sciendo

138 ENDOCRINE REGULATIONS, Vol. 58, No. 2, 138–143, 2024

doi:10.2478/enr-2024-0015

Statistically verified methods for determining predictors of development of arterial hypertension depending on endothelial nitric oxide synthase T786C gene promoter polymorphism using lipid profile indicators

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Objective. Polymorphism investigation of T786C gene promoter of endothelial nitric oxide synthase (eNOS/NOS3) in the arterial hypertension is a promising field for determining the relationship between heredity, hypertension, and dyslipidemia, which still remains controversial. The purpose of the study was to investigate the lipid profile, which depends on the NOS3 T786C gene promotor region polymorphism in patients with arterial hypertension.

Methods. The study involved 86 patients with arterial hypertension. The control group consisted of 30 basically healthy individuals. The lipid profile in the blood serum of the studied patients was measured by commercially available kits using Biochem FC-200 analyzer (HTI, USA). The allelic polymorphism of NOS3 T786C gene promoter was studied using a polymerase chain reaction technique with electrophoretic detection of the results.

Results. An increase at the level of all atherogenic fractions in the blood was found in the group of patients carrying the CC genotype compared with carriers of the TT genotype of the NOS3 gene. The total cholesterol serum level in the group of carriers of the CC genotype of NOS3 T786C gene promoter increased by 33.3% compared with carriers of the TT genotype and it was almost twice as high as the control values. In the group of carriers in the CC genotype of the NOS3 gene, the serum level of triglycerides was statistically significantly higher (2.9 times) than in the group of carriers of the TT genotype. The low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) serum levels significantly increased in patients with arterial hypertension with the CC genotype by 1.6 and 4.6 times, respectively, compared with the TT genotype carriers. The high-density lipoprotein (HDL) serum level, as an antiatherogenic factor, was statistically significantly lower (by 45.8%) in the group of the CC genotype carriers of the NOS3 gene than in the group with carriers of the TT genotype (0.58±0.06 vs. 1.07±0.03 mmol/l.)

Conclusions. The increase in all atherogenic and decrease in antiatherogenic lipid parameters of the lipidogram of patients with arterial hypertension and the deepening of dyslipidemia in carriers of the CC genotype compared with carriers of the TT genotype of the NOS3 T786C gene promoter is crucial in the development of dyslipidemia.

Key words: arterial hypertension, endothelial nitric oxide synthase T786C promotor region polymorphism, lipidogram

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Arterial hypertension is a multifactorial disease, the development of which is caused by a complex interaction of internal genetic, metabolic, and external factors (environment). In recent years, genetic factors have been attracting more and more attention from scientists, but they have not been thoroughly studied yet. Recent studies have shown that genetic factors play a significant role in the development and course of the arterial hypertension (Friso et al. 2015).

The study of endothelial nitric oxide synthase (eNOS/NOS3) T786C promotor region polymorphism in hypertension is a promising area for determining the relationship between heredity, hypertension, and dyslipidemia and it still remains controversial. Therefore, it is advisable to investigate the lipid profile depending on NOS3 T786C promotor region polymorphism in patients with hypertension (Salvi et al. 2017).

The aim of the present study was to investigate the lipid profile depending on the NOS3 T786C gene promotor region polymorphism in patients with arterial hypertension.

Material and Methods

Study population. We examined 86 patients with arterial hypertension aged from 45 to 76 years, of which 47 (55%) were women and 39 (45%) were men. The mean age was 61.35±13.30 years. Thirty people without signs of hypertension formed the control group. The inclusion criterion for the study was the presence of arterial hypertension of 1–3 degrees. The diagnosis of arterial hypertension was established in accordance with the orders of the Ministry of Health of Ukraine No.54 and 436 and the Recommendations of the Ukrainian Association of Cardiologists on the prevention and treatment of arterial hypertension, which was based on anamnesis, complaints, physical, and clinical examinations.

The study did not include patients with a history of myocardial infarction and stroke, secondary arterial hypertension, congenital or acquired heart disease, rhythm and conduction disorders, NYHA III–IV functional class heart failure, chronic obstructive pulmonary disease, diabetes mellitus, chronic kidney disease, cancer, and mental illness.

