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Original article

The effect of food elimination and probiotic supplementation in asthmatic children with food allergy

Background: *Both bronchial asthma and food allergy are comorbidities of increase prevalence and growing concern worldwide.* ***Objectives:*** *to detect the prevalence of food allergy in children with bronchial asthma, the effect of food elimination and probiotic supplementation on the clinical outcome of asthma and the quality of life (QOL).* ***Methods:*** *This randomized controlled trial included 226 children aged from 4 to 18, suffering from bronchial asthma, 88 of whom had associated food allergy. Patients who suffered food allergy as diagnosed by history, prick to prick test food elimination and oral food challenge test, were randomly divided into four groups, each comprised 22 children. Group (1): received pharmacological treatment only, group (2): received pharmacological treatment and probiotic supplementation, group (3): practiced food elimination and received pharmacotherapy, and Group (4): practiced food elimination and received probiotic supplementation and pharmacological therapy. For patients in all groups, grading of asthma severity, measurement of total IgE and Pediatric Asthma quality of life questionnaire (PAQLQ) were performed before and after 6 months at the end of the study.* ***Results:*** *There were significant statistical improvements of severity of asthma, total serum IgE level and QOL for all groups before and after intervention. The best outcome was achieved in children who practiced avoidance of food allergen(s) and took probiotic supplementation in addition to the pharmacological therapy (p <0.001).* ***Conclusion:*** *Diagnosis of food allergy in asthmatic children is mandatory and combining pharmacological therapy, avoidance of the offended food allergen and intake of probiotics are encouraged.*

Keywords: Pediatric asthma, food allergy, probiotic supplementation.

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INTRODUCTION

Both bronchial asthma and food allergy show increase in prevalence worldwide, this makes the management of children with food allergy and asthma a growing concern.[1] Population studies have shown that an early food sensitization or food allergy in the first year of life may precede the development of asthma.[2,3] When asthma and food allergy coexist, they adversely influence the course of each other. Asthma attack can be elicited by food allergens in sensitized children.[4]

The cornerstone of the nutritional management of food allergies is an individualized allergen avoidance management plan. In children, the main goals are to prevent the occurrence of acute and chronic symptoms by avoiding the offending food(s), whilst providing an adequate, healthy and nutritionally balanced diet and maintaining optimal growth.[5]

The role of the intestinal microbiota in the development of immune tolerance to food is increasingly appreciated. The commensal gut microbiota targets different cellular components of the innate and adaptive immune compartments to promote oral tolerance.[6] One mechanism by which the commensal microbiota influences the outcome of the allergic response is by modulating the innate lymphoid cells (ILC) to secrete IL-12. The commensal microbiota also targets the adaptive immune response to promote tolerance through promoting the differentiation of induced T regulatory (iTreg) cells from naive CD4+ T cell.[7]

Probiotics are living bacteria intended to have health benefits, they are not only a driver of growth but also a modulator of the immune system and prevention of many diseases.[8,9,10] The most commonly used probiotics are the strains of lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus* (*S.)*

thermophilus. Other organisms including *Enterococci* and yeasts have also been used as probiotics. Specifically, important, probiotics can induce a milieu of tolerogenic immune responses to food.[11]

In this work we aimed to detect the prevalence of food allergy in children with bronchial asthma, the effect of elimination of food allergens on the clinical outcome of asthma and the quality of life of these children and, also, we aimed to detect the effect of probiotic supplementation on quality of life and clinical manifestation in these children.

METHODS

The study was held in Allergy and Immunology Unit (the unit deals with both adult and pediatric allergic patients), Medical Microbiology and Immunology and Pediatric departments, Faculty of Medicine, Zagazig University from June 2019 to September 2020.The Institutional review board approved the study according to the ethical principle of the declaration of Helsinki, written informed consent was taken from patients. The IRB approval number was 5390.

This randomized controlled interventional study included 226 consecutively recruited children aged from 4 to 18 years, suffering from bronchial asthma from pediatric outpatient clinic (Diagnosis was based on GINA guidelines)[12], patients with different grades of asthma and different levels of control were included. From among these children, those who had associated food allergy were included in the study.

Food allergy was first suspected by history, then food sensitization was indicated by the presence of specific IgE (detected either by skin prick test or by serum specific IgE). Food allergy confirmed diagnosis was based on oral food challenge test.

