Probiotic Supplementation and Systemic Inflammation in Relapsing-Remitting Multiple Sclerosis: A Randomized, Double-Blind, Placebo-Controlled Trial

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Research

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Abstract

Background: Multiple sclerosis (MS) is a complex inflammatory disease in which demyelination occurs in the central nervous system affecting approximately 2.5 million people worldwide**.** Intestinal microbiome changes play an important role in the etiology of chronic diseases. This study aimed to investigate the effect of probiotic supplementation on systemic inflammation in patients with MS.

Methods: A twenty-four-week double-blind clinical trial study was designed and seventy patients with MS were randomly divided into two groups receiving probiotics and placebo. Patients in the intervention group received two capsules containing multi-strain probiotics daily and patients in the control group received the same amount of placebo. Factors associated with systemic inflammation were assessed at the beginning and end of the study.

Results: Sixty-five patients were included in the final analysis. There was no significant difference between the two groups in terms of baseline variables except for the duration of the disease (P>0.05). At the end of the study, probiotic supplementation compared to the placebo caused a significant reduction in the serum levels of CRP (-0.93± 1.62 vs. 0.05 ± 1.74, P=0.03), TNF-a (-2.09 ± 1.88 vs. 0.48 ± 2.53, P=0.015) and IFN-γ (-13.18± 7.33 vs. -1.93± 5.99, P<0.001). Also, we found a significant increase in the FOXP3 and TGF-β levels in the intervention group (P<0.05).

Conclusion: The results of our study showed that supplementation with probiotics can have beneficial effects on serum levels of some factors associated with systemic inflammation.

Trial registration: Approved by the Ethics Committee of the Ahwaz Jundishapur University of Medical Sciences, Ahvaz, Iran. This study was registered within Iranian Registry of Clinical Trials (IRCT) (http://www.irct.ir) under the number IRCT20181210041918N2.

Introduction

Multiple sclerosis (MS) is described as a long-lasting central nervous system inflammatory neurodegenerative disease that affects young and middle-aged adults from 20 to 55 years of age[1]. The prevalence of this disease is higher among women, so that more than 60% of cases are women[2]. The predominant feature of this disease is the progressive demyelinating of central nervous neurons (CNS) following T cell mediated autoimmune processes[3].

The etiology of this disease has poorly understood, however, in recent years a set of genetic and environmental factors have been suggested which involved in the MS etiology[4]. Various researchers have reported that MS is the result of an imbalance between inflammatory and anti-inflammatory conditions. The permeability of the Blood-Brain Barrier (BBB) can be increased by pro-inflammatory cytokines, enabling neurodegeneration and demyelination of the CNS, while anti-inflammatory cytokines can suppress the release of pro-inflammatory cytokines[5]. For these reasons, inflammation is one of the main components involved in the pathogenesis of MS and degeneration of brain axons and neurons[6].

Among the various inflammatory factors, some proinflammatory cytokines, such as nterleukin-2 (IL-2), tumor necrosis factor-a(TNF-a) or interferon-g(IFN-g) which are secreted by T helper 1 (Th1) cells, and some anti-inflammatory cytokines such as TGF-β1 and interleukin 10 (IL-10) play an important role in the MS pathogenesis[7].

It has been reported that in these patients, we see an increase in the differentiation of naive CD4+ T into inflammatory cells and an increase in the ratio of cytotoxic T cells to immune-protective regulatory T (Treg) cells[8, 9]. Forkhead box P3 (FoxP3)+Treg cells play an essential role in immune system hemostasis and maintenance of self-tolerance[10] and the results of some studies have shown that FoxP3 expression is impaired in these patients[11, 12].

In recent years, much attention has been paid to the association of intestinal microbiome with neurodegenerative diseases. Several studies have shown that microbiome integrity is impaired in patients with MS and the bacterial balance shifts to pathogenic bacteria[13, 14]. Some animal studies have also shown that the progression of experimental allergic encephalomyelitis (EAE), which is an animal model of MS, is more severe in Germ-free mice[15].

On the other hand, probiotic supplementation in some studies has significantly reduced the level of inflammatory factors and exerted inhibitory effects on inflammatory pathways[16, 17]. Considering that inflammation is an integral part of the MS pathogenesis and reducing the level of inflammation can be effective in the treatment of this disease, this study aimed to investigate the effect of probiotic supplementation on systemic inflammation in patients with MS.

