Role of the Autonomic Nervous System and Endothelial Vasoregulators in the Development of Primary Arterial Hypotension in Children

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Abstract—The aim of the study was to investigate the role of the autonomic nervous system and endothelial vasoregulators in the development of primary arterial hypotension (PAH) in children. The cardiointervalography results of 113 children with PAH were compared with 88 healthy children of comparable age (7–11 years). The findings revealed that children with PAH had higher activity of the sympathetic ($p < 0.001$) and parasympathetic ($p < 0.001$) divisions of the autonomic nervous system at the initial (resting) position of clinoorthostatic test. The activity of these divisions of the autonomic nervous system correlated with the activity of cardiac pacemaker. The change of position from horizontal into vertical was accompanied by a rise only in sympathetic activity ($p < 0.001$). However, there was a decline in the sympathetic nervous system ($p < 0.001$) compared to the indices of the initial (resting) position is registered in the tenth minute of the vertical position. This decrease may be due to the adaptational or compensatory abilities of the sympathetic nervous system. The parasympathetic division of the autonomic nervous system based on heart rate variability showed high activity in all positions of the clinoorthostatic test in the patients with PAH compared with healthy children. The activity of the parasympathetic nervous system was associated with increased synthesis of endothelial factors (e.g. nitric oxide, endothelins) in blood. In conclusion, the results of this study indicate that inadequate response of the autonomic nervous system to **clinoorthostatic** test in children with PAH is associated with disorders of both divisions of the autonomic nervous system as well as vascular endothelial factors.
Keywords—primary arterial hypotension; autonomic nervous system; cardiointervalography; clinoorthostatic test; nitric oxide; endothelial vasoregulators; endothelin

I. Introduction

The regulation of blood pressure is accomplished by short and long-acting mechanisms. The short-acting mechanisms involve the activity of baroreceptors, whereas the long-acting regulators are nervous and renin-angiotensin-aldosterone systems, as well as endothelial factors.

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The main regulator of integrative functions (including blood pressure) is the autonomic nervous system (ANS). The ANS ensures regulated interaction amongst the organs and systems of the body, maintenance of metabolic processes and interaction with the environment [1 – 3]. The ANS also interact with endothelial factors through α2 and β receptors of endothelial cells. Under normal conditions, this interaction is maintained at equilibrium [4, 5]. The endothelial vasoregulatory factors can either have a dilatory or constrictor effect on the blood vessels. Thus, the sympathetic division of the ANS stimulates the release of endothelin-1 (ET-1), which has a vasoconstrictor effect, while the parasympathetic division of the ANS, mainly through acetylcholine, increases the synthesis of nitric oxide (NO), which promotes vasodilation [6, 7]. Furthermore, NO attenuates the vasoconstrictor effect of the sympathetic nervous system [8]. Consequently, NO represent a crucial endothelial factor that equilibrates the sympathetic and parasympathetic activity of the autonomic nervous system [4, 6]. While the involvement of the ANS and endothelial factors in the development of atherosclerosis, coronary heart disease and arterial hypertension has been widely reported in the literature [8–11], there is dearth and inconsistency of data on the role of ANS in the formation of arterial hypotension. Moreover, there is no information on endothelial factors in the pathogenesis of this disease. Therefore, the aim of this study was to investigate the role of ANS and vasoregulators of the endothelium in the development of primary arterial hypotension in children.

II. Methods

A. Ethics and Research Committee

The medical ethics and research committee approved the study protocol before parents and children were contacted.

B. Inclusion Criteria

1. Only children that met the criteria for PAH were included in the study.
2. Children who participated in the study freely gave consent or assent in addition to their parents' agreement.

C. Exclusion Criteria

1. Children who did not show willingness or refused participation were not involved in the study.

2. Children that cannot stand up actively without support were not involved in the study.
3. Patients with moderate to severe anemia, metabolic acidosis, heart failure, hypotensive shock, electrolyte imbalance, severe kidney injury, severe cardiac disease, tachycardia, fractures of the extremity, and coma were not involved in the study.

