Original Article

Clinical and Metabolic Reaction to Probiotic Supplement in Children Suffering Attention-Deficit Hyperactivity Disorder: A Randomized, Double-Blind, Placebo-Controlled Experiment

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Abstract

Aim: This paper aimed at assessing the influence that probiotic supplement had on mental health and metabolic conditions of children suffering attention-deficit hyperactivity disorder (ADHD). **Materials and Methods:** A number of 34 children with ADHD were selected randomly. Participants were randomly allocated into a group receiving 8×10^9 CFU/g probiotic supplements ($n = 17$) and a group receiving placebo ($n = 17$) during an 8-week time period. Clinical symptoms were recorded applying the rating scale of ADHD (ADHD-RS), Children's Depression Inventory (CDI), and Hamilton Anxiety Rating Scale (HAM-A) both at onset of the study and after the 8-week interval. Moreover, samples of blood were also taken at the beginning and after the 8-week interval so that the metabolic information could be evaluated. **Results:** The probiotic supplementation could bring about a considerable decrease in total ADHD-RS (β −3.31; 95% confidence interval [CI]: −5.60, −1.02; $P = 0.006$) and HAM-A (β −1.91 [0.18]; 95% CI, −3.41, −0.41; $P = 0.01$) than that of the placebo. In addition, probiotic supplementation brought about a considerable decrease in high sensitivity C-reactive protein (hs-CRP) of serum (β −2.05 mg/L; 95% CI, −3.57, −0.52; $P = 0.01$) as well as a substantial rise in plasma overall antioxidant volume (TAC) (β 66.26 mmol/L; 95% CI, 36.83, 95.68; $P < 0.001$) than that of the placebo. No meaningful effects were observed on CDI and other metabolic features after the intake of probiotic supplements. **Conclusions:** Taking probiotic by children suffering ADHD could affect ADHD-RS, HAM-A, hs-CRP of serum, and TAC levels in plasma, while it did not show any effects on CDI and other metabolic profiles.

Keywords: Attention-deficit hyperactivity disorder, inflammation, mental health, oxidative stress, probiotic

Introduction

The disorder of attention-deficit hyperactivity disorder (ADHD) can be described as impairment of unreasonable levels of abnormally high activity/impulsivity or lack of attention or both.[1] The prevalence of ADHD was reported in 5.3% of children, and it is more common in boys than girls.[2] Several genetic and environmental factors have been proposed to be risk factors for ADHD.[3,4] Inflammatory markers due to positive findings regarding inflammation-related genes may be influential in the pathogenesis of ADHD.[5,6]

Clinical findings have indicated that probiotic administration in early stages of life would improve subsequent outcomes

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such as decreased risk of diagnosis with ADHD at the age of 13 years.[7] In addition, application of antibiotics during the 1st-year period of life may have associations with higher risk of ADHD.[8] Furthermore, undergoing cesarean section surgery, as one of the major influencing factors on the early microbiota colonization, is supposed to act as a risk factor regarding ADHD.[9] Therefore, according to the existing results, patients with ADHD may have probably experienced a change in the composition of their gut microbiota,[10] which may be subsequently associated with their maladaptive behaviors. In addition, the advantageous impacts of probiotics on inflammation as well as oxidative stress biomarkers were already confirmed through several studies. Alipour *et al*.[11] indicated in their study that probiotic supplementation for an 8-week period to patients suffering rheumatoid arthritis reduced inflammatory cytokines. Furthermore, taking 150 g/day fermented goat milk with 3×10^{11} CFU/day lactobacilli for 3 weeks by healthy participants could improve antiatherogenicity parameters and increase total antioxidant activity.[12] Two other trials among healthy subjects demonstrated that probiotic supplementation was useful in reducing oxidative stress.[13,14]

Since probiotics offer antioxidant as well as anti-inflammatory impacts,[15-18] the hypothesis was raised that probiotic supplementation may benefit children suffering ADHD. As a result, this study was performed in order to determine the influencing features of probiotic supplement on clinical symptoms, biomarkers of inflammation as well as oxidative stress in children with ADHD.

