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ENDOTHELIAL NITROGEN OXIDE SYNTHASE GENE POLYMORPHISM IN ADOLESCENTS WITH DIENCEPHALIC SYNDROME OF PUBERTY AND ARTERIAL HYPERTENSION

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Abstract

The purpose of this study was to improve the early diagnosis of cardiovascular lesions in adolescents with diencephalic syndrome of puberty and arterial hypertension. It is known that hypothalamic syndrome is the most common endocrine disorder of puberty. This syndrome is caused by hypothalamic-pituitary dysfunction and characterized with puberty shifts in the neuroendocrine system. This syndrome is characterized by carbohydrate and lipid metabolic disorders and of varying degrees of obesity. Arterial hypertension may be included in the diencephalic syndrome of puberty and occurs in half of patients with this disease. In the case of diencephalic syndrome of puberty, the absence of proper monitoring and treatment methods may become progressive and lead to resistant hypertension, abdominal obesity, and metabolic disorders which can eventually damage target organs. Thus, it is important to perform proper diagnostics of diencephalic syndrome of puberty and arterial hypertension in young people to prevent cardiovascular disorders.

The aim of this research was to improve early diagnosis of cardiovascular disorders in adolescents with diencephalic syndrome of puberty and arterial hypertension by studying endothelial nitric oxide synthase gene polymorphism and serum homocysteine level in different genotypes.

The purpose of this study was to improve the early diagnosis of cardiovascular lesions in adolescents with diencephalic syndrome of puberty and arterial hypertension. The authors studied the gene polymorphism of endothelial nitric oxide synthase - 4/5x repeat 27 nucleotide pairs in the gene 4 (4b, 4a) – in adolescents with diencephalic syndrome of puberty and arterial hypertension.

The level of homocysteine in the blood serum in different genotype patients with diencephalic syndrome of puberty was assessed. The highest level of homocysteine in the blood serum was identified in 4b4b and 4b4a genotypes in patients with diencephalic syndrome of puberty. This fact may suggest high-risk vascular complications due to the influence of hyperhomocysteinemia in patients with such genotypes.

It was found that teens with genotype 4a4a have the highest levels of serum insulin, triglycerides and very low density lipoprotein. This fact deserves attention, as hyperinsulinemia is an early marker of insulin resistance, which, in turn, leads to blood lipid spectrum disbalance. All mentioned above makes young people with diencephalic syndrome of puberty and genotype 4a4a a subpopulation with the highest risk of developing atherosclerosis and progressive metabolic disorders.

Keywords: *gene polymorphism, endothelial NO-synthase, adolescents, arterial hypertension.*

Introduction

The advance of modern genetics over the last decades has created a possibility to search for candidate genes coding proneness to various diseases. Nitric oxide (NO) synthase gene of the 3rd type (eNOS3) is considered to be one of candidate genes, determining the development of cardiovascular diseases. eNOS3 gene codes an enzyme, namely nitric oxide synthase, whose function is to produce NO. The latter belongs to the most crucial biological mediators, participating in multiple physiological and pathophysiological processes. Thus, NO assists

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with relaxation of vascular smooth muscles, regulation of their growth, transmission of nerve impulses, reduction in adhesion of platelets, immune reactions et cetera [*Karvonen J et al., 2002; Persu A et al., 2002*]. NO is produced from L-arginine through the action of NO-synthase enzyme, whose three forms are coded by different genes [*Kotovskaya YV et al., 2002; Persu A et al., 2002*]. Endothelial NO-synthase is a product of eNOS3 gene, located on 7q36 chromosome. eNOS3 gene polymorphism in the 4th intron is represented by two alleles, particularly by allele b, which is 5-repeat with fragments of 27 nucleotide pairs in size, and allele a, which is just 4-repeat. In population 5-repeat alleles are known to be found more frequently than 4-repeat ones [*Kotovskaya YV et al., 2002*]. Individuals with rare allele homozygote and *4a/4a* genotype have higher levels of nitrates and nitrites in blood, which has a direct relation to the rate of NO production by vascular endothelium. The latter is indicative of a potential genetic role of *4a/4a* genotype as a risk factor of atherosclerosis development, as well as diseases leading to impairment of normal NO production [*Mishchenko LA, 2006*]. Significant frequency of allele a (4-repeat) was observed in patients with myocardial infarction as compared to healthy subjects in Japanese (p=0.007) and Korean (p=0.00487) populations [*Persu A et al., 2002*]. Significant frequency of allele *a* was found in patients with essential hypertension in Japanese population, in a group of patients with arterial hypertension (AH) and left ventricular hypertrophy as compared to healthy subjects [*Luma GB, Spiotta RT, 2006*].

