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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