Exclusion criteria were children less than two years, those who had received immunosuppressants, antibiotics, systemic corticosteroids within 4 weeks, children who had immunodeficiency disease, children who used probiotic preparations within 4 weeks, those suffering from broncho-pulmonary disorders or infectious diseases, non-cooperative patients and patients who had received immunotherapy before the start of the study.

Study design

Patients who suffered from both bronchial asthma and food allergy were randomized in four different groups depending on a blind selection of colored cards, yellow for group I and green for group II, red for group III and blue for group IV. Each group comprised 22 patients as follows: group (1): were given pharmacological treatment only; group (2): were given pharmacological treatment and probiotic supplementation; group (3): practiced food elimination and received pharmacotherapy, and group (4): practiced food elimination and received probiotic supplementation and pharmacological therapy.

Patients were followed up after 3 months to step up or stepdown treatment according to deterioration or improvement of symptoms, then another final assessment was done after 6 months of study.

Asthma severity grading

Patients were classified in all four groups into intermittent, mild persistent, moderate persistent, and severe persistent according to symptoms, nighttime awakening, drugs used, interfering with normal activity, lung function.[13] Initial grading was done at the beginning of the study, patients were followed up at 3 months and then at 6 months for final grading and this was used to compare with the grading at the initial presentation.

Total IgE assay

Total serum IgE levels were measured using commercially available kits (Biocheck Total IgE kit) from Biokit (South San Francisco, CA 94080). It was done for the four groups according to the manufacturer guidelines. The minimum detectable concentration of IgE by this assay is estimated to be 5.0 IU/ml

Pediatric Asthma quality of life questionnaire (PAQLQ)

This questionnaire used for following up at baseline and after 6 months. The questionnaire was in standardized Arabic version supplied by Elizabeth Juniper (MCSP, MSc, Professor, England, received by mail) and filled by the parents of children. It included 23 questions to assess activity and quality of life of these children as questions 4,6,8,10,12,14,16,18,20 and 23 were about symptoms, questions 1,2,3,19 and 22 were about activity while questions 5,7,9,11,13,15,17 and 21 asked about emotional effect. High score of the questionnaire indicated better control of asthma and better quality of life.

Detection of specific IgE for food allergens

Prick to Prick test was done unless patients suffered from unstable asthma, un-cooperative children or parents' preference of serum specific IgE assay.

A) **Prick to prick skin testing:** The food used for testing fruit and vegetables should be fresh (not tinned or cooked in order not to alter allergenicity). For fruit / vegetables a lancet was pushed into fleshy part of the food then a small

amount of the food substance was placed onto the skin. Then lancet was pushed through the surface layer of the skin with an angle of 90° angle through the food. For foods like peanut; it was grinded to make a paste using sterile saline, the tip of the lancet was put in this paste and a small part of the paste was placed on the skin before pricking through it with the lancet.[14] The test was done according to the recommendations of European society of Allergy and Clinical Immunology (EAACI); the negative control was saline solution and the positive control was histamine dihydrochloride (10mg/ml) (Omegadiagnostic, Canada), positive test was considered when the test material produced a wheal >3mm above the wheal produced by the negative control.[15] Determination of the type of the food used in the test was based on careful history taking.

B) Serum specific IgE assay for food allergen (AllergyScreen / AlleisaScreen Spec. IgE) made in Germany. Immunblot assay for the quantitative determination of specific IgE in human serum. It was done for groups III and IV. Interpretation of the results were based on: none found or hardly exists: <0.35 IU/ml, Low: 0.35-0.69 IU/ml, Increased: 0.70-3.49 IU/ml, Significantly increased: 3.50-17.49 IU/ml, High: 17.50-49.99 IU/ml, Very high: 50.00-100 IU/ml, and extremely high: >100.00 IU/ml.

Food elimination

Food elimination was based on detailed history and or results of specific IgE detection. For all groups, patients suspected to have food allergy by history or positive specific IgE, were instructed to eliminate this food for 2-4 weeks from their diet. After this period food was reintroduced again under medical supervision through open oral food challenge test (OFC) to confirm food allergy diagnosis.[16] Patients in group groups III and IV were instructed to avoid food which they prove to be allergic for six months (the end of the study).

