

Streszczenie w języku angielskim

ATHENA Study - ArTerial HypErtension PAtogenesis - microRNA as a missing link in the disease development in children.

Introduction:

Arterial hypertension (HTN) occurs in 3–5% of children, and approximately half of the cases are primary hypertension (PH). Most cases of HTN are asymptomatic. Serious complications are rare in children and adolescents with HTN. Subclinical organ damage, such as left ventricular hypertrophy, is more common. The pathogenesis of PH is multifactorial (genetic and environmental factors, the sympathetic nervous system, the renin–angiotensin–aldosterone system, and substances produced by the endothelium, e.g., nitric oxide).

MicroRNAs are small, single-stranded ribonucleic acid molecules that are formed in the cell nucleus in the process of transcription from a DNA template. These molecules bind to specific mRNAs, leading to their degradation or inhibition of translation - this phenomenon is known as post-transcriptional regulation of gene expression. Disturbances in the above process may impair the function of individual cells in the body, leading to the development of many diseases. MicroRNA molecules that have been well studied in adults with HTN include microRNA-16, microRNA-21, microRNA-27a/b, microRNA-133a, and microRNA-145. Regular physical activity is one of the non-pharmacological methods of preventing and treating HTN. Both short-term and regular aerobic exercise reduce blood pressure in adults. There is limited data on this phenomenon in pediatric patients. There are no data on the relationship between microRNAs and PH and hypertension-mediated organ damage (HMOD) in children, nor on the relationship between microRNAs and blood pressure after a single session of aerobic exercise in patients with HTN.

Objectives:

1. Assessment of the expression of microRNA-16, microRNA-21, microRNA-27a, microRNA-27b, microRNA-133a, and microRNA-145 in children and adolescents with PH and in healthy peers.
2. Assessment of the relationship between the expression levels of the above microRNAs and blood pressure in pharmacologically untreated children with primary hypertension.
3. Assessment of the relationship between microRNA expression levels and hypertension-mediated organ damage: left ventricular mass index (LVMI) assessed by echocardiography, common carotid artery intima-media thickness (cIMT), and arterial stiffness in children with primary hypertension.
4. Assessment of circulating microRNAs as potential biomarkers of impaired cardiac systolic function expressed as left ventricular global longitudinal strain (LV GLS) in children with primary hypertension.
5. Assessment of the effect of a single session of standardized aerobic exercise on blood pressure and microRNA expression in children and adolescents with PH and in healthy peers.

Materials and Methods:

The studies were observational, partly cross-sectional and partly pre-post studies following a single session of standardized aerobic exercise. The analyses included children and adolescents with newly diagnosed, pharmacologically untreated PH and healthy peers constituting the control group. The study included 56 children with primary hypertension (15.4 ± 1.9 years, 42 boys, 14 girls) and 59 healthy peers (15.3 ± 1.6 years, 41 boys, 18 girls). The diagnosis of arterial hypertension was established according to the current European Society of Hypertension guidelines and confirmed by ABPM.

Basic anthropometric parameters, including height, body weight, body mass index (BMI), and their Z-score values, were assessed in all participants. Office blood pressure measurements were performed using the oscillometric method, and ABPM was performed with simultaneous analysis of hemodynamic parameters and central blood pressure. The analyzed parameters included mean systolic, diastolic, and mean peripheral blood pressure values (SBP, DBP, MAP) and central blood pressure (AoSBP, AoDBP, AoMAP).

As part of the assessment of HMOD, echocardiographic examinations were performed with assessment of left ventricular mass (LVM) and left ventricular mass index (LVMI). In

addition, left ventricular global longitudinal strain (LV GLS) was assessed using speckle-tracking echocardiography. Common carotid artery intima–media thickness (cIMT), carotid–femoral pulse wave velocity (cfPWV), and the albumin-to-creatinine ratio in a morning urine sample (ACR) were also measured.

The main study analyzed the effect of a single standardized session of aerobic exercise on microRNA expression and blood pressure parameters. For this purpose, all participants underwent cardiopulmonary exercise testing (CPET) to individually determine exercise intensity. Subsequently, after 2–6 weeks, they performed 60 minutes of supervised exercise on a cycle ergometer at an intensity corresponding to 90–110% of the workload achieved at the anaerobic threshold (AT), in accordance with the American College of Sports Medicine recommendations concerning moderate-intensity exercise. Blood samples were collected in the morning, after fasting, before and after physical exercise.

