Efficacy of synbiotic supplementation in improving rheumatoid arthritis

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Abstract

Background and purpose: Today, improving rheumatoid arthritis (RA) as a chronic inflammatory disease is attributed to the proper status of the gut microbiota. Although some supplements containing beneficial live microorganisms (probiotics) can reduce inflammation by altering the bacterial composition of the gut, there is limited information on the effect of synbiotic (probiotics mixed with prebiotics) supplements on RA. Therefore, this study aimed to evaluate the anti-inflammatory effects of a synbiotic supplement as an adjuvant therapy in rheumatic patients. Moreover, for the first time, it was attempted to investigate whether addition of a synbiotic (1000 mg/day) to the combination of methotrexate and prednisolone increases the effectiveness of these antirheumatic drugs.

Experimental approach: Eligible patients (186 subjects) were randomly divided into two groups. Both groups received their standard routine antirheumatic drugs, methotrexate and prednisolone. Moreover, the first group received a daily oral synbiotic supplement (1000 mg) for 3 months while the second group received a placebo. Various parameters indicating RA status were evaluated at baseline (time 0) and 3 months after the treatment.

Findings / Results: The results showed the changes in the level of RA indicators, including tender joint count with a range of 0 to 28 joints, swollen joint count with a range of 0 to 28 joints, visual analog scale, erythrocyte sedimentation rate, CRP, and disease activity score based on 28 joints, after 3 months

Conclusion and implications: Overall, no significant differences in the measured parameters were observed between synbiotic and placebo groups probably due to the short duration of the treatment period, and it is suggested to extend the treatment period to six months.

Keywords: Disease activity; Rheumatoid arthritis; Synbiotics.

INTRODUCTION

Rheumatoid arthritis (RA) with a worldwide prevalence of about 0.5 to 1% among adults is a chronic, systemic, and autoimmune disease which is characterized by progressive joint damages, significant pain, and functional disabilities (1-3). The aggressive use of disease-modifying antirheumatic drugs (DMARDs) in combination with biological agents can dramatically reduce RA-related disabilities (4-8). Methotrexate (MTX) is one of the initial DMARDs preferred by most rheumatologists because not only is the patient's response to this drug more persistent, but also its toxicity can be controlled by proper monitoring and serious side effects of taking this medicine are avoidable (9-10).
For over half a century, glucocorticoids have been used as one of the most well-known anti-inflammatory drugs for the treatment of RA. However, the long-term administration of glucocorticoids should be limited due to their extensive toxicity and devastating side effects (11). The recommended treatment for RA is the temporary use of glucocorticoids to rapidly relieve inflammation and concomitant use of DMARD, which will continue even after glucocorticoid discontinuation (12-15).

Probiotics are beneficial live microorganisms that provide the host's health when they are taken adequately (16). Probiotics consume special compounds called prebiotics which are often supplied by the host's food and converted to substances that are beneficial to both the host and the microbe. Prebiotics are mixtures typically composed of non-digestible carbohydrates (17). A product containing both probiotics and prebiotics is named synbiotic, which is available today in the form of commercial supplements (18,19).

Several studies have reported that probiotics play a determinant role in modulating the immune system during inflammations (20). Dysbiosis (disruptions in the microbiome), especially in the gut lumen, alters the host's secondary responses resulting in a variety of diseases including RA (21,22). For example, the destruction of the joints in mice as an indicator for RA, was observed as a result of the replacement of segmented filamentous bacteria instead of the beneficial intestinal microbiome. This substitution could induce antibody production due to the migration of T helper (Th)17 cells to peripheral immune compartments and conversion of the activated B cells to plasma cells with antibody-producing abilities, which could be a possible mechanism for RA development (23). Moreover, substantial alterations in the gut microbiota have been identified in patients in the early stages of RA, which is consistent with their pathogenic role (24-29). Probiotics reduce the level of Th1, Th17, and nuclear factor kappa B (NF-KB). They also increase the amount of interleukin (IL)10, an anti-inflammatory cytokine, and decrease inflammatory cytokines such as IL2, tumor necrosis factor alpha (TNFα), interferons, and IL17. Butyrate is one of the components of short-chain fatty acids (SCFA), which plays an important role in modulating the immune system by inducing regulators of T-cell production. Prebiotics are SCFA precursors that are fermented by gastrointestinal flora, especially probiotics. Lactobacillus and Bifidobacterium are among the SCFA producers which play an important role in modulating the immune system (30-32).

