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O.K. Koloskova, O.G. Hryhola, T.M. Bilous, Ye.P. Ortemenka

FEATURES OF EXERCISE INDUCED BRONCHIAL ASTHMA COURSE IN SCHOOLCHILDREN WITH POLYMORPHISMS IN GLUTATION-S-TRANSFERASE GENES

Bukovinian State Medical University, Chernivtsy, Ukraine

Abstract. With respect to the principles of bioethics, on the base of allergy department of Chernivtsi Regional Children's Hospital it has been conducted a comprehensive clinical and paraclinical examination of 51 children with symptoms of exercise induced asthma phenotype depending upon the presence or the absence of genes polymorphism of the enzymes of glutation-S-transferase – $GSTT_1M_1$. The study showed that the course of the disease was more severe in the patients with deletions of the mentioned genes, though with more rapid effect of standard reliever treatments as compared to the patients with genotype $GSTT_1+M_1+$. The patients with exercise-induced asthma in the presence of deletion genes polymorphisms of $GSTT_1M_1$ (GSTT1delM1del, GSTT1+M1del or $GSTT_1delM_1+$) require a special attention to their environmental enhancement and normalization of their life-style.

Key words: children, exercise-induced bronchial asthma, glutation-S-transferase genes polymorphisms.

Introduction. Bronchial asthma is a widespread chronic inflammatory respiratory disease, which occurs in children population ranging from 3.4% to 37,6 % [1, 9, 12], and, therefore, requires the direct and indirect expenses that, in developed countries, amount to billions of dollars [6]. Against the background of hereditary predisposition, this disease manifests by recurrent attacks of reversible bronchial obstruction due to the contact with the certain stimuli (triggers), among which phisical exercise is of a great importance [13]. Exercise-induced asthma (EIA) is considered as a heterogeneous group of disease with different pathogenic variants [10, 11]. While an ordinary bronchial spasmodic response in patients with impaired patency of airways occurs during exercise, for patients with exercise-induced asthma its appearance is typical at the end of the exercises or within next 10-15 minutes after it. As a result, often enough doctors and/or parents exclude from daily life any types of exercise considering it only as an asthma trigger, which leads to physical inactivity, hypodynamia, and, finally, results in the deterioration of the quality of life through a radical change in its style. However it is known that most patients with EIA can fully exercise with prior usage of medications that prevent the development of bronchial spasm.

According to current views, the contribution of genetic component in the development and course of the disease reaches 40-60 % of all risk factors, and the genes associated with the disease (‘candidate genes’), have been found in most chromosomes [9]. In recent years the close attention of the researchers is attracted toward a polymorphism in xenobiotic metabolizing genes that affects their functional ability. Specifically, the genes family of glutation-S-transferase (GST), which encodes enzymes grouped into α, μ, π and τ - classes (*GSTA, GSTM, GSTP* and *GSTT*), has a great influence on the oxidative stress in the lungs and thus, on the pathogenesis of bronchial asthma [5, 14]. However, the peculiarities of exercise-induced bronchial asthma under the presence of glutation-S-transferase genes polymorphism ($GSTM_1$ and $GSTT_1$) are still imperfectly studied, especially in matters of the control of the disease.

The aim: To study the clinical and anamnestic features of exercise induced bronchial asthma in schoolchildren with polymorphisms of genes GSTM1 and GSTT1 for improvement of asthma management efficacy.

Material and methods. With respect to the principles of bioethics, on the base of allergy department of Chernivtsi Regional Children's Hospital it has been conducted a comprehensive clinical and paraclinical examination of 51 children with symptoms of exercise induced asthma phenotype. In all examined patients using multiplex polymerase chain reaction (PCR), the polymorphisms of promoter region of genes GSTT1 and GSTM1 has been determined. As a positive control of the PCR quality the amplification of gene BRCA1 fragments was used.

Analysis of PCR has been performed by electrophoresis in 2 % agars’ gels by Maniatis et al., 1984. Homozygous deletion forms of both copies of the genes GSTT1 and GSTM1 (T1del, M1del) have been identified in the absence of the corresponding fragment, visualized by the electrophoregram. Instead, the presence of these fragments on the electrophoregrams testified to homo- or heterozygosity for a normal copy of the gene, designated such genotype as T1+ and M1+ respectively.

Depending on the presence or absence of polymorphisms in studied encoding glutathione-S-transferase genes, two comparison group have been formed. The first (I) clinical group consisted of 19 children with exercise induced bronchial asthma, who did not have deletion polymorphism of the studied genes, and that is why their genotype was determined as $GSTT_1+ M_1+$. Their average age was 11,8±0,65 years, and among them there were 57,9 % of males and 42,1 % of females. One third (31,6 %) of these patients was admitted to the hospital for a regular check-up, but the rest of children (68,4 %) were hospitalized because of asthma exacerbation. The second (II) comparison group contained 32 patients with EIA, who had deletion polymorphism of

studied genes of detoxification enzymes in both homozygous and heterozygous variants that were represented as GSTT1delM1del, GSTT1+M1del or $GSTT_1delM_1+$ genotypes. The number of boys in this group was 65,43 % (P>0,05), and the mean age of patients was equal to 10,6±0,5 years (P>0,05), and at the same time the rate of rural residents was 46,9 % (P>0,05), but only one forth (25,0 %) of children was hospitalized for a regular check-up (P>0,05).Consequently, the main clinical characteristics of the comparison groups and subgroups were comparable.

