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RESEARCH

Efficacy of oral folinic acid supplementation in children with autism spectrum disorder: a randomized double-blind, placebo-controlled trial

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Abstract

Oral folinic acid has shown potential to improve symptoms in children with autism spectrum disorder (ASD). However, randomized controlled trials (RCTs) are limited. This double-blind, placebo-controlled RCT aimed to compare changes in Childhood Autism Rating Scale (CARS) scores in children with ASD aged 2–10 years, among folinic acid (2 mg/kg/day, maximum of 50 mg/day) and placebo groups at 24 weeks, in comparison with baseline. Both the groups received standard care (ABA and sensory integration therapy). Secondary objectives included changes in behavioral problems measured by the Child Behavior Checklist (CBCL) and serum levels of anti-folate receptor autoantibodies and folic acid, correlated with changes in autism symptom severity. Out of the 40 participants recruited in each group, 39 and 38 participants completed the 24-week follow-up in the folinic acid and placebo groups, respectively. The change in CARS score was higher in the folinic acid group (3.6 ± 0.8) compared to the placebo group (2.4 ± 0.7, $p < 0.001$). Changes in CBCL total score and CBCL internalizing score were also better in the folinic acid group (19.7 ± 9.5 vs. 12.6 ± 8.4 and 15.4 ± 7.8 vs. 8.5 ± 5.7, $p < 0.001$ for both). High-titer anti-folate receptor autoantibodies were positive in 32/40 and 33/40 cases in the folinic acid and placebo groups, respectively ($p = 0.78$). In the placebo group, improvement in CARS score was comparable regardless of autoantibody status ($p = 0.11$), but in the folinic acid group, improvement was more pronounced in the high-titer autoantibody group ($p = 0.03$). No adverse reactions were reported in either group.

Conclusions: Oral folinic acid supplementation is effective and safe in improving ASD symptoms, with more pronounced benefits in children with high titers of folate receptor autoantibodies.

Trial registration: CTRI/2021/07/034901, dated 15–07-2021.

What is Known:
- *Folate receptor autoantibodies are more prevalent in children with autism spectrum disorder (ASD) compared to typically developing children.*
- *Folate receptor autoantibodies play a significant role in the neuropathogenesis of autism spectrum disorder.*

What is New:
- *Add-on oral folinic acid supplementation is safe and effective in reducing the severity of symptoms in children with ASD.*
- *The clinical benefits are more pronounced in children with high titers of folate receptor autoantibodies.*

Keywords Neurodevelopmental disorders · Autism · Nutritional supplementation · Behavior · Sensory profile

Introduction

Autism spectrum disorders (ASD) are a spectrum of neurodevelopmental disorders, which involve difficulties in interacting and communicating with others [1]. The etiology of ASD is complex, involving a variety of factors, including maternal, environmental, nutritional, and genetic influences

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[2]. Despite extensive research, the precise molecular mechanisms underlying ASD remain elusive [3]. However, disruptions in folate metabolism and elevated oxidative stress have been suggested as potentially significant contributors to the development of ASD.

Various abnormalities in folate metabolism have been associated with ASD [4]. The transportation of 5-methyltetrahydrofolate across the choroid plexus epithelium occurs via its binding to folate receptor-α (FRα) followed by endocytosis [5]. There is evidence suggesting that cerebral folate deficiency is connected to autism, particularly in children with Rett syndrome, a disease considered part of the autism spectrum [6]. Folate is one of the cofactors in one-carbon metabolism, which is crucial for the development of the nervous system during pregnancy in the fetus, as well as in infancy and early childhood [7].

Women who have pregnancies affected by neural tube defects or have children with ASD are not generally folate deficient; however, maternal folic acid intake during pregnancy has been shown to reduce the risk of both conditions [8]. Folate is transported across the placenta and into the brain through a complex system. This system involves a primary carrier called FRα, which has a high capacity for folate, and a secondary carrier called RFC, which has a much lower capacity [9]. Antibodies against FRα, known as FRαAb, can interfere with the transport of folate across the placenta and into the brain, which relies on the FRα carrier protein [10]. Studies have shown that FRαAb are more commonly found in children diagnosed with autism spectrum disorder than in typically developing children of the same age [11].

