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ASSOCIATION OF GLUTATHIONE-S-TRANSFERASE M_1/T_1 GENOTYPES WITH AIRWAY REMODELING IN CHILDREN WITH BRONCHIAL ASTHMA

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In this work, a study of individual clinical, laboratory and spirographic features of bronchial remodeling in children with bronchial asthma with an alternative $GSTT_1$ and $GSTM_1$ genotype was carried out. It has been established that in children with bronchial asthma, deletions and the null genotype of $GSTT_1$ and $GSTM_1$ are associated with worse spirographic indices of disease control (odds ratio – 4.0). The presence of bronchial remodeling markers (MMP-9, VEGF) in sputum is associated with deletion polymorphism or null genotype of $GSTT_1$ and $GSTM_1$, since such patients have a significantly higher risk of airway restructuring, which reflects the accumulation of MMP-9 > 5.4 ng/m (odds ratio – 3.0) and VEGF more than 143.2 ng/mL (odds ratio – 3.9).

Key words: bronchial asthma, children, MMP-9, VEGF, $GSTT_1$, $GSTM_1$

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АСОЦІАЦІЯ ГЕНОТИПІВ ГЛЮТАТІОН-S-ТРАНСФЕРАЗИ M_1/T_1 ІЗ ПРОЦЕСОМ РЕМОДЕЛЮВАННЯ ДИХАЛЬНИХ ШЛЯХІВ У ХВОРИХ НА БРОНХІАЛЬНУ АСТМУ ДІТЕЙ

В роботі проведено дослідження окремих клініко-лабораторних та спірографічних особливостей ремоделювання бронхів у хворих на бронхіальну астму дітей, які мають альтернативний генотип $GSTT_1$ і $GSTM_1$. Встановлено, що у хворих на бронхіальну астму дітей делеції та нульовий генотип $GSTT_1$ і $GSTM_1$ асоціюють з гіршими спірографічними показниками контролю над захворюванням (співвідношення шансів – 4,0). Наявність у мокротинні маркерів ремоделювання бронхів (MMP-9, VEGF) асоціює із делеційним поліморфізмом або нульовим генотипом $GSTT_1$ і $GSTM_1$, так як у таких хворих достовірно вищий ризик структурної перебудови дихальних шляхів, що відображує накопичення MMP-9>5,4 нг/мл (співвідношення шансів – 3,0) та VEGF>143,2 нг/мл (співвідношення шансів – 3,9).

Ключові слова: бронхіальна астма, діти, MMP-9, VEGF, $GSTT_1$, $GSTM_1$

The study is a fragment of the research project "Modern epidemiological, clinical, paraclinical and diagnostic features of the most common inflammatory diseases of infectious and non-infectious nature in children", state registration No. 0122U002208.

Bronchial asthma (BA) is a common chronic disease of childhood, characterized by inflammation of the respiratory tract, the development of its remodeling, and hypersensitivity to external stimuli [6]. Environmental risk factors are one of the reasons for the occurrence and exacerbation of asthma, as they contribute to the development of inflammation due to damage to the epithelial cells of the respiratory tract, which leads to the hyperreactivity and remodeling [5].

According to researchers [3], the main processes that lead to airway remodeling in asthma can be presented as follows: (1) epithelial inflammatory reactions, including the production of cytokines and inflammatory factors; (2) activation of lymphocytes, neutrophils, eosinophils, mast cells, and monocytes/macrophages and their production of cytokines/inflammatory factors; (3) the proliferation of fibroblasts and smooth muscle cells caused by the above processes. Associated with bronchial remodeling, their chronic persistent inflammation is usually activated under the influence of environmental pollutants, such as cigarette smoke, which contribute to the development of oxidative stress [13]. Exposure to environmental xenobiotics stimulates the production of reactive oxygen species, which further enhances oxidative stress. In contrast, antioxidant enzymes such as glutathione-S-transferase prevent the development of oxidative stress by neutralizing excess oxidants. In cases when the function of the antioxidant enzyme is absent, active forms of oxygen can accumulate and cause oxidative stress [10]. It has been shown that bronchial asthma is closely associated with an increase in oxidative stress [15], which, in turn, is an important characteristic of airway inflammation due to its ability to intensification and persistence.

Thus, with insufficient functioning of antioxidant systems, xenobiotics, damaging the epithelium of the respiratory tract disrupt the functioning of the mucous secretion, contribute to stable obstruction of the bronchi and their hyperreactivity, which finally leads to structural changes in the bronchial wall. Reactive oxygen forms and other pathogenic factors are usually neutralized by the xenobiotic biotransformation system. However, polymorphism of the genes encoding the enzymes of this system can

contribute to the development of a child's susceptibility to constant toxigenic oxidative stress [1]. The genes of glutathione-S-transferase family of enzymes ($GSTT_1$ and $GSTM_1$), which are involved in the pathogenesis of many diseases, act as modifiers and risk factors (propensity) in pathology associated with adverse environmental influences [2].

