Effect of administering kefir on the changes in fecal microbiota and symptoms of inflammatory bowel disease: A randomized controlled trial

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Cite this article as: Yılmaz İ, Dolar ME, Özpınar H. Effect of administering kefir on the changes in fecal microbiota and symptoms of inflammatory bowel disease: A randomized controlled trial. Turk J Gastroenterol 2019; 30(3): 242-53.

ABSTRACT

Background/Aims: Kefir is a kind of fermented probiotic dairy product. The objective of the present study was to investigate the effects of kefir consumption on the fecal microflora and symptoms of patients with inflammatory bowel disease (IBD).

Materials and Methods: Kefir was serially diluted and inoculated into de Man, Rogosa, and Sharpe agar and incubated at 37°C for 48 to 72 h under anaerobic conditions. This was a single-center, prospective, open-label randomized controlled trial. Forty-five patients with IBD were classified into two groups: 25 for treatment and 20 for control. A 400 mL/day kefir was administered to the patients for 4 weeks day and night. Their stool Lactobacillus, Lactobacillus kefiri, content was quantitated by real-time quantitative polymerase chain reaction before and after consumption. Abdominal pain, bloating, stool frequency, stool consistency, and feeling good scores were recorded in diaries daily by the patients.

Results: A 5×10^7 CFU/mL count of lactic acid bacteria colony forming units was found in a kefir sample as the total average count. Lactobacillus bacterial load of feces of all subjects in the treatment group was between 10^4 and 10^9 CFU/g, and the first and last measurements were statistically significant ($p=0.001$ in ulcerative colitis and $p=0.005$ in Crohn's disease (CD)). The L. kefiri bacterial load in the stool of 17 subjects was measured as between 10^4 and 10^6 CFU/g. For patients with CD, there was a significant decrease in erythrocyte sedimentation rate and C-reactive protein, whereas hemoglobin increased, and for the last 2 weeks, bloating scores were significantly reduced ($p=0.012$), and feeling good scores increased ($p=0.032$).

Conclusion: According to our data, kefir consumption may modulate gut microbiota, and regular consumption of kefir may improve the patient's quality of life in the short term.

Keywords: Inflammatory bowel disease, probiotics, kefir, Lactobacillus, Lactobacillus kefiri

INTRODUCTION

Inflammatory bowel disease (IBD), encompassing both ulcerative colitis (UC) and Crohn's disease (CD), is characterized by a chronic and relapsing inflammation of the gastrointestinal (GI) tract. UC and CD are generally described as chronic IBDs, although they are distinct diseases that differ in both symptoms and inflammation pattern (1).

The term probiotic means "for life". A viable mono or mixed microorganism culture that can be applied to an animal or a human being positively affects the host by improving the properties of the native microflora (2).

Kefir is a sour, carbonated and fermented milk product. It is a natural probiotic that contains live active cultures of the normal intestinal flora. Most lactic acid bacteria (LAB) in kefir have been considered as probiotic bacteria, such as *Lactobacillus kefiri*, *Lactobacillus casei*, *Lactobacillus kefiranofaciens*, *Pediococcus acidilactici*, and *Lactococcus lactis*, and they have potentially imparting health benefits (3-4).

According to the Turkish Food Codex, kefir is a kind of fermented dairy product containing starter cultures or examples of kefir. These cultures use specific forms of *L. kefiri*, *Leuconostoc*, *Lactococcus*, and *Acetobacter* with lactose fermenting (*Kluyveromyces marxianus*) and nonfermenting (*Saccharomyces unisporus*, *Saccharomyces cerevisiae*, and *Saccharomyces exiguous*) bacteria. *L. kefiri* is a heterofermentative bacterium that determines one of the flavor characteristics of kefir drink (5-6).

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Received: **March 30, 2018** Accepted: **July 24, 2018** Available online date: **November 21, 2018**

DOI: **10.5152/tjg.2018.18227**

CD is a serious immunity inflammatory disease that affects any part of the GI system, and the reason is still unknown. None of the treatments can heal the disease completely, but it is possible to keep it under control and enhance the quality of life.

UC is a chronic disease that is located in the mucosa of the large bowel and has recurrence and remission characteristics accompanied by inflammation and ulceration that can occur without any reason.

The human gut microbiota has a community of >100 trillion microbial cells that has been linked with GI conditions, such as IBD. For UC cases in the intestinal flora, *Lactobacillus* and *Bifidobacterium* decrease, whereas *Bacteroides vulgatus* and *Fusobacterium* increase. In addition, a decrease was reported in *Lactobacillus* and *Bifidobacterium* in the CD data (7). The most consistent change is the reduction in *Firmicutes* (8).

It is thought that in the enteropathogenesis of these illnesses, apart from genetic susceptibility, mucosal immune response disorder and the breakdown of the balance of the intestinal flora play significant roles. Probiotics are becoming increasingly popular. The use of oral probiotic cultures may improve intestinal disorders, such as UC (9).

Lactobacillus is the dominant flora of kefir and has probiotic properties, and *L. kefiri* is the characteristic microorganism of kefir, so they were selected for the study.

The aim of the present study was to determine the effects of kefir on CD and UC patient's *Lactobacillus* flora and their biochemical properties as well as symptoms and quality of life.

MATERIALS AND METHODS

Subjects

The study was performed as an open-label randomized control, single-center, prospective trial. From May 2015 to December 2016, 45 (25 treatment and 20 control group) patients participated in this trial. Three patients left the trial willingly. A total of 45 (25 treatment and 20 control group, 23 male and 22 female) patients completed the study. The trial protocol was assessed and approved by the ethics committee. Written informed consent was obtained from all the participants before the entry into the trial.

