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OPTIMIZATION OF BRONCHIAL ASTHMA TREATMENT ACCORDING TO POLYMORPHISM IN THE LEUKOTRIENE-C4 SYNTHASE GENE

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The aim of the study *was to investigate the effectiveness of different schemes of basic asthma therapy depending on the polymorphism of the LTC4 gene.*
Materials and methods. *181 patients with asthma were recruited to participate in the study. All patients included in the study underwent a general clinical study, spirometry, the level of asthma control was determined by Asthma control questionnaire 5 (ACQ-5), by studying the polymorphism of the LTC4 gene, it was determined that patients belong to the A/A, A/C and C/C genotypes.*
Results. *Allelic -444C polymorphism of the LTC4-S gene (rs 730012) had the following genotype frequency among asthma patients: A/A – 77 people (42.6 %), A/C – 73 people (40.3 %) and C/C – 31 people (17.1 %).*
In groups of patients with genotypes A/A and A/C, during treatment with a low dose of ICS and montelukast, there was a significant improvement in FEV1 and the score according to the ACQ-5 questionnaire (asthma control level). However, in the group of patients with the C/C genotype, there were no significant changes in FEV1 and the score according to the ACQ-5 questionnaire.
In the groups of patients with genotypes A/A and C/C on the background of increasing the dose of ICS to medium in a fixed combination with LABA compared with the results of treatment with low-dose ICS and montelukast, there was a significant improvement in FEV1 and asthma control – ACQ- 5. At the same time, in the group of patients with genotype A/C, there were no reliable indicators of FEV1 and ACQ-5 score changes.
Conclusions. *The frequency of genotypes A/A, A/C and C/C for LTC4S polymorphism in the studied population are 42.6 %, 40.3 % and 17.1 %, respectively.*
Patients with genotypes A/A and C/C have a significant response to treatment with a low dose of ICS and montelukast in the form of an improvement in FEV1 and ACQ-5 score.
Increasing the dose of ICS up to moderate in a fixed combination with β2-LABA in groups of patients with genotypes A/A and C/C leads to a significant improvement in the FEV1, as well as in the asthma control level - ACQ-5
Keywords: *bronchial asthma, -444C-polymorphism of the LTC4-S gene, genotype, aspirin asthma, antileukotriene therapy, inhaled corticosteroids*

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1. Introduction

Bronchial asthma (BA) is one of the most common chronic inflammatory diseases of the respiratory tract, which occurs under the influence of genetic and environmental factors [1]. Asthma has a large number of clinical phenotypes, due to the heterogeneity of the disease itself. The focus of modern medicine on the personification of treatment dictates the need to take into account the genotypic and phenotypic features of the disease in the selection of therapy in each individual case.

An important role in the induction of bronchoconstriction, airway edema, increased mucus secretion and eosinophilic infiltration is played by proinflammatory mediators – cysteinyl leukotrienes (cysLT) [2]. Leukotriene C4 synthase (LTC4S) is an enzyme that limits the rate of cysLT synthesis. It is proved that the expression of LTC4S mRNA is higher in the eosinophils of the blood of patients with asthma than in control subjects [3]. In addition, aspirin bronchial asthma (ABA), a specific subtype of asthma that affects 3 % to 20 % of adult asthma patients, is characterized by overproduction of cysLT [4] and overexpression of the LTC4S enzyme at the level of the bronchial mucosa compared with aspirin-tolerant patients with BA and control [5]. In patients with ABA, aspirin and other nonsteroidal anti-inflammatory drugs, non-selective cyclooxygenase inhibitors, direct the metabolism of arachidonic acid to the lipoxygenase pathway, which leads to increased release of cysLT into the airways and causes bronchospasm [6]. That is why the LTC4S gene is considered an important predictor gene for asthma.

Despite the large number of studies examining the diversity of BA phenotypes and the effectiveness of therapy for different types of asthma, there are currently no recommendations for personalized treatment of BA based on genotypic and phenotypic characteristics of the patient, especially LTC4S gene polymorphism.

The aim of the study was to investigate the effectiveness of different schemes of basic BA therapy depending on the polymorphism of the LTC4 gene.

2. Materials and methods

The study was conducted on the basis of Municipal Non-Commercial Enterprise of Sumy Regional Council "Sumy Regional Clinical Hospital" during 2013–2020. 181 patients with BA (135 women and 46 men) were involved in the study. The mean age of patients with BA was 46.5±11.0 years.

Criteria for inclusion of patients in the study: age 18 years and older; consent to participate in the study; the presence of a diagnosis of persistent BA with moderate course according to the GINA recommendations (2017) [1];

Exclusion criteria: the patient has severe comorbidities; period of pregnancy or lactation; constant intake of system CS; mental disorders and diseases of the nervous system; systemic connective tissue diseases; acute infectious diseases, exacerbation of chronic infectious diseases; acute cerebrovascular disorders in the anamnesis.

