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Endothelium function biomarkers and carotid intima-media thickness changes in relation to NOS3 (rs2070744) and GNB3 (rs5443) genes polymorphism in the essential arterial hypertension

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Objective. The aim of the present study was to clarify the endothelial function biomarkers and carotid "intima media" thickness (IMT) changes in relation to *GNB3* (rs5443) and *NOS3* (rs2070744) genes polymorphism in the essential arterial hypertension (EAH).

Methods. One-hundred EAH patients (48 – control) participated in the case-control study. Soluble vascular cell adhesion molecule (sVCAM-1), total NO metabolites (NO_2^-+NO_3^-), transcriptional activity of *NOS3* gene, endothelium-dependent flow-mediated dilation of the brachial artery (FMD BA), and carotid IMT were studied. *GNB3* (rs5443) and *NOS3* (rs2070744) genotyping was performed by TaqMan probes (CFX96™Real-Time PCR).

Results. The connection of *NOS3* (rs2070744) with decreased total NO metabolites (F=71.11; p<0.001), reduced *NOS3* genes transcription activity (F=8.71; p<0.001) and increased sVCAM-1 (F=6.96; p=0.002), especially in the *C*-allele carriers (particularly in *CC*-genotype patients with lower NO – 16.46% and 40.88%; p<0.001), lowered the transcription activity of NOS3 gene – 46.03% 7 times (p<0.001), and become higher sVCAM-1 – 35.48% and 89.48% (p<0.001), respectively. ANOVA did not confirm the association of *GNB3* (rs5443) gene with endothelial function and carotid IMT. Severe EAH was associated with increased carotid IMT – 50.0% (p<0.001) and 57.14% (p=0.007), wider carotid arteries – 17.36% (p=0.012) and 21.79% (p=0.004), and decreased NOS3 genes transcription activity – 34.54% (p=0.003). Atherosclerotic plaques were unilateral – 24.77% (χ^2=5.35; p=0.021) or bilateral – 27.62% (χ^2=5.79; p=0.016). IMT>0.9 mm was followed by a higher BP (p<0.001), FMD BA 11.80% decrease with compensatory increase in carotid arteries diameters – 17.38% and 21.99% (p<0.001) and sVCAM-1 by 20.49% (p=0.005).

Conclusion. *NOS3* (rs2070744), but not *GNB3* (rs5443), gene associated with the essential arterial hypertension severity relying upon the endothelial function impairment and *NOS3* genes reduced transcription activity.

Key words: *NOS3* (rs2070744), but not *GNB3* (rs5443), gene associated with the essential arterial hypertension severity relying upon the endothelial function impairment and *NOS3* genes reduced transcription activity.

The vascular endothelium is an important paracrine organ and a potential target in cardiovascular pathology and metabolic disorders (metabolic syndrome, diabetes mellitus, etc.). The healthy endothelium produces and releases potent vasodilators with powerful anti-atherosclerotic and

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anti-inflammatory properties and diminishes the vascular resistance. On the other hand, in the case of endothelial dysfunction (ED), the endothelium-dependent vasodilatation becomes attenuated with endothelial inflammatory activation. Therefore, ED is associated with vasoconstriction, subclinical atherosclerosis, and an increased risk of cardiovascular diseases (CVD) (Brandes 2014). Despite the growing number of scientific evidence supporting the associations between ED and CVD, little is known about the relationships between the ED and the genetic background in hypertensive patients (Williams et al. 2018; Visseren et al. 2021). Moreover, to assess the development of CVD including arterial hypertension (AH) from the standpoint of ED is one-sided and incompletely reproduces the pathogenesis of AH, in general. Therefore, an effort to translate these findings into an effective ED and CVD prevention strategies still remains limited.

The renal and central control of the blood pressure (BP) via the renin-angiotensin-aldosterone system (RAAS) and metabolic and local neural factors have a much stronger effect on BP in general and local vascular tone. In this way, each organ independently controls its perfusion through the local factors maintaining BP. However, systemic BP control is preserved mostly in conditions associated with ED, smoking, hypercholesterolemia, etc., even if it may eventually result in AH development (Johnson et al. 2013).

