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Effect of Probiotic Supplementation on Glycemic and Inflammatory Markers in Children with Type 1 Diabetes: A Double-Blind Clinical Study

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Abstract

Background: Type 1 diabetes mellitus (T1DM) is a prevalent metabolic disorder in childhood, marked by autoimmune destruction of pancreatic β-cells leading to insulin deficiency, hyperglycemia, and chronic inflammation. Achieving optimal glycemic control in children remains a challenge despite insulin therapy. Probiotics, with their immunomodulatory and metabolic benefits, have recently emerged as potential adjunctive agents for improving glycemic and inflammatory outcomes in T1DM.

Objectives: This study aimed to evaluate the effects of oral probiotic supplementation on glycemic control, inflammatory markers, and metabolic parameters in children with T1DM.

Methods: In this randomized, double-blind, placebo-controlled clinical trial, 60 children with type 1 diabetes mellitus were randomly assigned to receive either BioDiab® probiotic capsules (3×10^9 CFU/capsule) containing Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium lactis, Bifidobacterium bifidum, Bifidobacterium longum, and Bacillus coagulans or identical placebo capsules daily for three months. HbA1c, high-sensitivity C-reactive protein (hs-CRP), insulin dose, lipid profile, anthropometric, thyroid, and renal parameters were assessed at baseline and at 3, 6, and 9 months. Data were analyzed using repeated measures ANOVA and other appropriate statistical tests.

Results: HbA1c levels significantly decreased in the probiotic group compared with an increase in the placebo group (P=0.017). Median hs-CRP levels demonstrated a downward trend in the probiotic group, with significant within-group changes over time (P=0.026), though intergroup differences were not statistically significant (P>0.05). No significant effects were observed on lipid profile, thyroid indices, or daily insulin dose. However, blood urea nitrogen (BUN) levels were significantly lower in the probiotic group at 6-month follow-up (P=0.027).

Conclusion: Oral probiotic supplementation in children with T1DM improved glycemic control and modulated inflammatory markers without adverse effects. While other metabolic parameters remained unchanged, probiotics may serve as a safe and beneficial adjunct to conventional diabetes management.

Key Words: Glycemic control, Inflammation, Probiotics, Type 1 diabetes mellitus.

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1- INTRODUCTION

Type 1 diabetes mellitus (T1DM) is one of the most common autoimmune diseases in childhood, characterized by immune-mediated destruction of pancreatic β-cells and lifelong dependence on insulin therapy (1, 2). Despite advances in insulin treatment and glucose monitoring technologies, achieving optimal glycemic control remains challenging, and many children continue to experience chronic low-grade inflammation and an increased risk of long-term vascular complications (3-11). Growing evidence suggests that alterations in the gut microbiota contribute to the pathogenesis and progression of T1DM. Intestinal dysbiosis may impair gut barrier integrity, increase microbial translocation, and promote immune activation, thereby contributing to systemic inflammation and autoimmune responses (12-23). Studies have demonstrated reduced abundance of beneficial bacteria, including *Lactobacillus* and *Bifidobacterium*, together with decreased production of short-chain fatty acids, changes that may adversely affect immune regulation and metabolic homeostasis in children with T1DM (24-26). Probiotics have therefore attracted considerable interest as a potential adjunctive therapy for T1DM because of their ability to restore gut microbial balance, enhance epithelial barrier function, and modulate both innate and adaptive immune responses (19, 27). Experimental studies have demonstrated favorable effects of probiotic supplementation on glucose metabolism, oxidative stress, and inflammatory pathways (4, 28, 29). In addition, several randomized clinical trials have reported improvements in glycemic control and selected metabolic parameters following supplementation with *Lactobacillus*- and *Bifidobacterium*-containing probiotics or synbiotics in patients with diabetes. However, the magnitude and consistency of these effects remain variable (2, 14, 20, 30, 31). Differences in probiotic strains, dosages, intervention duration, and study populations may partly explain these inconsistent findings (1, 15, 26, 32, 33). Although the therapeutic potential of probiotics has been extensively investigated in type 2 diabetes, evidence in children and adolescents with T1DM remains limited, and their effects on inflammatory markers and other metabolic outcomes are still unclear (2,15,21). Therefore, the present randomized, double-blind, placebo-controlled clinical trial was conducted to evaluate the effects of oral probiotic supplementation on glycemic control, inflammatory markers, and selected metabolic parameters in children and adolescents with T1DM.

