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Probiotic as an adjuvant therapy in chronic urticaria: a blinded randomized controlled clinical trial

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Key words

Chronic spontaneous urticaria; probiotic; prebiotic; symbiotic; antihistamine; RCT; UAS score; DLQI; urticaria; CSU; trial.

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Impact statement

Based on UAS7 score, combination of probiotic + antihistamines had no statistically significant different efficacy than antihistamine alone, associating with higher reduction rate of itch, number of urticaria and total UAS7 score.

Summary

Background. *Chronic spontaneous urticaria (CSU) is a common and treatment challenging disorder which may involve about 2% of normal population and in 50% do not respond properly, even to the second line therapies. We aimed to evaluate the efficacy and safety of a symbiotic (prebiotic + probiotic) named as Lacto-Care in treatment of CSU in the RCT for the first time.* ***Methods.*** *This blinded RCT conducted on 42 patients (21 patients in control antihistamine group and 21 in intervention antihistamine + probiotic group) with CSU during 8 weeks. The efficacy was assessed by Urticaria Activity Score (UAS7) and quality of life measured by Persian validated Dermatology Life Quality Index (DLQI).* ***Results.*** *Before and after, in control group UAS7 score was 35.33 ± 7.81 and 16.86 ± 13.54, respectively. There was 53% score reduction in control group. Before and after, in intervention group UAS7 score was 32 ± 7.84 and 11 ± 11.41, respectively. There was 66% score reduction in intervention group. In control and intervention group improvement of DLQI was 44% and 66%, respectively. At the end, UAS7 score reduction and DLQI improvement was statistically significant in both groups.* ***Conclusions.*** *Probiotics are effective, safe and satisfactory adjuvant therapy for CSU. Combination of probiotic and antihistamines had no statistically significant different efficacy than the antihistamine alone, based on UAS7 score. But patients with combination therapy may experience higher reduction rate of itch, number of urticaria and total UAS7 score that is clinically of great value and is really practical by itself. Patients with combination therapy experienced more improvement of quality of life (DLQI).*

Introduction

It seems that up to 20% of individuals at some point in their life may be affected by urticaria and angioedema. Episodes lasting for less than 6 week are considered acute, whereas those occurring on most days for more than 6 week are considered as chronic urticarial (CU). The etiology, mechanism, causes and therapeutic options are different in acute and chronic urticarial; therefore, the distinction is considered of great importance. Acute urticaria is a self-limited condition, and mast cell activation with an allergen, the main activator. Moreover, foods, drug, insect venom or sting and viral infections are the main causes (1-8).

Chronic urticaria has significant burden and great impact on patient's quality of life and is associated with much psychological comorbidity (9-11). The most common form of management and treatment system of chronic urticaria is symptomatic therapy. In 5 to 50 percent of patients with chronic urticaria, the first-line treatment (one type of antihistamine regimen) may not result in satisfying disease control. Patients with refractory chronic urticaria require a 4-fold increase in the H1 antagonist dose or continue treatment with omalizumab, cyclosporine, or montelukast if urticaria persists. In patients with chronic spontaneous urticaria (CSU) resistant to four-fold usage dose of antihistamines with higher dose consumption, only 49% of patients reported a decrease or resolution of symptoms, and in 20% of patients, side effects were appeared (12-16).

Studies have been shown that *Lactobacillus salivarius* LS01 and compound of 2 probiotics (*Lactobacillus salivarius* LS01 and *Bifidobacterium breve* BR03) are capable of producing and releasing pro-Th-2 cytokines from Th-1 cells, and help to improve T-helper cells type Th1/Th2 (17-19).

Probiotics are viable microorganisms that have beneficial effects on the body when consumed in sufficient quantities, and as their great feature, they are safe and secure for the host (20).

Probiotics have been studied with promising results in the treatment of atopic dermatitis (AD), acne, eczema, allergic diseases, skin aging, bacterial and fungal infections, chronic wound healing like diabetic foot ulcers (21-26).

Th-2 cells play a critical role in the pathogenesis of allergic reactions and production of urticarial-related antibodies.

The high prevalence of CU and its great effects on the quality of life of patients and their family as well as the partially failure of current treatments led us to design and implement the following study.

