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Original Article

The adjunctive use of Bifidobacterium Quadruple Viable Bacteria with interferon in pediatric acute enteritis: inhibition of gut hormones, pain mediators, and systemic inflammation

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Abstract: Objective: To evaluate the adjunctive efficacy of Bifidobacterium Quadruple Viable Bacteria combined with recombinant human interferon α1b in pediatric acute enteritis (PAE). Methods: This retrospective propensity score matching (PSM) analysis included children aged 1-6 years with PAE admitted from March 2024 to March 2025. Patients receiving conventional therapy alone were assigned to the control group, while those receiving Bifidobacterium Quadruple Viable Bacteria plus recombinant human interferon α1b on the basis of conventional therapy were assigned to the observation group. A total of 128 children were included, with 64 cases in each group. Serum gut hormones, intestinal barrier markers, pain mediators, inflammatory factors, and oxidative stress indicators were compared before and after treatment. Results: After treatment, the observation group showed greater reductions in motilin and gastrin and a more marked increase in somatostatin than the control group. Substance P (SP) and prostaglandin E2 (PGE2) were also lower in the observation group, indicating reduced pain mediator release. In addition, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and high-sensitivity C-reactive protein (hs-CRP) declined more prominently, while superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) increased and malondialdehyde (MDA) decreased more substantially, suggesting alleviation of systemic inflammation and oxidative stress. Conclusion: In this retrospective PSM analysis, Bifidobacterium Quadruple Viable Bacteria combined with interferon α1b improved gut hormone balance, intestinal barrier function, pain-related mediators, inflammatory responses, and oxidative stress in PAE.

Keywords: Probiotics, interferon, pediatric acute enteritis, gastrointestinal hormones, inflammatory response

Introduction

Pediatric acute enteritis (PAE) is a frequently diagnosed gastrointestinal disorder in the pediatric age group, triggered predominantly by viral (e.g., rotavirus, norovirus) or bacterial infections [1]. Epidemiologic data indicate that children aged under 5 experience an average of 2.5 to 3.5 AE episodes annually. In about 30% of instances, the condition may become recurrent or persistent due to dysregulated gut motility, abnormal pain perception, and uncontrolled systemic inflammatory responses [2]. Recent research advances in intestinal microecology and inflammation regulation mechanisms have led to greater attention on adjuvant treatments that combine probiotics with immunomodulators [3, 4]. Yet, whether these combinations affect the key pathophysiologic indicators of PAE still requires thorough, systematic validation.

Contemporary research has suggested a close connection between the pathological progression of PAE and gut hormone imbalance, pain mediator release, and systemic inflammatory responses. Specifically, infections can stimulate the intestinal mucosa to secrete motility-promoting hormones like motilin (MTL) and gastrin (GAS), while inhibiting the production of inhibitory hormones such as somatostatin (SS) - ultimately leading to abnormal intestinal peristalsis [5]. Damage to intestinal epithelial cells activates nerve endings, prompting the release

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of pain mediators (e.g., substance P [SP], prostaglandin E2 [PGE2]) and exacerbating abdominal pain [6]. Additionally, pathogen-associated molecular patterns (PAMPs) trigger the innate immune response, inducing the massive secretion of inflammatory factors (interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α], high-sensitivity C-reactive protein [hs-CRP]) to form an "inflammatory cascade", which further impairs the intestinal mucosal barrier [7]. Nevertheless, current studies on PAE treatment tend to focus solely on clinical efficacy [8, 9], with few reports documenting changes in the aforementioned objective clinical indicators.

Against this backdrop, the present study centered on objective clinical markers (gut hormones, pain mediators, inflammatory factors, etc.) to explore how Bifidobacterium Quadruple Viable Bacteria-assisted interferon influences the pathophysiology of PAE. These insights establish a more objective foundation for refining future PAE treatment strategies. They also enable clinicians to assess the therapy's true effect on mucosal healing and pain control by tracking dynamic indicator changes, thereby helping to prevent over-medication. Furthermore, the results may pinpoint new targets for investigating the role of the "microbe-neuro-immune" axis in PAE, driving the shift from empirical treatment to precision medicine in this field.

