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ORIGINAL ARTICLE

Folinic Acid as Adjunctive Therapy in Treatment of Inappropriate Speech in Children with Autism: A Double-Blind and Placebo-Controlled Randomized Trial

Neda Batebi[1] · Hossein Sanjari Moghaddam[1] · Alireza Hasanzadeh[1] · Yousef Fakour[1] · Mohammad Reza Mohammadi[1] · Shahin Akhondzadeh[1,2]

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Abstract

This is a double-blind, placebo-controlled randomized trial to investigate the potential therapeutic effects of folinic acid/placebo as an adjuvant to risperidone on inappropriate speech and other behavioral symptoms of autism spectrum disorder (ASD). Fifty-five ASD children (age (mean ± standard deviation) = 13.40 ± 2.00; male/female: 35/20) were evaluated for behavioral symptoms at baseline, week 5, and week 10 using the aberrant behavior checklist-community (ABC-C). Folinic acid dosage was 2 mg/kg up to 50 mg per day for the entire course of the study. The repeated measures analysis showed significant effect for time × treatment interaction on inappropriate speech (F = 3.51; df = 1.61; P = 0.044), stereotypic behavior (F = 4.02; df = 1.37; P = 0.036), and hyperactivity/noncompliance (F = 6.79; df = 1.66; P = 0.003) subscale scores. In contrast, no significant effect for time × treatment interaction was found on lethargy/social withdrawal (F = 1.06; df = 1.57; P = 0.336) and irritability (F = 2.86; df = 1.91; P = 0.064) subscale scores. Our study provided preliminary evidence suggesting that folinic acid could be recommended as a beneficial complementary supplement for alleviating speech and behavioral symptoms in children with ASD.

Clinical trial registeration: This trial was registered in the Iranian Registry of Clinical Trials (www.irct.ir; No. IRCT20090117001556N114).

Keywords Autism spectrum disorder · Inappropriate speech · Aberrant behavior checklist-community · Folate metabolism · Folinic acid

Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders in early childhood, affecting approximately 2.47% of children and adolescents in the United States [1, 2]. The clinical symptoms of ASD are generally categorized into core and associated symptoms. The core symptoms of ASD include social communication impairments and inappropriate, restrictive, and stereotypic behavior. In contrast, the main associated symptoms of ASD are irritability, hyperactivity, aggressiveness, and cognitive deficits [3–7]. Among the associated symptoms, management of inappropriate speech has gained significant attention as it complicates development, learning, and communication of ASD children, necessitating its pharmacological treatment. Review of literature shows that ASD is associated with phonological [8], semantic [9], productive morphology and syntax [10], prosody (i.e. lexical and affective intonation) [11, 12], and pragmatics [11, 12] impairments, severely affecting the quality of life in these patients. In fact, studies estimate that even after receiving clinical and educational interventions, 30% of children with ASD remain minimally verbal [13, 14]. The beneficial effects of risperidone on associated symptoms of ASD, particularly inappropriate speech, have been substantiated in several clinical trials [15–17].

Neda Batebi, Hossein Sanjari Moghaddam and Alireza Hasanzadeh have contributed equallt to this study.

✉ Shahin Akhondzadeh
s.akhond@neda.net

[1] Psychiatric Research Center, Roozbeh Hospital, Tehran University of Medical Sciences, Tehran, Iran

[2] Psychiatric Research Center, Roozbeh Psychiatric Hospital, Tehran University of Medical Sciences, South Kargar Street, 13337 Tehran, Iran

Nonetheless, the therapeutic effects and tolerability of risperidone have not been universally ideal [18]. For instance, studies have reported that risperidone is associated with several adverse events, including, but not limited to, gastrointestinal symptoms, parkinsonism, weight gain, and prolactinemia [19]; thus, designing novel treatment strategies is an absolute necessity for optimal pharmacological treatment of ASD associated symptoms.