All patients underwent the following examinations: measurement of body weight and height, office blood pressure, electrocardiography (ECG), lipid profile, and NOS3 T786C promotor region polymorphism.

Laboratory data. The serum levels of total cholesterol, triglycerides, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) were determined by commercially available kits using Biochem FC-200 analyzer (HTI, USA).

Genotyping of NOS3 T786C gene promotor region polymorphism. The allelic polymorphism of NOS3 T786C promotor region was studied by polymerase chain reaction with electrophoretic detection of the results using SNP-EXPRESS reagent kits (Litex Ltd).

Ethical details. During the research, we followed the principles of bioethics set out in the Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", "the Universal Declaration on Bioethics and Human Rights (UNESCO)" and the Order of the Ministry of Health of Ukraine "On Approval of the Procedure for Conducting Clinical Trials of Medicines and Examination of Clinical Trial Materials and the Model Regulations on Ethics Commissions" No.69 of 23.09.2009. Written informed consent for the study was obtained from all patients (Conclusion of the Biomedical Ethics Committee of the Ternopil I.Y. Gorbachevsky National Medical University No.69 of 12.04.2022).

Statistical analysis. Statistical processing of the study materials was performed using biostatistical analysis methods implemented in the licensed software packages Microsoft Office 2010 Professional Plus (Microsoft Access 2010, Microsoft Excel 2010) - registration number 49521210; STATISTICA 6.1 software product (StatSoft Inc., serial number AGAR909E415822FA). Quantitative variables following a normal distribution were described using mean (M) ± standard deviation (SD), 95% confidence interval (95% CI) for the mean were estimated. Quantitative variables following a non-normal distribution were described using median (Me) and lower and upper quartiles (Q1–Q3). Comparisons of three or more groups on a quantitative variable whose distribution differed from normal were made using the Kruskal-Wallis test and Dunn's criterion with Holm correction as a post-hoc method. Mann-Whitney U-test was used to compare two groups on a quantitative variable whose distribution differed from the normal distribution.

Results

The state of lipid metabolism in patients with arterial hypertension depended on NOS3 T786C promotor region polymorphism.

Analyzing the lipid status parameters, we found the presence of pathological changes in lipid

metabolism in all patients with arterial hypertension, but in patients with CC genotype compared with TT genotype carriers of NOS3 T786C promotor region polymorphism, lipid homeostasis disorders were more profound, as evidenced by a statistically significant increase in the serum levels of total cholesterol, triglycerides, LDL, and VLDL.

When analyzing the lipid profile of patients with arterial hypertension, higher total cholesterol serum levels were noted in all groups compared with controls (Figure 1). According to the results of the comparative analysis of total cholesterol, a statistically significant difference was observed when comparing the control group [Me (IQR): 4.1 (4.0; 4.2)] with the groups of patients with hypertension who are carriers of NOS3 gene polymorphism, namely the TT genotype [Me (IQR): 5.0 (4.9; 5.0)], TC genotype [Me (IQR): 7.0 (7.0; 7.1)] and CC genotype [Me (IQR): 7.5 (7.5; 7.6)] (Figure 1).

An increase in the level of all atherogenic blood fractions was found in the group of patients carrying the CC genotype compared with carriers of the TT genotype of NOS3 gene. Thus, the level of total cholesterol in the group of carriers of the CC genotype of NOS3 T786C promotor region polymorphism increased by 33.3% compared with carriers of the TT genotype, and was almost twice as high as the control values.

According to the results of the comparative analysis, a statistically significant difference in triglycerides serum levels was observed when comparing the control group [Me (IQR): 1.0 (0.9; 1.0)] with the groups of patients with hypertension who are carriers of NOS3 gene polymorphism, namely the TT genotype [Me (IQR): 1.1 (1.1; 1.1)], TC genotype [Me (IQR): 2.5 (2.5; 2.6)], and CC genotype [Me (IQR): 3.2 (3.2; 3.3)] (Figure 2). In the group of carriers of the CC genotype of NOS3 gene, the triglycerides serum levels were statistically significantly higher than in the group of carriers of the TT genotype, namely 2.9 times.