Although there are various reports on the effects of probiotics on RA (24-29), the possible therapeutic impacts of synbiotics on RA have not been studied well (33). Therefore, the goal of this clinical research was to elucidate whether the combination of probiotics and prebiotics as the oral synbiotic supplement causes clinical improvement in patients with RA.

MATERIALS AND METHODS

Synbiotic supplement and drugs

Synbiotic and placebo supplements were purchased from the Zist-Takhmir company (Tehran, I.R. Iran) in capsule forms which were completely identical in shape, size, number, and appearance of opaque bottles. The synbiotic supplement (Familact®) was a 500 mg capsule containing a prebiotic (fructooligosaccharides) and probiotics including Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus rhamnosus, Bifidobacterium breve, Bifidobacterium longum, and Streptococcus thermophiles (the concentration of each bacteria were $10^9$ CFU/ mL). Seven point five to twenty mg/week of MTX (Ebetrex®, EBEWE Pharma, Unterach am Attersee, Austria) and 0-20 mg/day of prednisolone (Pred; Nisopred®, Iran Hormone, Tehran, I.R. Iran) were used as routine DMARDS. Calcium carbonate (500 mg/day), and 1 mg/day of folic acid (Iran Daru Company, Tehran, I.R. Iran) were also added to the therapeutic regimen of all patients.

Study type and setting

This prospective, randomized, placebo-controlled, double-blind, clinical trial study
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was performed in the outpatient rheumatology clinic of the internal medicine department, Al-Zahra Hospital of Isfahan, Iran, from January 2018 to January 2019.

The sample size of the examined population was determined using the Altman nomogram. A power analysis indicated that a total sample size of about 180 patients (90 patients in each group) would provide 80% power to detect a difference of 0.6 in disease activity score based on 28-joint count (DAS28). To account for possible dropouts and loss to follow up of 5 to 10%, we anticipated enrolling 200 patients.

**Ethics consideration**

This study was carried out based on the ethical principles of the declaration of Helsinki, and all study procedures were approved by the Isfahan University of Medical Science Ethics Committee (Code No. IR.MUI. MED.REC.1397.281). The study was registered in Iranian Registry of Clinical Trials (IRCT) with code number: IRCT20121216011763N37. The written informed consent forms were signed by all participants.

**Patient selection**

Patients with RA who fulfilled the 2010 American College of Rheumatology (ACR) criteria (34) were eligible to participate in this study. Randomization procedures to stratify patients into synbiotic or placebo groups were performed by STATA 12 statistical software. This work followed a study protocol based on the consolidated standards of reporting trials (CONSORT) statement (35).

Inclusion criteria were disease duration more than 3 months, age between 17 to 85 years, and the existence of at least 4 swollen joints which should be in a stable status for at least 1 month on the consumption of MTX and Pred. Exclusion criteria were hypersensitivity to MTX, Pred, synbiotic or placebo, creatinine clearance less than 40 mL/min per 1.73 m2, aspartate and alanine aminotransferase levels greater than twice the upper limit of normal, active hepatitis or cirrhosis, inflammatory bowel disease, perforated bowel, plan for surgery in the next 3 months, sybiotic consumption in the past 2 weeks, current sybiotic user, thyroid disorders, Cushing's syndrome, malignant tumors, inadequately controlled diabetes mellitus or arterial hypertension, serious infections, serious cardiac or respiratory diseases, leukopenia or thrombocytopenia, inadequate contraception, pregnancy or a plan for it in the next 3 months, breastfeeding, osteoporosis, use of cytotoxic or immunosuppressive drugs for 3 months before inclusion, current substance or alcohol abuse, psychological illnesses or intellectual disorders that would preclude adherence to the study protocol.

