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Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis

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ABSTRACT

Objectives: Rheumatoid arthritis (RA) is an inflammatory autoimmune disease in which the gut microbiota is altered. Probiotics are microorganisms that can normalize gut microbiota; thus, they may help to alleviate RA symptoms. The objective of the present clinical trial was to assess the effects of probiotic supplementation on disease activity and inflammatory cytokines in patients with RA.
Methods: Forty-six patients with RA were assigned into two groups in this randomized, double-blind, placebo-controlled clinical trial. The patients in the probiotic group received a daily capsule that contained a minimum of 10^8 colony-forming units of *Lactobacillus casei* 01 for 8 wk. The placebo group took capsules filled with maltodextrin for the same time period. Questionnaires, anthropometric measurements, and fasting blood samples were collected, and the participants were assessed by a rheumatologist at baseline and at the end of the trial.
Results: Disease activity score was significantly decreased by the intervention, and there was a significant difference between the two groups at the end of the study ($P < 0.01$). Three of the assessed serum proinflammatory cytokines (tumor necrosis factor-α, interleukin-6, and interleukin-12) significantly decreased in the probiotic group ($P < 0.05$); however, serum levels of interleukin-1 β were not significantly affected by the probiotic ($P = 0.22$). The serum level of regulatory cytokine (interleukin-10) was increased by the supplementation ($P < 0.05$). The proportion of interleukin-10 to interleukin-12 was significantly increased in the probiotic group as well.
Conclusions: L. casei 01 supplementation improved the disease activity and inflammatory status of patients with RA. Further studies are warranted to confirm these results, and such confirmation may lead to the introduction of probiotics as adjunctive therapy for this population.

Introduction

Rheumatoid arthritis (RA) is a relatively common disabling autoimmune disease that is characterized by progressive joint disorder, significant pain, and functional disability. This systemic inflammatory disease of unknown cause has a prevalence of 0.5% to 1% among adults worldwide and continues to cause significant morbidity and premature mortality [1,2]. Although many effective pharmacologic agents are available today to alleviate RA symptoms, side effects have been reported to accompany the benefits derived from these therapies [1,3]. In addition, therapies that target the modifiable probable underlying causes of RA and that may most efficiently bring the disease under control are still being sought. There is some evidence from human studies that gut microbiota is altered in patients with RA and that imbalanced gut microbiota may contribute to the initiation of the disease [4–7].

E.V.M. conceived and designed the study; generated, collected, assembled, analyzed, and interpreted the data; and drafted and revised the manuscript. B.A. generated and collected the data. A.H.R. conceived and designed the study; analyzed and interpreted the data; and approved the final version of the manuscript. S.K.S. generated and collected the data. M.A.J. analyzed the data. S.Z. collected the data.

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As defined by the Food and Agriculture Organization of the United Nations and the World Health Organization, probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [8]. Probiotics have been suggested to be effective against a number of disorders, and the modulation of immune system function is among the most studied properties of probiotics. Many in vivo and in vitro studies have demonstrated that some strains of probiotics can either stimulate or downregulate immune system function in a strain- and dose-specific manner; patients with diseases that result from downregulated immune systems may benefit from the first property, whereas those with hyperactive immune systems may profit from the probiotic strains that cause such downregulation [9,10].

The effect of probiotic administration for either the prevention or treatment of RA has been investigated in a limited number of animal and human studies. Animals fed *Lactobacillus casei* had improved clinical manifestations, reduced proinflammatory cytokines (i.e., interleukins-1 β, 2, 6, 12, and 17 [IL-1 β, IL-2, IL-6, IL-12, and IL-17, respectively], interferon-γ [IFN-γ], and tumor necrosis factor-α [TNF-α]), and increased regulatory cytokines (interleukin-10 [IL-10] and transforming growth factor-β [TGF-β]) [11–14]. Yogurts fermented with *Lactobacillus bulgaricus* and live or sacrificed *Lactobacillus rhamnosus GG* (LGG) reduced arthritis clinical scores in Lewis rats [15]. *Escherichia coli* strain O83 (Colinfant), when administered in combination with methotrexate, significantly inhibited both inflammation and destructive arthritis-associated changes [16]. Human studies have applied different strains of probiotics, and all have reported functional improvement or subjective well-being in those receiving the treatment. However, disease activity or inflammatory biomarkers were not significantly modified by the interventions [17–19]. The aim of the present randomized clinical trial was to investigate the effects of *L. casei* 01 supplementation on the disease activity and inflammatory cytokines of patients with RA.