LDL and VLDL serum levels in hypertensive patients with the CC genotype significantly increased by 1.6 and 4.6 times, respectively, compared with carriers of the TT genotype (Figure 3 and 4).

According to the results of the comparative analysis, a statistically significant difference in LDL was observed when comparing the control group [Me (IQR): 2.7 (2.6; 2.7)] with the groups of patients with hypertension who are carriers of NOS3 gene polymorphism, namely the TT genotype [Me (IQR): 3.5 (3.4; 3.6)], TC genotype [Me (IQR): 4.8 (4.7; 4.8)], and CC genotype [Me (IQR): 5.9 (5.5; 6.1)] (Figure 3).

According to the results of the comparative analysis, a statistically significant difference in VLDL was observed when comparing the control group [Me (IQR): 0.2 (0.2; 0.3)] with the groups of patients with hypertension who are carriers of NOS3 gene polymorphism, namely the TT genotype [Me (IQR): 0.4 (0.3; 0.5)], TC genotype [Me (IQR): 0.7 (0.7; 0.8)], and CC genotype [Me (IQR) 1.9 (1.8; 2.0)] (Figure 4).

According to the results of the comparative analysis, a statistically significant difference in HDL was observed when comparing the control group [Me

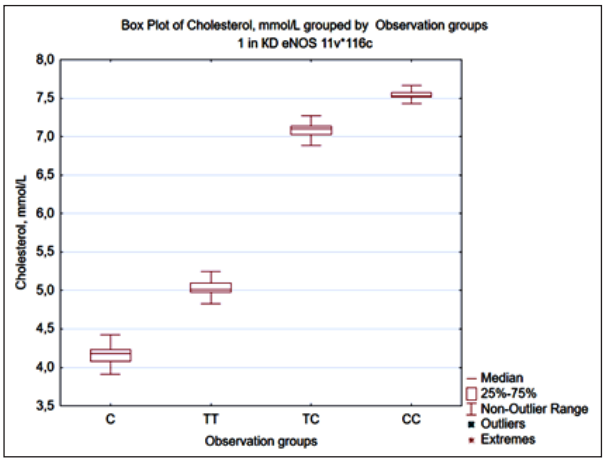

Figure 1. Changes in the total cholesterol serum level in patients with arterial hypertension depending on the polymorphism of endothelial nitric oxide synthase (NOS3) T786C promotor region of the TT, TC, and CC receptor types.

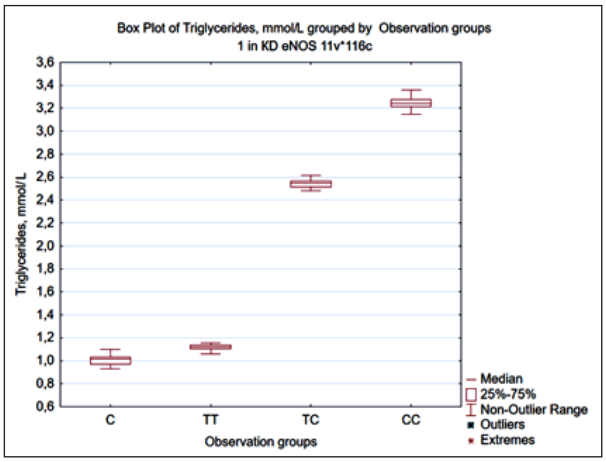

Figure 2. Changes in the triglyceride (TG) serum level in patients with hypertension depending on the polymorphism of endothelial nitric oxide synthase (NOS3) T786C promotor region of the TT, TC, and CC receptor types.

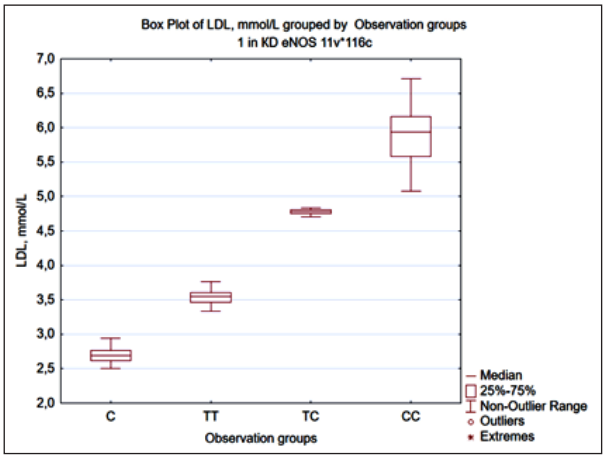

Figure 3. Changes in the low-density lipoprotein (LDL) serum level in patients with arterial hypertension depending on the polymorphism of endothelial nitric oxide synthase (NOS3) T786C promotor region of the TT, TC, and CC receptor types.

