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Synbiotic supplementation in type one diabetes mellitus: A Randomized Controlled Clinical Trial

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Abstract

Background: limited studies have been conducted on the effect of synbiotics supplementation on Type 1 Diabetes Mellitus (T1DM). The current study aimed to evaluate the impacts of synbiotic supplementation on glycemic parameters, lipid profile, and vitamin D levels in children with T1DM.

Methods: In this double-blind, randomized controlled trial, 86 T1DM patients aged 4-18 were randomly divided into two equal groups. One group received insulin and a synbiotic supplement once daily for 12 weeks; another group received insulin and a placebo. FBS (fasting blood sugar), HbA1C (hemoglobin A1c), triglycerides, cholesterol, HDL (High-density lipoprotein), LDL (low-density lipoprotein), and vitamin D levels were measured at the beginning and end of the study in both groups.

Results: The trend of FBS, HbA1C, triglycerides, cholesterol, HDL, LDL, and vitamin D changes was not significant over time in both groups. Based on the analysis of covariance, the means of FBS and HbA1C were lower in the intervention group (p=0.048 and 0.025, respectively). However, no significant changes in triglycerides, cholesterol, HDL, LDL, and vitamin D levels were observed between the two groups (p= 0.291, 0.291, 0.952, 0.140, and 0.557, respectively).

Conclusion: It's suggested that insulin treatment in combination with synbiotic supplementation could improve FBS and HbA1C in T1DM children but had no effects on lipid profile and vitamin D levels.

Key Words: Glycemic parameters, Lipid profile, Synbiotics, Type 1 Diabetes Mellitus.

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1- INTRODUCTION

Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease that mostly affects people under the age of 20 (1). Various factors, such as the individual's genome, diet, and gut microbiota are associated with the occurrence of T1DM (2). The incidence of T1DM has increased significantly in recent years, so its prevalence has increased by 3 to 4 percent yearly (3) and has become a significant health problem worldwide (4).

This rapid change cannot be explained by genetics alone. Therefore, it is hypothesized that recent changes in the living environment may have affected exposure to pathogenic microbes and the composition of the gut microbial flora regulating immunity and metabolism (5).

It has been shown in previous studies that the composition of gut microbiota is different between healthy controls and T1DM patients and also those with b-cell autoantibody who are at risk of T1DM (6-8). In fact, this difference is called dysbiosis, and it has been shown that T1DM patients had less abundance of bifidobacteria and more abundance of Gram-negative bacteria (6).

Prebiotics, selectively fermented ingredients, lead to specific changes in the activity and composition of gastrointestinal microbiota (9). For example, they can increase the abundance of bifidobacteria and Faecalibacterium prausnitzii and thus may help to correct defects in the gut microbial environment associated with the development and progression of T1DM (10). Some mechanisms have been offered for this action, such as increasing the amount of incretins and insulin secretion (11), affecting the intestinal mucosal barrier (12), reducing gut permeability, and increasing insulin sensitivity (13).

On the other hand, probiotics, as live microorganisms which can colonize the human gastrointestinal tract, can also positively affect diabetes. They can regulate intestinal microbiota and reduce insulin resistance, blood sugar levels, and diabetes-related complications (14).

Therefore, a mixture of probiotics and prebiotics as synbiotics could have beneficial effects against diabetes (15). Several animal and clinical studies suggest that synbiotic use may help control inflammatory factors, oxidative stress biomarkers, and metabolic profiles (16).

Few studies have been conducted on the effect of synbiotics supplementation on T2DM, concluding that it could improve lipid metabolism and glucose homeostasis in these patients (17). For instance, Zare Javid et al. conducted a study in this field, concluding that synbiotics can effectively improve FBS (fasting blood sugar), HbA1c (hemoglobin A1c), insulin, hs-CRP (C-Reactive Protein High-Sensitivity), and TAC (total antioxidant capacity) (18). However, previous research in T1DM is limited, and more studies with larger sample sizes are needed to prove its effects. Therefore, the current study aimed to evaluate the impacts of synbiotic supplementation on glycemic parameters and lipid profile in children with T1DM.