Materials and Methods

Subjects

The target population of the present study was women with RA who were referred to the rheumatology clinic of Sina Hospital in Tabriz, Iran, or Sheykholrayis Polyclinic in Tabriz, Iran. A rheumatologist listed patients who had been to her office who met the inclusion criteria and recorded their phone numbers. Subjects were contacted a day before commencing the supplementation, and the study was thoroughly explained to them. Patients entered the study if they were interested. The inclusion criteria consisted of being diagnosed with RA on the basis of American College of Rheumatology criteria, for more than one year; having inactive to moderate RA (i.e., a disease activity score of <5.1); not receiving nonsteroidal anti-inflammatory drugs (NSAIDs) or cytokine inhibitors; following a stable medication regimen for ≥ 3 mo before the intervention; having a body mass index (BMI) of <40 kg/m^2; being between 20 and 80 y old; and being willing to participate in the study. The exclusion criteria of the study included being pregnant or lactating; being under hormone therapy or receiving oral contraceptives; having diabetes mellitus, thyroid disorders, kidney or hepatic diseases, or Cushing syndrome; having inflammatory bowel disease or other inflammatory disorders; having digestive tract disorders or lactose intolerance; taking antioxidant, vitamin, or fiber supplements ≤ 3 wk before the interventions; using antibiotics 1 mo before the intervention; being on a weight-reduction diet; smoking or being exposed to cigarette smoke; and using other probiotic products.

The sample size for the study was calculated on the basis of the results (mean ± SD) for IL-12 as reported by Pineda et al. [19], with a confidence level of 95% and a power of 80%; this was found to be 22 patients. Taking into account the probable withdrawal of patients during the intervention course as well as those who may not adhere to the study protocol, 30 patients with RA were recruited for each group.

Study design and measurements

The present study was a double-blind, randomized, placebo-controlled trial in which the patients were randomly allocated into either the probiotic supplement group or the placebo group on the basis of menopausal status and BMI. The patients were asked to attend the rheumatology clinic of Sina Hospital on a particular date after an overnight fasting of at least 12 h. A sample of 8 mL of blood was taken from each participant's antecubital vein; the participants were then weighed using a Seca scale (Seca, Germany) with a precision of 500 mg while wearing minimal clothes and no shoes. A tape measure with a precision of 0.1 cm was used to measure the height of the patients while they were not wearing shoes. BMI was then calculated by dividing weight (kg) by height squared (m^2). In addition to a demographic questionnaire, the International Physical Activity Questionnaire and the Spielberger State-Trait Anxiety Inventory Form Y (STAI-Y) were filled in for the patients at baseline and at the end of the study. Physical activity was categorized as high, moderate, or low, and the women were categorized as having no or minimum, mild, moderate, or severe state and trait anxiety on the basis of the scores obtained from the STAI-Y questionnaires.

A visual analog scale (VAS) questionnaire was also completed for participants to assess global health (GH). A 24-h dietary recall questionnaire was used and two series of food record forms, each of which consisted of forms for two working days and one holiday, were given to the participants, who were asked to fill in the forms when they were contacted; three food record questionnaires were to be filled during the first wk of the intervention course, and the others were to be filled out during the last wk of the study period. Necessary explanations were provided about how to estimate food intake and record the estimations. The patients were then visited by the rheumatologist, and their tender and swollen joints were counted. Study capsules (60 capsules in each container) were provided to the patients after this process. The capsules and the containers for the probiotic supplements and the placebo capsules were identical; the patients, the rheumatologist, the person who filled in the questionnaires for the patients, and the laboratory staff were blinded to the treatment of each group. Instructions were given regarding how to store and take the capsules. Participants were asked to keep the capsules refrigerated and to take 1 each d on empty stomach after drinking a glass of water (for the dilution of gastric acid). The women were asked not to change their dietary intake or physical activity level during the study period, and they were contacted every other w to confirm that they were taking the capsules correctly and to ask about possible side effects of the treatment. After 8 wk of intervention, the patients attended the same clinic in a fasting state and another 8 mL of blood was drawn. The same measurements were performed and the same questionnaires were completed, and the joints were examined by the rheumatologist. The food records were handed over by the participants. The remaining capsules were also obtained from the patients to make sure that they had taken at least 70% of the administered supplements.

