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Studying the T-786C Polymorphism NO Synthase Gene of Chronic Heart Failure with Kidney Dysfunction

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Abstract The aim of our study was to evaluate the role of NO synthase and T786C polymorphism of the NO synthase gene in patients with chronic heart failure (CHF). In patients with CHF 114 Uzbeks have been studied the alleles and genotypes T786C polymorphism of the NO synthase gene. The progression of kidney dysfunction in patients with CHF was accompanied by a decrease in the expression of NO synthase, as evidenced by a decrease in the activity of endothelial NO synthase, NO metabolites (NO2-NO3), highly significant in the group of patients with eGFR <60 ml / min. On the basis of genetic models in Uzbek patients with CHF, the contribution of the T / C genotype T786C polymorphism of the NO-synthase gene increases the risk of disease progression in the group of patients with an eGFR <60 ml / min and makes it possible to determine this genotype as an in-dependent marker of severe kidney dysfunction in CHF patients.

Keywords Chronic heart failure, Kidney disfunction, NO synthase gene

1. Introduction

According to various studies, the prevalence of kidney dysfunction in chronic heart failure (CHF) ranges from 25% to 60%. Impaired kidney function is the most important predictor of a poor prognosis in patients with CHF, even more significant than the severity of CHF and left ventricular ejection fraction (LVEF). Similarly, left ventricular ejection fraction (LVEF) in CHF, a decrease in glomerular filtration rate (GFR) and creatinine level (Cr) are considered as independent signs of a poor prognosis. With GFR <60 ml / min / 1.73 m2, the risk of mortality increases by 2.1 times, with reduced systolic LV function, the risk of death in patients with renal lesions increases 3.8 times, with unchanged systolic function - 2.9 times [1]. The formation of cardiorenal syndrome (CRS) in patients with CHF is a natural manifestation of a functionally interrelated process at the organ level. Moreover, impaired kidney function is a common and independent factor in the progression of the disease, a high incidence of cardiovascular events and death in a population of patients with asymptomatic and / or clinically manifested CHF [2]. The persistence of subclinical kidney dysfunctions during treatment, even with the achievement of control of risk factors and regression of organ damage, can adversely affect the patient's prognosis. Assessment of the functional state of the kidneys is important for the selection of preventive and therapeutic measures [3].

However, molecular genetic testing can detect features of etiopathogenesis and course the most common cardiovascular disease in each patient. Endothelial dysfunction is given an important role in the development and progression of CHF. With CHF, pronounced endothelial dysfunction develops, primarily due to hyperactivation of the sympathoadrenal and renin-angiotensin-aldosterone systems, as well as overproduction of pro-inflammatory cytokines, which leads to a decrease in the production of vasodilating substances and an increase in the synthesis of vasoconstrictor substances Nitric oxide is a strong vasodilatory and anti-inflammatory signaling molecule that plays diverse roles in maintaining vascular homeostasis. Nitric oxide produced by endothelial cells is a critical regulator of this balance, such that endothelial dysfunction is defined as a reduced capacity for nitric oxide production and decreased nitric oxide sensitivity. This ultimately results in an imbalance in vascular homeostasis leading to a prothrombotic, proinflammatory, and less compliant blood vessel wall. Endothelial dysfunction is central in numerous pathophysiologic processes [4,5].

Over the past decade, genome-wide association studies (GWAS) have considerably improved our understanding of the genetic basis of kidney function and disease. Population-based studies, used to investigate traits that define chronic kidney disease (CKD), have identified >50 genomic regions in which common genetic variants associate with estimated glomerular filtration rate or urinary albumin-to-creatinine ratio [6]. One of the genes whose role in the development of endothelial dysfunction has been widely discussed in recent years is the gene for endothelial

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NO synthase (eNOS). The endothelial NO synthase (eNOS) gene is responsible for the synthesis of nitric oxide (NO) by the endothelium and is a key enzyme in the regulation of blood vessel tone, in the work of vascular wall smooth muscle and thrombus formation processes [7].

