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EFFECTIVENESS OF PROBIOTIC SUPPLEMENTATION IN ENHANCING IRON THERAPY FOR IRON DEFICIENCY ANEMIA IN CHILDREN AGED 1 TO 15 YEARS: A RANDOMIZED CONTROLLED TRIAL

DR. CHETHAN S *, DR. RADHA KUMAR[1], DR. CHAUDHARY DEVANAND GULAB[2], DR. SHAMI KUMAR[3], DR. MAITHREE MAHESH[4], DR. NAVIN UMAPATHY[5], DR. PRAKASH. D[6]

[1,2,3,4,5]INSTITUTION: DEPARTMENT OF PAEDIATRICS, SAVEETHA INSTITUTE OF MEDICAL SCIENCES.
[6]PROFESSOR, DEPARTMENT OF ORAL & MAXILLOFACIAL SURGERY , SREE BALAJI DENTAL COLLEGE & HOSPITAL, CHENNAI, INDIA

Abstract

Background:

Iron deficiency anemia (IDA) remains a leading cause of childhood morbidity worldwide. While oral iron therapy is the standard treatment, its efficacy is often limited by gastrointestinal side effects and suboptimal absorption. Probiotics, particularly *Lactobacillus rhamnosus GG*, may improve iron uptake and gastrointestinal tolerance, but pediatric data remain limited.

Objective:

To evaluate the efficacy of probiotic (*Lactobacillus rhamnosus GG*) supplementation as an adjunct to oral iron therapy in improving hematological outcomes and reducing gastrointestinal side effects in children with IDA.

Methods:

In this prospective, randomized controlled trial, 100 children aged 1–15 years with confirmed IDA were randomized to receive either oral ferrous sulfate alone or ferrous sulfate plus *Lactobacillus rhamnosus GG* for 8 weeks. Primary outcome was change in hemoglobin concentration. Secondary outcomes included changes in serum ferritin, symptom resolution, and gastrointestinal side effects. Statistical comparisons were made using t-tests, chi-square tests, and multivariate logistic regression.

Results:

Both groups were comparable at baseline. After 8 weeks, the probiotic + iron group showed a significantly greater increase in hemoglobin (mean rise: 3.4 ± 0.8 g/dL vs. 2.1 ± 0.9 g/dL; $p < 0.001$) and serum ferritin (Δ = +15.4 ng/mL vs. +9.4 ng/mL; $p < 0.001$). Clinical symptoms such as fatigue, appetite loss, pallor, and headache resolved more frequently in the probiotic group ($p < 0.05$). Incidence of abdominal pain and constipation was significantly lower in the probiotic group. Multivariate analysis identified probiotic use as the sole significant predictor of hemoglobin rise >3 g/dL (aOR = 4.6; $p < 0.001$).

Conclusion:

Probiotic supplementation with *Lactobacillus rhamnosus GG* significantly enhances hematologic response and reduces gastrointestinal side effects in children receiving iron therapy for IDA. This combined approach offers a safe, effective, and well-tolerated strategy to optimize pediatric anemia management, particularly in resource-limited settings.

Keywords:

Iron deficiency anemia, probiotics, *Lactobacillus rhamnosus GG*, pediatric, hemoglobin, iron supplementation, randomized controlled trial

INTRODUCTION

Iron deficiency anemia (IDA) is the most prevalent micronutrient deficiency globally, with children in low- and middle-income countries bearing the greatest burden. It is estimated that more than 40% of children under five years of age are anemic worldwide, with nutritional iron deficiency accounting for the majority of cases (1,2). The consequences of untreated IDA in childhood are profound, encompassing impaired cognitive and psychomotor

development, decreased academic performance, heightened susceptibility to infections, and overall poor growth and wellbeing (3,4).

Although oral iron supplementation remains the cornerstone of IDA management due to its low cost and wide availability, its effectiveness is often compromised by poor adherence resulting from gastrointestinal side effects such as nausea, abdominal pain, constipation, and diarrhea (5). In addition, iron absorption is influenced by various host-related and environmental factors, including the composition and integrity of the intestinal microbiota (6). Dysbiosis—common in undernourished or anemic children—can hinder iron uptake, while pathogenic overgrowth may exacerbate intestinal inflammation and promote iron sequestration (7).

