Abstract

114 patients of school age were observed at the serum and pulmonary department of Chernivtsi regional child clinical hospital with the aim of detecting the correlation between GSTT1 and GSTM1 gene deletion and non-specific bronchial hyperresponsiveness in children, suffering from eosinophilic bronchial asthma. Eosinophilic asthma (I clinical group) was diagnosed in 68 patients. 46 patients formed the II clinical group (non-eosinophilic asthma). It has been found that genotype T1delM1+ was more often registered with eosinophilic BA, and genotype T1+ M1del – in patients with non-eosinophilic phenotype of the disease. Coincidentally, genotype T1delM1del equifrequently occured in patients with different types of respiratory inflammation, and in patients with eosinophilic asthma was connected with more severe course of the disease. In patients, suffering from eosinophilic bronchial asthma, who were the carriers of defective alleles of GSTT1 and GSTM1 genes in homozygous state, there appeared a tendency to increased bronchial lability due to more distinctive bronchial spasm, and the index of bronchial hyperresponsiveness proved to be higher than in children with functionally adequate alleles of these genes. Thus, the genetically determined absence of activity of certain xenobiotic-metabolizing enzymes, namely, GSTT1 and GSTM1, may cause the more definite bronchial lability.

Thus, genotype determination of glutathione S-transferases in patients with eosinophilic bronchial asthma with due regard to bronchial hyperresponsiveness allows to predict the subsequent course of the disease and to devise the individualized therapeutic approach.

Introduction

Bronchial asthma (BA) is one of the most common conditions of a human in all age groups, the severe forms of which lead to disability and falling off in patients’ health-related quality of life, thus creating a serious sociomedical problem. It’s a typical multifactor disease, rested on a complex character of interrelation between genetic and environmental factors [1].

BA prevention is built with due regard to attainments of modern allergology but is still characterized by a quite low efficiency. In fact, the weaknesses of the current therapy and prevention complexes, presented as treatment regimen and prescriptions, depend significantly on the fact of existing of different variations of BA and different phenotypes of the disease. All these facts demand the development of the individual approach to BA therapy. In this dimension, a complex account of respiratory ways hyperresponsiveness indices and the character of inflammation, the factors which mainly determine BA polymorphism, may seem promising as to the development of individual therapy and prevention measures.

Given the ecological and toxicological basis of many multifactor diseases [2], including asthma, it seems appropriate to study the involvement of genes abnormal effects of which on phenotypic level occur under the influence of factors of chemical nature. These genes include genes of enzymes of biotransformation of xenobiotics (XBE) and, in particular, glutathione-S-transferase (GST). It is established that GST in humans is encoded in a large multigene family including more than 20 genes [3, 4], the function of many of which still needs further study. Relatively well studied is the cytoplasmic
isomorph GSTT1 and GSTM1, taking part in the detoxification of many toxins, products of oxidative stress, carcinogens and drugs.

In human populations widespread are polymorphisms in the genes GSTM1 and GSTT1, which are associated with deletions of both large fragments of genes, making them functionally deficient (null allele T1del and M1del). It has been proved that in homozygous individuals for the deletion of GSTT1 genes GSTM1 enzymatic activity of the respective isomorph is lost [5, 6].

Special literature describes studies on the interrelation between polymorphisms of genes GSTT1, GSTM1 and asthma in children and adults [7–9]. However, they are ambiguous and do not take into account the peculiarities of different phenotypes of asthma that arise from the interaction of environmental factors and genetic susceptibility to the disease. Particularly now the question remains as to phenotypic features of the disease and their interrelation of polymorphisms of genes XBE.

**The purpose of the study**

To find out the relationship between the presence of deletions in the genes GSTM1 and GSTT1 and non-specific bronchial hyperresponsiveness in children with eosinophilic asthma.