Recently, several RCTs have demonstrated the effectiveness of high-dose oral folinic acid in enhancing verbal communication in children with ASD [10, 12, 13]. However, the long-term tolerability profile of high-dose folinic acid remains unknown [14]. The EFFET trial, on the other hand, showed the efficacy of a lower dose of oral folinic acid of twice daily 5 mg dose in treating ASD in a country without food fortification [13]. No RCT has been conducted in the Indian subcontinent, and prior RCTs have not thoroughly investigated the impact of folinic acid on cognition, sleep abnormalities, and other comorbidities such as hyperactivity and aggressive behavior.

Methods

This placebo-controlled, double-blind randomized trial was conducted between January 2022 and January 2024 in the Pediatric Neurology Division, AIIMS, Rishikesh, India. Approval from the institute ethics committee was obtained (AIIMS/IEC/20/161), and the clinical trial protocol was registered on the Clinical Trials Registry of India (CTRI/2021/07/034901) before commencing the trial. Written informed consent was obtained from the parents.

The primary objective of the study was to evaluate the efficacy of add-on oral folinic acid supplementation with standard care, compared to standard care only in improving symptom severity in children with ASD aged 2–10 years after 24 weeks of treatment, measured by the Childhood Autism Rating Scale (CARS). Secondary objectives included comparing changes in the severity of behavioral problems measured by the Child Behavior Checklist (CBCL), cognition measured by the Vineland Social Maturity Scale (VSMS), sensory processing issues measured by Sensory Profile 2 (SP-2), and sleep problems measured by the Childhood Sleep Habit Questionnaire (CSHQ). We also aimed to compare serum levels of anti-folate receptor autoantibodies and folic acid in both groups and correlate them with changes in autism symptom severity.

Inclusion criteria for the trial were children with ASD (meeting DSM-V criteria) aged 2–10 years. Other inclusion criteria included children already on antipsychotic medications, with no history of dose or medication changes in the 8 weeks before screening for trial recruitment, and parents willing to comply with the study medications for 24 weeks and adhere to regular clinical follow-up protocols. Children were excluded from the study if they had severe gastroesophageal reflux, chronic hepatic or renal problems, were on medications affecting serum folate levels, had known genetic conditions linked to folate metabolism, or experienced a recent clinical seizure within 6 months. We also intended to exclude children whose parents informed us about the child receiving other complementary and alternative treatments, such as a casein-free, gluten-free diet (GFCF), apart from the standard of care behavioral therapy.

Based on the study by Frye et al. in 2018 [12], it was projected that the folinic acid group would show a 5.7 standardized point difference compared to the placebo group in CARS scores, reflecting a large effect size (Cohen's *d* of 0.70). To achieve a 0.05 alpha error and 80% power, a sample size of 35 children per group was required. Considering a potential 10% dropout rate, it was decided to include 40 children for each group.

Block randomization was implemented using a 1:1 ratio with variable block sizes and computer-generated random numbers. An investigator not involved in patient follow-up or outcome assessment managed the randomization process. Participants were assigned to either the folinic acid group (arm-A) or the placebo group (arm-B), with both groups receiving standard care. Randomization numbers were enclosed in opaque, sealed envelopes and were only opened when participants were enrolled. Envelopes were opened sequentially after recording the name of the participant, enrollment number, and other relevant details. Each participant was given a serial number as per their randomization

code, which remained concealed from the investigators and participants. Standard care was continued for all participants, and allocation concealment was maintained using the numbered opaque sealed envelopes.

Participants in the folinic acid group received oral folinic acid at 2 mg/kg daily (50 mg daily maximum dose) in a single dose for 24 weeks, while participants in the placebo group received a placebo tablet daily for 24 weeks. The placebo tablet was similar in color, appearance, size, and consistency to the folinic acid formulation.

The mainstay of standard care was behavioral intervention. The primary modes of behavioral intervention were Applied Behavioral Analysis (ABA), structured teaching, and sensory integration, used on an individualized basis [15]. Certain features like comorbidities (irritability, aggressive behavior, hyperactivity) and challenging behaviors were managed by pharmacotherapy when necessary [16]. However, we did not change the dose of any medication, including risperidone and aripiprazole, during the randomization period. We only continued the existing medications the child was already receiving before randomization, throughout the clinical trial period. Other modes of therapy, including complementary and alternative medicine and dietary modifications, were not advised for any participants.