There are no data concerning similar investigations in children in available literature. At the same time cardiovascular diseases remain one of the leading causes of disability and mortality in adults, provided that AH, which is frequently accompanied by obesity, holds a significant position in its structure [*Luma GB, Spiotta RT, 2006; Maidannyk VG, 2006; Mishchenko LA, 2006; Kislyak OA, 2009; Sirenko YN, 2010*]. The number of children with obesity in the world doubles every three decades and, over the last 20 years, the incidence of obesity in adolescents aged from 12 to 19 has increased almost 3-fold (from 5 to 14%) [*Sirenko YN, 2010*]. The incidence of diencephalic syndrome of puberty (DSP), including obesity of different degrees, AH, carbohydrate and lipid metabolism disorders increased [*Beaudin AE., Stover PJ.* 2007*; Kislyak OA, 2009*]. The findings show that AH transits into adulthood almost in 1/3 of children and adolescents with DSP [*Maidannyk VG, 2006*].

The above mentioned strengthens the relevance of and holds promise for a study of endothelial nitric oxide synthase gene polymorphism in overweight adolescents with AH, which can increase opportunities for individual follow-up and timely prevention of cardiovascular complications. A study of the relationship between different genotype variants and serum homocysteine level in adolescents with AH also holds promise as the latter belongs to independent modifiable risk factors of cardiovascular diseases [*Shevchenko OP, Olefrienko GA, 2002*].

Material and Methods

The study was conducted at the Regional Children Clinical Hospital and the Department of Pediatrics and Neonatology No.1 of Kharkiv National Medical University and involved 101 adolescents (88.1±3.1% boys and 11.9±3.2% girls) at the age from 14 to 17 (median age 15.8±0.66 years). All the patients were divided into 3 groups according to the genotypes. When studying endothelial nitric oxide synthase gene polymorphism the subjects under investigation were found to have the following genotypes: genotype *4b4b* in 47 adolescents (46.5±7.3%), genotype *4b4a* in 43 children (42.5±7.6%), genotype *4a4a* in 11 adolescents (11±9.9%). All the patients were admitted to hospital due to attacks of increased blood pressure in their clinical record.

The patients' clinical records and clinical picture were examined and physical development with waist measurement (WM), hips measurement (HM), abdominal obesity (WM/HM ratio) and body mass index (BMI) were assessed. Daily monitoring of blood pressure (DMBP) using MDplus unit (Russia, Novosibirsk) was implemented to verify blood pressure changes and confirm diagnosis. Arterial hypertension was diagnosed in com-

pliance with ICD 10 (code I-15.2). The state of cardiovascular system was assessed by standard Doppler echocardiography, recommended by Association of Echocardiography [*Vorobiev AS, 2010*]; the patients underwent ECG examination as well. Endothelial nitric oxide synthase gene polymorphism was studied by polymerase chain reaction. Study of serum homocysteine level in patients with different genotype was performed by means of the immune-enzyme analysis on automatic biochemical analyzer "Cobas c111 Roche" (Germany), "Human" chemical agents (Germany). Levels of insulin in the blood serum as a result of immune response were assessed by the immune-enzyme analysis on automatic biochemical analyzer "Cobas c111 Roche" (Germany), DRG chemical agents (Germany). Blood lipids were examined by the immune-enzyme analysis on automatic biochemical analyzer "Cobas c111 Roche" (Germany), Roche chemical agents (Germany). Statistical data processing was run by STATISTICA 6.0 software using parametric and non-parametric methods.