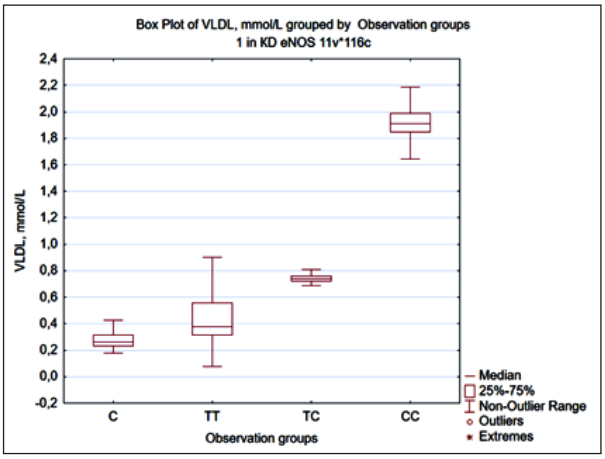

Figure 4. Changes in the serum level of very low-density lipoprotein (VLDL) in patients with hypertension depending on the polymorphism of endothelial nitric oxide synthase (NOS3) T786C promotor region of the TT, TC, and CC receptor types.

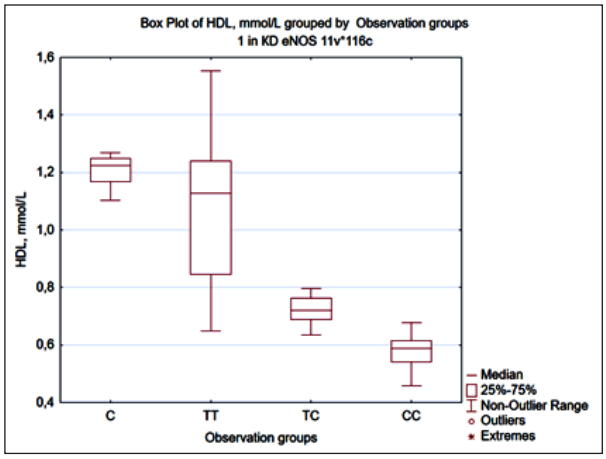

Figure 5. Changes in the serum level of high-density lipoprotein (HDL) in patients with hypertension depending on the polymorphism of endothelial nitric oxide synthase (NOS3) T786C promotor region of the TT, TC, and CC receptor types.

(IQR): 1.2 (1.1; 1.2)] with the groups of patients with hypertension who are carriers of NOS3 gene polymorphism, namely the TT genotype [Me (IQR): 1.1 (0.8; 1.2)], TC genotype [Me (IQR): 0.7 (0.7; 0.8)], and CC genotype [Me (IQR): 0.6 (0.5; 0.6)] (Figure 5).

Regarding the level of HDL as an antiatherogenic factor, in the group of carriers of the CC genotype of NOS3 gene, this indicator was statistically significantly lower by 45.8% than in the group with carriers of the TT genotype and was 0.58±0.06 vs. 1.07±0.03 mmol/l, respectively.

Discussion

Cardiovascular diseases, including arterial hypertension, are accompanied by changes in the blood lipid composition. In patients with arterial hypertension, compared with healthy people, the content of HDL-C in blood plasma is reduced, while the content of LDL-C and their oxidized forms, and triglycerides are increased (Prodan et al. 2023). A shift in the lipid profile to the atherogenic side is a risk factor for cardiovascular disease (stroke, myocardial infarction). LDL-C and their oxidized forms activate not only immune cells, but also endothelial cells, which begin to produce a variety of cytokines and chemokines that attach (attract) monocytes and neutrophils to the site of lipoprotein deposition (Virdis et al. 2014).