Some evidence indicates that ED is accompanied by an increase of inflammatory biomarkers in the hypertensive patient: soluble fms-like tyrosine kinase-1 (sFLT-1), adhesion molecules (ICAM-1 and VCAM-1) (Dorr et al. 2014), elevation of endothelial vasoconstriction (endothelin-1 and angiotensin II, thromboxane A2, and especially cyclooxygenase) (Virdis et al. 2013; Jiang et al. 2020), which promote formation of the vascular reactive oxygen species (ROS), prostaglandin H2, releasing and activation of the nuclear κB factor, activator protein-1, Nox NADPH oxidases (Takac et al. 2012; Bruder-Nascimento et al. 2020), inflammatory cytokines TNF-a, IL-β, IL-6, etc. (De Ciuceis et al. 2005). Under these conditions, the excessive production of the endothelial vasoconstriction factors is associated with a parallel decrease of NO availability, reduced endothelial NO production, and depressed endothelial nitric oxide synthase (eNOS) expression (Carrizzo et al. 2020). Therefore, there is an assumption that the rate of these processes can be, at least partially, determined by genes that regulates RAAS activity or code the enzymes expression or synthesis associated with hypertrophy and remodeling of the vascular smooth muscles (Sydorchuk et al. 2013, 2015, 2020a; Ji et al. 2017; Charoen et al. 2019; Smyth et al. 2019; Dzhuryak et al. 2020; Tegegne et al. 2020; Repchuk et al. 2021; Semianiv et al. 2021). The relationship between ED markers, vessels wall thickness, and genetic predisposition in hypertensive patients has not been fully revealed yet. These associations are of considerable interest because may allow the identification of hypertensive subjects, who might benefit from the targeted genetically predisposed interventions.

In this respect, the objective of our research was to clarify the endothelial function biomarkers and carotid "intima media" thickness (IMT) changes depending on the guanine nucleotide-binding protein beta-3 (*GNB3*, rs5443) and endothelial nitric oxide synthase (*NOS3*, rs2070744) genes polymorphism in essential arterial hypertension.

Materials and methods

Compliance with bioethics. The study fully adhered to European Convention on Human Rights and Biomedicine, GCP, GLP principles, EUC directive #609 and other EU and international legislations on bioethics. The study protocol has been approved by the Ethics' Committee of the Bukovinian State Medical University (Protocol No. 2 from October 10, 2019). The Research is defined as a prospective case-control study.

Inclusion/exclusion criteria. The study included EAH patients with hypertensive-mediated organ damage (HMOD) estimated according to the European Societies of Hypertension and Cardiology recommendations (ESH/ESC 2018): target-organs damage – 2nd stage (asymptomatic AH), from the 1st through to the 3rd grade of blood pressure elevation (BP); moderate-high cardiovascular (CV) risk; aged from 45 to 65 years.

Exclusion criteria have been described previously (Dzhuryak et al. 2020; Repchuk et al. 2021; Semianiv et al. 2021; Sydorchuk et al. 2020a, b). The patients with EAH stage 3 (established CV disease, chronic kidney diseases, CKD) – with estimated glomerular filtration rate (eGFR) decline <30 ml/min/1.73 m^2), secondary arterial hypertension, EAH patients with complications of HMOD, chronic heart failure (CHF) higher than II functional class (NYHA III–IV), diabetes mellitus type I (T1DM), sub- and de-compensated diabetes mellitus type 2 (T2DM) (with diabetic target-organ damage), malignant or uncontrolled arterial hypertension, sub- and de-compensated liver diseases (triple growth over the

normal level of aspartate aminotransferase, alanine aminotransferase), bronchial asthma, chronic obstructive pulmonary disease of III–IV stage with C or D risk value (GOLD 2019), exacerbated infectious diseases or during unstable remission, psychological disorders, oncologic problem of any location, administration of oral corticosteroids or contraceptives, and pregnancy or lactation were excluded.

After screening the matching inclusion and exclusion criteria, 100 patients were selected for further examination (75% women, 25% men, mean age 59.87±7.98 years). The genetic examination was performed in 72 patients. The control group included 48 practically healthy individuals who were not relatives of the patients and without reliable differences of gender distribution (62.5% females, 37.5% males) and mean age (49.13±6.28 years) with a study group. All enrolled subjects signed a consent form to participate in the study.

Essential arterial hypertension assessment. Hypertension was defined as office systolic BP (SBP) values ≥140 mmHg and/or diastolic BP (DBP) values ≥90 mmHg at least for three measurements during a month, according to the European Societies of Hypertension and the Cardiology (ESH/ESC) recommendations (Williams et al. 2018; Visseren et al. 2021).

All enrolled patients underwent a complex of examinations: general clinical examinations, complete blood count, creatinine, glucose, total cholesterol (TC) levels, triglycerides (TG), and low/high density level cholesterol (LDL-C, HDL-C), atherogenicity Index [AI=(TC–HDL-C)/HDL-C, Unit], body mass index (BMI, kg/m^2) for evaluation of overweight and abdominal obesity (AO), waist-to-hip ratio (WHR), office measurement of SBP, DBP, heart rate (HR), GFR calculation [according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation with Creatinine level], ECG in 12 leads, EchoCG, and consultations of ophthalmologist and neurologist according to the European recommendations (ESC 2018, 2021).