According to the study design, all patients involved in the study underwent a general clinical study, spirometry. The level of asthma control was determined by the questionnaire Asthma Control Questionnaire-5 (ACQ-5). The affiliation of patients to genotypes A/A, A/C and C/C was determined by studying the polymorphism of the LTC4 gene. At the study and primary screening stage, all patients received a low daily dose of inhaled corticosteroid (ICS) (budesonide 160 μg) and a prolonged-acting β_2-long-acting beta-adrenoreceptor agonist (β_2-LABA) (formoterol 4.5 μg) in a fixed combination twice daily. Patients (176 patients) who did not meet the criteria for asthma control (ACQ-5> 0.75) and require correction of therapy according to GINA recommendations were selected for inclusion in the study [1]. In the first phase of the study, treatment modification was performed – all patients received a low daily dose of ICS (budesonide 200 μg twice daily) and antileukotriene therapy (ALT) (montelukast 10 mg) once daily. After 3 months, an analysis of the treatment effectiveness was performed at the first stage of the study to determine the level of asthma control using FEV1 indicators and the ACQ-5 questionnaire. Patients who achieved asthma control at this stage remained on the selected treatment regimen (low daily dose of ICS and ALT), and those who did not achieve asthma control (123 patients) were included in the second stage of the study. In the second stage of the study, a treatment modification was performed again – a fixed combination of β_2-LABA (formoterol 9 μg) with ICS in an increased to average daily dose (budesonide 320 μg) twice a day.

Examination of patients included: standard clinical examination with blood pressure measurement, anthropometric examination to determine body mass index (BMI), spirography using a diagnostic complex "Cardioplus" (manufactured in Ukraine) with a test of reversibility of bronchial obstruction after inhalation of 400 μg salbutamol, determination of allelic -444C polymorphism of the LTC4 gene by polymerase chain reaction (PCR) followed by restriction fragment analysis using DIAtom DNA Prep 100 kits (Isogene, Russia). The level of BA control was evaluated using the ACQ-5 questionnaire [7].

The study was conducted in accordance with the requirements of the Helsinki Declaration of the World Medical Association, the Council of Europe Convention on Human Rights and Biomedicine, Good Clinical Practice (GCP) and was approved by the Commission on Bioethics of Medical Institute of the Sumy State University (Protocol №1 / 02 from 09.02.2021). Patients were included in the clinical trial after signing the informed consent.

Statistical analysis of the obtained data was performed using the program SPSS Statistics 21.0. Quantitative data are presented as mean values (M) and standard deviations (SD). When testing the null hypotheses, the critical value of the statistical significance criterion was set at 0.05. To compare the central parameters of the groups, parametric and nonparametric methods were used: Student's t-test, Wilcoxon (W) and Mann-Whitney (MW) tests. For pairwise comparison of groups, the U-Mann-Whitney (MW) test was used.

3. Research results

Allelic -444C polymorphism of the LTC4S gene (rs 730012) was determined by polymerase chain reaction. The frequency of genotypes A/A, A/C and C/C among patients with asthma is presented in Table 1.

According to the design of the study at the first stage, all patients (176) who did not achieve proper control (ACQ <0.75) on the background of treatment with low daily dose of ICS and β_2-LABA, changed the treatment regimen to a low daily dose of ICS (budesonide) twice per day and antileukotriene therapy (montelukast) once a day. The dynamics of ACQ-5 and FEV1 after 3 months of treatment are presented in Table 2.

Table 1

Frequency distribution of LTC4S gene genotypes in patients with BA

Genotype	Number of people	Specific gravity, %
Genotype A/A	77	42.6
Genotype A/C	73	40.3
Genotype C/C	31	17.1
Total	181	100

Table 2

Dynamics of FEV1 and BA control on the background of treatment with montelukast

Genotype A/A, n =76				
Indicators		Before treatment	In 3 months	p
FEV1, appropriate %		66.5±5.7	74.9±6.4	< 0.01
ACQ, points		1.3±0.2	0.9±0.2	< 0.01
Controllability of BA, absolute number of patients / share in genotype, %	partially controlled "1"(ACQ > 0.75 and < 1.5)	72/94.7	51/67.1	< 0.01
	uncontrolled "2"(ACQ > 1.5)	4/5.3	0/0	0.06014
Genotype A/C, n =71				
Indicators		Before treatment	In 3 months	p
FEV1, appropriate %		68.4±6.5	75.4±7.2	<0.01
ACQ, points		1.2±0.3	0.9±0.3	<0.01
Controllability of BA, absolute number of patients / share in genotype,, %	partially controlled "1"(ACQ > 0.75 and < 1.5)	64/90.1	49/69.0	<0.01
	uncontrolled "2" (ACQ > 1.5)	7/9.9	0/0	<0.01
Genotype C/C, n =29				
Indicators		Before treatment	In 3 months	p
FEV1, appropriate %		68.3±7.6	71.9±6.6	0.1247
ACQ, points		1.2±0.3	1.0±0.2	0.2174
Controllability of BA, absolute number of patients / share in genotype,, %	partially controlled "1" (ACQ > 0.75 and < 1.5	25/86.2	23/79.3	0.7613
	uncontrolled "2"(ACQ > 1.5)	4/13.8	0/0	0.0564