2- MATERIALS AND METHODS

2-1. Design

This study was a single-center, double-blind, randomized, placebo-controlled clinical trial with a parallel design and random allocation of patients in two groups with a 1:1 allocation ratio.

2-2. Sampling

2-2-1. Sample size

In order to determine the sample size, a formula was used according to repeated measurements and variable changes in Blood Sugar (BS). Considering two repetitions, correlation of 0.50, statistical power of 80%, error level of 0.05, and an standard deviation obtained from previous

studies equal to 87.81 (FBG changes)(18), the minimum sample size by applying a 10% drop was to be 86 and 43 people in each group.

$$n_0 = \frac{2(z_{\frac{\alpha}{2}} + z_{\beta})^2 \sigma^2 (1 + (m-1)\rho)}{md^2} = \frac{2(1.96 + 0.84)^2 81.87^2 (1 + (1)0.5)}{2(45.35)^2} = 38.33 n = n_0 \times \frac{1}{1-f} \cong 43$$

2-2-2. Participants

The study population was T1DM patients aged 4-18 referred to the Endocrine and Metabolic Clinic of 17 Shahrivar Hospital in Rasht, during August 2022 to November 2022. Overall, 97 patients were invited to participate, and finally, 86 met the conditions for entering the study and completed the informed consent form. These patients were randomly divided into two equal groups through a block randomization method with a block size of 4 prepared by a statistician and according to the random sequences designed through the allocation of closed envelopes.

The statistician performed blinding, and the patients and the research team did not know about the blinding procedure and allocating synbiotics or placebos to patients. The first group received insulin and a synbiotic supplement (one sachet of Kidilact) once daily for 12 weeks. The second group received insulin along with a placebo.

2-2-3. Inclusion and exclusion criteria

Inclusion criteria were type 1 diabetes, 4-18 years of age, diabetes duration of at least six months, being treated with insulin, and having completed the informed consent. Patients with concomitant kidney, gastrointestinal, cardiovascular, and lung diseases; taking antibiotics, anti-inflammatory, and immunosuppressive drugs in the last month; consuming products containing probiotics and prebiotics or antioxidants within the previous month; and failure to follow the diet plan were not included.

2-3. Procedure

2-3.1. Preparation of Synbiotic Supplementation and placebo

The intervention group received KidiLact Sachet (Zist Takhmir Co., Iran) with 10^9 Colony Forming Units (CFUs) containing strains of Lactobacillus rhamnosus, Lactobacillus reuteri, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Bifidobacterium infantis, Bifidobacterium brue, Bifidobacterium bifidum, Bifidobacterium lactis, Streptococcus thermophilus, and fructooligosaccharide. The control group received a placebo with similar shape, size, and color. It was made from the combination of lactose monohydrate 80 mesh, inulin, talc, magnesium stearate, colloidal silicon dioxide, sucralose, corn starch, and xanthan gum in the school of Pharmacy at Guilan University of Medical Sciences. Both synbiotic and placebo were provided in powder form in pre-weighed foil packs.

2-3-2. Data collection

At the beginning, the demographic characteristics of patients were recorded, and five ml of blood samples were taken from each participant for routine tests, including FBS (fasting blood sugar), HbA1C (hemoglobin A1c), triglycerides, cholesterol, HDL (High-density lipoprotein), LDL (low-density lipoprotein), and vitamin D. The participants were instructed to mix one sachet with 250 ml of water and drink it 15-20 minutes before the evening meal (Of course, based on the age of the child, the volume of water consumed by children

could be less than 250 ml, but the synbiotic sachet was completely mixed in that amount of water) for 12 weeks. Participants were asked to take only half of the dose for the first two weeks to minimize gastrointestinal side effects, followed by the full dose for the remaining ten weeks. Monthly, a research team member called the participants to encourage consumption compliance and record side effects. At the end of the 12 weeks, five ml blood samples were taken to check the FBS, HbA1C, TG, cholesterol, HDL, LDL, and vitamin D.