Materials and methods

Research design

A retrospective propensity score matching (PSM) cohort design was used to enroll AE children admitted to our hospital from March 2024 to March 2025. Following screening against the inclusion and exclusion criteria, children diagnosed with acute AE who were deemed eligible were recruited into the trial. Inclusion criteria: Diagnosis of PAE confirmed by clinical manifestations [10] and laboratory tests; Age range: 1-6 years; Acute onset (symptom duration ≤72 hours) with no prior use of probiotics, interferon, or other immunomodulators before admission. Exclusion criteria: Comorbidity with organic intestinal diseases (e.g., intussusception, intestinal obstruction); Immunodeficiency, malignant tumors, or long-term use of glucocorticoids/immunosuppressants; Allergies to Bifidobacterium Quadruple Viable Bacteria (containing Lactobacillus, Bifidobacterium, etc.) or interferon; Severe heart, liver, or kidney failure, or genetic metabolic diseases; Premature withdrawal from the study, loss to follow-up, or poor compliance (e.g., failure to take medications as prescribed). The study has been approved by the ethics committee of The Second Affiliated Hospital of Hunan University of Traditional Chinese Medicine (No. HNUCN11-2409-139).

Treatment

All children received conventional treatment upon admission, with pathogen-specific medications administered as needed. In addition, some children received Bifidobacterium Quadruple Viable Bacteria (S20060010, Hangzhou Yuanda Bio-Pharmaceutical Co., Ltd., 2 capsules per dose, twice daily) and recombinant human interferon α1b injection (1 μg/kg per dose, once daily) in addition to conventional therapy. The treatment was discontinued upon symptom resolution (diarrhea frequency ≤2 episodes daily and relief from abdominal discomfort) or until completion of the 7-day maximum treatment period.

Study population

We used the children who received only conventional treatment as the control group, and the children who received Bifidobacterium Quadruple Viable Bacteria plus recombinant human interferon α1b as the observation group. Propensity score matching was performed using the 1:1 nearest neighbor matching method, and the matching variables included all baseline characteristics: age, sex, time from onset to admission, etc. The matching caliper value was set to 0.05, and the matching process did not allow repeated matching. Standardized mean differences (SMDs) were used to evaluate covariate balance before and after matching, and an SMD <0.20 was considered to indicate acceptable balance. Before matching, SMDs for the baseline variables ranged from 0.384 to 0.784. Through PSM, we obtained 128 children with matched baseline information from 231 children with AE (64 children in the observation group and 64 in the control group) (P>0.05; **Table 1**). Following approval from our hospital's Ethics Committee, informed consent was obtained from participants' guardians.

Table 1. Baseline data sheet for the children

Group	Age	Time from onset to admission (h)	Boys	Girls	First onset of illness		Pathogenic bacteria	
					Yes	No	Virus	Bacteria
Control group (n=64)	3.78±1.79	40.45±20.44	36 (56.25)	28 (43.75)	58 (90.63)	6 (9.38)	34 (53.13)	30 (46.88)
Observation group (n=64)	3.47±1.75	35.89±21.75	40 (62.50)	24 (37.50)	52 (81.25)	12 (18.75)	38 (59.38)	26 (40.63)
t or χ^2	1.001	1.223	0.518		2.327		0.508	
P	0.319	0.224	0.472		0.127		0.476	
SMD	0.135	0.196	0.128		0.172		0.126	

Note: Data are presented as mean ± standard deviation or n (%). PSM, propensity score matching; SMD, standardized mean difference; h, hours. Continuous variables were compared using independent-samples t-tests, and categorical variables were compared using chi-square tests. An SMD <0.20 was considered to indicate acceptable post-matching balance.

Sample collection

Fasting venous blood samples were collected from each child before and after treatment for subsequent laboratory testing.

Laboratory testing

ELISA: The samples were first allowed to sit undisturbed for 30 minutes. Subsequent centrifugation was carried out at 3000×g for 15 minutes to complete the serum isolation. Prior to loading into pre-coated microplate wells, standards, and samples were diluted as instructed by the manufacturer. The loaded plates were incubated at room temperature for 2 hours on a shaker, after which the wells were rinsed three times using a wash solution. Enzyme-labeled secondary antibodies were added, followed by a 1-hour incubation in darkness and a 15-minute color development with a chromogenic reagent. Upon termination of the reaction by stop solution addition, absorbance was measured at 450 nm. Serum concentrations of MTL (CSB-E08207h, Wuhan Huamei Biological Engineering Co. LTD), GAS (CSB-EL009270HU, Wuhan Huamei Biological Engineering Co. LTD), SS (CSB-E08203h, Wuhan Huamei Biological Engineering Co. LTD), DAO (CSB-E10137h, Wuhan Huamei Biological Engineering Co. LTD), D-LA (ARD3-N139, Guangzhou Orida Biotechnology Co., LTD), i-FABP (CSB-E08024h, Wuhan Huamei Biological Engineering Co. LTD), SP (EH3806, Wuhan Fine Biological Technology Co., LTD), PGE2 (CSB-E07965h, Wuhan Huamei Biological Engineering Co. LTD), IL-6 (CSB-E04638h, Wuhan Huamei Biological Engineering Co. LTD), TNF-α (CSB-E09315h, Wuhan Huamei Biological Engineering Co. LTD), SOD (CSB-EL022399HU, Wuhan Huamei Biological Engineering Co. LTD), MDA (CELI-66086h, Wuhan VCTS Technology Co., LTD), and GSH-Px (XK-E1802, Shanghai Thresholding Biotechnology Co., LTD) were calculated from standard curves. Pre-analysis handling: Serum samples were placed on ice immediately after collection and centrifugation was completed within 2 hours. To avoid repeated freeze-thaw cycles, frozen samples were thawed slowly in a 37°C water bath. Quality control: Each microplate included both positive (blank matrix with known concentrations) and negative (blank matrix) controls. Intra- and inter-batch precision was verified using BioRad quality control materials, with all coefficient of variation (CV) values remaining below 15%.

