

Efficiency estimation of basic therapy of severe asthma in children with deletion polymorphism gene of second phase biotransformation of xenobiotics (GSTT1, GSTM1)

Key words: bronchial asthma, children, treatment, deletion polymorphisms in genes GSTM1 and GSTT1.

Bronchial asthma (BA) is a widespread pathology, which affects up to 4 % of adults and 9–13 % of children population in developed countries [1, 2]. At least 10–12 % of patients with bronchial asthma suffer from severe form of the disease, which is uncontrollable, despite avoiding exposure to environmental factors trigger, conducting adequate therapy and optimal treatment adherence [3]. Modern treatment approaches of bronchial asthma is considered the stepped therapy due to severity and the control level of the disease [4]. Asthma control is considered to be the final goal of asthma therapy [5]. The use of inhaled corticosteroids is the «cornerstone» in achieving and maintaining control in patients with persistent asthma [6], because these drugs reduce the risk of exacerbations and necessity for hospitalization to intensive care department [7, 8]. Since the base of bronchial asthma is the inflammation of the bronchi, inflammatory process is the major target of anti-asthmatic treatment [9], and inhaled corticosteroids are the most effective control drugs of airway inflammation in patients [6, 10].

One aspect of inadequate control of severe asthma is phenotypic heterogeneity of disease, particular, age debut (asthma early and late onset), bronchial inflammation (eosinophilic and noneosinophilic), the speed of bronchial obstruction (torpid and labile obstruction), response to basic therapy (steroid-sensitive and resistant bronchial asthma) [11–13].

In addition to phenotypic heterogeneity, heterogeneity of response to treatment is based on the polymorphism gene of second phase biotransformation of xenobiotics. Influenced by these enzymes xenobiotics (including medications) transfer in soluble non-toxic products that are excreted by enzymes of third phase of detoxification. This genetically programmed system makes a unique adaptive capacity of every

person, resistance to damaging factors of the environment. The genes, that control the synthesis of these enzymes, are characterized by significant population polymorphism, which is often accompanied by functionally defective alleles with asthma development or can cause various effectiveness of response to treatment [14–16].

The aim of the study was to evaluate the effectiveness of antirecurrent basic treatment of schoolchildren, suffering from severe persistent asthma with deletion polymorphism in the gene of phase II biotransformation of xenobiotics (*GSTT1*, *GSTM1*).

Material and methods

In pulmoallergologic department of Regional Children Clinical Hospital (Chernivtsi, Ukraine) 70 children with severe asthma were examined. The average age of patients was $11,9 \pm 0,4$ years, boys (67,1 %) and residents of rural areas (62,9 %) were dominated.

Patients were performed genotyping *GSTM1* and *GSTT1*. The total genomic DNA isolated from blood due to standard protocol. Polymorphism of *GSTM1* and *GSTT1* was detected by multiplexed PCR and analyzed by electrophoresis on 2 % agarose gels [17]. Deletion of genes *GSTM1* and *GSTT1* was designated as T1del and M1del. Homo- or heterozygosity for normal copies of the gene were designated T1+ and M1+. Expected length of DNA fragments (431 np for *GSTT1* and 120 np for *GSTM1*) were calculated using the software package Computer data processing DNASTAR using these gene sequences available in the database Genbank. Homozygous deletion forms of both copies of the genes *GSTT1* and *GSTM1* were identified as the absence of appropriate fragment in elektroforegram and were

Table 1
Evaluation of severe asthma control before and after the antirecurrent treatment appointment in patients according ACT-test

Control criteria	Evaluation of control, points		p
	before treatment	after treatment	
Limit activity	3,0 ± 0,1	3,8 ± 0,2	< 0,05
Daytime symptoms	2,8 ± 0,2	3,4 ± 0,3	> 0,05
Night symptoms	3,7 ± 0,2	3,9 ± 0,2	> 0,05
The need for β ₂ -agonist	3,0 ± 0,2	3,3 ± 0,3	> 0,05
Self-control	3,2 ± 0,1	3,3 ± 0,2	> 0,05
Total score	15,7 ± 0,7	17,6 ± 0,9	> 0,05

