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Pharmacogenetic aspects of basic anti- inflammatory therapy of bronchial asthma in school-aged children under the deletion polymorphism of genes *Gstt$_1$* and *Gstm$_1$*

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Based on a comprehensive survey of 150 school-age children who suffer from bronchial asthma, there has been found that homozygosity for both alleles of the gene of glutathione transferase (*GSTT$_1$+ M$_1$+)* was associated with more frequent use both inhaled corticosteroids at 3 Step 3 of basic therapy and leukotriene modifiers as well as better asthma control level according to the ACT-test. Under the presence of such genotypes as *GSTT$_1$+ M$_1$-*, *GSTT$_1$-M$_1$+* or *GSTT$_1$-M$_1$-*, the basic treatment was more likely within 2-nd and 4-th Steps by GINA recommendations, but the level of asthma control was worse in these children.

Keyword: bronchial asthma, children, glutathione-S-transferase.

1. Introduction

The primary principles of the treatment of bronchial asthma are reduction of the airways inflammation, as well as achievement and maintenance of control of the disease [1]. According to existing guidelines for asthma management, stepwise approach to bronchial asthma therapy is definitely based on indication of disease severity, at that, inhaled corticosteroids (ICS) are the main basic anti-inflammatory drugs [2-3]. In most cases of childhood bronchial asthma after receiving basic therapy by ICS, reduction of the frequency of symptoms and improvement of respiratory function are observed. But in some patients, especially with severe asthma, inefficiency of corticosteroids occurs, that leads to insufficient asthma control [4].

However, the fact that the genetic factor is a one of significant components playing role in asthma control achievement remains the undisputed [5-6], because it forms a specific phenotype of bronchial asthma and, as well, causes both features of responses to stimuli of the environment and response to drug therapy [7]. Some authors observed relatively less efficacy of corticosteroids under the polymorphism of genes CDH1 and SERPINE1 [8-9], but the question about the effectiveness of ICS based on genetic characteristics in children, remains uncertain.

It is noted that this polymorphism of genes encoding enzymes of the second phase of detoxication of xenobiotics, affect the functionality of these enzymes in the lungs and other organs, which increases genetic susceptibility to oxidative stress and bronchial asthma [10]. These genes-modifiers include such genes of glutathione-S-transferase (GST) as $GSTM_1$, $GSTT_1$, and at the same time the association of genotypes $GSTT_1$-\ $GSTM_1$ increase the risk of bronchial asthma in children five times more compared to a population [11-12].

In most cases, the GST brings into detoxification of electrophilic substances, connecting them with glutathione and reducing the harmful effects of reactive oxygen species into essential protein components of cells [13]. However, research on the impact of the genetic components of children to asthma control achieve as well as characteristics of patients' needs for basic anti-inflammatory therapy are currently poorly understood [14-15].

Consequently, the aim of our research was to evaluate basic anti-inflammatory therapy of bronchial asthma in school-age patients to further optimization of the therapeutic approach and maintaining of asthma control.

2. Materials and Methods

2.1 Principle of randomization.

By the method of simple random sampling cohort of 150 school-age children with bronchial asthma was established. At the allergological department of the Chernovtsy Regional Children Clinical Hospital (Ukraine) the complex clinical, laboratory and instrumental investigations was performed for all patients. For establishment of the asthma therapy efficacy depending on the activity of second phase of detoxification of xenobiotics, polymorphism of genes of glutathione-S-transferase family ($GSTT_1$ and $GSTM_1$) was determined by the method of genetically-molecular analysis.

2.2 Methods for determination of polymorphism of GST genes

Determination of deletions in the genes $GSTM_1$ and $GSTT_1$ was conducted using multiplex polymerase chain reaction (PCR), and an amplification of fragments of BRCA1 was used as a positive control of successfulness of PCR. To visualize the DNA fragments, a gel was stained with ethidium bromide and then photogravured under ultraviolet light by the device GelDoc 2000 (BioRad, USA). To determine the length of the fragments, their electrophoretic mobility compared with the mobility of such DNA-marker as Gene Ruler DNA Leader Mix (Fermentas, Lithuania).

Homozygous forms with deletion of both copies of genes $GSTT_1$ and $GSTM_1$ were identified by the absence of the corresponding fragment, visualized by the diaelectrophoresis. These genotypes are designated as T_1- and M_1-. Accordingly, the presence of these fragments testified homo- or heterozygosity by a normal copy of the gene. The genotype of such patients designated as T_1+ and M_1+.