**Blinding**

With the exception of the main researchers, other people in this study including patients, intervention staff, residents, and medical students involved in the assessment of the outcomes remained blinded from the intervention. According to blinding strategy, the supplement bottles (synbiotic and placebo) were numbered for each patient using a computer in the manufacturer company. Then bottles were put in 2 black boxes by a research-blinded rheumatology clinic secretary to hide the sequence from the main researchers enrolling and assessing participants. To ensure adequate concealment from the researchers, drugs were administered to the patients by a research-blinded rheumatology clinic secretary. The outcomes were evaluated and documented by internal medicine residents. In case of disagreement, consensus between these residents and the attending physician determined the final scores. All were blinded to patient characteristics and treatment strategies.

**Assessments and follow up**

Different parameters indicating RA status were evaluated at baseline (time 0) and 3 months after the treatments with the synbiotic supplement or placebo. The parameters included complete blood count (CBC), C-reactive protein (CRP), serum alanine aminotransferase (ALT), creatinine (Cr), swollen joint count with a range of 0 to 28 joints (SJC28), tender joint count with a range of 0 to 28 joints (TJC28), visual analog scale (VAS) on pain with a range of 0 to 100 mm (100 mm signifies the worst score) and erythrocyte sedimentation rate (ESR) with a
range of 1 to 140 mm/h. DAS28 which is an index for disease activity with a range of 0 to 9.3, (9.3 signifying the highest disease activity) were calculated based on VAS, ESR, TJC28, and SJC28. A DAS28 score of less than 2.6 suggests disease remission. DAS28 values of 2.6 to 3.2, 3.3 to 5.1, and 5.2 or more indicate low disease activity, moderate disease activity, and high disease activity, respectively. The moderate response is when there is a DAS28 score reduction of 0.6 to 1.2, whereas a reduction of more than 1.2 means major response (36,37). During the three-month treatment period, patients and their general status including CBC, serum ALT, and Cr were monitored for possible adverse events including oral ulcers, nausea, renal impairment, hepatotoxicity, bone marrow suppression, and pneumonitis.

**Statistical analysis**

The statistical software SPSS 21.0 was used for data analysis. A $P$-value of less than 0.05 was considered to be statistically significant. For more precise comparison, four subgroups based on the dosage of MTX and Pred were determined in both synbiotic and placebo groups. The subgroups were patients who received RA drug combinations, including 15-20 mg MTX / 0 -2.5 mg Pred, 15-20 mg MTX / 5 -10 mg Pred, 7.5-10 mg MTX / 0 -2.5 mg Pred, and 7.5-10 mg MTX / 5 -10 mg Pred. For variables with normal distribution and homogeneity of variance, one-way ANOVA and Duncan’s multiple range tests were used to compare the mean values among different groups.

**RESULTS**

The results of the parameters indicating RA status in patients under routine RA pharmacotherapy, including different combinations of MTX and Pred, showed the changes in the level of TJC28, SJC28, VAS, ESR, CRP, and DAS28 after 3 months in both patient groups treated with the synbiotic supplement and placebo. However, no significant differences were observed in the amounts of ALT, glomerular filtration rate, and Cr in these patient groups (data not shown).

The results of the changes in the number of tender joints showed that after 3 months, there was a significant decrease ($P < 0.05$) in the amount of TJC28 in all patients treated with synbiotic, while the remarkable reduction ($P < 0.05$) in the placebo group was observed in patients who had received 7.5-10 mg MTX and 0-2.5 or 5-10 mg Pred (Fig. 1A). With the exception of patients treated with 15-20 mg MTX and 0-2.5 mg Pred, the level of SJC28 dropped dramatically ($P < 0.05$) after 3 months in other synbiotic groups. The significant decline of SJC28 ($P < 0.05$) in the placebo group belonged to the patients who had been given 7.5-10 mg MTX and 0-2.5 or 5-10 mg Pred (Fig. 1B).

