RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2024.2024-17
Cyprus J Med Sci 2024;9(5):323-331

The Effect of *Lactobacillus rhamnosus* GG in Infants with Food Protein-Induced Allergic Proctocolitis

Özgecan Avcı[1], Merve Usta[2], Ayşenur Kaya[3], Nesrin Kaya[4], Nafiye Urgancı[2]

[1]Clinic of Pediatrics, Atatürk State Hospital, Sinop, Türkiye
[2]Clinic of Pediatric Gastroenterology, University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, Türkiye
[3]Department of Pediatric Allergy and Immunology, İstinye University Faculty of Medicine, İstanbul, Türkiye
[4]Department of Neonatology, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, İstanbul, Türkiye

Abstract

BACKGROUND/AIMS: Most infants with food protein-induced allergic proctocolitis (FPIAP) achieve clinical tolerance between 1 and 3 years of age. The aim of this study was to investigate the effect of *Lactobacillus rhamnosus* GG on the development of tolerance in infants who are exclusively breastfed and diagnosed with FPIAP.

MATERIALS AND METHODS: Sixty infants with FPIAP were divided into two groups: group 1 (study); who received probiotic *Lactobacillus rhamnosus* GG for 3 months, and group 2 (control); who did not. Clinical characteristics, allergy tests, and tolerance development were examined. Randomized controlled study.

RESULTS: Thirty patients [mean age: 3.9±1.3, range: 2-6 months, male/female (M/F): 1.7] in group 1 and 30 patients (mean age: 4.1±1.3, range: 1.4-6 months, M/F: 0.7) in group 2 were included in the study. The reintroduction of trigger foods into the mothers' diet at 9 months was significantly higher in group 1 than in group 2 (63.3% versus 26.7% of the patients, respectively, p=0.004). No significant difference was observed in terms of the resolution time of symptoms and time of tolerance development between groups and subgroups.

CONCLUSION: A significant difference was observed in the mean time to reintroduce trigger foods into the maternal diet, the number of mothers who first reintroduced trigger foods back into their diet at 9 months, and the resolution of symptoms at 9 and 12 months in infants with multiple food allergies.

Keywords: Food protein-induced allergic proctocolitis, *Lactobacillus rhamnosus* GG, probiotics, tolerance, treatment

INTRODUCTION

Food protein-induced allergic proctocolitis (FPIAP) is usually characterized by fresh rectal bleeding and stool mucus in otherwise healthy, well-appearing infants during the first months of life. A definitive diagnosis is made by eliminating the trigger foods thought to be responsible from the diet and reintroduceing the foods after the symptoms improve.[1]

It has been reported that the deterioration of the gut microbiota may play a role in the development of food allergy by negatively affecting immune system development in the early stages of life.[2] Therefore, the gut microbiota may be a potential target for preventing food allergy and its treatment. It has been suggested that the relationship between diet, probiotics, the immune system, and gut microbiota determines the susceptibility to allergy.[3]

To cite this article: Avcı Ö, Usta M, Kaya A, Kaya N, Urgancı N. The Effect of *Lactobacillus rhamnosus* GG in Infants with Food Protein-Induced Allergic Proctocolitis. Cyprus J Med Sci. 2024;9(5):323-331

ORCID IDs of the authors: Ö.A. 0000-0002-7978-4930; M.U. 0000-0002-5086-6270; A.K. 0000-0002-8183-0190; N.K. 0000-0002-1504-0406; N.U. 0000-0003-4854-507X.

Address for Correspondence: Merve Usta
E-mail: mervekesim@yahoo.com
ORCID ID: orcid.org/0000-0002-5086-6270

Received: 01.03.2024
Accepted: 06.08.2024

Lactobacillus rhamnosus GG is a preferred probiotic for the prevention and treatment of intestinal microbiota degradation by strengthening intestinal epithelial barriers, binding to mucous surfaces stronger due to its surface exopolysaccharide and piles, preventing the attachment of pathogenic bacteria, and contributing to the regulation of the immune system.[4,5]

Although there are studies about the addition of probiotics such as *Lactobacillus rhamnosus* GG or *Bifidobacterium breve* to formula feed in formula-fed infants,[6-10] there are few studies about the addition of probiotics exclusively in breastfed infants. The aim of this study was to determine whether *Lactobacillus rhamnosus* GG has an effect on the development of tolerance in exclusively breastfed infants with FPIAP.

MATERIALS AND METHODS

Sixty infants who were exclusively breastfed and followed up for diagnosis of FPIAP at the departments of pediatric gastroenterology and pediatric allergy between 2018 and 2019 were enrolled in this single-center, prospective, randomized, controlled clinical study.

The diagnosis of FPIAP was based on detailed history and physical examination findings according to the food allergy and anaphylaxis guidelines recommended by The European Society for Pediatric Gastroenterology Hepatology and Nutrition and European Academy of Allergy and Clinical Immunology (EAACI).[11,12]

The exclusion criteria were; the patients younger than 1 month and older than 6 months, those fed with formula, those given prebiotics or probiotics during the 4 weeks before enrollment, those with known allergies to probiotics, patients with chronic diseases, immunodeficiency, a central venous catheter, and other diseases causing gastrointestinal findings or bleeding.

The patients were divided into two groups: Group 1 (study); exclusively breastfed infants whose mothers were on an elimination diet and received probiotic *Lactobacillus rhamnosus* GG (Maflor® 1x5 drops once a day) for 3 months, and group 2 (control); whose mothers were on only milk and dairy products elimination diet. The control group did not receive any placebo. These groups were divided into two separate subgroups: Those diagnosed in the first 3 months (groups 1a and 2a) and those diagnosed in the last 3 months (groups 1b and 2b). Our aim in dividing the patients into two subgroups was to homogenize both groups as we switched to complementary foods in both groups at 6 months.