Identification of genetic risk factors for cardiovascular disease and to assess their contribution to the development of disease— one of the main problems of molecular cardiology. Genetic risk factors, as opposed to a clinical, bio-chemical, environmental and others. Testing of susceptibility genes allows, first of all, to form a group of persons of high cardiovascular risk for the purpose of therapeutic and preventive measures aimed at reducing the extent of the risk under the supervision of a physician. Identification of the genetic predisposition to any disease can be carried out well before the onset of clinical symptoms, which can effectively prevent its development or postpone deadlines demonstrations [8].

The aim of our study was to evaluate the role of NO synthase and T786C polymorphism of the NO synthase gene in patients with chronic heart failure (CHF).

2. Materials and Methods

Were examined 127 patients of Uzbek nationality aged 35-60 years. Patients were divided into three groups of functional class (FC) of CHF according to the New York Heart Classification (NYHA) according to the test of 6-minute walk: the first group consisted of 27 patients with CHF FC I, the second group 56 patients with FC II and 3 group — 44 patients with FC III classification NYHA and All patients were determined by the level of NO synthase and its metabolites - NADPH-diaphorase (eNOS), nitrite reductase (iNOS), endothelin-1. The assessment of kidney function was carried out using the calculation formula SKD-EPI. According to GFR-EPI, the patients were divided into 2 groups: group 1 consisted of 54 patients with GFR - < 60 ml / min and group 2 of 73 patients with GFR > 60 ml / min.

In 114 patients with CHF of Uzbek nationality, genetic determinants of DE development were studied - alleles and genotypes of T786C of the NO synthase gene. The control group consisted of 75 healthy individuals - men of Uzbek nationality. The age groups were comparable. The study was performed according to the standards of Good Clinical Practice (Good Clinical Practice) and the Declaration of Helsinki. The study protocol was approved by the ethics committees of all participating clinical centers. Before inclusion in the study all participants provided written informed consent. Study polymorphism T786C of the NO synthase gene was conducted using polymerase chain reaction on programmable thermocycler CG-1–96 «Corbett Research» (Australia) and 2720 «Applied Biosystems» (USA), using kits LLC “Medlab” (St. Petersburg), according to the manufacturer’s instructions. In our work allele polymorphism T786C revealed after digestion of the amplified fragment of 206 bp containing the polymorphic site. Evaluation of deviation of the distribution of genotypes of studied polymorphisms of DNA from the canonical distribution of Hardy-Weinberg equilibrium was performed using the computer program for the analysis of genetic data “GenePop” (“Genetics of Population”). To calculate the “odds ratio” (OR — odds ratio) with 95% confidence intervals (CI— confidenceinterval), $\chi 2$ and pvalues used statistical package statistical software package «OpenEpi 2009, Version 2.3».

3. Results

Evaluation of endothelial dysfunction in patients with CHF was characterized by a decrease in the expression of NO-synthase, as evidenced by a decrease in eNOS by 14.9% ($P < 0.05$) in patients with FC I, by 29.9% in patients with FC II and by 38.6% in patients with FC III CHF ($P < 0.001$), accompanied by a decrease in NO metabolites (NO2-NO3). In patients with FC I, this indicator was 14.9% lower than in the control group ($P < 0.05$). In patients with FC II and III CHF, the decrease in this indicator was 31.5 ($P < 0.05$) and 39.4% ($P < 0.001$) compared with the group of healthy individuals, respectively. At the same time, the level of nitrite reductase - iNOS increased in patients with FC I CHF by 2.1 times, and in patients with FC II CHF by 3.4 times and FC III CHF by 4.2 times in relation to the control group.