![Fig. 1](image-url)

**Fig. 1.** The results of the changes in (A) TJC28 and (B) SJC28 in patients treated with the synbiotic supplement and placebo who also received different combinations of rheumatoid arthritis drugs including MTX and Pred. Values have been obtained at two different times, the time 0 (baseline) and 3 months after treatments, and represent mean ± SEM; $n = 88$ and 98 for the synbiotic the placebo groups, respectively. The similar lowercase letters indicate insignificant differences between the groups. The groups that differed significantly from each other were marked with dissimilar letters, $P < 0.05$. TJC28, Tender joint count with a range of 0 to 28 joints; SJC28, swollen joint count with a range of 0 to 28 joints; MTX, methotrexate; Pred, prednisolone.
Fig. 2. The results of the changes in VAS in patients treated with the synbiotic supplement and placebo who also received different rheumatoid arthritis drug combinations including MTX and Pred. Values have been obtained at two different times, the time 0 (baseline) and 3 months after treatments, and represent mean ± SEM; n = 88 and 98 for the synbiotic group and n=98 for the placebo groups, respectively The similar lowercase letters indicate insignificant differences between the groups. The groups that differed significantly from each other were marked with dissimilar letters, \( P < 0.05 \). VAS, Visual analog scale; MTX, methotrexate; Pred, prednisolone.

As Fig. 2 illustrates, the amount of VAS was reduced considerably in synbiotic groups \( (P < 0.05) \). This result was also observed for placebo groups except for the drug group of 15-20 mg MTX and 0-2.5 mg Pred.

However, after 3 months, there was no significant change in ESR in both patients treated with the synbiotic supplement and placebo (Fig. 3A). The only remarkable decrease in CRP level \( (P < 0.05) \) belonged to the patients treated with synbiotic and 15-20 mg MTX and 5-10 mg Pred (Fig. 3B).

The results of the DAS (Fig. 4) showed a significant decrease in DAS28 in the synbiotic and placebo groups after 3 months \( (P < 0.05) \). In addition, there were no significant differences between patients treated with the synbiotic supplement and placebo, except groups given 7.5-10 mg MTX and 5-10 mg Pred \( (P < 0.05) \). Since a DAS28 value more than 5.1 corresponds to a high disease activity and a DAS28 value between 3.2 and 5.1 represents a moderate disease activity, only in the synbiotic groups receiving 15-20 mg MTX, the status of the disease activity was changed from high (DAS28 ~ 5.8 on average) to moderate (DAS28 ~ 4.8 on average). However, the moderate disease activity was observed in all placebo groups after 3 months, whose DAS28 was about 4.5 on average.

Fig. 3. The results of the changes in (A) ESR and (B) CRP in patients treated with the synbiotic supplement and placebo who also received different rheumatoid arthritis drug combinations including MTX and Pred. Values have been obtained at two different times, the time 0 (baseline) and 3 months after treatments, and represent mean ± SEM; n = 88 and 98 for the synbiotic the placebo groups, respectively The similar lowercase letters indicate insignificant differences between the groups. The groups that differed significantly from each other were marked with dissimilar letters, \( P < 0.05 \). ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; MTX, methotrexate; Pred, prednisolone.
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Fig. 4. The results of the changes in DAS28 in patients treated with the synbiotic supplement and placebo who also received different rheumatoid arthritis drug combinations including MTX and Pred. Values have been obtained at two different times, the time 0 (baseline) and 3 months after treatments, and represent mean ± SEM; n = 88 and 98 for the synbiotic the placebo groups, respectively. The similar lowercase letters indicate insignificant differences between the groups. The groups that differed significantly from each other were marked with dissimilar letters, *P* < 0.05. DAS28, disease activity score based on 28 joints count; MTX, methotrexate; Pred, prednisolone.