Infants were followed up monthly for the first 3 months after diagnosis and every 3 months thereafter until 1 year of age at outpatient clinics of pediatric gastroenterology. Allergy tests were performed by the same trained allergist during the follow-up period after six months of age. Allergy tests were performed in those with non-IgE symptoms, family history of atopy, and who accepted the allergy tests.

Food-Specific Immunoglobulin E and Skin Prick Test

Sensitization in patients with a preliminary diagnosis of FPIAP, especially those with concomitant atopic diseases such as atopic dermatitis and recurrent wheezing, was evaluated by serum food-specific immunoglobulin E (IgE) and/or skin prick test (SPT). SPT was performed on the forearm by microneedling method using commercial extracts for common offending food proteins, including cow's milk, soy, egg, wheat, fish, sesame, and peanut (Stallargenes SA. Antony, France). The prick-to-prick method was used for the other suspected foods, particularly those reported by the parents. Histamine (10 mg/mL) was used as the positive control, and physiological saline was used as the negative control. Skin reactions were evaluated after 20 min, and a wheal >3 mm was considered positive SPT.

Serum-specific IgE concentrations in suspected foods were measured using the ImmunoCAP system (PHADIA AB, Uppsala, Sweden). A serum specific IgE concentration >0.10 kU/L was considered sensitization.

Atopy Patch Test

One drop (50 µL) of milk, scrambled eggs, soy milk, and 1 g of flour dissolved in 10 mL of water and physiological saline as a negative control were adsorbed with filter paper and placed in 12 mm diameter aluminum chambers ("Finn chamber") on adhesive tapes. The patch adhered to the patient's back. Reactions were evaluated at 48 and 72 h according to the European Task Force for atopic Dermatitis Consensus Report.[13]

Diagnostic Food Elimination

A maternal elimination diet was started with the most common offending triggers, milk and dairy products. Food elimination was explained in detail to the mothers and caregivers. In patients with persistent symptoms, other foods such as egg and wheat were also excluded from the diet, based on frequency, parent's suspicion, and symptoms. The occult blood test was not performed, and the evaluation was performed with the resolution of bloody stool.

Oral Food Challenge Test

After a food elimination diet for 2-4 weeks and the disappearance of the complaints, the patients underwent oral food challenge test (OFC) at home or in the hospital, depending on the severity of the patient's symptoms. Provocation tests and protocols were performed according to the recommendations of the World Allergy Organization Food Allergy Working Group and EAACI.[11,14,15] An open food challenge test was performed in all patients. The dosing intervals of the given foods were 15 minutes and were given gradually increasing doses as; peanut and walnut 6, 12, 48, 240, 480, 1200, and 2000 mg; hazelnut 12, 25, 100, 500, 860, 2500, and 4000 mg; cow's milk 0.1,0.5, 1, 3, 10, 30, 50, 100, and 200 mL; egg 0.5, 1, 3, 6, 10, 15, 16, and 32 g. If symptoms appeared at any stage of the test, the test was considered positive. When no reaction developed, the diet was opened to trigger food and was given daily as cooked. Each child was observed for 4 hours after provocation. The families contacted by telephone at 24, 48, and 72 hours and 1 week after the provocation to ask whether any symptoms had occurred. The mothers were also asked to keep an allergy diary at home.

Tolerance Development

All patients on the elimination diet were reevaluated at 3-6 months intervals. OFC was performed in patients who had resolved their symptoms and healthy growth and development. No symptoms were observed after OFC, which was considered as tolerance achieved.

The study was approved by the Ethics Committee of University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital (approval number: 911, date: 08/07/2018). Written informed consent was obtained from all parents.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 15.0 package program (SPSS Inc, Chicago, Illinois, U.S.A.). The descriptive statistics; were given as mean, standard deviation, minimum and maximum for numerical variables, and numbers and percentages for categorical variables. Comparisons of the ratios between the groups were made using the chi-square test. Because the normal distribution condition was not met, numerical variables in the groups were compared using the Mann-Whitney U test. P values of <0.05 were considered statistically significant.

RESULTS

Thirty patients [mean age: 3.9±1.3, range: 2-6 months, male/female (M/F): 1.7] in group 1 and 30 patients (mean age: 4.1±1.3, range: 1.4-6 months, M/F: 0.7) in group 2 were included in the study. The demographic and clinical characteristics of the patients are presented in Table 1.

22.7% (n=5) of the patients in group 1 and 44.8% (n=13) in group 2 had food-specific IgE positivity (p=0.10). SPT was performed in 22 (73.3%) patients in group 1 and 23 (79.3%) patients in group 2. Approximately 13.6% of the patients in group 1 and 8.7% in group 2 had positive SPT (p=0.66). Atopy patch test (APT) was positive in 7 of 13 (53.8%) patients in group 1 and 3 of 13 (23.1%) of 13 patients in group 2 (p=0.10). No significant difference was observed in the APT and SPT results according to the food allergens between the groups and subgroups (Table 1).

Eleven (36.7%) patients with multiple food allergies in group 1 and 11 (36.7%) patients in group 2 reacted to egg, cow's milk, and dairy products (p=1.00). Six common food allergens (nuts, wheat, fish/seafood, chicken, and veal) were restricted from the diet of 8 (26.7%) patients in group 1 and 6 (20%) in group 2 (p=0.54). Six (54.5%) patients in group 1a, 2 (40%) in group 2a, 5 (26.3%) in group 1b, and 9 (36%) in group 2b reacted to both egg, cow's milk, and dairy products (p>0.05). Six food allergens were restricted from diet in 2 (18.2%) patients in group 1a, 2 (40%) in group 2a, 6 (31.6%) in group 1b, and 4 (16%) in group 2b (p>0.05).

