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სარჩევი CONTENT

Significance of certain genotypic characteristics to form exercise-induced bronchial asthma in children

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Introduction. According to a topical up-to-date definition suggested in PRACTALL [1], exercise-induced bronchial asthma (EIBA) is a separate variant of bronchial asthma (BA) when transient bronchial obstruction associated with physical activity is detected. Its occurrence in children without BA is determined as "bronchial spasm" or "exercise-induced bronchial constriction" [2, 3]. Exercise-induced bronchial spasm (EIBS) is objectively determined as 10% reduction of FEV1 (forced expiratory volume in 1 second) and more than that of the initial value after appropriate bronchial provocation test [4, 5]. Although the most frequent trigger of EIBA is intensive and relatively long physical activity, this disease is characterized by a multi-trigger character of exacerbations altering the course of BA into more severe one [6]. According to these authors in addition to physical activity the following triggers of BA are: meteorological factors (32,9%), acute respiratory viral infection (ARVI) of effect of cold (22,3%), pollen (19,0%), animal epidermis (14,4%), house dust (11,0%), and exposure to tobacco smoke (8,0%). At the same time there are certain data proving the role of a genetic component in the development of BA as a multifactorial disease [7-8]. These investigations deal with the study of the role of deletion gene polymorphism coding the activity of glutathione-S-transferase for the course of BA in children [9-10].

At the same time, it should be noted that pathogenesis of BA phenotype has not been studied completely yet, which is evident in unsolved questions of diagnostics and individualized approaches to treatment. Modern scientific literature available does not present sufficient evidence concerning the value of genetic component, and deletions of $GSTT_1$ and $GSTM_1$ genes in particular, and/or mutation polymorphism of e*NOS* gene in the formation of exercise-induced bronchial spasm.

Objective of the investigation: to study the value of deletion ($GSTT_1$ and $GSTM_1$ genes) and mutation (e*NOS* gene) polymorphism in the formation of bronchial instability in children suffering from exercise-induced bronchial asthma in order to optimize individualized therapeutic-preventive recommendations.

Materials and methods. To achieve the purpose of the study 102 children of a school age suffering from BA were examined at the Pulmonological-allergological department of the Regional Children Clinical Hospital in Chernivtsi. With the aim to verify EIBA the tolerance of patients to physical activity was studied, as well as bronchial instability was determined in response to graduated running and bronchial-motor test with the inhalation of 200 mcg of Salbutamol [11-12]. At the same time, the results obtained were presented in the following indices: bronchial lability index (BLI, %) and its components – bronchial spasm index (BSI, %) and bronchial dilation index (BDI, %). Provocative spirography test with graduated exercise was performed under conditions of rejection of medicines able to change non-specific reactivity of the bronchi to indirect (graduated running) bronchial spasm stimuli.

2 clinical groups were formed on the basis of examination of children. The first group (I, the main one) included 50 school children diagnosed with EIBA, and the group of comparison (II group) included 52 patients with BA without signs of exercise-induced bronchial spasm (EIBS). An average age of children in the I group was 11,2±0,4 (95% confidence interval (CI) 10,3-12,1) including 22 girls (44,0%) and boys – 28 (56,0%). There were 27 rural residents (54,0%), and urban ones – 23 patients (46,0%). An average duration of the disease was 6,18±0,45 (95% CI 5,3-7,1). In the II clinical group there were 16 girls (30,8%), and 36 boys (69,3%). An average age of the patients in the II clinical group was 12,02±0,46 (95% CI 11,1-12,9). 25 children were rural residents (48,1%), and urban ones – 27 patients (51,9%). An average duration of the disease was 6,77±0,55 (95% CI 5,7-7,9). The data available are the evidence to consider that by the main clinical characteristics the groups of comparison did not differ much, and thus they were comparable.

Genetic examinations to study polymorphism of $GSTT_1$, $GSTM_1$ and e*NOS* genes were conducted

in the certified laboratory at the Department of Molecular Genetics and Biotechnology, Yuriy Fedkovych Chernivtsi National University using the method of polymerase-chain reaction.

The study was performed in the parallel groups formed according to the principle of a simple random sampling method "experiment-control". The examinations were conducted during the period without attacks of the disease. Provocative spirography tests with graduated exercise were performed under conditions of rejection of medicines able to change non-specific reactivity of the bronchi to direct (histamine) and indirect (graduated running) bronchial spasm stimuli.