Results and discussion

Clinical symptoms analysis in patients with different genotypes revealed that 90% of children had complaints of recurrent high blood pressure, regardless of a genotype. 67.4% of the examined subjects suffered from headache and cardialgia. The analysis of anthropometric indices did not reveal any differences in the growth rate.

Children with genotype *4a4a* were found to have abdominal obesity and an increase in BMI (p=0.01). Moreover, stable forms of AH were predominant in the group of adolescents with genotype *4a4a*.

Blood serum homocysteine level analysis revealed significant differences between the groups of patients with a different genotype (p=0.0001). (Fig. 1)

Thus, serum homocysteine level made up: in patients with genotype 4b4b - ME=11,8 (LQ (25%) - 6.9; UQ (75%) - 17.3 *μmol/l*); 4b4a - ME=12.2 (LQ (25%) 7.9; UQ (75%) - 19.3 *μmol/l*); 4a4a - ME=2.9 (LQ (25%) 2.5; (UQ (75%) - 4.6 *μmol/l*). Patients with genotype 4a4a were found to have significant differences (p=0.0001) in homocysteine levels which can be explained by its interrelation with intracellular NO concentration.

An increase in homocysteine level is known to be connected with oxidative stress as this amino acid is a precursor of the main cellular antioxidant compound glutathione. Oxidative stress results in the formation of free radicals that change the course of oxidoreduction processes within the cell and cause apoptosis [*Coppola A et al., 2002]*. On the other hand, endogenic NO can regulate methyl cycle by the flow of iron and activation of cystathionine-β-synthase. The latter has been proved by research of the last decade, showing that a decrease in intracellular NO concentration leads to an increase in iron homeostasis and is indicative of negative correlation between endogenic NO and intracellular level of iron [*Shevchenko OP, Olefrienko GA, 2002*].

In other words, NO induces suppression of ceramide pro-oxidative and pro-apoptosis effects, preserving homeostasis of intracellular iron.

It can be assumed that a decrease in nitric oxide level changes methyl cycle. Oxidative stress, directly or by redox-sensitive transcription factors, can change the activity of cystathionine-β-synthase and methionine synthase**,** switching methyl cycle pathways from re-methylation to trans-sulphuration, preserving intracellular store of glutathione by means of adaptation processes that are important for cell viability. The above mentioned ex-

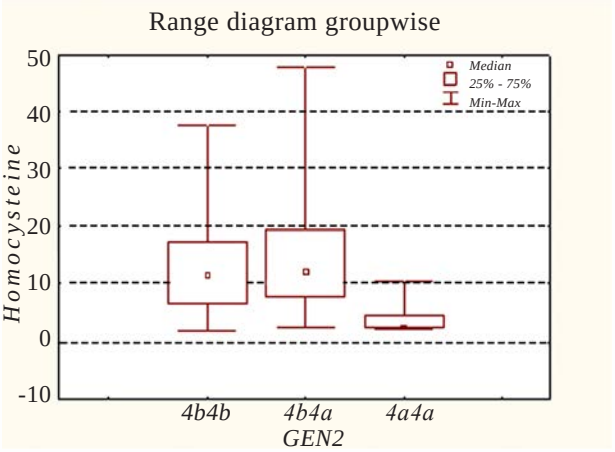

Figure 1. *Serum homocysteine level in patients with different genotypes.*

Notes: *GEN2 – endothelial nitric oxide synthase gene polymorphism: genotypes 4b4b, 4b4a, 4a4a.*