Determining the asthma severity and the level of asthma control, as well as management approach were conducted according to the orders of Health Ministry of Ukraine № 767 and № 128 [2, 3], according to the recommendations of the Global Initiative for (GINA-2006 and its subsequent versions) [1, 8]. Assessment of a severity of bronchial obstruction during asthma attack was performed by the score scale in the comparison group [4].

These survey results were analyzed by the methods of biostatistics and clinical epidemiology, and using the software package "STATISTICA 7.0" StatSoft Inc. and Excel XP for Windows on a PC, by parametric and nonparametric methods of calculation.

Results and discussion. Severity of asthma was also corresponded on average in patients of the comparison groups. Thus, at the first clinical group persistent mild, moderate and severe course of the disease occurred in 5,3 %, 36,8 % and 57,9 % of patients, respectively. At the second clinical comparison group mild persistent asthma was not registered at all,but moderate and severe course of the disease occurred in 40,6 % and 59,4 % of cases respectively (in all cases P>0,05).

We found no differences in age of the disease onset and then in the duration of the EIA. Thus, early-onset bronchial asthma has been registered in 36,8 % of children in the 1st group and in 34, % of cases in the 2nd comparison group, but late-onset asthma phenotype (over the age of 6 years) has been observed in 47,4 % and 43,8 % of cases in corresponding clinical groups. However, aged 3 to 6 years onset of asthma clinical manifestation has been noticed in 15,8 % and 21,9 % of patients of comparison groups respectively (in all cases P>0,05).

According to the anamnesis' survey there were no reliable differences in frequency of breastfeeding in the comparison groups. Thus, until six months of age 36,8 % of children in the I-st group and 43,3 % of patients in the II-nd comparison group were exclusively breastfed, and at the same time 52,6 % and 40,0 % of patients respectively were breastfed until the age of 1 year, the rest of them were bottle-fed (in all cases P>0,05).

The frequency of birth of patients during the period of the year, when there is greater concentration of pollen allergens in the air, associated with the maximum plants flowering (April-September), there were no significant differences in the comparison group though most of the patients (59,3 %) of the 2nd clinical group were born in other seasons of the calendar year, in contrast to the 1st comparison group, where such cases were only 47,1 % (P>0,05).

At the same time, clinical signs of exudative diathesis, associated in early life mainly with symptoms of atopic dermatitis, have been recorded only in 36,8 % of patients in the 1st group, but more than in half (56,3 %) representatives of the 2nd comparison group (P>0,05). Concomitant allergic disease was observed in 57,9 % of children with EIA in the absence of gene polymorphism of glutathione-S-transferase and in 62,5 % of cases in the comparison group (P>0,05). Not aggravated individual allergic history occurred in 26,3 % of children with genotype GSTT1+M1+ and twice less frequently among the comparison group (13,3 % of cases, P>0,05). Significantly greater number of EIA patients with polymorphisms of genes GSTT1M1 had allergic reactions to household triggers (43,3 % versus 15,8 % cases in the 1st group, P<0,05) as well as to food allergens (10,0 % vs. 5,3 % respectively; P>0,05).

From the above data, one can make a conclusion about greater clinical expression of atopic constitutional type in children with polymorphisms of genes of detoxification that may be explained by external (including household allergens and tobacco smoke) factors overloading. To be exact, among the pathogenic factors of the environment that influenced the course of the exercise-induced asthma in clinical comparison group, the most impressive was the fact that only every other child (50,0 %) in the 1st group was not under a harmful effect of tobacco smoke, being a passive or/and active tobacco smoker. Thus, in this group 37,5 % of patients were considered as passive smokers, and 12,5 % of the school-aged children had already had a personal experience of smoking at the time of the survey. In the

Table

Scoring severity of bronchial obstruction in the comparison group during hospitalization due to asthma exacerbation (M±m)

Clinical groups	Day of hospital stay / score						
	1 day	2 day	3 day	4 day	5 day	6 day	7 day
I group	14,3±1,7	13,4±1,7	11,3±1,7	9,0±1,5	7,5±1,8	6,2±0,3	4,9±0,6
II group	11,4±1,3	11,1±1,2	9,2±1,02	7,3±0,8	5,5±0,6	4,2±0,3	3,2±0,4
P	>0,05	>0,05	>0,05	>0,05	>0,05	<0,05	<0,05

Note. P – Student's criterion

comparison group 37,5 % of patients had never had continuous exposure to tobacco smoke (P>0,05), but the same percentage of children were passive smokers (P>0,05), while a quarter of asthma patients with EIA (25,0 %; P>0,05) were active smokers during the study.