Materials and Methods

The design and population of the research

This is a randomized double-blinded placebo controlled clinical trial, which has been recorded on the website to register clinical experiments performed in Iran at http://www.irct.ir: IRCT2017090133941N16. Participants of the research have been 34 children aging 8–12 years, who suffered from ADHD, and diagnosis has been established with regard to clinical diagnostic DSM-IV-TR criteria. The research lasted from October to December 2017. The Committee of Research Ethics in Kashan University of Medical Sciences (KAUMS) confirmed the study design. Conscious written consent forms have been given to the participants. Moreover, parental consents have been gained for each participant as well. Exclusion criteria were as following: bi-polar abnormality, kefir, mental disorders, and autism that was not related to our study, the use of probiotic yogurt, pervasive developmental abnormalities, the use of the fermented food, probiotic, antioxidant, and/or anti-inflammatory supplementary regimens, including Vitamin C, omega-3 fatty acids, the use of antibiotics, and Vitamin E.

At first, each child has been randomized with regard to body mass index (BMI) (<20 and ≥20 kg/m^2) and gender (males vs. females) for declining the probable side effects. Allocating participants in each group has been done into two treatment groups for taking either 8×10^9 CFU/day probiotic sachet with *Lactobacillus reuteri, Lactobacillus acidophilus, Lactobacillus fermentum, and Bifidobacterium bifidum* (each 2×10^9) ($n = 17$) or placebo ($n = 17$) during 8 weeks through the balanced block randomization. Each participant received Ritalin dose of 0.1 mg/kg of body weight. Participants pursued taking medicine during the trial. Takgen Company (Tehran, Iran) has been selected to purchase probiotics and the respective placebos (starch) sachets. The packages have been the same for the placebo sachets and supplements. It should be noted that the participants and authors did not know the package contents till the end of research. The participants have been randomly assigned by the computer-generated random numbers as blindness that has been done by a trained employee at the psychiatry clinic. Researchers, personnel at the clinic, children, and laboratory personnel have been masked to the treatment assignment during the research. Containers with sachets have been examined to comply with the placebo and supplement intake. Moreover, a daily reminder message was sent to each parent's cellphone for regular uptake of the supplement. Each parent reported following a pattern of 3-day dietary intake information at the 1st, 4th, and 8th weeks of the intervention. This research applied Nutritionist IV software (First Databank, San Bruno, CA) to obtain the nutritional intake by the children in accordance to 3-day food records. The software has been customized for the Iranian food pattern.

Outcomes

The major result has been the rating scale of ADHD (ADHD-RS). Minor outcomes have been Hamilton Anxiety Rating Scale (HAM-A), Children's Depression Inventory (CDI), and inflammation as well as oxidative stress biomarkers.

Clinical evaluation

This paper employed parent-rated 18-item ADHD-RS IV for assessing ADHD-RS.[19] CDI applied a self-rating scale for adolescents and children's depression, which contained 27 items that evaluate affective, behavioral, and cognitive symptoms of depression considering scores ranging 0–2.[20] HAM-A included 31 items by scoring in the range between 0 and 52 and cutoff point of 14. It has been completed by a single clinician.[21] Clinical symptoms have been evaluated in a blind mode by a single clinician at baseline and at the end of trial.