Detailed neuropsychological assessments using DSM-V criteria, CARS, CBCL, VSMS, SP-2, and CSHQ, along with neurological examinations, were conducted once at baseline and repeated after 24 weeks. Assessment of serum levels of folic acid and folate receptor autoantibodies was done using specific chemiluminescent immunoassay (CLIA) and enzyme-linked immunosorbent assay (ELISA) methods, respectively, and was performed at baseline and 24 weeks.

For estimating serum folate receptor autoantibody levels, 96-test ELISA kits from ELK Biotechnology (ELK3046) were used. Based on the cut-offs provided in the ELISA kit manual, we categorized each group into those with high folate receptor autoantibody (≥ 100 ng/ml) levels and those with low (< 100 ng/ml) or undetectable levels.

All patients were followed up at 4-, 8-, 12-, and 24-week post-randomization on an outpatient basis. At each visit, compliance and any treatment-emergent adverse effects were checked. Patients were instructed to return empty medication packets at follow-up visits. A record of the number of tablets dispensed and returned was maintained, and drug compliance was assessed by counting the tablets in the bag and discussing any discrepancies with the family. We also assessed the nature and severity of adverse effects by using a prespecified check list during each follow-up visit on an outpatient basis.

The primary outcome measure was the change in CARS score at 24 weeks from baseline. Secondary efficacy outcomes included changes in CBCL total score, internalizing and externalizing scores, CSHQ score, and social quotient (SQ) measured by VSMS at 24 weeks compared to baseline in both groups. For sensory issues measured by SP-2, we determined the number of participants with significant hyposensitivity or hypersensitivity to auditory, visual, touch, and oral sensory stimuli at baseline and 24 weeks in both folinic acid and placebo groups. These tests and assessments were carried out at baseline and after 24 weeks of oral folinic acid supplementation by trained pediatric neurologists who were unaware of the allocation arm. The change in CARS score was also compared between subgroups with high and low titers of folate receptor autoantibody levels in both the folinic acid and placebo groups.

Throughout the study, participants did not undergo any additional hospital visits or extra blood draws beyond their routine visits related to dietary therapy. Blood samples were handled and disposed of following universal safety protocols. The research was conducted in accordance with Good Clinical Practice (GCP) standards and the principles outlined in the Declaration of Helsinki [17].

Statistical analysis

Data analysis was conducted using IBM SPSS software. The normality of the data was checked using the Kolmogorov–Smirnov test. All participants were included in the analysis, regardless of whether they completed the study (ITT approach). Information about groups was presented as percentages with confidence intervals, and differences between groups were compared using Fisher's exact test. Numerical data was summarized as averages with standard deviations for normally distributed data, or as medians with ranges for other data types.

To compare average values between the two groups at the study's start and after 24 weeks, researchers used either Student's *t*-test or the Wilcoxon rank-sum test. The choice of test depended on whether the data followed a normal distribution.

The analysis followed an intention-to-treat approach, with missing values for participants lost to follow-up addressed using the last observation carried forward (LOCF) method. Statistical significance was determined with a p value < 0.05. The researchers examined the connection between the levels of folic acid in the blood and changes in CARS scores by calculating the Pearson correlation coefficient.

Given the longitudinal nature of the data and potential confounders, a mixed-effects regression model was employed. In this model, age, gender, baseline autism severity measured by CARS score, baseline social quotient, folate receptor autoantibody level, and treatment group (folinic acid or placebo) were independent variables, and the change in CARS score at 24 weeks compared to baseline was the dependent variable.

Results

In this clinical trial, a total of 109 patients were screened. Out of these, 29 children with autism were excluded: 7 had Rett syndrome, 12 had uncontrolled epilepsy, 6 had complex autism (characterized by evidence of problems in early life morphogenesis, such as noticeable dysmorphism or microcephaly), 3 had tuberous sclerosis, and 1 had neurofibromatosis (Fig. 1). A total of 80 children were included in the clinical trial, with 40 in each group. A total of 39 and 38 patients in the folinic acid and placebo groups, respectively, completed the 24-week follow-up. All participants who completed the follow-up period had good compliance, and no protocol violations were reported. Baseline sociodemographic and clinical variables were comparable between both groups (Table 1).