2.3 General characteristic of comparative groups of patients.

In total cohort the genotype $GSTT_1$+M_1+ was observed in 69 patients (46.0%), the genotype $GSTT_1$-M_1+ was determined in 19 (12,7%) children, but the $GSTT_1$+M_1- and $GSTT_1$-M_1- genotypes were identified in 48 (32.0%) and 14 (9.3%) cases correspondingly. Based on the results, all of the patients were divided into two clinical groups. The first (I) clinical group consisted of 69 children with bronchial asthma, who did not have deletion polymorphism of the studied genes, and so their genotype was determined as $GSTT_1$+ M_1+. Theirs average age was 10.71±0.35 years, among them there were 48 boys (69.6%). The second (II) comparison group entered 81 patients, who had deletion polymorphism of studied genes of detoxification enzymes in both homozygous and heterozygous variants, that were represented as $GSTT_1$+M_1-, $GSTT_1$-M_1+ and $GSTT_1$-M_1- genotypes. The number of boys in this group was 53 (65.43%), and mean age of patients was equal to 10.75±0.33 years. There were no differences in place of residence of residence surveyed children. Thus, 34 (49.28%) patients of the first clinical group and 35 (43.21%) children from the II-nd group

lived in the city (P>0.05). 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 7.25%, 49.28% and 43.48% of patients, respectively. At the second clinical comparison group intermittent asthma was registered in 1 child (1.24%), 2 patients (2.47%) had mild persistent asthma, but moderate and severe course of the disease occurred in 46.91% and 49.39% of cases respectively (in all cases P>0.05). Consequently, the main clinical characteristics of the comparison groups and subgroups were comparable, while established minor differences did not affect significantly the results of the study, indicating so a minimal risk of systemic error.

2.4 Principles of bronchial asthma management.

Treatment of asthma conducted according to the GINA-2011 recommendations and the order of Health Ministry of Ukraine № 767. The level of asthma control was scored by ACT test [16, 17].

2.5 Statistical Analysis

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.

2.6 Compliance with bioethics.

The survey was carried out in parallel clinical comparison groups formed on the basis of a simple randomization by the "case-control" method with a strict observance of the bioethical requirements (GCP ICH and the Helsinki Declaration of the World Medical Association on biomedical research, in which a person acts as its object, as well as the order of the Health Ministry of Ukraine № 960 of 23.09.2009).

3. Results and Discussions.

Prescribed basic anti-inflammatory treatment by corticosteroids was accepted by 79.1% patients of the first clinical group and 85.0% of patients with polymorphisms of studied genes (P>0.05). At once, 20.9% of children without $GSTT_1$ and $GSTM_1$ gene polymorphism, as well as 15.0% of patients of II-nd group (P>0.05) did not receive recommended management, and that was evidence of low compliance. According to step approach to asthma management (by GINA-2011 recommendations) [18] prescribed treatment in comparison groups was distributed as follows (Table 1).

Table 1: Stepwise approach (according to GINA-2011) for managing asthma in children of comparison groups (P±m)

Clinical groups	Number of children	Step 1	Step 2	Step 3	Step 4
I group	69	20,9±4,89	20,28±5,01	52,94±6,01	5,88±2,85
II group	81	15,0±3,96	34,72±5,34	33,36±5,3	16,92±3,83
P		>0,05	=0,05	<0,05	=0,05

Note: P – Student's criterion.

So, in every second child with preserved functional activity of the studied genes and in every third patient with a deletion polymorphism basic therapy corresponded to Step 3 by the GINA recommendations. However, in patients of the II-nd group one and a half times more often low doses of ICS as well as monotherapy by leukotriene modifiers were used. At the same time, at this clinical group twice as often Step-4 approach, that considered an active anti-inflammatory treatment (a medium or high doses of ICS in combination with two other drugs from different groups: long-acting β_2- agonists, antagonists of leukotriene receptor modifiers and/or sustained release methylxanthines), was used.

However, the average daily dose of inhaled corticosteroids taken by representatives of clinical comparison group showed a trend to higher doses of these drugs in patients without deletion polymorphism of the studied genes. Thus, the average daily dose of ICS (by

beclomethasone) was 301.51±22.41 mg at the first group, and 265.15±20.25 mg at the comparison group (P>0.05).

The qualitative distribution of usage frequency of low, medium and high doses of ICS in comparison groups given in Table 2.

Table 2: The frequency of usage of equipotential (by beclomethasone dipropionate) doses of ICS as a basic asthma treatment in children of comparison groups (P±m)

Clinical groups	Number of children	ICS were not used	Low doses of ICS	Medium doses of ICS	High doses of ICS
I group	69	20,9±4,89	29,83±5,54	36,23±5,79	13,04±4,05
II group	81	15,0±3,96	45,67±3,95	29,63±5,07	9,7±3,32
P		>0,05	<0,05	>0,05	>0,05

Notes: P – Student's criterion, ICS- inhaled corticosteroids

Thus, low doses of ICS were used frequently in asthmatic children with deletion polymorphism of $GSTT_1$ and $GSTM_1$ genes, but patients of the first clinical group had a tendency to predominance of high and medium doses of corticosteroids. Despite the lack of differences in the frequency of use of ICS during the day by children of the clinical groups (1.58±0.12 vs 1.6±0.1 times/day at the comparison groups, respectively; P>0.05), qualitative distribution of this index testified about trend towards more frequent use of these drugs in patients without deletion polymorphism of $GSTT_1$ and $GSTM_1$ genes. Namely, inhaled corticosteroids once a day were used by 17.91% of children in the first group and 18.75 % of II-nd clinical comparison group (P>0.05), twice a day - by 43.28% and 58.75% children, respectively (P> 0.05) and three times a day – by 17.91% and 7.5% representatives, respectively (P<0.05).