For molecular analysis, venous blood plasma was collected using Vacutainer systems. MicroRNA isolation was performed using commercially available mirVana PARIS diagnostic kits, followed by reverse transcription and quantitative real-time PCR (qPCR) using TaqMan assays and probes. The expression of selected microRNAs was analyzed: microRNA-16, microRNA-21, microRNA-27a, microRNA-27b, microRNA-133a, and microRNA-145. Relative expression was assessed using the $2^{-\Delta\Delta C_t}$ method.

Results:

In the first publication, which included the results of the pilot study, the effect of physical exercise on the expression of selected circulating microRNAs was assessed in 22 children with primary hypertension (PH). It was demonstrated that changes in microRNA expression after physical exercise correlated with parameters of HMOD: an increase in microRNA-133a expression was associated with greater left ventricular mass (LVMI, $r = 0.491$, $p = 0.020$), whereas an increase in microRNA-145 expression correlated with lower systolic blood pressure values in 24-hour monitoring ($r = -0.430$, $p = 0.046$) and with a lower severity of arterial damage as assessed, among others, by common carotid artery intima–media thickness (cIMT, $r = 0.445$, $p = 0.033$) and pulse wave velocity (cfPWV, $r = -0.557$, $p = 0.007$).

In the second, main publication, in 56 patients with primary hypertension and 59 healthy children, a different expression profile of selected circulating microRNAs was demonstrated in children with PH compared with healthy peers. The expression of microRNA-27b and microRNA-133a was significantly reduced in patients with PH. The expression levels of

microRNA-16, -21, -27a, and -145 also correlated positively with blood pressure values. Furthermore, in a multivariable logistic regression model, high baseline microRNA-16 expression was an independent factor distinguishing patients with higher (>2.1) versus lower DBP Z-score values. No significant correlations were found between the expression levels of the investigated microRNAs and markers of HMOD.

The same study also demonstrated a significant reduction in blood pressure following exercise in children with PH, which was not observed in the control group, as well as between-group differences in the exercise-induced responses of selected microRNAs. In the control group, exercise increased microRNA-27b, microRNA-133a, and microRNA-145 expression, whereas in children with PH, only microRNA-145 expression increased significantly.

In the third publication, echocardiographic parameters were assessed in relation to circulating microRNA expression levels in 50 patients with primary hypertension and 57 healthy controls. It was demonstrated that children with PH were characterized by less negative left ventricular global longitudinal strain values (LV GLS, $-19.28\% \pm 3.02$ vs. $-20.99\% \pm 3.11$, $p = 0.002$). In addition, a significant correlation was found between microRNA-16 expression and LV GLS values ($R = 0.305$; $p = 0.031$).

Conclusions:

1. Children with primary hypertension are characterized by a different expression profile of selected circulating microRNAs compared with healthy peers, which may indicate the involvement of epigenetic mechanisms in the pathogenesis of arterial hypertension in the pediatric population. The expression of microRNA-27b and microRNA-133a is reduced in children with primary hypertension.
2. In untreated children with primary hypertension, higher expression of microRNA-16, microRNA-21, microRNA-27a, and microRNA-145 correlates positively with blood pressure values, which may suggest the involvement of these molecules in the pathogenesis of primary hypertension. High microRNA-16 expression is associated with higher diastolic blood pressure values and may constitute a potential biomarker of hypertension severity in children.
3. In children with primary hypertension, there is no correlation between classical parameters of organ damage and the expression of microRNA-16, microRNA-21, microRNA-27a, microRNA-27b, microRNA-133a, and microRNA-145.

4. MicroRNA-16 may constitute a potential biomarker of early systolic damage of the left ventricle, which may indicate the involvement of this molecule in the early stages of cardiovascular remodeling in children with primary hypertension.
5. A single session of standardized aerobic exercise, unlike in healthy peers, leads to a significant reduction in blood pressure values in children with primary hypertension and affects the expression of selected microRNAs, which highlights the potential importance of physical activity in the modulation of mechanisms associated with the development of arterial hypertension in the pediatric population.