Based on our literature review, this is the first blinded RCT to evaluate the efficacy and safety of symbiotic for treatment of chronic urticaria.

Materials and methods

The present interventional study was a parallel study; analyst blinded randomized controlled clinical trial conducted on 42 patients with chronic urticaria at the allergy and dermatology department of Rasool Akram Medical Complex of Iran University of Medical Sciences, between February and December 2019. Sample size was calculated according to the study of Nettis *et al.*, (16) with regard to P1 = 71.1, P2 = 28.9, α = 0.05 and power = 90%, then, 36 patients needed to be enrolled in the study. Fifty-two patients were enrolled but 10 of them discontinued the study and finally 42 patients finished (each group: 21 patients).

Eligibility criteria

Patients with chronic non-autoimmune non-vasculitic urticaria (at least two days a week more than 6 weeks), aged 18 to 45 years, without any serious co-morbidities (such as malignancies, mental illness, hepatitis, endocrine, rheumatologic or other acute and chronic systemic diseases), not treated with any drugs other than antihistamines, no history of acute gastrointestinal illnesses such as indigestion and mal-absorption and not taking any corticosteroids for any reason were included in the study.

Patient recruitment

Informed consent was filled prior to recruitment. Liver, thyroid, kidney function in addition to peripheral cell analysis and autoimmunity screening was done. Assays before allocation is listed as: CBC Diff, BUN/Cr, ALT, AST, ALP, ANA, RF, ESR, CRP, TSH, ANTI TPO, U/A, S/E, stool *H. pylori* Ag, and in the case of abnormality detection due to probability of systemic disorder, they did not enrolled in the study. The recruitment phase was finished within 5 months. It should be notified that patients were free to exit the study at any time by any reasons such as unwillingness to continue the study or due to side effects especially which did not respond to routine approaches including dose reduction or changing drug type.

Random sequencing and allocation

Patients were assigned to control (only antihistamine) and intervention groups (antihistamine + symbiotic) through computerized randomization with a 1:1 allocation ratio.

Follow-Up and evaluation

Study duration was 8 weeks with 2 visits as the first day visit and final visit after 8 weeks. The safety profile for the probiotic strain over 8 weeks of treatment in our patients was consistent with previous observations in patients treated with probiotic supplement (27). Questionnaire 1 was completed for all eligible participants at the beginning of the study including demographic data of patients (age, sex, occupation, weight, marital status, urticaria number, severity, duration, characteristics and its triggering factors, number of involved days per week, previous antihistamine use and its type). By supervision of the main study investigator, in questionnaire 2 and 3, patients recorded the number and severity of urticaria based on Urticaia Activity Score (UAS7) questionnaire (28) and their quality of life based on Persian validated Dermatology Life Quality Index (DLQI)

questionnaire (29), respectively. The number and severity of urticaria and also quality of life (questionnaires 2 and 3) were recorded by patients in 8th week visit, again.

Detailed information of questionnaires

Questionnaire 2 filled based on the patient's condition at last week which was designed in two columns, the first column was for itch: 0 (no itching), 1 (slight no annoying itching), 2 (medium annoying itching but does not interfere with daily activities), 3 (severe itching that is annoying and interfere with sleep or daily activities) and the next column was related to the number and severity of urticaria: 0 (no lesion), 1 (less than 20 lesions during 24 hours), 2 (20-50 lesions during 24 hours), 3 (over 50 lesions during 24 hours or a large area of the body involved by large interconnected lesions) (28). *UAS7 score = (Itch score + Urticarial number score) × (number of involved days per week).* Range (0-42), where 0 = no lesion, 1-6 = well controlled, 7-15 = mild, 16-27 = moderate, 28-42 = severe. Based on previous studies, at the end of study, the percentage of UAS7 score reduction was calculated and a ≤ 10%, 11-30%, 31-90% and > 90% score reduction was considered as no response, mild response, significant response and complete response, respectively. DLQI score: 10 item. Range (0-30) (29).

Blinding

There was no blinding of patients or investigator in the study so that only data analyst was blind.