No significant difference was observed in the resolution time of the first symptom (bloody stool) between groups (p=0.78) (Table 2). A statistically significant difference was observed in the mean time from the reintroduction of trigger food into the maternal diet between groups (9.1±2.1 months in group 1, and 10.3±1.5 in group 2, p=0.041).

The reintroduction of trigger foods into the mothers' diet at 9 months was significantly higher in group 1 than in group 2 (63.3% versus 26.7% of the patients, respectively, p=0.004), whereas it was observed higher in group 1b than in group 2b (p<0.001) (Tables 2, 3). The distribution of patients according to the reintroduction of trigger foods into the maternal diet and tolerance development is shown in Figure 1.

When the mothers who only had egg and milk elimination diet were evaluated, no significant difference was observed in terms of the resolution time of all symptoms and the reintroduction of trigger food

Table 1. Demographic and clinical characteristics of the patients

	<3 months at diagnosis		p	≥3 months at diagnosis		p
	Group 1a	Group 2a		Group 1b	Group 2b	
	Probiotics (+)	Probiotics (-)		Probiotics (+)	Probiotics (-)	
	(n=11)	(n=5)		(n=19)	(n=25)	
Age at diagnosis (mean ± SD, months)	2.5±0.3 (2-2.9)	2.0±0.6 (1.4-2.8)	0.12	4.7±0.8 (3.3-6)	4.5±1.0 (3-6)	0.61
Gender (M/F)	1.2 (6/5)	0.6 (2/3)	1.00	2.1 (13/6)	0.78 (11/14)	0.10
Type of birth						
Normal spontaneous vaginal	4 (36.4%)	1 (20%)	1.00	11 (57.9%)	11 (44%)	0.36
Cesarean	7 (63.6%)	4 (80%)		8 (42.1%)	14 (56%)	
Duration of symptoms (mo)	1.1±0.5 (0.5-2)	1.1±0.7 (0.5-2)	0.81	2.2±1.1 (0.5-4.5)	2.0±1.0 (0.5-4)	0.53
Gestational age						
Term	10 (90.9%)	5 (100%)	1.00	19 (100%)	25 (100%)	1.00
Post-term	1 (9.1%)	-		-	-	
Birth weight	3502±466.8 (2760-4380)	3420±255 (3000-3680)	0.73	3288.4±452 (2540-3990)	3380.6±372 (2800-4200)	0.48
Birth weight classification						
Normal	9 (81.8%)	5 (100%)	1.00	19 (100%)	24 (96%)	1.00
LGA	2 (18.2%)	-		-	1 (4%)	
SGA	-	-		-	-	
Smoking	1 (9.1%)	1 (20%)	1.00	3 (15.8%)	5 (20%)	1.00
Antibiotics	4 (36.4%)	1 (20%)	1.00	2 (10.5%)	8 (32%)	0.14
Consanguineous marriage	-	-		1 (5.3%)	2 (8%)	1.00
Family history of atopy	11 (100%)	3 (60%)	0.08	15 (78.9%)	17 (68%)	0.41
Mother	6 (54.5%)	3 (60%)	1.00	8 (42.1%)	10 (40%)	0.83
Father	5 (45.5%)	2 (40%)	0.24	11 (57.9%)	15 (60%)	0.57
Symptoms on admission						

Table 1. Continued

	<3 months at diagnosis		p	≥3 months at diagnosis		p
	Group 1a	Group 2a		Group 1b	Group 2b	
	Probiotics (+)	Probiotics (-)		Probiotics (+)	Probiotics (-)	
	(n=11)	(n=5)		(n=19)	(n=25)	
Vomiting	3 (27.3%)	2 (40%)	1.00	7 (36.8%)	10 (40%)	0.83
Diarrhea	4 (36.4%)	3 (60%)	0.59	11 (57.9%)	13 (52%)	0.69
Constipation	1 (9.1%)	1 (20%)	1.00	2 (10%)	3 (12%)	1.00
Bloody stool	6 (54.5%)	1 (20%)	0.30	10 (52.6%)	14 (56%)	0.82
Stool with mucus	10 (90.9%)	4 (80%)	1.00	18 (94.7%)	24 (96%)	1.00
Colic	10 (90.9%)	4 (80%)	1.00	13 (68.4%)	13 (52%)	0.27
Breastfeeding refusal	4 (36.4%)	1 (20%)	1.00	10 (52.6%)	7 (28%)	0.09
Skin rash	6 (54.5%)	3 (60%)	1.00	11 (57.9%)	7 (28%)	0.04
Diaper dermatitis	8 (72.7%)	4 (80%)	1.00	7 (36.8%)	13 (52%)	0.31
Persistent cough/wheezing	2 (18.2%)	1 (20%)	1.00	5 (26.3%)	3 (12%)	0.26
Low weight gain	1 (9.1%)	-	1.00	2 (10.5%)	8 (32%)	0.14
Physical examination						
Atopic dermatitis	6 (54.5%)	3 (60%)	1.00	5 (26.3%)	9 (36%)	0.49
Seborrheic dermatitis/eczema	8 (72.7%)	3 (60%)	1.00	14 (73.7%)	13 (52%)	0.14
Failure to thrive	-	-		-	-	
Laboratory examinations						
Anemia	-	-		3 (15.8%)	1 (4.2%)	0.30
Eosinophilia (n, %)	4 (36.4%)	3 (60%)	0.59	7 (36.8%)	15 (60%)	0.12
Eosinophil count (µ/L)	360.9±238 (330)	624±556.7 (300)	0.61	325±179.8 (270)	450.8±277 (320)	0.11
Total IgE positivity	6 (54.5%)	-	0.09	2 (10.5%)	5 (20%)	0.68
Serum total IgE (U/L)	108±309.4 (16)	7.1±4.4 (6.2)	0.39	20.7±55.3 (4.4)	14.5±23.6 (6.4)	0.61
Skin prick test positivity	1 (12.5%)	-	1.00	2 (14.3%)	2 (10.5%)	1.00
Cow's milk	-	-	-	1 (7.1%)	1 (5.3%)	1.00
Egg	1 (12.5%)	-	1.00	1 (7.1%)	1 (5.3%)	1.00
Soy	-	-		-	-	
Wheat	-	-		-	-	
Nuts	-	-		1 (8.3%)	-	0.42
Sea food/chicken	-	-		-	-	
Specific IgE positivity	2 (22.2%)	2 (40%)	0.58	3 (23.1%)	11 (45.8%)	0.28
Cow's milk	1 (11.1%)	1 (20%)	1.00	2 (15.4%)	8 (34.8%)	0.27
Egg	1 (12.5%)	1 (20%)	1.00	1 (8.3%)	8 (40%)	0.10
Soy	-	-		-	-	
Wheat	-	-		-	-	
Nuts	-	-		1 (50%)	-	1.00
Sea food/chicken	-	-		-	-	
Atopy patch test positivity	3 (50%)	1 (33.3%)	1.00	4 (57.1%)	2 (20%)	0.16
Cow's milk	1 (16.7%)	-	1.00	1 (14.3%)	2 (20%)	1.00
Egg	2 (40%)	1 (33.3%)	1.00	4 (57.1%)	1 (11.1%)	0.10
Soy	-	-		-	-	
Wheat	1 (20%)	-	1.00	-	-	
Nuts	-	-		-	-	
Sea food/chicken	-	-		-	-	