The majority of recruited children were boys, belonged to lower or middle socio-economic status, and resided in rural areas. Autism severity, as measured by the CARS score, improved significantly more in the folinic acid group (3.6 ± 0.8) in comparison to the placebo group (2.4 ± 0.7, $p < 0.001$) (Fig. 2). The change in CBCL total score and CBCL internalizing depicting the severity of behavioral abnormalities also improved more in the folinic acid group in comparison to the placebo group (19.7 ± 9.5 vs. 12.6 ± 8.4 and 15.4 ± 7.8 vs. 8.5 ± 5.7, $p < 0.001$ for both). However, the change in social quotient, number of patients with significant abnormalities in SP-2, and CSHQ score were comparable within the two groups ($p = 0.94$, 0.24, and 0.91, respectively) (Table 2).

Both groups showed significant improvement in CARS score, CBCL total score, CBCL internalizing and externalizing score, number of participants with significant sensory processing problems, and CSHQ score at 24 weeks in comparison to baseline ($p < 0.05$ for all), suggesting that even standard care such as applied behavior analysis and sensory integration therapy can cause improvement in autistic symptoms. However, oral folinic acid further enhances this improvement (Table 3). Regarding individual domains of sensory processing problems in the folinic acid and placebo groups, auditory processing, touch processing, and oral sensory processing problems were the most commonly noted sensory processing abnormalities. In both folinic acid and placebo groups, the number of participants with significant sensory processing abnormalities (either hypersensitivity or hyposensitivity) in all individual domains numerically reduced at 24 weeks compared to baseline (Fig. 3). None of the participants in either group developed any study medication–related adverse effects.

In the two study groups, the anti-folate receptor autoantibodies were positive in high titers in 32/40 and 33/40 cases, respectively, with the difference being not significant statistically ($p = 0.78$). Improvement in CARS score in those with high-titer autoantibody positivity and without high-titer autoantibody was comparable in the placebo group ($p = 0.11$). However, in the folinic acid supplementation group, the improvement in the high-titer autoantibody group in terms of reduction in CARS score at 24 weeks in comparison with baseline was more pronounced compared to their counterparts ($p = 0.03$) (Table 4). Serum folic acid levels did not have any significant correlation with improvement

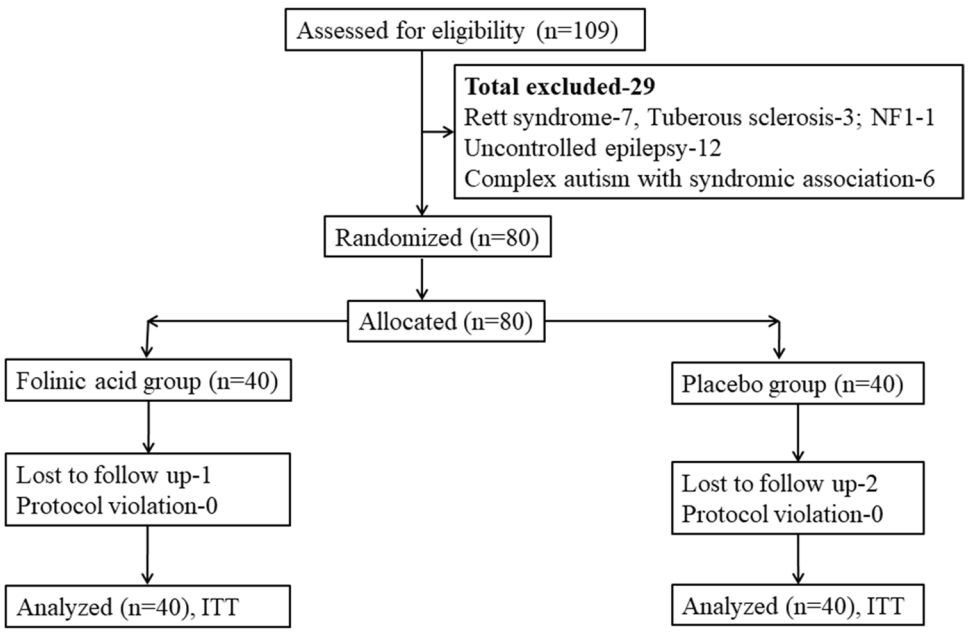

Fig. 1 Study CONSORT diagram

Table 1 Comparison of baseline sociodemographic, clinical, and diagnostic variables between folinic acid and placebo group