In the groups of patients with genotypes A/A and A/C on the background of treatment with low-dose ICS and montelukast compared with the results of treatment with low-dose ICS and β_2-LABA, there was a significant improvement in FEV1 by 12.6 % and 10.2 %, respectively (p=0.0001). The change in the treatment regimen in these groups was accompanied by a probable change in points on the ACQ-5 questionnaire by 30.8 % in the A/A genotype and by 25.0 % in the A/C genotype (p=0.0001), which in turn led to achievement of BA control in 25 patients in the A/A genotype group and 22 patients in the A/C genotype group (p <0.05). In the group of patients with genotype C/C no probable changes in FEV and ACQ-5 point were detected, BA control at this stage of the study was achieved only by 6 patients (p> 0.05). Thus, in the treatment of patients with BA with a low daily dose of ICS (budesonide 200 μg) twice a day and antileukotriene drug (montelukast 10 mg) once a day, 32.9 % of patients with genotype A/A, 31 % of patients with genotype A/C and 20.7 % of patients with C/C genotype.

In the second stage of the study, patients who did not achieve control in previous BA treatment regimen were transferred to a fixed combination of β_2-LABA (formoterol) with ICS (budesonide) in an increased to average daily dose, followed by monitoring the effectiveness of treatment after 3 months, the results are given in Table 3.

Increasing the dose of ICS to the average dose in a fixed combination with β_2-LABA compared with treatment with low daily doses of ICS and montelukast in groups of patients with genotypes A/C and C/C led to an improvement in FEV1 by 3.1 % (p>0.05) and 8.5 % (p <0.05), respectively, as well as the control indicator of asthma – ACQ-5 by 11.1 % (p>0.05) and 20.0 % (p<0.05), respectively, that was accompanied by the achievement of control in 8 and 6 patients, respectively (p>0.05). In the group of patients with genotype A/A significant changes in FEV1 and ACQ-5 by 2.4 % and 11.1 %, respectively (p<0.05) were determined, BA control was achieved in 11 patients (p>0.05). Thus, treatment of patients with asthma who did not achieve control of symptoms on the background of therapy with low daily dose of ICS (budesonide) twice a day in combination with the antileukotriene drug montelukast once a day and were transferred to treatment with average daily dose of ICS and β_2-LABA in one inhaler, 14.5 % of patients with genotype A/A, 11.2 % of patients with genotype A/C and 20.7 % of patients with genotype C/C achieved control. Patients with uncontrolled BA did not remain in any group. The number of cases of BA symptoms control during the transition to average daily doses of ICS in combination with β_2-LABA in the group of patients with genotypes A/A and A/C decreased by 1.5 and 1.9 times, respectively, and in the group of genotype C/C – increased 1.3 times.

Table 3

Dynamics of FEV1 and asthma control on the background of treatment with the average daily dose of ICS and β_2-LABA

Genotype A/A, n=76				
Indicators		In 3 months	In 6 months	P
FEV1, appropriate %		74.9±6.4	76.7±5.8	0.016
ACQ, points		0.9±0.2	0.8±0.2	< 0.01
Controllability of BA, absolute number of patients / share in genotype, %	partially controlled "1" (ACQ > 0.75 and < 1.5)	51/67.1	40/52.6	0.0714
	uncontrolled "2" (ACQ > 1.5)	0/0	0/0	
Genotype A/C, n =71				
Indicators		In 3 months	In 6 months	P
FEV1, appropriate %		75.4±7.2	77.7±6.4	0.2231
ACQ, points		0.9±0.3	0.8±0.2	0.2056
Controllability of BA, absolute number of patients / share in genotype, %	partially controlled "1" (ACQ > 0.75 and < 1.5)	49/69.0	41/57.8	0.1909
	uncontrolled "2" (ACQ > 1.5)	0/0	0/0	
Genotype C/C, n =29				
Indicators		In 3 months	In 6 months	P
FEV1, appropriate %		71.9±6.6	78.0±5.7	< 0.01
ACQ, points		1.0±0.2	0.8±0.2	< 0.01
Controllability of BA, absolute number of patients / share in genotype, %	partially controlled "1" (ACQ > 0.75 and < 1.5)	23/79.3	17/58.6	0.1845
	uncontrolled "2" (ACQ > 1.5)	0/0	0/0	