Automated Hematology Analyzer (Beckman Coulter DxH 800): The collected blood samples underwent mild inversion 5-8 times for homogenization, avoiding vigorous shaking that may induce hemolysis. They were then centrifuged (3000×g, 10 minutes), and plasma was separated into sterile EP tubes while preventing repeated freeze-thaw exposure. The analyzer was calibrated using matching calibrators before daily testing to ensure a linear range of 0.1-320 mg/L and a precision of CV <5%. A 20 μL aliquot of plasma was automatically drawn into a reaction cup. hs-CRP concentrations were then quantified by the analyzer using dual-wavelength turbidimetric detection (570/800 nm), with concentrations determined from a built-in standard curve. Pre-analysis handling: Samples were centrifuged within 30 minutes of collection to prevent fibrin precipitation or platelet activation. The permitted storage conditions for plasma are as follows: up to 4 hours at ambient temperature, a maximum of 72 hours between 2-8°C, or no longer than one month at -20°C. Each sample underwent no more than one freeze-thaw cycle, and thawing was conducted gradually in a 37°C water bath.

Quality control: Prior to daily testing, both high- and low-level quality control materials were run. All testing strictly followed the Westgard multirules ($1_3S/2_2S/R_4S/4_1S/10X$; warning limit: target value ±2 SD; out-of-control limit: target value ±3 SD).

Adverse reactions

Safety monitoring was conducted throughout the 7-day treatment period and 2-week follow-up. All adverse events possibly related to study medication were recorded, including type, onset time, severity, and outcome. Severity was graded using the National Institutes of Health Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (pediatric adaptation). Only grade 1-2 mild adverse reactions were analyzed, as no grade 3 or higher severe adverse events occurred in this study.

Clinical efficacy evaluation

The primary clinical endpoint was the total effective rate at 7 days of treatment, defined as: Cure: Diarrhea, abdominal pain and other clinical symptoms completely disappeared, stool routine returned to normal; Effective: Clinical symptoms were significantly relieved, stool frequency decreased to ≤3 times/day, stool routine was basically normal; Ineffective: No significant improvement in clinical symptoms or worsening of the condition. Total effective rate = (Cure cases + Effective cases)/Total cases × 100%. Secondary endpoints included the time to diarrhea cessation and time to abdominal pain relief.

Statistical analysis

Data analyses were carried out with SPSS 30.0. Categorical data [n (%)] were compared using the chi-square test. For continuous data, the Shapiro-Wilk test was first used to assess normality. The analysis of normally distributed data (mean ± standard deviation [SD]) was conducted using independent samples t-tests (between groups) and paired t-tests (within groups). Non-normally distributed data, presented as [M (P25, P75)], were compared using the Mann-Whitney U test (between groups) and Kruskal-Wallis H test (among multiple groups). Exploratory subgroup analyses were performed according to pathogen type, including viral and bacterial infections. Within each pathogen subgroup, we compared diarrhea cessation time, abdominal pain relief time, and favorable changes in key biomarkers between the observation and control groups. Statistical significance was established if *P*-value was less than 0.05.

Results

Effects of Bifidobacterium Quadruple Viable Bacteria-assisted interferon therapy on gut hormones

Pre-treatment assessment identified no significant baseline differences in MTL, GAS, or SS concentrations across the groups (P>0.05). After treatment, the observation group showed 22.38% and 24.37% reductions in MTL and GAS levels, respectively, together with a 38.99% elevation in SS levels (P<0.05). This indicates that the combined treatment inhibited excessive gastrointestinal motility disorders by regulating gut hormone balance. In contrast, the control group only exhibited slight decreases in MTL (14.14%) and GAS (16.27%) and a modest increase in SS (23.12%), with significantly weaker improvements than the observation group (P<0.05) (**Figure 1**).