Nutritionist IV software (First Databank, Hearst Corp, San Bruno, CA) was used to assess the participants' diets. The blood samples were centrifuged at 3500 rpm for 10 min (Orum Tadjhiz Centrifuge, Iran) at room temperature to separate serum, which was then aliquoted into 1-mL microtubes. The microtubes were immediately frozen at −70°C until the assays could be performed. Blood samples were analyzed at the Drug Applied Research Center (Tabriz University of Medical Sciences, Tabriz, Iran). Turbidometric assay and commercial kits (Parsazmun, Iran) were applied to measure serum levels of high-sensitivity C-reactive protein (hs-CRP) in the present study, and hs-CRP concentrations were read by an autoanalyzer (Abbott, model Alcyon 300, Philippines) at a wavelength of 500 nm. Enzyme-linked immunosorbent assays and commercial kits (DIASource, Belgium) were applied to measure the cytokines IL-1 β, IL-6, IL-10, IL-12, and TNF-α. An enzyme-linked immunosorbent assay plate reader (Awareness, Statfax-2100 model, USA) at a wavelength of 450 nm was used to determine cytokine concentrations in the sera. Serum IL-10/IL-12, IL-10/IL-6, and IL-10/TNF-α proportions were also calculated and compared within the groups for changes that occurred from baseline until the end of the study. Between-group differences at the end of the study duration were assessed as well.

Disease activity score (DAS28) was calculated on the basis of the tender and swollen joint count, the serum hs-CRP level, and the VAS for GH with the use of the online calculator DAWN (Radboud University Nijmegen, Netherlands). The following formula was applied [20]:

DAS28 (CRP) = 0.56 SQRT (TJC28) + 0.28 SQRT (SJC28) + 0.36 ln (CRP + 1) + 0.014 GH + 0.96 (SQRT: square root; TJC: tender joint count; SJC: swollen joint count; ln: logarithm (natural); CRP: C-reactive protein; GH: global health)

The present study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki, and all procedures involving human persons were approved by the Ethics Committee of Tabriz University of Medical Sciences (no. 9149). Written informed consent was obtained from all patients, and the study was registered with the Iranian Registry of Clinical Trials (http://www.irct.ir) and given the identification no. IRCT201206234105 N9.

Intervention

Hard yellow gelatin capsules were used as delivery vehicle in the present study. *L. casei* 01 (Chr. Hansen, Denmark) was the active agent of the probiotic capsules, and maltodextrin was used as the excipient. The placebo capsules contained only maltodextrin. The capsules were cultured with the use of MRS

agar via serial dilution and the pour plate technique at baseline as well as in the middle and at the end of the intervention period. Bacterial enumeration of the capsules showed that the capsules contained a minimum of 10^8 colony-forming units of *L. casei* 01 at the three time sections. Capsules were identical in all aspects, and the containers were exactly the same. Capsule count was performed by the researcher at the end of the study to evaluate compliance.

Statistical analyses

The statistical analyses were performed per protocol with the use of SPSS version 20.0 software (SPSS Inc, Chicago, IL, USA). The results were expressed as mean (SD) for normally distributed quantitative data, median (25th and 75th percentiles) for quantitative data not normally distributed, and frequency (percent) for qualitative data. The normality of distribution of data was checked via the Kolmogorov-Smirnov test. To compare the two groups for background characteristics and baseline measures, the chi-squared test, the independent samples *t* test, and the Mann-Whitney U test were used for qualitative (nominal), normally distributed quantitative data and quantitative data not normally distributed, respectively. The Fisher exact test was used instead of the chi-squared test to compare the two groups for qualitative variables that did not follow Cochrane criteria. The Mann-Whitney U test was used to compare the results for ordinal qualitative variables, physical activity, and state and trait anxiety categories between the two intervention groups at baseline and at the end of the study. Analysis of covariance (ANCOVA) was used to compare the two groups for the measures at the end of the study after adjusting for the baseline measures and covariates (i.e., changes in BMI and state and trait anxiety scores throughout the study as well as menopausal status). Comparisons between the baseline and final results for the measures within each group were made with the use of paired samples *t* tests and Wilcoxon signed-rank tests for normally and not normally distributed data. The sign test was used to compare the results for ordinal qualitative variables throughout the study within groups. Results with two-sided *P* values of <0.05 were considered statistically significant.