P<0.05 is statistically significant. LGA: Large for gestational age, SGA: Small for gestational age.

back into the maternal and infant diets at 9 and 12 months between groups (p>0.05). Five (100%) mothers in group 1b versus 2 (22.2%) mothers in group 2b who were on egg and milk elimination diet only had the first reintroduction of trigger food back into their diet at 9 months (p=0.021). The resolution of all symptoms at 12 months was achieved in 5 (100%) patients in group 1b and 2 (22.2%) patients in group 2b who were on egg and milk elimination diet (p=0.021). Three patients (60%) in group 1b who received egg and milk elimination diet switched to normal diet at 12 months, but none of the patients in group 2b achieved tolerance (p=0.02).

When patients who were on elimination diet with six trigger food allergens in groups and subgroups were compared, no statistically significant difference was observed in the first time of reintroducing trigger foods back into the maternal diet, resolution of all symptoms, and time of tolerance development. No probiotic-related adverse effects were observed.

Table 2. Comparison of tolerance development between groups

	Group 1, (n=30)	Group 2, (n=30)	p
Resolution time of first symptom*	22.7±16.3 (21)	22±17.3 (17.5)	0.78
First resolved symptom			
Vomiting	3 (10%)	2 (6.7%)	0.2
Diarrhea	1 (3.3%)	6 (20%)	
Bloody stool	13 (43.3%)	8 (26.7%)	
Stools with mucus	3 (10%)	6 (20%)	
Colic	2 (6.7%)	4 (13.3%)	
Breastfeeding refusal	2 (6.7%)	-	
Skin rash	5 (16.7%)	4 (13.3%)	
Diaper dermatitis	1 (3.3%)	-	
Introduction of trigger foods back into maternal diet*	9.1±2.1 (9)	10.3±1.5 (10)	0.04
At 9 months	19 (63.3%)	8 (26.7%)	0.004
At 12 months	28 (93.3%)	24 (80%)	0.25
Resolution of all symptoms*	10.1±2.1 (10)	10.8±1.6 (12)	0.42
At 9 months	5 (16.7%)	4 (13.3%)	1.00
At 12 months	15 (50%)	12 (40%)	0.43
Time for free diet*	11.1±1.4 (12)	11.9±0.4 (12)	0.24
At 9 months	2 (6.7%)	-	0.49
At 12 months	14 (46.7%)	7 (23.3%)	0.05
Multiple food allergies	(n=19)	(n=17)	
Introduction of trigger foods back into maternal diet*	9.0±1.8 (9)	10.3±1.7 (10)	0.05
At 9 months	13 (68.4%)	5 (29.4%)	0.01
At 12 months	17 (89.5%)	14 (82.4%)	0.65
Resolution of all symptoms*	9.6±2.2 (10)	11.8±0.5 (12)	0.09
At 9 months	5 (26.3%)	-	0.04
At 12 months	11 (57.9%)	4 (23.5%)	0.03
Time for free diet*	10.7±1.5 (11)	12 (1)	0.008
At 9 months	2 (10.5%)	-	0.48
At 12 months	9 (47.4%)	1 (5.9%)	0.008

*Mean ± standard deviation (mean). P<0.05 is statistically significant.

DISCUSSION

In breastfed children with FPIAP, no significant difference was detected in the time to resolution of bloody stools or the first symptoms between the groups in terms of probiotic use. Bloody stools were not the only symptoms in the babies, and other accompanying digestive system symptoms, such as sucking refusal, vomiting, and colic, could occur even if there was no bloody defecation when a trigger food was added. Mothers could state that they avoided going on a diet. One limitation of our study was perhaps the lack of evaluation of multiple symptoms. We performed an evaluation based on the family's major symptoms when bringing them to the hospital. Tolerance assessments were conducted every 3 months based on the child's symptoms and anthropometry during physical examination.

