

*Марусык У.И.*

*Высшее учебное заведение Украины «Буковинский государственный медицинский университет», г., Черновцы, Украина, доцент кафедры педиатрии и детских инфекционных болезней, кандидат медицинских наук*

## **ВЛИЯНИЕ НУКЛЕИНАТУ НА ФУНКЦИОНАЛЬНУЮ АКТИВНОСТЬ ГРАНУЛОЦИТОВ КРОВИ У ШКОЛЬНИКОВ, БОЛЬНЫХ БРОНХИАЛЬНОЙ АСТМОЙ, В ЗАВИСИМОСТИ ОТ АЦЕТИЛЯТОРНЫХ ФЕНОТИПОВ**

### **THE EFFECT OF THE NUCLEINAT ON FUNCTIONAL ACTIVITY BLOOD GRANULOCYTES IN SCHOOL-AGE CHILDREN WITH ASTHMA, DEPENDING ON ACETYLATION PHENOTYPE**

*Marusyk U.I.*

*Higher education institution Ukraine "Bukovinian State Medical University", c. Chernivtsi, Ukraine, Assistant professor, Department of pediatrics and children's infectious diseases, PhD*

#### **АННОТАЦИЯ**

**Актуальность.** Бронхиальная астма - одно из самых распространенных заболеваний в мире, количество больных которого постоянно увеличивается, особенно среди детей. Недостаточная эффективность контролирующей терапии астмы обусловила необходимость применения у детей новых медикаментов, способствующих улучшению течения данного заболевания.

**Цель исследования.** Оценить влияние нуклеината в комплексном лечении БА на показатели функциональной активности гранулоцитов крови детей школьного возраста в зависимости от их ацетиляторных фенотипов.

**Материал и методы.** В периоде ремиссии комплексно обследовано 36 школьников, больных БА. Все школьники в комплексе базисной терапии принимали нуклеинат в дозе 0,25 г в сутки в течение 21 дня. За типом ацетилирования больных распределяли на две клинические группы. В первую клиническую группу вошли 16 пациентов, у которых определяли медленный тип ацетилирования (средний процент ацетилированного сульфадимезина в моче составлял менее 75,0%). Вторую клиническую группу сформировали 20 школьников с быстрым характером ацетилирования (средний процент ацетилированного сульфадимезина в моче составлял более 75,0%). Всем детям определяли фагоцитарную активность и фагоцитарное число нейтрофилов крови, анализировали кислородозависимый метаболизм эозинофилов и нейтрофилов крови за показателями спонтанного и стимулированного НСТ-теста.

**Результаты.** Относительный риск выявления абсолютного числа фармазанположительных нейтрофилов в спонтанном НСТ-тесте меньше 0,42 усл. ед. у школьников при медленном типе ацетилирования после проведенной терапии составил 1,9 (95% ДИ 1,6-2,4) и абсолютный риск - 0,3.

Использование нуклеината в комплексе базисной противовоспалительной терапии у детей с быстрым ацетиляторным фенотипом, по сравнению с медленными-ацетиляторами, сопровождалось риском недостаточного контроля БА, то есть возможностью активации иммунной системы (СШ = 3,3, 95% ДИ 1,7-6,4).

**Заключение.** В большинства детей с медленным ацетиляторным фенотипом отмечено снижение показателей кислородозависимой микрообидности эозинофилов крови, за счет возможного снижения активности воспалительного процесса в организме.

Минимальное количество больных, у которых необходимо применять нуклеинат в комплексной терапии при быстром типе ацетилирования, для достижения хотя бы одного случая повышения уровня контроля БА составляет 4.

#### **ABSTRACT**

**Introduction.** Bronchial asthma is one of the most common diseases in the world, the number of patient with an ever increasing, especially among children. Inadequate efficiency of controlling asthma therapy necessitated the application of new drugs in children which have improved the course of the disease.

**Objective.** To assess the Nucleinat effect in the treatment of asthma on indicators of the functional activity of blood granulocytes school-age children depend of their atsetylyatornyh phenotypes.

**Materials and Methods.** 36 school-age children with bronchial asthma in remission were complex examined. All pupils in the complex basic therapy received Nucleinat 0,25 g per day for 21 days. Due to the type of acetylation patients were divided into two clinical groups. The first clinical group included 16 patients who determined the slow type of acetylation (average percentage acetylated sulfadimezin of urine was less than 75,0%). 20 students formed the second clinical group of, which is marked the rapid nature of acetylation (average percentage acetylated sulfadimezin urine was more than 75,0%).

All children conducted by determining phagocytic activity and phagocytic number of blood neutrophil, analis of oxygen-dependent metabolism of eosinophilic and neutrophilic blood granulocytes due to parameters of spontaneous and stimulated Nitroblue tetrazolium test.

**Results.** This definition of relative risk above the absolute number of neutrophils in farmazan positive spontaneous NBT-test less than 0,42 S. U. in pupils with the slow type of acetylation after therapy was 1,9 (95% CI 1,6-2,4) and absolute risk – 0,3.

