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EFFECT OF POLYMORPHISM T786C OF NOS3 GENE ON LEVELS OF NITRIC OXIDE METABOLITES AMONG PATIENTS WITH PAROXYSMAL ATRIAL FIBRILLATION ON THE BACKGROUND OF CORONARY HEART DISEASE COMBINED WITH HYPERTENSION

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ЭФФЕКТ ПОЛИМОРФИЗМА T786C ГЕНА NOS3 НА УРОВЕНЬ МЕТАБОЛИТОВ ОКСИДА АЗОТА У ПАЦИЕНТОВ С ПАРОКСИЗМАЛЬНОЙ ФИБРИЛЛЯЦИЕЙ ПРЕДСЕРДИЙ НА ФОНЕ ИШЕМИЧЕСКОЙ БОЛЕЗНИ СЕРДЦА И ГИПЕРТОНИЧЕСКОЙ БОЛЕЗНИ

Summary. The aim of the study was to detect effect of polymorphism T786C of NOS3 gene on levels of nitric oxide metabolites among patients with paroxysmal atrial fibrillation, **coronary heart disease combined with hypertension.**

Material and methods. To achieve this goal, a prospective study was conducted on the basis of the communal uncommercial company "City Hospital № 10" of Zaporizhzhia City Council. The sample of patients was conducted in the period from 2014 to 2019. The results of the study are based on data from a comprehensive examination and dynamic monitoring of 176 patients with paroxysmal AF on the background of coronary heart disease **combined with** hypertension, of which 98 were from the city of Zaporozhzhia and 78 were from rural areas. Almost healthy 31 volunteers were examined on an outpatient basis.

Results

1. Decreased nitric oxide metabolites occur in patients with paroxysmal atrial fibrillation on the background of coronary heart disease combined with hypertension compared with the healthy group.

2. The level of NO3 in the group of patients from the city was significantly lower than in the group of patients from the rural areas.

3. The level of NO2 was lower among patients with the C7 allele of the T786C polymorphism of the NOS3 gene, whereas the value of NO3 did not depend on this polymorphism.

Аннотация. Целью исследования: выявить влияние полиморфизма T786C гена NOS3 на уровень метаболитов оксида азота у пациентов с пароксизмальной фибрилляцией предсердий на фоне ишемической болезни сердца и артериальной гипертензии.

Материалы и методы. Для достижения этой цели было проведено проспективное исследование на базе коммунального некоммерческого предприятия «Городская больница № 10» Запорожского городского совета. Выборка пациентов проводилась в период с 2014 по 2019 год. Результаты исследования основаны на данных комплексного обследования и динамического наблюдения 176 пациентов с пароксизмальной ФП на фоне ишемической болезни сердца в сочетании с артериальной гипертензией, из которых 98 из города Запорожья, 78 из сельской местности. В амбулаторных условиях было обследовано 31 практически здоровых добровольца.

Полученные результаты

1. У пациентов с пароксизмальной фибрилляцией предсердий на фоне ишемической болезни сердца в сочетании с артериальной гипертензией наблюдается снижение метаболитов оксида азота по сравнению со здоровой группой.

2. Уровень NO3 в группе пациентов из города был значительно ниже, чем в группе пациентов из сельской местности.

3. Уровень NO2 был ниже у пациентов с аллелем С7 полиморфизма T786C гена NOS3, тогда как значение NO3 не зависело от этого полиморфизма.

Key words: atrial fibrillation, nitric oxide, NOS3 gene, T786c polymorphism, coronary heart disease, hypertension

Ключевые слова: фибрилляция предсердий, оксид азота, ген NOS3, полиморфизм T786C, ишемическая болезнь сердца, гипертоническая болезнь.

Introduction. Atrial fibrillation (AF) is one of the most important medical and social problems of modern society, which is a common cause of ischemic stroke and leads to disability. The incidence of embolic complications is about 2.1% per year among the patients with paroxysmal AF, and is currently considered as a potentially dangerous arrhythmia with a significant increase in the incidence of serious complications [1].

Atrial fibrillation is a multifactorial disease. In its development such factors are leading, as old age, arterial hypertension, environmental factors, as well as genetic predisposition. The risk of development increases in those who have a history of at least one parent with this arrhythmia [2].

The assessment of endothelial dysfunction is one of the new and most perspective areas in the study of the pathogenesis of AF, which occurs in comorbid pathology. Recent studies have convincingly demonstrated the independent role of the endothelium in the development of cardiovascular disease. To date, the determination of the concentration of nitric oxide metabolites in blood plasma is one of the markers of endothelial dysfunction [3].

