A correction of a gut microflora composition for the allergic bronchial asthma complex therapy

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ABSTRACT

The efficacy of a gut microbiota control was investigated for patients with atopic asthma. 45 patients with atopic asthma were included in the study. The results of our clinical and lab tests, pulmonary function tests and the lactulose hydrogen breath tests have been presented to evaluate small intestine bacterial overgrowth (SIBO). Under the standard SIBO's therapy (long-acting beta-agonists, inhaled glucocorticoids), the first group (15 patients) had being tested with Rifaximin for the SIBO therapy during 7 days. The second group (15 patients) had been tested with Rifaximin and with a succeeding probiotics therapy for three months (*B. bifidum, B. longum, B. infantis, L. rhamnosus*). SIBO was diagnosed for 30 (67%) patients. We have detected a higher IgE level ($P<0.01$), a higher eosinophils level ($P<0.001$) in sputum and more significant decrease of FEV_1 ($P<0.01$) in SIBO(+). The IgE level in patients was decreased ($P<0.01$) after the complex SIBO therapy both for the Rifaximin therapy group ($P<0.05$) and for the Rifaximin + Probiotic therapy group ($P<0.05$). A dramatic decrease of the IgE level ($P<0.05$) had been induced by probiotics and it was confirmed by the control testing results with a high statistical accuracy for the observed groups of patients. We did not detect any changes for the patients without SIBO ($P=0.46$), those who had been treated with a standard therapy. A decrease in the number of patient hospitalization was defined by the treatment with probiotics after SIBO therapy ($P<0.05$). So, SIBO is a significant factor aggravating the atopic asthma in patients. The gut microflora correction with probiotics therapy has been accompanied by a statistical reliability improvement for the immune response and spirometry, as well as by a decrease in the number of hospitalizations for these patients during the year.

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Introduction

Over the last few decades, allergic diseases have been becoming some of the most serious healthcare issues. A prevalence of asthma, food allergy and atopic dermatitis is increasing in the world. The Th-2 immune response has been formatted by the large number of allergens in patients. An increasing of the Th-2 depended cytokines (IL-4, Il-5 and IL-13) secretion and the products of the allergic-specified IgE have introduced the allergic reaction activation.[1] Certainly, a stability of the immune Th1/Th2 balance is defined by genetic factors and this is regulated by the transcription functions of GATA-3 (Th2) and T-bet (Th1). Nevertheless, one of the main factors to support the human immunity is the gut microbiome which is also important for the adaptive possibilities of the organism.

The human body is provided with microbiome with the biggest antigenic stimulus source, which is programming a post-natal immunity evolution. An influence of the normal microflora on the immune regulation out of the digestive system was established. And this phenomenon has been actively investigated by different scientists.

Signals have been transmitted by synanthropic bacteria and their metabolites are interacting with Toll-

like receptors (TLRs), inducing effector functions have been bound to an expression of the nuclear transcription factor, Dendritic cells, T regulatory lymphocytes, chemokines, and cytokines. So, this hypothesis has been actively discussed at present - the microbiota bacteria are affecting the important aspects of lung immunity and susceptibility to allergens. As it was demonstrated by different investigations, the probiotic preparations can modulate the congenital and adaptive immunity. By activating the TLR, microorganisms are initiating the immune response, which could introduce the system effects.[2-4] So, this fact gives a base to investigate an opportunity to use probiotics for an integrated treatment and prevention of atopic asthma.

The aim of our work is the research and analysis of the efficacy of the gut microflora correction on a disease development in patients with atopic asthma.

Materials and Methods

Forty-five allergic atopic asthma patients, who had not been taking any antibacterial drugs for three months before the testing, were examined in our investigation. The clinical and biochemical analyses of the patients' blood, sputum and urine, level of immunoglobulins (A, G, E classes) were examined in this study. The chest X-ray and a spirometry with a bronchodilation test were performed on all patients.

Patients with less than 80% of the predicted forced expiratory volume (FEV_1) per second and with a positive test with a bronchodilator (more than 15% rising from the initial FEV_1) were tested.