Selection of patients

Patients with IBD participated in the study. In the trial, CD Activity Index for CD and Truelove-Witts scoring systems for UC were used for disease assessment scores (10-11). If the score was <450, patients with CD were admitted to the study. If the score was higher, patients with UC were not admitted to the study. Volunteers also had to be >18 years old. Patients with alcohol consumption >20 g/day, allergies or intolerance to milk, antibiotic treatment within the last 1 month, column or bowel operation history up to 3 months before the start of the study, and the presence of active infection within 1 month prior to the start of the study or during the study were excluded from the study. In addition, if a patient requested to leave on his/her own will, or if kefir was not consumed continuously for 2 weeks, the trial protocol was assessed and was not approved.

Treatment of patients

Eligible patients were selected randomly to receive one of the following treatments: 400 mL/day kefir was administered twice a day to the patients for 4 weeks, which contains a total of 2.0×10^{10} CFU/mL viable *Lactobacillus* bacteria (treatment group, 25 patients). Treatment was interrupted in case of disease relapse, occurrence of side effects, and poor compliance. Patients were requested to fill out the symptoms diary that has questionnaires of bowel habits. Abdominal pain and bloating were rated on a four-point scale with 0=none, 1=mild, 2=moderate, and 3=severe. Stool consistency results were rated on a daily basis as slurry/watery=0, mash consistency=1, medium watery=2, normal=3, hard feces=4, and very hard or lumpy=5. Feeling good score was rated as very poor=1, worse=2, moderate/normal=3, good=4, and very good=5.

All patients underwent blood analysis (hemoglobin (Hgb), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR)), and the clinical activity index was calculated before and after the treatment.

The control group did not consume placebo because it was not possible to prepare a control product with a similar flavor, texture, and taste as those of kefir. Ayran and yogurt were similar to kefir, but they also have *Lactobacillus* and can affect the microbiota results.

Sample collection of feces

For measurement of the initial *Lactobacillus* quantity of feces, samples were obtained and stored at -20°C. After 4 weeks, patients were asked for a sample, and the stool was stored at -20°C at appropriate conditions for analysis.

Microbiological analysis of kefir

Kefir was serially diluted and inoculated into de Man, Rogosa and Sharpe (Oxoid CM361) agar and incubat-

ed at 37°C for 48 to 72 h under anaerobic conditions (anaerobic jars, Anaerocult C Merck) for LAB and Potato Dextrose Agar (Oxoid CM139) at 22°C for 5 days for yeast.

Isolation and identification of kefir Lactobacillus

The colonies obtained in the tests were cultured and purified. Pure bacterial cultures for species identification of *Lactobacillus* isolates were performed by Vitek® MS MALDI-TOF mass spectrometer (BioMerieux, Marcy l'Etoile, France).

At the same time, the isolates were identified using the API 50 CHL (BioMerieux) test.

PCR analysis of feces

Quantification of *Lactobacillus* bacteria from human stool samples was performed via real-time quantitative polymerase chain reaction (qPCR) in a culture-independent manner.

Total DNA of the stool was extracted using the Stool DNA Isolation Kit (QIAamp DNA Stool Kit) according to the manufacturer's instructions. *Lactobacillus* primers' specificity and optimization were performed by PCR amplification on a Thermal Cycler T-100 (Bio-Rad, Istanbul, Turkey). The amplified products were analyzed via gel electrophoresis according to size. The amplified region was confirmed as *Lactobacillus* by the Sanger sequencing method. All of the samples were studied on real-time PCR for both *Lactobacillus* and *L. kefiri* quantification separately. The *Lactobacillus* quantity in the stool samples was analyzed using the Roche LightCycler Nano Software (Bio-Rad) on real-time q-PCR device. For quantification of experiments, a standard curve of positive controls at different known concentrations was used. For positive control, the *Lactobacillus* gene *Lactobacillus rhamnosus* strain with the code CECT278ATCC7469 was used. The *L. kefiri* strain used is ATCC35411 in standard curve experiments. Primers for *Lactobacillus* spp. were designed according to Wang et al. (2011). *L. kefiri* primers were designed to be flat/reverse by taking the nucleotide sequences in the National Center for Biotechnology Information as reference (12).

The reaction conditions for PCR amplification were 95°C for 10 min; 40 cycles of 95 °C for 1 min, 50°C for 1 min, and 72°C for 1 min; and final elongation at 72°C for 10 min. The quantification protocol used to identify the abundance of fecal *L. kefiri* was according to Castillo et al. (13).

Statistical analysis of symptom diaries

Statistical analysis of symptom diary data was made using the SPSS 23.0 statistical package program. The Shapiro-Wilk test was used to determine whether the test was normal or not. The Mann-Whitney U test was used for normal distribution data. The Wilcoxon signed-rank test was used to compare dependent samples. The Fisher's exact, chi-square, and Fisher-Freeman-Halton tests were used to examine the categorical data. For analysis of repeated measures, percent change value (percent change=(last measurement-first measurement)/first measurement) according to the initial measurement was calculated and compared among the groups. Significance level was set at $\alpha=0.05$.

RESULTS

In IBDs, it is necessary to improve the quality of life. In our study, we aimed to elucidate the effects of kefir, which is a kind of probiotic food, on the intestinal microflora. In addition, we aimed to find kefir's effects on the quality of life of patients who have CD and UC that have not been investigated in humans previously with IBDs.

We investigated and compared the effects of fermented kefir drink on the changes of feces *Lactobacillus* flora and *L. kefiri* of patients with CD and UC and the effects of their biochemical parameters and symptoms. We found that regular kefir usage may improve both the symptoms and the quality of life in the short term in patients with CD and have a positive effect on the biochemical parameters of patients, such as Hgb, ESR, and CPR.