2-4. Data analysis

Descriptive statistics such as frequency, percentage, mean, and standard deviation were used to describe the obtained data. In order to check the assumptions of the parametric tests, the normality of the quantitative variables was checked using the Kolmogorov Smirnov test and homogeneity of variances was checked using the Levene's test. In order to analyze the data of the research, paired samples T-test was used, and to adjust the confounding effects, analysis of covariance (ANCOVA) was implemented. It should be added that these calculations were made through IBM statistics version 24 software and the significance level was considered as 0.05 in all tests.

3- RESULTS

A total of 97 patients were evaluated for eligibility and 86 eligible patients were compared in the two groups. The patients were randomly assigned to both insulin and synbiotic supplement (n=43) or insulin and placebo (n=43) groups (**Fig. 1**).

Table 1 shows the patients' baseline characteristics in the two groups. Results showed no significant difference between the groups regarding sex, diabetes duration, and weight. Patients in the placebo group were older than those in the intervention group (p= 0.011). The trend of FBS, HbA1C, TG, cholesterol, HDL, LDL, and vitamin D changes was not significant over time in both groups. As the means of FBS and HbA1C were lower in the intervention group (**Fig. 2** and **Fig. 3**), there was a significant difference in these variables between the two groups (p=0.048 and 0.025, respectively). However, no significant changes in TG, cholesterol, HDL, LDL, and vitamin D, were observed in the two groups (**Table 2**).

4- DISCUSSION

The present study evaluated the effects of symbiotic supplementation in combination with insulin on the blood glucose level, HbA1C, lipid profile, and vitamin D in T1DM children. Our results indicated a significant difference in HbA1c and FBS between the two groups (p=0.048 and 0.025, respectively), and they were lower in the intervention group. However, based on the analysis of covariance, no changes in TG, cholesterol, HDL, LDL, and vitamin D were observed in the two groups. Any changes in diet, gastrointestinal tract infections, or overuse of antibiotics can lead to changes in the gut microbiota and its shift from the normal state, called dysbiosis. Since the gut microbiota can affect the host's immune system, changes in it can lead to inflammatory or autoimmune diseases (19, 20). Although previous studies have shown that any changes in the intestinal microbiome composition or microbial metabolite production might affect T2DM, it was recently demonstrated that it might also affect disease susceptibility of T1DM, amplify inflammatory responses, and hence accelerate the course of T1DM pathogenesis (21).

Recent studies have reported a significant difference between the intestinal microbial profile of T1DM patients and healthy individuals. It has been suggested that the development and progression of T1DM may be correlated with the intestinal microbial profile (22).