Results and discussion. Considering literary data concerning the participation of $GSTT_1$ and $GSTM_1$ genes in realization of oxidative stress in the respiratory tract [13], the frequency of their deletion polymorphism was studied in children of the comparison groups (Table 1).

Table 1

Frequency of combinations of $GSTT_1$ and $GSTM_1$ gene polymorphism in children of the comparison groups

Clinical groups	Number of patients	Distribution of $GSTT_1$ and $GSTM_1$ genotypes							
		$T_1+ M_1+$		$T_1del M_1+$		$T_1+ M_1del$		$T_1del M_1del$	
		A	B	A	B	A	B	A	B
I group	50	21	42,0	9	18,0	15	30,0	5	10,0
II group	52	28	53,8	7	13,5	15	28,8	2	3,9
Pφ		>0,05		>0,05		>0,05		<0,05	

Note: A – absolute number of patients; B - % percentage from the total number of patients

Therefore, deletion polymorphism of $GSTT_1$ and $GSTM_1$ genes was detected in practically every second child suffering from BA. At the same time, in 1/3 of patients of the I clinical group (28,0%) deletions of $GSTT_1$ gene were detected, and in the similar part of children from the group of comparison (32,7%) – $GSTM_1$ gene. In addition, patients with EIBA phenotype presented practically three times as frequent "zero" genotype of the genes indicated above (10,0% against 3,85%, P<0,05). It gives the ground to think that deletion in $GSTT_1$ genes can influence upon the pronunciation of EIBS development.

Table 2 presents bronchial instability indices in children of the I clinical group depending on deletion polymorphism of $GSTT_1$ and $GSTM_1$ genes.

Table 2

Bronchial instability indices in patients suffering from EIBA depending on deletion polymorphism of $GSTT_1$ and $GSTM_1$ genes (P±m)

Genotype	BSI, %	BDI, %	BII, %
$GSTT_1+M_1+$	21,0±2,44	10,1±2,16*	31,1±3,67
$GSTT_1-M_1+$	24,2±4,98	11,2±4,24*	35,4±5,64
$GSTT_1+M_1-$	18,4±0,9	21,0±2,72	39,3±2,86
$GSTT_1-M_1-$	19,4±1,7	12,8±4,92	32,1±4,83
P	>0,05	-	>0,05

Note: * - P<0,05 relatively to GSTT1+M1-

The presented results enable to consider that EIBS is the most pronounced in children with *GSTM*$_1$+genotype, especially in combination with deletions of *GSTT*$_1$ gene (*GSTT*$_1$*delM*$_1$+genotype). In case patients with EIBA possess *GSTM*$_1$+genotype, especially in combination with *GSTT*$_1$+ (*GSTT*$_1$+M$_1$+genotype), the least pronounced bronchial motor test with Salbutamol is detected, that might reflect re-modulation of the respiratory tract [14]. The afore-mentioned gives the reasons to consider that availability of *GSTM*$_1$+ genotype in patients with EIBA is accompanied by a potential risk to develop pronounced EIBS and weak bronchial motor reaction (according to the data of BDI) caused by a probable re-modulation of the bronchi. Table 3 presents the indices of bronchial hypersensitivity (BHS) and bronchial hyperactivity (BHA) to histamine in children of the comparison groups.

Table 3

Indices of hypersensitivity and hyperactivity of the bronchi in children of the comparison groups (M±m)

Spirography parameters	I group	II group	P
$PC_{20}H$, mg/ml	1,25±0,24	1,26±0,18	>0,05
$PD_{20}H$, mg	0,29±0,05	0,52±0,14	>0,05
DDC (dose-dependent curve)	2,29±0,2	1,76±0,11	=0,01

The data obtained give the grounds to suggest that bronchial sensitivity to histamine in the groups of comparison does not differ considerably, while their reactivity was reliably pronounced in children with EIBA. At the same time it should be noted that representatives of the I clinical group demonstrated pronounced sensitivity and reactivity of the bronchi to histamine inhalations more frequently. Thus, provocative concentration of histamine ($PC_{20}H$) less than 0,2 mg/ml was found in the patients in 23,5% of observations, provocative dose ($PD_{20}H$) more than 0,9 mg was found only in 5,9% of cases, and DDC more than 2,5 – in 37,5%. In the group of comparison the frequency of the given indices of hypersensitivity and hyperactivity of the bronchi was 6,9% (P<0,05), 20,9% (P<0,05) and 11,6% (P<0,05) respectively.