The development of AF against the background of multifactorial diseases such as coronary **heart** disease (CHD) and hypertension can be promoted by gene polymorphisms. The genetic predisposition of AF has a strong inherited component that is independent of concomitant cardiovascular disease. Up to a third of patients with this arrhythmia have common genetic variants that predispose to AF, although with a relatively low additional risk [4, 5].

The expression level of NO synthase is associated with various diseases, such as hypertension, coronary heart disease, atherosclerosis. At the same time, the NOS3 gene has allelic polymorphisms that are associated with different nitric oxide production activity. Changes in the amino acid sequence of the enzyme eNOS can lead to a decrease in its catalytic activity and, as a result, low NO production in those situations where it is locally necessary to participate in the implementation of protective or regulatory mechanisms [6, 7].

Research interest in eNOS polymorphisms in AF has increased in recent years, but the results of some studies are conflicting due to their small sample size. According to the results of a meta-analysis of Y.Q. Zhang et al. eNOS C786T gene locus polymorphism is related to the risk of AF. The results have shown that 786T / C polymorphism significantly reduces the risk of AF in white people in the control population, but the authors conclude that further studies are needed for further evaluation. [8].

Air pollution is an acute cause of AF, which is likely to contribute to the adverse cardiac effects associated with pollution observed in epidemiological studies. An important aspect is to discover the interaction of genes with the environment for better understanding the various influencing factors. Determining the interaction of genes and the environment is useful for understanding cardiovascular disease [9, 10].

Studies of endothelial function among patients with paroxysmal atrial fibrillation on the background of coronary heart disease combined with hypertension have a great practical interest for cardiologists. The relationship between genetic and environmental factors will determine the different phenotypes of the disease. Therefore, in connection with the above, there is an interest to determine the levels of plasma nitric oxide metabolites in different populations of patients with paroxysmal AF and to compare those with the C786T polymorphism of the eNOS gene, which has determined the purpose of this work.

The aim of the study was to detect effect of polymorphism T786C of NOS3 gene on levels of nitric oxide metabolites among patients with paroxysmal atrial fibrillation, **coronary heart disease combined with hypertension.**

Material and methods. To achieve this goal, a prospective study was conducted on the basis of the communal uncommercial company “City Hospital № 10” of Zaporizhzhia City Council. The sample of patients was conducted in the period from 2014 to 2019. The results of the study are based on data from a comprehensive examination and dynamic monitoring of 176 patients with paroxysmal AF on the background of coronary heart disease **combined with** hypertension, of which 98 were from the city of Zaporozhzhia and 78 were from rural areas. Almost healthy 31 volunteers were examined on an outpatient basis.

Criteria for inclusion in the study: male and female patients aged 45 to 70 years; recurrence of paroxysmal atrial fibrillation; verified stable coronary heart disease combined with stage II hypertension with known disease duration of more than 1 year; the patient's consent to participate in the study.

Criteria for exclusion from the study: atrioventricular block II-III degree; ventricular arrhythmias; circulatory failure more than II class of NYHA; oncological diseases; thyroid dysfunction; diabetes; hemodynamically significant heart defects; drug addiction, alcohol dependence, the presence of mental disorders; refusal of the patient from further observation.

Screening and division of patients into groups. Verification of the diagnosis of paroxysmal atrial fibrillation was performed in accordance with the AF treatment guidelines of the European Society of Cardiology 2016 [11]. The presence of AF was determined by recording the ECG changes in the patient during the examination. The division of patients into groups was performed after establishing the compliance of patients with the inclusion / exclusion criteria of the study depending on the place of residence, and on subgroups depending on the combination of genotype polymorphism T786C.

- the first group included 98 patients with AF from the city (the median of age was 61.0 [45.0; 70.0] years);
- the second group included 78 patients with AF from rural areas (the median of age was 60.0 [46.0; 69.0] years);

- the third group included 31 almost healthy volunteers (the median of age was 58.0 [45.0; 66.0] years).

Determination of the level of nitrate and nitrite ions in blood plasma was performed by a method based on the reduction of nitrates to nitrites with a further determination of the latter by reaction with Gris reagent. the optical density was measured on a spectrophotometer SF-46 (Russia) at a wavelength of 540 nm. The calculation of the amount of nitrites was carried out according to the calibration graph based on nitrogen nitrite. The study obtained three results: the content of nitrite ions (NO2) (μmol / l), the content of nitrate ions (NO3) (μmol / l) and the total content of nitrite and nitrate ions (NO2 + NO3) (μmol / l).