Twenty-five patients as treatment group and twenty patients as control group completed a total of 4 weeks. A 5×10^7 CFU/mL count of LAB colony forming units was found in a kefir sample as the total average count. A 2.1×10^4 CFU/mL yeast was found in a kefir sample as the total average count.

Identification of Lactobacillus *strains of kefir*

Overall, 10 Gram-positive, catalase-negative, rod-shaped isolates were obtained from kefir drink. The LP 1, LP 6, and LP 5b isolates were identified as *Lactobacillus pentosus*, LB 2 and LB 3 isolates were identified as *Lactobacillus brevis*, LPL 4 and LPL 5 isolates were identified as *Lactobacillus plantarum*, LF 7 isolate was identified as *Lactobacillus fermentum*, LK 9 isolate was identified as *L. kefiri*, and LL 10 isolate was identified as *Lactobacillus lindneri*, respectively, using the API 50 CHL test and Vitek® MS MALDI-TOF mass spectrometer. Therefore, we found six different strains of lactobacilli consisting of *L.*

Table 1. Comparison between patients fecal *Lactobacillus* population and *Lactobacillus kefiri* count before and after kefir consumption CD treatment groups and control groups

		Crohn's Disease Treatment Group (log10)				Crohn's Disease Control Group (log10)	
No	Microorganisms	0.day	28.day	No	Microorganisms	0.day	28.day
CDT1	*Lactobacillus*	4.65	7.95	CDC1	*Lactobacillus*	0	7.39
	Lactobacillus kefiri	3.41	5.95		*Lactobacillus kefiri*	0	4.88
CDT2	*Lactobacillus*	0	6.62	CDC2	*Lactobacillus*	4.69	4.92
	Lactobacillus kefiri	0	6.15		*Lactobacillus kefiri*	0	0
CDT3	*Lactobacillus*	0	6.65	CDC3	*Lactobacillus*	5.47	5.63
	Lactobacillus kefiri	0	0		*Lactobacillus kefiri*	4.29	4.20
CDT4	*Lactobacillus*	4.2	6.36	CDC4	*Lactobacillus*	5.37	4.2
	Lactobacillus kefiri	0	0		*Lactobacillus kefiri*	0	0
CDT5	*Lactobacillus*	0	6.43	CDC5	*Lactobacillus*	0	0
	Lactobacillus kefiri	0	4.69		*Lactobacillus kefiri*	0	0
CDT6	*Lactobacillus*	0	6.91	CDC6	*Lactobacillus*	4.71	4.95
	Lactobacillus kefiri	0	4.45		*Lactobacillus kefiri*	2.70	0
CDT7	Lactobacillus	4.47	6.63	CDC7	*Lactobacillus*	6.43	6
	Lactobacillus kefiri	0	4.59		*Lactobacillus kefiri*	0	0
CDT8	*Lactobacillus*	0	6.53	CDC8	*Lactobacillus*	4.65	4.64
	Lactobacillus kefiri	0	5.04		*Lactobacillus kefiri*	4.2	3.95
CDT9	*Lactobacillus*	0	6.89	CDC9	*Lactobacillus*	5.89	5.18
	Lactobacillus kefiri	0	5.48		*Lactobacillus kefiri*	0	0
CDT10	*Lactobacillus*	5.99	7.82	CDC10	*Lactobacillus*	5.83	5.43
	Lactobacillus kefiri	5.69	5.98		*Lactobacillus kefiri*	0	0

pentosus, *L. brevis*, *L. plantarum*, *L. fermentum*, *L. kefiri*, and *L. lindneri*.

Analysis of feces (treatment and control groups of patients) results

The composition of the fecal *Lactobacillus* microflora of 25 patients was monitored before and after the administration of kefir containing *Lactobacillus* (daily dose, 2.0×10^{10} CFU/day). After 1 month of kefir administration, the *Lactobacillus* amount in the stool of all subjects was between 10^4 and 10^9 CFU/g. The *L. kefiri* bacterial load in the stool of 17 subjects was measured between 10^4 and 10^6 CFU/g. The total amount of *Lactobacillus* in the treatment group of patients with CD was 10^6-10^7 CFU/g for all subjects and between 0 and 10^6 CFU/g for *L. kefiri*. The amount of *Lactobacillus* in the control group of patients shown in Table 1 and Figure 1. with CD was found to be between 0 and 10^7 CFU/g. *L. kefiri* was found in the range of 0-10^3 CFU/g. It was not found in 7 out of 10 patients. The amount of *Lactobacillus* in the treatment group of patients with UC was found to be 10^4-10^9 CFU/g for all subjects and 0-10^5 CFU/g for *L. kefiri*. The amount of *Lactobacillus* in the control group of are shown in Table 2 and Figure 2. with UC was found to be 0-10^5 CFU/g for all subjects. *Lactobacillus* was found to be 0-10^6 CFU/g in 18 patients for kefir. *L. kefiri* was not found in 6 out of 10 patients. Demographic and clinic properties of treatment groups are displayed in Table 3.

Biochemical parameters and symptom diary results

For biochemical parameters, patients with CD showed statistically significant differences in terms of all variables after kefir use. There was a significant decrease in ESR and CRP, whereas patients with CD showed an increase in Hgb after kefir use. The increase in Hgb measurements was found to be higher in patients with CD than in the CD control group (p=0.024 and p=0.029) which is shown in Table 5.