Fig. 5. The percentage of improved patients (men and women) under the co-treatment of rheumatoid arthritis medicines and synbiotic/placebo supplements. MTX, methotrexate; Pred, prednisolone.

The percentage of patients responding to co-treatment of routine RA medicines and synbiotics or placebo indicated the status of response to synbiotics in comparison with placebo. The results (Fig. 5) showed that an almost identical percentage of patients improved in response to both treatments of synbiotics (65.9%) and placebo (65.3%). The percentage of patients who responded moderately to the treatments was greater in the
synbiotic group than the placebo group (23.86% vs 16.3%). In contrast, a lower percentage (42.04%) of the synbiotic group showed a major response in comparison with the placebo group (48.97%). Moreover, the percentage of improved men in the synbiotic group (13.63%) was more than that in the placebo group (8.16%), whereas a greater percentage of improved women were in the placebo group (57.14% for placebo vs 52.27 for synbiotic). Although the remission was observed only in the synbiotic group, its percentage was too low (2.27%).

**DISCUSSION**

A significant decrease in TJC28 and SJC28 values in the patients treated with both the synbiotic supplement and routine RA drugs (MTX and Pred) implied the positive effects of the treatments on these clinical indicators of joint inflammation and damage. However, these changes were independent of the dosage of RA medicines. In addition, the comparison of these indicators between synbiotic and placebo groups demonstrated that the effect of co-treatment of the synbiotic supplement and RA medicines is similar to or even less than RA drugs used alone. In other studies investigating the effects of probiotics on RA, no significant differences in the amounts of TJC28 and SJC28 were observed between placebo and probiotic groups (25).

The remarkable reduction in the VAS, a scoring system for the degree of the pain, confirmed the affirmative results of antirheumatic drugs in relieving rheumatic pain, not the effectiveness of synbiotic supplement. VAS results also indicated that treatment with the synbiotic supplement in some cases (such as patients receiving 5-10 mg Pred) probably can decrease the effectiveness of the antirheumatic drugs in reducing pain, which could be related to the interactions between RA drugs and intestinal bacteria in drug absorption. Interactions between probiotics and some drugs have been previously studied. For example, warfarin can interact with probiotics (38). Moreover, patients who receive immune-suppressants, such as cyclosporine, tacrolimus, azathioprine, and chemo-therapeutic agents, should not consume probiotics due to inducing an unexpected infection in the patients by probiotics (39).

The lack of significant changes in ESR values in the synbiotic and placebo groups may be owing to a very slow rate of response to the drugs in reducing this parameter. Former studies confirmed that ESR does not change quickly at the beginning of the inflammatory process because some plasma proteins have long half-life and response to treatments may take a longer time (40). In the case of CRP, only the combination of the synbiotic supplement with 15-20 mg MTX and 5-10 mg Pred was able to bring this parameter to the normal level, which was consistent with a decrease in inflammation. The reason why this special drug combination could decrease CRP is that the synbiotic supplement has likely changed the absorption of antirheumatic drugs, therefore these doses of RA drugs were more effective than other dosages in reducing CRP. In another study, a decrease in CRP of patients receiving synbiotic supplements has been reported, though the dose of RA drugs has not been mentioned in that study (33).

Since DAS28 depends on the values of TJC28, SJC28, VAS, and ESR the effect of treatments of RA drugs and the synbiotic supplement on these parameters determined the levels of RA activity and response to the treatments. In this study, the efficacy of the combination of RA drugs and synbiotic was almost similar to the effects of RA drugs when they were used alone, which means the synbiotic supplement has not affected the reduction of disease activity and the conversion of disease activity from the high level to the moderate one. Thus, this disease status conversion was attributed to the performance of RA drugs in a dose-independent manner. In contrast, Zamani et al. reported the improvement of DAS28 by synbiotic supplementation. They used a synbiotic supplement containing Lactobacillus acidophilus, Lactobacillus casei, and Bifidobacterium bifidum (2 × 10⁹ colony-forming units/g each) plus 800 mg inulin (33). The discrepancy between our results and the results of Zamani and colleagues can be related to the differences in the composition of supplements.
used in these two experiments. The dissimilar effects of various probiotics and prebiotics on health have already been proven (33,41).

