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## Beta-adrenergic reactivity of erythrocyte membranes in adolescents with supraventricular and ventricular arrhythmias before and after radiofrequency ablation

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### ABSTRACT

**Aim.** To evaluate  $\beta$ -adrenergic reactivity of erythrocyte membranes ( $\beta$ -ARM) in adolescents with ventricular and supraventricular arrhythmias before and after radiofrequency ablation (RFA) of heart rhythm disturbances.

**Materials and methods.** The study included 49 adolescents aged 11–17 years, of which 15 had Wolff – Parkinson – White pattern (WPW), 13 – WPW syndrome, 10 – atrioventricular nodal reentry tachycardia (AVNRT), and 11 – ventricular arrhythmia (VA). The control group consisted of 11 adolescents without cardiovascular pathology. All patients received surgical treatment for heart rhythm disturbances (HRD) using RFA. In patients with HRD,  $\beta$ -ARM was determined by a set of reagents BETA-ARM AGAT (AGAT LLC, Russia) before RFA and 3 days after it. In the control group, the parameter was determined at the stage of inclusion in the study.

**Results.** In adolescents with supraventricular arrhythmias, median values of  $\beta$ -ARM did not differ significantly from the control group. RFA in adolescents in these groups did not affect the value of  $\beta$ -ARM on day 3 after the surgery. In adolescents with VA, the median value of  $\beta$ -ARM was initially higher than in the control group ( $p = 0.026$ ). On day 3 after RFA, an increase in  $\beta$ -ARM was noted in this group ( $p = 0.028$ ) compared to baseline values.

**Conclusion.** Activation of the sympathetic nervous system plays a significant role in the pathogenesis of VA in adolescence. The study showed the possibility of using  $\beta$ -ARM to assess the state of the sympathetic nervous system in patients with methodological limitations in analyzing heart rate variability.

**Keywords:** radiofrequency ablation, heart rhythm disturbance, adolescents,  $\beta$ -adrenergic reactivity of erythrocyte membranes

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## Адренореактивность мембран эритроцитов у подростков с суправентрикулярными и желудочковыми аритмиями до и после радиочастотной абляции

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### РЕЗЮМЕ

**Цель.** Оценить показатель  $\beta$ -адренореактивности мембран эритроцитов ( $\beta$ -АРМ) у подростков с суправентрикулярными и желудочковыми аритмиями до и после выполнения радиочастотной коррекции нарушения ритма.

**Материалы и методы.** В исследование включено 49 подростков от 11 до 17 лет, из них 15 с феноменом Вольфа – Паркинсона – Уайта (ВПВ), 13 с синдромом ВПВ, 10 с атриовентрикулярной узловой реципрокной тахикардией и 11 с желудочковой аритмией (ЖА). Группу контроля составили 11 подростков, не имеющих патологии сердечно-сосудистой системы. Всем пациентам проведено оперативное лечение нарушения ритма сердца (НРС) методом радиочастотной абляции (РЧА). Пациентам с НРС определение  $\beta$ -АРМ эритроцитов с использованием набора реагентов БЕТА-АРМ АГАТ (ООО «АГАТ», Россия) выполняли перед проведением РЧА и через 3 сут после нее. В контрольной группе показатель определяли на этапе включения в исследование.

**Результаты.** У подростков в группах с суправентрикулярными аритмиями показатели  $\beta$ -АРМ значительно не отличались от группы контроля. Проведение РЧА у подростков этих групп не повлияло на величину показателя  $\beta$ -АРМ эритроцитов на 3-и сут после оперативного вмешательства. У подростков с ЖА показатель  $\beta$ -АРМ исходно превышал значение в группе контроля ( $p = 0,026$ ). На 3-и сутки после РЧА в этой группе отмечено увеличение  $\beta$ -АРМ эритроцитов ( $p = 0,028$ ) относительно исходных значений в группе.

**Заключение.** Активация симпатического отдела вегетативной нервной системы занимает существенное место в патогенезе желудочковых аритмий в подростковом возрасте. Выполненное исследование показало возможность использования показателя  $\beta$ -АРМ эритроцитов для оценки состояния симпатической нервной системы у категории пациентов с методическими ограничениями выполнения анализа вариабельности сердечного ритма.