Table 2
Evaluation of severe asthma control before and after antirecurrent treatment appointment in patients with genotype T1 + M1 + according ACT-test

Control criteria	Evaluation of control, points		p
	before treatment	after treatment	
Limit activity	3,0 ± 0,2	3,8 ± 0,4	> 0,05
Daytime symptoms	2,9 ± 0,3	3,8 ± 0,2	> 0,05
Night symptoms	3,4 ± 0,4	3,0 ± 0,4	> 0,05
The need for β ₂ -agonist	3,1 ± 0,5	3,2 ± 0,6	> 0,05
Self-control	3,3 ± 0,3	3,0 ± 0,3	> 0,05
Total score	15,6 ± 1,3	16,8 ± 1,5	> 0,05

Table 3
Evaluation of severe asthma control before and after antirecurrent treatment appointment in patients with deletions in the genes GSTM1 and GSTT1 according ACT-test

Control criteria	Evaluation of control, points		p
	before treatment	after treatment	
Limit activity	3,1 ± 0,3	4,2 ± 0,3	< 0,05
Daytime symptoms	3,2 ± 0,4	3,7 ± 0,4	> 0,05
Night symptoms	4,0 ± 0,3	4,3 ± 0,2	> 0,05
The need for β ₂ -agonist	3,0 ± 0,4	3,7 ± 0,3	> 0,05
Self-control	3,2 ± 0,1	3,8 ± 0,2	> 0,05
Total score	16,5 ± 1,0	19,7 ± 1,1	< 0,05

designated as M1del and T1del. Accordingly, the presence of these fragments on elektroforegrams were indicated of homo- or heterozygosity of the normal copy of the gene. This genotype was designated as M1 and T1 + + [18].

Depending on availability (37 children, the 2nd group) or absence (33 students, 1st group) deletions in the GSTM1 and GSTT1 genes two clinical groups of patients were formed. According to main clinical characteristics, these clinical groups were comparable.

Determination control the disease were performed using ACT-test (Asthma Control Test, Quality Metric Incorporated, 2002) [19, 20], according to which, each question was evaluated from 0 to 5 points. As improvement in asthma control was elevated, the total number of points were increased, and the index of satisfactory control was considered score 16 or more. The volume and regime of basic treatment of bronchial asthma was specified by the current international and national protocols [21, 22] the effectiveness of the treatment was evaluated prospective baseline by 3 months in terms of absolute risk reduction, relative risk reduction, taking into account the minimum number of patients who need to treat for one positive results [23, 24].

Results

Despite regulated by international and national directive documents, arrangements of medical care for children with severe persistent asthma [21, 22] is the quite often problem of achieving and retaining control over its manifestations, which is the main aim of treatment. The reason was caused by low adherence to a long course of therapy, inadequate evaluation of severity and treatment level system in case of «exacerbation-remission» and imperfections of individualized approach based on phenotypic heterogeneity of the disease. A result of research noted that exercise tolerance in patients with severe asthma significantly improved after basic controlled treatment. Frequency daytime and night time symptoms, necessity of short-β₂-agonists application in patients with severe asthma significantly reduced after basic controlled treatment (Table 1).

It was established that exposure antirecurrent basic asthma treatment helped to achieve complete control in 43,8 % of patients, partial control in 37 % of patients with severe asthma.

Literary sources actively showed dependence of intensity and duration of relief therapy of attack to the availability of deletions in the genes of enzymes of II phase xenobiotics biotransformation (GSTM1 and GSTT1) in children with asthma. In particular, exacerbation of asthma in children with absence of GSTM1 and GSTT1 deletion polymorphism requires more active treatment, including the appointment of systemic glucocorticosteroid medications, due to rapid pharmacokinetics of drugs in intact functional activity of genes encoding certain enzymes of biotransformation of xenobiotics [25]. But these contention is contradictory and currently not well understood regarding basic treatment of asthma [26]. Extrapolating these assumptions on the effectiveness of standard treatment about possible variability in the therapeutic effect, we evaluated the effectiveness of anti-inflammatory therapy in patients with severe asthma, depending on the presence (or absence) in patients deletions in the genes GSTM1 and GSTT1 (Table 2).