The using of Nucleinat with basic anti-inflammatory therapy in children with rapid type of acetylation compared to slow type of acetylation, accompanied by the risk of inadequate asthma control, namely the ability to increase the activity of the immune system (OR = 3,3, 95% CI 1,7-6,4).

**Ключевые слова:** бронхиальная астма; дети; ацетилирование; нуклеинат; эозинофильные гранулоциты крови; нейтрофильные гранулоциты крови.

**Keywords:** asthma; children; acetylation; nucleinat; eosinophilic granulocytes blood; neutrophilic granulocytes blood.

**Introduction.** It has been known that asthma belongs to the multifactorial disease. Etiology and pathogenesis of which are determined by a complex interaction of genetic factors and environmental factors [3,6]. The study of molecular-genetic characteristics of hereditary predisposition to asthma, which held recently focused mainly, are identifying to the role of some genes and their encoded compounds in the pathogenesis of this disease [1,2].

At the heart of bronchial asthma (BA) are changes in the immune response, leading to sensitization of the organism, the development of allergic (immune) airway inflammation [7]. Having determined the characteristic inflammatory changes identified the importance of medication anti-inflammatory drugs using in children with asthma [5]. However, during the application of the basic treatment of this disease in children is not always possible to achieve complete control of the BA [11]. The lack of anti-inflammatory therapy effectiveness, in some cases, [12], necessitated the application new drugs in children, such Nucleinat which would help improve the course of disease.

Nucleinat has immunomodulatory and anti-inflammatory effects [4] that's why we suggests that its application in the complex basic therapy of asthma in children accompanied decreased activity of the immune system.

Considering literature data on the association of severe asthma with genetic polymorphisms N-acetyltransferase - an enzyme that determines the feature of metabolic processes [10], we thought it appropriate to assess the impact Nucleinat in the complex base treatment on features of the immune response in children with asthma, according to their acetylation phenotypes.

**The aim of the study.** To assess the Nucleinat effect in the treatment of asthma on indicators of the functional activity of blood granulocytes school-age children depend of their atsetylyatornyh phenotypes.

**Investigation's material and methods.** 36 school-age children with bronchial asthma in remission were complex examined in pulmonology department of Chernivtsi Regional Children's Clinical Hospital. All pupils in the complex basic therapy received Nucleinat 0,25 g per day for 21 days. Due to the type of acetylation patients were divided into two clinical groups. The first (I) clinical group included 16 patients who determined the slow type of acetylation (average percentage acetylated sulfadimezin of urine was less than 75,0%). 20 students formed the second (II) clinical group of, which is marked the rapid nature of acetylation (average percentage acetylated

sulfadimezin urine was more than 75,0%). For the main clinical signs groups were comparison.

The first clinical group consist of 66,7% boys and 33,3% girls, the average age of patients was  $11,7 \pm 0,6$  years. The second group formed 70,0% ( $P > 0,05$ ) boys and 30,0% girls ( $P > 0,05$ ), the average age of patients was  $11,4 \pm 0,6$  years ( $P > 0,05$ ). The first clinical group included 50,0% of urban and rural residents. Another group formed 90,0% of the cities residents and urban areas and 10,0% patients ( $P > 0,05$ ) who live in villages.

However, in the first clinical group mild asthma was observed in 16,7% patients, moderate - in 50,0% children and severe - in 33,3% pupils. Severity of asthma in the group of childrens, which were marked rapid acetylation nature, was determined according to 10,0%, 70,0% and 20,0% patients ( $P > 0,05$ ).

Thus, by gender, age, place of residence and severity of asthma clinical comparison group did not differ significantly.

All children received asthma treatment according to the ordered Ministry of Public Health of Ukraine from 08.10.2013 number 868 "On approval and introduction of medical and technological documents on standardization of care in asthma."

All children conducted by determining phagocytic activity - FA (percent) and phagocytic number - FN (S.U.) of blood neutrophil, analysis of oxygen-dependent metabolism of eosinophilic and neutrophilic blood granulocytes due to parameters of spontaneous and stimulated Nitroblue tetrazolium test (NBT-test), which were expressed as the relative content (percent) farmazan positive neutrophilic and eosinophilic blood granulocytes.

The rate of acetylation processes that characterized the features of II phase biotransformation of xenobiotics system was studied by Prebstynh GavriloVA, in modification.

Statistical analysis of the results of research carried out by methods of variation statistics using statistical program StatSoft Statistica v5.0., and from the perspective of clinical epidemiology determining the absolute (AR), relative (RR) risks and odds ratios (OR) indicating 95% confidence interval (95% CI).

**Results and discussion.** After treatment in patients with both clinical comparison group were recorded changes in redox processes of neutrophilic and eosinophilic blood granulocytes, which was confirmed according to the NBT-test in spontaneous and stimulated options. In the children of both clinical groups it was marked downward trend to oxygen-dependent metabolism of neutrophils. However, in

patient of I clinical group compared with children from second phagocytic activity was decreased.