Results

Sixty female patients with RA were recruited in the present clinical trial, and of these 60, 46 women completed the study: 10 patients (6 in the probiotic group and 4 in the placebo group) withdrew from the study for reasons irrelevant to the treatment (i.e., not willing to continue the treatments, being on vacation, having changed their location and thus not accessible), and 4 patients (2 in the probiotic group and 2 in the placebo group) were dropped out of analyses because they had not followed the study protocol (i.e., 2 had changed their medications, 1 had changed her physical activity level, and 1 had caught a severe cold). Capsule counts showed good compliance on the part of the participants who completed the study, and no adverse effects were reported. Baseline characteristics of the patients are presented in Table 1; there were no significant differences between the two groups with regard to any of the baseline characteristics. Within-group changes for weight and BMI were insignificant in both the probiotic group ($P = 0.851$ and $P = 0.891$, respectively) and the placebo group ($P = 0.647$ and $P = 0.477$, respectively) by the end of the study. Table 2 presents the results for state and trait anxiety and physical activity. No statistically significant differences were observed for physical activity and STAI-Y scores between the two intervention groups either at baseline or at the end of the study period; within-group changes were statistically insignificant as well. The analysis of dietary questionnaires, which is presented in Table 3, revealed that the two groups had no significant differences for energy and macronutrient intakes either at baseline or at the end of the study course. The micronutrient intakes of the patients in both intervention groups remained constant through the study period as well. Thus, dietary factors could not confound the results for disease activity scores and inflammatory cytokine levels in the sera. Tender and swollen joint counts decreased significantly in the probiotic group by the end of study course ($P = 0.02$ and $P = 0.03$, respectively). No statistically significant changes were observed for the tender and swollen joint counts in the placebo group ($P = 1.00$ and $P = 0.317$, respectively). VAS score decreased by 43.96% in the probiotic group and 5.99% in the placebo group at the end of the study as compared with baseline, and there was a significant difference between the two groups at the end of the study ($P < 0.01$). A significant decrease in the serum hs-CRP level was seen only in the probiotic group ($P < 0.01$). Figure 1 presents the results of the DAS28 scores of the two groups throughout the study. The scores were not significantly different between the two groups at baseline ($P = 0.39$). At the end of the study, DAS28 significantly decreased in the probiotic group ($P < 0.01$), but it did not change significantly in the placebo group ($P = 0.33$). The DAS28 score was significantly lower in the probiotic group as compared with the placebo group at the end of the study ($P = 0.03$).

The serum levels of inflammatory biomarkers at baseline and at the end of the study are presented in Table 4. TNF-α and IL-12 decreased significantly only in the probiotic group by the end of

Table 1
Baseline characteristics of the study participants

	Placebo group ($n = 24$)	Probiotic group ($n = 22$)	*P* value*
Age (y)†	44.29 (9.77)	41.14 (12.65)	0.347
Weight (kg)†	68.56 (11.96)	69.29 (11.47)	0.833
BMI (kg/m^2)†	28.08 (4.03)	27.70 (4.16)	0.753
Duration of RA (y)‡	4.75 (3.0, 9.0)	5.25 (3.75, 10.0)	0.566
Menopausal status§			
Premenopausal	17 (70.8)	15 (68.2)	0.845
Postmenopausal	7 (29.2)	7 (31.8)	
Current medication§			
Methotrexate	20 (83.3)	15 (68.2)	0.229
Hydroxychloroquine	18 (75.0)	18 (81.8)	0.725
Prednisolone	23 (95.8)	21 (95.5)	1.000

BMI, body mass index; RA, rheumatoid arthritis

* Independent Student t test for age, height, and BMI; Mann-Whitney U test for duration of RA; chi-squared test for menopausal status and methotrexate; and the Fisher exact test for hydroxychloroquine and prednisolone. Values are expressed as:

† Mean (SD).