The pathophysiology of ASD is associated with several factors, such as genetic, epigenetic, and environmental factors. However, the underlying molecular mechanisms of ASD are poorly understood [20, 21]. Among these underlying mechanisms, abnormal folate metabolism and increased oxidative stress might have significant importance in the development of ASD. There are lots of aberrations in folate metabolism that have been linked to ASD [22]. Folate binds folate receptor-α (FRα) to transport across the choroid plexus epithelium by endocytosis [23]. There are some pieces of evidence in the literature showing that cerebral folate deficiency is related to ASD (specially in individuals with Rett syndrome, a disorder closely associated with ASD) [24, 25]. Folate supplementation during conception and prenatal period can decrease ASD risk in children [26, 27]. Treatment with folinic acid, a reduced form of folate, was demonstrated to improve ASD and other neurological symptoms [23, 28]. In this regard, Ramaekers et al. [23] examined four patients with late-infantile autism and idiopathic cerebral folate deficiency with high titers of blocking autoantibodies Treatment with folinic acid led to improved communication and neurologic abnormalities in the two younger children with ASD that received early treatment for cerebral folate deficiency (at the ages of two and three years), while the two older children, who were treated beginning at the ages of 5 and 12 years, had a poorer clinical outcome. In another study, Frye et al. [28] investigated the therapeutic effects of folinic acid (leucovorin) in 93 children with ASD (84 male and 9 female children, mean age = 7 years 3 months, SD = 3 years 1 month) that had a high prevalence (75.3%) of folate receptor autoantibodies. Compared with ASD children without folinic acid treatment, improved outcomes were observed in treated children over a mean period of four months in verbal communication, receptive and expressive language, attention and stereotypical behavior.

Furthermore, a robust bulk of evidence suggests that ASD is linked to aberrant redox metabolism [29, 30]. In this regard, it was shown that the mean concentration of free reduced glutathione (GSH), a crucial intracellular antioxidant, was significantly reduced, and the oxidized form of glutathione (GSSG) was significantly augmented, leading to decreased glutathione redox status and increased oxidative stress [29, 30]. Folinic acid has been substantiated to alleviate oxidative stress in patients with ASD, which might lead to better immune function, increased detoxification capacity, and membrane redox signaling [31–34].

Previous animal studies have reported potential beneficial effects of folinic acid on ASD-related behavioral impairments. In a recent study by Sequeira et al. [35] on rats, it was substantiated that FRα antibodies during gestation and weaning lead to severe behavioral impairments in rats. In another study, Desai et al. [17] administered antibodies against rat FRα during pregnancy, which led to deficits in communication, learning, and cognitive functions. They further demonstrated that folinic acid could prevent these deficits in their rat model. Given the protective role of folinic acid in the pathophysiology of ASD and previous clinical reports showing beneficial effects of folinic acid in verbal communication, receptive and expressive language, attention and stereotypical behavior in ASD patients with cerebral folate deficiency, we postulated that compared to risperidone plus placebo treatment, adjunctive treatment with risperidone and folinic acid might lead to significant improvement in clinical outcomes in inappropriate speech and other behavioral symptoms of ASD. In this randomized clinical trial, we aimed to assess the therapeutic effects of risperidone plus folinic acid on inappropriate speech and other behavioral symptoms of ASD patients.

Methods

Trial Design and Settings

This is a double-blind, placebo-controlled randomized clinical trial carried out at the autism clinic in children's outpatient clinic of Roozbeh Hospital (Tehran University of Medical Sciences, Tehran, Iran) from November 2018 to April 2019. Written informed consent was acquired from the parents or guardians of all patients before inclusion into the trial. The participants were randomly categorized into two parallel folinic acid and placebo groups. The enrolled patients were examined prospectively at baseline/screening, week 5, and week 10. The study was carried out in accordance with the ethical principles laid down in the Declaration of Helsinki and its later amendments World Medical Association (2013) [36]. Moreover, the trial was approved by the institutional review board and the ethics committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.503). This trial is registered in the Iranian Registry of Clinical Trials with IRCT20090117001556N114 as the registration code.

Participants

The participants were chosen from both male and female outpatients referred to the autism clinic in the children's outpatient clinic of Roozbeh Hospital from different parts

of Iran with probable autistic signs and symptoms. Eligible participants were 4-12-year-old children with symptoms compatible with the Diagnostic and Statistical Manual of Mental Disorders, fifth edition [37]. Diagnosis and severity of ASD were also verified by a qualified child psychiatrist based on the behavioral observations and semi-structured interviews with the parents (Autism Diagnostic Interview-Revised) [38]. Children with symptoms that were not severe enough to be considered for treatment with risperidone were excluded from the study. Other exclusion criteria were: (a) concurrent prominent psychiatric disorders (e.g. depression, mania, anxiety, and bipolar disorder), (b) preexistent medical or disease conditions such as epileptic disorders and febrile seizures, (c) severe intellectual disability (i.e. intelligence quotient < 70), (d) tardive dyskinesia, or (e) history of antipsychotic or behavioral therapy during the past six months prior to registration. Due to ethical guidelines and to avoid asking patients to stop taking any medication prior to entry, only participants were enrolled who were medication-free for at least six weeks before registration due to other reasons (e.g. discontinuation of drugs by their parents).