Probiotics—live microorganisms that confer health benefits—have recently gained attention as a supportive strategy to enhance iron therapy. Certain strains, particularly *Lactobacillus rhamnosus GG*, have demonstrated the ability to improve gut mucosal integrity, reduce intestinal inflammation, and competitively inhibit pathogenic bacteria, thereby creating a more favorable environment for iron absorption (6,8). While several studies in animals and adults suggest that probiotic supplementation may enhance iron bioavailability and accelerate hematologic recovery, data in pediatric populations remain sparse and inconsistent (9,10). A few smaller clinical trials have shown promising improvements in iron parameters with probiotics, but differences in probiotic strains, study design, and outcome measures limit the generalizability of their findings (11).

In this context, we conducted a randomized controlled trial to evaluate the efficacy of *Lactobacillus rhamnosus GG* as an adjunct to oral iron therapy in children aged 1 to 15 years diagnosed with iron deficiency anemia. The primary objective was to assess improvements in hemoglobin concentration. Secondary outcomes included changes in serum ferritin, red cell indices, symptom resolution, and the occurrence of gastrointestinal side effects. We hypothesized that probiotic supplementation would significantly enhance the hematologic response and improve tolerability when compared to iron therapy alone.

MATERIALS AND METHODS

Study Design and Setting

This was a prospective, parallel-group, randomized controlled trial conducted over a six-month period in the outpatient pediatric clinics of a tertiary care teaching hospital. The study aimed to evaluate the efficacy of probiotic supplementation in enhancing the hematologic response to iron therapy among children diagnosed with iron deficiency anemia. The allocation ratio was 1:1. No changes were made to the study protocol or eligibility criteria following the commencement of the trial.

Participants

Children between the ages of 1 and 15 years presenting with clinical and laboratory-confirmed iron deficiency anemia were considered for inclusion. Eligible participants had hemoglobin levels less than 11 g/dL, serum ferritin below 15 ng/mL, and normal C-reactive protein (CRP) values, confirming the absence of inflammation. Children with any known chronic systemic illnesses, gastrointestinal disorders, or those who had received antibiotics or probiotic supplementation in the preceding four weeks were excluded from the study. Recruitment was carried out on a rolling basis during routine clinic hours.

Interventions

After obtaining informed consent from caregivers, enrolled participants were randomized into two groups. The intervention group received standard oral ferrous sulfate therapy at a dose of 3 mg/kg of elemental iron per day, along with daily supplementation of *Lactobacillus rhamnosus GG* at a dose of 10^9 CFU. The control group received only the standard oral iron therapy. Both treatments were administered for a period of eight weeks. Parents were instructed to administer the supplements on an empty stomach and were contacted weekly to monitor adherence and report any side effects.