‡ Median (25th and 75th percentiles).

§ Frequency (percent).

Table 2
State and trait anxiety and physical activity results for the two experimental groups at baseline and throughout the study

	Placebo group ($n = 24$)		Probiotic group ($n = 22$)		*P* value*
	Baseline	End of study	Baseline	End of study	
State anxiety					
Not at all	13 (54.2)	13 (54.2)	10 (45.5)	8 (36.4)	0.495†
Low	9 (37.5)	10 (41.7)	9 (40.9)	12 (54.5)	0.218‡
Intermediate	1 (4.2)	0 (0.0)	1 (4.5)	2 (9.1)	
Very much	1 (4.2)	1 (4.2)	2 (9.1)	0 (0.0)	
P value§	1.000		1.000		
Trait anxiety					
Not at all	4 (16.7)	4 (16.7)	6 (27.3)	6 (27.3)	0.866†
Low	15 (62.5)	16 (66.7)	9 (40.9)	10 (45.5)	0.922‡
Intermediate	5 (20.8)	4 (16.7)	5 (22.7)	4 (18.2)	
Very much	0 (0.0)	0 (0.0)	2 (9.1)	2 (9.1)	
P value§	1.000		1.000		
Physical activity					
Low	16 (66.7)	16 (66.7)	17 (77.3)	16 (72.7)	0.392†
Moderate	7 (29.2)	7 (29.2)	5 (22.7)	6 (27.3)	0.602‡
High	1 (4.2)	1 (4.2)	0 (0.0)	0 (0.0)	
P value§	1.000		1.000		

Frequency (percent) is reported for the measures

* Mann-Whitney U test.

† Between-group differences at baseline.

‡ Between-group differences at the end of the study.

§ Sign test.

Table 3
Energy and macronutrient intake in the two experimental groups at baseline and throughout the study

	Placebo group ($n = 24$)	Probiotic group ($n = 22$)	P value
Energy (cal)			
Baseline	1699.68 (416.49)	1689.82 (358.32)	0.932*
End of study	1696.41 (423.30)	1694.82 (329.17)	0.909†
P value‡	0.890	0.769	
Protein (g)			
Baseline	51.35 (16.73)	51.86 (15.80)	0.917*
End of study	53.00 (14.23)	51.21 (14.17)	0.389†
P value‡	0.619	0.811	
Fat (g)			
Baseline	51.06 (17.25)	55.60 (11.12)	0.299*
End of study	55.85 (18.13)	59.80 (14.02)	0.690†
P value‡	0.174	0.185	
PUFAs (g)			
Baseline	10.37 (4.07)	12.36 (3.15)	0.072*
End of study	12.37 (5.59)	12.86 (4.87)	0.915†
P value‡	0.122	0.639	
MUFAs (g)			
Baseline	18.02 (7.05)	19.58 (5.07)	0.398*
End of study	20.34 (7.81)	20.41 (5.67)	0.916†
P value‡	0.195	0.552	
SFAs (g)			
Baseline	11.63 (6.14)	11.15 (5.60)	0.784*
End of study	12.52 (5.51)	13.98 (6.46)	0.530†
P value‡	0.535	0.135	
Fiber (g)			
Baseline	14.54 (7.82)	11.80 (5.90)	0.190*
End of study	11.52 (4.48)	11.85 (4.56)	0.405†
P value‡	0.580	0.953	

MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids
Mean (SD) are presented for the measures
* Independent Student t test.
† Based on analysis of covariance adjusted for baseline measures.
‡ Paired Student t test.

the trial ($P < 0.01$), and a statistically significant increase in serum IL-10 was observed in the probiotic group at the end of the study as compared with baseline ($P = 0.02$). According to ANCOVA, except for IL-1 β ($P = 0.18$), there was a statistically significant difference between the two intervention groups, for the measured inflammatory cytokines at the end of the study that was in favor of the probiotic group ($P < 0.05$).