**Ключевые слова:** радиочастотная абляция, нарушение ритма, подростки,  $\beta$ -адренореактивность мембран эритроцитов

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## INTRODUCTION

Heart rhythm disturbances (HRD) are some of the most common (60–70%) cardiovascular pathologies in children and adolescents. In recent years, an increase in the total number of various types of HRD in pediatric

population has been noted [1–3]. It may be caused, on the one hand, by an improvement in the diagnosis and, on the other hand, by long asymptomatic development of arrhythmia in children and untimely referral to specialists. In children, several developmental periods are distinguished which are characterized by the

highest risk of developing arrhythmia: the neonatal period; 4–5 years of age; 7–8 years of age; 12–13 years of age [4, 5]. In adolescence, the most common HRD is pacemaker migration (13.5%). Other forms are much less common: bradycardia (3.5%), atrial tachycardia (2.7%), extrasystole (1.9%), Wolff – Parkinson – White (WPW) pattern and first-degree atrioventricular block (AVB) (0.5% each), and long QT interval (0.3%) [3].

The limited effect of existing drug therapy for the control of supraventricular tachycardia (SVT) in children and adolescents is recognized [6]. It is noted that the impossibility of treating such HRDs as WPW syndrome, atrioventricular nodal reentry tachycardia (AVNRT), and ventricular arrhythmia (VA) with drugs in school-age children can cause the development of life-threatening arrhythmias, arrhythmogenic cardiomyopathy, and even death [7]. In these circumstances, radiofrequency ablation (RFA) is becoming the method of choice in the treatment of drug-refractory HRD in children [8].

A distinctive feature of the heart is an autonomic nervous system (ANS). At the body level, it controls interaction with the conducting system of the heart [9]. In this regulation, special importance is attributed to maintaining the balance between the sympathetic and parasympathetic divisions of ANS, as well as to the sensitivity of  $\beta$ -adrenergic receptors [9]. The dominance of the sympathetic division results in life-threatening HRDs and is considered as an independent risk factor for a lethal outcome [10]. This circumstance indicates the relevance of a timely assessment of the sympathetic division of the ANS in children with congenital heart disease (CHD), including after treatment of these disorders by RFA. A promising approach that allows for monitoring of the state of the sympathetic division of the ANS, including in the presence of life-threatening CHD, is  $\beta$ -adrenergic reactivity of erythrocyte membranes ( $\beta$ -ARM) [11, 12].

The aim of the study was to assess  $\beta$ -ARM in adolescents with ventricular and supraventricular arrhythmias before and after radiofrequency ablation (RFA) of HRD.

## MATERIALS AND METHODS

The study included 49 children aged 11–17 years. VA was detected in 11 patients. WPW pattern was detected in 15 patients, WPW syndrome and AVNRT

were detected in 13 and 10 patients, respectively. All patients were receiving planned treatment at the Department of Pediatric Cardiology of Cardiology Research Institute of Tomsk NRMC. The control group consisted of 11 age-matched children who did not have cardiovascular pathology.

The study was carried out in compliance with the ethical principles of the Declaration of Helsinki (“Ethical principles of research involving humans” as amended in 2000) and the Rules of Clinical Practice in the Russian Federation approved by the Order of the Healthcare Ministry of Russia No. 266 of 19.06.2003. The study was approved by the Bioethics Committee at Cardiology Research Institute of Tomsk NRMC (Protocol No. 208 of 20.01.2021).

The inclusion criteria were the following: absence of congenital heart defect, presence of persistent paroxysmal tachycardia, sustained tachycardia, permanent tachycardia, frequent supraventricular extrasystoles (SVE) accounting for more than 15% of the total number of heartbeats per day, frequent ventricular extrasystoles (VES) constituting more than 15% of the total number of heartbeats per day, absence of acute infections and exacerbations of chronic diseases, absence of laboratory signs of myocarditis, a signed informed consent.

The exclusion criteria were the presence of congenital heart defects, acute infections and exacerbations of chronic diseases, laboratory signs of myocarditis and primary electrical heart diseases. When determining the indications for RFA, national guidelines and guidelines of the American and European associations of arrhythmologists and pediatric cardiologists were used [13, 14].

Upon admission, all patients underwent clinical examinations, including taking a history and complaints, an objective examination of a child, 12 lead electrocardiography (ECG), Holter ECG monitoring (HM ECG), echocardiography (echo). In groups of adolescents with arrhythmias, ECG, HM ECG, and echo were repeated 3 days after RFA. Based on the results of HM ECG, the following parameters were assessed: maximum, minimum, and daily average heart rate (HR) during the day, the total number of SVE and VES.