Oral food challenge test (OFC)

Open OFC test was performed. On the day of the test, a child should be in a good health, chronic allergic conditions such as asthma, atopic dermatitis (eczema) and allergic rhinitis (hay fever) have to be well controlled in order not to interfere with the interpretation of any symptoms, and antihistamines have to be stopped before the OFC since they might mask mild early symptoms. Emergency treatment such as epinephrine, antihistamines or inhaled asthma rescue medications should be available. The test was done according to the EACCI guidelines. OFC was preceded by elimination of the suspected food. Briefly, children were given increasing amount of the tested food, on interval of 25-20 min. Doubling of dose continued until the child had the regular intake amount. Before each dose all the vital signs of the child were recorded and compared with the base line measures before the beginning of the test. The test was considered positive if allergic manifestations were detected after any given dose.[17]

Probiotic supplementation

Probiotic supplements containing *Lactobacillus acidophilus* LB Strain 10 billion (Lyophilized microbial bodies), Lactose monohydrate, calcium carbonate, silicic acid, banana-orange flavour, saccharose, q.s. 800mg were given for children in groups II and IV. Children received probiotics as one sachet Bid x 2 days, for three months.

Statistical Analysis

Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures were coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 22.0) software for analysis. For data comparison, The Chi square test (X), Kruskal Wallis test (K), percentage of change and Fisher exact test (F) were used. A p-value equal or less than 0.05 was considered significant, highly significant values were recognized when P was less than 0.01.

RESULTS

Prevalence of food allergy among asthmatic children

Out of 226 asthmatic children, 88 children had been diagnosed with food allergy. Diagnosis of food allergy was based on the result of OFC test. The prevalence of food allergy in asthmatic children was about 38.9%. Detected food allergens were cow milk and casein 68 (77%), followed by chicken 56 (65%), egg 52(59%), banana 52 (59%), fish 52 (59%), wheat 48(54%) and peanut 28(31%).

Age, sex, Severity of asthma, Quality of life and total IgE in the four groups before treatment

At the beginning of the study, there were no statistical significance differences between the studied groups regarding age, sex, severity of asthma, total IgE and QOL. (table 1).

Severity of asthma, Quality of life and total IgE for each group before and after treatment

There was statistically significant decrease in the severity of asthma in all groups except group 1. However, IgE levels, and QOL showed high statistically significant improvement (Table 2 and figure 1).

Severity of asthma, quality of life and total IgE among four groups after treatment

Our results showed that there was a statistically significant increase in frequency of mild cases among group IV compared to groups I and II. Total IgE level significantly decreased with treatment in group IV compared to groups I and II , in group III compared to groups I and II and in group II compared to group I. There was a statistically significant increase in mean QOL score after treatment of group IV compared to groups I and II and also among group III compared to group I (table 3). However, comparing the individual categories of Qol regarding scores of symptoms, activity and emotion revealed no statistically significant difference P >0.05 (table 4).

Table 1. Differences in severity of asthma, total IgE and quality of life among four groups before treatment

Variable	Group I (*n*=22)	Group II (*n*=22)	Group III (n=22)	Group IV (n=22)		P
Age **(years)**					KW	
Mean ± SD	7.91± 2.80	8±3.13	9.45±3.70	8.55±3.14	1.86	0.60
Range	4-13	4-13	4-14	3-12		NS
Sex					χ^2	
Male: n (%)	16 (72.7)	14 (63.6)	12 (54.5)	12 (54.5)	1.05	0.79
Female: n (%)	6 (27.3)	8 (36.4)	10 (45.5)	10 (45.5)		NS
Severity: **n (%)**						
Mild	2 (9.1)	0 (0)	0 (0)	1 (9.1)	χ^2	0.87
Moderate	12 (54.5)	4(18.2)	4 (18.2)	1(9.1)	2.51	NS
Severe	8 (36.4)	18 (81.8)	18 (81.8)	18(81.8)		
IgE: Pre						
Mean ± SD	246.36 ± 292.02	404 ± 448.94	317.73 ± 405.38	301.36 ± 383.8	KW	0.99
Range	5 – 970	7 - 1000	10 – 1000	10 - 900	0.13	NS
QOL: Pre						
Mean ± SD	52.36 ± 5.48	53.45 ± 3.21	50 ± 3.66	49.91 ± 3.94	F	0.13
Range	40 – 60	48 – 59	43 - 56	44 - 57	1.98	NS

SD: Standard deviation, KW: Kruskal Wallis test, F:Fisher exact test , $X^{2:}$ Chi square test; NS: Non-significant (P>0.05)

Table 2. Grading of asthma severity pre and post treatment among the studied group.