Interventions

Risperidone was administered for both folinic acid and placebo groups twice daily (Risperdal; Janssen Pharmaceuticals, Beerse, Belgium) initiating at a dose of 0.5 mg with a dose increase of 0.5 mg per week (for the first three weeks). The risperidone dosage was maintained at the dose of risperidone at the end of week 3 for the rest of the study. If needed, the dose of risperidone was adjusted based on the clinical assessment of the participants. The maximum risperidone dose for children below 20 kg was 1 mg/day, and for children equal to 20 kg or heavier was 2 mg/day, respectively. Folinic acid ($C_{20}H_{23}N_7O_7$) dosage was 2 mg/kg up to 50 mg per day for the entire course of the study. The dosage of folinic acid was based on previous clinical trials [39]. At the same time, the control group received placebo capsules. Folinic acid and risperidone were initiated simultaneously. No other concomitant medication was allowed for neither of the trial groups. The adherence to treatment was assessed via checking with parents and pill-counting.

Outcomes and Tools

For full description regarding the design, administration, and scoring of aberrant behavior checklist-community (ABC-C) refer to the studies by Ghaleiha et al. [40, 41]. Briefly, ABC-C is a questionnaire with 58 items for the assessment of behavioral symptoms in developmental disorders such as ASD. ABC-C is a valid and reliable tool designed to investigate five domains of behavioral disruptions, including inappropriate speech, lethargy/social withdrawal, stereotypic behavior, hyperactivity/noncompliance, and irritability [16, 17]. The primary outcome measure of this study was the mean change in the score for inappropriate speech subscale from baseline/screening to study endpoint. The secondary outcome measures comprised of mean changes in lethargy/social withdrawal, stereotypic behavior, hyperactivity/noncompliance, and irritability subscales of ABC-C and the frequency of adverse events. The parents completed the ABC-C after education by the investigators.

Adverse Events

Adverse events were either observed by a trained rater or reported by parents in week 5 and 10 events. A checklist containing 25 possible side effects of trial medications was used to record the side effects [42]. The Extrapyramidal Symptom Rating Scale (ESRS) was used to record the potential extrapyramidal symptoms, including Parkinsonism, akathisia, and dystonia [43]. Parents were also inquired about any adverse events other than those in the checklist. A 24-hour accessible phone line was provided for the parents to report any unexpected adverse events requiring immediate medical intervention.

Sample Size

Based on the results of a pilot study and previous clinical trials, 3.5 score variation in ABC-C inappropriate speech subscale between the folinic acid and placebo groups with a standard deviation (SD) of 3.5 was considered for calculation of sample size. Considering a power of 90%, a 2-sided significance level of 5%, and an attrition rate of 20%, the total sample size of the trial was 50 patients.

Randomization and Blinding

The trial participants were equally randomized into two groups of folinic acid and placebo in a 1:1 ratio. Using the Microsoft Office Excel software, a specific random code was allocated to each patient. The randomization and allocation were conducted using block randomization (with blocks of size 4) by the primary investigator of the study, who was not involved in the diagnosis and follow-ups. The allocations were kept in confidential and sealed opaque envelops and were exposed at the end of the trial. Separate individuals implemented randomizations, drug administration, rating, data entry, and statistical analysis. Moreover, patients, parents, and researchers were blinded to the allocations. Placebo capsules were completely indistinguishable to folinic acid regarding shape, size, color, and taste. Both folinic acid and placebo capsules were administered by a psychiatry resident.

Statistical Methods

The Statistical Package of Social Science Software (SPSS version 20, IBM Company, USA) was used to compare the trial groups based on ABC-C subscale scores and the frequency of adverse events. Statistical analysis of this trial was performed by the intent-to-treat approach. Continuous variables are demonstrated as mean (standard deviation), and categorical variables are displayed as frequency (percentage). The general linear model (GLM) repeated measures analysis was implemented to explore the time, treatment, and time × treatment effects. Greenhouse-Geisser correction for degrees of freedom was reported if Mauchly's test of sphericity was significant. Effect sizes were reported as partial eta squared (η_p^2) [44]. Student T-test (two-tailed) was used to compare the mean change of the five ABC-C subscales scores from baseline to the study endpoint between folinic acid and placebo groups and Cohen's d effect sizes were reported [45]. The categorical variables, including the number of adverse events, the Chi-square test was applied to compare the frequency between two trial groups at the study endpoint. P-value of < 0.05 was considered as statistically significant.

Results

Participants

From a total of 95 patients with ASD who were screened for the eligibility criteria, 66 patients were included and randomly divided into two groups of risperidone plus folinic acid or risperidone plus placebo in a 1:1 ratio (Fig. 1). Eleven patients (risperidone plus folinic acid = 5, risperidone plus placebo = 6) were excluded at week 5 because of consent withdrawal. Finally, 55 patients completed the 10-week course of the study and were chosen for statistical analysis. The demographic features of the participants are shown in Table 1. The patients in folinic acid and placebo groups were comparable based on age, sex, and body weight at baseline.