A higher percentage of improved men in the synbiotic group in comparison with the placebo group and the opposite of this case for women demonstrated that the synbiotic was more effective for men than women. The hormonal difference between women and men may be responsible for mechanisms underlying the gender-dependent responses to synbiotics (42,43). Differences in physiologic mechanisms and gut microbiota state in women compared to men can be another reason for dissimilar effects of synbiotics in men and women. Moreover, the reduction of inflammation in patients with RA might be determined by the normal gut microbial ecosystem, which depends on gender (44-46).

CONCLUSION

Overall, no significant difference was observed between synbiotic and placebo groups in the terms of the measured parameters and disease improvement. Such a result was probably due to the short duration of the treatment period with the synbiotics. Therefore, it is suggested to extend the treatment period to at least 6 months. In addition, although the relatively identical percentage of the improved patients in both the synbiotic and placebo groups was another confirmation of the ineffectiveness of the synbiotic supplement applied in our research, the existence of a very low percentage of patients who showed remission is a promising window for the future research on the use of the optimized synbiotic supplements.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHORS’ CONTRIBUTION

M. Salesi and H. karimzadeh designed the project. F. Esmaeili, M. Salesi, and A. Esmaeilisharif performed experiments, clinical studies, and collected data. F. Esmaeili collected sample of patients. B. Shojaje, M. Salesi, and F. Esmaeili performed literature search, analysed data, interpreted the results, and wrote the manuscript. B. Shojaje and M. Salesi revised the manuscript. G. Askari helped to choose and prepare the synbiotic supplement. M. Maracy helped to set up experiments and randomly categorize patients by STATA 12 statistical software into synbiotic and placebo groups. All authors read and approved the final manuscript.

REFERENCES

1. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res. 2002;4(S3):S265-S272. DOI: 10.1186/ar578.
2. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365(23):2205-2219. DOI: 10.1056/NEJMra1004965.
3. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American college of rheumatology/european league against rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-2581. DOI: 10.1002/art.27584.
4. Emery P. Treatment of rheumatoid arthritis. BMJ. 2006;332(7534):152-155. DOI: 10.1136/bmj.332.7534.152.
5. Burke RA, White ND. Biologic disease-modifying antirheumatic drugs. PSAP. Chronic illnesses II; 2014, pp. 9-31.
6. Choy EH, Smith C, Dore CJ, Scott DL. A meta-analysis of the efficacy and toxicity of combining disease-modifying anti-rheumatic drugs in rheumatoid arthritis based on patient withdrawal. Rheumatology (Oxford). 2005;44(11):1414-1421. DOI: 10.1093/rheumatology/kei031.
7. Benjamin O, Lappin SL, Bansal P, Goyal A. Disease modifying anti-rheumatic drugs (DMARD). Treasure Island (FL): StatPearls Publishing; 2020.
8. Emami J, Ansarypour Z. Receptor targeting drug delivery strategies and prospects in the treatment of rheumatoid arthritis. Res Pharm Sci. 2019;14(6):471-487.
10. Weinblatt ME. Methotrexate in rheumatoid arthritis: a quarter century of development. Trans Am Clin Climatol Assoc. 2013;124:16-25.

11. Moreland LW, O’Dell JR. Glucocorticoids and rheumatoid arthritis. Pharmacol Rep. 2006;58(4):473-492.

12. Smolen JS, Steiner G. Therapeutic strategies for rheumatoid arthritis. Nat Rev Drug Discov. 2003;2(6):473-488.

13. O’Dell JR. Therapeutic strategies for rheumatoid arthritis. N Engl J Med. 2004;350(25):2591-2602. DOI: 10.1056/NEJMrad040226.

14. Bakker MF, Jacobs JW, Welsing PM, Verstappen SM, Tekstra J, Ton E, et al. Low-dose prednisone inclusion in a methotrexate-based, tight control strategy for early rheumatoid arthritis: a randomized trial. Ann Intern Med. 2012;156(5):329-339. DOI: 10.7326/0003-4819-156-5-201203060-00004.