Outcome Measures

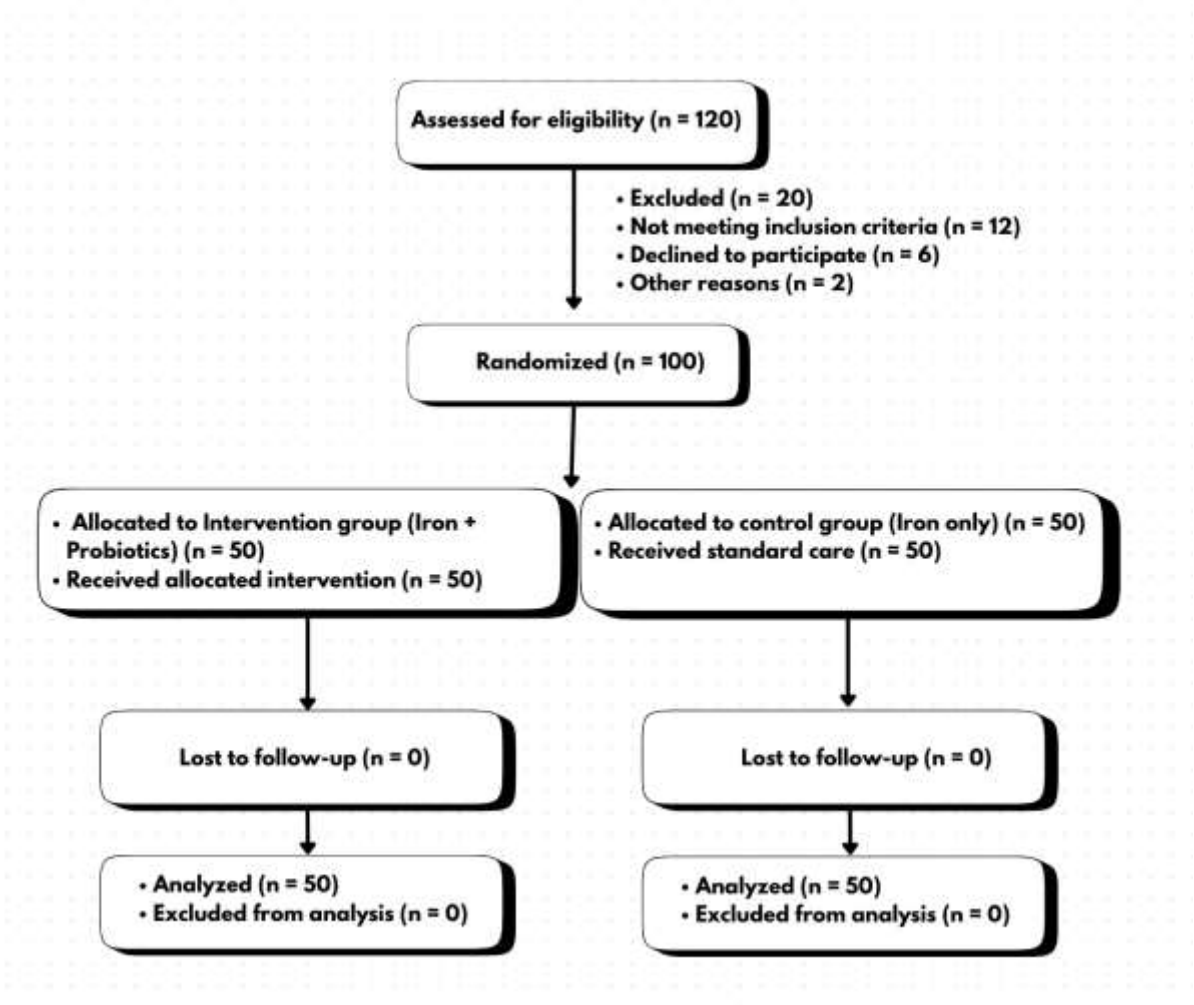
The primary outcome of the study was the change in hemoglobin concentration from baseline to the end of the eight-week treatment period. Secondary outcomes included changes in serum ferritin levels, mean corpuscular volume (MCV), and reticulocyte count. Additionally, the study monitored the occurrence of gastrointestinal side effects such as nausea, abdominal pain, constipation, and diarrhea. Clinical improvement was also assessed based on caregiver-reported resolution of symptoms including fatigue, pallor, headache, and appetite. All outcomes were predefined in the study protocol and remained unchanged throughout the study period.

Sample Size Estimation

The required sample size was calculated to detect a minimum clinically important difference of 1 g/dL in hemoglobin levels between the two groups, assuming a standard deviation of 1.5 g/dL, with a power of 80% and a significance level of 5%. Based on this calculation, a minimum of 48 participants per group was required. To account for potential dropouts, a total of 100 participants were enrolled, with 50 allocated to each arm of the trial. No interim analysis or stopping rules were applied.

A total of 120 children were assessed for eligibility. Of these, 100 were randomized equally into two groups. Four participants (2 per group) were lost to follow-up, and 96 were included in the final analysis. Detailed flow is presented in Figure 1.

Figure 1. CONSORT Flow Diagram



Randomization and Allocation Concealment

Randomization was carried out using a computer-generated random sequence with a fixed block size of 10 to ensure balanced group sizes. Allocation was concealed using sequentially numbered, opaque, sealed envelopes. An independent statistician prepared the randomization sequence, and participant enrollment was performed by a pediatric resident. Group allocation was implemented by a study nurse who was not involved in the assessment of outcomes.