In this work, we hypothesized that children with a predetermined genotype of glutathione-S-transferase M_1/T_1 (*$GSTM_1/GSTT_1$*) [7] have peculiarities in the formation of structural changes in the airways associated with differences in detoxification processes [8].

The purpose of the study was to establish clinical and instrumental features and the content of airway remodeling markers in sputum in children with bronchial asthma with an established genotype of glutathione-S-transferases ($GSTT_1$ and $GSTM_1$) to improve therapeutic and preventive measures and disease management.

Material and methods. 65 children with bronchial asthma in the non-attack period with the genotype of glutathione-S-transferases ($GSTT_1$ and $GSTM_1$) established at the preliminary stages of examination and treatment were examined using a simple random sampling method in the pulmo-allergological department of the Regional Clinical Non-Profit Enterprise "Chernivtsi Regional Children's Clinical Hospital". Depending on the results of a genetic examination of children with the expression of the $GSTT_1$ and $GSTM_1$ genes or their deletion or null variants, two clinical comparison groups were formed. The first (I) clinical group included 27 patients with genotypes $GSTM_1+$, $GSTT_1+$, and the second (II) group consisted of 38 patients with deletion polymorphism of these genes or null genotype (genotypes $GSTM_1+GSTT_1-$, $GSTM_1-GSTT+$, respectively). The general characteristics of the comparison groups are shown in Table 1.

Table 1

The general characteristics of the comparison groups

Groups	Mean age, years	Boys, %	Residents of the village, %
I group (n=27)	10.9±0.6	57.9	60.5
II group (n=38)	10.7±0.5	66.6	48.1
p	>0.05	>0.05	>0.05

Note: p – significance of differences

Assessment of the severity of the disease permitted to establish that intermittent, persistent mild, moderate and severe course of bronchial asthma occurred in 3.7 %, 18.5 %, 33.3 % and 44.4 % of patients in group I. At the same time, in children of group II, these indices occurred in 2.6 % ($p\varphi>0.05$), 10.5 % ($p\varphi>0.05$), 28.9 % ($p\varphi>0.05$), 57.9 % ($p\varphi>0.05$) of cases, respectively. The mean duration of the disease in group I was 4.7±0.7 years, and in children of group II – 4.7±0.6 years ($p>0.05$). Therefore, according to the main clinical data, the comparison groups did not differ, it can be assumed that they did not affect our results.

After obtaining the informed consent of the parents, children with asthma were examined using laboratory and instrumental methods of investigation. Asthma control was assessed using a clinical-instrumental assessment scale (CIS), according to which a score of 10 or less points correspond to sufficient control of asthma symptoms, a range of 11–16 points means partial control is achieved, and 17 points or more means loss of control over the disease [5].

The content of angiogenesis mediators in the sputum supernatant was determined as follows:

1. VEGF – three-stage "sandwich" method of enzyme-linked immunosorbent assay using standard mono- and polyclonal antibodies (VEGF-VectorBest A-8784).

2. MMP-9 – using the "sandwich-ELISA" method ("Affymetrix eBioscience" BMS 2016/2/BMS2016/2TEN ("Bender MedSystems", GmbH, Austria).

A spirographic study was performed in the non-attack period in compliance with all requirements, and bronchial lability was determined by evaluating the reactions of the bronchi to dosed running and inhalation of 200 μg of salbutamol with the calculation of the bronchospasm index (BSI, %), bronchodilation index (BDI) and the total index of bronchial lability (BLI, %).

The results of the scientific study were analyzed with computer programs Statistica 6.0 and Excel using clinical and epidemiological analysis with the determination of absolute (AR) and relative (RR) risks, odds ratio (OR) with their 95 % confidence interval (95 % CI). Using the methods of parametric and non-parametric statistics, the probable differences between the absolute numbers were determined by Student's criterion (P), relative indices – by Fisher's criterion (Pφ). Examination of children was carried out taking into account the principles of the Declaration of Helsinki of the World Medical Association "Ethical

principles of medical research involving a person as an object of research". Informed consent of parents and children was obtained for the study.

Results of the study and their discussion. The working hypothesis of this scientific study was to establish the peculiarities of the formation of bronchial remodeling in patients with alternative genotypes of glutathione-S-transferases ($GSTT_1$ and $GSTM_1$), which can cause insufficient inactivation of reactive metabolites with cytotoxic properties. Table 2 shows the results of assessing disease control in children of clinical comparison groups using a clinical instrumental scale.