Variables	Folinic acid group ($n=40$)	Control group ($n=40$)	p value
Age (years)	5.2 ± 1.8	5.3 ± 1.9	0.81
Gender			
Male	37 (92%)	38 (95%)	0.93
Female	3 (7%)	2 (5%)	
Socioeconomic status			
Lower	11 (27%)	12 (30%)	0.94
Middle	29 (72%)	28 (70%)	
Residence			
Rural	5 (12%)	4 (10%)	0.72
Urban	35 (87%)	36 (90%)	
Use of atypical antipsychotics	16 (40%)	15 (37%)	0.95
CARS score	34.5 ± 3.9	35.1 ± 4.3	0.51
CBCL total score	69.8 ± 11.4	68.5 ± 10.7	0.60
CBCL internalizing score	54.6 ± 10.9	55.2 ± 11.3	0.80
CBCL externalizing score	15.3 ± 8.5	13.7 ± 8.4	0.39
Number of patients with significant sensory processing abnormalities	29 (72%)	27 (68%)	0.81
CSHQ score	38.5 ± 6.9	37.9 ± 7.2	0.71
Social quotient	63.6 ± 11.8	64.7 ± 12.6	0.68
Serum folic acid level (ng/ml)	11.1 ± 5.2	10.9 ± 5.4	0.86
Serum folate receptor autoantibody level (ng/ml)	317.4 ± 149.2	323.8 ± 153.4	0.85
High levels of serum folate receptor autoantibody level	32 (80%)	33 (82%)	0.78

CARS Childhood Autism Rating Scale, *CBCL* Child Behavior Checklist, *CSHQ* Children's Sleep Habits Questionnaire

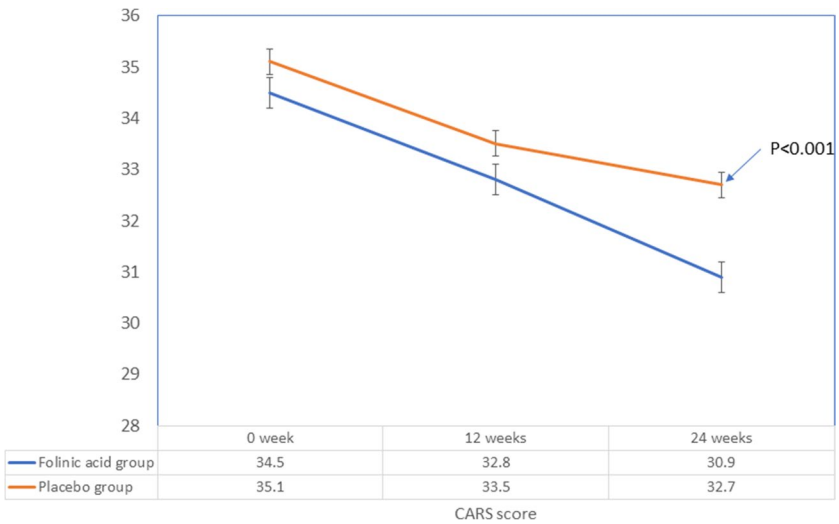

Fig. 2 Change in CARS score over study period in both groups

in CARS score in either the placebo or folinic acid supplementation group ($p=0.23$ and 0.47, respectively).

In the mixed-effects regression model, treatment with folinic acid was the only significant predictor of improvement in autism severity over 24 weeks ($p=0.01$) (Supplementary table). Even the folate receptor autoantibody level was not a significant predictor in the multivariate regression ($p=0.18$).

Discussion

The current trial showed that oral folinic acid has a role in improving the core features of ASD, with more pronounced improvement in the subset with high titers of FRAAs. This suggests that pediatricians may consider testing for these autoantibodies and administer oral folinic

Table 2 Comparison of primary and secondary efficacy outcomes between folinic acid and placebo groups

Change in scores	Folinic acid group ($n=40$)	Control group ($n=40$)	p value
CARS score	3.6 ± 0.8	2.4 ± 0.7	< 0.001
CBCL total score	19.7 ± 9.5	12.6 ± 8.4	< 0.001
CBCL internalizing score	15.4 ± 7.8	8.5 ± 5.7	< 0.001
CBCL externalizing score	4.3 ± 2.9	4.1 ± 2.7	0.75
Number of patients with significant sensory processing abnormalities	17 (42%)	18 (45%)	0.94
CSHQ score	4.6 ± 3.8	3.7 ± 3.1	0.24
Social quotient	3.4 ± 3.1	3.5 ± 2.9	0.91

CARS Childhood Autism Rating Scale, *CBCL* Child Behavior Checklist, *CSHQ* Children's Sleep Habits Questionnaire

acid to children who test positive. As this trial did not show any adverse effects causally related to folinic acid in any participants, it appears that oral folinic acid can be safely administered universally to all children with ASD. However, even the presence of folate receptor autoantibody was not a significant predictor of change in CARS score in participants. This could be because only some of the participants with high titers of folate receptor autoantibodies received folinic acid, while others in the placebo group received only standard care (ABA).

