

STUDY ON IMMUNOMODULATORY EFFECTS OF PROBIOTICS IN ASTHMA

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Abstract: Background and purpose: Asthma is based on adjusting the balance of Th1/Th2 in the direction of augmented production of Th2 cytokines. The interest in the therapeutic potential of probiotics in the case of allergic diseases is revealed from the fact that they enhance the intestinal permeability and reduce the level of inflammatory cytokines. According to the latest research, altered production of Th2 cytokines, by the induction of Th1 responses can occur both in prophylaxis and also in the treatment of allergic diseases. Materials and methods: Patients included in the study were given a combination of probiotics, daily for four weeks. Biological evaluation of patients who received immunomodulator treatment with probiotics included the determination of blood eosinophilia, by determining the peripheral blood picture and the total immunoglobulin E. In order to evaluate the allergic inflammation, plasma levels of interleukin-4 (IL-4) and Interferon gamma (IFN γ) were determined in serum. Laboratory measurements were made at the inclusion in the study and were repeated after four weeks of treatment. Results: Despite short-term consumption, probiotics was able to reduce the IL-4 levels in association with an increased production of IFN γ . Correlation between decreased peripheral eosinophil and that of total IgE was direct, statistically significant; correlation between the value changes of IFN γ and IL-4 was indirect. Conclusions: Our results confirm that probiotics might have a beneficial effect against allergic diseases by modulating the TH1/TH2 response. Clinical trial assessment is required to elucidate whether this modulation might be useful in the prevention and treatment of allergic diseases.

INTRODUCTION

In this moment, over 20% of the global population suffers from some form of allergy with a prevalence that tends to increase. Although the exact etiology remains ambiguous regarding the allergic diseases, most researchers believe that the essential factor in the onset of allergies is represented by an epiphenomenon of exposure to the environment.(1)

In this study, I started from the premise of hygiene hypothesis, according to which, the increase in the prevalence of the allergic diseases occurs due to a decrease in exposure to microbes in early in life.(2)

By early contact with microbes in immune and metabolic programming, even during the fetal and childhood period, there appear new opportunities to improve the health of pediatric patient and to reduce subsequently the risk of exposure to various diseases.(3) According to the hygiene hypothesis, early development of the immune system is influenced both qualitatively and quantitatively by the microbiota bacteria. Intestinal microbiota is extremely variable in response to diet and environmental factors and, at the same time, it governs a number of aspects of immune function in the body. The role of bacteria in the intestinal flora in modulating the balance of Th1/Th2 is demonstrated herein, as a result of striking a balance between pro-inflammatory cytokines and anti-inflammatory cytokines.

A great part of the research on allergic diseases have focused on intestinal microbiota, allergic diseases in children being partially associated with imbalances in the intestinal microflora.(4) In the case of dysbiosis, rebalancing the microbial balance can be achieved by administering probiotics "live microorganisms administered in adequate amounts confer a health benefit for the host".(5)

The use of probiotics in preventing or treating allergic pathology is a new concept. Studies in recent years have been focused on a potential pediatric nutrition supplements with probiotics, some studies demonstrating control of inflammatory responses, a change in the cytokine profile, in the spirometric parameters, or clinical evolution in allergic patients of pediatric age.(6)

PURPOSE

The main objective of this paper was to study the induced alterations on serum markers of allergic inflammation in patients with occasional wheezing, recurrent and especially asthma of immunomodulatory treatment with probiotics. In this aspect, I intended to evaluate the dynamics of IL-4, IFN γ , serum eosinophil and total IgE in these patients after treatment with probiotics.

MATERIALS AND METHODS

To achieve the proposed objectives, there was selected a group of 80 children of both genders diagnosed with occasional wheezing, recurrent and asthma in Sibiu Pediatric Hospital, Clinic of Pediatrics, 2013-2015.

Patients included in the study received a combination of probiotics, daily for four weeks. Biological evaluation of patients who received immunomodulator treatment with probiotics included the determination of blood eosinophilia, by determining the peripheral blood picture and the total immunoglobulin E. In order to evaluate the allergic inflammation, there was determined in serum, the plasma levels of IL-4 and IFN γ . Laboratory measurements were made at the inclusion in the study and were repeated after four weeks of treatment.