Laboratory processes

Weeks zero and eight were selected to take 5 ml blood samples at Kashan reference laboratory located in Kashan, Iran. Quantification of the levels of high-sensitivity C-reactive protein (hs-CRP) in serum has been performed using an ELISA kit (LDN; Nordhorn; Germany) with intra-assay and inter-assay coefficient variances (CVs) to be <6%. Inter-assay and intra-assay CVs of <5% have been used to evaluate nitric oxide (NO) of plasma through Griess procedure,[22] glutathione (GSH) through Beutler *et al*.'s[23] technique, total antioxidant capacity (TAC) through the ferric decreasing antioxidant power technique which Benzie and Strain[24]

designed, and levels of malondialdehyde (MDA) through thiobarbituric acid reactive substance technique.[25]

Sample size

The formula proposed to clinical randomization has been used to calculate the size of the sample. Errors of Type 1 (α) and Type 2 (β) have been described as 0.05 and 0.20 with a power of 80%. According to the recent research,[26] this research applied 6.4 as SD. In addition, 6.5 as effect size (the mean difference) of the beck depression inventory (BDI) has been examined. According to this inventory, the research required 16 children in all treatment groups. It should be noted 20% of the children has been dropped out in each group; therefore, the resulting sample size has been specified as twenty participants in each group.

Statistical analyses

A comparison was made between macronutrient and micronutrient dietary intakes and anthropometric measures for both groups. Independent sample *t*-test has been used to make the comparison. Multiple linear regression model has been applied for evaluating the therapeutic impacts on the research results after setting for confounding variables such as baseline values of results, age, and body mass index at baseline. The effect size has been provided as the mean difference with 95% confidence interval (CI). Kolmogorov–Smirnov test has been employed for testing the model residual normality. The outcome variable has been log-transformed if the model residual distributed abnormally (CDI). $P < 0.05$ has been identified statistically significant. SPSS 18 has been applied to do statistical analyses (SPSS Inc.; Chicago; Illinois; USA).

Results

Three participants of the supplemented and placebo groups had to be removed due to individual matters [Figure 1]. Consequently, the final analytical procedures were administered taking 34 children into account. In general, there was a considerable rate of agreement, since over 90% of sachets had been consumed during the study period in the two groups under study. After administration of probiotic in children suffering ADHD during the research period, no adverse events were observed.

Mean of age, height, weight as well as BMI showed no statistical differences between the two groups at the start point and at the end of the study [Table 1].

There was not a considerable change regarding the mean dietary macro- and micro-nutrient intakes in both groups during the experiment [Table 2].

Probiotic supplementation could significantly reduce the total ADHD-RS (β −3.31 [0.35]; 95% CI, −5.60, −1.02; $P = 0.006$), inattention (β −1.97 [0.39]; 95% CI, −3.42, −0.52; $P = 0.009$), and hyperactivity-impulsivity (β −1.33 [0.25]; 95% CI, −2.56, −0.10; $P = 0.03$) [Table 3] relative to that of the placebo. Probiotic supplementation also reduced HAM-A (β-1.91 [0.18]; 95% CI, −3.41, −0.41; $P = 0.01$) and hs-CRP of serum (β −2.05 mg/L; 95% CI, −3.57, −0.52; $P = 0.01$) significantly, while plasma TAC (β 66.26 mmol/L; 95% CI, 36.83, 95.68; $P < 0.001$) was significantly increased in comparison with the placebo. There were no significant effects on CDI and other metabolic features after the intake of probiotic supplements.