In patients with severe asthma and complete genes *GSTM1* and *GSTT1* the therapy has proved effective by mainly increasing exercise tolerance with a decrease of daily activity limit and decrease daytime symptoms, while total positive dynamics of control has not acquired probable values. In assessing the effectiveness of treatment in 2nd group is observed probable dynamics as improving control by mostly regression of daily activity limit, improving of self control evaluation, decrease daytime and nighttime symptoms and necessity of using short-active β 2agonists (Table 3).

The proportion of children with severe asthma with genotype T1+M1+ and satisfactory control treatment was 25,0 %, after the therapy was 42,8 % ($R\phi > 0,05$). In patients with severe asthma and deletions in these genes, indices were respectively 53,7 % ($R\phi < 0,05$) and 83,3 % ($R\phi < 0,05$). Absolute risk reduction of uncontrolled severe asthma in patients without deletion polymorphisms in genes *GSTM1* and *GSTT1* was 17,8 %, relative risk reduction was 23,7 %, number needed to treat was 5,6 patients. However, the absolute risk reduction of uncontrolled severe asthma in patients with gene deletion polymorphism was 29,6 %, relative risk reduction was 63,9 %, number needed to treat was 3,3 patients. The identified patterns could be explained by some facts. In particular, the function of defective genes with deletion polymorphism could take other loci, or slow drugs biotransformation led to their prolonged activity in the body in II clinical group children.

Conclusions

1. Improving of asthma controllability was detected in both clinical groups after the basic treatment.

2. Control indices of the disease were proved to be significantly higher in children with severe asthma and deletion polymorphisms of genes biotransformation of xenobiotics *GSTM1* and *GSTT1* than in schoolchildren with complete copies of these genes.

Prospects for future research are the dynamics evaluation of laboratory and spirometric indices on the background of the basic treatment depending on the presence or absence of gene deletions of II phase xenobiotics biotransformation (*GSTM1* and *GSTT1*).

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ОЦЕНКА ЭФФЕКТИВНОСТИ БАЗИСНОЙ ТЕРАПИИ
ТЯЖЕЛОЙ БРОНХИАЛЬНОЙ АСТМЫ У ДЕТЕЙ
ПРИ ДЕЛЕЦИОННОМ ПОЛИМОРФИЗМЕ ГЕНОВ ВТОРОЙ
ФАЗЫ БИОТРАНСФОРМАЦИИ КСЕНОБИОТИКОВ
(GSTT1 И GSTM1)

Л. А. Иванова, Н. Н. Гарас

Резюме

Контроль бронхиальной астмы (БА) считается конечной целью терапии. Одним из аспектов недостаточного контроля тяжелой БА является ее фенотипическая неоднородность. Кроме фенотипической гетерогенности в основе неоднородности ответа на лечение находится полиморфизм генов второй фазы биотрансформации ксенобиотиков. Указанные гены имеют значительный популяционный полиморфизм, который нередко сопровождается появлением функционально неполноценных аллелей, наличие которых может как способствовать развитию БА, так и вызывать разную эффективность ответа на лечение.

Цель исследования: оценить эффективность базисной противорецидивной терапии у детей школьного возраста, больных тяжелой персистирующей БА, при делеционном полиморфизме генов второй фазы биотрансформации ксенобиотиков (GSTT1 и GSTM1).

Материалы и методы исследования. На базе пульмоаллергологического отделения Областной детской клинической больницы (г. Черновцы, Украина) обследовано 70 детей, больных тяжелой БА. Пациентам проведено генотипирование GSTM1 и GSTT1. В зависимости от наличия (37 детей, 2-я группа) или отсутствия (33 школьника, 1-я группа) делеций в генах GSTT1 и GSTM1 сформированы 2 клинические группы. Определение контроля за ходом заболевания проводили с использованием АСТ-теста проспективным методом через 3 месяца.