2- MATERIALS AND METHODS

2-1. Study Design

This randomized, double blind clinical trial was conducted in the Pediatric Endocrinology and Metabolism Clinic of Akbar Hospital, Mashhad University of Medical Sciences, Iran. Recruitment started after approval by the institutional ethics committee and registration in the Iranian Registry of Clinical Trials (IRCT20240617062159N1). Participants were followed for 9 months.

2-2. Sample Size

Sample size was calculated using G-Power software based on a previous study (2), with HbA1c as the main outcome. With $\alpha=0.05$, power=80%, and effect size d=0.8, 26 participants per group were required. Considering a 20% dropout rate, 30 participants per group (n=60 total) were recruited. Ultimately, 106 patients were screened; 60 were randomized, with 1 dropout in the probiotic group and 7 in the placebo group (Figure 1).

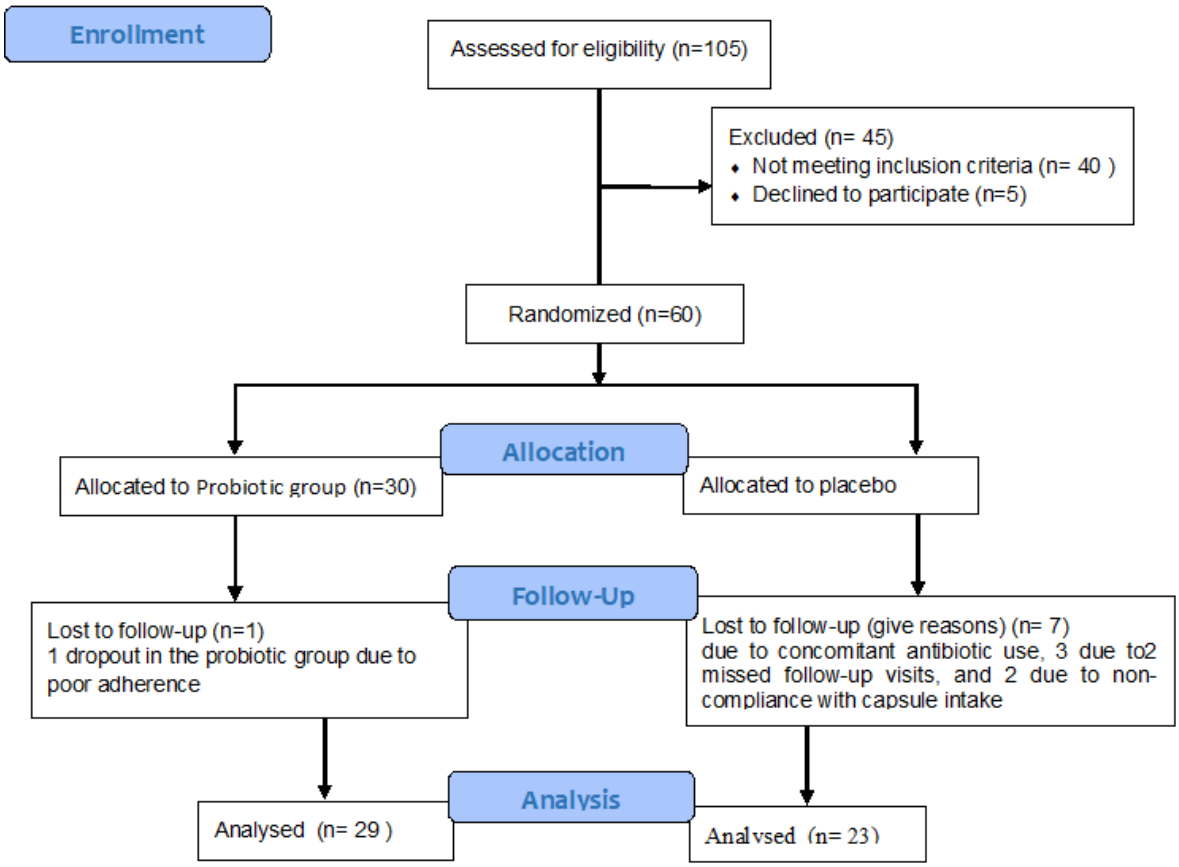

Figure-1: CONSORT 2010 flow diagram.