D. Patients

The children that met the inclusion criteria were treated in designated children hospitals of the Belarusian State Medical University in Minsk (Belarus). Following ethical approval, parents of the children were approached and explained the purpose of the study, as well as risks and benefits of participation in the study. Consent was obtained from parents without any financial inducement. Children to be involved in the study were also informed, in a developmentally acceptable way, regarding the purpose, risks and benefits of the study. To minimize discomfort, fear and any other risk, all children were told that participation was voluntary, and that withdrawal was acceptable at any point of the study. Only children who gave their assent were involved in the study. The dignity, privacy and confidentiality of all involved children were maintained at all times. Of the 211 children that were approached 10 declined. The study involved 88 healthy children and 113 children with PAH. The age range of all groups of children was 7–11 years.

E. Evaluation of Autonomic Nervous System: Computer Cardiointervalography (CGI) with Clinoortostatic Test

The divisions of the autonomic nervous system were evaluated with computer cardiointervalography (CGI) (Equipment, company, country) in conjunction with clinoortostatic test. Heart rhythm was studied with CGI and clinoortostatic test. The test was performed in the same time of the day in a quiet room with stable temperature and humidity for all participants. They were requested to drink coffee or eat two-three hours prior to the experiment. The test started following 30 minutes after the participants have entered to room. During this test, participants were requested to rest for 10 minutes at the in the supine position. Thereafter, they were requested to stand upright for ten minutes. During this active standing, CGI indices in the resting position (supine), first minute, fifth, tenth minute and at the first, fifth minute of repeated supine position using a mercury sphygmomanometer with an appropriately sized cuff. The following indices were analyzed with the CGI: Mo (seconds) – mode; AMo (%) – amplitude of the mode; ΔX (seconds) – variation range, which is the difference between the value of the largest and the smallest cardiac interval (CI); IAR is an autonomic index of rhythm; IT – index of tension of regulatory systems; and IAE is the index of autonomic equilibrium.

F. Investigation of Endothelial Vasoregulators: Enzyme Immunoassay

A right forearm vein was cannulized 10–15 minutes before commencing the study and blood samples for investigation were drawn in the last minute of resting position and in the last minute of upright position. The level of endothelin-1 & -2 (ET-1 and ET-2), nitric oxide (NO), prostaglandin F2 (PF2), bradykinin (BR) of blood plasma of all participants were investigated by the enzyme immunoassay in the biochemistry section of Central Scientific Research Laboratory of the Belarusian State Medical University using the equipment (DRG International, Inc, USA).

G. Statistics

Data processing was carried out using the program Statistica 10.0 for Windows. Quantitative data that were normally distributed are presented as mean and error of the mean (M ± m), whereas data that differed from the normal distribution are presented as median and quartiles. The reliability of the differences was estimated by calculating the Student t-test and Mann-Whitney test. The probability value for significance was set at $p < .05$.

III. Results And Discussion

The result of CIG investigation of the healthy children and those with PAH showed the following results in the resting (supine) position (Table 1).

TABLE I. CARDIOINTERVALOGRAM INDICES OF HEALTHY CHILDREN (CONTROL) AND CHILDREN WITH PAH IN THE RESTING POSITION

Indices	PAH n=113	Control n=88
Mo, seconds	0.72±0.06	0.68±0.04
AMo, %	17.2±0.45***	13.1±0.32
X, seconds	0.54±0.03***	0.30±0.03
IAR	2.6±0.31***	5.5±0.18
IT	22.3±3.7***	37.4±2.8
IAE	31.8±3.7**	46.7±4.1

PAH – primary arterial hypotension; Mo – mode; AMo – amplitude of the mode; X – variation range, which is the difference between the value of the largest and the smallest cardiac interval (CI); IAR – index of autonomic rhythm; IT – index of tension of regulatory systems; IAE – index of autonomic equilibrium.* – p<0.05; ** – p<0.01; *** – p<0.001.