Effects of Bifidobacterium Quadruple Viable Bacteria-assisted interferon therapy on intestinal barrier function

Regarding intestinal barrier function, the observation group showed post-treatment reductions of 39.93%, 35.78%, and 35.15% in DAO, D-LA, and i-FABP levels, respectively (P<0.05) - demonstrating that the combined treatment significantly alleviated intestinal mucosal epithelial damage and intestinal bacterial translocation. In the control group, modest declines were observed in DAO (22.41%), D-LA (17.55%) and i-FABP (20.08%) (P<0.05). The significantly less pronounced improvement in intestinal barrier function in the control group versus the observation group (P<0.05) indicated a constrained effectiveness of conventional treatment in promoting intestinal barrier repair (**Figure 2**).

Effects of Bifidobacterium Quadruple Viable Bacteria-assisted interferon therapy on pain mediators

Pre-treatment SP and PGE2 levels were comparable between groups (P>0.05). Therapeutic

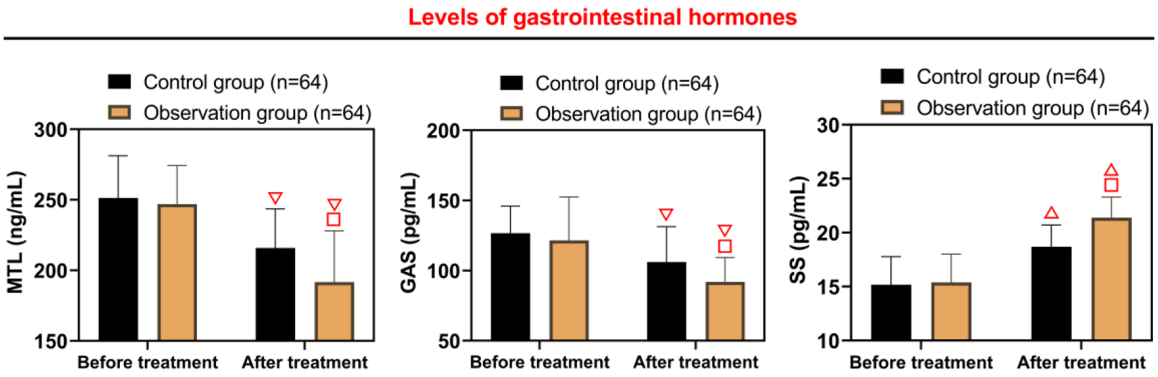

Figure 1. Changes and comparison of gastrointestinal hormones (MTL, GAS, SS) before and after treatment. Note: MTL, motilin; GAS, gastrin; SS, somatostatin. Data are presented as mean ± standard deviation. Within-group before-after comparisons were performed using paired t-tests, and between-group comparisons at the same time point were performed using independent-samples t-tests. △ indicates higher levels than before treatment (P<0.05); ▽ indicates lower levels than before treatment (P<0.05); □ indicates a significant difference compared to the control group (P<0.05).

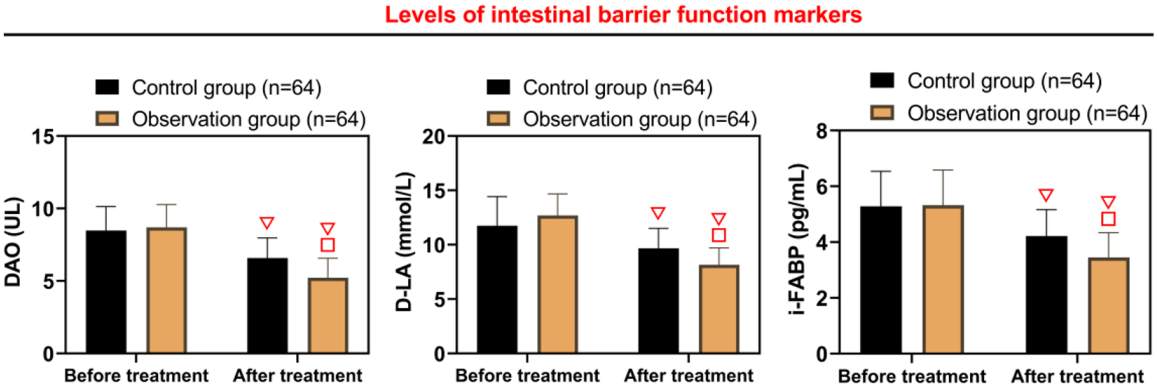

Figure 2. Changes and comparison of intestinal barrier function markers (DAO, D-LA, i-FABP) before and after treatment. Note: DAO, diamine oxidase; D-LA, D-lactic acid; i-FABP, intestinal fatty acid-binding protein. Data are presented as mean ± standard deviation. Within-group before-after comparisons were performed using paired t-tests, and between-group comparisons at the same time point were performed using independent-samples t-tests. ▽ indicates lower levels than before treatment (P<0.05); □ indicates a significant difference compared to the control group (P<0.05).

intervention resulted in a measurable decrease of both biomarkers in each group, with the observation group demonstrating conclusively lower concentrations of SP and PGE2 compared to controls (P<0.05) (**Figure 3**).