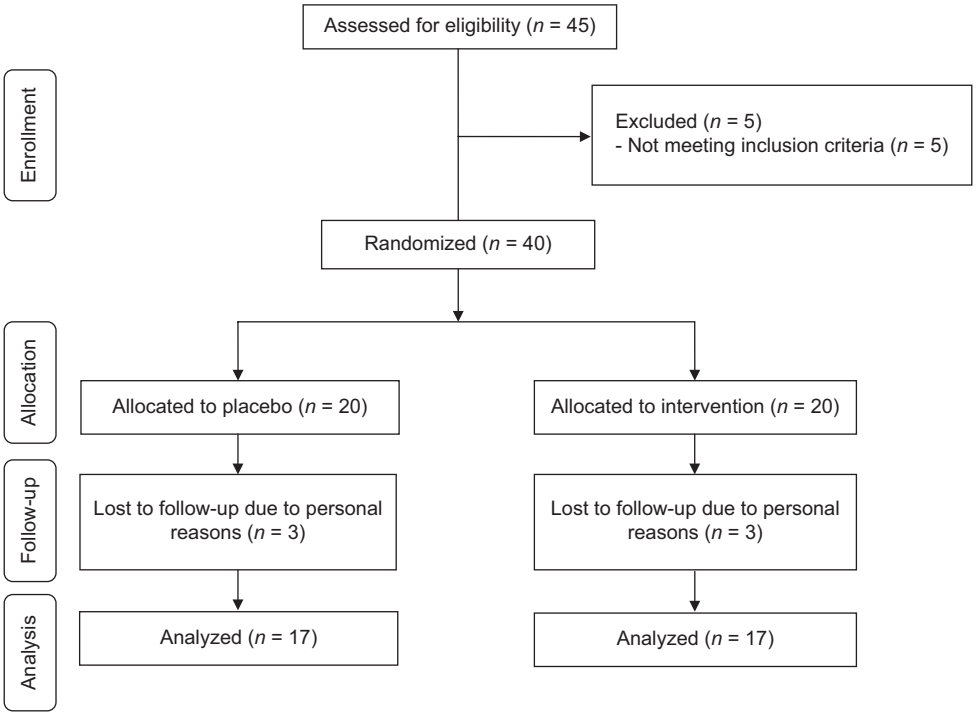

Figure 1: Summary of patient flow

Table 1: General characteristics of study participants

	Placebo group (n=17)	Probiotic group (n=17)	P[a]
Age (years)	8.9±1.0	9.3±1.3	0.38
Height (cm)	128.5±10.5	133.2±11.2	0.22
Gender, *n* (%)			
Male	13 (76.5)	14 (82.4)	>0.999[†]
Female	4 (23.5)	3 (17.6)	
Weight at study baseline (kg)	36.2±7.4	36.5±12.6	0.92
Weight at end-of-trial (kg)	36.2±7.3	36.8±12.7	0.86
Weight change (kg)	0.1±1.4	0.3±0.8	0.54
BMI at study baseline (kg/m^2)	22.3±6.3	20.5±6.5	0.41
BMI at end-of-trial (kg/m^2)	22.4±6.4	20.7±6.5	0.43
BMI change (kg/m^2)	0.1±0.9	0.1±0.5	0.73
Ritalin intake (mg/kg BW per day)	3.6±0.7	3.7±1.2	0.92

Data are means±SD. [a]Obtained from independent *t*-test, [†]Obtained from Fisher's exact test. BMI: Body mass index; BW: Body weight, SD; Standard deviation

Table 2: Mean dietary intakes of study participants at week 1, 4, and 8 of the study

	Placebo group (n=17)	Probiotic group (n=17)	P[a]
Energy (kcal/day)	1900±250	1943±318	0.65
Carbohydrates (g/day)	251.1±55.8	267.3±56.2	0.40
Protein (g/day)	77.3±26.7	69.6±16.3	0.32
Fat (g/day)	68.2±13.3	68.9±16.7	0.89
SFA (g/day)	20.5±4.8	20.2±6.6	0.89
MUFA (g/day)	21.5±5.3	23.3±6.8	0.37
PUFA (g/day)	18.9±6.1	19.1±6.4	0.92
TDF (g/day)	15.4±5.2	13.9±4.3	0.34
Selenium (µg/day)	49.2±6.3	50.0±5.7	0.69
Magnesium (mg/day)	215.3±54.5	227.9±47.5	0.48
Manganese (mg/day)	1.5±0.8	1.7±0.8	0.45
Iron (mg/day)	10.9±2.8	12.0±3.0	0.26
Zinc (mg/day)	8.2±2.7	7.9±2.8	0.80
Calcium (mg/day)	928.5±189.6	959.4±213.3	0.65
Vitamin D (µg/day)	2.2±0.7	2.3±0.8	0.62
Vitamin C (mg/day)	48.9±5.2	49.5±4.5	0.72