These parameters were selected in accordance with GINA-2017 (Global Initiative for Asthma) and they corresponded to the medium level of atopic asthma disease in this investigation. Salbutamol (dosing: 200-800 mcg) was used to confirm reversible bronchoconstriction in the patients, the patient's reactions had been measured for 15 min. Patients did not take any medicines before the testing: short-acting beta agonists - 6 h before; long-acting beta-2 agonists - 12 h before; theophylline - 24 h before.

To define the small intestine bacterial overgrowth (SIBO) we used the hydrogen breath test with a lactulose (*Bedfont*, GB). The test was positive, when the hydrogen concentration had increased to 12 ppm over from the initial level, even if this increasing happened one time during the test.

All patients were treated with the standard base therapy, which consisted in the long-acting beta-2 adrenomimetics and inhaled glucocorticoids (Salmeterol + Fluticasone, Budesonide + Formoterol, Beclometasone + Formoterol) for atopic asthma.

Fifteen patients took Rifaximin with dose 800 mg/day for seven days, other patients took Rifaximin with the same dose and after that they took probiotics (*Bifidobacterium bifidum* not less than 1×10^9 CFU; *Bifidobacterium longum* not less 1×10^9 CFU; *Bifidobacterium infants* not less 1×10^9 CFU; *Lactobacillus rhamnosus* not less 1×10^9 CFU) one capsule three times a day for a month. The control spirometry was performed twice on all patients after the 14th day and for a month after the treatment. The second hydrogen test and lab analysis control was repeated for a month after the treatment. The patients' hospitalization frequency was also analyzed over the following year.

Statistical data were calculated with the STATISTICA 10 program (StatSoft Inc., USA). The non-parametric statistical methods were used to analyze the obtained data. An evaluation of the medium data was made with t-tests, χ^2 and Fisher's criteria, the significance level was considered as acceptable at $P<0.001$, $P<0.01$, $P<0.05$.

Results

Forty-five patients with atopic asthma (21 males, 24 females) were included in the study. SIBO was diagnosed for 30 (67%) patients, these patients were included in a group SIBO (+). There are 16 females and 14 males among them. 15 (33%) patients were without SIBO and they were included in the second group SIBO (-), there were 8 females and 7 males among them.

The groups SIBO (+) and SIBO (-) were compared according to the following parameters of age (41.4±12.49 years *vs* 45.1±12.92, P=0.22), body mass index (25.3±3.7 kg/m^2 *vs* 25.6±3,97, P=0.44), duration of anamnesis (20.06±10.2 year *vs* 18.8±8.6, P=0.37) and severity of the disease (P>0.05). The next results were obtained for the IgE level (348.4±110.16 IU/mL *vs* 237.1±103.51, P=0.006), for the IgA level (1.18±0.29 IU/mL *vs* 1.24±0.11, P=0.146), for the IgG level (13.40±1.60 IU/mL *vs* 14.05±1.62, P=0.156), for the sputum eosinophils level (7.4±3.03 pic. *vs* 3.06±1.55, P=0.0002), for the blood eosinophils level (3.6±2.01% *vs* 3.28±1.11, P=0.306) and for the most significant decrease of FEV_1 (64.6±5.31% *vs* 69.6±5.47, P=0.011) for these groups.

When Rifaximin was added to the base treatment course, the IgE level decreased with a good statistical reliability (348.4±110.16 *vs* 249.0±89.38, P<0.01) for patients with SIBO. We did not detect any changes in patients without SIBO (237.1±103.5 *vs* 241.0±90.2, P=0.46), these patients were treated with a standard therapy.

Fifteen patients were treated with Rifaximin + probiotic and other 15 patients were treated only with a Rifaximin therapy for the SIBO (+) group (30 patients) in this investigation. The IgE level was not significantly different between the two groups before the therapy (308.4±139.28 *vs* 368.5±95.3; P=0.13).

The IgE level on the background of SIBO treatment had clearly decreased in both groups (P<0.05) (Table 1). Moreover, after a control testing, the IgE level turned out to have decreased dramatically in the group treated with probiotic therapy compared to the group that was treated only with a Rifaximin (192.5±60.5 *vs* 280.83±81.57, P<0.05).

A change in the spirometry parameters demonstrated that breath function had improved during the treatment of diseases in both groups (Figures 1 and 2).