Effects of Bifidobacterium Quadruple Viable Bacteria-assisted interferon therapy on systemic inflammatory responses

Among inflammatory factors, the observation group exhibited post-treatment decreases of 42.12%, 27.83%, and 32.63% in IL-6, TNF-α, and hs-CRP levels, respectively (P<0.05). The control group only had corresponding reductions of 31.20%, 16.34%, and 18.96% in these factors (all P<0.05). The observation group thus demonstrated a more pronounced inhibitory effect on the inflammatory cascade (**Figure 4**).

Effects of Bifidobacterium Quadruple Viable Bacteria-assisted interferon therapy on oxidative stress status

At baseline, SOD, MDA, and GSH-Px values were comparable between the groups (P>0.05). Post-treatment, the observation group displayed a marked rise in SOD and GSH-Px accompanied by a decline in MDA (P<0.05). Conversely, the control group showed only slight elevations in SOD and GSH-Px without

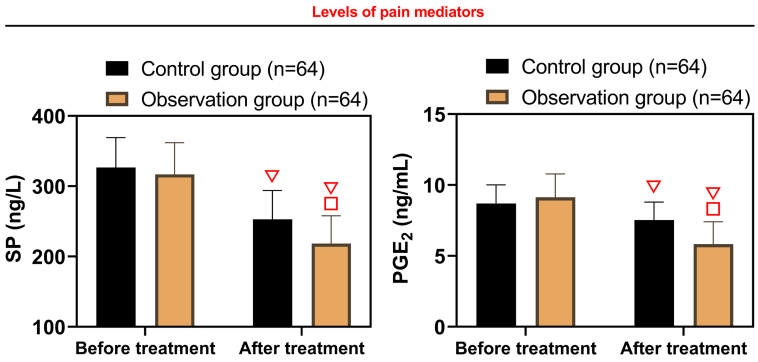

Figure 3. Changes and comparison of pain mediators (SP, PGE_2) before and after treatment. Note: SP, substance P; PGE2, prostaglandin E2. Data are presented as mean ± standard deviation. Within-group before-after comparisons were performed using paired t-tests, and between-group comparisons at the same time point were performed using independent-samples t-tests. ▽ indicates lower levels than before treatment (P<0.05); □ indicates a significant difference compared to the control group (P<0.05).

significant alteration in MDA levels (P>0.05) (**Figure 5**).

Safety profile of combined therapy

No grade 3 or higher severe adverse events, treatment-related discontinuations or dose adjustments were observed in either group during the entire study period. The total incidence of adverse reactions in the observation group was 26.56%, which was significantly higher than 12.50% in the control group (P=0.046). There were no significant differences in the incidence of individual adverse reactions (mild diarrhea, nausea/vomiting, abdominal distension and rash) between the two groups (P> 0.05). All adverse reactions were transient and well-tolerated by the pediatric patients (**Table 2**).

Clinical efficacy and symptom relief time

At 7 days of treatment, the total effective rate in the observation group was 93.75%, which was significantly higher than 75.00% in the control group (P=0.003). The cure rate in the observation group was 65.63%, which was significantly higher than 43.75% in the control group (P<0.05). In terms of symptom relief time, the observation group had significantly shorter diarrhea cessation time (P<0.001) and abdominal pain relief time (P<0.001) compared to the control group. These results indicated that the combined treatment was associated with faster clinical recovery in children with acute enteritis (**Table 3**).

Subgroup analysis by pathogen type

In the exploratory pathogen-stratified analysis, the observation group showed shorter diarrhea cessation time and abdominal pain relief time than the control group for both the viral and bacterial infection subgroups (**Table 4**). No significant treatment-by-pathogen interaction was observed for diarrhea cessation time or abdominal pain relief time, suggesting that the symptomatic benefit of the combined regimen was not clearly dependent on pathogen type.

Discussion

As a common pediatric disease, pediatric acute enteritis (PAE) involves multi-dimensional pathophysiologic processes, including gut hormone imbalance, intestinal barrier disruption, pain mediator release, and systemic inflammatory responses [11]. By focusing on changes in multi-dimensional objective indicators in children, this study provides new insight for clinical practice.