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CLINICAL ASPECTS

The patients received a combination containing 3 probiotic strains of bacteria. Each capsule contains 5.04 mg of freeze-dried bacteria (at least 1.2×10^7 units forming colonies in which: 4.5×10^6 Lactobacillus acidophilus 3.0×10^6 , Bifidobacterium infantis, 4.5×10^6 Enterococcus faecium). Excipients, capsule content: lactose, dextrin, potato starch, magnesium stearate, the capsule shell: hypromellose, titanium dioxide, gelling agents and water.

The serum of the patients was analyzed by enzyme linked immunosorbent assay (ELISA) method, for total IgE, IL-4 and IFN γ . Data were loaded and processed using SPSS 18.0 statistical functions.

RESULTS AND DISCUSSIONS

This study aimed at investigating the effects of probiotics on TH1/TH2 paradigm. The current study revealed that treatment with a combination with Lactobacillus acidophilus, Bifidobacterium infantis, Enterococcus faecium induced increase of IL4 level and reduction of IFN γ level, and this could be explained by polarization of immune system of allergic patients towards TH2, more IL4 and less IFN γ

After four weeks of treatment, there was a decrease of eosinophils (expressed as percentage), statistical significant ($p = 0.05$). At the beginning of the treatment, there was a mean value of 4.03% eosinophils, this value decreased after a month, with 2% to the value of 2.03%. The mean values of total IgE decreased by 26.66kU/l. The decrease in total IgE levels was significantly correlated with the decrease in the number of eosinophils ($p=0.05$). A decrease in the mean value of eosinophils, respectively total IgE, was recorded, observing higher differences in males (-2.82% vs. -2.02% respectively -29.38 kU/l vs. -23.47 kU/l). Among age groups, there was not underlined any significant decrease of eosinophils number (-3.82 vs. -1.02%; $p = 0.08$) and total IgE levels (-31.30 vs. -21.90 kU/l).

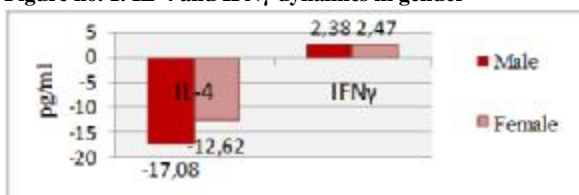
When assessing patients after four weeks of treatment, there has been noted a statistically significant decrease in serum levels of IL-4. Thus, at the beginning of the treatment, there has been recorded a mean value of IL-4 to 16.85pg/ml, this value decreased during the treatment to 6.44 pg/ml, a statistically significant decrease ($p = 0.005$).

The evolution of IL-4 and IFN γ is indirectly correlated, the mean values of IL-4 decreases by 10.4pg/ml, while that of IFN γ increases by 2.42 g/ml. According to the literature data, treatment with probiotics increases the levels of IFN γ in parallel with the decrease in IL-4 levels, such effects are dependent on the used strain, indicating a change in the balance of IFN γ and IL-4.(7)

In many clinical studies, there has been underlined the probiotic potential, that to induce the release of cytokine Th1, and also IFN γ (8) and to inhibit the production of cytokines Th2, and also IL-4.(9,10) Although this balance changes Th1/Th2 after the treatment with probiotics, it is still controversial.(11)

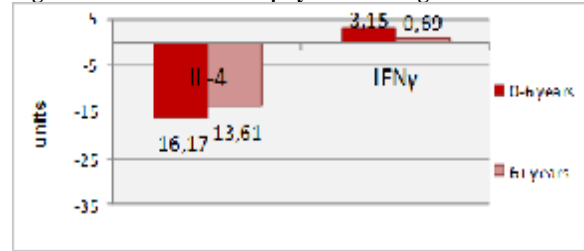
In genders, there were underlined decreases of values IL-4 (-17.08 vs. -12.62 pg/ml) and increases of the values IFN γ (2.38 vs. 2.47 ng/ml) greater in males.

Figure no. 1. IL-4 and IFN γ dynamics in gender



Analogously, in age, there were highlighted decreases of the values IL-4 (-16.17 vs. -13.61 pg/ml) and increased values of IFN γ (3.15 vs. 0.69 pg/ml).