Markers of the endothelium function. The serum level of the soluble vascular cell adhesion molecule (sVCAM-1) (CD 106) was determined by Enzyme Linked-Immuno-Sorbent Assay (ELISA) according to the Manufacturer's Guidelines with a highly sensitive sVCAM-1 ELISA KIT® (Diaclon SAS, France) on a "StatFax 303" device (USA). The sVCAM-1 assay has a sensitivity of 0.6 ng/ml.

The monoxide nitrogen metabolites (NO_2^-/NO_3^-) concentration was evaluated in serum stabilized with EDTA (1 mg/ml) by colorimetric method with Total $NO/NO_2^-/NO_3^-$ Assay Kit (RDS, UK) on a 550 nm Spectrophotometer (TS8210, China). Detection principle: after recovery of nitrate to nitrite by nitrate-reductase enzyme, nitrite reacts with chromogenic agent producing light red azo-compound and the content of nitrite can be calculated by measuring the OD value at 550 nm.

Transcriptional activity of NOS3 gene in the peripheral blood was validated by pathway-specific PCR array with Maxima SYBR Green/ROX qPCR MasterMix (2X) set (ThermoScientific™, USA). Total RNA was isolated from blood leucocytes using NucleoZOL (Macherey-Nagel, Germany) according to the Manufacturer's Guidelines. The quality and value of the isolated total RNA was determined using NanoDrop spectrophotometer (Thermo Scientific™, USA). The purified RNA underwent reverse transcription (RT) with a cDNA conversion RT^2 Strand Kit (OT-1, Syntol™, RF). RNA samples within the range of –2.0–2.2 were selected. Amplification was performed on samples in triplicate. Samples were assigned to both groups: control and studied ones. Quantity detection of mRNA performed by calculating the relative normalized amount of cDNA of the *NOS3* gene, when the control group data were taken as "1" and the data of the study/test group were determined relative to the control group. Relative cDNA values were normalized and based on ΔΔCt method with the reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The data for analysis were uploaded to the GeneGlobe portal and computed fold change/expression using delta-delta Ct method (ΔΔCt) as: ΔCt study/test group (ΔCt *NOS3* gene) – delta Ct control group (ΔCt average of *GAPDH* reference gene) with following calculation of fold change using 2^(–delta–delta Ct) equation.

Statistical analysis of PCR array data. The RT2 Profiler PCR Array Data Analysis software does not perform any statistical analysis beyond the calculation of p-values using a Student's t-test (two-tail distribution and equal variances between the two samples) based on the triplicate 2^(–ΔCt) values of *NOS3* gene between patients and the control group. The Microarray Quality Control indicated that sample numbers and p-value calculation was sufficient to demonstrate reproducible results across microarrays and PCR Arrays, including the RT2 Profiler PCR Arrays.

Carotid intima-media thickness and endothelium-dependent flow-mediated dilation of the brachial artery ultrasound assessment. Endothelial-dependent flow-mediated dilation of the brachial artery (FMD BA) measurement according to the

FMD assessment Guidelines (Corretti et al. 2002; Thijssen et al. 2019; Maruhashi et al. 2020; Holder et al. 2021) using ultrasonographic complex "ACCUVIX A30" (Samsung Medison, South Korea) with duplex scanning of brachial arteries (BA), high-frequency vascular transducer, color and spectral Doppler and an internal ECG monitor. A blood pressure cuff was applied to the forearm and inflated to a pressure that was 50 mm Hg above the baseline SBP for 5 min. From 30 s before to 2 min after cuff deflation, the BA diameter was recorded on ultrasound. Cuff deflation induces a reactive hyperemia – a brief high-flow through the brachial artery to accommodate the dilated resistance vessels. An increase of internal BA diameter was expressed in the percentage of the baseline BA diameter. The increase diameter less than 10% was determined as endothelial dysfunction (ED), of FMD insufficiency.

Carotid intima-media thickness (C-IMT) and carotid plaque, including mean-maximal or composite IMT measures of common carotid artery (CCA) and internal carotid artery (ICA) from both sides in a region free of plaque was assessed on ultrasound complex "ACCUVIX A30" (Samsung Medison, South Korea) in B-mode regime with high-frequency vascular transducer, color and spectral Doppler and an internal ECG monitor according to the Mannheim CIMT Consensus Report (2012) and ESC Recommendation (2021) (Den Ruijter et al. 2012; Touboul et al. 2012; Ravani et al. 2015; Kablak-Ziembicka et al. 2021; Visseren et al. 2021). An upper limit of 0.9 mm has been used as a cut off value that denotes an increased IMT. Plaque was defined as the presence of a focal wall thickening that is ≥50% greater than the surrounding vessel wall, or as a focal region with an IMT measurement ≥1.5 mm that protrudes into the lumen (ESC 2021).