**Materials and methods**

114 patients of school age were observed at the serum and pulmonary department of Chernivtsi regional child clinical hospital. The average age of patients was 11.1 ± 0.3 year; surveyed was 79.4 % of boys. All children underwent a comprehensive survey, which included evaluation of non-specific bronchial hyperresponsiveness. The diagnosis of asthma was set on international standards GINA-2009 [10] and the Order of the Ministry of Health of Ukraine [11]. Taking into account the published data on the probability of the existence of different phenotypes depending on the type of airway inflammation, which determine the features of asthma in children, 2 phenotypes of the disease in a cohort of patients indicated were established [10, 12, 13, 14]. Eosinophilic asthma phenotype was defined in patients whose sputum contained 3 % or more of eosinophilic leukocytes (I clinical group). Non-eosinophilic inflammation of the bronchial tree contained 3 % or more of eosinophilic leukocytes (I clinical group). Non-eosinophilic inflammation of the bronchial tree was assessed by spirometric tests with histamine considering as hypersensitivity to it, it was thought advisable to study gene polymorphism of glutathione-S-transferase M1 and T1 in patients with different types of respiratory tract inflammation (Table 1).

The results of genotyping give reason to believe that the genotype T1delM1+ is more (15.5 %) often registered with eosinophilic asthma than the type of neutrophilic airway inflammation (11.6 %). Genotype T1+ M1del was more often recorded in children with the non-eosinophilic disease phenotype (32.6 %) than in their peers suffering from eosinophilic asthma (28.9 %). Genotype T1delM1del, which leads to the simultaneous loss of activity of the two isomorphs of GST, was equally recorded in patients with different types of airway inflammation. Therewith the severe form of the disease was detected in 4 of 5 T1delM1del genotype carriers (80 %) in patients with eosinophilic asthma and in 2 of 5 carriers (40 %) with neutrophilic type of airway inflammation. At the same time, in T1+M1+ genotype carriers severe form of the disease was diagnosed in 12 of 21 (57.1 %) patients with eosinophilic asthma and in 8 of 19 (42.1 %) with non-eosinophilic type of airway inflammation. Thus, in patients with eosinophilic asthma phenotype, having T1delM1del genotype, the disease often ran into severe.

**Detection of deletions in the genes GSTM1 and GSTT1** was performed using multiplexed polymerase chain reaction (PCR). As a positive control of PCR success amplification of gene fragments BRCA1 was used. Analysis of PCR was performed by electrophoresis in 2 % agarose gels [17]. To visualize DNA fragments they were stained with ethidium bromide gel and photographed under ultraviolet light using the facility GelDoc 2000 (BioRad, USA). To determine the length of the fragments obtained their electrophoretic mobility was compared with the mobility of DNA Marker Gene Ruler DNA Ladder Mix (Fermentas, Lithuania).

Expected length of DNA fragments (431 np for GSTT1 and 120 np for GSTM1) were calculated using the software package DNASTAR computer data using sequences of genes GSTT1 and GSTM1, which are available in the database Genbank. Homozygous deletion forms of both copies of the genes GSTM1 and GSTT1 were identified in the absence of appropriate fragment on electrophoregram. These genotypes designated as T1del and M1del. Accordingly, the presence of these fragments on electrophoregram showed homo- or heterozygosity in normal copies of the gene. The genotypes of patients were designated as T1 + and M1 + (Fig. 1).

**Figure 1.**

Non-specific bronchial hyperresponsiveness was assessed by spirometric tests with histamine considering as hypersensitive airways (RK20H) and their hyperactivity, according to a dose-dependent curve (DDC) [18]. Bronchial lability was studied as bronchial lability index (BLI), which was an estimate of spasm after dosed physical activity (IBS — index of bronchospasm) and bronchodilation following inhalation of salbutamol (IBD — index of bronchodilatation) [18].

Statistical analysis of the results was performed on a personal computer using the application package Statistica 5.0.

**Results and Discussion**

Taking into account the fact that BA is a typical multifactor disease in the development of which the significant role is played by both environmental factors and genetic predisposition to it, it was thought advisable to study gene polymorphism of glutathione-S-transferase M1 and T1 in patients with different types of respiratory tract inflammation (Table 1).
In general, it should be noted that in almost half of the patients of both groups there was a deletion of genes of glutathione-S-transferases.

Taking into consideration the presence of deletions in the genes of xenobiotics biotransformation enzymes in children surveyed, it could be suggested the slowdown in detoxification processes, leading to changes in non-specific bronchial hyperresponsiveness.