The hospitalization cases decreased for patients under the SIBO treatment on the average up to one time per year in comparison with 2-3 times annually before the SIBO therapy. In the SIBO (+) group, 19 patients (63% from the total number of the SIBO (+) group patients) needed one hospitalization. 15 patients (79%) were taking only Rifaximin, and the other 4 patients (21%) were taking Rifaximin + probiotic (P<0.05 exact Fisher's criteria). Patients without any probiotic therapy were hospitalized more frequently both in the 6-month period and in the 6-12-month period after starting the tests (Fisher's criteria P<0.05). Hospitalizations were not needed for 11 patients (37% from the total quantity of the SIBO (+) group patients) in the next year. All of them had a long-term SIBO therapy (Rifaximin + probiotic).

All patients (15 people - 100%) from SIBO (-) group had received a base therapy and were hospitalized two or more times in the following year.

Discussion

It is also known that the changing of microflora-has been confirmed by the published clinical and experimental results. This change could introduce an organism's susceptibility toward an exacerbation of atopic asthma and could cause enforcing a bronchial obstruction, so this could influence the final therapy result.[2,5-11]

Our results have shown a frequent concurrence of the bacterial overgrowth syndrome and asthma in this research. 67% of patients with atopic asthma had SIBO.

When the atopic asthma patients had SIBO, and therefore, their organisms were more susceptible to allergens, in that case the high levels of IgE (P<0.01), eosinophils in sputum (P<0.001) have been combined with a more significant decrease of FEV_1 (P<0.01). In our opinion, these results could confirm a clear expressed immune's activity simultaneously with the gut microbiota change and IgE immune response formation in patients.

The pathogenesis of the immune interactions influence on the airway hyperactivity and this phenomenon may be related with several factors. First of all,

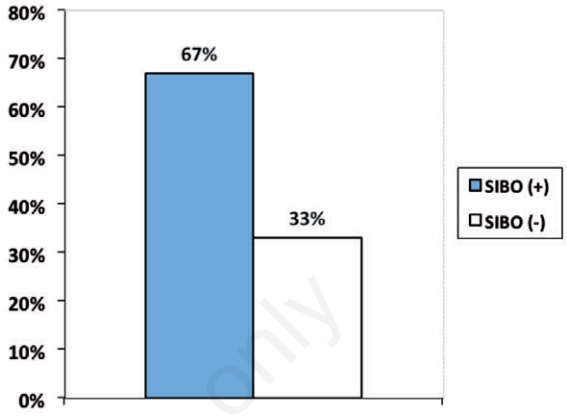

Figure 1. A frequency of small intestine bacterial overgrowth (SIBO) detection in the patients with the atopic asthma.

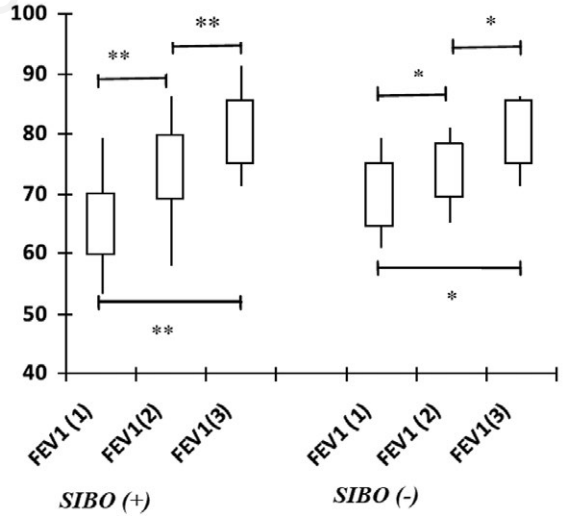

Figure 2. A changing of spirometry parameters in patients with atopic asthma (FEV_1 (1) - before a treatment, FEV_1 (2) - for two weeks after the treatment, FEV_1 (3) - for four weeks after the treatment). **P<0.001; *P<0.05.

Table 1. Immunoglobulin E in small intestine bacterial overgrowth (SIBO) (+) groups before and after the SIBO's therapy.

Therapy	IgE (IU/mL), before treatment, m±σ	IgE (IU/mL), after treatment, m±σ	P
Rifaximin + Probiotic (n=15)	308.4±139.28	192.5±60.5	<0.05
Rifaximin (n=15)	368.5±95.3	280.83±81.57	<0.05

the IgA secretory level has been decreased under SIBO, its deficiency was associated with an increasing of the atopy risk.[9] A change in *Lacto-* and *Bifidobacterium* levels was accompanied by the bacterial contamination and translocation. So, endotoxins penetrated into microcirculation channel through destroyed epithelial barrier and contributed to the activation of the inflammatory reactions and modulation of immune cells.