First, significant reductions in MTL and GAS levels were observed in the observation group, along with a marked increase in SS. These outcomes are consistent with probiotic functionality, wherein competitive adhesion to intestinal epithelium inhibits motility-inducing hormone secretion, bile acid metabolism is adjusted, and short-chain fatty acid (SCFA) generation is boosted [12]. For instance, previous studies have suggested that Bifidobacterium may influence SS secretion through GPCR43-related mechanisms [13], which may partly explain the elevated SS levels observed in this study. Notably, the control group had only a slight increase in SS; however, any involvement of interferon-related JAK-STAT signaling in SS regulation remains speculative and requires experimental verification.

Additionally, the significant decreases in DAO, D-LA, and i-FABP in the observation group indicated the combined treatment's ability to effectively mitigate intestinal mucosal damage. Intestinal barrier integrity heavily depends on

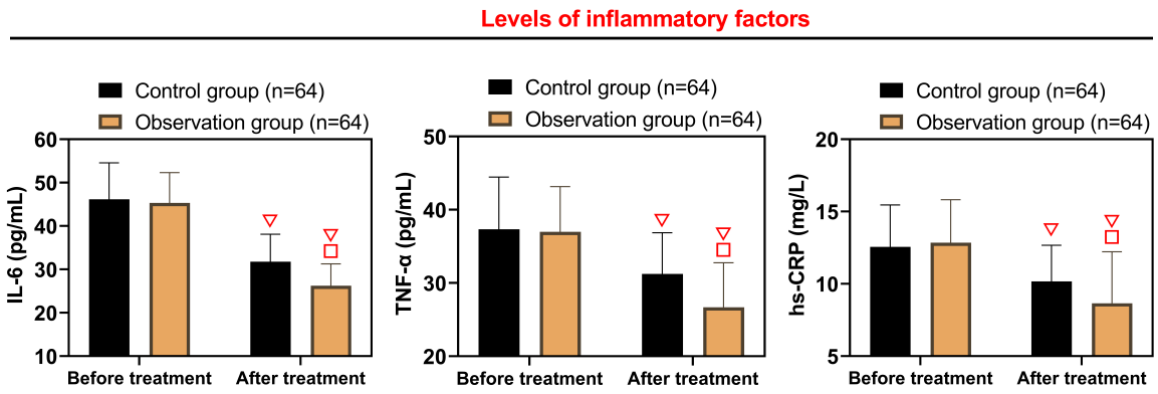

Figure 4. Changes and comparison of inflammatory factors (IL-6, TNF-α, hs-CRP) before and after treatment. Note: IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; hs-CRP, high-sensitivity C-reactive protein. Data are presented as mean ± standard deviation. Within-group before-after comparisons were performed using paired t-tests, and between-group comparisons at the same time point were performed using independent-samples t-tests. ▽ indicates lower levels than before treatment (P<0.05); □ indicates a significant difference compared to the control group (P<0.05).

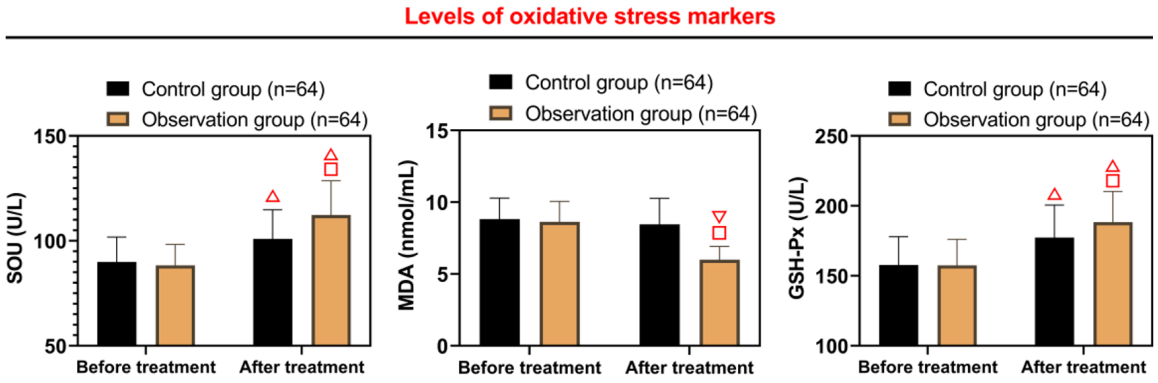

Figure 5. Changes and comparison of oxidative stress markers (SOD, MDA, GSH-Px) before and after treatment. Note: SOD, superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase. Data are presented as mean ± standard deviation. Within-group before-after comparisons were performed using paired t-tests, and between-group comparisons at the same time point were performed using independent-samples t-tests. △ indicates higher levels than before treatment (P<0.05); ▽ indicates lower levels than before treatment (P<0.05); □ indicates a significant difference compared to0 the control group (P<0.05).

the presence and function of tight junction proteins. Probiotics can support the preservation of this barrier by upregulating occludin mRNA expression [14]. The marked decrease in D-LA (a metabolite of intestinal bacteria) in this study suggests that probiotics may indirectly repair the intestinal barrier by inhibiting the overgrowth of pathogenic bacteria. This finding also complements a study by Jena et al., which reported that interferon α reduces intestinal epithelial cell apoptosis [15].