Table 2. Comparison between patients fecal *Lactobacillus* population and *Lactobacillus kefiri* count before and after kefir consumption UC treatment groups and control groups

		Ulcerative Colitis Treatment Group (log10)				Ulcerative Colitis Control Group (log10)	
No	Microorganisms	0.day	28.day	No	Microorganisms	0.day	28.day
UCT1	*Lactobacillus*	4.93	5.54	UCC1	*Lactobacillus*	5.91	0
	Lactobacillus kefiri	0	0		*Lactobacillus kefiri*	4.83	0
UCT2	*Lactobacillus*	0	9.17	UCC2	*Lactobacillus*	3.93	4.31
	Lactobacillus kefiri	0	5.8		*Lactobacillus kefiri*	0	4.4
UCT3	*Lactobacillus*	4.65	5.59	UCC3	*Lactobacillus*	3.81	6.29
	Lactobacillus kefiri	0	0		*Lactobacillus kefiri*	2.95	4.4
UCT4	*Lactobacillus*	0	6.22	UCC4	*Lactobacillus*	3.85	4.6
	Lactobacillus kefiri	0	4.55		*Lactobacillus kefiri*	0	0
UCT5	*Lactobacillus*	4.6	5.81	UCC5	*Lactobacillus*	4.92	4.2
	Lactobacillus kefiri	4.07	5.04		*Lactobacillus kefiri*	0	0
UCT6	*Lactobacillus*	0	6.11	UCC6	*Lactobacillus*	4.65	4.07
	Lactobacillus kefiri	0	5.97		*Lactobacillus kefiri*	2.80	3.00
UCT7	*Lactobacillus*	0	6.46	UCC7	*Lactobacillus*	5.66	5.58
	Lactobacillus kefiri	0	0		*Lactobacillus kefiri*	4.82	0
UCT8	*Lactobacillus*	5.58	5.66	UCC8	*Lactobacillus*	4.07	5.81
	Lactobacillus kefiri	0	0		*Lactobacillus kefiri*	2.85	2.80
UCT9	*Lactobacillus*	4.68	4.91	UCC9	*Lactobacillus*	5.47	5.15
	Lactobacillus kefiri	4.54	4.2		*Lactobacillus kefiri*	0	0
UCT10	*Lactobacillus*	0	4.71	UCC10	*Lactobacillus*	4.55	5.39
	Lactobacillus kefiri	0	0		*Lactobacillus kefiri*	0	0
UCT11	*Lactobacillus*	0	5.38				
	Lactobacillus kefiri	0	5.15				
UCT12	*Lactobacillus*	6.43	6.65				
	Lactobacillus kefiri	0	5.78				
UCT13	*Lactobacillus*	6.14	6.17				
	Lactobacillus kefiri	0	5.53				
UCT14	*Lactobacillus*	0	5.18				
	Lactobacillus kefiri	0	0				
UCT15	*Lactobacillus*	0	5.83				
	Lactobacillus kefiri	0	5.15				

Demographic and clinic properties of treatment and control groups of ulcerative colitis are shown in Table 4. According to the symptoms diary for patients with CD, the last 2 weeks of bloating was significantly reduced (p=0.012). At the same time, the feeling good score improved in the last 2 weeks, and patients' conditions improved (p=0.032). The feeling good score was significantly higher in patients with CD as the abdominal pain score was significantly lower in patients with CD in the last 2 weeks than in patients with UC. No statistically significant difference was found between weeks 1 and 2 in patients with UC in terms of abdominal pain, bloating, frequency of stools, defecation consistency, and feeling good. A statistically significant difference was observed between

Table 3. Demographic and clinic properties of treatment groups

	Ulceratif Colitis (n=15)	Crohn's Disease (n=10)	p
Age (median(min-max)) year	33 (19;68)	33 (24;65)	0.643
Gender			
Male	9 (%60)	4 (%40)	0.428
Female	6 (%40)	6 (%.60)	
Involment place (Localition)			
Colon	15 (%100)	1 (%10)	<0.001
Ileum	0 (%0)	6 (%60)	
Colon+Ileum	0 (%0)	3 (%30)	
Age of illness (median (min-max)) year	4 (1;12)	2 (1;9)	0.129
Total Kefir consumption (median(min-max)) liter	11.2 (9.4;11.2)	11.2 (9.6;11.2)	0.683
Lactobacillus first measurement (Range)	0 (0-271.3)	0 (0-99.1)	1.000
Lactobacillus last measurement (% change)	1.87 (0.19-53.4)	3.4 (0.44-13.7)	0.914
HGB first measurement	11.7 (10.6-15.8)	12.7 (9.3-15.5)	0.723
HGB last measurement (% change)	0.05 (-0.09;0.17)	0.08 (-0.04;0.24)	0.567
ESR firs measurement	25 (5;59)	29 (12;59)	0.428
ESR last measurement (% change)	-0.15 (-.71;1.18)	-0.20 (-0.53;0.69)	0.643
CRP first measurement	0.33 (0.3;6)	1.1 (0.3;8)	0.048
CRP last measurement (% change)	-0.06 (-0.94;2.52)	-0.60 (-0.96;0.72)	0.103

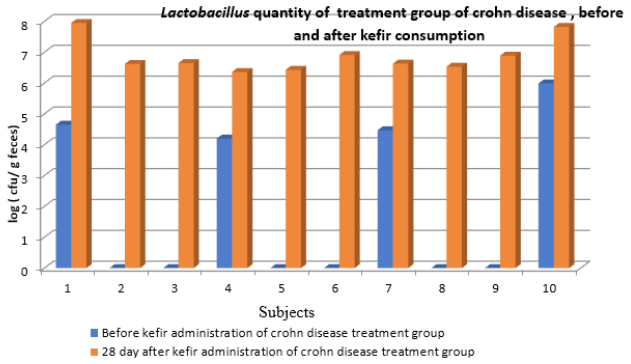

Figure 1. *Lactobacillus* quantity of treatment group Crohn disease treatment group; before and after kefir consumption