Genotyping of the endothelial nitric oxide synthase (NOS3, rs2070744) and guanine nucleotide-binding protein beta-3 (GNB3, rs5443) genes polymorphisms. DNA isolation, amplification and genotyping. Venous blood was collected in a sterile vacutainer, stabilized by K2-EDTA. DNA was isolated from the whole venous blood lymphocytes nuclei of participants and purified according to GeneJET Genomic DNA Purification Kit Manufacturers Guidance (Thermo Fisher Scientific, USA). DNA fragments of analyzed genes amplified by Quantitative Real-Time PCR (qRT-PCR) with specific for each gene TaqMan probes and genotyping with TaqMan Genotyping Master Mix on CFX96 Touch™ RT-PCR Detection System (Bio-Rad Laboratories, Inc., USA). The genotyping protocol was described in our previous publications (Dzhuryak et al. 2020; Sydorchuk et al. 2020 a, b; Kamyshna et al. 2021; Repchuk et al. 2021; Semianiv et al. 2021). Alleles' discrimination of *NOS3* (rs2070744) and *GNB3* (rs*5443*) genes polymorphisms was analyzed by licensed CFX96 RT-PCR Detection System Software (Microsoft, USA).

Statistical analysis. Statistical analysis was performed using StatSoft Statistica v.7.0 software (StatSoft Inc., USA). To verify the differences between groups, we applied the Students t-test (two-tail distribution and equal variances between the two samples), ANOVA, Pearsons χ^2 test, or the Wilcoxon-Mann-Whitney U-test (in case of uneven data distribution according to W-Shapiro-Wilk or Kolmogorov-Smirnov test results). Differences were regarded as significant at $p<0.05$ values.

Results

The parameters of the endothelial function and IMT depending on EAH severity (after BP value) are presented in Table 1. With more severe EAH course of 2nd – 3rd grades of BP elevation the IMT of the CCA and ICA were found – by 50.0% ($p<0.001$) and 57.14% ($p=0.007$) larger, and the diameter (D) of CCA and ICA – 17.36% ($p=0.012$), and 21.79% ($p=0.004$) wider with lower expression of *NOS3* gene by the level of mRNA – 34.54% ($p=0.003$), than in patients with BP value of the 1st grade (SBP/DBP <160/<100 mmHg). Moreover, patients with more severe course of EAH (SBP/DBP ≥160/≥100 mmHg) presented atherosclerotic plaques relatively more often irrespective of their location: with unilateral – by 24.77% ($\chi^2=5.35$; $p=0.021$) more often, and bilateral – by 27.62% ($\chi^2=5.79$; $p=0.016$), respectively. BP values did not reliably influence on the size of plaques, level of NO total metabolites in the blood (with a tendency to irreversible correlation) and sVCAM-1 content. It should be noted that atherosclerotic plaques on the ICA were found 10.0% ($p>0.05$) more frequently than on the CCA, and they were a little larger in size ($p>0.05$).

Parameters of the endothelial function and IMT depending on the polymorphic variants of *NOS3* (rs2070744) gene are presented in Table 2. Hypertensive patients with minor *C*-allele (stronger in *CC*-genotype carriers) presented lower blood level of total NO metabolites – by 16.46% and 40.88% ($p_{TT}<0.001$), and lower transcription *NOS3* gene activity after the level of mRNA – by 46.03% ($p_{TT}<0.001$) and 7 times ($p_{TT}<0.001$), with higher sVCAM-1 blood content – by 35.48% ($p_{TT}<0.001$) and 89.48% ($p_{TT}<0.001$), respectively, than in the *TT*-genotype

Table 1
Biomarkers of endothelial function, carotid "intima-media" thickness and signs of atherosclerosis depending on the hypertension severity