In addition, a significantly greater number of patients of the 1st group (36,4 %) lived under more environmentally favorable conditions but only 13,3 % of children of comparison group (P<0,05), while 18,2% of children with genotype GSTT1+M1+ and a third (33,3 %) of patients with EIA deletions of studied genes (P> 0,05) lived in conditions with expressive ecological pollution. This contributed to the year-round clinical symptoms of EIA in the representatives of 2ndclinical group in 12,5 % of cases, although in the 1stgroup the fall and spring seasonality of asthma attacks predominated in 70,6 % of patients.

Scoring evaluation of asthma attack severity, which was carried out for patients admitted to the department because of asthma exacerbation, revealed differences in the severity of bronchial obstruction syndrome regardless of the presence or absence of gene polymorphism of xenobiotics' biotransformation enzymes (table).

In particular, while the representatives of the 1stclinical group at the time of admission to the hospital because of asthma exacerbation had only pronounced tendency of a more severe bronchial obstruction syndrome, within a week of in-patient treatment their symptoms of airways' obstruction acquired statistically significant scores.

Thus, in children with deletion polymorphism of genes GSTT1M1, suffering from EIA, the greater expressiveness of allergization of the organism is more common, at the same time, almost 63,0 % of these children are under the influence of tobacco smoke, and one fourth of them are active smokers, when 86,7 % of the patients are living in unfavorable ecological surrounding. It should be noted that asthma attacks in these children are much milder, and the effect of bronchodilator treatments is more rapid as compared to the EIA patients with genotype $GSTT_1+M_1+$.

Conclusions

1. The presence of deletion polymorphism of genes $GSTT_1M_1$ is associated with more severe course of EIA, though with more rapid effect of standard reliever treatments as compared to the patients with genotype $GSTT_1+M_1+$.

2. The patients with EIA in the presence of deletion genes polymorphism of $GSTT_1M_1$ require a special attention on their environmental enhancement and normalization of their life-style.

The prospect of further research is to study the characteristics of local inflammation in the bronchi in children with exercise induced asthma with the gene polymorphism of enzymes for biotransformation of xenobiotics.

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ОСОБЕННОСТИ ТЕЧЕНИЯ БРОНХИАЛЬНОЙ АСТМЫ ФИЗИЧЕСКОГО НАПРЯЖЕНИЯ У ШКОЛЬНИКОВ ПРИ ПОЛИМОРФИЗМЕ ГЕНОВ ГЛУТАТИОН-S-ТРАНСФЕРАЗЫ

Е.К. Колоскова, Е.Г. Григола, Т.М. Белоус, Е.П. Ортеменка

Резюме. Проведено комплексное обследование школьников, страдающих бронхиальной астмой физического напряжения, в зависимости от наличия либо отсутствия полиморфизма генов ферментов глутатион-S-трансферазы

– $GSTT_1M_1$. Отмечено, что астма протекает тяжелее у больных с делециями указанных генов, чаще имеют место аллергические реакции, однако дезобструктивный эффект во время приступов достигается быстрее. Показано, что у больных бронхиальной астмой физического напряжения при наличии делеционного полиморфизма генов $GSTT_1M_1$ следует обратить особенное внимание на оздоровление окружающей среды и нормализацию способа жизни.

Ключевые слова: дети, бронхиальная астма физического напряжения, глутатион-S-трансфераза.

ОСОБЛИВОСТІ ПЕРЕБІГУ БРОНХІАЛЬНОЇ АСТМИ ФІЗИЧНОГО НАПРУЖЕННЯ В ШКОЛЯРІВ ІЗ ПОЛІМОРФІЗМОМ ГЕНІВ ГЛУТАТІОН-S-ТРАНСФЕРАЗИ

О.К. Колоскова, О.Г. Григола, Т.М. Білоус, Є.П. Ортеменка

Резюме. Проведене комплексне обстеження школярів, які хворіють на бронхіальну астму фізичного напруження, залежно від наявності чи відсутності поліморфізму генів ферментів глутатіон-S-трансферази – *$GSTT_1M_1$*. Відмічено, що астма проходить тяжче у хворих із делеціями у вказаних генах, частіше мають місце алергічні реакції, проте дезобструктивний ефект під час нападів досягається швидше. Показано, що у хворих на бронхіальну астму фізичного напруження за наявності делеційного поліморфізму генів *$GSTT_1M_1$* слід особливу увагу звернути на оздоровлення навколишнього середовища та нормалізацію способу життя.

Ключові слова: діти, бронхіальна астма фізичного напруження, глутатіон-S-трансфераза.

Буковинський державний медичний університет (м. Чернівці)

Рецензент – проф. Т.В. Сорокман

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