Patients with HRD had their blood sampled before RFA and 3 days after it to determine  $\beta$ -ARM. In the control group, blood samples were taken once at the stage of inclusion in the study.

Beta-ARM in the blood samples was determined using the beta-ARM AGAT reagent kit (AGAT LLC, Russia). The method is based on increasing the osmotic stability of erythrocytes (inhibition of hemolysis) in hypo-osmotic buffer in the presence of the  $\beta$ -blocker 1-(1-isopropylamino)-3-(1-naphthylxy)-2-propanol hydrochloride.

The degree of hemolysis inhibition, expressed as a percentage, was determined by the ratio of the optical density of the supernatant in the sample with the addition of the  $\beta$ -blocker to the incubation medium (test sample) to the optical density of the supernatant in the sample without the addition of the  $\beta$ -blocker to the incubation medium (control sample). Hemolysis inhibition percentage was taken as conventional units (conv. units) of  $\beta$ -ARM. The reference values were the  $\beta$ -ARM values recommended by the manufacturer within the range from 2 to 20 conv. units. At the same time, the  $\beta$ -ARM values over 20 conv. units indicated an increase in the degree of erythrocyte hemolysis and a decrease in  $\beta$ -ARM following desensitization of  $\beta$ -ARM in response to a persistent increase in the activity of the sympathoadrenal system.

Statistical processing of the obtained data was performed using the STATISTICA 10 software. Qualitative variables were presented as absolute and relative values  $n$  (%). The difference in frequencies in independent groups of patients was determined using the Pearson's  $\chi^2$  test. Quantitative variables were checked for normality of distribution using the Shapiro – Wilk test. Quantitative data were presented as the median and the interquartile range ( $Me [Q_1; Q_3]$ ). Given non-normal distribution of quantitative variables, the statistical significance of differences in three or more independent groups was assessed using the Kruskal – Wallis test. If statistically significant differences between the groups were detected, the post-hoc test was applied. Comparison of dependent data in individual groups of diseases was performed using the Wilcoxon test. The differences were considered statistically significant at  $p \leq 0.05$ .

## RESULTS

Table 1 presents the clinical and demographic characteristics of the sample with account of specific features of HRD. The formed groups had no significant differences in age, gender, body weight, and height. The history of HRD in all groups lasted

from 1 to 2 years. Processing the HM ECG results revealed no significant differences in the daily average, minimum, and maximum HR between the studied groups of adolescents with HRD and the controls. In patients with supraventricular arrhythmia, single SVE and VES were recorded. For patients with VA, the presence of a large number of VES in the context of single SVE was noted.

Echo did not show any significant differences in cardiac parameters between the patients with HRD and healthy adolescents in left ventricular end-diastolic volume (EDV), left ventricular ejection fraction (LVEF), and left and right atrial volumes (LAV, RAV).

The results obtained in determining  $\beta$ -ARM are presented in Table 2. At the stage of inclusion in the study, the adolescents appeared to have similar values for the WPW pattern, WPW syndrome, and AVNRT that did not differ significantly from the values in the control group. In contrast, high baseline  $\beta$ -ARM values were found in the group of adolescents with VA. In this group of patients,  $\beta$ -ARM significantly exceeded the value in the control group ( $p = 0.026$ ). In the meantime, no significant differences were found between  $\beta$ -ARM values in the groups with supraventricular arrhythmia and VA.

RFA in adolescents with WPW syndrome, WPW pattern, and AVNRT had virtually no effect on  $\beta$ -ARM on day 3 after the procedure (Table 2). On the contrary, in the group of children with VA, RFA resulted in an increase in  $\beta$ -ARM. Therefore, on day 3 after RFA, the parameter value increased significantly ( $p = 0.028$ ).

## DISCUSSION

Emergence and development of HRD in children and adolescents in most cases are not associated with organic changes in the heart, which complicates the determination of their etiology and pathogenesis. The functioning of the cardiac conducting system is regulated by ANS. The method for studying heart rate variability which allows to assess the activity of the sympathetic and parasympathetic divisions of ANS is widely used in clinical practice. At the same time, application of this method is limited in patients with ventricular ectopic beats.

Currently, the presence of  $\alpha$ -,  $\beta$ 1-, and  $\beta$ 2-adrenergic receptors on erythrocyte membranes has been proven [15, 16].