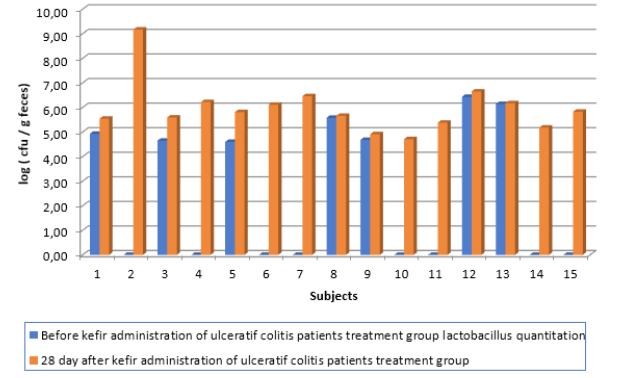

Figure 2. *Lactobacillus* quantity of treatment group ulcerative colitis disease treatment group; before and after kefir consumption

the abdominal pain score (p=0.049) and the feeling good score (p=0.019) in the last 2 weeks when the symptom diary data were compared between patients with CD and UC. According to this, the rate of feeling good was significantly higher in patients with CD as the abdominal pain score was lower in the last 2 weeks than in patients with UC. None of the patients in either of the groups had worsening of disease symptoms. No side effects were observed in all of the subjects.

According to the results we obtained, it was determined that in some patients using kefir, there was a statistically significant improvement in abdominal pain, bloating, and quality of life when compared with the control group. The feeling good score was significantly higher in patients with CD when the abdominal pain score was significantly lower in patients with CD than in patients with UC in the last 2 weeks. A statistically significant difference was found in terms of bloating and feeling good when the symptom log data of the first 2 weeks and the last 2 weeks of patients with CD were examined.

DISCUSSION

The mean count of lactobacilli in some studies was similar to our study, with 8 log CFU/mL, 7.2 log CFU/mL, and 1.2×10^7 CFU/mL of lactobacilli, respectively, in kefir (14,15,16). A 5×10^7 CFU/mL count of LAB colony forming units was found in a kefir sample as the total average count in our study.

In the present study, *L. pentosus*, *L. brevis*, *L. plantarum*, *L. fermentum*, *L. kefiri*, and *L. lindneri* were isolated from

Table 4. Demographic and clinic properties of treatment and control groups of ulcerative colitis

	Ulcerative Colitis Treatment Group Group (n=15)	Ulcerative Colitis Control Group Group (n=10)	p
Age (median(min-max)) year	33 (19;68)	43.5 (29;76)	0.041
Gender			
Male	9 (%60)	4 (%40)	0.428
Female	6 (%40)	6 (%60)	
Involment Place (Localition)			
Colon	15 (%100)	10 (%100)	-
Ileum	0 (%0)	0 (%0)	
Colon + Ileum	0 (%0)	0 (%0)	
Age of illness (median (min-max)) year	4 (1;12)		
Total Kefir Consumption (median(min-max)) liter/ 4 week	11.2 (9.4;11.2)	-	-
Lactobacillus first measurement (Range)	0 (0;271.3)	4.04 (0.65;81.3)	0.048
Lactobacillus last measurement (% change)	1.87 (0.19;53.4)	0.62 (-1;299.02)	0.428
HGB first measurement	11.7 (10.6;15.8)	12.35 (8.5;15)	0.531
HGB last measurement (% change)	0.05 (-0.09;0.17)	0.02 (-0.06;0.49)	0.807
ESR first measurement	25 (5;59)	25.5 (17;64)	0.367
ESR last measurement (% change)	-0.15 (-0.71;1.18)	-0.19 (-0.6;0.88)	1.000
CRP first measurement	0.33 (0.3;6)	0.78 (0.31;18.70)	0.461
CRP last measurement (% change)	-0.06 (-0.94;2.52)	-0.44 (-0.93;5.48)	0.531

Table 5. Demographic and clinic properties of treatment and control groups of Crohn's disease

	Crohn Disease Treatment Group (n=10)	Crohn Disease Control Group (n=10)	p
Age (median(min-max)) year	33 (24;65)	42 (21;66)	0.529
Gender			
Male	4 (%40)	6 (%60)	0.656
Female	6 (%.60)	4 (%40)	
Involment Place (Localition)			
Colon	1 (%10)	0 (%0)	0.628
Ileum	6 (%60)	10 (%100)	
Colon + Ileum	3 (%30)	0 (%0)	
Age of illness (median (min-max)) year	2 (1;9)	2 (1;10)	0.971
Total Kefir Consumption (median(min-max)) Liter/4 week	11.2 (9.6;11.2)	-	-
Lactobacillus first measurement (Range)	0 (0;99.1)	14.26 (0;271.3)	0.143
Lactobacillus last measurement (% change)	3.4 (0.44;13.7)	-0.6 (-0.93;0.74)	0.024
HGB first measurement	12.7 (9.3;15.5)	13.2 (10.6;15.9)	0.481
HGB last measurement (% change)	0.08 (-0.04;0.24)	-0.01 (-0.13;0.15)	0.029
ESR first measurement	29 (12;59)	20.5(3;89)	0.353
ESR last measurement (% change)	-0.20 (-0.53;0.69)	-0.09 (-0.77;0.67)	0.393
CRP first measurement	1.1 (0.3;8)	0.40 (0.31;10.80)	0.481
CRP last measurement (% change)	-0.60 (-0.96;0.72)	-0.18 (-0.97;4.48)	0.190

kefir. The most common lactobacilli isolated from kefir grains as reported by other studies are: *L. brevis*, *L. kefir*, *Lactobacillus acidophilus*, *L. plantarum*, *L. kefiranofaciens*, *Lactobacillus kefirgranum*, and *Lactobacillus parakefiri*. The LAB isolated from kefir in our study were the same as the following studies: *L. fermentum* Witthuhn et al. (16); *L. kefiri* Bosch et al. (17), Kesmen and Kacmaz (19), and Magalhaes et al. (20); *L. plantarum* Garrote et al. (18) and Witthuhn et al. (16); and *L. brevis* Simova et al (21). and Witthuhn et al. (16).