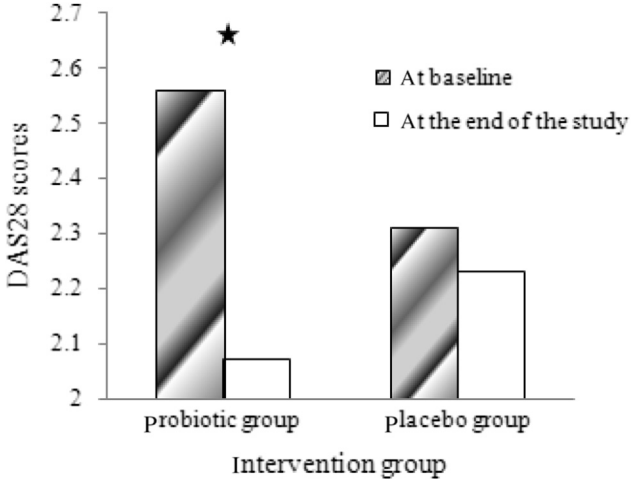

Fig. 1. Effect of 8 wk of *Lactobacillus casei* 01 supplementation as compared with placebo on the disease activity scores of female patients with rheumatoid arthritis. ★, Statistically significant change observed via a paired Student t test.

Table 4
Effect of 8 wk of probiotic supplementation as compared with placebo on inflammatory biomarkers in female patients with rheumatoid arthritis

	Placebo group ($n = 24$)	Probiotic group ($n = 22$)
TNF-α (pg/ml)		
Baseline	3.60 (2.32, 5.10)	5.00 (2.75, 9.60)
End of study	3.65 (2.05, 5.40)	4.05 (1.60, 6.37)*,†
IL-1 β (pg/ml)		
Baseline	4.10 (2.65, 10.57)	12.70 (3.02, 118.07)
End of study	4.50 (3.25, 9.87)	12.80 (4.25, 108.07)
IL-6 (pg/ml)		
Baseline	8.80 (1.52, 122.67)	22.30 (1.65, 43.05)
End of study	11.55 (0.00, 141.02)	20.55 (0.90, 41.22)†
IL-10 (pg/ml)		
Baseline	1.70 (0.25, 3.07)	1.20 (0.00, 3.32)
End of study	0.90 (0.00, 3.65)	0.90 (0.00, 7.82)*,†
IL-12 (pg/ml)		
Baseline	187.25 (106.40, 374.80)	422.85 (162.40, 574.80)‡
End of study	236.60 (121.00, 429.90)*	342.25 (143.70, 555.50)*,†

IL-1 β, interleukin-1 β; IL-6, interleukin-6; IL-10, interleukin-10; IL-12, interleukin-12; TNF-α, tumor necrosis factor-α
All values are expressed as median (25th and 75th percentiles)
* Significant difference within group throughout the study ($P < 0.05$, Wilcoxon test).
† Significant difference between groups after the intervention ($P < 0.05$, analysis of covariance adjusted for baseline measures, changes in body mass index and state and trait anxiety scores throughout the study, and menopausal status).
‡ Significant difference between groups at baseline ($P < 0.05$, Mann-Whitney U test).

The IL-10/IL-12 proportion significantly increased in the probiotic group ($P = 0.01$); the changes were not statistically significant in the placebo group ($P = 0.054$) (Fig. 2). At the end of the study, a significant difference was seen between the two groups for the IL-10/IL12 proportion ($P = 0.04$). The IL-10/IL-6 proportion was not significantly affected by the study treatment in the probiotic group ($P = 1.00$), and a significant decrease was observed in the placebo group ($P < 0.05$); ANCOVA revealed a nonsignificant difference between the two groups at the end of the study for the IL-10/IL-6 proportion ($P = 0.27$). A significant increase was found in the IL-10/TNF-α proportion in the probiotic