Endothelial dysfunction in patients with CHF, characterized by a decrease in the expression of NO-synthase, in the group of patients with eGFR <60 ml / min decreased by 42.7% ha ($P < 0.001$) and in the group of patients with eGFR> 60 ml / min by 24.7% ($P < 0.05$) compared with the indicators of the control group and amounted to 6.29 ± 0.07 and 8.31 ± 0.09 μmol / min / L (Table 1). This was accompanied by an increase in the index of nitrite reductase - iNOSning: in patients with eGFR <60 ml / min, this indicator was 3.4 times higher than in the control group and amounted to 2.04 ± 0.05 μmol / min / L, respectively. In patients in the group with eGFR> 60 ml / min, this parameter was 1.57 ± 0.07 μmol / min / and was 2.6 times higher than in the control group. The endothelin index - 1 in the group of patients with eGFR <60ml / min, 2.3 times ($P < 0.01$) and in the group with eGFR> 60ml / min, 1.6 times ($P < 0.05$) higher than the indicators the control group, respectively. A correlation was found between endothelin 1 and eGFR ($r = -0.64$, $p < 0.05$).

The population frequency of distribution of alleles for RHV in the control group was: T = 0.85; C = 0.15. Expected frequency of distribution of genotypes for RHV in the control group: T / T = 0.73; T / C = 0.25; C / C = 0.022. The observed frequency of distribution of genotypes for RHV in the control group: T / T = 0.71; T / C = 0.29; C / C = 0.0. In patients with CHF, the frequency of distribution of alleles for RHV in the group of patients with T = 0.77; C = 0.23. Expected frequency of distribution of genotypes for RHV in the group of patients: T / T = 0.59; T / C = 0.36; C / C = 0.05. The observed frequency of distribution of genotypes for

RHV in the group of patients: T / T = 0.56; T / C = 0.43; C / C = 0.01. Reliability: X2 = 4.1; P = 0.04 (Table 1).

Table 1. Expected and observed frequencies of distribution of alleles and genotypes T786C of NO synthase gene polymorphism (rs 198389) in the group of patients with CHF

Alleles	Allele frequency				
T	0.77				
C	0.23				
Genotype	Genotype frequency				
	Observed Ho	Expected He	$\chi 2$	p	df
T/T	0.59	0.56	4,1	0.04	1
T/C	0.36	0.43			
C/C	0.05	0.01			
Total	1.0	1.0			

The relative deviation of the expected heterozygosity from the observed (D) polymorphism - T786C of the NO synthase gene was calculated using the formula: D = (hobs – hexp) / hexp, where hobs and hexp are the expected and observed heterozygosity, respectively.

The distribution frequency of T alleles in patients with CHF was 77.2% and C alleles - 22.8%. In the group of healthy individuals, this parameter was 85.3% and 14.7%. The distribution of T786C genotypes in patients with CHF was as follows: T / T - 55.5% and T / C - 43.2%. In patients of the control group, this indicator was - T / T - 70.7% and T / C - 29.3%. At the same time, in the examined patients, the frequency of alleles depending on FC CHF was characterized as follows: T alleles were found in 83.3% of cases in patients with FC I of CHF, in 73.3% in patients with FC II and 78.2% in patients with FC III of CHF.

The distribution of T786C genotypes in patients with CHF was as follows: T / T - in patients with I FC CHF was 66.6%, in patients with FC II - 50% and III FC - 56.4%. T / C genotypes in patients with FC I - 33.3%, FC II - 46.6% and FC III CHF - 43.6%.

Comparative analysis of differences in the frequency of genotypic variants of T786C polymorphism of the NO synthase gene between the control group and the subgroup of patients with GFR <60 ml / min. Namely, it was statistically established that there is a tendency towards a decrease in the protective effect of the homozygous C / C genotype in relation to the formation of kidney disorders, which was expressed by its highest frequency in group 2 (15.2% versus 25.5%; $\chi 2 = 1.9$; p = 0.1; OR = 0.5; 95 % CI: 0.21-1.32). Along with this, a very high statistically significant difference was found in the distribution of the mutant genotype T / T, the frequency of which among patients was 2.6 times higher (43.5% versus 22.5%; $\chi 2 = 6.7$; p = 0.01; OR = 2.6; 95% CI: 1.25-5.6) in the absence of significant differences in the frequency of distribution of the heterozygous C / T genotype (41.3% versus 52.0%; $\chi 2 = 1.4$; p = 0.2; OR = 0.6; 95% CI: 0.32-1.31).