Comparative analysis of the results healthy children and those with PAH in Table showed that patients with PAH had increased activity of both the sympathetic and parasympathetic divisions of the autonomic nervous system. So, in healthy children AMo was 13.1% and the variation range was 0.3 second, whereas children with PAH had an AMo of 17.2% ($p < 0.001$) and variation range of 0.54 second ($p < 0.001$). The children with PAH had lower values of cardiointervalogram indices (IT and IAR) in the patients indicated the predominance of the parasympathetic nervous system over the sympathetic nervous system.

Correlation analysis of cardiointervalogram indices in healthy children did not reveal any statistically significant

relationship. However in children with PAH, there was a significant correlation between AMo and X ($r = –0.62$) ($p < 0.05$). This suggests an interference of the central regulatory mechanisms with autonomic channel of regulation (not shown in Table). This may be the reason of increased level of index of tension (i.e. tension in regulation of the heart rhythm) in children with PAH even at the resting position already in the resting position, indicating that sinus node experiences high activity of both the sympathetic and parasympathetic divisions of the autonomic nervous system.

Transition of healthy children from the resting (supine) to the vertical position was accompanied by a reliable decrease in the vagal activity, and an increase in the tone of the sympathetic division of the autonomic nervous system. This reaction to active orthostasis was absent in children with PAH; they only a significant increase in the AMo. The other indices of cardiointervalogram such as Mo, X, IAR, IT, and IAE did not change during the first minute of active orthostasis (not shown in Table, but IT dynamics in Figure 1).

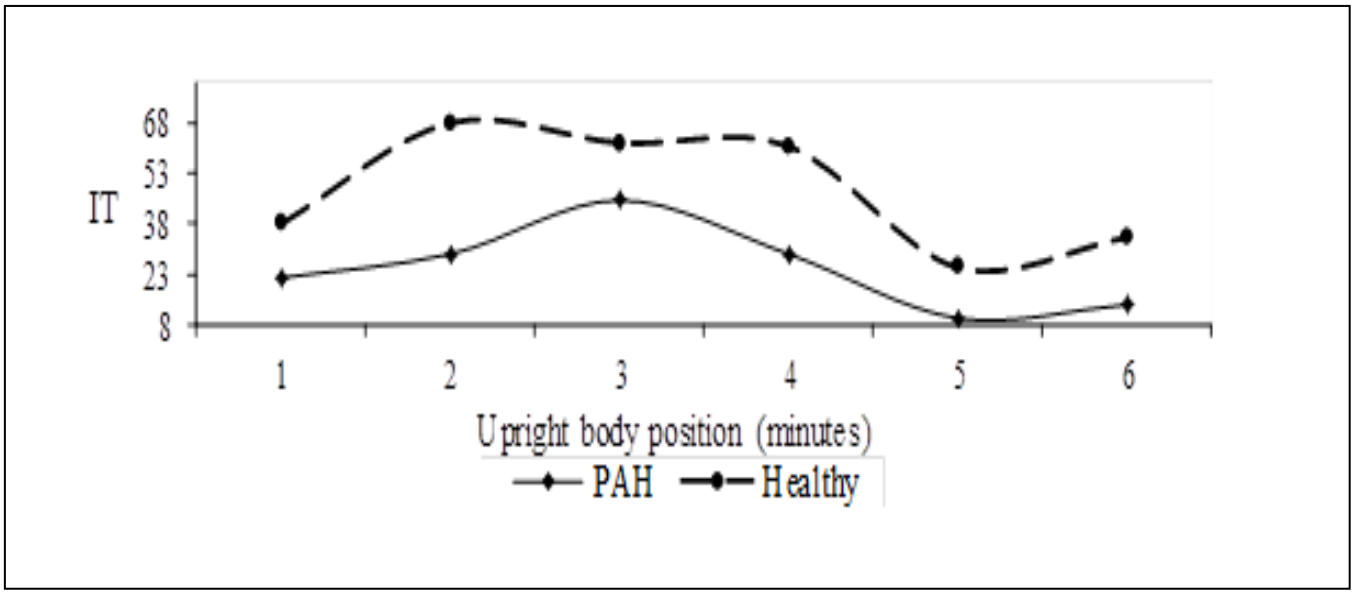

Fig. 1. Dynamics of indices in children with PAH (primary arterial hypotension) under conditions of clinoortostatic test.

