and higher shear stress typical for HTN may contribute to weaker interaction of the large VEGF isoforms with heparan sulfate proteoglycan which causes higher VEGF concentration in serum in patients with -634 GG genotype.

Elevation of free VEGF concentration in hypertensive patients occurs in response to tissue ischaemia, increased mechanical stretching and/or angiotensin II over expression [13, 16]. Local fatty tissue hypoxia in obesity may promotes VEGF expression and vessels growth which leads to better cells oxygenation, improved insulin sensitivity and suppression of inflammatory reactions [8]. Our results suggest that augmentation of VEGF concentration is typical for patients with combination of HTN and obesity and isn’t seen in patients with isolated HTN. Probably in this case increased VEGF level first of all reflects local fatty tissue hypoxia and only secondary mechanical stretching and angiotensin II over expression.

Except stimulation of erythropoiesis EPO has direct or indirect positive effects to fats and carbohydrates metabolism. In hypoxic conditions at high altitude there are negative correlations between EPO concentration, BMI, glucose
and insulin levels [2]. Obesity and insulin resistance characterized by low level of EPO receptors in white fatty tissue and hypothalamus [35].

In our study EPO over expression in patients with combination of HTN and obesity most probably reproduce organism’s reaction to fatty tissue hypoxia and serves for metabolic processes normalization. It shout be noted that much higher EPO level in patients with first grade HTN was observed in both male and female which excluded the estrogen-dependent character of the elevated EPO expression. Higher level of EPO contributes to growth of haematocrit, circulating blood volume, spasm and remodelling of vessels, higher sensitivity to angiotensin II and catecholamines which provokes aggravation of HTN [6, 32].

Increased EPO and EPO receptors content usually accompany by elevation of VEGF level and neovascularisation [22, 34]. Concerted growth of VEGF and EPO expression in patients with combination of first grade HTN and obesity may be considered as compensatory reaction pointed at reduction of local and system hypoxia, metabolic processes normalization, cardio- and neuroprotection.

Conclusions
1. Overage VEGF and EPO concentration in serum in patients with first grade HTN is higher in the presence of concomitant obesity than in the absence of it when VEGF and EPO levels don’t differ from persons with normal BP.
2. Neither changes in the sVEGFR1 and PlGF concentration in serum nor peculiarities of genetic polymorphism in -634 G/C and -2578 C/A promoter region of VEGF gene were revealed in the patients with first grade HTN in comparison with individuals without BP elevation.

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