Values are means±SD. [a]Obtained from independent samples *t*-test. MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, SFA: Saturated fatty acids, TDF: Total dietary fiber, SD; Standard deviation

Discussion

This study investigated the influence that probiotic supplement could have on clinical as well as metabolic status in children suffering ADHD. According to the findings, taking probiotic during an 8-week interval by children with ADHD showed positive effects on ADHD-RS, HAM-A, hs-CRP of serum, and TAC levels in plasma, but it neither influenced CDI nor other metabolic features. To the authors' knowledge, the present paper is the first publication on the effects probiotic supplement may have regarding clinical and metabolic status of children suffering ADHD.

Effects on clinical symptoms

The present paper indicated that administration of probiotic in children with ADHD during an 8-week period could significantly reduce ADHD-RS and HAM-A in comparison with the placebo, while it did not affect CDI. Previous studies have suggested that the presence of gut microbiota, especially it's certain beneficial bacteria make change the function of nervous system.[27,28] Earlier, we showed that administration of probiotic supplements during an 8-week period in patients experiencing major depressive disorder had advantageous outcomes for BDI.[26] Furthermore, supplementation with probiotic bacteria for a 4-week period to healthy women had beneficial impacts on brain regions that are responsible for central processing of emotion and sensation.[29] Furthermore, a meta-analysis research confirmed that probiotic consumption in healthy human affected the psychological symptoms of depression, anxiety, and perceived stress positively.[30] In another study in an animal model, *Lactobacillus rhamnosus* intake improved stress-induced corticosterone and anxiety- and depression-related behavior.[31] Nevertheless, no meaningful changes were reported in mental health after administration of *Lactobacillus rhamnosus* along with *Bifidobacterium animalis* during a 14-week period among patients who had schizophrenia.[32] It was reported that between 15% and 35% of patients with ADHD or an anxiety disorder had both at the same time so that ADHD was reported as the most prevalent externalizing comorbidity for anxiety[33,34] which was correlated with considerable social impairments, along with challenges in school context.[35] Consequently, probiotics may be beneficial in controlling neurological symptoms probiotics because of their positive impacts on indices of mental health. Probiotic administration seems likely to enhance mental health indices by changing the composition and/or activity of the gastrointestinal microbiota,[36] effects on the mucosal immune system,[36] and reduced tryptophan levels.[37] Multiple mental disorders including anxiety and depression are associated with increased inflammatory markers.[38] Therefore, altering microbial composition of the gut, reducing pro-inflammatory cytokines production, decreasing inflammation, and having a favorable effect on gut-brain signaling as a result is one of the possible ways to improve mental health parameters.[38,39]

Table 3: The effect of probiotic supplementation on mental health parameters and metabolic status in children with attention-deficit hyperactivity disorder

Variables	Placebo group ($n=17$)		Probiotic group ($n=17$)		Difference in outcome measures between probiotic and placebo treatment groups[a]	
	Baseline	Week 8	Baseline	Week 8	β (95% CI)	P^b
ADHD-RS	29.6±7.7	30.1±7.5	28.3±10.8	25.8±11.1	−3.31 (−5.60–−1.02)	0.006
CDI	4.1±1.8	3.9±1.8	5.0±2.5	4.2±1.3	−0.27 (−1.03-0.48)	0.97
HAM-A	22.8±10.9	22.5±9.6	24.9±9.6	22.7±9.9	−1.91 (−3.41–−0.41)	0.01
Hs-CRP (mg/L)	3.4±1.7	5.2±3.1	3.1±2.1	2.7±1.9	−2.05 (−3.57–−0.52)	0.01
NO (µmol/L)	35.4±4.5	34.6±2.7	32.0±2.4	32.5±2.4	−0.80 (−2.59-0.98)	0.36
TAC (mmol/L)	838.4±67.3	839.3±42.4	845.6±51.1	904.8±51.3	66.26 (36.83-95.68)	<0.001
GSH (µmol/L)	617.1±138.5	630.9±70.2	531.0±66.5	629.8±101.4	−9.57 (−78.16-59.02)	0.77
MDA (µmol/L)	2.6±0.2	2.6±0.2	2.8±0.2	2.6±0.2	−0.03 (−0.19-0.11)	0.62