Treatment regimens

Among these three oral antihistamines (cetirizine 10 mg, desloratadine 5 mg and fexofenadine 180 mg), two of them were selected and given twice a day (for example cetirizine + desloratadine or desloratadine + fexofenadine, *etc*.). The selection criteria were based on the patient's preference and appropriate previous clinical response. This treatment strategy was the same for both groups. But in intervention group, in addition to similar antihistamines regimen, patients received twice daily oral probiotic capsules named LactoCare, manufactured by Iranian Bio Fermentation Company. LactoCare capsule is a synbiotic (probiotic + prebiotic), which contains high amounts of many beneficial and safe bacterial strains (*Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Streptococcus thermophilus*) plus fructooligosaccharides as prebiotic.

Laboratory tests

CBC Diff, BUN/Cr, ALT, AST, ALP, ANA, RF, ESR, CRP, TFT, ANTI TPO, U/A, S/E, stool *H. pylori* Ag, test only at the beginning of the study for all participants.

Statistics and data analysis

Descriptive results for values are presented as mean ± SD or percent. Independent t-test and Mann-Whitney test were used to compare the two means. Cochran test was used to investigate the differences between the binary qualitative variables during the measured time. Repeated measures ANOVA and LSD were used to evaluate the quantities over time and between groups. P-values less than 0.05 were considered statistically significant. All data were analyzed using SPSS 21 software as intention to treat.

Ethics and study registration

All stages of this research are committed to the Helsinki Declaration and all patients' information is protected. The ethical code of this study was: IR.IUMS.FMD.REC.1398.129 and the IRCT number was: IRCT20190825044613N1.

Results

The present study was performed on 42 patients with chronic spontaneous urticaria (21 patients in intervention symbiotic antihistamine group and 21 patients in control antihistamine group). At first, among demographic data, only the mean WBC count and lymphocyte percentage in the intervention group was significantly lower and higher than the control group, respectively (6800 *vs* 8400 p = 0.047; 46% *vs* 37% p = 0.038) (**table I**).

The mean UAS7 score was not statistically different between groups, before therapy. Although after therapy, a statistically significant score reduction was observed in both treatment groups (p < 0.001) and this score reduction was higher in intervention group, with using the independent t-test, final mean UAS7 score and reduction rate of UAS7, were not significantly different between treatment groups (p > 0.05) (**table II**). There was not any statistically significant difference between two groups regarding the type of antihistamines regimen and the final UAS7 score (p > 0.05). The severity of itch, at first and after 8 weeks of therapy was evaluated and the results showed that at the end of the study the itch severity had a significant decreased in both control and intervention group (p = 0.047; p < 0.001, respectively). Itching severity was significantly lower in the intervention group than the control (p < 0.05), but rate of itching decline during the study, was not statistically different between two groups (p = 0.162).

The urticaria number, at first and after 8 weeks of therapy was evaluated and the results showed that at the end of the study, the urticaria number had a significant decreased in control and intervention group (p = 0.001; p < 0.001, respectively). The urticaria number was significantly lower in the intervention group than the control (p < 0.05), but the rate of urticaria number decline during the study, was not statistically different between two groups (p = 0.073).

Based on UAS7 categories, in the control group, firstly 15 (71.42%) and 6 (28.57%) of the patients had severe and moderate CSU, respectively which at the end of the study changed to 6 (28.57%), 2 (9.52%), 2 (9.52%) and 11 (52.38%) as sever, moderate, mild and well-controlled CSU, retrospectively.

Based on UAS7 categories, in intervention group, firstly 19 (90.47%) and 2 (9.52%) of the patients had severe and mod-

Table I *- Comparison between control and intervention group regarding basic demographic characteristics.*