Table 2

Dynamics of bronchial asthma control in patients of observation groups before the appointment of basic treatment

Indices of bronchial asthma control	Clinical groups		p
	group I	group II	
Clinical symptoms of disease			
Day symptoms	1.2±0.2	2.1±0.2	>0.05
Night symptoms	1.3±0.2	1.2±0.1	>0.05
β_2-agonist as needed	1.5±0.2	1.7±0.2	>0.05
Physical activity – limitation	2.2±0.2	2.4±0.2	>0.05
Frequency of hospitalizations	2.2±0.2	2.0±0.1	>0.05
Frequency of exacerbations	2.2±0.2	2.6±0.2	>0.05
Unscheduled visit to an allergist	1.2±0.1	1.4±0.1	>0.05
Total points (1)	11.8±0.9	13.4±0.7	>0.05
Indices of spirography			
FEV_1 (% of the norm)	1.2±0.2	2.6±0.2	<0.05
PEF (% of the norm)	1.4±0.2	4.7±0.3	<0.05
Total points (2)	2.6±0.3	7.0±0.5	<0.05
Total points	14.4±1.2	21.6±1.2	<0.05

As can be seen from the above data, in children with asthma with deletion polymorphism or null $GSTT_1$ and $GSTM_1$ genotypes, spirographic indices show significantly worse control of the disease than in representatives of clinical group I. The risk indices of worse disease control in terms of the total scores of the CIS ≥17 points in patients of group II compared to children of group I were: AR – 32.9 %, RR – 1.9 (95 % CI:1.2–2.9) with OR – 4.0 (95 % CI:2.1– 7.5). At the same time, the mean assessment of clinical indices, reflecting the control of the disease, coincided in the clinical comparison groups.

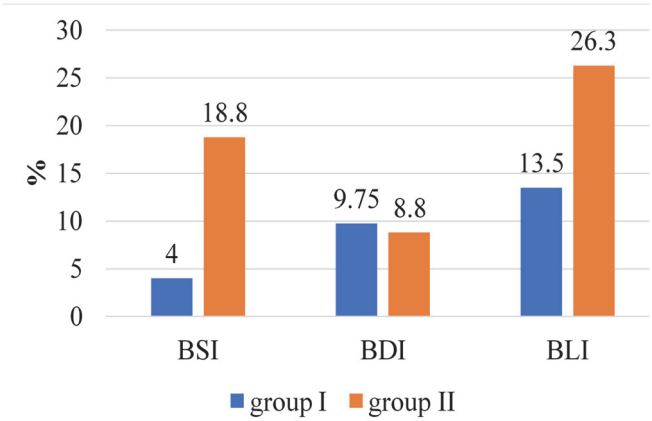

Fig. 1. Indices of bronchial lability in patients of observation groups

The results of this scientific study demonstrate that the null genotype and deletions of these genes are not only susceptibility factors for developing asthma, but also clearly associated with an increase in the sensitivity of the bronchi to physical activity in the form of significantly higher bronchospasm index (BSI) and bronchi lability index (BLI) (Fig. 1).

Indices of clinical and epidemiological risk of exercise-induced bronchospasm, exceeding the mean value for patients of group I (BDI> 4 %), in patients with a null genotype or deletions of the studied genes were: AR – 24.4 %, RR – 1.7 (95 %CI:1.3–2.1) at OR–2.7 (95 %CI:1.5–4.9). Similar risks were typical for children of the II clinical group in relation to their peers with a full-fledged genotype and were determined by us according to BLI> 13.5 %: AR –11.5 %, RR – 1.3 (95 % CI:1.1–1.5) at OR – 1.6 (95 %CI:1.0–2.9). Therefore, in patients with deletion polymorphism or null genotype of $GSTT_1$ and $GSTM_1$, there is a more pronounced reactivity of the bronchi in response to a provocation of dosed physical activity, which contributes to increased lability of the bronchi. At the same time, the response to bronchodilator inhalation in clinical groups coincided.

The obtained results are consistent with the data of other researchers in whose works lower level of FVC and FEV_1 indices were demonstrated due to the polymorphism of the genes of the glutathione-S-transferase family [6].

In our own studies of previous years [1, 3], we showed a higher level of bronchial hypersusceptibility to bronchial provocation factors due to the neutrophilic phenotype of asthma and the deletion polymorphism of the $GSTT_1$ and $GSTM_1$ genes.