Gene polymorphism was determined by polymerase chain reaction (PCR). Genomic DNA was isolated from peripheral blood leukocytes using a standard DNA-express blood test system (Litech, Russia) according to the manufacturer's instructions. Determination of SNP (Single Nucleotide Polymorphism) polymorphisms C786T of gene eNOS was performed by real-time PCR using the amplifier "Rotor-Gene 6000", Australia. The structure of primers from standard sets "SNP-express-RV" (Litech) was used.

Statistical analysis. The analysis of distribution on each studied indicators by means of Shapiro-Wilk criterion was carried out. The obtained data were presented as the median and interquartile range of Me [Q25; Q75]. When testing statistical hypotheses, the null hypothesis was rejected at a level of statistical significance (p) below 0.05. To compare it was used the analysis of variance (One-way ANOVA) followed by aposteriori tests (post-hoc analysis). Equality of variances was checked using the Levene's test. In the case of equality of variances in the studied groups, the Scheffe criterion was used. In the absence of equality of variances, the Tamhane's T2 test was used. In the case of distribution of data other than normal, when comparing independent variables used an analogue of analysis of variance - Krukal-Wallis method (H-test) followed by post-hoc analysis using Dunn's test (Dunn). For statistical data processing the statistical software package PSPP (version 1.2.0, GNU Project, 1998-2018, license GNU GPL) was used.

Results and Discussion. Levels of nitric oxide metabolites in the blood plasma of the subjects were determined. Table 1 presents the distribution depending on the groups of respondents.

Table 1.

Levels of nitric oxide metabolites in the blood plasma of subjects (Me [25 ; 75], n = 207)

Variable	Groups of surveyed persons		
	AF city (n = 98)	AF rural areas (n = 78)	Healthy individuals (n=31)
	1	2	3
NO_2, μmol / l	7,02 [5,74 ; 8,14]	6,77 [5,47 ; 7,98]	8,46 [7,45 ; 9,45]
P-value	$p_{1-2} = 1,0$	$p_{2-3} < 0,001$	$p_{1-3} < 0,001$
NO_3, μmol / l	10,36 [8,14 ; 13,32]	11,69 [10,36 ; 15,33]	13,36 [11,85 ; 15,35]
P-value	$p_{1-2} = 0,04$	$p_{2-3} = 0,08$	$p_{1-3} < 0,001$
NO_2+ NO_3, μmol / l	17,60 [14,80 ; 20,35]	18,02 [15,83 ; 21,78]	21,56 [20,38 ; 24,53]
P-value	$p_{1-2} = 0,22$	$p_{2-3} < 0,001$	$p_{1-3} < 0,001$

NO2 levels in groups of patients from both urban and rural areas were significantly lower against the value of 8.46 [7.45; 9.45] μmol / l group of healthy individuals, 7.02 [5.74; 8.14] μmol / L and 6.77 [5.47; 7.98] μmol / l, respectively (p <0.05). There was no significant difference in the significant indicator between groups of patients (p> 0.05).

The lowest reliable level of NO3 was in the group of patients from the city 10.36 [8,14; 13.32] μmol / l, as opposed to the value of 11.69 [10.36; 15.33] μmol / l in the group of patients from the rural areas and against the group of healthy people - 13.36 [11.85; 15.35] μmol / l, (p <0.05). However, there was no statistically significant difference between the group of patients from the rural areas and the group of healthy volunteers (p> 0.05).

The highest value of NO2 + NO3 was in the group of almost healthy individuals - 21.56 [20.38; 24.53] μmol / l, as against the level of 18.02 [15.83; 21.78] μmol / l in the group of patients from the rural areas, and - 17.60 [14.80; 20.35] μmol / l group of patients from the city (p <0,05). There was no significant difference between groups of patients in the amount of nitric oxide metabolites (p> 0.05).

Further, the levels of nitric oxide metabolites in the plasma of the subjects were distributed depending on the T786C polymorphism of the NOS3 gene. The results obtained are presented in table 2.

Table 2.