It is proved that with eosinophilic asthma phenotype the proportion of children who are sick at the age of 6 years comprised 51,2 ± 5,4 %, and with non-eosinophilic asthma it was 52,6 ± 5,7 % (p > 0,05). Meanwhile, in the children with the “early-onset” asthma (up to 3 years) eosinophilic phenotype of the disease was more frequently recorded – in 32,1 ± 5,2 % of cases. In contrast, in patients with no n-eosinophilic asthma type “early onset” disease was recorded in 26,3 ± 5,0 % of observations (p > 0,05). Despite the fact that light and severe courses of the disease were recorded more often with eosinophilic asthma phenotype, the proportion of children with moderate course of disease was more likely with non-eosinophilic type of airway inflammation (55,3 ± 5,7 %) compared with eosinophilic phenotype (39,3 ± 5,3, p < 0,05).

The study of sputum revealed that the percentage of eosinophils in patients of I clinical group was 12,7 ± 1,7 %, and in the comparison group – 1,57 ± 0,1 %.

The obtained results suggest that the definition of GST genotypes in patients with eosinophilic asthma based on their bronchial hyperresponsiveness will predict the future course of the disease and to develop individualized treatment approaches.
### Conclusions

1. Genotype *T1*M1+ is more often registered with eosinophilic asthma and genotype *T1*M1del – in patients with non-eosinophilic phenotype of the disease.

2. Genotype *T1delM1del* is equifrequent recorded in patients with different types of airway inflammation.

3. The presence of genotype *T1delM1del* in eosinophilic asthma patients associated with severe disease course.

4. Among patients with eosinophilic asthma there was a tendency of increasing the index of bronchial lability due to more expressive bronchospasm and bronchial hyperreactivity rate was significantly higher than in children with functional full alleles of these genes.

### References


### Table 3

<table>
<thead>
<tr>
<th>Genotype GSTT1 and GSTM1</th>
<th>Bronchial lability indices, %</th>
<th>Bronchial hyperresponsiveness indices</th>
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<td>T1+M1+ n = 21</td>
<td>12,5 ± 2,4</td>
<td>21,6 ± 3,5</td>
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<tr>
<td>T1M1del: T1delM1+; T1delM1del n = 25</td>
<td>11,4 ± 2,0</td>
<td>24,5 ± 4,1</td>
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|  | Bronchodilation index | Bronchial hyperresponsiveness index, RK20H, (mg/ml) | Hyperreactivity index DDC, (RVU) |
|--------------------------|-----------------------------------|----------------------------------------|
| T1+M1+ n = 21            | 0,25 ± 0,1                        | 1,3 ± 0,4                              |
| T1M1del: T1delM1+; T1delM1del n = 25 | 2,0 ± 0,2                        | 1,9 ± 0,2                             |

### Table 4

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<td>24,5 ± 4,1</td>
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У больных с эозинофильной формой БА чаще наблюдается генотип T1delM1+, а генотип T1+M1del – у больных с нейтрофильной формой заболевания. В то же время, генотип T1delM1del с одинаковой частотой регистрировался у больных с разным типом воспаления дыхательных путей, а у пациентов с эозинофильной формой БА, но не эозинофильной, он был менее распространен. У больных с эозинофильной формой БА, которая ассоциировалась с тяжелым вариантом заболевания, генотип T1delM1del с одинаковой частотой регистрировался у детей с инфекционным, у детей с нейтрофильным и у детей с эозинофильным типом воспаления дыхательных путей. Кроме того, у детей с эозинофильной формой БА, у которых были дефектные аллели генов GSTT1 и M1 в гомозиготном состоянии, регистрировалась тенденция к повышению лабильности бронхов за счет более выраженного бронхоспазма, а показатель гиперреактивности бронхов у них был достоверно выше, чем у детей с функционально полноценными аллелями этих генов.

Таким образом, генетически обусловленная хромосомная патология системы биотрансформации ксенобиотиков, в частности GSTT1 и M1, может бать причиной более выраженной лабильности бронхов. Определение генотипов глутатион-S-трансфераз у больных с эозинофильной формой БА позволит прогнозировать дальнейшее течение заболевания и разработать индивидуализированные подходы к лечению.

Ключевые слова: дети, бронхиальная астма, гены системы биотрансформации ксенобиотиков.