We isolated *L. kefiri* from kefir. Pintado et al. isolated *L. kefiri* from Portuguese kefir by using API 50 as the same. Chen et al. also identified *L. kefiri* from the kefir in Taiwan (22,23).

Our data indicated that the selected LK 9 *L. kefiri* strains were colonized in the gut of this study of patients. As similarly seen in the study by Toscano et al., after 1 month of *L. kefiri* LKF01 administration, the *Lactobacillus* strain was detected in the feces of all subjects participating in our study with a bacterial load of 10^5-10^6 CFU/g. According to the same study, *L. kefiri* showed a strong ability to modulate the gut microbiota composition, leading to a significant reduction of several bacterial genera directly involved in the onset of proinflammatory response and GI diseases (24).

According to Braat et al., there was a decrease in the number of CRP levels of patients with CD consuming *L.*

lactis for 1 week (25). In our study, CRP levels decreased after a 28-day kefir consumption of patients with CD, and it was statistically significant (p=0.015). The number of studies evaluating the immunomodulatory properties of probiotics is increasing. The immunomodulatory properties of kefir may be due to the direct action of the microbicide or may be indirect through different bioactive compounds produced during the fermentation process (25). The immunomodulatory effect of kefir may be attributed to its ability to reduce or repair intestinal permeability of these probiotics. Thus, contact between the antigens in the host and intestinal lumen is reduced, which can reduce the inflammatory response (26). IBD is associated with the intestinal microflora. In humans with IBD, there are a low number of lactobacilli and bifidobacteria and a large number of anaerobic bacteria. Treatment is performed using probiotics to help the patient maintain the remission period (27). In the intestines of individuals with IBD, the numbers of *Lactobacillus* and *Bifidobacterium* are lower, and anaerobes are higher. Probiotics do not cure the disease; however, after some time, they may prolong the remission period. This increases the quality of life of patients (25). According to data from our study, a statistically significant difference was observed in abdominal pain score (p=0.049) and feeling good score (p=0.019) for patients who consumed kefir, which contains probiotics. They have positive effects on diseases caused by an imbalance of the intestinal microflora (28).

Some studies show that probiotics have effects on patients with UC and CD (29). According to Tursi et al. (2010), VSL # 3 probiotic mixture reinforcement is safe and can reduce the UC Disease Activity Index (UCDAI) scores in patients affected by mild to moderate UC treated with 5-aminosalicylic acid and/or immunosuppressants. In addition, it improves rectal bleeding and regenerates remission in patients with recurrent UC after 8 weeks of treatment. However, these parameters do not reach statistical significance (30).
The study was performed in a small open-label study in patients with active UC. Compared with 10 patients treated with inactivated bacteria given live *L. plantarum* 299v, 6 out of 9 patients reached remission (31).

Patients with relapses with mild to moderate UC were treated with 3×250 mg/day probiotic *Saccharomyces boulardii* for 4 weeks. A 68% remission rate was observed (32).

Patients with UC who were on remission in a placebo-controlled study using fermented pills containing 1×10^{10} CFU *Bifidobacterium breve*, *Bifidobacterium bifidum*, and *L. acidophilus* were given 100 mL milk for 12 months. At the end of the study period, 73% of patients in the fermented milk group remained in remission, whereas the number was 10% for the placebo group, and a significant difference was detected in clinical remission; however, no difference was found 1 year after colonoscopy (33).

One of the other studies was the one which forty patients with clinical and endoscopic remissions participated in the randomized, placebo-controlled trial. VSL # 3 was infected with 6 g/day for 9 months. Fecal samples showed significantly increased fecal concentration of *Lactobacillus*, bifidobacteria, and *Streptococcus thermophilus* after pretreatment and treatment ($p<0.01$) only in baseline levels in the VSL # 3 treated group (34).

We also found that the amount of *Lactobacillus* in patients' feces at the end of 1 month of kefir consumption was between 10^4 and 10^9 CFU/g for all subjects. For *L. kefiri*, it was found to be between 10^4 and 10^6 CFU/g in 17 patients, and the change in the amount of *Lactobacillus* was significant.

In one study related to lactose intolerance, a group of subjects were fed low-fat milk, and another group was fed with kefir. The subjects have lactose intolerance. Lactose intolerance is caused by low β-galactosidase (lactase) activity in the intestine. Diarrhea and pain in the abdomen were observed in the milk group, but these effects were not observed in the kefir group (35). In lactose intolerance, individuals have an osmotic effect by lactose fermentation, which is not digested due to enzyme deficiency, and lactose and methane, hydrogen, and organic acids emerge, which cause discomfort. Dairy products can cause gas and bloating in patients with CD and UC. Nevertheless, since kefir has *Lactobacillus* that degrades lactose in the intestines, no one complained about lactose intolerance symptoms, such as abdominal pain and gas, in our study (36). Patients with CD and UC who cannot consume dairy products can easily consume kefir, and they do not feel uncomfortable and cannot stay away from calcium source.