Data are mean±SD. [a]"Outcome measures" refers to the change in values of measures of interest between baseline and week 8. β (difference in the mean outcomes measures between treatment groups [probiotic group=1 and placebo group=0]), [b]Obtained from multiple regression model (adjusted for baseline values of each biochemical variables, age and baseline BMI). ADHD-RS: Attention-deficit hyperactivity disorder rating scale, CDI: Children's depression inventory, GSH: Glutathione, HAM-A: Hamilton Anxiety Rating Scale, Hs-CRP: High-sensitivity C-reactive protein, MDA: Malondialdehyde, NO: Nitric oxide, TAC: Total antioxidant capacity, SD; Standard deviation, CI: Confidence interval

Effects on inflammation and oxidative stress

It was found that administration of probiotic in children with ADHD during an 8-week interval could significantly reduce the serum hs-CRP, while TAC levels were significantly increased in comparison with the placebo; however, plasma NO, GSH, and MDA levels were not affected. It was already illustrated that probiotic along with Vitamin D co-supplementation after a 12-week period to diabetic patients suffering coronary heart disease could positively affect the levels of hs-CRP of serum, NO in plasma, and TAC, while it showed no effects on the levels of plasma GSH and MDA. Furthermore, in a meta-analysis research, supplementing with probiotic significantly decreased serum C-reactive protein levels,[40] probiotic soy milk intake for an 8-week period among diabetic kidney disease patients could also improve few oxidative stress parameters.[41] Taking 300 g/day probiotic yogurt fermented with 7×10^6 CFU/day *Bifidobacterium lactis* and 6×10^6 CFU/day *Lactobacillus acidophilus* for 6 weeks by diabetic patients increased total antioxidant status and superoxide dismutase, and MDA levels decreased.[42] Furthermore, in another study by Hariri *et al*.,[43] consuming 200 ml/day probiotic soy milk containing 10^7 CFU/day *Lactobacillus plantarum* for 8 weeks by diabetic patients significantly decreased oxidative stress parameters. However, taking probiotics supplements for 6 and 12 months by patients undergoing laparoscopic sleeve gastrectomy did not affect inflammatory and clinical outcomes.[44] Ebrahimi-Mameghani *et al*.[45] found that the administration of 900 billion/day probiotics to critically ill patients for 1 week did not affect oxidative stress parameters. The discrepancy between the results of previous studies may be in part due to the different strains of probiotics and dosage of the probiotic used. Inflammatory cytokines have also been reported to play a key function in tryptophan metabolism and dopaminergic pathways in the brain, which in turn may implicate ADHD.[46] There is an increasing number of studies indicating that increasing oxidative stress might be correlated with the pathophysiology of psychiatric disturbances as cause and/or consequence of abnormal brain signaling.[47] Therefore, probiotics might reduce complications related to ADHD because of their anti-inflammatory and antioxidative features. Decreasing inflammation and oxidative stress following administration of probiotic might be due to their effects on scavenging superoxide and hydroxyl radicals, decreasing gene expression of interleukin 6 and nuclear factor-κB in adipocytes, decreasing adiposity, and higher levels of short chain fatty acids produced in the colon.[48,49]

This study confronted with several limitations. First of all, fecal bacteria loads had not been assessed before and after probiotic administration. Besides, the evaluation of expression of genes associated with the inflammation as well as oxidative stress in order to clarify the possible mechanisms seems a fascinating issue.

Conclusions

Totally, the present study indicated that administration of probiotic in an 8-week period by children suffering ADHD could affect ADHD-RS, HAM-A, serum hs-CRP, and plasma TAC levels positively, while it neither affected CDI nor other metabolic features.

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Conflicts of interest

There are no conflicts of interest.

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