15. Gaffo A, Saag KG, Curtis JR. Treatment of rheumatoid arthritis. Nat Rev Rheum. 2002;46(10):2553-2563. DOI: 10.1038/nrd1109.

16. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506-514.

17. Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, et al. Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods. 2019;8(3):E92,1-27. DOI: 10.3390/foods8030092.

18. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and symbiotics-approaching a definition. Am J Clin Nutr. 2001;73(2):361S-364S. DOI: 10.1093/ajcn/73.2.361s.

19. Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and symbiotics-a review. J Food Sci Technol. 2015;52(12):7577-7587. DOI: 10.1007/s13197-015-1921-1.

20. Isolauri E, Sütas Y, Kankaanpää P, Arvilommi H, Salminen S. Probiotics: effects on immunity. Am J Clin Nutr. 2001;73(2):444S-450S. DOI: 10.1093/ajcn/73.2.444s.

21. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis. 2015;26:26191-26199. DOI: 10.3402/mehd.v26.26191.

22. Horta-Baas G, Romero-Figueroa MDS, Montiel-Jarquín AJ, Pizano-Zárate ML, García-Mena J, Ramírez-Durán N. Intestinal dysbiosis and rheumatoid arthritis: a link between gut microbiota and the pathogenesis of rheumatoid arthritis. J Immunol Res. 2017;2017:4835189,1-13. DOI: 10.1155/2017/4835189.

23. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity. 2010;32(6):815-827. DOI: 10.1016/j.immuni.2010.06.001.

24. Hatakka K, Martio J, Korpela M, Herranen M, Poussa T, Laasamen T, et al. Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis-a pilot study. Scand J Rheumatol. 2003;32(4):211-215. DOI: 10.1080/0300974031003695.

25. Mohammed AT, Khattab M, Ahmed AM, Turk T, Sakr N, M Khalil A, et al. The therapeutic effect of probiotics on rheumatoid arthritis: a systematic review and meta-analysis of randomized control trials. Clin Rheumatol. 2017;36(12):2697-2707. DOI: 10.1007/s10067-017-3814-3.

26. Alipour B, Homayouni-Rad A, Vaghef-Mehrabany E, Sharif SK, Vaghef-Mehrabany L, Asghari-Jafarabadi M, et al. Effects of Lactobacillus casei supplementation on disease activity and inflammatory cytokines in rheumatoid arthritis patients: a randomized double-blind clinical trial. Int J Rheum Dis. 2014;17(5):519-527. DOI: 10.1111/1756-185X.12333.

27. Mandel DR, Eichas K, Holmes J. Bacillus coagulans: a viable adjunct therapy for relieving symptoms of rheumatoid arthritis according to a randomized, controlled trial. BMC Complement Altern Med. 2010;10(1):1-7. DOI: 10.1186/1472-6882-10-1.

28. Wang P, Tao JH, Pan HF. Probiotic bacteria: a viable adjuvant therapy for relieving symptoms of rheumatoid arthritis. Inflammopharmacology. 2016;24(5):189-196. DOI: 10.1007/s10787-016-0277-0.

29. Vaghef-Mehrabany E, Vaghef-Mehrabany L, Asghari-Jafarabadi M, Homayouni-Rad A, Issazadeh K, Alipour B. Effects of probiotic supplementation on lipid profile of women with rheumatoid arthritis: a randomized placebo-controlled clinical trial. Health Promot Perspect. 2017;7(2):95-101. DOI: 10.15171/hpp.2017.17.

30. Viljanen M, Pohjavedo E, Haantela T, Korpela R, Kuitunen M, Sarnesto A, et al. Induction of inflammation as a possible mechanism of probiotic effect in atopic eczema-dermatitis syndrome. J Allergy Clin Immunol. 2005;115(6):1254-1259. DOI: 10.1016/j.jaci.2005.03.047.