In an experiment on 10 patients with IBD, VSL # 3 probiotic mixture was administered to the patients for 2 months, and the stool was analyzed by PCR. As a result, colonization of *S. thermophilus*, *Bifidobacterium infantis* Y1, and *B. breve* Y8 strains was found to be similar to healthy individuals (37).

One study was conducted to directly detect *S. thermophilus* in human feces, except culture-based techniques or

DNA isolation and purification procedures with culture-independent PCR protocol. The persistence of *S. thermophilus* in the intestines of 10 healthy subjects who were given VSL # 3 or yogurt was investigated. The bacteria sought after 3 days of administration were detected and continued to be found 6 days after treatment suspension.

Manichanh et al. (38) found a significant decrease in the *Clostridium* family in patients with CD using the DNA microarray-based analysis method, but no significant variation was found in the *Bacteroides* family.

A 16S rDNA-column library index method was used in the study by Gophna et al. (39) for the analysis of IBD intestinal microbiota. In conclusion, a decrease in the number of *Bacteroidetes* and *Proteobacteria* in CD, but a decrease in the *Clostridium* family, was observed.

The general composition of the intestine is considered most relevant in the etiology and pathogenesis of IBD. However, microbiota analyses are long and labor intensive, and as a result, only cultivable bacterium can detect 20%-30% of microbiota. Owing to complex anaerobic environment requirements, the rest cannot be cultured. Therefore, molecular approaches are widely used for microbiota analysis (40).

In a study investigating whether the fecal microbiome of patients with UC and CD differed from healthy individuals, studies using terminal restriction fragment length polymorphism analysis showed differences. However, the intestinal microbiology of patients with inactive UC is similar to that of healthy individuals. Identification of the intestinal mechanisms of these patients and changes in microbiota structure may contribute to the development of new treatment options for patients with UC and CD (40).

When constantly consumed, the lactobacilli in the kefir settle in the intestines and produce acid components that correct the microflora against the pathogenic bacteria, thus the diseased bacteria can be removed (41).

Although pathogenic bacteria, such as *Salmonella* and *Shigella*, have been associated with the presence of kefir starter, these pathogens have not been developed (42). In addition, LAB and yeast present in the microflora have an inhibitory effect on kefir intestinal microorganisms (43). Kefir reduces the time of transit time by allowing feces to be easily thrown away. When antibiotic therapy is applied, it improves the irregular bowel flora (41).

Patients with UC and CD who started to use kefir in our study were seen to have been colonized by kefir probiotics according to the first week and the last 2 weeks when they started to establish a positive balance in the gut. Since the results in the literature are mostly obtained by different symptom evaluation methods, we are unable to make a direct comparison with data from our study.

In our study, the decrease in abdominal pain and bloating scores in the IBD group compared with the control group was similar to Nagendra and Shah (44).

The effect of *S. boulardii* was also investigated in a study on the effect of CD. Patients who were in remission from CD have been treated with idiopathic remedies. In this treatment, mesalamine was administered to a group of 3×g/day. The other group was *S. boulardii* for 1 month and 2×1 g/day mesalamine for 6 months. The remission rate in the group administered only mesalamine was 38%. The remission rate for mesalamine and *S. boulardii* was 94% (32).

In patients with CD, there are experiments with *Lactobacillus salivarius* UCC118 and *Lactobacillus* GG as probiotics. The results obtained for these patients are not sufficient, nonetheless promise future work.

In a meta-analysis, probiotics, which failed to prevent remission in CD and prevent clinical and endoscopic recurrence, have been recommended to use probiotic preparations containing a mixture of *Lactobacillus*, *Escherichia coli*, or *Saccharomyces* (45).

A pilot study by Gupta et al. showed that *Lactobacillus* GG can increase the intestinal barrier function in children with mild to moderate active CD (46).

In a double-blind, randomized, controlled study with *Lactobacillus* GG, children with CD did not prolong their recurrence time (47).

Saccharomyces boulardii with mesalazine has been found to be effective only in the recurrence control group when administered mesalazine (32).

In the study conducted by Steed et al. in 2010, by reviewing patients with active CD, they were given a symbiotic containing *Bifidobacterium longum* and as a result found to be effective when compared with the placebo. In the treatment of CD, randomized, controlled trials have proven the effectiveness of probiotics (48).

In our study of the microbial analysis of feces, the kefir treatment group showed significantly higher fecal lactobacilli count than the control group. This has been attributed to their ability to survive at low pH and high bile concentration as in in vitro experiments. These potentially probiotic bacteria colonizing the intestinal mucosa provide a barrier to pathogens through various mechanisms, competition for nutrients, and the production of antimicrobials.

According to Toscane et al. (24), *L. kefiri* appears to be effective and safe to maintain remission in patients with UC and may be a good treatment option for preventing relapse in this group of patients. *L. kefiri* LKF01 demonstrated a strong ability in modulating the intestinal microbiota composition, leading to a significant decrease in several bacterial generations at the onset of direct proinflammatory response and GI disorders.

Although the etiology of CD is uncertain, evidence suggests the involvement of intestinal bacteria, and studies have shown that bacterial, fusobacteria, enterococci, *E. coli*, and fewer bifidobacteria, lactobacilli, eubacteria, *Clostridium coccoides*, and *Clostridium leptum* showed higher concentrations in patients with CD. In *Faecalibacterium prausnitzii* and remission from healthy individuals, populations of fecal bacteria changed (48).