2-3. Participants

Eligible participants were children aged 6 to 18 years with a confirmed diagnosis of T1DM for at least two years, beyond the honeymoon phase. Diagnosis was established by a pediatric endocrinologist using clinical criteria and autoantibodies (ICA, GAD, IAA). Inclusion criteria were: HbA1c between 6.5% and 10%, willingness and ability to continue treatment, and absence of severe renal, gastrointestinal, or pulmonary diseases. Exclusion criteria included discontinuation of study medication for ≥1 week, adverse drug reactions, irregular follow-up, new comorbidities affecting outcomes, or need for antibiotics/anti-inflammatory or immunosuppressive drugs during the study.

2-4. Randomization and Blinding

Consecutive sampling was applied, and 60 eligible patients were randomly allocated to probiotic (n=30) or placebo (n=30) groups using balanced block randomization (blocks of six) generated by Random Allocation Software. Allocation concealment was ensured using opaque, sealed, numbered envelopes. The trial was double blinded: participants, investigators, and outcome assessors were unaware of group assignments.

2-5. Intervention

The intervention group received one BioDiab probiotic capsule daily for 3 months (3×10^9 CFU, Tak-Gen Darou Co.), containing Lactobacillus acidophilus, L. casei, Bifidobacterium lactis, B. bifidum, B. longum, and Bacillus coagulans. The control group received identical placebo capsules.

2-6. Outcomes

Primary outcomes were glycemic control (HbA1c) and inflammatory status (Hs-CRP). Secondary outcomes included

lipid profile (cholesterol, triglycerides), anthropometrics (BMI percentile), thyroid function (TSH, T4), and renal function (BUN, creatinine). Assessments were performed at baseline, 3, 6, and 9 months. All laboratory tests were performed in the same reference laboratory (Mashhad Jihad Daneshgahi) to ensure consistency.

2-7. Compliance and Safety Monitoring

Adherence was assessed by capsule counts at each visit. Adverse events (e.g., bloating, diarrhea, skin rash) were monitored using a standard checklist and reported to the study physician.

2-8. Ethical Considerations

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1403.191; approval date: June 18, 2024) and registered in the Iranian Registry of Clinical Trials (IRCT20240617062159N1; registration date: August 29, 2024). Written informed consent was obtained from all participants and/or their guardians. Confidentiality of patient data was maintained throughout the study.

2-9. Statistical Analysis

Data were analyzed with SPSS V.26.0. Continuous variables were expressed as mean±SD, categorical variables as frequency and percentage. Between-group comparisons used independent t-tests or Mann-Whitney U tests as appropriate. Within-group changes over time were analyzed with repeated-measures ANOVA. Categorical variables were compared using chi-square tests. Linear regression was applied to assess correlations. A two-tailed $P<0.05$ was considered statistically significant.

3-RESULT

3-1. Demographic and Anthropometric Characteristics

A total of 52 patients with type1 diabetes completed the trial (29 in the probiotic group and 23 in the placebo group). The two groups were comparable in baseline demographic characteristics, including mean age, age at diagnosis, disease duration, and sex distribution, with no statistically significant differences. Anthropometric indices also remained similar across groups. Mean height increased slightly in both groups during the intervention, but between-group differences were not significant. Likewise, mean weight showed a mild upward trend in both groups, without statistically meaningful differences. The percentage of underweight patients was comparable (10.3% in placebo vs. 8.7% in probiotic). BMI percentiles before and after the intervention also showed no significant differences, confirming baseline homogeneity between the two groups (Table1).

Table-1. Demographic characteristics and anthropometric indicators in the two groups.