Table 1

Clinical and laboratory characteristics of patients						
Parameter	Groups of patients					p
	WPW		AVNRT	VA	Controls	
	pattern	syndrome				
Total number of patients, n	28		10	11	11	
	15	13				
Men, n (%)	11 (73.3)	8 (61.5)	2 (20.0)	6 (54.5)	6 (50.0)	0.200
Women, n (%)	4 (26.7)	5 (38.5)	8 (80.0)	5 (45.5)	6 (50.0)	
Age, years, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	13 [10; 15]	14 [10; 14]	14.5 [13; 15]	13 [11; 15]	14 [12; 16]	0.754
Body weight, kg, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	52 [38; 60]	51 [39; 60]	56 [40; 63]	54 [33; 61]	58 [43; 71]	0.880
Height, cm, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	164 [140; 172]	165 [149; 172]	160 [156; 172]	160 [144; 167]	170 [151; 179]	0.997
BMI, kg / m <sup>2</sup> , Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	19.7 [16.7; 22.1]	19.4 [16.6; 20.3]	19.7 [16.3; 23.0]	19.2 [15.4; 23.7]	20.6 [17.9; 21.6]	0.991
Functional class (NYHA) I–IV, n (%)	I, 15 (100.0)	I, 13 (100.0)	I, 10 (100.0)	I, 11 (100.0)	–	–
Age of HRD detection, years, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	12 [7; 13]	8 [6; 12]	13 [10; 15]	11 [11; 13]	–	0.119
History of HRD, years, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	1 [0; 2]	2 [1; 6]	1 [0; 1.75]	1 [1; 2]	–	0.875
Drugs taken, n (%) *	1 (6.66)	2 (15.38)	1 (10.00)	2 (18.18)	–	0.815
<i>HM ECG findings</i>						
Daily average HR, beats / min, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	77 [75; 90]	82 [69; 87]	84 [75; 87]	81 [75; 93]	74 [69; 81]	0.938
Minimum HR, beats / min, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	50 [48; 54]	50 [44; 54]	51 [47; 54]	51 [45; 54]	42 [40; 53]	0.564
Maximum HR, beats / min, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	151 [147; 165]	152 [140; 163]	161 [153; 165]	158 [157; 163]	153 [129; 178]	0.461
Total number of SVE at baseline	single	single	single	single	–	–
Total number of VES at baseline, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	single	single	single	18,395.0 [12,066.0; 36,871.0]	–	–
Features of QRS morphology during HM ECG	Deformation due to permanent / intermittent preexcitation	Deformation due to permanent / intermittent preexcitation	–	–	–	–
<i>ECG findings</i>						
EDV, ml, %**, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	77 [57; 89], 103 [96; 105]	78 [62; 90], 101 [97; 109]	79 [69; 94], 100 [94; 104]	75 [51; 96], 104 [91; 115]	94 [67; 99], 103 [96; 109]	0.816 0.847
LVEF (b), %, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	65 [63; 67]	65 [63; 67]	63.5 [63; 65]	63 [62; 64]	66, [66; 68]	0.375
LAV, ml, %**, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	33 [19; 38], 102 [96; 118]	33 [25; 42], 101 [93; 102]	37 [28; 41], 105 [99; 108]	33 [21; 380], 99.1 [94; 109]	38 [22; 39], 98 [93; 106]	0.943 0.457
RAV, ml, %**, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]	30 [21; 34], 105 [100; 117]	31 [19; 39], 103 [102; 117]	34 [28; 40], 112 [107; 119]	31 [21; 40], 108 [95; 119]	29 [19; 41], 105 [103; 113]	0.949 0.722

\* drugs taken at the time of hospitalization, including the ones to treat the underlying disease; \*\* percentage from the individual predicted norm.

Table 2

Beta-ARM values in adolescents with VA and supraventricular arrhythmia before and after RFA, conv. units, Me [Q <sub>1</sub> ; Q <sub>3</sub> ]					
Parameter	Control group (healthy children), n = 11	WPW pattern, n = 15	WPW syndrome, n = 13	AVNRT, n = 10	VA, n = 11
β-ARM before RFA	13.1 [8.8; 16.5]	17.6 [13.3; 22.6] p <sub>1</sub> = 0.192	16.1 [11.5; 18.2] p <sub>1</sub> = 0.600	15.5 [12.9; 27.7] p <sub>1</sub> = 0.465	19.01 [14.3; 21.7] p <sub>1</sub> = 0.026
β-ARM after RFA	–	18.6 [13.2; 22.8] p <sub>2</sub> = 0.886	16.2 [9.7; 17.9] p <sub>2</sub> = 0.661	14.6 [10.1; 30.8] p <sub>2</sub> = 1.000	29.6 [23.2; 31.7] p <sub>2</sub> = 0.028

Note. Statistical significance of differences in the value of the parameter between the pre-RFA group and the control group – p<sub>1</sub>, statistical significance of differences in the value of the parameter in the group before and after RFA – p<sub>2</sub>.