Variable	**Pre-treatment**	**Post-treatment**	**% of change**	**P**
Group I (*n*=22)				
Mild persistent n (%)	2 (9.1)	4 (18.2)	-3.03%	0.45
Moderate persistent n (%)	4 (18.2)	6 (27.3)		NS
Severe persistent n (%)	16 (72.7)	12 (54.5)		
Group II (*n*=22)				
Mild persistent n (%)	0 (0)	2 (9.1)	-22.73%	0.02
Moderate persistent n (%)	4 (18.2)	14 (63.6)		
Severe persistent n (%)	18 (81.8)	6 (27.3)		
Group III (*n*=22)				
Mild persistent n (%)	0(0)	10 (45.5)	-37.88%	0.008
Moderate persistent n (%)	4 (18.2)	8 (36.4)		**
Sever persistent n (%)	18 (81.8)	4 (18.2)		
Group IV (*n*=22)				
Mild persistent n (%)	2 (9.1)	12 (54.5)	-43.94%	0.002
Moderate persistent n (%)	2 (9.1)	10 (45.5)		* *
Severe persistent n(%)	18(81.8)	0 (0)		

NS: Non-significant (P>0.05) ,* * Highly significant

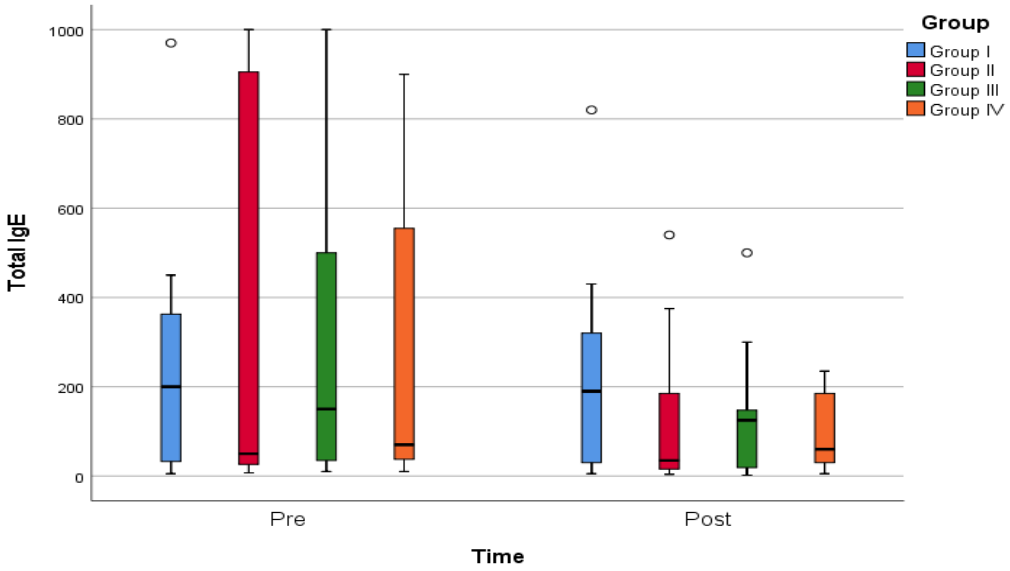

Figure 1. Total IgE level pre & post treatment among the studied groups.