Variable			Placebo Group (Mean ± SD)	Probiotic Group (Mean ± SD)	p-value
Age(years)			0.654	11.17±3.17	10.75± 3.38
Duration of diabetes (years)			0.361	11.17±3.17	3.58± 2.14
sex	**Female n(%)**		16(55.2)	13(56.5)	0.922
	Male n(%)		13(44.8)	10(43.5)	
Height (cm)	**Baseline**		140.65 ± 16.54	137.37 ± 19.48	0.582
	After Intervention		143.60 ± 15.81	140.08 ± 18.75	0.531
Weight (kg)	**Baseline**		36.97 ± 12.95	33.10 ± 12.64	0.664
	After Intervention		40.30 ± 12.51	35.24 ± 11.96	0.628
BMI Percentile	**Baseline**	**Under Weight**	3(10.3)	2(8.7)	0.956
		Normal	24(82.8)	19(82.6)	
		Over weight	2(6.9)	2(8.7)	

3-2. Inflammatory Marker (Hs-CRP)

Table 2 presents the comparison of hs-CRP levels between the probiotic and placebo groups at four consecutive time points. The median (IQR) levels of hs-CRP showed a decreasing trend in the probiotic group over time, whereas in the placebo group, values tended to remain stable or slightly increase. Based on the Mann-Whitney U test, no statistically significant difference was observed between the two groups at any measurement point ($p > 0.05$). However, the Friedman test revealed a significant change in hs-CRP levels over time within the probiotic group ($p=0.026$), indicating a within-group reduction after the intervention (Table2).

3-3. Glycemic Control (HbA1c)

HbA1c values demonstrated distinct trends in the two groups. In the probiotic group, a mild but consistent reduction was observed across four consecutive measurements, declining from 8.85± 1.29 at baseline to 8.59 ± 1.23 after the intervention. In contrast, the placebo group exhibited an increase from 9.18 ± 1.09 at baseline to 9.81 ± 1.87 post intervention. The coefficient of variation was lower in the probiotic group (12–14%) than in the placebo group (12–19%), indicating greater stability of glycemic control. Repeated measures ANOVA confirmed a significant group effect ($F= 6.059$, $P= 0.017$, $\eta^2= 0.108$) as well as a significant time × group interaction ($F= 3.377$, $P= 0.020$, $\eta^2= 0.169$). Linear contrast analysis further supported these findings ($F= 4.718$, $P= 0.035$), suggesting that probiotics contributed to a stable and sustained reduction in HbA1c, while the placebo group experienced progressive worsening of glycemic control (Table 3, Figure 2).

Serum triglyceride concentrations showed no significant differences between groups, with both groups remaining stable throughout the study. Similarly, serum cholesterol levels decreased slightly in the probiotic group but increased modestly in the placebo group. The between-group difference after intervention (approximately 18.9 mg/dL) did not reach statistical significance ($P= 0.078$).

Table-2. Comparison of Hs-CRP levels at four consecutive time points in the probiotic and placebo groups.

Time	Probiotic median(IQR)	Placebo median(IQR)	p-value*
Hs-CRP			
pre- intervention	0.20 (0.10-0.30)	0.20 (0.10-0.30)	0.772
post- intervention (3 months)	0.15 (0.10-0.25)	0.16 (0.10-0.30)	0.224
post- intervention (6 months)	0.15 (0.10-0.25)	0.18 (0.10-0.30)	0.202
post- intervention (9 months)	0.15 (0.10-0.26)	0.20 (0.10-0.45)	0.268
p-value**	0.026	0.261	

* Mann-Whitney U; ** Friedman Test

Table-3. Comparison of HbA1C at four consecutive time points in the probiotic and Placebo.

Time	Probiotic (Mean ± SD)	Placebo (Mean ± SD)	Mean Difference	CV (%)
pre- intervention HbA1C	8.85 ± 1.29	9.18 ± 1.09	-0.33	14.6 / 11.9
post- intervention HbA1C (3 months)	8.73 ± 1.07	9.70 ± 1.89	-0.97	12.2 / 19.5
post- intervention HbA1C (6 months)	8.71 ± 1.20	9.75 ± 1.88	-1.04	13.8 / 19.3
post- intervention HbA1C (9 months)	8.59 ± 1.23	9.81 ± 1.87	-1.22	14.3 / 19.1

- The coefficient of variation (CV) was calculated using the formula SD / Mean × 100.
- The CV values in the table are written in the order of probiotic group /placebo group.