Material And Methods

The method of this study was based on ethical standards from the Helsinki Declaration, approved by the ethics committee of Ahwaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1398.865) and registered on the Iranian Registry of Clinical Trials (IRCT) (http://www.irct.ir: IRCT20181210041918N2). Informed consent was obtained from all patients.

2.1. Study design and participants

The present study was a 24-week randomized, double-blind clinical trial which conducted between July 2019 and April 2020. The participants enrolling in this trial were patients referred to the MS association of Khuzestan, Iran, who were included in the study if they met the following criteria: age range between 18 to 50, clinical definite MS according to McDonald criteria and an Expanded Disability Status Scale (EDSS) score £4.5. Exclusion criteria included the presence of any concomitant disease such as rheumatoid arthritis, Inflammatory bowel disease (IBD), rheumatoid arthritis, systemic lupus, type 1 diabetes and other autoimmune diseases, use of anti-inflammatory drugs, omega-3 and probiotic supplements or other antioxidant and anti-inflammatory supplements, corticosteroid therapy, current smoker, disease duration of less than 1 year, malnourishment (BMI under 18.5) or morbid obesity (BMI>35), impaired Th1/Th2 balance, (such as asthma, rheumatoid arthritis and type 1 diabetes mellitus), pregnancy and lactation. All

patients were under the guidance of two neurologists and no restrictions were set on concomitant immunomodulatory treatment (i.e. Interferon beta-1a (IFN β-1a) (Avonex), glatiramer acetate, or natalizumab).

Finally, 70 patients with MS were randomly allocated in the intervention and control groups, so that 35 patients were divided into the intervention group and 35 patients into the control group. Randomization lists were computer-generated by a statistician and given to the interviewer. The randomization process took place in a way that researchers, neurologist, staff and patients were blinded.

Patients which participated in the study were randomly divided into two groups receiving probiotic supplement and placebo. Patients in the intervention group received 2 multi-strain probiotic capsules/day for six months, each containing minimum 2 billion live microor-ganisms (2×10^9 CFU/capsule), equivalent to 10 billion live microorganisms per gram (1 × 1010 CFU/gram) of14 strains (Bacillus subtilis PXN 21, Bifidobacterium bifidum PXN 23, Bifidobacterium breve PXN 25, Bifidobacterium infantis PXN 27, Bifidobacterium longum PXN 30, Lactobacillus acidophilus PXN 35, Lactobacillus delbrueckii ssp. bulgaricus PXN 39, Lactobacillus caseiPXN 37, Lactobacillus plantarum PXN 47, Lactobacillusrhamnosus PXN 54, Lactobacillus helveticus PXN 45,Lactobacillus salivarius PXN 57, Lactococcus lactis ssp.lactis PXN 63, Streptococcus thermophilus PXN 66), cellulose (bulking agent) and vegetable capsule (Hydroxy-propylmethyl Cellulose). Participants in the control group received the same amount of placebo for 6 months, and the placebo capsules contained microcellules, so that there was no difference in appearance or smell between probiotic and placebo capsules.

At the beginning of the study, after fully explaining the study protocol and receiving informed consent from patients, patients' demographic information was recorded and then probiotic and placebo boxes were given to patients for 6 weeks. Subsequent probiotic and placebo boxes were presented to patients in the sixth, twelfth and eighteenth weeks. During the 24-week intervention period, patients were reminded of the use of probiotics and placebo by texting and calling. Patients who did not consumed more than 10% of the given capsules in each period were excluded from the study.

Anthropometric measures, dietary intake and physical activity

To evaluate anthropometric variables, at the beginning and after week 24, patients' height and weight were measured by a trained researcher. Patients 'weight was measured using an Inbody device (Inbody BDM370, Korea) without shoes, light clothing with the precision of 0.1 kg and patients' height was measured using a Seca scale without shoes to the nearest 0.5 cm. The standard formula was also used to calculate body mass index (BMI).

To evaluate the dietary intake of the study participants, 3 days 24 hours' dietary recall on one holiday and two working days at the beginning and end of the study was recorded from all participants. The analysis of 24-h food recall questionnaires was done using Nutritionist IV (N4) (First Databank, Hearst Corp, San Bruno, CA, USA).

Physical activity was assessed by the metabolic equivalent of task (MET) questionnaire at

the beginning and the end of the study.