Comparing the indices of the cardiointervalograms of the children of the two groups in the first minute of active orthostasis showed that the mode was 0.55 second, amplitude of the mode was 15.3%, and variation range was 0.2 second in the group with healthy children. The children with PAH had higher values of these indices. So, in the group of children with PAH, mode, amplitude of mode and variation range were 0.67 second ($p < 0.01$), 19.3% ($p < 0.001$), and 0.49 second ($p < 0.001$) respectively. The other indices of cardiointervalogram (IAR, IT, and IAE) were also reduced. This result indicates increased activity of the regulatory channels of parasympathetic division of the autonomic nervous system on the heart rhythm.

The correlation analysis of the values of the indices at the upright position showed that there was a significant correlation ($r = + 0.71$) ($p < 0.05$) instead of the insignificant correlation observed in the resting position. However, this correlation was negative in children with PAH ($r = –0.61$; $p < 0.05$). This result point to the fact that change in position (from supine to upright) does not affect the tension of the parasympathetic division of the autonomic nervous system in children with PAH. It also suggests that the activity of the sympathetic division is not able to provide adequate autonomic activity to regulate the activity of cardiac structures. The maximum influence on the heart rhythm is due to the activity of the parasympathetic division of the autonomic nervous system.

The analysis of the dynamics of the cardiorhythm from the fifth to the tenth minute of the upright position revealed a significant change in AMo in children of both groups. While the amplitude of the mode in healthy children decreased only by 1.3% ($p < 0.05$), in children with PAH, the decrease was 7.1% ($p < 0.001$). Decrease in sympathetic activity at the tenth minute of active orthostasis compared with baseline value in children with PAH may indicate depletion of adaptive-compensatory capabilities of the sympathetic nervous system during active orthostasis. However, the tone of the parasympathetic nervous system did not change in the patients with PAH. The maximum vagal effect on the sinus heart rhythm was confirmed by a significant decrease in IAE in children with PAH from 57.4 in the fifth minute to 35.7 at the tenth minute of upright position ($p < 0.001$). In children of the control group, IAE remained the same in all positions of the clinoortostatic test. Thus, active orthostasis in healthy children is accompanied by a stable tone of the parasympathetic nervous system, and moderate activity of the sympathetic nervous system. Children with PAH exhibit sinus rhythm dysregulation. The high tone of the parasympathetic nervous system in these patients is compensated by the high activity of the sympathetic nervous system. However, thereafter, the adaptive-compensatory capabilities fail and the sympathetic influence on the sinus node decreases to the basal (resting) level.

Transition of children from active orthostasis to a repeated supine position led to a significant increase in Mo, X and a decrease in AMo, and IT in both groups. At the same time, the amplitude of the mode and the variation range in children with PAH during the first and fifth minute in the lying position remained significantly increased in comparison with the healthy children. The autonomic index of equilibrium showed differences in the two groups. For example, the IAR of the children with PAH was 1.85 compared to 4.81 in the healthy children ($p < 0.001$); IT was 14.3 versus 33.7 ($p <0.001$) respectively. The result indicate high activity of the parasympathetic nervous system in children with PAH in all positions of the clinoortostatic test. These results suggest abnormal influence of autonomous regulatory circuit on the heart rhythm in children with PAH in upright position. But the increase in activity of the sympathetic nervous system in children with PAH can be considered an adaptive-compensatory mechanism, which is insufficient for a long upright position.