Figure no. 2. IL-4 and IFN γ dynamics in age



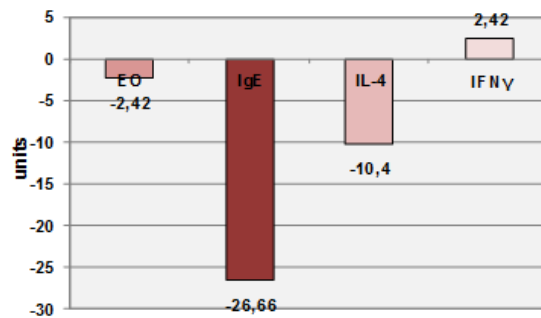
Decrease of values IL-4 and total IgE is, in both cases, higher in patients with recurrent wheezing compared with those with asthma, the lowest decrease being recorded in patients with occasional wheezing, differences between the initial values and those recorded after the administration of probiotics is significantly higher in these situations.

From a statistical point of view, the analysis of mean values of allergic inflammation markers post-treatment concluded that the highest values of IgE, eosinophils and IL-4 are found in children with asthma ($p < 0.05$), while in children with occasional wheezing, there were noted the highest mean values of IFN γ ($p = 0.001$).

If at the beginning of the study, there were noted significant correlations, direct between the values of IgE and the number of eosinophils ($r = +0.192$; $p = 0.042$), between the levels of IgE and IL-4 ($r = +0.415$; $p = 0.025$), between the number of eosinophils and the values IL-4 ($r = +0.670$; $p = 0.001$) and indirect between the values of eosinophils and the values of IFN γ ($r = -0.226$; $p = 0.048$), at the end of the study, the correlations were not statistical significant.

Correlation between decreased number of peripheral eosinophils and total IgE levels was direct, statistically significant, but moderate in intensity ($r = +0.278$; $R^2 = 0.0775$; $p = 0.012$).

Figure no. 2. Evolution of inflammatory markers

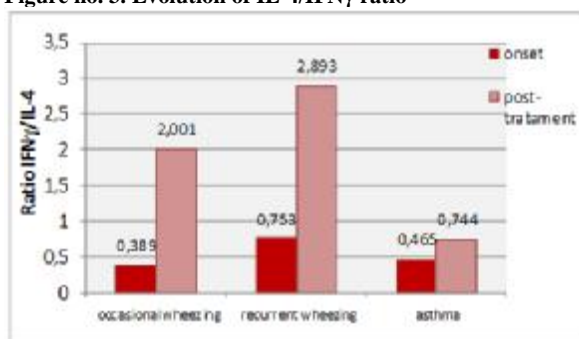


The correlation between the changes of IFN γ and IL-4 levels was indirect, moderate in intensity, being underlined by the fact that 25.6% of patients associated increased values of IFN γ with the decrease of IL-4 levels ($r = -0.256$; $R^2 = 0.0653$; $p = 0.022$).

Cytokines' report value IFN γ /IL-4 initially, ranged from 0.006 in patients with occasional wheezing up to 5.13 in those with recurrent wheezing, without being recorded significant differences between the medium values on the basis of the diagnosis. The highest average ratio value IFN γ /IL-4 was observed in patients with recurrent wheezing (0.753 vs. 0.389 occasional wheezing and respectively 0.465, asthma).

CLINICAL ASPECTS

Figure no. 3. Evolution of IL-4/IFN γ ratio



After the treatment, the report of cytokine values IFN γ /IL-4 varied from 0.03 in patients with asthma up to 26.88 in those with recurrent wheezing, registering significant differences of mean values depending on the diagnosis ($p=0.027$). The highest mean values of the IFN γ /IL-4 report were underlined in patients with wheezing (2.001 occasional wheezing vs. 2.893 with recurrent wheezing and respectively, 0.744 with asthma), suggesting an achievement of new equilibrium of Th1 and Th2 profile.

CONCLUSIONS

Asthma was described as being the result of a deficit of bacterial stimulation during childhood. Our results confirm and extend previous observations that probiotics might have a beneficial effect against allergic diseases by reducing TH2 cytokine production. Despite short-term consumption, probiotics were able to reduce the IL-4 levels in association with an increased production of IFN γ .

Based on the limitations of this study, extrapolations to clinical effects must be considered with caution. These data support the study of probiotics consumption in a clinical trial to further demonstrate its potential beneficial effect.

Therefore, clinical trial assessment is required to elucidate whether this modulation might be useful in the prevention and treatment of allergic diseases.

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