Table 1

**Indicators of functional activity of neutrophils of children in the comparison group**

Group	N (number of the patient)	NBT-test (%)				FA (%)		FN (S.U.)	
		spontaneous		stimulated		before treatment	after treatment	before treatment	after treatment
		before treatment	after treatment	before treatment	after treatment				
I group	16	42,6±5,9	37,6±7,2	58,8±5,2	54,0±8,3	84,0±4,3	65,2±2,7	8,9±0,9	8,0±0,9
II group	20	39,5±5,7	27,6±4,3	59,8±5,4	50,5±3,9	85,2±3,3	74,6±5,2	9,7±1,4	6,3±1,1

P>0,05

It should also be noted that the sheer number of farmazan positive neutrophils in spontaneous NBT-test less than 0,42 S. U. was determined in 16,6% of patients with slow type of acetylation before treatment and in 83,3% of patients after (Rφ<0,05). Then, as in the second clinical group the number of school-age children with present value of NBT-test before and after treatment was 50,0% and 60,0% respectively (Rφ>0,05). This definition of relative risk above the absolute number of neutrophils in farmazan positive spontaneous NBT-test in pupils with the slow type of acetylation after therapy was 1,9 (95% CI 1,6-2,4) and absolute risk – 0,3.

Thus, the using of the Nucleinat together with basic therapy in children with asthma was more effective then in patients with slow type of acetylation, probably due to long-lasting effect of the drug.

However, absolute number of farmazan positive neutrophils of blood, due to stimulated NBT-test after therapy, decreased in 83,3% patients from the first clinical group while in 60,0% pupils in the second clinical group only (Rφ>0,05). Thus, the risk of reducing the number of farmazan positive neutrophils in patient I clinical group was higher relative to the

second, and the odds ratio was 3,3 with 95% confidence interval 1,7-6,4.

The using of Nucleinat with basic anti-inflammatory therapy in children with rapid type of acetylation compared to slow type of acetylation, accompanied by the risk of inadequate asthma control, namely the ability to increase the activity of the immune system (OR = 3,3, 95% CI 1,7-6,4 ). This relative risk reduction (RR) inadequate asthma control in patients of first clinical group was 28,0%, absolute risk (AR) – 23,3%, and the minimum number of patients who should be used in the treatment Nucleinat to achieve at least one case of improvement of quality control asthma, equal to 4 cases.

Lack of efficiency Nucleinat together with anti-inflammatory therapy for children with rapid type of acetylation is probably due to a faster withdrawal of the drug from the body.

It should be noted that after treatment in children I clinical group it was marked downward trend to oxygen-dependent metabolism of eosinophils, and in children from II clinical group, on the contrary - its growth, in terms of spontaneous and stimulated NBT-test.

Table 2

**Indicators of functional activity of eosinophils of children in the comparison group**

Group	N (number of the patient)	NBT-test (%)			
		spontaneous		stimulated	
		before treatment	after treatment	before treatment	after treatment
I group	6	29,1±5,7	18,0±3,7	29,0±6,8	20,6±5,8
II group	10	16,5±3,4	18,8±2,6	16,1±3,1	20,5±3,2

P>0,05

The reducing of the percentage by farmazan positive eosinophils in the blood of patients from firch clinical groups indicates a pointed to possible reduction of chronic inflammation in the body. Conversely, activation of these cells underlies the development of severe asthma [9] and observed in patients of the second clinical group probably associated with insufficient basic treatment efficacy in pupils with fast type of acetylation.

However, in patients with slow type of acetylation after the therapy absolute number of farmazan positive eosinophilic granulocytes of blood, according to NBT-test in stimulated version above 0,27 S.U. determined

at 83,3% of cases and only 10,0% children (Pφ<0,05) in the second clinical group. The reduction of relative risk (RR) inadequate asthma control in patients from I clinical group was 88,0%, absolute risk (ARR) – 73,3%, and the minimum number of patients who should be used in the treatment Nucleinat to achieve at least one case of improvement of quality control asthma, equal to 1,4. The findings give reason to consider that the increase of this granulocytes in patients with slow acetylation type indicated to possible saving of allergic inflammation in children of this group [8].

Thus, the using of the Nucleinat in treatment of bronchial asthma, reduced inflammation in children, but pupils with slow acetylation phenotype the effectiveness of standard treatment was slightly higher. It should be noted that in patients with in the complex therapy of asthma received Nucleinat not observed side effects of the therapy.

**Conclusions.** 1. An application of the Nucleinat with basic treatment leads to reduce the severity of chronic inflammation.

2. The significantly higher number of children with slow acetylation phenotype observed decrease oxygen-dependent metabolism eosinophilic granulocytes blood due to possible reduction of inflammatory activity in the body.

3. The minimum number of patients who should be used in the treatment Nucleinat to achieve at least one case of improvement of quality control asthma, was 4.

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