During the last decade, some authors [11–15] have established a close relationship between autonomic nervous system activity and level of endothelial factors. High sympathetic activity stimulates the synthesis of endothelin, and parasympathetic activity stimulates the synthesis of nitric oxide [16, 17]. Results of investigation of the level of the most significant peripheral vasoregulators of the endothelium of blood vessels in PAH children and healthy children are shown in Table 2. Comparative analysis of the levels of endothelial factors did not reveal a significant difference in the concentrations of ET-1, ET-2, PF2α and BR. So in the group of children with PAH, the ET-1 value was 0.72 ng / mL (0.65-0.80), ET-2 – 0.90ng / mL (0.81–1.26), PF2α – 95.0 pg / mL (59.5–110.0), BR – 5.7 ng / mL (3.8–8.4). The healthy children had the following values of vasoregulatory factors: ET-1 – 0.7 ng / mL (0.60–0.74) ($p < 0.1$), ET-2 – 0.84 ng / mL (0.76–0.96) ($p < 0.1$), PF2α – 95.0 pg / mL (54.0–125.0) ($p < 0.1$), BR – 4.1 ng / mL (1.6–6.2) ($p < 0.1$). Nevertheless, we established an increase in the level of NO in children with PAH more than seven times compared to baseline values. Thus, the concentration of NO in the children with PAH was 11.4 μmol / L (6.4–22.5) compared to 1.6 μmol / L (0.9–3.8) in healthy children ($p <0.001$). Importantly, we also observe differences in the ratio of NO / E1 in the two groups of children. The healthy children had NO / E1 value of 2.28, whereas the children with PAH had a value of 15.8. Such high concentrations of plasma NO in children with PAH, in our opinion, cannot only lead to vasodilation of the vessels (which may result to PAH), but also cause stress due to excessive release of NO in the vascular endothelium.

TABLE II. THE LEVEL OF VASOACTIVE SUBSTANCES IN CHILDREN WITH PAH, ME (25–75%)

Groups	ET-1 ng/mL	ET-2 ng/mL	NO μmole/L	$PF_{2\alpha}$ pg/mL	BR ng/mL
Children with PAH	0.72 (0.65–0.80)	0.90 (0.81–1.26)	11.4 (6.4–22.5)	95.0 (59.5–110.0)	5.7 (3.8–8.4)
Healthy children	0.70 (0.60–0.74)	0.84 (0.76–0.96)	1.6 (0.9–3.8)	95.0 (54.0–125.0)	4.1 (1.6–6.2)
Significance	–	–	Z=4.6, p<0.001;	–	–

Therefore, high blood plasma nitric oxide in children with PAH are associated with high activity of the parasympathetic nervous system and stimulation of NO synthesis by acetylcholine. The excess nitric oxide causes dilatation of the blood vessels, a negative inotropic effect on myocardial contractility, and a decrease in peripheral vascular resistance, which ultimately form the hemodynamic component of PAH in children [8]. Thus, children with PAH may not only have dysfunctions in autonomic nervous system functions, but endothelial dysfunction, in which the formation of vasodilatators (NO) predominates over the synthesis of vasoconstrictors (ET- 1, ET-2, PF2α).

IV. CONCLUSION

Autonomic homeostasis in children with PAH according to cardiointervalography is characterized by high activity of the sympathetic and parasympathetic division of the autonomic nervous system in each position of active clinoortostasis. The indices of variation range of PAH patients exceed the values of healthy children and ranged from 0.2 second ($p < 0.001$) in the third to 0.41 second ($p < 0.001$) in the sixth minute of active clinoorthostasis. The conduction of clinoorthostatic test increases the vagal effect on the sinus node of the heart and increases X of the cardiointervalogram by 0.19 second ($p < 0.001$). The drop in AMo of the cardiointervalogram of PAH by 24.3% at the fifth minute of active orthostasis to 18.2% in the tenth minute of the upright position ($p < 0.001$) indicates depletion of the adaptive-compensatory capabilities of the sympathetic nervous system and may be the cause of syncope in this category of patients. Increase in blood nitric oxide level by more than seven times ($p < 0.001$), high NO / E1 ratio, but normal indices of ET-1, ET-2, PF2α, BR in children with PAH strongly suggest that endothelial dysfunction is due to the predominance of the synthesis of vasodilators (NO) over the synthesis of vasoconstrictors (ET-1, ET-2, PF2α).

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