In our study, mothers in the probiotic-treated group were able to open their diet earlier in the 9th month, but we did not find a significant difference in the resolution of all symptoms. It may take longer in groups with multiple food allergies. In different studies, it was reported that 30.4-31.5%, that is, one third of the cases with FPID, have multiple food allergies.[16,17] Approximately one-third of patients with food allergies have multiple food allergies. In non-IgE-mediated food allergy, a single nutrient (the most common cow's milk protein) is responsible in the majority of cases (65-80%), and in 10%, three or more nutrients are responsible.[18] Recent studies conducted in our country reported multiple food allergies at rates of up to 50%.[17] In addition, the responsible allergenic foods may vary according to country and culture. Cultural differences may also affect altitude. Moreover, we were one of the reference centers for pediatric allergy and gastroenterology at that time, and it was a center to which many patients were referred. Our rate of allergy testing was high; thus, we may have found it to be too high compared with the literature. The diagnosis of multiple food allergies has been more common since we performed allergy testing for study purposes at that time when we did not perform routine allergy testing to diagnose FPIAP.

In the absence of clinical response to cow's milk protein elimination within 2 weeks in FPIAP and 4-8 weeks in food protein-related enteropathy,[19] food protein-related enterocolitis syndrome within hours,[20] and recurrence of findings after intake of certain foods, multiple food allergies should be considered, and allergenic foods should be avoided. Gradual elimination from the diet should be planned.

Cows' milk was the most common trigger food in this study, similar to the other studies,[21-23] followed by eggs. Family history of atopy was reported in 86.7% of patients in group 1 and 66.7% in group 2, which was higher than that reported in the literature.[24,25] Multiple food allergies were reported to be 4-42.9% in studies from our country,[21,22,24,25] whereas it was found to be 60% in our study.

It has been suggested that gut microbiota play an important role in immune system maturation, maintaining the Th1/Th2 balance, which is the key mechanism involved in allergic diseases and tolerance acquisition.[3,6,26,27] The gastrointestinal microbiota may also modulate mucosal physiology, barrier function, and systemic immunologic and inflammatory responses.[3-5] Yang et al.[3] reported that sIgE, sIgG1, interleukin 4 (IL-4), IL-5, and IL-13 levels were significantly decreased, resulting in improved diarrhea in mice with food allergy who were administered the probiotic *Bifidobacterium* infantis. It has been reported that the administration of probiotics in early-life stimulate Th1 cytokines to reverse Th2 imbalance.[28]

Table 3. Comparison of tolerance development between subgroups

	<3 months at diagnosis		p	≥3 months at diagnosis		p
	Group 1a	Group 2a		Group 1b	Group 2b	
	Probiotics (+)	Probiotics (-)		Probiotics (+)	Probiotics (-)	
	(n=11)	(n=5)		(n=19)	(n=25)	
Resolution time of first symptom (day)*	21.4±1 (1.9)	23.2±17.3 (15)	0.9	23.4±1 (8.6)	21.8±17.6 (20)	0.77
First resolved symptom						
Vomiting	1 (9.1%)	1 (20%)	0.19	2 (10.5%)	1 (4%)	0.32
Diarrhea	-	2 (40%)		1 (5.3%)	4 (16%)	
Bloody stool	4 (36.4%)	-		9 (47.4%)	8 (32%)	
Stools with mucus	1 (9.1%)	1 (20%)		2 (10.5%)	5 (20%)	
Colic	1 (9.1%)	-		1 (5.3%)	4 (16%)	
Breastfeeding refusal	-	-		2 (10.5%)	-	
Skin rash	4 (36.4%)	1 (20%)		1 (5.3%)	3 (12%)	
Diaper dermatitis	-	-		1 (5.3%)	-	
Introduction of trigger foods back into maternal diet*	9.7±2.2 (9)	9.2±1.6 (9)	0.67	8.8±2.1 (9)	10.6±1.4 (11)	0.008
At 9 months	5 (45.5%)	4 (80%)	0.30	14 (73.7%)	4 (16%)	<0.001
At 12 months	9 (81.8%)	5 (100%)	1.00	19 (10%)	19 (76%)	0.12
Resolution of all symptoms*	9.7±2.2 (9)	9.2±1.6 (9)	0.84	10.4±2.1 (11)	11±1.6 (12)	0.49
At 9 months	3 (27.3%)	1 (20%)	1.00	2 (10.5%)	3 (12%)	1.00
At 12 months	5 (45.5%)	2 (40%)	1.00	10 (52.6%)	10 (40%)	0.40
Time for free diet*	10.8±1.8 (12)	12±0.0 (12)	0.33	11.3±1.1 (12)	11.8±0.4 (12)	0.50
At 9 months	1 (9.1%)	-	1.00	1 (5.3%)	-	0.43
At 12 months	5 (45.5%)	2 (40%)	1.00	9 (47.4%)	5 (20%)	0.05
Multiple food allergies	**(n=8)**	**(n=4)**		**(n=11)**	**(n=13)**	
Introduction of trigger foods back into maternal diet*	9.5±2.5 (9.5)	9.3±1.9 (8.5)	1.00	8.7±1.3 (9)	10.7±1.5 (11)	0.006
At 9 months	3 (37.5%)	3 (75%)	0.54	10 (90.9%)	2 (15%)	<0.001
At 12 months	6 (75%)	4 (100%)	0.51	11 (100%)	10 (76.9%)	0.22
Resolution of all symptoms*	9.3±1.9	-		10±2.3	12±0.0	
At 9 months	3 (37.5%)	-	0.48	2 (18.2%)	-	0.19
At 12 months	4 (50%)	1 (25%)	0.57	7 (63.6%)	3 (23.1%)	0.09
Time for free diet*	10.5±1.9 (11)	12±0.0		10.8±1.3 (11)	-	0.01
At 9 months	1 (12.5%)	-	1.00	1 (9.1%)	-	0.45
At 12 months	4 (50%)	1 (25%)	0.57	5 (45.5%)	-	0.01

*Mean ± standard deviation (median). P<0.05 is statistically significant.