31. So JS, Kwon HK, Lee CG, Yi HJ, Park JA, Lim SY, et al. Lactobacillus casei suppresses experimental arthritis by down-regulating T helper 1 effector functions. Mol Immunol. 2008;45(9):2690-2699. DOI: 10.1016/j.molimm.2007.12.010.

32. Zamani B, Golkar HR, Farshbaf S, Emadi-Baygi M, Tajabadi-Ebrahimi M, Jafari P, et al. Clinical and metabolic response to probiotic supplementation in patients with rheumatoid arthritis: a randomized,
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double-blind, placebo-controlled trial. Int J Rheum Dis. 2016;19(9):869-879. DOI: 10.1111/1756-185X.12888.
33. Zamani B, Farshbaf S, Golkar HR, Bahmani F, Asemi Z. Synbiotic supplementation and the effects on clinical and metabolic responses in patients with rheumatoid arthritis: a randomised, double-blind, placebo-controlled trial. Br J Nutr. 2017;117(8):1095-1102. DOI: 10.1017/S000711451700085X.
34. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American college of rheumatology/european league against rheumatism collaborative initiative. Ann Rheum Dis. 2010;69(10):1892-1900. DOI: 10.1136/ard.2010.138461.
35. Moher D, Hopewell S, Schulz KF, Montori V, Gotzsche PC, Devereaux P, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c869. DOI: 10.1136/bmj.c869.
36. Aletaha D, Ward MM, Machold KP, Nell VP, Stamm T, Smolen JS. Remission and active disease in rheumatoid arthritis: defining criteria for disease activity states. Arthritis Rheum. 2005;52(9):2625-2636. DOI: 10.1002/art.21235.
37. Aletaha D, Martinez-Avila J, Kvien TK, Smolen JS. Definition of treatment response in rheumatoid arthritis based on the simplified and the clinical disease activity index. Ann Rheum Dis. 2012;71(7):1190-1196. DOI: 10.1136/annrheumdis-2012-201491.
38. Lindh J. Possible interaction between probiotics and warfarin. Lakartidningen. 2010;107(13-14):917.
39. Vyas U, Ranganathan N. Probiotics, prebiotics, and synbiotics: gut and beyond. Gastroenterol Res Pract. 2012;2012:872716,1-16. DOI: 10.1155/2012/872716.
40. Bray C, Bell LN, Liang H, Haykal R, Kaiksow F, Mazza II, et al. Erythrocyte sedimentation rate and C-reactive protein measurements and their relevance in clinical medicine. WMJ. 2016;115(6):317-321.
41. Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 2017;9(9):E1021,1-30. DOI: 10.3390/nu9091021.
42. Lang TJ. Estrogen as an immunomodulator. Clin Immunol. 2004;113(3):224-230. DOI: 10.1016/j.clim.2004.05.011.
43. Inoue K, Inoue E, Imai Y. Female sex hormones ameliorate arthritis in SKG mice. Biochem Biophys Res Commun. 2013;434(4):740-745. DOI: 10.1016/j.bbrc.2013.03.111.
44. Haro C, Rangel-Zúñiga OA, Alcalá-Díaz JF, Gómez-Delgado F, Pérez-Martínez P, Delgado-Lista J, et al. Intestinal microbiota is influenced by gender and body mass index. PLoS One. 2016;11(5):e0154090,1-16. DOI: 10.1371/journal.pone.0154090.
45. Fransen F, van Beek AA, Borghuis T, Meijer B, Hugenholtz F, van der Gaast-de Jongh C, et al. The impact of gut microbiota on gender-specific differences in immunity. Front Immunol. 2017;8:754-767. DOI: 10.3389/fimmu.2017.00754.
46. Yurkovetskiy L, Burrows M, Khan AA, Graham L, Volchkov P, Becker L, et al. Gender bias in autoimmunity is influenced by microbiota. Immunity. 2013;39(2):400-412. DOI: 10.1016/j.immuni.2013.08.013.