A polarization of the immune response to the side of Th2 type leads to an activation and synthesis of IgE and IgG4 by B-lymphocytes, as well an activation of eosinophils cells and a subsequent release of histamine, leukotrienes (C4, D4, E4), prostaglandin D, interleukins (IL-3, -4, -5, -6, -7, -8), neutrophil and eosinophilic chemotactic factors, tumor necrosis factor - alfa (TNF-α).[2,3,9]

The SIBO influence on the development of allergic reactions could be bound up not only with a damaged biofilm and the direct contact of allergens with mucus, but also with the oversupplying biogenic amines. Experimental investigations have shown that the gut microbiota takes part in the histamine metabolism, which is one of the most important allergic mediators. In case of increased permeability of the intestine and in case of a decrease in the histamine production by the damaged mucus membrane, histamine is actively absorbed into blood, intensifying the phenomenon of bronchospasm.[12]

An added pathogenic factor is a disturbance of the ventilation-perfusion ratio of a lung under atopic asthma, which defines a decrease in CO_2 and H_2 release out from the organism. This can cause a shifting of the oxidation-reduction potential of the intraluminal environment providing an active growth of opportunistic anaerobes, in particular conditionally pathogenic strains of bacteroides.[13]

As it was published, the gut microbiota can change the human biorhythms by influencing the serotonin, dopamine, and melatonin production.[10] According to the opinion of some investigators, this could be one of the reasons for atopic asthma exacerbation and even for a different response to the therapy.[11]

The IgE level had decreased both in patients after the SIBO therapy with Rifaximin ($P<0.05$), and in the group taking Rifaximin + Probiotic ($P<0.05$) too. A dramatic decrease of the IgE level ($P<0.05$) was induced by probiotics and it was confirmed by the control tests results with a high statistical accuracy under the groups comparison.

As it was seen on the therapy background, breath function improved during the treatment of diseases in all patients ($P<0.001$, $P<0.05$). This result was evaluated as positive after the therapy inward. Nevertheless, we can define a higher statistical reliability level for the result in the group with atopic asthma after the SIBO's correction ($P<0.001$).

So, the effective correction of the gut microbiota contributed to a normalization of the immune response indicators. This fact was observed again upon further examination of patients. We have defined a decrease in the number of patients' hospitalizations because of the treatment with probiotics after SIBO therapy ($P<0.05$). We have assumed the long term SIBO therapy with probiotics has played a significant role in the obtained results.

Probiotics can modulate a gut microbiome composition. Probiotics contribute to the Toll-like receptors activation launching the cascades of reactions, including the proteins and transcription factors activation, simultaneously inducing a secretion of cytokines. The probiotics efficacy could be achieved from the external environment change in the lumen of the intestine, the pH level decrease, and competitions for nutrients. At the same time, it may be caused by a bounding of the specific receptors, which have caused a restriction of the physiological conditions for the growth of pathogenic bacteria.[11] Furthermore, the probiotic bacteria help to restore the epithelial barrier and mucus secretions, moreover they produce the bacteriocins, which suppress a pathogenic bacteria growth also. Probiotics produce a lot of short-chain fatty acids (SCFAs) during a fermentation process of the dietary fibers, these acids render a very powerful anti-inflammatory influence.[2,6,7]

Conclusions

One of the experimental limitations was the small quantity of patients included in the groups tested for this study. Nevertheless, based on the results of this research, we can state the following: for the tested groups of patients, the correction of the gut microflora composition had been accompanied by an improvement of statistical reliability in terms of immune response and breath function, as well as by a decrease in the number of hospitalizations during the year. So, the bacterial overgrowth syndrome is a significant aggravating factor for a course of atopic asthma disease, because it plays an important role for a development and supporting for the patients' sensibilization. At present, an application of the probiotic bacteria has demonstrated a very encouraging result in a complex therapy both in terms of prevention and of treatment of allergic diseases. However, the reasonableness of these conclusions should be confirmed by further randomizing experiments.

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