Variable	Group	Mean	SD	P-value
Age (year)	Control	40.21	13.74	
	Intervention	36.50	10.75	0.352
Weight (kg)	Control	73.31	11.17	
	Intervention	67.37	14.06	0.181
WBC (count)	Control	8376.11	2164.57	
	Intervention	6830.38	2529.42	0.047
Hb	Control	13.09	1.79	
	Intervention	13.56	2.16	0.564
Neutrophil (%)	Control	50.21	12.36	
	Intervention	46.58	14.43	0.418
Lymphocyte (%)	Control	37.15	12.19	
	Intervention	45.94	12.16	0.038
Eosinophil (%)	Control	3.62	2.57	
	Intervention	3.02	2.39	0.483
Urticaria duration (week)	Control	19.81	50.661	0.931
	Intervention	18.76	22.430	
Days involved with urticaria per week (day)	Control	6.43	2.014	0.729
	Intervention	6.24	1.480	
		-	**+**	**P-value**
H. pylori (%)	Control	14 (66.7)	7(33.3)	0.215
	Intervention	9 (42.9)	12(57.1)	
		-	**+**	**P-value**
ANA (%)	Control	20 (95.2)	1(4.8)	> 0.05
	Intervention	20 (95.2)	1(4.8)	
		Female	Male	**P-value**
Gender (%)	Control	15 (71.4)	6(28.6)	0.633
	Intervention	15 (71.4)	6(28.6)	
Previous therapeutic regimens (%)		**Steroids**	**Steroids + Antihistamines**	**P-value**
	Control	11 (52.4)	10(47.6)	0.197
	Intervention	16 (76.2)	5(23.8)	
Types of prescribed antihistamines regimens (%)		**Cetirizine + Fexofenadine**	**Cetirizine + Desloratadine**	**P-value**
	Control	11 (52.4)	10(47.6)	> 0.05
	Intervention	11 (52.4)	10(47.6)	

erate CSU, respectively, which at the end of study changed to 3 (15.78%), 4 (19.04%), 7 (33.33%) and 7 (33.33%) as sever, moderate, mild and well-controlled CSU, retrospectively.

So, in the control group, 3 (14%), 3 (14%), 11 (53 %) and 4 (19%) of the patients had no, mild, significant and complete response, respectively, and in the intervention group 2 (9%), 12 (58%) and 7 (33%) of the patients had mild, significant and complete response, respectively. In overall, 19% and 33% of the patients in two groups, respectively had complete therapeutic response.

At the baseline of the study, there was no significant difference between groups regarding distribution of prescribed antihistamines combination regimen ($p > 0.05$) and by χ^2 test it was showed that this regimen did not have a confounding effect on the study results.

In **table III** you can see the mean final patients' quality of life which increased significantly in both treatment groups and in intervention group, quality of life improvement was significantly higher than the control ($p < 0.05$).

In **figure 1**, we showed the main change of UAS7 Score (response) and patients' quality of life, before and after therapy in the control and intervention and group.

We did not find any serious or irreversible side effects among all participants of the study. Some patients had complaint of

Table II *- Mean UAS7 score before and after therapy in both groups and compare the final UAS7 score between the groups.*

	Group	Mean	N	SD	Score reduction (%)	P-value
Control	UAS7 Before	35.33	21	7.81	53%	< 0.001
	UAS7 After	16.86	21	13.54		
Symbiotic	UAS7 Before	32.00	21	7.84	66%	< 0.001
	UAS7 After	11.00	21	11.41		
	Group	**N**	**Mean**	**SD**		**P-value**
Final UAS7 score in 8th week	Control	21	16.86	13.54		0.137
	Symbiotic	21	11.00	11.41		

Table III *- Mean DLQI of patients in control and intervention group before and after therapy and comparing the final DLQI score between 2 groups.*

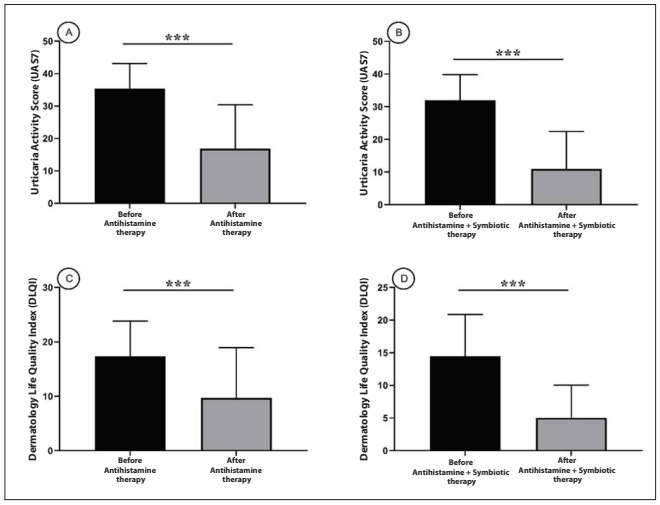
	Group	Mean	N	SD	Improvement of quality of life (%)	P-value
Control	DLQI Before	17.33	21	6.49	44%	< 0.001
	DLQI After	9.71	21	9.24		
Symbiotic	DLQI Before	14.47	21	6.40	66%	< 0.001
	DLQI After	5.04	21	5.00		
	Group	**N**	**Mean**	**SD**		**P-value**
Final DLQI in 8th week	Control	21	9.71	9.24		0.049
	Symbiotic	21	5.04	5.00		

drowsiness and few patients in symbiotic group experienced mild GI discomfort that was not sustainable.