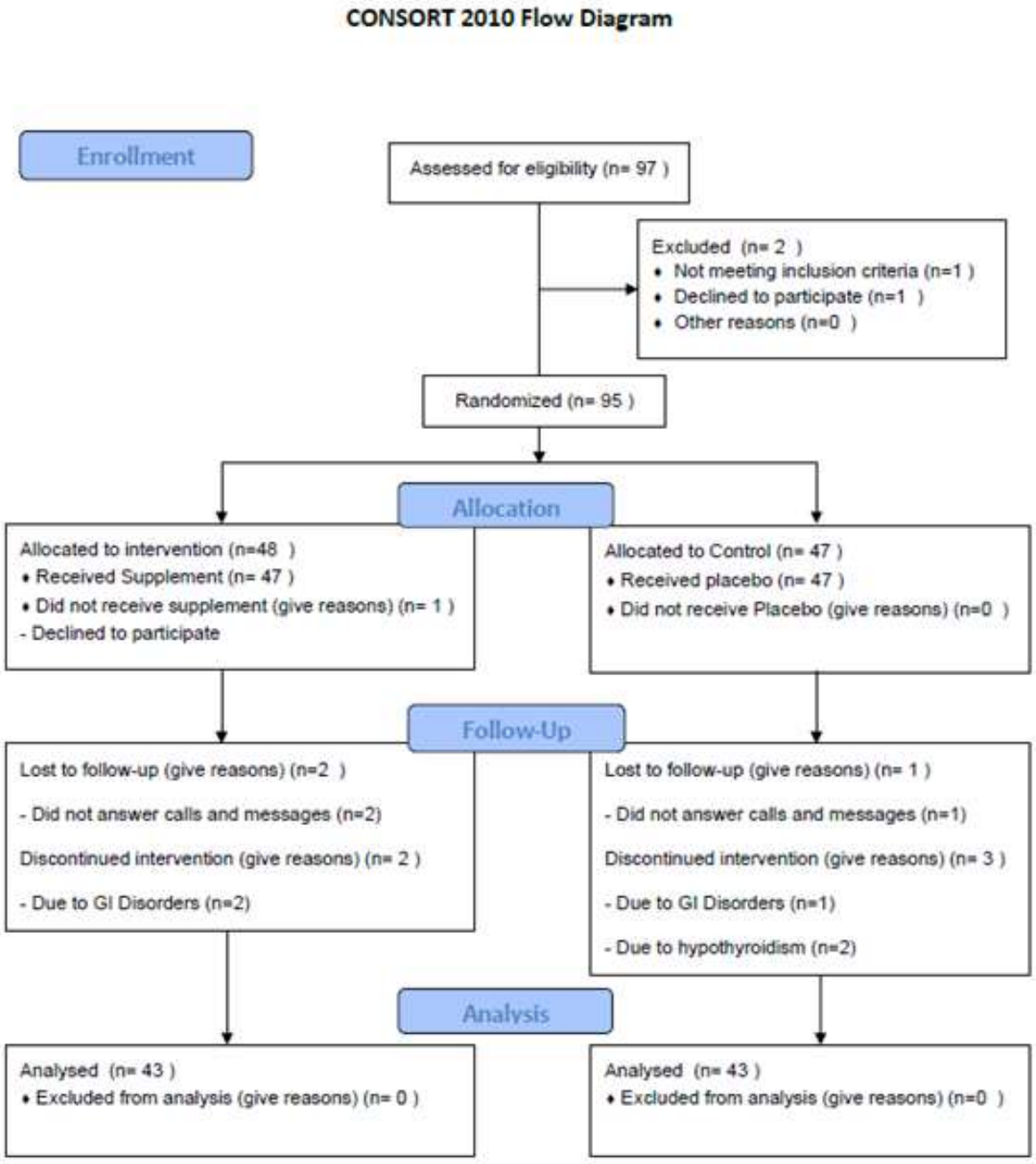

Fig. 1: Consort flow diagram

Table-1: Baseline characteristics of patients

Variables	Intervention group	Placebo group	P-value
Age (year)	10.53 (3.13)	12.4 (2.97)	0.011*
Female N (%)	48.5	58.1	0.443#
Diabetes duration (month)	38.03 (27.56)	35.30 (24.98)	0.350*
Weigh (kg)	41.71 (14.33)	44.08 (16.25)	0.316*

Values are presented as mean (standard deviation) or as percentages; P-values are the result of comparing two study groups; *: Independent Samples Test; #: Chi-Square test

Table-2: The changes of variables over time in the two groups

Variables		Intervention group	Placebo group	P-value
FBS	baseline	160.55 (50.81)	156.60 (59.30)	0.025#
	end	160 (75.57)	176.50 (77.50)	
	P-value	0.973*	0.327*	
HbA1C	baseline	7.04 (1.23)	7.63 (1.65)	0.048#
	end	6.84 (0.96)	7.30 (0.98)	
	P-value	0.603*	0.432*	
Triglycerides	baseline	100.14 (57.45)	97.50 (44.54)	0.291#
	end	119.29 (121.99)	97.88 (32.69)	
	P-value	0.511*	0.974*	
Cholesterol	baseline	164 (39.92)	156 (27.65)	0.291#
	end	164.50 (26.53)	158.43 (31.91)	
	P-value	0.961*	0.648*	
LDL	baseline	80.50 (16.89)	96.34 (29.84)	0.140#
	end	89.50 (17.93)	92 (28.79)	
	P-value	0.095*	0.349*	
HDL	baseline	49.57 (9.96)	44.30 (7.54)	0.952#
	end	52 (14.13)	46.67 (11.91)	
	P-value	0.580*	0.665*	
Vitamin D	baseline	41.10 (20.92)	32.72 (15.90)	0.557#
	end	40.93 (20.10)	45.82 (19.24)	
	P-value	0.983*	0.157*	