Parameter	Control group (n=48)	Hypertensive patients SBP /DBP (mmHg)	
		<160/<100 (n=42)	≥160/≥100 (n=30)
FMD BA (%)	11.14±0.74	8.75±0.57 p=0.013	6.53±0.50 (p=0.002; p_1=0.005)
IMT CCA (mm)	0.55±0.04	0.68±0.10	1.02±0.13 (p<0.001; p_1<0.001)
IMT ICA (mm)	0.52±0.05	0.63±0.11	0.99±0.14 (p<0.001; p_1=0.007)
Atherosclerotic plaques on the CCA (n, %)			
Unilateral	3 (6.25)	26 (61.90)	26 (86.67)
Bilateral	2 (4.16)	22 (52.38)	24 (80.0)
Size of atherosclerotic plaques (mm)			
CCA	1.44±0.05	1.62±0.15	1.82±0.27
ICA	1.41±0.08	1.72±0.20	1.86±0.33
D (mm)			
CCA	6.61±0.24	6.74±0.28	7.91±0.35 (p<0.001; p_1=0.012)
ICA	5.54±0.19	5.69±0.42	6.93±0.36 (p<0.001; p_1=0.004)
Monoxide nitrogen metabolites (NO_2^-/NO_3^-) (μmol/l)	27.88±2.25	23.23±2.70	19.86±1.95 (p<0.001)
sVCAM-1 (ng/ml)	909.1±75.54	1152.0±150.31	1453.3±129.55 (p=0.007)
Transcriptional activity of NOS3 gene after mRNA (U)	1.68±0.23	0.55±0.06 (p<0.001)	0.36±0.07 (p<0.001; p_1=0.003)

Abbreviations: FMD BA – flow-mediated dilation of the brachial artery; IMT CCA, ICA – intima-media thickness of common carotid artery, internal carotid artery; D – mean diameter of CCA and ICA in systole and diastole; NO_2^-/NO_3^- – total nitrite/total nitrate; sVCAM-1 (CD 106) – soluble vascular cell adhesion molecule. Data are presented as mean ± S.D. p – significance of differences with control group; p_1 –significance of differences with group of patients with BP <160/<100 mmHg.

carriers. The above-mentioned parameters deteriorated reliably in homozygous mutational *C*-allele carriers: by the decrease of total NO metabolites level – 29.23% (p_{TC}<0.001) and *NOS3* gene expression – 3.78 times (p_{TC}<0.001), and by the increase of sVCAM-1 – 38.44% (p_{TC}<0.001), respectively.

In addition, a similar tendency of the above showed parameters changes was found in the control group for the *C*-allele and *CC*-genotype carriers of *NOS3* gene (rs2070744): FMD BA decrease by 11.78% (p_{TT}=0.004), lower level of total NO metabolites – 20.06% and 27.83% (p_{TT}<0.001), as well as transcription activity of *NOS3* gene – 27.88% and 46.15% (p_{TT}<0.001) with higher sVCAM-1 blood content – 35.51% (p_{TT}=0.002) and 39.10% (p_{TC}=0.003), respectively (Table 2).

The one-way ANOVA analysis proved certain relations between *NOS3* (rs2070744) gene in hypertensive patients and decreased content of total NO metabolites (F=71.11; p<0.001), reduced transcription activity of *NOS3* gene (F=8.71; p<0.001) and increase of sVCAM-1 (F=6.96; p=0.002). In the healthy subjects, ANOVA analysis confirmed a threshold association of *NOS3* (rs2070744) gene with FMD BA reduction (F=3.06; p=0.052), decrease of the total NO metabolites in the blood (F=50.42; p<0.001) and *NOS3* gene expression (F=21.98; p<0.001) and increase of sVCAM-1 (F=5.67; p=0.006).

The markers of the endothelial function and carotid IMT depending on the polymorphic variants of *GNB3* (rs5443) gene (Table 3) showed lower total NO metabolites blood level in hypertensive patients with *TT*-genotype than in *C*-allele carriers – 10.90% (p_{CC}=0.046) and 16.02% (p_{TC}=0.014) with lower expression of *NOS3* gene after the mRNA – 34.29% (p_{CC}=0.035) and 47.73% (p_{CT}=0.007) and

Table 2
Biomarkers of endothelial function and carotid "intima-media" thickness depending on the polymorphic variants of *NOS3* gene (rs2070744)