Serum biochemical measurement

To evaluate biochemical variables, at the beginning and end of the study, 10 cc of blood was taken from patients after 12 hours of fasting. Blood samples were allowed to clot at room temperature (20-25°C) for20 min in a vertical position. Then, the samples were centrifuged at 3000 rpm for 10 min and serum samples were frozen at −70°C, until biochemical marker measurement. ELISA kits (Diaclone Research, Besançon, France) were used to assess the concentration of Interferon gamma (IFNγ), Interleukin 17 (IL-17) and Interleukin 35 (IL-35). Also, for evaluation serum levels of TGF-β and FOXP3, we used from the Crystal Day Elisa kits (Shanghai Crystal Day Biotech, China).

Statistical analysis

All statistical analyses were performed with SPSS 19 software. Kolmogorov–Smirnov test was used to assess the normality of the data. Numerical data were presented as mean ± SD. Mann- Student'st-test was used to compare the continuous data and alternative non-parametric tests were used if the data not normally distributed. Categorical data were compared using the Chi-square test. Paired t-test or McNemar test was applied for intra-group comparison. Also, analysis of covariance was used to remove confounding variables. All of the analysis were adjusted for the age, sex, disease duration and calorie intake. P< 0.05 was considered significant.

Result

The procedure of the trial is depicted in Figure 1 which summarizes the Consolidated Standards of Reporting Trials (CSRT). At the end of the 24 weeks of the intervention, out of 70 patients included in the study, three patients in the intervention group and two patients in the control group were excluded from the study for reasons such as travel, unwillingness to continue participating in the study and other reasons and finally 65 patients included in the final analysis[18]. Of 65 patients witch included in final analysis, 33 participants (7 men and 26 women) in the probiotic group and 32 participants (10 men and 22 women) in the control group completed intervention period.

The demographic information of the study participant is reported in Table 1. There was no significant difference between the participants in the intervention and control groups in terms of baseline variables including age (P=0.59), sex (P=0.71), race (P=0.47), weight (P=0.65), height (P=0.59), BMI (0.28) and MET (P=0.34). In terms of EDSS score, the mean score of EDSS was 1.45± 0.9 in the intervention group and 1.39 ± 1.03 in the control group, but no significant difference was observed between the two groups (P=0.74). In terms of disease duration, participants in the intervention group had higher disease duration in comparison to the control group (7.75± 3.99 y vs 5.66 ± 2.53 y;P= 0.014).

At the beginning of the study, the mean calorie intake by study participants in the intervention group was 1836.27± 464.71 kcal/day and in the control group was 1895± 398.07 kcal, which was not statistically significant (P=0.58). Also, there wasn't any significant difference between two groups in terms of calorie intake and macronutrients at the beginning and end of the study (P>0.05). Moreover, there was no significant difference between the two groups in term of physical activity at the beginning and end of 24 weeks of intervention (P>0.05).

Effect of probiotic supplementation on serum levels of inflammatory markers

Effects of probiotics supplementation on the serum levels of inflammation related biomarkers are presented in the Table 2. As shown in Table 2, probiotic supplementation compared to the placebo caused a significant reduction in the serum levels of CRP (-0.93± 1.62 vs. 0.05 ± 1.74, P=0.03), TNF-a (-2.09 ± 1.88 vs. 0.48 ± 2.53, P=0.015) and IFN-γ (-13.18± 7.33 vs. -1.93± 5.99, P<0.001).

About anti-inflammatory cytokines, the results of our study showed that there weren't any significant differences between two groups in terms of IL-17 (P=0.19) and IL-35 (P=0.08) concentration. After adjusting the results for the confounding variables, no difference was observed in the significance of the results.

Effect of probiotic supplementation on serum levels of TGF-β and FOXP3

The results of the effects of probiotic supplementation on serum levels of TGF-β and FOXP3 are shown in Table 3. The results of our study showed that probiotic supplementation caused a significant increase in the serum concentration of TGF-β (0.53 ± 0.67 pg/dL vs. -0.07 ± 0.58 pg/dL) compared to the placebo. Also, it has been reported that participants in the intervention group had significantly higher levels of FOXP3 (0.25 ± 0.41 pg/dL vs. -0.02 ± 0.53 pg/dL, P=0.014).