The established differences in the distribution of the wild C / C genotype, the low frequency of which among CHF patients with GFR <60 ml / min (15.2%) indicate a decrease in its protective role, i.e., the protective effect of this genotype in relation to the development of severe CHF. The revealed, at the same time, a statistically significant difference in the frequency of distribution of the unfavorable homozygous T / T genotype among CHF patients with GFR <60 ml / min compared to the control group ($\chi 2 = 6.7$; P = 0.01) allows us to distinguish that the genotypic variant of T The / T locus of the T786C gene of the NO synthase gene plays an important role in the development of severe CHF with GFR <60 ml / min as an independent genetic marker. According to the odds ratio, the risk of developing CHF with GFR <60 ml / min with the carriage of this genotypic variant can increase 2.6 times. A comparative analysis of differences in the frequency of variants of genotypes of T786C polymorphism of the NO-synthase gene between groups 1 and 2 revealed significant differences in the distribution of the wild C / C genotype, the frequency of which was higher in group 1 of patients (30.7% versus 15.2%; $\chi 2 = 3.8$; p = 0.05; OR = 0.4; 95% CI: 0.16-1.02) compared with its frequency in group 2. The established fact can be explained by the possible protective role of this genotype in relation to kidney disorders and a decrease in GFR in patients with CHF in group 1.

4. Discussion

With the development of CHF, LV dysfunction is the main trigger, and LVEF is the main factor determining the prognosis of CHF, impaired renal function is also the most important predictor of a poor prognosis in patients with CHF, even more significant than the severity of HF and LVEF [9]. The kidneys are an integral and significant part of the microcirculatory system of the body, an important organ of metabolic and humoral processes; therefore, a decrease in GFR is considered as a marker of an unfavorable prognosis for CVD. The results of epidemiological and population studies indicate that even the earliest subclinical renal dysfunction is an independent risk factor for CVC and death [10].

Endothelium is strategically located at the interface between blood and interstitial tissues, placing thus endothelial cell as a key player in vascular homeostasis. Endothelial cells are in a dynamic equilibrium with their environment and constitute concomitantly a source, a barrier, and a target of defensive mediators. Endothelium is both a target and a driver of kidney and systemic cardiovascular complications. Emerging therapeutic strategies that target the renal endothelium may lead to improved outcomes for both rare and common renal diseases [11].

Researchers analyzed PubMed and Embase databases were searched for relevant articles on eNOS T-786C and CKD before February 28, 2021. After screening with eligibility criteria, 15 papers were included and eventually combined in a meta-analysis. The result of the TSA showed that the sample size for Caucasians was adequate to ascertain

the correlation between eNOS T-786C and CKD but was insufficient for Asians. eNOS T-786C genetic polymorphism was of conclusive significance in the association with CKD among Asians in our meta-analysis. Our case-control samples play a decisive role in changing conclusions from indefinite to definite [13].

5. Conclusions

The progression of kidney dysfunction in patients with CHF was accompanied by a decrease in the expression of NO synthase, as evidenced by a decrease in the activity of endothelial NO synthase, NO metabolites (NO2-NO3), highly significant in the group of patients with eGFR <60 ml / min. On the basis of genetic models in Uzbek patients with CHF, the contribution of the T / C genotype T786C polymorphism of the NO-synthase gene increases the risk of disease progression in the group of patients with an eGFR <60 ml / min and makes it possible to determine this genotype as an independent marker of severe renal dysfunction in CHF patients.

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