Parameters	Group	Genotypes of *NOS3* gene		
		TT-	TC-	CC-
FMD BA (%)	Controls	10.95±0.34	10.31±0.29	9.66±0.23 (p_{TT}=0.004)
	Hypertensive patients	6.78±0.20 (p<0.001)	6.72±0.25 (p<0.001)	6.88±0.23 (p<0.001)
IMT CCA (mm)	Controls	0.59±0.04	0.60±0.03	0.58±0.02
	Hypertensive patients	0.90±0.05 (p<0.001)	1.03±0.07 (p<0.001; p_{TT}=0.041)	1.00±0.09 (p<0.001)
IMT ICA (mm)	Controls	0.54±0.02	0.56±0.03	0.53±0.02
	Hypertensive patients	0.87±0.06 (p<0.001)	0.96±0.05 (p<0.001; p_{TT}=0.044)	0.97±0.09 (p<0.001)
D CCA (mm)	Controls	6.89±0.25	7.21±0.17	6.73±0.16 (p_{TC}=0.045)
	Hypertensive patients	7.45±0.21 (p=0.017)	7.65±0.27	7.73±0.30 (p=0.004)
D ICC (mm)	Controls	5.92±0.21	6.14±0.17	5.75±0.14 (p_{TC}=0.051)
	Hypertensive patients	6.71±0.20 (p=0.002)	7.04±0.30 (p=0.004)	7.20±0.35 (p<0.001)
Total NO metabolites (NO_2^-/NO_3^-) (μmol/l)	Controls	30.90±0.69	24.70±0.35 (p_{TT}<0.001)	22.30±0.78 (p_{TT}<0.001; p_{TC}=0.007)
	Hypertensive patients	22.48±0.55 (p<0.001)	18.78±0.33 (p<0.001; p_{TT}<0.001)	13.29±0.44 (p<0.001; $p_{TT,TC}$<0.001)
sVCAM-1 (ng/ml)	Controls	819.18±39.35	798.08±60.28	1110.1±90.04 (p_{TT}=0.002; p_{TC}=0.003)
	Hypertensive patients	1047.50±72.07 (p<0.001)	1419.20±137.65 (p<0.001; p_{TT}<0.001)	1964.8±111.7 (p<0.001; $p_{TT,TC}$<0.001)
Transcriptional activity of *NOS3* gene after mRNA value (U)	Controls	2.08±0.08	1.50±0.10 (p_{TT}<0.001)	1.12±0.07 ($p_{TT,TC}$<0.001)
	Hypertensive patients	0.63±0.07 (p<0.001)	0.34±0.07 (p<0.001; p_{TT}<0.001)	0.09±0.00 (p<0.001; $p_{TT,TC}$<0.001)

Abbreviations: FMD BA – flow-mediated dilation of the brachial artery; IMT CCA, ICA – intima-media thickness of common carotid artery, internal carotid artery; D – mean diameter of CCA and ICA in systole and diastole; NO_2^-/NO_3^- – total nitrite/total nitrate; sVCAM-1 (CD 106) – soluble vascular cell adhesion molecule. Data are presented as mean ± S.D. p – significance of differences with control group; p_{TT}, p_{TC} – significance of differences with TT-genotype and TC-genotype carriers in corresponding group.

higher sVCAM-1 content – 18.76% (p_{CC}=0.035) and 44.67% (p_{CT}<0.001), respectively. In the contrary, in the control group among the *TT*-genotype carriers of *GNB3* (rs5443) gene 22.24% (p_{CC}=0.007) higher than in *CC*-genotype subjects' level of the total NO metabolites was found. At the same time, ANOVA analysis of variance did not confirm association of *GNB3* (rs5443) gene with the endothelial function markers and carotid IMT in hypertensive patients.

To find out the dependence of the analyzed data on the carotid IMT, the hypertensive patients (n=100) were divided into those with carotid IMT <0.9 mm (n=51) and >0.9 mm (n=49) (Table 4). SBP and DBP values were higher in patients with IMT CCA >0.9 mm than those with IMT CCA <0.9 mm – 8.54% and 5.85% (p_I<0.001), respectively. The rest of the parameters was not associated with the carotid IMT.

Dependence of lipids metabolism and glucose level changes in EAH patients on the IMT CCA value was not found (Table 5). The parameters of the endothelial function depend on the IMT CCA value (Table 6): in hypertensive patients with IMT CCA >0.9 mm the FMD BA value was 11.80% (p_1<0.001) lower, wider CCA and ICA diameters – 17.38% and 21.99% (p_1<0.001) and higher sVCAM-1 blood level – 20.49% (p_1=0.005), which indicates of a probable subclinical chronic vascular inflammation.

Discussion

The endothelial cells of vessels in physiological conditions under the compression factors resulted in a compensatory vasodilation (FMD) maintaining the essential NO synthesis level and did not express adhesion molecules (Versari et al. 2009; Carrizzo et al. 2020; Puddu et al. 2000). This was confirmed by our results of the NO and sVCAM-1 values in the control group. In the case of the essential hypertension, the endothelial dysfunction led to a decreased nitric oxide bioavailability, impairing the endothelium-dependent vasodilatation, which might precede a vascular dysfunction, as it was proved by our research. Moreover, ED might also be a predictor of the premature atherosclerosis development.