Few studies have explored the association between FRAAs and clinical features in children with ASD. A significant inverse relationship between elevated blocking FRAA levels and decreased 5-MTHF cerebrospinal fluid concentrations has been observed [9]. The impact of age on blocking FRAA levels is inconsistent, with reports of both increases and decreases over time. The clinical presentation of ASD varies depending on whether individuals produce blocking or binding FRAAs. Binding FRAAs are associated with higher serum B12 levels, while blocking FRAAs are linked

Table 3 Comparison of key outcome variables in the folinic acid and placebo groups at baseline and 24 weeks

Variable	Folinic acid group			Placebo group		
	Baseline ($n=40$)	24 weeks ($n=40$)	p value	Baseline ($n=40$)	24 weeks ($n=40$)	p value
CARS score	34.5 ± 3.9	30.9 ± 2.2	< 0.001	35.1 ± 4.3	32.7 ± 3.2	0.005
CBCL total score	69.8 ± 11.4	50.1 ± 10.7	< 0.001	68.5 ± 10.7	45.9 ± 9.1	< 0.001
CBCL internalizing score	54.6 ± 10.9	49.2 ± 9.6	0.02	55.2 ± 11.3	46.8 ± 9.8	< 0.001
CBCL externalizing score	15.3 ± 8.5	10.9 ± 6.4	0.009	13.7 ± 8.4	9.6 ± 5.7	0.01
Number of patients with significant sensory processing abnormalities	29 (73%)	17 (42%)	0.01	27 (68%)	18 (45%)	0.005
CSHQ score	38.5 ± 6.9	33.9 ± 4.3	< 0.001	37.9 ± 7.2	34.2 ± 5.9	0.01
Social quotient	63.6 ± 11.8	60.2 ± 10.5	0.17	64.7 ± 12.6	61.2 ± 11.6	0.20

CARS Childhood Autism Rating Scale, *CBCL* Child Behavior Checklist, *CSHQ* Children's Sleep Habits Questionnaire

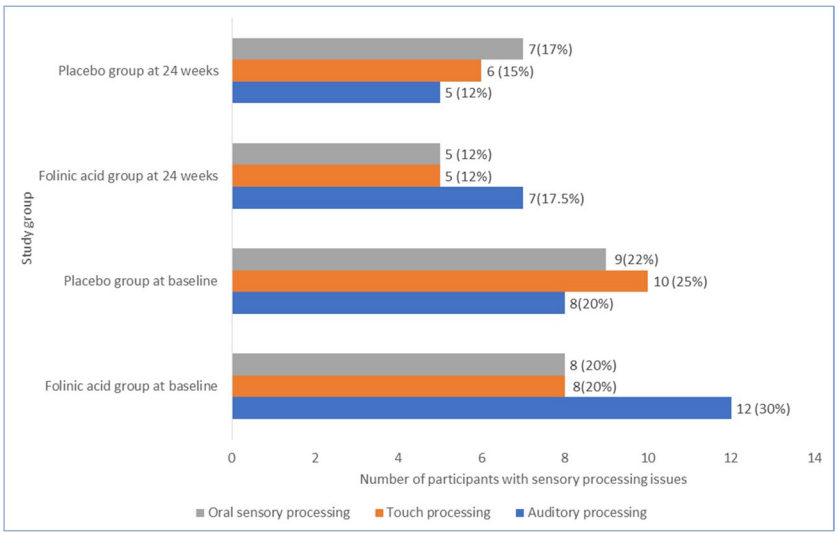

Fig. 3 Sensory processing problems in both groups at baseline and after 24 weeks

Table 4 Comparison of change in CARS score in the folinic acid and placebo groups in those with and without high titers of anti-folate receptor autoantibody

	Folinic acid group ($n=40$)			Placebo group ($n=40$)		
	High-titer anti-folate receptor antibody ($n=32$)	Low-titer anti-folate receptor antibody ($n=8$)	p value	High-titer anti-folate receptor antibody ($n=33$)	Low-titer anti-folate receptor antibody ($n=7$)	p value
Change in CARS score from baseline to 24 weeks	4.3 ± 1.3	3.2 ± 1.1	0.03	2.2 ± 0.8	2.7 ± 0.9	0.11