The results obtained in this work are of particular value, since they are fully consistent with the modern concept of bronchial remodeling in asthma. Thus, over the years, scientists have obtained evidence pointing to new signaling mechanisms in bronchial smooth muscle cells that control various functions [11], in particular: 1) contractility and relaxation; 2) cell proliferation and apoptosis; 3) synthesis and modulation of extracellular components; 4) release of pro- or anti-inflammatory mediators and factors that regulate immunity, as well as the function of other types of airway cells, such as epithelium, fibroblasts and nerve cells. These various processes inherent in muscle tissue lead to the predominance of bronchoconstriction over bronchodilation, the formation of airway hyperreactivity, thickening and fibrosis of the bronchial wall [15].

From this point of view, based on the results obtained, one could assume the presence of a more pronounced bronchial remodeling in representatives of the II clinical group. To confirm this hypothesis, the content of such recognized markers of airway remodeling as matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) was studied in the supernatant of patients' sputum in clinical comparison groups.

It was assumed that matrix metalloproteinase (MMP) enzymes, in particular MMP-9, are associated with structural rearrangements of the airways in asthma, since they have the ability to degrade proteoglycans, increase airway fibrosis and proliferation of smooth muscles through the release and activation of growth factors [12] and also cause degradation of collagen [6]. In our research study, the mean content of MMP-9 in the supernatant of sputum in the representatives of group I was 4.0±0.7 ng/ml, and in patients of group II it was 5.7±0.9 ng/ml ($p>0.05$). The proportion of patients in whom the content of MMP-9 in the supernatant of sputum exceeded 5.4 ng/ml in group II was 50.0 % of cases, and in clinical group I – only 25.0 % of all cases ($p\varphi<0.05$). Thus, null genotypes or deletion polymorphism of the $GSTT_1$ and $GSTM_1$ genes were associated with an increased concentration in sputum of the MMP-9 remodeling marker, which indicates a high risk of bronchial restructuring [1]. Indices of clinical and epidemiological risk associated with an increase in the concentration of MMP-9>5.4 ng/ml in the supernatant of sputum in patients with deletion polymorphism or null genotype $GSTT_1$ and $GSTM_1$ compared to patients of group I were: AR – 26.6 %, RR – 1.7 (95 %CI:1.1–2.5), OR – 3.0 (95 %CI:1.6–5.5).

Considering that remodeling is caused not only by thickening of the subepithelial layer and hyperplasia of the smooth muscles of the respiratory tract, but also by neoangiogenesis, and increased vascularization of the bronchial mucosa is closely associated with the expression of angiogenic endothelial growth factor, we studied the content of this remodeling marker directly in the sputum of patients. Thus, the mean content of VEGF in the representatives of group I reached 100.0±15.2 ng/ml, and in patients of group II it was 143.2±22.7 ng/ml ($p>0.05$). Risk indices of VEGF registration > 143.2 ng/ml in children with deletion polymorphism or null $GSTT_1$ and $GSTM_1$ genotype compared to representatives of group I were: AR – 32.1 %, RR – 1.8 (95 % CI:1.2– 2.9) at OR – 3.9 (95 % CI:2.0–7.4), and indicated a higher risk of airway remodeling.

Conclusions

1. In this scientific study, some clinical, laboratory and spirographic features of bronchial remodeling were studied in children with bronchial asthma with an alternative genotype $GSTT_1$ and $GSTM_1$.

2. In children with asthma, deletions and null $GSTT_1$ and $GSTM_1$ genotypes are associated with worse spirographic (but not clinical) indices of disease control and four times increase the chances of loss of control.

3. In children with asthma with a null genotype or a deletion polymorphism of the $GSTT_1$ and $GSTM_1$ genes, there is a statistically significant risk of a bronchospastic response to physical activity (OR – 2.7–4.9), as well as an increase in bronchial lability by more than 13.5 % (OR – 1.6), which emphasizes the association of these genetic factors with nonspecific bronchial hyperreactivity.

4. The content of bronchial remodeling markers (MMP-9, VEGF) in sputum is associated with deletion polymorphism or null genotype of $GSTT_1$ and $GSTM_1$, since such patients have a significantly

higher risk of airway restructuring, which reflects the accumulation of MMP-9 >5.4 ng/ml (OR – 3.0) and VEGF >143.2 ng/ml (OR – 3.9).

5. To improve disease management in childhood, it should be taken into account that in patients with alternative variants of the $GSTT_1$ and $GSTM_1$ genotypes and accumulation of bronchial remodeling markers in sputum, the clinical characteristics and response to bronchodilator inhalation are the same and do not allow distinguishing children with structural restructuring of the airways.

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