Regardless of the fact that chronic stress, long excessive RAAS activation, ROS formation, inflammatory mediators release (IL-1β and IL-6, lipopolysaccharides, TNF-α, IFN-γ, etc.) intensified the sVCAM-1 expression with following leukocytes

Table 3
Biomarkers of endothelium function and carotid "intima-media" thickness depending on the polymorphic variants of *GNB3* gene (rs5443)

Parameters	Group	Genotypes of *GNB3* gene		
		CC-	CT-	TT-
FMD BA (%)	Controls	10.03±0.32	10.13±0.26	9.61±0.28
	Hypertensive patients	6.67±0.24 (p<0.001)	6.91±0.33 (p<0.001)	6.64±0.14 (p<0.001)
IMT CCA (mm)	Controls	0.58±0.03	0.59±0.02	0.60±0.02
	Hypertensive patients	0.99±0.08 (p<0.001)	0.94±0.09 (p<0.001)	0.98±0.07 (p<0.001)
IMT ICA (mm)	Controls	0.55±0.025	0.55±0.02	0.53±0.03
	Hypertensive patients	0.96±0.08 (p<0.001)	0.91±0.06 (p<0.001)	0.94±0.07 (p<0.001)
D CCA (mm)	Controls	7.03±0.23	7.03±0.19	6.68±0.21
	Hypertensive patients	7.62±0.25 (p=0.014)	7.61±0.28 (p=0.019)	7.49±0.22 (p=0.028)
D ICC (mm)	Controls	5.96±0.20	6.04±0.16	5.72±0.15
	Hypertensive patients	7.01±0.26 (p<0.001)	6.93±0.31 (p=0.004)	6.99±0.25 (p=0.004)
Total NO metabolites (NO_2^-/NO_3^-) (μmol/l)	Controls	25.36±0.99	27.58±1.42	31.0±0.53 (pCC=0.007)
	Hypertensive patients	18.71±0.83 (p<0.001)	19.85±0.96 (p<0.001)	16.67±0.92 (p<0.001) (p_{CC}=0.046, p_{TC}=0.014)
sVCAM-1 (ng/ml)	Controls	929.1±71.51	889.9±76.50	910.8±59.84
	Hypertensive patients	1489.6±130.9 (p<0.001)	1222.8±111.8 (p=0.002) (pCC=0.008)	1769.0±89.92 (p<0.001) (pCC=0.05; p_{CT}<0.001)
Transcriptional activity of *NOS3* gene after mRNA value (U)	Controls	1.56±0.12	1.75±0.14	1.80±0.11
	Hypertensive patients	0.35±0.06 (p<0.001)	0.44±0.07 (p<0.001)	0.23±0.04 (p<0.001) (pCC=0.035; pCT=0.007)

Abbreviations: FMD BA – flow-mediated dilation of the brachial artery; IMT CCA, ICA – intima-media thickness of common carotid artery, internal carotid artery; D – mean diameter of CCA and ICA in systole and diastole; NO2-/NO3- – total nitrite/total nitrate; sVCAM-1 (CD 106) – soluble vascular cell adhesion molecule. Data are presented as mean ± S.D. p – significance of differences with control group; p_{CC}, p_{CT} – significance of differences with CC-genotype and CT-genotype carriers in corresponding group.

Table 4
Clinical and anthropometric value depending on the carotid "intima-media" thickness

Parameters	Control (n=48)	Carotid IMT <0.9 mm (n=51)	Carotid IMT >0.9 mm (n=49)
SBP (mmHg)	117.0±2.32	148.82±3.95 (p<0.001)	161.53±4.33 (p, p_1<0.001)
DBP (mmHg)	76.05±2.79	92.16±2.13 (p<0.001)	97.55±1.82 (p, p_1<0.001)
BMI (kg/m^2)	24.82±1.25	31.44±1.54 (p<0.001)	31.13±1.75 (p<0.001)
WHR (U)	0.83±0.03	0.91±0.03 (p=0.004)	0.91±0.02 (p<0.001)
Creatinine blood value (μmol/l)	71.60±3.22	76.43±4.17	72.98±3.12
eGFR (CKD-EPI after creatinine value) (ml/min/1.73 m^2)	105.73±9.68	80.74±5.78 (p=0.002)	82.83±4.53 (p=0.008)

Abbreviations: IMT – "intima-media" thickness; SBP, DBP – systolic, diastolic blood pressure; BMI – body mass index; WHR – waist-to-hip ratio; eGFR – estimated glomerular filtration rate; CKD-EPI - Chronic Kidney Disease Epidemiology Collaboration. Data are presented as mean ± S.D. p – significance of differences with control group; p_1 – significance of differences with patients' group with carotid IMT <0.9 mm.