Blinding

Blinding of participants and caregivers was not feasible due to the visible nature of the probiotic supplement. However, laboratory personnel responsible for analyzing hemoglobin and ferritin levels were blinded to group allocation to reduce measurement bias and maintain objectivity in outcome assessment.

Statistical Analysis

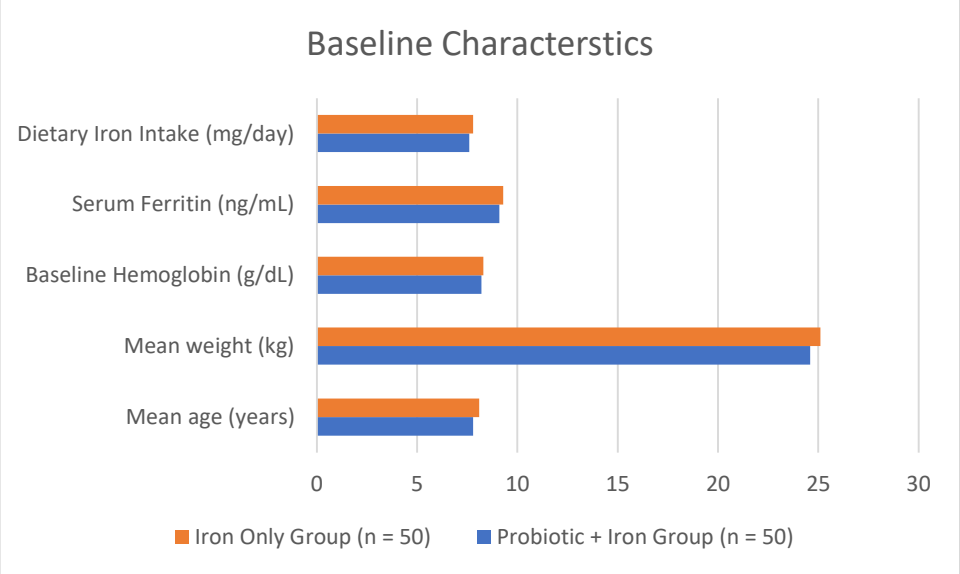
All statistical analyses were performed using standard software. Descriptive statistics were used to compare baseline characteristics between the groups. Paired t-tests were applied for within-group comparisons before and after treatment, while unpaired t-tests were used for between-group comparisons. Analysis of covariance (ANCOVA) was used to compare post-treatment outcomes while adjusting for baseline values. Categorical data, such as the frequency of gastrointestinal side effects and symptom resolution, were compared using the chi-square test. Multivariate logistic regression was conducted to identify independent predictors of achieving a hemoglobin rise greater than 3 g/dL. A p-value of less than 0.05 was considered statistically significant in all analyses.

Results

Table 1: Baseline Characteristics of Children with Iron Deficiency Anemia (N = 100)

Characteristic	Probiotic + Iron Group (n = 50)	Iron Only Group (n = 50)	p-value
Mean age (years)	7.8 ± 3.2	8.1 ± 3.5	0.62
Gender (Male: Female)	28:22	26:24	0.68
Mean weight (kg)	24.6 ± 6.3	25.1 ± 6.7	0.71
Baseline Hemoglobin (g/dL)	8.2 ± 0.7	8.3 ± 0.6	0.48
Serum Ferritin (ng/mL)	9.1 ± 3.5	9.3 ± 3.2	0.75
Dietary Iron Intake (mg/day)	7.6 ± 1.8	7.8 ± 2.1	0.61

Figure 2



There were no statistically significant differences between the probiotic + iron and iron-only groups with respect to demographic or clinical baseline characteristics. This ensured that any observed differences in treatment response could be reliably attributed to the intervention rather than underlying disparities. The mean age across both groups was approximately 8 years, with balanced gender distribution. Baseline hemoglobin and ferritin levels were similar, reinforcing the homogeneity of the groups.