Discussion

Chronic spontaneous urticaria is a common and treatment challenging disorder which may involve about 2% of normal population and in 50% of cases do not respond properly even to the second line therapies (2, 3, 5, 13-16). Regarding these facts, there are many trends to find and evaluate safe therapeutic options with better or additive response rate. The skin is the largest organ in the body that carries hundreds of microorganisms called the skin microbiota. Colonized bacteria react with toll like receptors on the intestinal epithelial cells and dendritic cells which cause activating and signaling of immune cells including macrophages, NK cells, B cells, T helper cells, cytotoxic T cells and regulatory T cells. Intestinal microbiota imbalance can lead to allergic reaction, thus regulating immune system through intestinal microbiota can affect chronic urticaria (17, 18). There is evidence suggesting that alteration of the composition and/or size of the gut microflora may modulate the IgE response to allergens (19). Probiotics are viable microorganisms that have beneficial effects on the body when consumed in sufficient quantities and their great feature is that they are safe and secure for the host (20). Nowadays, there are many promising data regarding the benefits of microbiota regulation and use of prebiotics, probiotic and symbiotic in various fields of medicine including dermatology (21-26). Whereas modern lifestyles have

Figure 1 *- The mean change of UAS7 Score (response) and patients' quality of life, before and after therapy in control and intervention groups.*



contributed to changes in the composition of the intestinal microflora, diet supplementation with probiotics may counterbalance the Th-2 activity by promoting Th-1 cytokines production and down regulate IgE production via inhibition of IL-4 and IL-5 production (28-30). In cases of CSU, in which autoreactive IgG antibodies against FcεRI, IgE, or both or autoreactive IgE antibodies against autoallergens are found, these autoantibodies are causative factors, and IgE, FcεRI, and mast cells are unambiguously at the centre of the pathologic process. For the remaining cases of CSU, IgE, FcεRI, and mast cells are also likely to play essential pathologic roles, although the causative factors have not been identified. Autoimmune processes might be the primary cause of most cases of CSU. Thus, for those cases with a clear autoimmune cause, the reduction of the IgE by the action of probiotics yields the observed therapeutic efficacy. Even for those cases that involve autoimmune response and autoreactive IgE antibodies subtly, they still involve the central pathologic axis of IgE-FcεRI-mast cells, and probiotics similarly render therapeutic effects (31, 32). However, in recent years, several lines of evidence suggest that some bacterial probiotics can modulate the skin immune system (33).

Since there are many evidences regarding the role of microbiota in pathogenesis and course of the CSU specially gut flora, also little case series or trials focusing on this entity, we designed the first RCT to evaluate the efficacy and safety of a symbiotic (prebiotic + probiotics) named as LactoCare in treatment of CSU.

Specific studies about effects of microbiota and symbiotics in course and treatment of chronic urticaria are really rare, so we discuss about the role of symbiotics in other dermatoses at first and then discuss about urticaria with more detail.

In a review study by Notay *et al.* (34), conducted in 2017 entitled "Probiotics, prebiotics, and synbiotics for the treatment and prevention of adult dermatological diseases" the results indicated that studies were optimistic about the use of some probiotic and prebiotics strains to improve clinical response in symptomatic AD, also as a treatment for acne. In addition, this review emphasis on further research to evaluate how probiotics and prebiotics could be better used in dermatology.

In a study by Rezazadeh *et al.*, (35) from Iran, authors investigated the protective effect of *Lactobacillus* and *Bifidobacterium* against chronic urticaria. In this study, stool samples of 20 patients with chronic urticaria were compared with 20 age- and sex-matched healthy controls in terms of *Lactobacillus*, *Bifidobacterium* and *Bacteroides* contents. The results showed that there was no significant difference between the frequencies of these bacteria between groups.