One of the predictors of arterial hypertension is endothelial dysfunction, which leads to an increase in the peripheral vascular tone and is manifested in an imbalance in the production of vasoconstrictors and vasodilators, primarily endothelin and nitric oxide (NO) (Yaremchuk et al. 2020). eNOS is responsible for the synthesis of NO in the endothelial cells. There are 11 polymorphisms in the gene encoding eNOS synthesis, one of the most well-studied is the polymorphism in the 786T>C promoter region. High levels of NO as a source of reactive nitrogen species contribute to protein and DNA damage and inflammation of the vascular walls. The increase in plasma levels of LDL-C, VLDL-C, and NO, found in our study, confirms the rare data presented in the literature indicating that the NO levels and changes in lipid profile are interrelated (Hammad et al.

2012). NO can directly or indirectly affect the lipid spectrum. For example, exogenous NO has been shown to activate lipase in some bacteria and yeast (Taskin et at. 2016).

Lipoprotein lipase (LPL) plays a significant role in the lipid metabolism. LPL breaks down triglycerides of the largest and most lipid-rich plasma lipoproteins - chylomicrons and VLDLs. A decrease in its activity can lead to dyslipidemia. In the culture of adipocytes that produce large amounts of tumor necrosis factor (TNFα), an increase in NO levels, and inhibition of LPL activity in cells has been observed (Van de Voorde et al. 2013). The suppressive effect of the increased NO synthesis on the activity of this enzyme was attenuated by the addition of NOS inhibitors to the culture medium. Since the cGMP inhibitor also reduced the activity of LPL, the authors concluded that elevated NO levels affect the activity of this enzyme by regulating cGMP production. Arterial hypertension is accompanied by an inflammatory process, in which an increase in the level of pro-inflammatory cytokines in blood plasma is observed. Pro-inflammatory cytokines activate inducible nitric oxide synthase (NOS2), which leads to NO production increase (Forstermann and Sessa 2012). As a result, in hypertension, elevated levels of NO are likely to inhibit LPL activity. However, not only the high level of NO produced by NOS2 activity, but also its reduced content due to the inhibition of NOS3 function in hypertensive rats contribute to a decrease in LDL activity and an increase in the level of triglycerides, LDL-C, free fatty acids, and phospholipids.

Another mechanism, by which NO can affect the level of various lipid fractions is through the regulation of the transcriptional activity of genes encoding pro-inflammatory proteins (Dziubanovskyi et al. 2021). As mentioned above, under conditions of inflammation, NO can activate the transcription factors NF-κb, AP-1, which regulate the synthesis of pro-inflammatory proteins and NOS2 (Shin et al. 2020). Pro-inflammatory cytokines, such as interleukin 6 and TNFα, in turn, can modulate the activity of a number of lipid metabolism-related enzymes and affect the synthesis and metabolism of triacylglycerols, fatty acids, and cholesterol. The increased levels of TNFα in the blood increase the production of reactive oxygen species and reactive nitrogen species, which contribute to lipid hyper oxidation (Liu et al. 2023). In addition, in conditions of inflammation, there is an increase in the surface of endothelial cells of adhesion molecules and endothelial permeability to leukocytes and LDL-C (Mundi et al. 2018). All of this contributes to damage and inflammation of the vessel walls, and increased blood pressure. It is important that patients with the CC genotype, compared with TT and TC genotypes, have a lower content of the antiatherogenic lipid fraction (HDL-C). Thus, the carriage of allelic variations of the CC genotype may affect the ratio of atherogenic and antiatherogenic lipid fractions, which is likely to be one of the mechanisms of involvement of this polymorphic marker in the development of arterial hypertension.

Conclusion. The increase in all atherogenic and decrease in antiatherogenic lipid parameters of the lipidogram of patients with arterial hypertension and the deepening of dyslipidemia in carriers of the CC genotype compared with carriers of the TT genotype of NOS3 T786C promotor region polymorphism are determinative in the development of dyslipidemia.

Acknowledgements

The authors would like to thank all our participants and technical staff of hospitals for their cooperation.

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

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