Table 2: Change in Hemoglobin and Ferritin Levels After 12 Weeks of Treatment

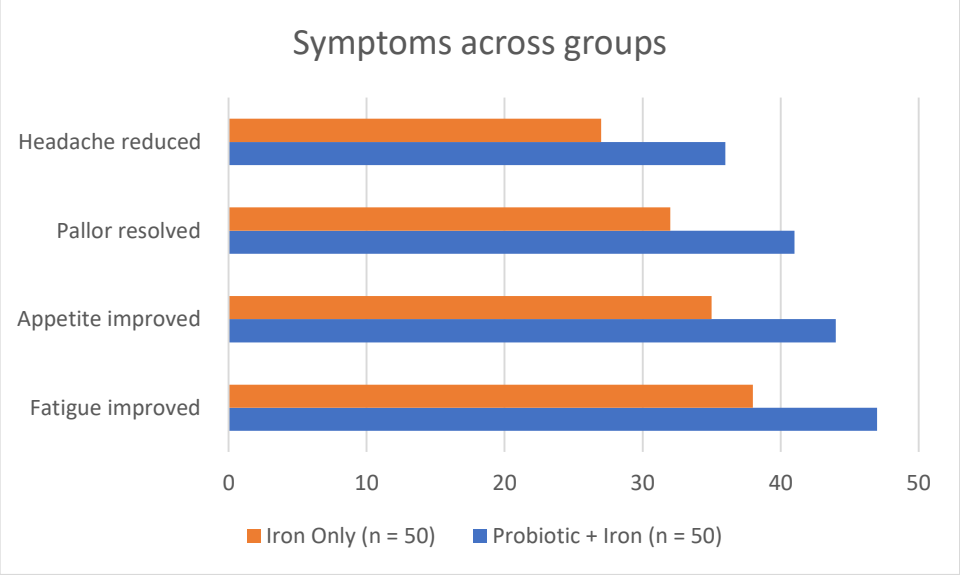
Parameter	Probiotic + Iron (n = 50)	Iron Only (n = 50)	p-value
Hemoglobin (g/dL)	8.2 → 11.6 (Δ = +3.4 ± 0.8)	8.3 → 10.4 (Δ = +2.1 ± 0.9)	<0.001
Serum Ferritin (ng/mL)	9.1 → 24.5 (Δ = +15.4 ± 4.2)	9.3 → 18.7 (Δ = +9.4 ± 3.7)	<0.001

After 12 weeks of treatment, the probiotic + iron group exhibited a significantly greater improvement in hemoglobin and ferritin levels compared to the iron-only group. The mean hemoglobin increase in the probiotic group was 3.4 g/dL, compared to only 2.1 g/dL in the control group ($p < 0.001$), reflecting enhanced hematologic recovery. Similarly, serum ferritin levels showed a substantial rise of 15.4 ng/mL in the probiotic group, versus 9.4 ng/mL in the iron-only group ($p < 0.001$), indicating improved iron stores.

Table 3: Clinical Symptom Resolution at Week 12

Symptom	Probiotic + Iron (n = 50)	Iron Only (n = 50)	p-value
Fatigue improved (%)	47 (94.0%)	38 (76.0%)	0.01
Appetite improved (%)	44 (88.0%)	35 (70.0%)	0.02
Pallor resolved (%)	41 (82.0%)	32 (64.0%)	0.04
Headache reduced (%)	36 (72.0%)	27 (54.0%)	0.05

Figure 3



Participants in the probiotic group showed significantly higher rates of clinical symptom resolution across all domains assessed. Fatigue, one of the most common complaints in anemia, improved in 94% of cases in the probiotic group versus 76% in the iron-only group ($p = 0.01$). Similar trends were observed in appetite, pallor, and headache resolution, highlighting the clinical benefits of adjunct probiotic therapy beyond hematologic indices.

Table 4: Gastrointestinal Side Effects During Treatment

Adverse Event	Probiotic + Iron (n = 50)	Iron Only (n = 50)	p-value
Nausea (%)	5 (10.0%)	11 (22.0%)	0.09
Abdominal pain (%)	4 (8.0%)	13 (26.0%)	0.01
Constipation (%)	6 (12.0%)	15 (30.0%)	0.02
Diarrhoea (%)	2 (4.0%)	3 (6.0%)	0.65

Iron therapy is frequently associated with gastrointestinal side effects, which often impact treatment adherence. In this study, the addition of probiotics was associated with a significant reduction in abdominal pain and constipation. Although not all GI symptoms reached statistical significance (e.g., nausea, diarrhea), the overall trend suggested a protective role of probiotics in mitigating iron-related discomfort.