Discussion

The results of the present study showed that probiotic supplementation compared with placebo significantly reduced the concentration of CRP, TNF-a and IFN-γ factors and also increased the level of FOXP3 and TFB-β. However, we not found any significant differences between two groups in serum concentration of IL-17 and IL-35.

MS is one of the most important inflammatory diseases and inflammation plays an important role in the pathogenesis of this disease. It has been reported that the destruction of the intestinal microbiome is one of the main causes of chronic inflammation and can accelerate the progression of MS[19, 20]. One of the hypotheses that has been suggested in connection with the high prevalence of MS in most countries is the excessive use of antibiotics and especially the destruction of the intestinal microbiome following inappropriate changes in dietary patterns. In fact, the results of various studies have shown that intestinal microbiome dysbiosis has been associated with the development of various immune-related diseases such as MS, rheu-matoid arthritis, type 1 diabetes, and inflammatory bowel disease[19, 21, 22].

The present clinical trial demonstrated that the supplementation of multistrain probiotics resulted in a significant reduction in serum concentration of some inflammatory biomarkers including CRP, TNF-a and IFN-γ. Our finding was similar to that reported in some previous studies. Most studies that have evaluated the effect of bacterial strains on the severity of MS attacks and inflammatory biomarkers have been studies on experimental model of MS (EAE).

Salehipour et al. in an animal study evaluated the effect of administering several bacterial strains, especially plantarum A7 on experimental model of MS, and the results showed that mice receiving probiotics had a significant improvement in the level of anti-inflammatory factors, including TFB-β and FOXP3 and significant reduction in the serum levels of IL-17 and IFN-γ[23]. Also, Secher et al. have shown that probiotic administration in mice model with EAE led to a significant reduction in the serum levels of IL-17, IFN-γ, and TNF-α. They suggested that the anti-inflammatory effects of probiotics could be exerted by reducing the number of total numbers of CD4+ and MOG-specific CD4+ T- cells in the spinal cord (SC) regulatory T cells and increasing lethal T cells [24]. In another animal study, Kwon et al. showed that administration of five bacterial strains in mice with EAE slowed the progression of disease and decreased the expression of inflammatory factors[25]. Also, Lavasani et al. showed that administration of multi strain probiotics in mice with EAE was more effective in reducing inflammatory factors than single bacterial strain[26]. Tamtaji et al. in a human study showed that probiotic supplementation in 20 patients with MS down-regulated the expression of interleukin-8 and TNF-α genes[27].

Various human and animal studies have shown that probiotics exert anti-inflammatory and immune-boosting effects through a variety of mechanisms, such as maintaining mucosal barrier integrity, improving mucus secretion, decrease in the amount of lipopolysaccharides (LPS) and some other mechanisms[28-30]. LPS play an important role in the exacerbation of MS by binding to the toll-like receptors (TLR2, 4) on endothelial cells (ECs), DCs (dendritic cells) and macrophage cells (MQs). Stimulation of TLR2 and TLR4 by LPS increases the production and secretion of inflammatory cytokines[31, 32]. In fact, probiotic supplementation has been shown to significantly increase the differentiation of native T cells to the Th2 which improve production and secretion of anti-inflammatory cytokines such as IL-10 and IL-4[25, 26]. It has been reported that Lactobacillus species oral administration in mice with EAE led to IL- 10-dependent activation of Tregs in the CNS followed by reduction of IFN-γ, TNF-α, and IL-17. and they concluded that improving the gut microbial profile could reduce chronic inflammation[33].

Some studies have reported that administration of probiotics, especially Lactobacillus strains, induces anti-inflammatory effects by reducing the number of intermediate monocytes[34]. Also, some metabolites produced by probiotics, such as butyrate, induce anti-inflammatory effects by inhibition of the NF-kB pathway as well as inhibiting the secretion of lipopolysaccharide-induced TNF-and IL-6[35]. Zhang et al. showed that administration of lactobacillus rhamnosus in Caco-2 cells causes a significant reduction in the TNF-α induced IL-8 secretion [36]. Also, Shimazu et al. in a cell study reported that porcine intestinal epithelial cells treatment with lactobacillus jensenii led to a significant anti-inflammatory effects by down-regulating the NF-kB and mitogen-activated protein kinase (MAPK) pathways[37].