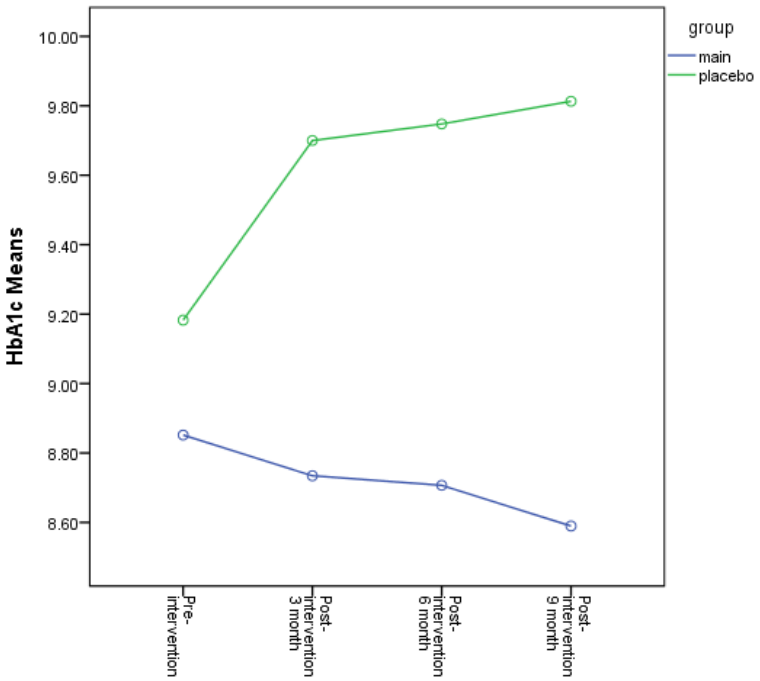

Figure-2: Comparison of mean HbA1C during the study between the two groups.

Despite the absence of significant effects, the trend suggests a possible cholesterol-lowering effect of probiotics that may require confirmation in larger samples.

3-4. Thyroid Function and Renal Function

Thyroid hormone levels, including T4 and TSH, did not differ significantly between groups at baseline or after the intervention. While mean T4 values showed only minor fluctuations, TSH levels exhibited wider variability, particularly in the probiotic group post intervention. The high variability (CV up to 323%) limits the robustness of these findings and indicates the need for further studies to clarify the potential effect of probiotics on thyroid function.

Serum creatinine levels were comparable in both groups before and after intervention, with no significant differences (P> 0.7). However, mean BUN levels showed a significant reduction in the probiotic group compared to placebo after the intervention (11.29 vs. 12.61 mg/dL, P= 0.027). This finding suggests that probiotic supplementation may have a modest but beneficial effect on renal function in children with type 1 diabetes (Table 4).

4- DISCUSSION

This randomized, double-blind, placebo-controlled clinical trial evaluated the effects of three months of probiotic supplementation, with follow-up assessments extending to nine months, on glycemic control, inflammatory markers, and selected metabolic parameters in children and adolescents with T1DM. The findings demonstrated that probiotic supplementation was associated with a gradual reduction in HbA1c, and this beneficial effect was maintained throughout the follow-up period. In contrast, HbA1c showed an increasing trend in the placebo group. In addition, hs-CRP demonstrated a favorable within-group trend in the probiotic group, whereas lipid profile, thyroid function, creatinine, and daily insulin dose remained largely unchanged. A modest reduction in BUN was also observed following probiotic supplementation.

Table-4. Comparison of laboratory parameters in the two study groups.

Variable	Probiotic (Mean ± SD)	Placebo (Mean ± SD)	Mean Difference	CV (%)	95% Confidence Interval of the Difference	P-value
Triglyceride						
pre- intervention	75.138 ± 38.94	74.722 ± 16.23	0.41	51.8	-19.10, 19.93	0.966
post- intervention	76.179 ± 35.37	80.889 ± 22.51	-4.7	46.4	-16.11, 16.94	0.960
Cholesterol						
pre- intervention	140.690 ± 26.02	154.625 ± 23.77	-13.93	18.5	-29.80, 1.93	0.084
post- intervention	139.138 ± 27.44	158.000 ± 24.36	-18.86	19.7	-29.50, 1.64	0.078
t4						
pre- intervention	7.900 ± 1.72	8.639 ± 1.80	-0.739	21.8	-1.72, 0.24	0.138
post- intervention	7.831 ± 1.67	8.174 ± 1.38	-0.342	20.7	-1.73, 0.25	0.140
tsh						
pre- intervention	2.190 ± 1.96	2.638 ± 2.05	-0.448	89.5	-4.32, 10.62	0.402
post- intervention	5.473 ± 17.70	2.326 ± 2.11	3.14	323	-3.64, 9.93	0.351
Creatinine (Cr)						
Pre-intervention	0.657 ± 0.174	0.666 ± 0.144	-0.009	26.5	-0.099, 0.082	0.847
Post-intervention	0.675 ± 0.168	0.689 ± 0.121	-0.015	24.9	-0.098, 0.069	0.726
BUN						
Pre-intervention	12.752 ± 4.86	12.783 ± 3.93	-0.031	38.1	-2.54, 2.48	0.980
Post-intervention	11.286 ± 1.82	12.609 ± 2.25	-1.322	16.1	-2.49, -0.16	0.027