Thus, probiotics may treat food allergy by restoring imbalanced indigenous microbiota and controlling inflammatory responses (activation of local macrophages, modulation of local and systemic IgA production, and alteration of the pro- and anti-inflammatory cytokine profile).[29]

Morisset et al.[30] reported a significant decrease in the proportion of positive SPT in cows' milk and a decrease in positive IgE tests against other foods than cow's milk in the probiotic group after 12 months. Berni Canani et al.[6] noted a risk reduction in infants receiving *Lactobacillus rhamnosus* GG for additional atopic diseases (eczema, asthma, rhinoconjunctivitis, and other food allergies) and a decrease in oral tolerance development in children with IgE-mediated cow's milk allergy in their two studies. Baldassarre et al.[7] showed significant improvement in hematochezia in infants fed an extensively hydrolyzed casein formula containing *Lactobacillus rhamnosus* GG. Cukrowska et al.[8], Kirjavainen et al.[9], and Majamaa and Isolauri[10] observed a decrease in the Severity Scoring of the Atopic Dermatitis Index in patients with atopic eczema and cows' milk allergy who received probiotics. Although Qamer et al. [31] stated that probiotics (*Lactobacillus rhamnosus* GG) can lead to an earlier acquisition of tolerance to cows' milk in children aged 36 months, and probiotic supplementation was not associated with an earlier resolution of hematochezia. In contrast to these studies, others reported no significant effects of probiotics on clinical tolerance, SPTs, and the rate of symptomatic food allergy.[32,33] Fiocchi et al.[34] reported very low quality of evidence for probiotics in preventing eczema, and Hojsak et al.[35] mentioned that probiotics cannot be recommended for the prevention of atopic diseases.

In our study, we observed that 10^9 *Lactobacillus rhamnosus* GG-containing drops added to the diet of exclusively breastfed infants diagnosed with FPIAP over a 3-month period shortened the average time to reintroduce trigger foods into the maternal diet, and more mothers in the study group were able to reintroduce trigger foods back into

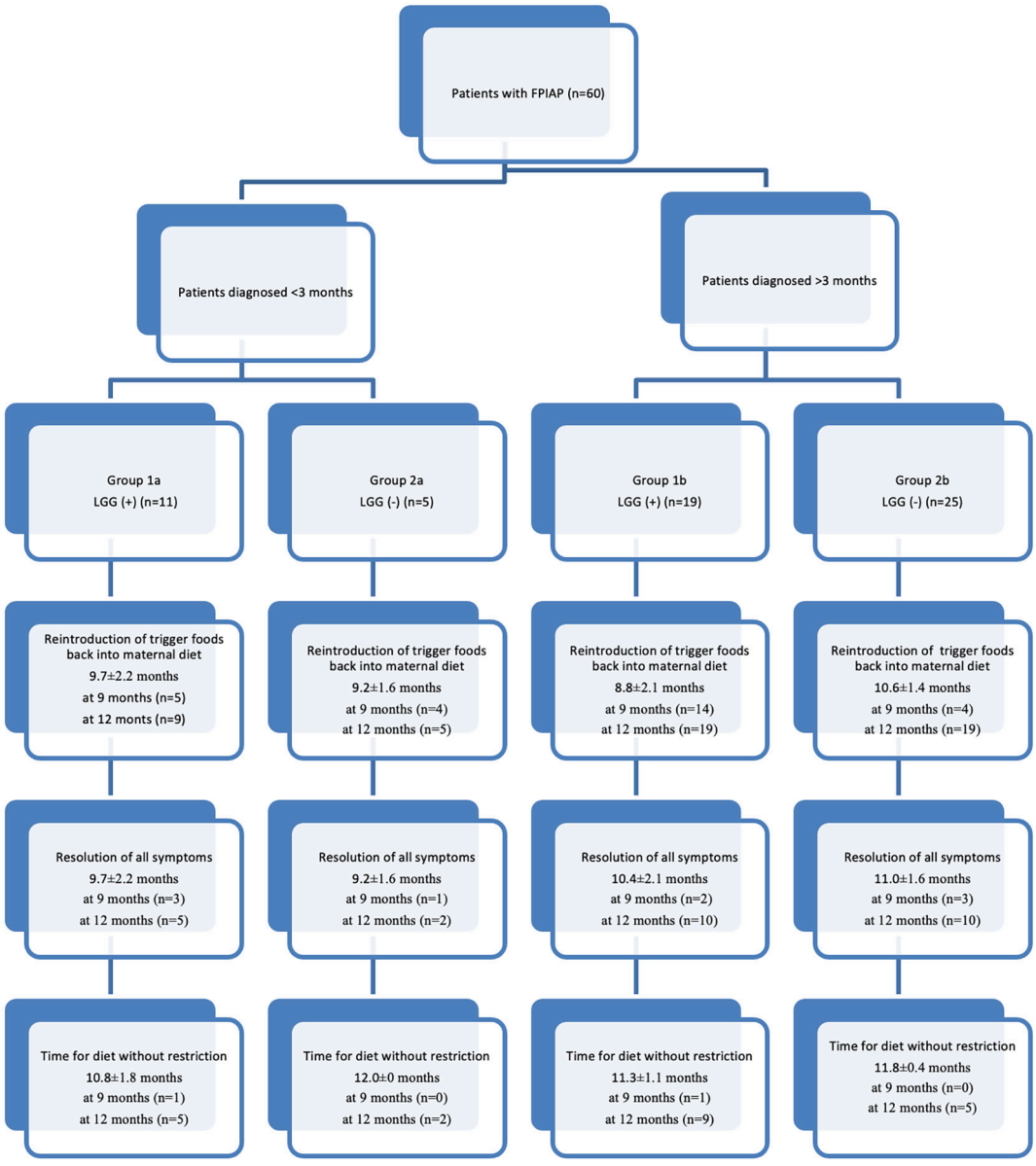

Figure 1. Distribution of patients according to tolerance development.