We found that probiotic supplementation for 24 weeks led to a significant increase in the serum levels of FOXP3 and TFB-β. As mentioned, MS results from the failure of the body's regulatory mechanisms, such as regulatory T cells against the spread of pathogenic T cells directed at myelin determinants. Among the regulatory cells that play a protective role against the progression of MS are $CD4^+CD25^+$ regulatory T cells. $CD4^+CD25^+$ cells have a unique function and support both central and peripheral tolerance in the body. These cells exert central tolerance in the thymus and also induce peripheral Tregs[38]. On the other hand, $CD4^+CD25^+$ cells play an important role in inhibiting effector T-cell proliferation as well as reducing the production and secretion of inflammatory cytokine in a cytokine-independent way requiring cell-to-cell contact. The FOXP3 transcription factor is one of the most important and sensitive indicator for evaluation of $CD4^+CD25^+$ regulatory T cells. It has been reported that in patients with MS, the expression and function of FOXP3 is significantly impaired[11]. On the other hand, the results of some studies have shown that any mutation in FOXP3-related genes disrupts the development of regulatory T cells and increases the risk of some autoimmune diseases such as MS, some inflammatory and allergic diseases[39].

In line with our findings, Smelt et al. in an animal study were reported that oral administration of L. plantarum WCFS1, L. salivarius UCC118, and L. lactis MG in healthy mice led to a significant increase in the FoxP3 T-cell responses in the small intestine and simultaneously inducing CD4 and CD8 T cell activation in the large intestine[40]. Also, Lavasani et al. in an animal study evaluated the effects of five bacterial strains in mice with EAE and showed that probiotic oral administration induced $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) in mesenteric lymph nodes and also improved TFB-β levels[26].

TFB-β is one of the most important regulatory cytokine that has a variety of functions in the immune system as an anti-inflammatory agent and influence the differentiation and function of T cells. One of the most important roles of insulin is to promote immune self-tolerance by regulation of lymphocyte proliferation, differentiation, and survival. Among T cells, $CD4^+CD25^+FOXP3^+$ T regs contain the main source of TGF-beta that suppresses immune responses in inflammatory sites[7, 41]. In line with our findings, some other studies showed the positive effects of probiotic administration on TFB-β concentration[6, 42, 43].

As mentioned, in recent years, limited studies have evaluated the effect of probiotics on patients with MS, and our study was one of the most recent trial in this field. One of the main strengths of our study was the use of multi-strain probiotic supplements instead of a single specific bacterial strain. Various studies have shown that the use of products containing several bacterial strains is more effective in improving inflammation and strengthening the immune system than a single bacterial strain[44-46]. Also, the duration of intervention in the present study was long and it seems that the duration of intervention of 24 weeks causes appropriate changes in the intestinal microbial profile. On the other hand, unlike some previous studies, the participants in this study included both men and women, and therefore the results of the study can be generalized to both sexes.

However, the present study had some limitations that should be considered in interpreting the results. One of the main limitations of our study was the lack of evaluation of changes in intestinal microbial profiles in fecal samples. Also, assessing the gene expression of some inflammatory factors, especially TFB-β and FOXP3, could increase the accuracy of the results.

Conclusion

In conclusion, the results of our study revealed that the modification of the gut microbiota to a more favorable composition may contribute to improved systemic inflammation in patients with MS. Despite the positive results in this study, more studies are needed to prove the findings of this study. Identification of the mechanisms involved in these beneficial effects can also be considered in the design of future studies.

Declarations

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Authors' contribution

MR: Conception and design, clinical implementation, assignment of participations, and collection of blood samples, manuscript writing; ShN: clinical implementation; NBJ: assignment of participations and Analysis of blood samples; DH: Analysis of blood samples and data, manuscript writing; NM: conception and design, analysis data; SAH: clinical implementation, Conception and design.

Conflict of Interest

None.

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Availability of data and materials

Data are available upon reasonable request.

Ethical approval and consent to participate

The protocol was approved by the Ahwaz Jundishapur University of Medical Sciences Ethics Committee, and all participants provided written informed consent. All patients were provided written informed

consent.

Consent for publication

Not applicable.

Competing interests

None of the authors have any conflicts of interest or financial ties to disclose.