Furthermore, we observed reduced SP and PGE2 concentrations in the observation group, implicating the modulation of intestinal neurogenic pain pathways as a potential therapeutic mechanism for the combined treatment. Research indicates that probiotics can suppress pro-inflammatory mediator production. This effect may involve inhibition of the NF-κB signaling pathway, which may contribute to reduced SP expression [16]. Additionally, interferon α can enhance endogenous analgesic effects by upregulating opioid receptor expression [17]. Although this study did not directly assess pain scores, the objective changes in pain mediators offer a more direct reference for clinical pain management strategies.

Moreover, the observation group's greater reductions in IL-6, TNF-α, and hs-CRP levels versus controls suggested a possible additive

Table 2. Comparison of adverse reaction incidence between the observation group and the control group

	Mild diarrhea	Nausea/vomiting	Abdominal distension	Rash	Total adverse reactions
Observation group (n=64)	6 (9.38%)	5 (7.81%)	4 (6.25%)	2 (3.13%)	17 (26.56%)
Control group (n=64)	3 (4.69%)	2 (3.13%)	2 (3.13%)	1 (1.56%)	8 (12.50%)
χ^2	1.076	1.371	0.696	0.341	4.026
P	0.300	0.242	0.404	0.559	0.045

Note: Data are presented as n (%). χ^2, chi-square statistic. Adverse events were graded according to the National Institutes of Health Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. Between-group comparisons were performed using chi-square tests.

Table 3. Comparison of clinical efficacy and symptom relief time between the observation group and the control group

	Clinical efficacy				Symptom relief time (d)	
	Cure	Effective	Ineffective	Total effective rate	Diarrhea cessation time	Abdominal pain relief time
Observation group (n=64)	42 (65.63%)	18 (28.13%)	4 (6.25%)	60 (93.75%)	2.66±0.67	2.09±0.56
Control group (n=64)	28 (43.75%)	20 (31.25%)	16 (25.00%)	48 (75.00%)	3.30±0.71	2.80±0.67
Statistical	-	-	-	χ^2=8.533	t=5.260	t=6.458
P	-	-	-	0.003	<0.001	<0.001

Note: Data are presented as n (%) or mean ± standard deviation. d, days; χ^2, chi-square statistic. Total effective rate = (cure cases + effective cases)/total cases × 100%. Total effective rate was compared using the chi-square test, and symptom relief times were compared using independent-samples t-tests.

Table 4. Subgroup analysis of symptom relief time according to pathogen type

Pathogen type	Outcome	Observation group	Control group	t	*P* value	P for interaction
Viral infection	Diarrhea cessation time (d)	2.71±0.73 (n=38)	3.35±0.73 (n=34)	3.714	<0.001	0.955
Viral infection	Abdominal pain relief time (d)	2.21±0.58 (n=38)	3.00±0.60 (n=34)	5.674	<0.001	0.492
Bacterial infection	Diarrhea cessation time (d)	2.58±0.58 (n=26)	3.23±0.68 (n=30)	3.863	<0.001	0.955
Bacterial infection	Abdominal pain relief time (d)	1.92±0.48 (n=26)	2.57±0.68 (n=30)	4.027	<0.001	0.492

Note: Data are presented as mean ± standard deviation. d, days. Between-group comparisons within each pathogen subgroup were performed using independent-samples t-tests. P for interaction was calculated using regression models including treatment group, pathogen type, and the treatment-by-pathogen interaction term.

anti-inflammatory effect of probiotics and interferon, although mechanistic synergy cannot be confirmed from this retrospective clinical dataset. Interferon α triggers antiviral protein production through JAK-STAT pathway activation [18], whereas probiotics suppress IL-6 release by modulating the Th17/Treg equilibrium [19]. Of note, the rapid decrease in hs-CRP, an acute-phase reactant, suggests that the combined treatment may accelerate inflammation resolution and shorten the disease course.

Finally, the combined treatment effectively alleviated oxidative stress damage, as evidenced by the lower MDA levels and higher SOD and GSH-Px activities in the observation group. Probiotics enhance redox homeostasis through the synthesis of reactive oxygen species (ROS)-scavenging enzymes (e.g., catalase) [20]. Meanwhile, interferon α can boost antioxidant defenses by upregulation of the Nrf2 signaling pathway [21]. This dual antioxidant mechanism may create a favorable microenvironment for intestinal mucosal repair.