Another study was conducted in 2017 by Nabizadeh *et al.* (36) to investigate the relationship between microbiota composition and chronic urticaria. In this study, 20 patients with chronic urticaria and 20 age-matched healthy controls were selected. The PCR of bacterial DNA genome results showed that the frequency of *A. muciniphila*, *C. leptum* and *F. prausnitzii* in the stool of healthy subjects was significantly higher than in patients with chronic urticaria ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively).

Nettis *et al.* (2016) (37), conducted 8 weeks clinical trial on 38 patients with severe CSU to evaluate efficacy and safety of probotics. They used UAS7 score as primary outcome measure also assessed the patients' quality of life. Patients received double dose of 3 or more types of antihistamines also received oral probiotics named as Bifiderm twice daily. Patients visited at the beginning, 4 weeks and 8 weeks after study started. Nine patients experienced mild clinical improvement (23.7%), one patient reported significant clinical improvement (2.6%) and one patient completely recovered (2.6%). Twenty-seven patients showed no signs of recovery (71.1%). In addition, no adverse effects were reported during the study. In this study we evaluated the clinical efficacy and safety of an intake of a capsule is a symbiotic (probiotic + prebiotic), which contains high amounts of many beneficial and safe bacterial strains (*Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Streptococcus thermophilus*) plus fructooligosaccharides as prebiotic in patients with CSU.

The Nettis *et al.*'s study (37) was a single arm before-after trial, but our study was a RCT; we compared the results of Nettis's study with the results of the intervention arm of ours that showed 2 (9%), 12 (58%) and 7 (33%) of our patients had mild, significant and complete response, respectively. Our positive therapeutic results were more excellent than the previous study and may be due to better case selection, randomization and the least confounders due to better study design, also all cases of Nettis's study initially had sever urticaria with highest UAS7 score but 90% of our CSU group had severe

urticaria with mean UAS7 score as 32. In our study improvement of quality of life was higher than the Nettis's study, logically due to better therapeutic response. The safely was comparable with Nettis's study. In a review article of Ghaffari *et al*. (2013) (38), the efficacy of similar therapeutic regimens has been mentioned, as that our study confirmed too. The DLQI was conversely related to urticarial severity that is to somehow similar to our findings that final DLQI was higher in the intervention group which had higher UAS7 score reduction. Baiardini *et al*. (2003) (39) found that the quality of life in CSU patients were lower than the respiratory allergies in various social, physical and psychological aspects, that is comparable with our results which showed an improvement of DLQI during the study by decreasing of UAS7 score. In our study, although based on the statistical analysis, there were not any significant differences between control and intervention group regarding UAS7 score (final UAS7 score and the reduction rate during the study), but there were many differences in severity category change and response rate of 2 treatment groups during time, that are really important in practical managements of patients. So that symbiotics may make the therapeutic results of urticaria better but for more exact interpretations, we need further studies with higher sample size. There are many articles in dermatology regarding potential role of microbiota and efficacy of prebiotics, probiotics and symbiotic in many dermatoses such as dermatitis, atopic eczema, acne and *etc*., that in this study we focused on chronic urticaria and designed the first blinded RCT, in this regard (40-44).

Conclusions

Probiotics and symbiotics are effective, safe and satisfactory adjuvant therapy for CSU, although combination of probiotic and antihistamine did not have significant efficacy difference compared with the antihistamine alone based on final mean UAS 7 score, but patients with combination therapy may experience higher mean reduction rate of UAS 7 (although insignificant) and also significantly higher reduction rate of itch and number of urticaria, that is clinically really important and practical. In conclusion, our study suggests that probiotics administered twice daily for 8 weeks might reduce the symptom scores and quality of life scores in a part of patients with CSU who remained symptomatic despite treatment with H1 antihistamine. The probiotic approach might represent a new well tolerated option in the treatment of CSU.

Limitations and recommendations

Loss to follow ups was one of the major limitation of this study, also our study was not patient blinded as we did not have any placebo. High cost of symbiotics was another limitation of our study. With higher sample size and well-designed study, it is probable to observe significant difference between routine and combination therapies (+ probiotic or symbiotics), regarding score reduction.

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Conflict of interests

The authors declare that they have no conflict of interests.

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