Table 4 presents the indices of non-specific hypersensitivity ($PC_{20}H$, $PD_{20}H$) and hyperactivity (DDC) of the bronchi as well as basic spirography index (Tiffeneau coefficient) reflecting permeability of the respiratory tract. Therefore, the data presented enable to consider, that none of the indices of bronchial hypersensitivity to histamine, used as diagnostic tests in verification of EIBA, does not exceed information value of a technically simple spirography test, that is, Tiffeneau coefficient. In case of positive results of the tests mentioned above availability of EIBA increases in the following way: $PC_{20}H$<0,2 mg/ml – on 27,0%, $PD_{20}H$ <0,05 mg – on 12,0%, DDC >2,5 - on 26,0% and Tiffeneau coefficient <80,0% from the standard one – on 35,2%. In case of negative results of the above mentioned diagnostic methods post-test probability of EIBA absence becomes 4,9%, 3,2%, 8,6% and 12,4% less respectively.

Table 4
Diagnostic value of certain spirography indices to verify EIBA

Spirography parameters	Diagnostic value, % (95% CI)				Likelihood ratio of	
	sensitivity	specificity	Prognostic value of		positive result	negative result
			positive result	negative result		
$PC_{20}H$ <0,2 mg/ml	23,5 (16-33)	93,0 (86-97)	77,0 (58-90)	54,9 (47-62)	3,36	0,82
$PD_{20}H$ <0,05 mg	26,5 (18-36)	83,7 (75-90)	61,9 (46-76)	53,2 (45-61)	1,62	0,88
DDC >2,5	37,5 (28-48)	88,4 (80-94)	76,4 (62-87)	58,6 (50-66)	3,23	0,71
Tiffeneau coefficient<80,0% from the standard	44,4 (34-55)	92,3 (85-97)	85,2 (72-94)	62,4 (54-70)	5,77	0,6

Note: $PC_{20}H$ – provocative concentration of histamine; $PD_{20}H$ – provocative dose of histamine; DDC – dose-dependent curve

The use of spirography indices as risk factors of EIBA availability is presented in Table 5. The results of evaluation of clinical-epidemiological risk of EIBA availability considering the presented spirography indices enable to believe that all of them except $PD_{20}H$ can be considered as probable criteria. At the same time, availability of initial bronchial obstruction in children reflecting basic values of Tiffeneau coefficient is not lower but even increases risk indices of a time-consuming method to evaluate sensitivity and reactivity of the bronchi to histamine considering verification of EIBA.

Table 5
Clinical-epidemiological risk of EIBA availability depending on certain spirography parameters

Spirography parameters	Odds ratio (95% CI)	Relative risk (95% CI)	Attributive risk
$PC_{20}H$ <0,2 mg/ml	4,08 (1,7-10,0)	1,71 (0,8-3,8)	0,32
$PD_{20}H$ <0,05 mg	1,85 (0,9-3,7)	1,32 (0,8-2,3)	0,15
DDC >2,5	4,57 (2,2-9,5)	1,84 (1,01-3,3)	0,35
Tiffeneau coefficient <80,0% from the standard	9,57 (4,2-22,0)	2,27 (1,1-4,63)	0,48

Note: $PC_{20}H$ – provocative concentration of histamine; $PD_{20}H$ – provocative dose of histamine; DDC – dose-dependent curve

Estimation of diagnostic value of bronchial inflammation activity indices and probably markers of their hypersensitivity connected with this process give the ground to consider that in case of sufficient specificity of these tests is usually accompanied a considerable part of false-negative results limiting post-test probability of EIBA in case of positive and negative results of the test. At the same time, numerous from the presented indices are indicative of a reliable although inconsiderable risk of EIBA availability in the patients examined.

The role of endothelial *NO*-synthase is known to participate in the development of inflammatory processes of the respiratory tract, and e*NOS* gene refers to the production of nitrogen monoxide in the early stage of inflammation resulting in relaxation of vessels and increasing their permeability as well as promotes "efflux" syndrome. Considering this fact to study the frequency of mutation polymorphism of this gene in the groups of comparison appears to be reasonable (Table 6).

Table 6
Frequency of eNOS genotypes in children of clinical groups of comparison

Clinical groups	Number of patients, n	Distribution of *eNOS* genotypes					
		GG		*GT*		*TT*	
		A	B	A	B	A	B
I group	28	15	53,6	11	39,3	2	7,14
II group	22	10	45,5	8	36,4	4	18,2
Pφ >0,05				>0,05		<0,05	

Note: A – absolute number of patients; B - % percentage from the general number of patients.