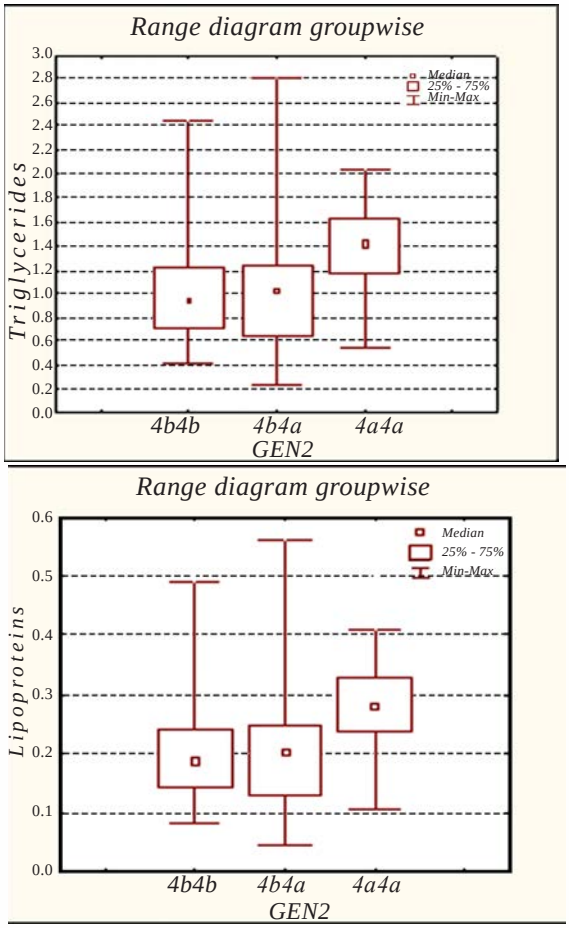

FIGURE 2. *Triglycerides (a) and very low-density lipoproteins (b) in blood serum of patients with different genotypes.*

NOTES: *GEN2 – endothelial nitric oxide synthase gene polymorphism: genotypes 4b4b, 4b4a, 4a4a.*

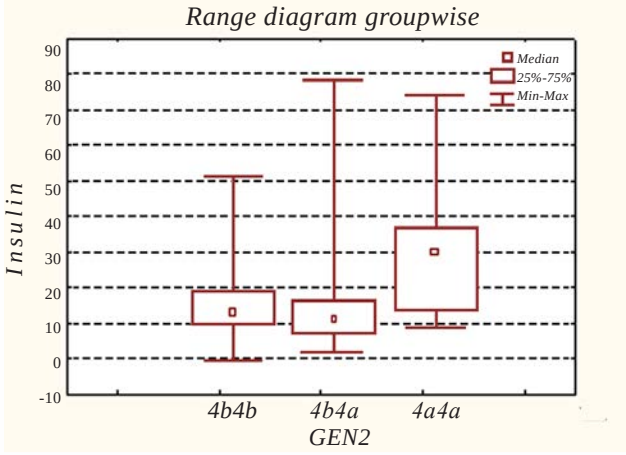

FIGURE 3. *Serum insulin level in patients with different genotype.*

NOTES: *GEN2 – endothelial nitric oxide synthase gene polymorphism: genotypes 4b4b, 4b4a, 4a4a.*

plains the presence of the smallest serum homocysteine contents in patients with genotype *4a4a*.

There are also significant differences between the indices in patients with different genotypes by the levels of triglycerides ($p<0.05$), very low-density lipoproteins ($p<0.05$) and insulin ($p<0.05$). (Fig. 2a,b, 3)

High levels of triglycerides and very low-density lipoproteins in adolescents with genotype *4a4a* most likely account for vascular wall degradation in the presence of oxidative stress and lead to progression of AH.

Thus, an increase in serum insulin in patients with genotype *4a4a* renders the unfavorable effect of hyperinsulinemia (HI) on the vascular wall. It results in a series of metabolic disorders that eventually lead to progression of atherosclerosis [*Karvonen J et al., 2002; Persu A et al., 2002*].

The analysis of the research findings corroborates the following conclusions:

It is advisable to determine the serum homocysteine level as an independent modifiable risk factor of cardiovascular diseases and study endothelial nitric oxide synthase gene polymorphism in overweight adolescents with arterial hypertension in order to predict and prevent complications of arterial hypertension in due time.

Significant differences in the serum homocysteine level, detected in patients with genotype *4a4a* ($p=0.0001$) as well as alterations in the level of triglyceride ($p<0.05$), very low-density lipoproteins ($p<0.05$) and serum insulin ($p<0.05$) determined in examined subjects of these group in comparison to adolescents with genotypes *4b4b* and *4b4a*, suggest that the adolescents with this genotype are at high risk of developing arterial hypertension and associated disorders of target organs and their subsequent progression.

Continuing research in this area to specify prognostic factors of cardiovascular disorders in overweight adolescents with arterial hypertension holds promise.

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