Table 5
Lipids' panel and glucose value depending on the carotid "intima-media" thickness

Parameters	Control (n=48)	Carotid IMT <0.9 mm (n=51)	Carotid IMT >0.9 mm (n=49)
Glucose (mmol/l)	5.08±0.17	7.50±0.99 (p<0.001)	7.36±0.98 (p<0.001)
TC (mmol/l)	5.57±0.22	5.44±0.34	5.85±0.36
TG (mmol/l)	1.64±0.16	2.20±0.23 (p<0.001)	1.73±0.29 (p=0.006)
LDL-C (mmol/l)	3.90±0.24	4.08±0.35	4.27±0.30
HDL-C (mmol/l)	1.40±0.09	1.22±0.08 (p=0.002)	1.31±0.08
AI (U)	2.99±0.34	3.64±0.35 (p=0.053)	3.59±0.30 (p=0.055)

Abbreviations: IMT – "intima-media" thickness; TC – total cholesterol; TG – triglycerides; LDL-C, HDL-C – low/high density level cholesterol; AI – Atherogenicity Index.
Data are presented as mean ± S.D. p – significance of differences with control group.

(except neutrophils) and NK-cells adhesion on the vascular endothelium surface, due to the interaction with very late leukocyte antigen (VLA-4), the regulatory mechanisms of keeping balance between eNO and endothelium-derived contracting factors release remain to be determined. Furthermore, sVCAM-1 participated in the leukocyte adhesion and their precursors outside of the vessels to the stromal cells of the bone marrow, B-cells, dendrite cells of the lymph node follicles causing inflammation. In such condition, the endothelium is transformed from a protective organ to a source of pro-inflammatory and pro-mitogenic mediators, pro-aggregatory molecules, and vasoconstrictors (Versari et al. 2009; Carrizzo et al. 2020). Therefore, the markers of the endothelial dysfunction such as total NO metabolites and sVCAM-1, FMD BA, *NOS3* gene expression and carotid IMT might have an important diagnostic and prognostic value in the predicting of severe vascular dysfunctions. Thus, early detection of ED, depending on the genetic background as we proved in our study, whereas in *C*-allele carriers of *NOS3* gene (rs2070744), especially in *CC*-genotype, significantly worse function impairment was found than in other hypertensive patient's endothelium, which might help to provide the appropriate therapeutic genotype-determined strategies to improve the endothelial function and finally decrease the mortality and the morbidity in the hypertensive patients.

Conclusion

Severe structural carotid IMT changes in EAH patients (IMT>0.9 mm) are followed by higher BP values (p<0.001) and deterioration of the endothelial function: FMD BA decrease by 11.80% with compensatory increase of carotid arteries diameters by 17.38% and 21.99% (p<0.001) and serum sVCAM-1 concentration by 20.49% (p=0.005) may indicate for a possible subclinical chronic vascular inflammation.

Table 6
Markers of endothelium function depending on the carotid "intima-media" thickness

Parameters	Control (n=48)	Carotid IMT <0.9 mm (n=51)	Carotid IMT >0.9 mm (n=49)
FMD BA (%)	11.14±0.74	7.29±0.22 (p<0.001)	6.43±0.19 (p, p_1<0.001)
D CCA (mm)	6.61±0.24	6.96±0.13	8.17±0.23 (p, p_1<0.001)
D ICA (mm)	5.54±0.19	6.23±0.22 (p=0.022)	7.60±0.29 (p, p_1<0.001)
Total NO metabolites (NO_2^-/NO_3^-) (μmol/l)	27.88±2.25	19.25±0.93 (p<0.001)	18.81±0.91 (p<0.001)
sVCAM-1 (ng/ml)	909.13±75.54	1281.4±142.19 (p<0.001)	1544.0±130.26 (p<0.001; p_1=0.005)
Transcriptional activity of *NOS3* gene after mRNA value (U)	1.68±0.23	0.39±0.07 (p<0.001)	0.36±0.06 (p<0.001)

Abbreviations: FMD BA – flow-mediated dilation of the brachial artery; D – mean diameter of CCA and ICA in systole and diastole; NO_2^-/NO_3^- – total nitrite/total nitrate; sVCAM-1 (CD 106) – soluble vascular cell adhesion molecule. Data are presented as mean ± S.D. p – significance of differences with control group; p_1 – significance of differences with patients' group with carotid IMT<0.9 mm.

It was ascertained the dependence of polymorphic site of *NOS3* (rs2070744) gene in hypertensive patients on decreased content of the total NO metabolites (F=71.11; p<0.001), reduced transcription activity of *NOS3* gene (F=8.71; p<0.001), increased sVCAM-1 (F=6.96; p=0.002), especially in the *C*-allele carriers, particularly in *CC*-genotype patients with a lower serum level of NO metabolites by 16.46% and 40.88% (p<0.001), lower transcription activity of *NOS3* gene by the level of mRNA by 46.03% and 7 times (p<0.001), and a higher sVCAM-1 content by 35.48% and 89.48% (p<0.001), respectively.

The association of *GNB3* (rs5443) gene with the endothelial function parameters and carotid IMT in hypertensive patients was not confirmed.

Conflict of interest: *The authors declare no conflict of interest.*

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