Probiotics can effectively protect UC remission, but little is known about their ability to induce remission. Adult patients with mild to moderate UC were randomized to receive 3.6×10^{12} CFU VSL # 3 (n=77) twice daily for 12 weeks and placebo (n=70). In the UCDAI, a reduction of 50% was achieved at 6 weeks. UCDAI is a measure of the degree of fecal incidence, rectal bleeding, mucosal appearance, and disease activity of the physician. The percentage of patients with a >50% improvement in the UCDAI score at week 6 was compared with the placebo-treated group (10%; 0.001) in the VSL # 3 given group (25% vs. 32.5%). At week 12, 33 (42.9%) patients receiving VSL # 3 entering remission were compared with 11 (15.7%) placebo patients ($p<0.001$). In addition, it was observed that the number of patients given VSL # 3 (40%; 51.9%) decreased by 3 points in UCDAI compared with placebo (13%; 18.6%) ($p<0.001$). The VSL # 3 group showed significantly greater reductions in UCDAI scores and symptoms at 6 and 12 weeks compared with the placebo group (49).

Other studies have confirmed that probiotic bacteria may increase the integrity of tight junctions between intestinal epithelial cells during infections or inflammatory conditions. For this reason, colonization with probiotic bacteria may cause exposure of immune cells to bacterial antigens believed to induce IBD. Experimental colitis showed that the protective effects of probiotic microorganisms (VSL # 3) in a dextran sulfate sodium model were mediated by DNA as recognized by the mucosal Toll-like receptor 9 receptor. This interaction subsequently led to increased endogenous production of bacterial survival beta-defensin and antibacterial peptides. In addition, it has been reported that treatment of VSL # 3 cultured intestinal epithelial cells leads to an increase in transepithelial electrical resistance, a change associated with reduced permeability. In the present study, incubation of intestinal epithelial cells with this probiotic consortium also induced the expression of various mucins, resulting in decreased adhesion of microorganisms and components to the epithelial surface (50). According to our study, probiotics have been evaluated in animal models and in some clinical trials. Oral administration of probiotics with VSL # 3 has been shown to normalize the interleukin 10 barrier function in IBD mice. VSL # 3 is a probiotic cocktail consisting of eight different Gram-positive organisms. Many studies on kefir's biological activities have revealed that kefir has anti-inflammatory, immunomodulatory, and antimicrobial activities and is a functional food (51). Regular kefir consumption is associated with lactose intolerance and tolerance; antibacterial effect; hypocholesterolemic effect; control of plasma glucose; antihypertensive and anti-inflammatory effects; antioxidant, anticarcinogenic, and anti-allergic activities; and healing effects. Much of the work supporting these findings has been made in vitro or in animal models (52). All studies show that probiotics may play an important role in the management of IBD in the future, despite the fact that current clinical trials do not have statistical power, probably due to limited data. The availability of new techniques to better understand bacterial and host interactions and to better define the microbiota modification in different clinical subclasses may be a key to the success of effective probiotic therapy in patients with IBD (50).

Study disadvantages and limitations

Our study has some limitations. Moreover, the literature on IBD data is insufficient to reach at definite conclusions about the changes in the quality of life. Short-term kefir consumption and changes in the quality of life in our study may not have been revealed by patients. Inadequate number of patients may prevent the statistical significance of the changes.

The small sample size and short time are major weak points of the present study; however, it is very difficult for

patients who have UC and CD to consume anything due to their illness. They especially want to know the effect of the symptoms of the diseases before consuming a different food. The lack of study on kefir was also questioned by the patients. One other limitation of our study was that the questionnaires were self-administered by the patients.

One advantage of our study was that we performed both feces analysis and concurrent assessment of bloating, defecation consistency, defecation, and feeling good scores with biochemical parameters at the same time. We also measured the severity of symptoms.

According to data from our study, regular kefir usage may improve both symptoms and quality of life in the short term in patients with IBD. The actual effects of probiotics on intestinal ecology are still to be discussed, as differences in microbial strains have a number of factors to be explored, such as their concentration and formulations.

Kefir has a tart, creamy flavor and apart from having a high nutritional value, it is also known to have a probiotic effect (53). Probiotic bacteria should be produced as an alternative to industrial probiotics through non-transgenic microorganisms isolated from natural food products such as kefir (54).

There are many useful probiotic microorganisms in kefir. It is easy to find and is inexpensive. We investigated the undefined effects of kefir in patients with IBD, *Lactobacillus* and *L. kefiri* flora of feces, and biochemical parameters and disease symptoms. Further studies are needed to evaluate the best dose-response effect of kefir, including monitoring patients to assess the persistence of potential beneficial effects in patients with CD and UC following kefir intervention. Unfortunately, countless human research conducted with kefir is often poorly designed. More human studies should be conducted to demonstrate the effect of kefir consumption and reduce the risk of disease. In addition, the actual effects of probiotics affecting intestinal ecology should be investigated, and advanced studies should be conducted on disease-specific food product formulations with customized studies on microbial strains in well-designed randomized clinical trials. The trials should continue on greater patient populations.

Ethics Committee Approval: Ethics committee approval was received for this study from the Uludağ University School of Medicine Clinical Research Ethics Committee (Decision No: B.30.2.ULU.0.20.70.02-050.99/440, Decision Date: 25.11.2013)

Informed Consent: Written informed consent was obtained from the subjects who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - İ.Y., M.E.D., H.Ö.; Design - İ.Y., M.E.D., H.Ö.; Supervision - İ.Y., M.E.D., H.Ö.; Materials - M.E.D.; Data Collection and/or Processing - İ.Y.; Analysis and/or Interpretation - İ.Y., M.E.D., H.Ö.; Literature Review - İ.Y., M.E.D.; Writing Manuscript - İ.Y.; Critical Review - İ.Y., M.E.D., H.Ö.

Conflict of Interest: The authors have no conflicts of interest to declare.

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

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