The improvement in HbA1c observed in the probiotic group is consistent with previous randomized clinical trials in patients with T1DM. Shabani-Mirzaee et al. (2) reported that oral probiotic supplementation significantly reduced HbA1c levels in children with T1DM, although no significant improvements were observed in inflammatory or lipid parameters. Likewise, Zhang et al. (14) demonstrated that multispecies probiotic supplementation improved glycemic control and metabolic profile in adults with T1DM. Similarly, Zare Javid et al. (20) found that synbiotic supplementation improved glycemic status and reduced oxidative stress biomarkers in patients with T1DM. Collectively, these findings support the hypothesis that modulation of the intestinal microbiota may contribute to improved metabolic control in individuals with T1DM.

Several biological mechanisms may explain the beneficial effects of probiotics on glycemic control. Restoration of gut microbial balance may improve intestinal barrier integrity, reduce endotoxin translocation, attenuate systemic inflammation, and enhance immune regulation (19, 26). In addition, probiotic bacteria produce short-chain fatty acids that improve epithelial function and may influence glucose metabolism through modulation of gut-derived hormones and immune pathways (25, 26). Although the present study did not directly evaluate gut microbiota composition or microbial metabolites, these mechanisms may partly explain the observed reduction in HbA1c.

Interestingly, HbA1c showed an increasing trend in the placebo group during follow-up. Because no active intervention was administered to this group, this finding may reflect the natural variability of glycemic control in children and adolescents with T1DM. Pubertal hormonal changes, fluctuations in insulin requirements, dietary habits, physical activity, and treatment adherence may all influence HbA1c over time. Since these variables were not specifically assessed during the present study, no causal relationship can be established.

The present study also demonstrated a favorable trend in hs-CRP levels within the probiotic group. Although the between-group difference did not reach statistical significance, the significant within-group reduction suggests that probiotics may exert modest anti-inflammatory effects. Similar findings have been reported in previous studies evaluating probiotics or synbiotics in diabetes, in which improvements in inflammatory biomarkers were generally smaller and less consistent than improvements in glycemic indices (20, 29, 34). The relatively small sample size and low baseline hs-CRP concentrations in our participants may also have limited the ability to detect statistically significant between-group differences.

No significant effects were observed on serum triglycerides, total cholesterol, thyroid hormones, creatinine, or daily insulin dose. Although total cholesterol increased modestly in the placebo group, the between-group difference was not statistically significant. This increase may be related to the deterioration of glycemic control observed during follow-up, as poorer glycemic control has been associated with less favorable lipid metabolism in patients with T1DM (5, 9). Nevertheless, because dietary intake and lifestyle-related factors were not evaluated, this finding should be interpreted cautiously. The significant reduction in BUN observed after probiotic supplementation may indicate a modest beneficial effect on renal metabolic status; however, considering the absence of changes in serum creatinine and the relatively short intervention period, this finding requires confirmation in larger studies.

Our findings are biologically plausible and are supported by growing evidence regarding the role of the gut microbiota in T1DM pathogenesis. Previous studies have shown that intestinal dysbiosis contributes to immune dysregulation, increased intestinal permeability, and chronic inflammation, all of which are involved in the development and progression of T1DM (13, 15, 19, 24, 35). Probiotic supplementation may restore microbial homeostasis, improve epithelial barrier function, and regulate immune responses, thereby contributing to better metabolic control (19, 26, 27).

Table-5. Summary of major clinical studies evaluating probiotic supplementation in patients with diabetes mellitus.