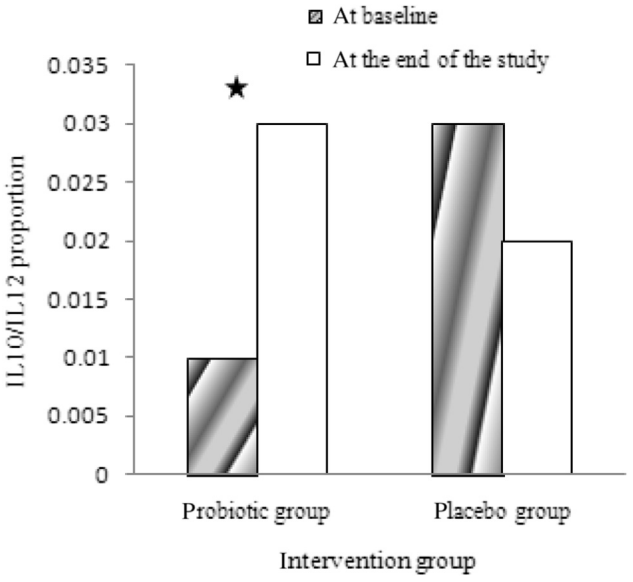

Fig. 2. Effect of 8 wk of *Lactobacillus casei* 01 supplementation as compared with placebo on proportion of interleukin-10 to interleukin-12 in female patients with rheumatoid arthritis. ★, Statistically significant change observed via a Wilcoxon signed-rank test.

group at the end of the study as compared with baseline ($P = 0.03$), but the proportion did not change significantly in the placebo group by the end of the study ($P = 0.20$); comparing the two groups at the end of the study by ANCOVA showed no significant difference between the groups ($P = 0.65$).

Discussion

The results of the present study showed that 8 wk of *L. casei* 01 supplementation reduced disease activity and proinflammatory cytokines (TNF-α, IL-6, and IL-12) while at the same time increasing the regulatory cytokine (IL-10) in patients with RA. IL-1 β was not significantly affected by the intervention. The IL-10/IL-12 proportion significantly increased in the probiotic group. However, the IL-10/IL-6 and IL-10/IL-TNF-α proportions were not significantly different between the two groups at the end of the study.

During the late 1970s, it was shown for the first time that germ-free rats were greatly susceptible to the development of RA, whereas those raised under conventional conditions had milder disease and a lower incidence in an adjuvant-induced arthritis model. The use of gnotobiotic animals confirmed the role of gut microbial communities in RA development in animal models as well [21]. It has also been revealed that patients with early RA have altered gut microbiota [5]. Patients with RA who consumed a vegan diet rich in lactobacilli for 1 mo had improved disease activity, which was attributed to the changes observed in their fecal microbiota; however, determinations of the type of changes and of whether there was a causal relationship were not possible [7]. Probiotics are capable of normalizing gut microbiota toward healthy bacteria. In addition, probiotic microorganisms have been shown to have anti-inflammatory properties, not only locally in the gut but also systematically [10]. All of this evidence together has encouraged researchers to consider whether probiotics could help to alleviate the symptoms of RA; a number of animal and human studies, although limited, have been conducted to address this question.

The ability to resist the intestinal environment and colonize the gut is essential for a probiotic to be able to confer its health effects. These characteristics have been shown to be strain specific [22–28]. It has been demonstrated that *L. casei* 01 can properly withstand a wide range of gastrointestinal pH levels [29]. *L. casei* 01 has also been found to have a good adhesion capacity with rat small intestine (IEC-6) epithelial cells, which is comparable to that of *L. acidophilus* La-5 [30]. *L. casei* 01 has also been reported to have good adhesion to Caco-2 cells; this adhesion is far less than that of *L. casei* Fyos but greater than that of *L. casei* Shirota, which is usually administered in clinical trials [23]. Although *L. casei* 01 has not been investigated for its antirheumatic properties, a couple of studies have found promising effects of *L. casei* subspecies for alleviating RA symptoms as well as for enhancing the inflammatory profile of animals with induced RA [11–14]. With all of these facts in mind, an *L. casei* subspecies was applied in this trial.