FPIAP: Food protein-induced allergic proctocolitis, LGG: Lactobacillus rhamnosus GG.

their diet at 9 months. Significant differences were also found in both groups and their subgroups who had multiple food allergies in terms of reintroducing trigger foods back into the maternal diet at 9 months in the study group, tolerance development, resolution of all symptoms at 9 and 12 months, and return to a normal diet at 12 months.

20% of breastfed infants with FPIAP have spontaneous resolution, and nearly all infants become tolerant to the trigger food by the age of 1-3 years.[1] Tolerance to trigger foods by 1 year of age in infants with FPIAP was reported to be 40% in Erdem et al.[25] The frequency of tolerance development was found to be higher in patients who had a single food allergy than in patients with multiple food allergies (86.6% vs. 44.7%, respectively, $p<0.001$)[22] and among the patients who developed tolerance at >24 months, most had multiple food allergies. In our study, no significant difference was observed in tolerance development between patients with single or multiple food allergies at the age of 1 year.

A significantly greater number of patients with single and multiple food allergies in both group 1 and group 1b who received probiotics and switched to a free diet at 12 months in this study. The tolerance was achieved in more patients with multiple food allergies who received probiotics.

Study Limitations

The limitations of the study were its single-center nature with a small number of participants, and being not placebo controlled.

CONCLUSION

It is important to achieve tolerance to trigger foods earlier to prevent inappropriate, unnecessary, or prolonged elimination diet intake, which may affect dietary nutritional intake, health-related quality of life, and growth in children with FPIAP. Further studies with larger sample sizes are needed to clarify the beneficial effects of probiotics on food allergy.

MAIN POINTS

- Most of the infants with food protein-induced allergic proctocolitis achieve clinical tolerance between 1 and 3 years of age.
- Most of patients with single and multiple food allergies who received probiotics, switched to free diet at 12 months in this study.
- The tolerance development was achieved in more patients with multiple food allergies who received probiotics.

ETHICS

Ethics Committee Approval: The study was approved by the Ethics Committee of University of Health Sciences Türkiye, Şişli Hamidiye Etfal Training and Research Hospital (approval number: 911, date: 08/07/2018).

Informed Consent: Written informed consent was obtained from all parents.

Authorship Contributions

Surgical and Medical Practices: Ö.A., M.U., A.K., N.K., N.U., Concept: Ö.A., M.U., Design: Ö.A., M.U., Data Collection and/or Processing: Ö.A., M.U., A.K., N.K., N.U., Analysis and/or Interpretation: M.U., A.K., N.K., N.U., Literature Search: Ö.A., M.U., Writing: Ö.A., M.U.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study had received no financial support.