This fact allows to think that  $\beta$ -ARM can reflect  $\beta$ -adrenergic reactivity of the body as a whole. It has been convincingly shown that an increase in the content of catecholamines in the blood is accompanied by desensitization of  $\beta$ -adrenergic receptors and a decrease in the response from regulated organs to the stimulating effect of neurohormones [11, 17, 18]. The information value of  $\beta$ -ARM in the treatment of HRD in adult patients is being studied [11, 12]. At the same time, we have not found any works devoted to the study of  $\beta$ -ARM in HRD in adolescents, including after RFA.

At the time of inclusion in the study, the formed groups were comparable in anthropometric parameters, age of HRD detection, and duration of HRD history. ECG parameters also did not differ significantly between the groups. With the considered duration of HRD history, the presence of supraventricular arrhythmia was not accompanied by a change in  $\beta$ -ARM compared to the controls. In adolescents with VA, the  $\beta$ -ARM value was significantly higher than in the control group.

Conducting RFA in groups of adolescents with supraventricular arrhythmia did not lead to changes in  $\beta$ -ARM. At the same time, surgical treatment of VA was accompanied by a significant increase in  $\beta$ -ARM on day 3 after surgery compared to baseline values in the group. An increase in  $\beta$ -ARM indicates desensitization of  $\beta$ -adrenergic reactivity in response to an increased stimulating effect of ANS mediators. The obtained results allow to consider the role of the sympathetic nervous system in the etiology and pathogenesis of VA and an increase in its functional stress in the early postoperative period.

In children with supraventricular arrhythmia, the morphofunctional arrhythmia substrate is presented by anomalies in the embryonic development of the cardiac conducting system. Therefore, the emergence of an arrhythmogenic focus in the atria in the first 6 months after birth may be associated with its embryonic origin. At the same time, a number of researchers note that it is possible to suppose an association between rhythm disturbances and features of postnatal development of the cardiac conducting system even in the absence of obvious congenital anomalies. [19]. Studying the electrophysiological characteristics of the atrioventricular node and accessory pathways during transesophageal electrocardiography and cardiac pacing in groups of

adolescents with WPW syndrome or pattern made it possible to establish a high degree of vagotonia in adolescents with WPW pattern [20]. This serves as an indirect confirmation of the results of our study on the absence of tension in the sympathetic division of ANS and desensitization of  $\beta$ -ARM in adolescents with supraventricular arrhythmias before and after RFA.

## CONCLUSION

For adolescents with VA, a significant increase in  $\beta$ -ARM was revealed before RFA, which indicates tension in the sympathetic division of ANS. A significant increase in  $\beta$ -ARM in the early postoperative period indicates a further increase in the activity of the sympathetic division, which is accompanied by further desensitization of  $\beta$ -ARM. The sympathetic division of ANS plays an essential role in the pathogenesis of VA in adolescents.

Supraventricular arrhythmias (WPW syndrome, WPW pattern, AVNRT) that developed in adolescence were not accompanied by a significant increase in  $\beta$ -ARM compared to the healthy controls. RFA in this category of patients did not result in significant changes in  $\beta$ -ARM.

The study showed the possibility of using  $\beta$ -ARM to assess the state of the sympathetic nervous system in patients with methodological limitations in the analysis of heart rate variability.

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## Authors' contribution

Rebrova T.Yu. – analysis and interpretation of the data, drafting of the article. Perevoznikova Yu.E. – collection and processing of clinical material, statistical analysis of the data. Svintsova L.I. – design of the study, critical revision of the manuscript for important intellectual content. Muslimova E.F. – processing of the biological material, analysis and interpretation of the data. Afanasiev S.A. – conception and design, final approval of the manuscript for publication. Dzhaffarova O.Yu. – selection and recruitment of patients, obtainment of informed consents, collection and processing of the clinical material.

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