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Tables

Table 1- Baseline characteristics of the participants

Variable[2]		Total (n=65)	Probiotic (n=32)	Control (n=33)	p-value
Age (years)		41.01 (10.45)	42.15 (11.98)	39.9 (8.76)	0.39
Height (cm)		165.56 (8.71)	164.96 (8.21)	166.13 (9.27)	0.59
Gender	Male	18 (27.7)	6 (18.75)	12 (36.36)	0.11
	Female	47 (72.3)	26 (81.25)	21 (63.64)	
Weight (kg)		68.7(13.07)	69.45(13.93)	67.96(12.27)	0.65
WC(cm)		86.58(11.86)	87.09(11.29)	86.09(12.54)	0.73
BMI (kg/m^2)		25.01(4.05)	25.48(4.54)	24.55(3.51)	0.36
MS duration (y)		6.69 (3.46)	7.75 (3.99)	5.66 (2.53)	0.014
EDSS Score		1.70(0.72)	1.68(0.71)	1.72(0.74)	0.82
MET-h/day at study baseline		34.16(4.84)	34.75(4.95)	33.60(4.75)	0.34

Data are presented as mean (SD) for quantitative and frequency (%) for qualitative variables. BMI: Body Mass Index; MS, Multiple sclerosis; EDSS, Expanded Disability Status Scale

Table 2. Comparison of inflammatory biomarkers between groups at the baseline and end of the study[1]

Variables[2]	Groups		P-value[3]	
	Intervention (n=33)	Control (n=32)		P- adjusted[4]
IL-17 (pg/dL)				
Before	25.43 ± 11.36	23.71 ± 9.65	0.24	
After	25.46 ± 14.49	25.04 ± 13.89	0.75	
Change	0.02 ± 1.19	1.32 ± 1.97	0.19	0.105
P-value[5]	0.32	0.18		
IL-35 (pg/dL)				
Before	10.29 ± 4.76	10.81 ± 6.12	0.16	
After	10.42 ± 5.16	11.16 ± 5.88	0.62	
Change	0.13 ± 0.65	0.35 ± 0.49	0.08	0.124
P-value[5]	0.376	0.182		
CRP (mg/dl)				
Before	3.62±2.12	3.24± 1.28	0.43	
After	2.69±1.78	3.29± 2.24	0.38	
Change	-0.93± 1.62	0.05 ± 1.74	0.03	0.034
P-value[5]	0.04	0.71		
TNF-a (pg/ml)				
Before	5.25 ± 3.28	4.68± 3.13	0.364	
After	3.16 ± 2.78	5.16 ± 3.62	0.089	
Change	-2.09 ± 1.88	0.48 ± 2.53	0.015	0.026
P-value[5]	0.021	0.367		
IFN-γ (pg/dL)				
Before	33.17±8.40	30.76± 7.46	0.17	
After	19.98 ± 2.57	28.83± 7.14	<0.001	
Change	-13.18± 7.33	-1.93± 5.99	<0.001	<0.001
P-value[5]	<0.001	0.12		

1. CRP, C-reactive protein; IFN-γ, Interferon gamma, IL-17, Interleukin 17; IL-35, Interleukin 35; TNF-α, Tumor necrosis factor-α
2. Data are presented as mean (SD) or geometric mean (SD).
3. Calculated using one-way ANOVA
4. Calculated using ANCOVA, adjusted for the effect of age, sex, disease duration and calorie intake
5. Calculated using paired sample t-test.

Table 3. Comparison of TGF-β and FOXP3 levels between groups at the baseline and end of the study[1]

Variables[2]	Groups		P-value[3]	
	Intervention (n=33)	Control (n=32)		P- adjusted[4]
TGF-β (pg/dL)				
Before	1.45 ± 0.52	1.83 ± 0.87	0.135	
After	1.98 ± 0.74	1.76 ± 0.65	0.55	
Change	0.53 ± 0.67	-0.07 ± 0.58	0.023	0.036
P-value[5]	0.03	0.16		
FOXP3 (pg/dL)				
Before	1.42 ± 0.92	1.54 ± 0.73	0.182	
After	1.67 ± 0.91	1.52 ± 0.88	0.309	
Change	0.25 ± 0.41	-0.02 ± 0.53	0.014	0.026
P-value[5]	0.02	0.8		

1. FOXP3, forkhead box P3; TGF-β, Transforming growth factor beta
2. Data are presented as mean (SD) or geometric mean (SD).
3. Calculated using one-way ANOVA
4. Calculated using ANCOVA, adjusted for the effect of age, sex, disease duration and calorie intake
5. Calculated using paired sample t-test.