CARS Childhood Autism Rating Scale

to improvements in oxidative stress, inflammation, communication, and repetitive behaviors [9]. Some studies have focused on serum folic acid levels without differentiating FRAA types, noting a higher prevalence of hypothyroidism in FRAA-positive children. Positive correlations between blocking FRAA titers and TSH levels have been reported [18, 19]. Although thyroid hormone levels often remain within normal limits, the interaction between TSH and thyroid hormones may be altered in some children with ASD. Notably, FRAAs have been found to bind to prenatal thyroid tissue, suggesting a potential role in the early programming of the hypothalamic-pituitary-thyroid axis [20].

In contrast to our study, previous research has reported excitement or agitation in 11.7% of cases, aggression in 9.5%, insomnia in 8.5%, increased tantrums in 6.2%, headaches in 4.9%, and gastroesophageal reflux in 2.8% of cases [13]. In studies combining folinic acid with other agents, adverse events included worsening behavior in 8.5% and aggression in 1.3%. Frye et al. [12] conducted a 12-week study that monitored folinic acid's impact on agitation and excitability every 3 weeks. Initially, adverse events were similar in both treatment and placebo groups, but by the ninth week, these symptoms significantly decreased in the treatment group. This suggests that some adverse events in prior studies may not be causally related to folinic acid.

Frye et al. [12] also conducted a double-blind, placebo-controlled RCT replicating previous findings. Improvements were observed in verbal communication, daily living skills, irritability, social withdrawal, stereotypic behaviors, hyperactivity, and inappropriate speech. Verbal communication improvements surpassed the clinical relevance threshold. A smaller trial involving 19 children also reported significant advancements in ASD severity and specific domains of social interaction and communication [13]. Additionally, a large retrospective analysis of 1286 ASD participants found that higher doses of folinic acid were linked to greater cognitive, attentional, and language improvements with minimal adverse effects [21]. These findings suggest oral folinic acid is effective in mitigating core and associated ASD symptoms, with different dosing strategies showing notable clinical benefits.

In contrast to our findings, Shi et al. [22] reported lower serum binding-FRAA levels in ASD children compared to typically developing peers, particularly in boys. While neither study found overall folate level differences between the groups, the current study observed no gender-based disparities, unlike Shi et al. [22]. The combination of nitrite and binding-FRAA showed potential as a diagnostic marker for ASD in Shi et al.'s study. These discrepancies highlight the need for further research with larger sample sizes to elucidate underlying mechanisms.

In children with ASD and cerebral folate deficiency (CFD), case series and reports indicate that oral folinic acid treatment leads to symptom improvement in approximately 67% of cases. Three studies separately assessed folinic acid's impact on irritability in ASD and CFD children. Among the ASD group, 58% showed reduced irritability, compared to 47% in the CFD-only group. Response rates varied significantly across studies: one reported an 88% response rate, while two others recorded much lower rates of 22% and 0% [23–25].

This RCT has limitations. The CARS, used in our study, relies on observations from parents and teachers. Notably, objective measures by impartial examiners tend to show large effect sizes in blinded studies, while parent-reported outcomes exhibit more modest effects. This highlights the challenge of the placebo effect in ASD research, especially in mildly affected children, where the placebo effect may be larger. This may explain the larger effect sizes in parent-reported outcomes in studies with non-treatment comparison groups compared to placebo-controlled trials.

Conclusions

Add-on oral folinic acid supplementation is safe and efficacious in improving the severity of symptoms in children with ASD. This clinical benefit is more pronounced in children with high titers of folate receptor autoantibodies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00431-024-05762-6.

Authors' contributions PKP, IKS, DG, AP and SS were involved in the conception, acquisition, analysis, and interpretation of data. SS and KM performed biochemical analysis. PKP, DG and AP drafted the initial draft, and PKP, IKS performed the statistical analysis and critically revised the manuscript. All the authors read and approved the final version of the manuscript.

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Data availability Data will be provided on reasonable request by the corresponding author.

Declarations

Ethical approval Ethical approval for the study was obtained from the Institutional Review Board of All India Institute of Medical Sciences, Rishikesh, and written informed consent was provided by the participants' caregivers.

Competing interest The authors declare no competing interests.

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