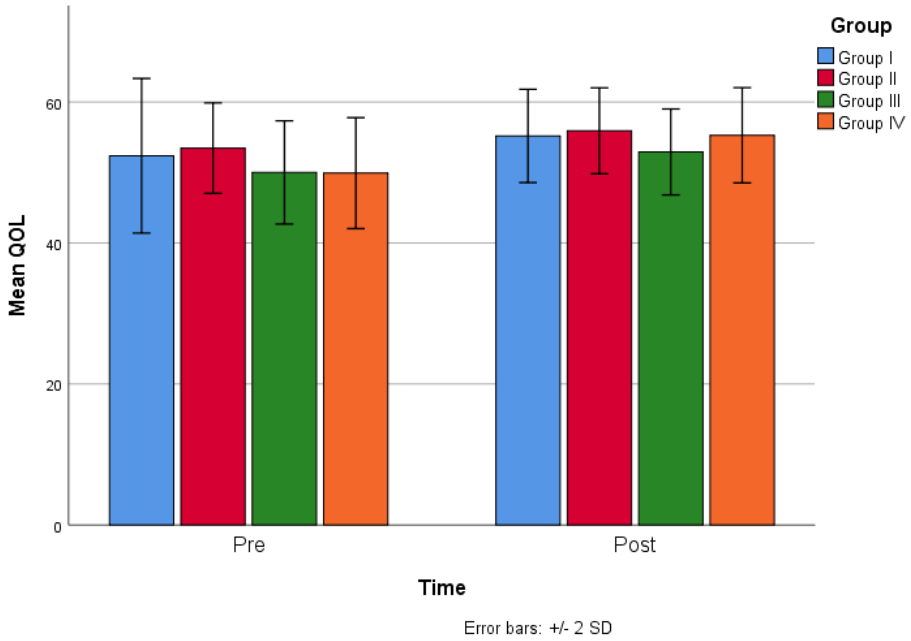

Figure 2. QOL score pre- and post-treatment among the studied groups.

Table 3. Severity of asthma, total IgE level, and QOL between four groups after treatment.

Variable	Group I (*n*=22)	Group II (*n*=22)	Group III (n=22)	Group IV (n=22)		P
Severity ***Mild* n (%)** ***Moderate* n (%)** ***Severe* n (%)**	 4 (18.2) 6 (27.3) 12 (54.5)	 2 (9.1) 14 (63.6) 6 (27.3)	 10 (45.5) 8 (36.4) 4 (18.2)	 12 (54.5) 10 (45.5) 0 (0)	 KW 13.52	 0.04*
IgE: Post ***Mean* ± *SD*** ***Range***	216.82 ± 252.39 5 – 820	174.91 ± 178.72 4 - 540	119.64 ± 140.25 2 – 500	103.18 ± 126.83 5 – 430	KW 8.99	<0.001 **
QOL **Mean ± SD** **Range**	 53.09 ± 4.39 45 – 60	 54.55 ± 3.05 49 – 60	 56.09 ± 3.81 49 – 60	 57.90 ± 3.24 49 - 60	F 3.52	0.02*

SD: Standard deviation, KW: Kruskal Wallis test, F:Fisher exact test , *: significant (P<0.05), **: highly significant (P<0.001)

Table 4. Changes in symptoms, emotion and activity scores of Qol for the four groups before and after treatment

		Group I	t P	Group II	t P	Group III	t P	Group IV	t P
Symptoms Mean±SD	Before	21.09 ±5.29	-0.47 0.32	21.45 ±1.07	-1.79 0.05	20.75 ±2.57	-1.6 .06	19.5 ±9.91	-1.27 0.10
	After	21.55 ±4.87	NS	22.27 ±1.22	NS	21.75 ±2.02	NS	20.92 ±4.99	NS
Activity Mean±SD	Before	10.82 ±4.36	-1.23 0.11	14 ±5.4	-0.22 0.41	9.25 2.2	-0.62 .27	10.33 ±8.61	-1.120 0.13
	After	12.17 ±9.06	NS	14.55 ±5.8	NS	9.6 1.16	NS	11.75 ±10.57	NS
Emotion Mean±SD	Before	20.45 ±16.9	- 1.074	18 ±5.2	-0.53 0.3	20 3.11	-1.40 .08	18 ±4.24	-1.356 0.094
	After	22.18 ±11.6	0.147 NS	19.18 ±5.07	NS	21.1 2.99	NS	20.33 ±3.8	NS

P: p value, t: paired t test, NS: non-significant

DISCUSSION

According to our results, out of 226 asthmatic children, 88 children had been diagnosed with food allergy, so the prevalence of food allergy in asthmatic children was about 38.9%. This study was consistent with another study reported that 48% of asthmatic patients had food allergy.[21] While other studies had found that 34% to 78% of asthmatic patients complained from food-related symptoms.[18,19,20,21] Also, Aba-Alkhail and El-Gamal, reported that the prevalence of clinical sensitivity to food was 29%. [22] Moreover, Friedlander et al., 2013 prospectively surveyed 300 asthmatic children, of whom 24% had food allergy out of them 12% had multiple food allergies.[23]

On the other hand, Krogulska et al, reported that IgE-related food allergy was present in 9.8% of children with asthma.[24] The difference in prevalence could be expected as a result of difference in food habits, genetic backgrounds and environmental conditions among different populations.