Figures

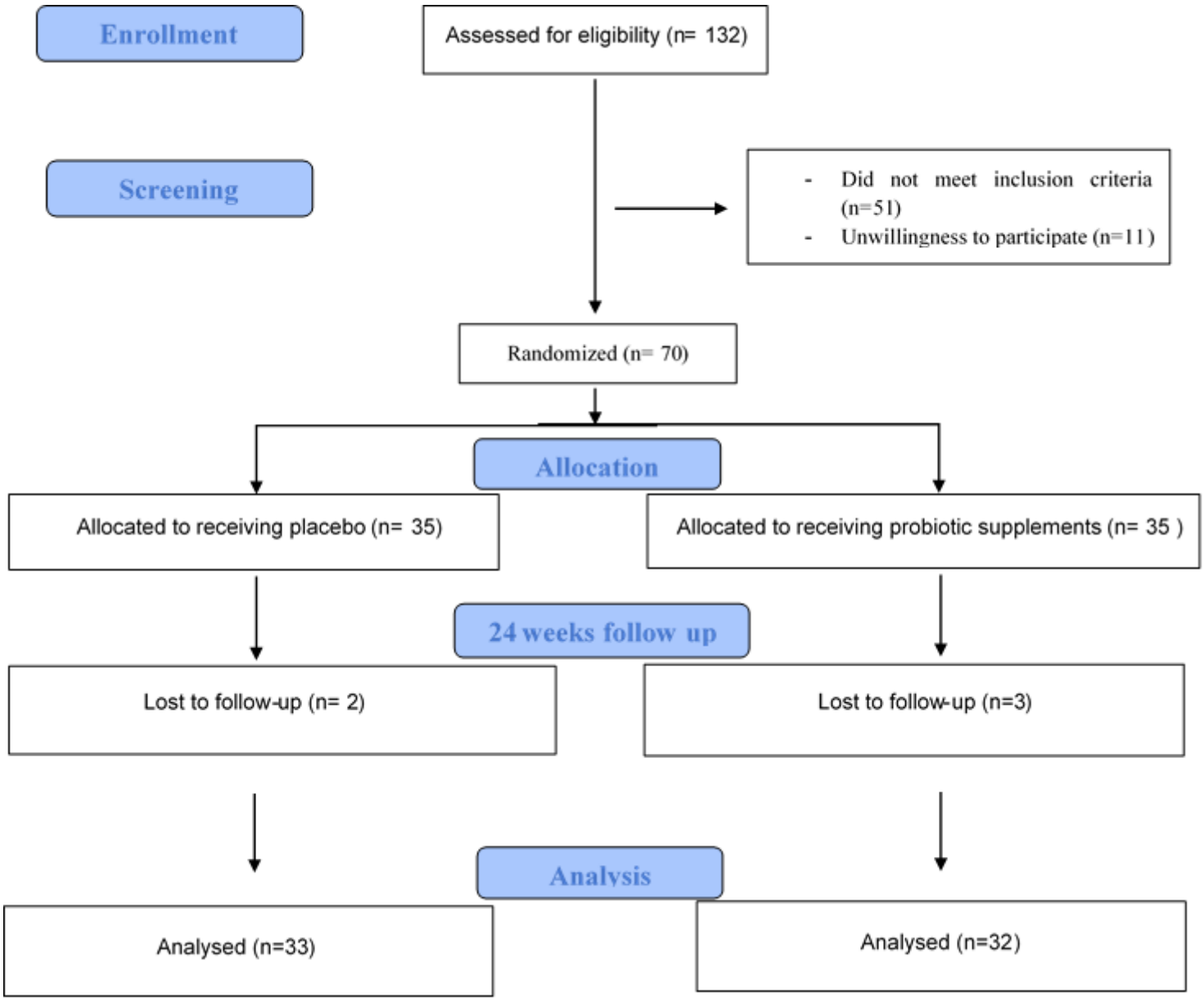

Figure 1

The procedure of the trial is depicted in Figure 1 which summarizes the Consolidated Standards of Reporting Trials (CSRT). At the end of the 24 weeks of the intervention, out of 70 patients included in the study, three patients in the intervention group and two patients in the control group were excluded from the study for reasons such as travel, unwillingness to continue participating in the study and other reasons and finally 65 patients included in the final analysis[18]. Of 65 patients witch included in final analysis, 33 participants (7 men and 26 women) in the probiotic group and 32 participants (10 men and 22 women) in the control group completed intervention period.