Levels of nitric oxide metabolites in the blood plasma of the subjects depending on the T786C polymorphism of the NOS3 gene (Me [25 ; 75], n = 176)

Variable	Subgroups		
	TT (n = 47)	TC (n = 99)	CC (n = 30)
	1	2	3
NO_2, μmol / l	7,86 [6,66 ; 8,88]	6,66 [5,18 ; 7,98]	5,81 [5,07 ; 6,72]
P-value	$p_{1-2} < 0,001$	$p_{2-3} = 0,20$	$p_{1-3} < 0,001$
NO_3, μmol / l	11,68 [8,88 ; 16,78]	11,10 [8,98 ; 14,36]	10,86 [9,64 ; 12,25]
P-value	p = 0,29		
NO_2+NO_3, μmol / l	20,35 [17,07 ; 25,16]	17,52 [15,37 ; 20,72]	16,41 [14,48 ; 17,93]
P-value	$p_{1-2} = 0,02$	$p_{2-3} = 0,33$	$p_{1-3} < 0,001$

The median NO2 level in the subgroup of homozygotes for the T allele was 7.86 [6.66; 8.88] μmol / l and was significantly higher than in the subgroup of heterozygotes TC - 6.66 [5,18; 7.98] μmol / l - and subgroup homozygotes for the C allele - 5.81 [5.07; 6.72] μmol / l (p <0.05). However, there was no significant difference between subgroups of heterozygotes and homozygotes for the C allele (p> 0.05). There was no statistically significant difference between subgroups of patients depending on the genotypes of T786C polymorphism when comparing NO3 levels (p> 0.05).

The largest value of the total nitric oxide metabolites was in the subgroup of homozygotes for the T allele - 20,35 [17,07; 25.16] μmol / l, against the level of 17.52 [15.37; 20.72] μmol / l subgroup heterozygotes TC, and - 16.41 [14.48; 17.93] μmol / l subgroup homozygotes for the C allele (p <0.05). However, there was no significant difference between subgroups of heterozygotes and homozygotes for the C allele (p> 0.05).

Nitric oxide is a universal regulator of metabolic processes in various human tissues. The results of our study showed that the levels of nitric oxide metabolites depend on various factors. Air pollution is a global problem associated with the development and progression of cardiovascular disease. One of the points of influence of air pollution is endothelial function [12].

Although there are currently limited data on the association of air pollution with atrial fibrillation, but there are such studies. Thus, according to a study by X. Liu et al. it has been determined that air pollution increases the risk of AF [13].

To date, the T786C polymorphism is the most studied for regulating NOS3 gene expression. It was determined that the presence of the C allele at position -786 of the promoter of the NOS3 gene leads to a decrease in its expression, and may be a factor in reducing the synthesis and release of nitric oxide and, consequently, endothelial dysfunction in patients with coronary heart disease [14].

However, the impact on the course of AF polymorphism T786C can be quite different depending on ethnicity. Thus, in a meta-analysis of H. Chen et al. it is noted that 786T / C polymorphism of the eNOS gene reduces the risk of AF for CC vs. T carriers among Caucasians, but not for mixed populations [15].

In general, the question of what the identified interpopulational differences are mainly related to, whether they are due to differences in environmental factors, or more to the fact that the effect of a particular locus can be modified by unaccounted for polymorphic variants affecting the phenotype, remains open and needs further research. Most likely, the AF association cannot be easily detected at one locus, even with a large sample size. This is consistent with the complex nature of this arrhythmia and clarifies the relatively minor role of gene polymorphism in the development of cardiovascular disease [16].

Thus, genetic testing is not currently used in routine clinical practice, but in the future, genomic analysis may provide an opportunity to improve the guidelines of the AF management. Modern scientific research is aimed at the problem of nitric oxide, new data on its role in various cardiovascular diseases, and arrhythmias in particular. The importance of nitric oxide and the need to correct its metabolism in patients with AF is a very important task, further study of this problem will assess and understand the versatility and importance of the functioning of the nitric oxide system in the body, which requires further research.

Conclusion

1. Decreased nitric oxide metabolites occur in patients with paroxysmal atrial fibrillation on the background of coronary heart disease combined with hypertension compared with the healthy group.

2. The level of NO3 in the group of patients from the city was significantly lower than in the group of patients from the rural areas.

3. The level of NO2 was lower among patients with the C7 allele of the T786C polymorphism of the NOS3 gene, whereas the value of NO3 did not depend on this polymorphism.

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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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ФАКТОРЫ РИСКА РАЗВИТИЯ СУДОРОГ У НОВОРОЖДЕННЫХ С ПЕРИНАТАЛЬНЫМ ГИПОКСИЧЕСКИМ ПОРАЖЕНИЕМ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ.

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RISK FACTORS FOR DEVELOPMENT OF SEIZURES IN NEWBORNS WITH PERINATAL HYPOXIC INJURY OF THE CENTRAL NERVOUS SYSTEM