It should be noted that combined with long-acting β_2-agonists (LABA) therapy received 14.92% of the patients without the polymorphism of $GSTT_1$ and $GSTM_1$ genes as well as one in four patients (25.0%, P=0.05) of comparison group. However, the doses of LABA were significantly higher (47.38±7.44 mg/day) at the group of children with the deletion polymorphism of $GSTT_1$ and $GSTM_1$ genes, compared to the representatives of the I-st clinical group (36.1±9.38 mg/day, P> 0,05).

As an additional per oral therapy asthma patients received sustained release theophyllines and antagonists of leukotriene receptors: 8.96% and 7,46% of the children of the I-st group, respectively, as well as 8.64% (P>0.05) and 3.75% (P>0.05) of the patients of comparison group, respectively.

Thus, the features of the basic anti-inflammatory treatment of children with bronchial asthma asthma who have no signs of deletion polymorphism of $GSTM_1$ and $GSTT_1$ genes, are more aggressive (use of medium and high dosages of ICS - within Step 3 by GINA recommendations) managing of asthma and twice as of often usage of leukotriene modifiers as a complementary therapy.

In contrast, in school-aged asthma patients under the presence of genotype $GSTT_1$+M_1-, $GSTT_1$-M_1+ or $GSTT_1$-M_1-, the basic controlling therapy (within 2-nd and 4-th Steps by GINA) received a few more children. Namely, within this clinical group in the 1,5 times more often low doses of ICS or monotherapy by antagonists of leukotriene receptors, and twice more often the aggressive anti-inflammatory complex treatment (according to Step 4) as well were used. These patients had lower doses of inhaled corticosteroids, but in every fourth patient ICS was combined with LABA, which dosage was higher than at the I-st comparison group.

The average results of ACT-test, that reflected the level of asthma control in patients of comparison groups, are shown in Table 3. Despite the lack of statistically significant differences in average ACT-test results, there were marked tendency towards better control of the disease in children without deletion polymorphism of the studied genes. However, the qualitative distribution of the points sum of the controlling criteria has been showed that the total score of ACT-test more than

Table 3: Comparative assessment of asthma by ACT questionnaire in children of clinical comparison groups (M±m)

Indices of asthma control (points)		Clinical groups		P
		I group	II group	
Children under 12 years old	Q 1	1,50±0,22	1,80±0,25	>0,05
	Q 2	1,17±0,48	1,8±0,29	>0,05
	Q 3	1,83±0,31	1,5±0,22	>0,05
	Q 4	2,0±0,26	1,6±0,34	>0,05
	Q for parents 1	3,17±0,31	2,5±0,43	>0,05
	Q for parents 2	3,33±0,56	2,9±0,35	>0,05
	Q for parents 3	3,5±0,22	3,5±0,27	>0,05
	Total:	**16,5±2,08**	**15,5±1,66**	**>0,05**
Children 12 years old and older	Q 1	3,29±0,2	2,97±0,18	>0,05
	Q2	3,13±0,21	2,97±0,24	>0,05
	Q 3	3,33±0,29	3,62±0,24	>0,05
	Q 4	3,13±0,28	2,72±0,22	>0,05
	Q 5	3,21±0,24	3,1±0,14	>0,05
	Total:	**16,0±0,94**	**15,41±0,68**	**>0,05**

Note: P – Student's criterion, Q- question.

20 points, which associate with controlled disease, was observed in 41.18% of children without evidence of polymorphism of $GSTM_1$ and $GSTT_1$ genes, but in 57.14% of cases in the second group (P>0.05). At the same time, partly controlled asthma (total score by the ACT-test is 16 to 19 points) and uncontrolled asthma (total score less than 15 points) was identified among the 29.41% and 29.41% cases, respectively, at the I-st group as well as 38,1% (P>0.05) and 4.76% (P<0,05) children of the comparison group, respectively.

4. Conclusion

At the complex basic anti-inflammatory treatment of bronchial asthma, more often medium and high doses of inhaled corticosteroids, that correspond to Step 3 management approach according to GINA recommendations, as well as twice as often antagonists of leukotriene receptors, were used under the presence of genotype $GSTT_1+M_1+$ in asthma school-aged patients. Under the presence of genotypes $GSTT_1+M_1-$, $GSTT_1-M_1+$ or $GSTT_1-M_1-$ stepwise managing asthma was within 2nd and 4th Steps (according to GINA) and every fourth patient used a combination of inhaled corticosteroids with long-acting β_2-agonists, which dosage was higher, however asthma control level was worse in these children.

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