The four study groups were comparable before treatment regarding age, gender and severity of asthma and QOL. Severe asthma has a great impact on the quality of life (QOL) of patients and their families. QOL is defined as the perception that individuals have of their position in life, in the context of the culture and system of values in which they live and in relation to their objectives, expectations, standards and concerns.[25] Before the study, total IgE in all four groups ranged from 5 to 1000 IU/ml. Similar range of serum IgE (5-996) IU/ml was also obtained from a previous study on Egyptian children.[26]

In this study, the standardized questionnaire of asthma quality control (PAQLQ) score did not differ significantly among the four groups before treatment; the ranges were very near for all groups. Similar results were obtained when the same questionnaire was applied on a random sample of Egyptian children where mild cases had scores ranging from 41 to 58.[27]

Asthma control showed significant improvement with treatment in all groups except the group that received pharmacological therapy only, (p values were 0.45, 0.02, 0.008 and 0.002 respectively), where there were increases in the number of mild persistent and moderate persistent asthma at the expense of those suffering from severe persistent asthma. The lack of statistical significance was probably due to small sample size, especially in view of the significant improvement of QOL and decrease of the total IgE level with treatment. The improvement observed in all three parameters of the other treatment groups illustrate that pharmacological therapy, avoidance of food that triggered asthma and the use of probiotics can have a significant positive impact on the course of the disease and the quality of life of these children. The impact of food elimination we obtained in this study, is consistent with Giovannini et al., who found that the avoidance of the offending food is the cornerstone of treatment of IgE mediated food allergy.[28] Elimination of the offending food must take place not only from the diet, but also from the environment in which the child lives. Asthmatic crises and the use of anti-asthmatic drugs decrease when the offending foods are no longer even cooked in the domestic environment and decrease the level of total IgE.[29]

The role of intestinal microbiota in the development of the host immune system and the induction of immune tolerance have gained much interest. Probiotics are considered safe for human consumption if sold as dietary supplements according to the Food and Drug Administration (FDA), as they fall under the broad category of food items. Despite the marked rise in food allergies and intolerances, probiotics remain

underutilized as a treatment despite an emerging body of research implicating the critical role of gut microbiota and their metabolites in the treatment of food allergies and intolerances.[30,31]

Asthmatic children who received probiotics supplementation and those who practiced food elimination and received probiotic showed improvement in the studied parameters. This agreed with Elazab et al., who found that in asthma, treatment with Lactobacillus rhamnosus GG (LGG) inhibited inflammatory cell infiltration.[32] In addition, in OVA-sensitized mice (Ova albumin), LGG reduced OVA-specific IgE levels in serum, suppressed the airway hyper-responsiveness to methacholine and decreased the number of infiltrating inflammatory cells and Th2 cytokines and IgE level in bronchoalveolar lavage fluid and serum.[33] This was consistent with the result of Wu et al., 2016 who showed that *Lactobacilllus gasseri A5* showed a significant reduction in symptoms of asthma and allergic rhinitis along with improvement of pulmonary functions as there was a significant reduction in the TNF-α, IFN-γ, IL-12, and IL-13 production by the peripheral blood mononuclear cells (PBMCs) following the probiotic treatment.[32,34]

It has been postulated that early exposure to commensal bacteria plays a crucial role in Th1/Th2 polarization and maturation of proper immune regulatory mechanisms. Probiotics may modulate toll-like receptors and the proteoglycan recognition proteins of enterocytes, leading to activation of dendritic cells and a Th1 response; the resulting stimulation of Th1 cytokines can suppress Th2 responses.[32] It is also suggested that probiotics induce a tolerogenic microenvironment and high IL-10, this might be employed for oral immunotherapy (OIT)) to treat allergy and autoimmunity in the future.[35,36,37]

With respect to the effect of treatment, we found that children who received all suggested lines of treatment (pharmacological therapy, food elimination and probiotic supplementation), showed the best outcome, followed by those who received pharmacological therapy and practiced food elimination then children who received pharmacological therapy and probiotic supplementation . Results herein intensify the importance of diagnosis of food allergy in asthmatic children which seems to be a common comorbidity and uniquely encourage combining pharmacological therapy, avoidance of the offended food allergen and intake of probiotic to make a significant improvement in the quality of life of pediatric population suffer from bronchial asthma.

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