In the present study, *L. casei* 01 supplementation significantly decreased VAS. This was in accord with the study conducted by Mandel et al. [18] in which *Bacillus coagulans* was administered to patients for 60 d and a significant improvement in pain score was reported. Pineda et al., who evaluated the effects of 3 mo of *Lactobacillus rhamnosus* and *Lactobacillus reuteri* supplementation in patients with RA, obtained enhanced Health Assessment Questionnaire scores from their subjects score as well [19]. However, no significant improvements in subjective well-being was reported in the study by Hatakka et al., in which RA patients received LGG for a year [17]. Tender and swollen joint counts of the patients in the probiotic group decreased significantly in the present study, which did not follow results obtained in human studies that were performed previously. Moreover, in contrast with previous work done in the field, a significant decrease in hs-CRP was found in the probiotic group by the end of the 8 wk trial course. Proinflammatory cytokine decreases and the regulatory cytokine increase were statistically significant in the present study. This was not in accord with studies for the probiotic group, by Hatakka et al. [17] and Pineda et al.[19], both of which reported either no significant changes or changes in favor of the placebo group.

The serum IL-10/IL-12 proportion was evaluated, because it has recently been used to successfully evaluate the immunomodulatory effects of probiotics, and it has been reported to be a good indicator of the clinical efficacy of the strains under investigation [31,32]. This was not assessed as part of the previous studies investigating the effects of probiotic supplementation in patients with RA. In the present study, the proportion was significantly increased in the probiotic group, thereby further emphasizing the anti-inflammatory properties of *L. casei* 01. The IL-10/IL-6 and IL-10/TNF-α proportions were also assessed, because they can present as the Th2/Th1 proportion, which has been found to decrease in the presence of autoimmune disease [33]. These proportions were also not assessed in the previous relevant studies. Although no differences were observed between the two groups at the end of the study for either the IL-10/TNF-α or IL-10/IL-6 proportions, the IL-10/TNF-α proportion was significantly increased in the probiotic group at the end of the study as compared with baseline.

The immunomodulatory properties of probiotics are strain specific, which has to be taken into account when it comes to opting for a strain that may exert the desired effects in a patient with a particular health condition. Thus, the disagreement between the results of the studies conducted during the last few years and those of the present clinical trial may in large part be the result of the probiotic strain administered in this study. Dose differences may be another factor that could cause varying results. Study duration was not likely to significantly impact the disagreement of the outcomes of the present study with those of the previous ones, however, because the study course of the present work was similar to two of the studies performed in the field that found no significant effects of probiotic supplementation.

The probable underlying mechanism through which probiotics affect systemic immunity is through the interaction of microorganism-associated molecular patterns and pattern-recognition receptors (including Toll-like receptors) on the dendritic cells (DCs), either at the gut lumen through the DC passed through the intestinal epithelial cells or at the dome region of the gut-associated lymphoid tissue. This results in an array of immunologic pathways that may stimulate or downregulate immune system function, depending on the probiotic strain. Some suggested mechanisms for how probiotics induce regulatory T cells (Tregs) include enhancing the potency of Tregs; diminishing the apoptosis of Tregs; repressing bacterial adenosine triphosphate, which prevents conversion to Th17 cells; converting gut DCs to induce Tregs; and inducing the maturation of tolerogenic DCs. A possible mechanism for the induction of Tregs by probiotics is proposed as being through all-*trans* retinoic acid [34]. Although the basic structure of the outer walls of the different species is similar, there are various modifications (e.g., glycosylation) that are assumed to be responsible for strain-specific properties of probiotics [35].

There were some limitations in the present study that resulted from limited funding sources. The short duration of the study was one drawback. Moreover, results could be better

interpreted if the measurements were performed at least once more (i.e., during the fourth wk). The evaluation of more cytokines and the cells that regulate the secretion of these biomarkers (e.g., natural killer cells) could be of great benefit as well. In addition, the assessment of the recovery of *L. casei* 01 in the feces could have more precisely ensured the compliance of the patients with the study protocol and confirmed the appropriate colonization of the probiotic in the gut.

Conclusions

This study demonstrated that *L. casei* 01 could improve disease activity and inflammation in patients with RA and suggested that this probiotic may be a beneficial adjunct therapy in this population of patients if the results are confirmed by future studies. It also indicates that different strains of *L. casei* and other species with various dosages should be studied.

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