The data presented give the possibility to consider that mutations of e*NOS* (genotypes *GT,* TT*)* occur in every second child suffering from bronchial asthma. Homozygous genotype *TT* occurred most frequently in children without EIBS signs, giving the grounds to suggest the effect of mutation polymorphism of *eNOS* gene on the indices of bronchial instability (Table 7).

Table 7
Indices of bronchial instability in patients suffering from EIBA depending on mutation of eNOS gene (P±m)

Genotype	BSI, %	BDI, %	BII, %
GG	20,3±2,57	19,8±3,49	40,0±3,85
GT	20,1±2,49	11,7±1,23*	31,3±4,9
TT	21,5±5,5	12,0±1,3	33,5±5,5
P	>0,05	-	>0,05

Note: * - P<0,05 concerning *GG* genotype

The data mentioned are indicative of the fact that mutation of *eNOS* gene does not affect considerably EIBS pronunciation at the same time decreasing results of bronchial-motor tests with salbutamol, probably due to availability of bronchial re-modulation in a child [15]. Therefore, analysis of genetic susceptibility of the children organism on the whole was indicative of the fact that exercise induced bronchial spasm increased significantly in case deletions in $GSTM_1$ gene were absent, and they could be partially considered as susceptibility factors to the development of bronchial obstruction after physical activity. In patients with EIBA pronunciation of bronchial motor reaction after salbutamol inhalation decreased considerably in case they possessed $GSTM_1$+ genotype and/or mutations of *eNOS* gene.

The data presented above stipulated reasonability to study the indices of diagnostic value of certain genotypic peculiarities in children to identify EIBA (Table 8).

Table 8
Diagnostic value of certain genotypic characteristics to verify EIBA

Genotypic characteristics	Diagnostic value, % (95% CI)				Odds ratio of	
	sensitivity	specificity	Prognostic value of		positive result	negative results
			positive result	negative result		
$GSTT_1delM_1$+	18,0 (11-27)	86,5 (78-92)	57,1 (38-74)	51,3 (43-59)	1,33	0,95
$GSTT_1$+M_1del	30,0 (21-40)	71,1 (61-80)	50,9 (37-64)	50,4 (42-59)	1,04	0,99
eNOS/GG	53,6 (43-64)	54,5 (44-64)	54,1 (44-64)	54,0 (44-64)	1,18	0,85
eNOS/GT	39,3 (29-49)	63,3 (53-73)	51,9 (40-64)	51,2 (42-60)	1,08	0,95

Therefore, only the absence of deletions in $GSTM_1$ gene of a child can be considered as a specific test to verify EIBA. Mutation polymorphism of *eNOS* gene according to the data above did not possess substantial information value to verify EIBA.

It should be noted that only absent deletions in $GSTM_1$ gene increased post-test probability of EIBA in case of a positive result on 7,0% do not considerably influencing on a negative post-test probability. Availability of other presented above genotypic characteristics in children did not influence considerably on the post-test probability in case of positive and negative results of these tests.

Conclusion. In children suffering from exercise induced bronchial asthma the least pronunciation of bronchial motor reaction after salbutamol inhalation was found in children with $GSTT_1$+M_1+ genotype and/or mutations of *eNOS* gene, that might reflect re-modeling of the respiratory tract and was indicative of a potential risk of bronchial obstruction development after physical activity.

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Summary. The value of deletion (*GSTT*, and *GSTM*, genes) and mutation (e*NOS* gene) polymorphism in the formation of bronchial instability in school children suffering from exercise-induced bronchial asthma was studied. Exercise induced bronchial spasm was found to be the most pronounced in children with *GSTM*,+ genotype especially in combinations with deletions of *GSTT*, gene (*GSTT*,*delM*,+ genotype), and in case patients with EIBA possess *GSTM*,+ genotype especially in combination with *GSTT*,+ gene (*GSTT*,+*M*,+genotype) the least pronounced bronchial motor test with salbutamol is found which probably reflects re-modulation of the respiratory tract. Mutation of *eNOS* gene does not affect considerably EIBS pronunciation at the same time decreasing results of bronchial-motor tests with salbutamol, probably due to availability of bronchial re-modeling in a child.

Key words: children, exercise induced bronchial asthma, gene polymorphism.