4. Discussion

The ability of inflammatory cells to generate leukotrienes depends on polymorphisms in the genes involved in their synthesis. The results of available genetic studies suggest that genes encoding the CysLT pathway may further or synergistically alter drug responses or asthma endophenotypes [8, 9]. It has been shown that LTC4S gene polymorphism is associated with aspirin intolerance in patients with asthma, which is manifested by respiratory reactions to aspirin and other cyclooxygenase inhibitors [10]. Particular attention is drawn to the nucleotide polymorphism, the size of 444 nucleotide pairs, located above the gene encoding leukotriene-C4 synthetase (LTC4S-444C, rs730012), as a strong risk factor for asthma and aspirin intolerance [11].

The distribution by belonging to the genotypes A/A, A/C and C/C according to the available studies varies in a fairly wide range, the frequency of the C allele by different authors is from 11.5 to 25 % in the group of patients with BA [11-14]. In our statistical sample of patients, the frequency of the studied genotypes A/A, A/C and C/C by LTC4S polymorphism was 42.6 %, 40.3 % and 17.1 %, respectively. The results of the study -444C polymorphism of the LTC4S gene suggest that the frequency of alleles depends significantly on the population in which the study was conducted. It is noteworthy that the C/C genotype is less widespread.

A significant proportion of patients with BA can not achieve complete control over BA and there is even absence of control. It is established that the variability of the response to a number of antiasthmatic drugs depends on the effect of interaction of many genes, which implies the presence of certain combinations of genetic variants in patients, as well as the influence of external factors on the formation of clinical features. Genetic determinism may be responsible for 60-80 % of variations in drug responses [12], and therefore genetic research is generally the most promising in terms of studying the pathogenesis of therapeutically resistant asthma, as well as in finding new diagnostic tests with high sensitivity and specificity.

The variability in response to montelukast ranges from 35 % to 78 % of patients receiving active treatment [13]. The mechanisms of variability in the response to montelukast are related to genetic changes. According to a study by Lima JJ et al., the presence of a homozygote for the C allele -444A/C polymorphism of the leukotriene C4 synthetase gene is associated with an increase in CysLT, a decrease in FEV1, which causes the severity of BA and affects the response to treatment with leukotriene receptor antagonists[13]. That was confirmed in our study as patients with C/C genotype did not have a probable response to FEV1 and ACQ-5 for the ICS treatment with the addition of montelukast for 3 months, also in this group the lowest number of patients achieved control of symptoms. However, this partly contradicts the results of Cai C. et al., who showed that carriers of C/C and A/C genotypes had higher FEV1 values compared to carriers

of A/A genotype after treatment with montelukast ($p < 0.01$) [14]. The inconsistency of the data indicates the need for further studies of the association of BA with the genotypic characteristics of patients.

The response to increasing the ICS dose in a fixed combination with β_2-LABA in terms of FEV1 and ACQ-5 in our studied population of patients with BA was also heterogeneous. Patients with A/A and C/C genotypes responded best to this modification of the treatment regimen. Unfortunately, there is currently insufficient information on the effect of the LTC4S polymorphism genotype on the efficacy of ICS treatment in patients with asthma.

The obtained results demonstrate the heterogeneity of response to different schemes of basic BA therapy and emphasize the need for a comprehensive study of genotypic and phenotypic features that affect the effectiveness of BA treatment and control of BA symptoms.

Study limitations. The presented fragment of the study aimed to study the only genotype effect on the LTC4S polymorphism on the effectiveness of BA patients treatment, without taking into account the phenotypic variations in the course of asthma.

Prospect for further research includes the study of the influence of phenotypic variants of different genotypes by LTC4S polymorphism on the effectiveness of treatment of patients with BA.

5. Conclusions

The allelic -444C polymorphism of the LTC4-S gene (rs 730012) determined by polymerase chain reaction had the following frequency of genotypes among patients with BA: A / A – 77 people (42.6 %), A/C – 73 people (40.3 %) and C/C – 31 persons (17.1 %).

In the groups of patients with genotypes A/A and A/C on the background of treatment with low daily dose of ICS and montelukast compared with the results of treatment with low daily dose of ICS and β_2-LABA there was a significant improvement in FEV1 and points on the ACQ-5 questionnaire (BA controllability). However, in the group of patients with the C/C genotype, no probable changes in FEV and ACQ-5 questionnaire point were detected.

Increasing the ICS dose to the average daily dose in a fixed combination with β_2-LABA compared low ICS doses treatment and montelukast in groups of patients with genotypes A/A and C/C led to a significant improvement in FEV1, as well as the BA control indicator – ACQ-5. At the same time, in the group of patients with genotype A/C no probable indicators of FEV1 and ACQ-5 were detected.

Conflict of interests

The authors declare that they have no conflicts of interest.

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