REFERENCES

1. Mennini M, Fiocchi AG, Cafarotti A, Montesano M, Mauro A, Villa MP, et al. Food protein-induced allergic proctocolitis in infants: Literature review and proposal of a management protocol. World Allergy Organ J. 2020; 13(10): 100471.
2. Gri G, Piconese S, Frossi B, Manfroi V, Merluzzi S, Tripodo C, et al. CD4 + CD25 + regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction. Immunity. 2008; 29(5): 771-81.
3. Yang B, Xiao L, Liu S, Liu X, Luo Y, Ji Q, et al. Exploration of the effect of probiotics supplementation on intestinal microbiota of food allergic mice. Am J Transl Res. 2017; 9(2): 376-85.
4. Wang Y, Liu L, Moore DJ, Shen X, Peek RM, Acra SA, et al. An LGG-derived protein promotes IgA production through upregulation of APRIL expression in intestinal epithelial cells. Mucosal Immunol. 2017; 10(2): 373-84.
5. Llewellyn A, Foey A. Probiotic Modulation of Innate Cell Pathogen Sensing and Signaling Events. Nutrients. 2017; 9(10): 1156.
6. Berni Canani R, Di Costanzo M, Bedogni G, Amoroso A, Cosenza L, Di Scala C, et al. Extensively hydrolyzed casein formula containing Lactobacillus rhamnosus GG reduces the occurrence of other allergic manifestations in children with cow's milk allergy: 3-year randomized controlled trial. J Allergy Clin Immunol. 2017; 139(6): 1906-13.e4.
7. Baldassarre ME, Laforgia N, Fanelli M, Laneve A, Grosso R, Lifschitz C. Lactobacillus GG improves recovery in infants with blood in the stools and presumptive allergic colitis compared with extensively hydrolyzed formula alone. J Pediatr. 2010; 156(3): 397-401.
8. Cukrowska B, Ceregra A, Maciorkowska E, Surowska B, Zegadło-Mylik MA, Konopka E, et al. The Effectiveness of Probiotic Lactobacillus rhamnosus and Lactobacillus casei Strains in Children with Atopic Dermatitis and Cow's Milk Protein Allergy: A Multicenter, Randomized, Double Blind, Placebo Controlled Study. Nutrients. 2021; 13(4): 1169.
9. Kirjavainen P, Salminen S, Isolauri E. Probiotic bacteria in the management of atopic disease: underscoring the importance of viability. J Pediatr Gastroenterol Nutr. 2003; 36(2): 223-7.
10. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol. 1997; 99(2): 179-85.
11. NIAID-Sponsored Expert Panel; Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. J Allergy Clin Immunol. 2010; 126(6 Suppl): 1-58.
12. Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, et al. European Society of Pediatric Gastroenterology, Hepatology, and Nutrition. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. J Pediatr Gastroenterol Nutr. 2012; 55(2): 221-9.
13. Darsow U, Ring J. Airborne and dietary allergens in atopic eczema: A comprehensive review of diagnostic tests. Clin Exp Dermatol. 2000; 25(7): 544-51.
14. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, Blanco C, Ebner C, Hourihane J, et al. European Academy of Allergology and Clinical Immunology. Standardization of food challenges in patients with immediate reactions to foods--position paper from the European Academy of Allergology and Clinical Immunology. Allergy. 2004; 59(7): 690-7.
15. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS, et al. Work Group report: oral food challenge testing. J Allergy Clin Immunol. 2009; 123(6 Suppl): 365-83.
16. Sicherer, S.H. and H.A. Sampson, Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. J Allergy Clin Immunol 2014; 133(2): 291-307.
17. Guler N, Cokugras FC, Sapan N, Selimoglu A, Turktas I, Cokugras H, et al. Diagnosis and management of cow's milk protein allergy in Turkey: Region-specific recommendations by an expert-panel. Allergol Immunopathol (Madr). 2020; 48(2): 202-10.
18. Zubeldia-Varela E, Barker-Tejeda TC, Blanco-Pérez F, Infante S, Zubeldia JM, Pérez-Gordo M. Non-IgE-Mediated Gastrointestinal Food Protein-Induced Allergic Disorders. Clinical Perspectives and Analytical Approaches. Foods. 2021; 10(11): 2662.
19. Feuille E, Nowak-Węgrzyn A. Food Protein-Induced Enterocolitis Syndrome, Allergic Proctocolitis, and Enteropathy. Curr Allergy Asthma Rep. 2015; 15(8): 50.
20. Nowak-Węgrzyn A, Chehade M, Groetch ME, Spergel JM, Wood RA, Allen K, et al. International consensus guidelines for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary-Workgroup Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2017; 139(4): 1111-26.
21. Arik Yilmaz E, Soyer O, Cavkaytar O, Karaatmaca B, Buyuktiryaki B, Sahiner UM, et al. Characteristics of children with food protein-induced enterocolitis and allergic proctocolitis. Allergy Asthma Proc. 2017; 38: 54-62.
22. Uncuoğlu A, Aydoğan M, Şimşek IE, Çöğürlü MT, Uçak K, Acar HC. A Prospective Assessment of Clinical Characteristics and Responses to Dietary Elimination in Food Protein-Induced Allergic Proctocolitis. J Allergy Clin Immunol Pract. 2022; 10(1): 206-14.
23. Buyuktiryaki B, Kulhas Celik I, Erdem SB, Capanoglu M, Civelek E, Guc BU, et al. Risk Factors Influencing Tolerance and Clinical Features of Food Protein-induced Allergic Proctocolitis. J Pediatr Gastroenterol Nutr. 2020; 70(5): 574-9.

24. Koksal BT, Barıs Z, Ozcay F, Yilmaz Ozbek O. Single and multiple food allergies in infants with proctocolitis. Allergol Immunopathol (Madr). 2018; 46(1): 3-8.

25. Erdem SB, Nacaroglu HT, Karaman S, Erdur CB, Karkıner CU, Can D. Tolerance development in food protein-induced allergic proctocolitis: Single centre experience. Allergol Immunopathol (Madr). 2017; 45(3): 212-9.

26. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018; 141(1): 41-58.

27. Pratap K, Taki AC, Johnston EB, Lopata AL, Kamath SD. A Comprehensive Review on Natural Bioactive Compounds and Probiotics as Potential Therapeutics in Food Allergy Treatment. Front Immunol. 2020; 11: 996.

28. Navarro-Tapia E, Sebastiani G, Sailer S, Toledano LA, Serra-Delgado M, García-Algar Ó, et al. Probiotic Supplementation During the Perinatal and Infant Period: Effects on Gut Dysbiosis and Disease. Nutrients. 2020; 12(8): 2243.

29. Vandenplas Y, Huys G, Daube G. Probiotics: an update. J Pediatr. 2015; 91(1): 6-21.

30. Morisset M, Aubert-Jacquin C, Soulaines P, Moneret-Vautrin DA, Dupont C. A non-hydrolyzed, fermented milk formula reduces digestive and respiratory events in infants at high risk of allergy. Eur J Clin Nutr. 2011; 65(2): 175-83.

31. Qamer S, Deshmukh M, Patole S. Probiotics for cow's milk protein allergy: a systematic review of randomized controlled trials. Eur J Pediatr. 2019; 178(8): 1139-49.

32. West CE, Hammarström ML, Hernell O. Probiotics in primary prevention of allergic disease-follow-up at 8-9 years of age. Allergy. 2013; 68(8): 1015-20.

33. Viljanen M, Kuitunen M, Haahtela T, Juntunen-Backman K, Korpela R, Savilahti E. Probiotic effects on faecal inflammatory markers and on faecal IgA in food allergic atopic eczema/dermatitis syndrome infants. Pediatr Allergy Immunol. 2005; 16(1): 65-71.

34. Fiocchi A, Pawankar R, Cuello-Garcia C, Ahn K, Al-Hammadi S, Agarwal A, Beyer K, et al. World allergy organization-McMaster University guidelines for allergic disease prevention (GLAD-P): probiotics. World Allergy Organ J. 2015; 8(1): 4.

35. Hojsak I, Fabiano V, Pop TL, Goulet O, Zuccotti GV, Çokuğraş FC, et al. Guidance on the use of probiotics in clinical practice in children with selected clinical conditions and in specific vulnerable groups. Acta Paediatr. 2018; 107(6): 927-37.