Values are presented as mean (standard deviation); FBS: fasting blood sugar; HbA1C: hemoglobin A1c; HDL: High-density lipoprotein; LDL: low-density lipoprotein; *: Paired Samples Test; #: ANCOVA

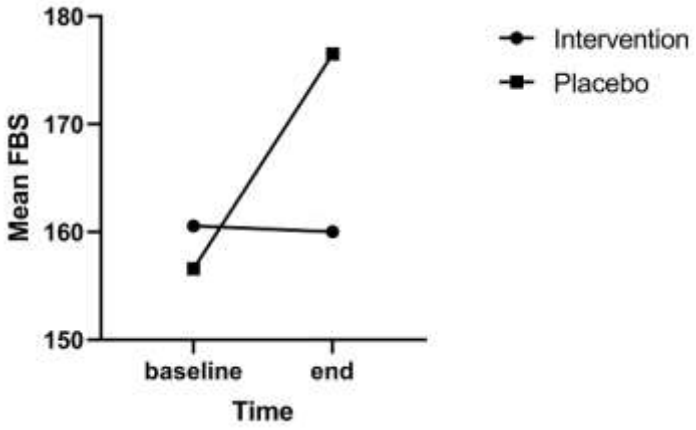

Fig. 2: Trend of FBS changes in intervention and placebo group

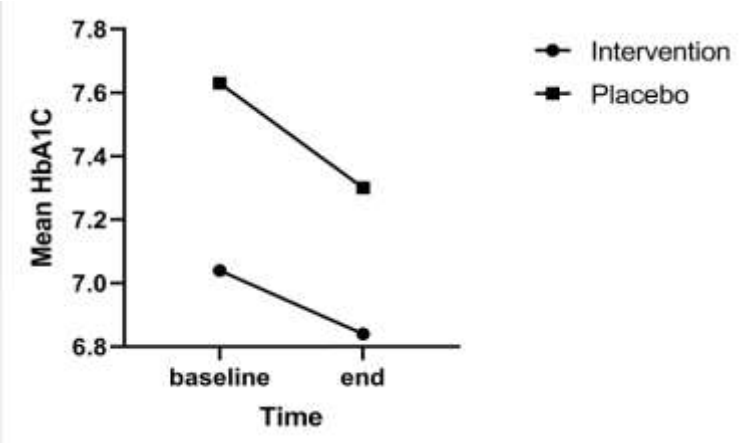

Fig. 3: Trend of HbA1C changes in intervention and placebo group

Murri et al. concluded that Actinobacteria, Firmicutes, Lactobacillus, Bifidobacterium, Blautia coccoides/Eubacterium rectal, Prevotella, and the ratio of Firmicutes to Bacteroidetes were lower, and the number of Bacteroidetes, Clostridium, and Veillonella were higher in T1DM patients than in healthy individuals. Also, they found that the amount of Bifidobacterium, Lactobacillus, and the ratio of Firmicutes to Bacteroidetes had a significant negative correlation with the plasma glucose level. In contrast, the amount of Clostridium had a significant positive correlation with the plasma glucose level in T1DM (23).