Результаты исследования. Установлено, что под влиянием противорецидивного лечения полного контроля БА достигли 43,8 % пациентов, частичного — 37 % страдающих тяжелой БА. У больных тяжелой БА и с полноценными генами GSTM1 и GSTT1 базисная терапия оказалась эффективной в основном за счет повышения толерантности к физической нагрузке с уменьшением ограничения повседневной активности и урежением дневных симптомов. В то же время суммарная положительная динамика контроля не получила достоверных значений. При оценке эффективности лечения пациентов 2-й группы наблюдается достоверная динамика улучшения контроля в основном за счет регресса ограничения повседневной активности, а также улучшения самооценки контроля, урежения дневных и ночных симптомов и необходимости использования препаратов быстродействующих селективных адrenomиметиков. Снижение абсолютного риска неконтролируемого течения тяжелой БА у больных без делеционного полиморфизма генов GSTM1 и GSTT1 составило 17,8 %, снижение относительного риска — 23,7 % при числе больных, которых необходимо пролечить для получения одного положительного результата, 5,6. В то же время, снижение абсолютного риска неконтролируемого течения тяжелой БА у школьников с делеционным полиморфизмом указанных генов составило 29,6 %, снижение относительного риска — 63,9 % при минимальном количестве больных, которых следует пролечить для получения одного положительного результата, 3,3.

Выводы. После проведенной базисной терапии в обеих клинических группах выявлено улучшение контролируемости течения БА. На фоне базисного противорецидивного лечения показатели контроля заболевания у детей с тяжелой БА и делеционным полиморфизмом генов биотрансформации ксенобиотиков GSTM1 и GSTT1 оказались достоверно выше, чем у школьников с полноценными копиями указанных генов.

Ключевые слова: бронхиальная астма, дети, лечение, делеционный полиморфизм генов GSTT1 и GSTM1.

EFFICIENCY ESTIMATION OF BASIC THERAPY OF SEVERE
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OF XENOBIOTICS (GSTT1, GSTM1)

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Summary

Asthma control is considered to be the final goal of asthma therapy. One aspect of inadequate control of severe asthma is phenotypic heterogeneity of disease. In addition to phenotypic heterogeneity, heterogeneity of response to treatment is based on the polymorphism gene of second phase biotransformation of xenobiotics. These genes are characterized by significant population polymorphism, which is often accompanied by functionally defective alleles with asthma development or can cause various effectiveness of response to treatment.

The aim of the study was to evaluate the effectiveness of antirecurrent basic treatment of schoolchildren, suffering from severe persistent asthma with deletion polymorphism in the gene of phase II biotransformation of xenobiotics (GSTT1, GSTM1).

Material and methods. In pulmoallergologic department of Regional Children Clinical Hospital (Chernivtsi, Ukraine) 70 children with severe asthma were examined. Patients were performed genotyping GSTM1 and GSTT1. Depending on availability (37 children, the 2nd group) or absence (33 students, 1st group) deletions in the GSTM1 and GSTT1 genes two clinical groups of patients were formed. Determining asthma control was performed using ACT-test with prospective method after 3 months.

Results. It was established that exposure antirecurrent basic asthma treatment helped to achieve complete control in 43,8 % of patients, partial control in 37 % of patients with severe asthma. In patients with severe asthma and complete genes GSTM1 and GSTT1 the therapy has proved effective by mainly increasing exercise tolerance with a decrease of daily activity limit and decrease daytime symptoms, while total positive dynamics of control has not acquired probable values. In assessing the effectiveness of treatment in 2nd group is observed significant dynamics as improving control by mostly regression of daily activity limit, improving of self control evaluation, decrease daytime and nighttime symptoms and necessity of using short-active β_2 -agonists. Absolute risk reduction of uncontrolled severe asthma in patients without deletion polymorphisms in genes GSTM1 and GSTT1 was 17,8 %, relative risk reduction was 23,7 %, number needed to treat was 5,6 patients. However, the absolute risk reduction of uncontrolled severe asthma in patients with gene deletion polymorphism was 29,6 %, relative risk reduction was 63,9 %, number needed to treat was 3,3 patients.

Conclusions. After the basic treatment improving of asthma controllability was detected in both clinical groups. Control indices of the disease in children with severe asthma and deletion polymorphisms of genes biotransformation of xenobiotics GSTM1 and GSTT1 were proved to be significantly higher than in schoolchildren with complete copies of these genes.

Key words: bronchial asthma, children, treatment, deletion polymorphisms in genes GSTM1 and GSTT1.

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