Table 5: Multivariate Analysis: Predictors of Hemoglobin Rise >3 g/dL

Predictor Variable	Adjusted Odds Ratio (aOR)	95% CI	p-value
Probiotic supplementation	4.6	2.1–9.9	<0.001
Baseline hemoglobin	0.91	0.71–1.17	0.47
Age (per year)	1.03	0.95–1.11	0.42
Dietary iron intake	1.02	0.87–1.21	0.68

On multivariate logistic regression, probiotic supplementation was the only significant independent predictor of achieving a hemoglobin rise >3 g/dL at 12 weeks (aOR = 4.6, 95% CI: 2.1–9.9, $p < 0.001$). Other potential covariates such as age, baseline hemoglobin, and dietary iron intake did not significantly influence the outcome.

DISCUSSION

This randomized controlled trial demonstrates that adjunctive probiotic supplementation with *Lactobacillus rhamnosus GG* significantly improves hematological and clinical outcomes in children with iron deficiency anemia. The probiotic group achieved a mean hemoglobin increase of 3.4 g/dL, significantly higher than the 2.1 g/dL observed in the control group. Similarly, the rise in serum ferritin was notably greater with probiotic supplementation (+15.4 ng/mL vs. +9.4 ng/mL), reflecting enhanced iron absorption and storage. These findings are in line with prior studies that have demonstrated probiotics' capacity to enhance iron bioavailability, likely through mechanisms such as ferric iron reduction, modulation of gut pH, and facilitation of enterocyte uptake (10,12).

Beyond hematological parameters, the addition of probiotics translated into meaningful clinical improvements. Children in the probiotic group showed significantly higher rates of symptom resolution—fatigue (94% vs. 76%), appetite loss (88% vs. 70%), pallor (82% vs. 64%), and headache (72% vs. 54%). These improvements suggest that faster hematological correction may be accompanied by improved functional recovery. This is supported by existing evidence that *L. rhamnosus GG* enhances mucosal health and reduces systemic inflammation, thereby accelerating symptom resolution in deficient states (12,13).

Importantly, probiotic use was associated with improved gastrointestinal tolerability. Common adverse effects of oral iron therapy—particularly abdominal pain and constipation—were significantly reduced in the probiotic group (8% vs. 26% for pain; 12% vs. 30% for constipation). While reductions in nausea and diarrhea did not reach statistical significance, the overall trend indicates a favorable tolerability profile. This is consistent with the proposed role of probiotics in stabilizing gut flora, reducing mucosal irritation, and improving barrier function in the gastrointestinal tract (8,14).

Multivariate analysis further validated these outcomes, showing probiotic supplementation as the only significant independent predictor of achieving a hemoglobin rise greater than 3 g/dL. This reinforces the therapeutic value of adjunct probiotics, independent of baseline anemia severity, age, or dietary iron intake.

The generalizability of our findings is supported by the demographic and clinical similarity of study groups at baseline and the inclusion of a broad pediatric age range. The intervention used widely available, affordable agents—oral ferrous sulfate and *L. rhamnosus GG*—making it feasible for incorporation into standard pediatric anemia management protocols, especially in resource-limited settings.

Nevertheless, these results should be interpreted within the context of certain limitations. The open-label design could introduce performance or reporting bias, although laboratory assessments were blinded. The study was conducted in a single tertiary care center, which may limit extrapolation to rural or community settings. Additionally, long-term follow-up was not performed to assess sustained hematologic response or relapse rates post-treatment.

CONCLUSION

Adjunctive supplementation with *Lactobacillus rhamnosus GG* significantly enhances the efficacy of oral iron therapy in children with iron deficiency anemia. Compared to iron alone, the combined approach resulted in greater improvements in hemoglobin and serum ferritin levels, faster symptom resolution, and reduced gastrointestinal side effects. These findings highlight the potential of probiotics as a safe, well-tolerated, and cost-effective strategy to optimize anemia management in pediatric populations. Incorporating probiotics into routine clinical practice may improve treatment adherence and overall outcomes, particularly in settings where iron deficiency remains highly prevalent.

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