Study	Population	Probiotic strain(s)	Duration	Main findings
Shabani-Mirzaee et al., 2023 (2)	Children with T1DM	Multi-strain probiotic (*Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum, B. lactis*)	12 weeks	Significant reduction in HbA1c; no significant changes in inflammatory markers
Zhang et al., 2023 (14)	Adults with T1DM	Multi-species probiotic	12 weeks	Improved HbA1c, fasting glucose, and metabolic profile
Zare Javid et al., 2020 (20)	Patients with T1DM	Synbiotic preparation	8 weeks	Improved glycemic status and oxidative stress biomarkers
Firouzi et al., 2017 (36)	Adults with T2DM	Multi-strain probiotics	6 weeks	Reduced fasting glucose and insulin resistance
Present study	Children with T1DM	*Lactobacillus acidophilus, L. casei, Bifidobacterium lactis, B. bifidum, B. longum, Bacillus coagulans*	3 months (9-month follow-up)	Reduced HbA1c, improved inflammatory trend (hs-CRP), no significant effect on lipid profile or thyroid function

However, differences among studies regarding probiotic strains, dosages, duration of supplementation, patient characteristics, and baseline microbiota composition probably explain the variability of reported outcomes (32, 34).

This study has several limitations. First, the sample size was relatively small, which may have limited the statistical power to detect significant changes in some secondary outcomes. Second, eight participants were lost during follow-up, most of whom belonged to the placebo group; therefore, a per-protocol analysis was performed. Third, dietary intake, physical activity, pubertal status, and gut microbiota composition were not assessed, precluding evaluation of the mechanisms underlying the observed effects. Nevertheless, the randomized, double-blind, placebo-controlled design, together with the extended follow-up period, strengthens the validity of the present findings.

4-1. Clinical Implications

Based on the results, oral probiotic supplementation can be considered a safe and noninvasive complementary strategy in the management of type 1 diabetes. The observed gradual reduction in HbA1C and stabilization of Hs-CRP suggest a supportive role of probiotics in glycemic control and modulation of inflammatory responses. These findings may serve as scientific evidence for pediatricians, pediatric endocrinologists, and diabetes care teams in optimizing individualized treatment plans. However, probiotics should not replace insulin therapy and must be used only as an adjunct. Selection of probiotic strains, dosage, and duration should be evidence-based and tailored to each patient's clinical condition.

Overall, these results may contribute to the development of integrated therapeutic approaches aimed at improving glycemic control and reducing long-term complications of type 1 diabetes, while highlighting the potential role of gut microbiota and probiotic supplementation in enhancing patient health.

4-2. Limitations

This study has several limitations. First, the sample size was relatively small, which may have limited the statistical power for detecting changes in some secondary outcomes. Second, eight participants were lost during follow-up, most of whom belonged to the placebo group; therefore, a per-protocol analysis was performed. Third, dietary intake, physical activity, and gut microbiota composition were not assessed, precluding evaluation of the mechanisms underlying the observed effects. Despite these limitations, the randomized double-blind design and the 9-month follow-up strengthen the validity of our findings.

5- CONCLUSION

In conclusion, this randomized, double-blind clinical trial demonstrated that three months of probiotic supplementation improved glycemic control in children and adolescents with type 1 diabetes, and this beneficial effect was maintained throughout the 9-month follow-up period. Probiotic supplementation was also associated with a favorable trend in inflammatory status, while no significant effects were observed on lipid profile, thyroid function, renal parameters, or daily insulin dose. These findings suggest that probiotics may serve as a safe and promising adjunct to standard insulin therapy in children with type 1 diabetes. Larger multicenter trials are warranted to confirm these findings and identify the most effective probiotic strains and treatment duration.

6- ACKNOWLEDGEMENT

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7- FUNDING

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8- ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1403.191; approval date: June 18, 2024). In the present study, all procedures were conducted in adherence to the pertinent guidelines and regulations, particularly the Declaration of Helsinki. Before their involvement, informed consent was procured from all participants. Participants were explicitly informed about their voluntary participation and their right to withdraw from the study at any stage. To ensure confidentiality and privacy, questionnaires were anonymously completed, employing unique identifiers for each respondent.

9- AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the research deputy of Mashhad University of Medical Sciences, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the corresponding author upon reasonable request and with the permission of the research deputy of Mashhad University of Medical Sciences.

10- COMPETING INTERESTS

The authors declare that they have no financial and non-financial competing interests.

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