Therefore, microbiota-based treatment to slow down disease progression or reverse T1DM has been taken into consideration. Including prebiotics and probiotics in the diet can improve gut permeability, decrease inflammation, and be useful for T1DM (24). A meta-analysis by Wang et al. concluded that in patients with T2DM and Gestational diabetes, probiotic /prebiotic/ synbiotic supplementation could be effective and helpful in improving glycemic profiles. Still, they did not affect T1DM patients (25). On the other hand, It has also been shown that using probiotics or prebiotics alone can improve glycemic parameters in T1DM (26). Javid et al.'s study, which was the first study on the effect of synbiotic supplements on T1DM, proved that synbiotics could effectively improve glycemic and inflammatory parameters in these patients (18). This result was in accordance with the findings of our study.

Previous studies have suggested some mechanisms to explain synbiotics effects on diabetes. Synbiotics have been reported to increase GLP-1, which decreases blood sugar, and GLP-2, which decreases intestinal permeability. Totally, they lead to hypoglycemia, HbA1c improvement, and weight loss (27). Synbiotics can replace the aggravating bacteria in the intestine, which can improve insulin resistance and lipid profiles (28). They can also increase lipolytic activity by producing carbon disulfide, methyl acetate, and short-chain fatty acids such as butyrate (29, 30). Increasing butyrate also could modulate glucose metabolism, leading to increased insulin sensitivity and decreased plasma glucose levels (30). So, according to the above mechanisms, synbiotics can improve the effects of endogenous insulin in T2DM and exogenous insulin in T1DM.

Similar to Asemi et al. (29), Ebrahimi et al.(31), and Javid et al.(18), our study found that synbiotics cannot significantly affect lipid profiles. However, contradictory to our results, another study has shown that synbiotics can improve

HDL-C levels (32). The type and quantity of bacteria used in studies, the target population, and the intervention duration might justify the contradictions observed in different studies.

HbA1C is a reliable parameter to assess T1DM patients; and the preset study indicated that synbiotics could improve its level. Recent studies have suggested glycemia metrics such as Time In Range (TIR) as useful factors for assessing the T1DM patient's condition and risk of complications. The metric TIR refers to the percentage of time that glucose concentrations are between 70 and 180 mg/dL (33). Although TIR and HbA1C have a reasonable correlation (34), this issue does not diminish the importance of measuring TIR in clinical studies; because a patient's HbA1C may be normal, but his TIR may not be within the normal range. It could indicate a higher risk of diabetes complications, which would be missed by measuring HbA1C alone (33). We did not measure TIR in our study, and of course, it was one of the limitations of this study, which is suggested to be considered in future studies.

4-1. Limitations of the study

This study had several limitations that should be considered in interpreting the data. First, it had a small sample size, and the findings need confirmation in larger RCTs with adequate follow-up duration and sample size. Second, we did not detect children's diet and the amount of physical activity. Finally, we did not measure the level of incretins, short-chain fatty acids, or other things that relate gut microbiota to T1DM.Thus, such possible mechanisms involved are to be proved in further studies.

5- CONCLUSION

Our study showed that insulin treatment in combination with a synbiotic supplement had better outcomes than insulin therapy alone. It is suggested that symbiotic supplementation can improve FBS and HbA1C in T1DM children, though it had no effects on lipid profile and vitamin D levels. We recommended larger RCTs with adequate follow-up duration and sample size to confirm these findings.

6- ETHICAL CONSIDERATIONS

This study was approved by the ethics committee of Guilan University of Medical Sciences (Number: IR.GUMS.REC.1400.595, Date: 2022-02-23). The study protocol was registered in the Iranian registry of clinical trials (Number: IRCT20210712051866N1, Date: 2022-05-04). Written Informed consent was obtained from all patients, parents, or guardians in this study, to whom the study procedure and possible risks were clearly explained.

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8- AUTHORS' CONTRIBUTIONS

S.D. Conceptualization, Methodology, Supervision; G.S.A. Methodology; S.K. Supervision; M.S. Data Curation, Investigation; M.A.E. Writing the manuscript, Methodology; J.A. Investigation; S.A.N. Investigation; A.H.R. Formal Analysis; H.H. Project Administration, Supervision

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