The safety analysis confirmed the favorable tolerability of Bifidobacterium Quadruple Viable Bacteria combined with interferon for the treatment of pediatric enteritis. Although the total incidence of mild adverse reactions was slightly higher in the observation group, all reactions were self-limiting and did not affect the treat-

ment process. Nevertheless, this increase warrants consideration when selecting this adjunctive regimen. The risk-benefit balance may be acceptable when faster symptom relief and improved clinical efficacy are expected, but treatment should be accompanied by parental counseling and close monitoring for transient gastrointestinal discomfort or rash. The mild gastrointestinal reactions observed are common and predictable side effects of probiotic supplementation in children, which are usually related to the temporary adjustment of intestinal flora. No serious allergic reactions or systemic adverse events occurred, indicating that the combined treatment has a good safety profile and is suitable for clinical application in children aged 1-6 years. The clinical efficacy analysis demonstrated that Bifidobacterium Quadruple Viable Bacteria combined with interferon significantly improved the treatment effect of pediatric enteritis, with a total effective rate of 93.75% and a cure rate of 65.63%. The combined treatment also shortened the diarrhea cessation time and abdominal pain relief time by approximately 38.6% and 35.9% respectively compared to conventional treatment alone. These clinical benefits are consistent with the changes in objective pathophysiological indicators observed in this study: the regulation of gut hormone balance alleviates abnormal intestinal peristalsis, the repair of intestinal barrier reduces intestinal permeability, and the inhibition of pain mediators and inflammatory cascade relieves abdominal pain and systemic inflammatory response. The clinical improvement observed in this study may be related to complementary antiviral, immunomodulatory, and barrier-protective mechanisms rather than a definitively proven synergistic interaction. The exploratory pathogen-stratified analysis showed similar symptomatic benefits in both viral and bacterial AE, although these subgroup findings should be interpreted cautiously because of the limited sample size. Previous studies have suggested that Bifidobacterium-related or probiotic/postbiotic interventions may modulate type I interferon-related antiviral responses during rotavirus infection [22, 23]. Type I interferons are important mediators of innate antiviral immunity, whereas probiotics may contribute to intestinal microecological regulation and mucosal barrier maintenance [24, 25]. Therefore, the combined regimen may act on multiple pathophysiologic links in pediatric enteritis; however, direct mechanistic confirmation requires further prospective and experimental studies.

This study has several limitations that need to be addressed in future research. First, the retrospective design inevitably introduced potential selection bias and residual confounding. Although PSM was used to improve baseline comparability, unmeasured or incompletely recorded factors, such as prior antibiotic exposure, hydration status, nutritional status, dietary intake during treatment, baseline disease severity, medication adherence, and differences in supportive care, may have influenced treatment selection and clinical outcomes. For example, despite a total sample of 128 cases, the limited subgroup sizes (n=72 for viral, n=56 for bacterial infections) may have weakened the ability to analyze pathogen-specific effects. Future investigations should prioritize larger cohorts to facilitate robust, stratified analyses by pathogen type. The second limitation was its seven-day post-treatment observation period, which fell short of evaluating long-term outcomes. Therefore, subsequent research is necessary to gather extended follow-up data to validate the treatment's lasting effectiveness. Additionally, while the study measured certain pathway-related markers, it did not conduct an in-depth investigation into the molecular mechanisms that drive the synergistic interaction between probiotics and interferons - for instance, the expression patterns of specific genes or the activation status of key signaling pathways. Future incorporation of techniques such as proteomics or transcriptomics would offer more thorough insights into these underlying processes. Lastly, the research did not document potential confounding factors like the dietary patterns of the pediatric participants and variations in probiotic colonization within the gut. Such omissions may introduce biases into the study results. Hence, standardizing dietary interventions and incorporating fecal microbiota analysis would be essential to help enhance the reliability of the collected data. Cost-effectiveness was not evaluated in this study. Future studies should consider medication costs, laboratory testing costs, hospitalization expenses, caregiver burden, and cost per day of symptom reduction to determine whether the additional clinical benefit justifies the added treatment cost.

Conclusion

In this retrospective PSM analysis, Bifidobacterium Quadruple Viable Bacteria combined with recombinant human interferon α1b was associated with improved clinical efficacy and shorter symptom relief time in PAE compared to conventional therapy alone. The combined regimen was also related to more favorable changes in gut hormones, intestinal barrier markers, pain mediators, inflammatory factors, and oxidative stress indicators. No severe treatment-related adverse events were observed, although mild adverse reactions were more frequent in the observation group. Given the retrospective design, limited sample size, and short follow-up period, these findings should be interpreted cautiously and require confirmation in larger prospective studies.

Disclosure of conflict of interest

None.

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