Outcomes

Table 2 shows the five subscale scores of ABC-C in folinic acid and placebo groups at baseline and two follow-up visits (weeks 5 and 10). There was no significant difference between the two trial groups based on any of ABC-C subscale scores at baseline and two follow-up visits (weeks 5 and 10) (Table 2). Nonetheless, patients in both folinic acid and placebo groups demonstrated significant improvements

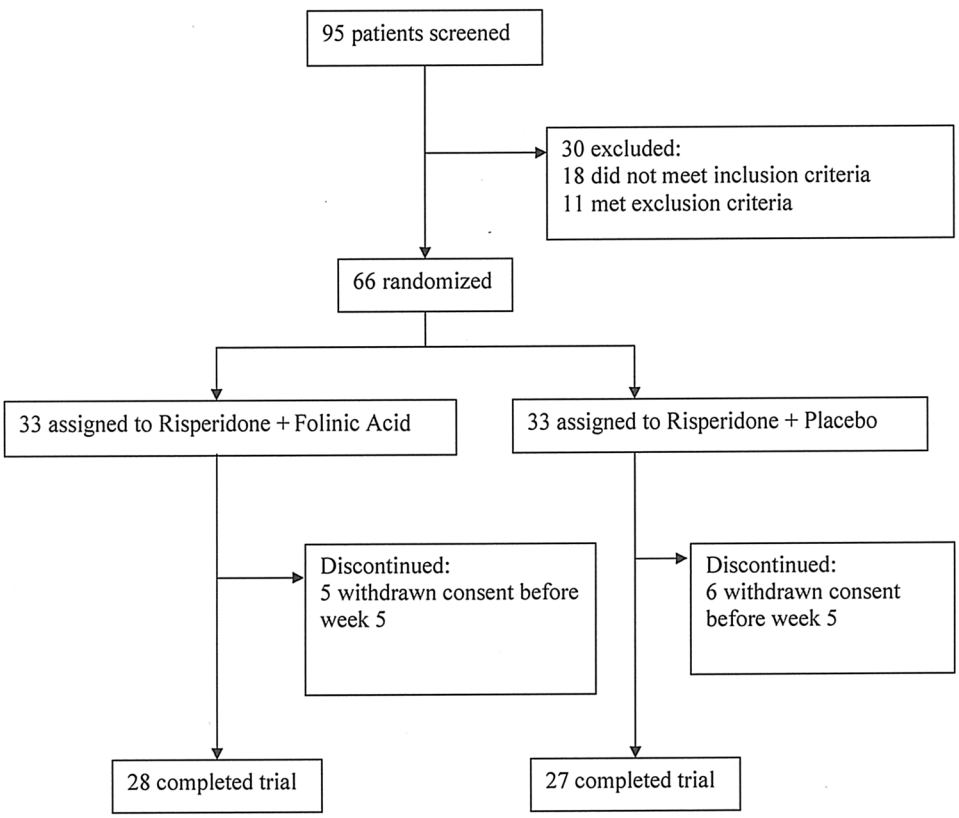

Fig. 1 Flow diagram of the study

Table 1 Baseline characteristics of the patients in the two trial groups

	Folinic acid group (n = 28)	Placebo group (n = 27)	P-value
Age [years; mean (SD)]	8.36 (1.81)	7.52 (1.84)	0.09[a]
Sex [n (%)]			0.403[b]
Male	16 (57.1%)	19 (70.4%)	
Female	12 (42.9%)	8 (29.6%)	
Baseline body weight [kg; mean (SD)]	36.71 (9.08)	32.89 (9.50)	0.13[a]
Baseline inappropriate speech [mean (SE)]	6.54 (0.327)	6.41 (0.437)	0.814[a]
Baseline stereotypic behavior [mean (SE)]	12.32 (0.766)	11.30 (1.057)	0.434[a]
Baseline hyperactivity/noncompliance [mean (SE)]	28.25 (0.949)	26.37 (1.595)	0.312[a]
Baseline lethargy/social withdrawal [mean (SE)]	20.57 (1.156)	20.37 (1.652)	0.920[a]
Baseline irritability [mean (SE)]	22.82 (0.922)	22.67 (1.122)	0.915[a]

P-value of < 0.05 was considered statistically significant

SD Standard deviation, *SE* standard error

[a]Student T-test

[b]Chi-square test

in all subscale scores of ABC-C from baseline to week 10 (study endpoint) (effect of time $p < 0.001$ for all subscales).

Primary Outcome Measure: *ABC-C Inappropriate Speech Subscale Score*

As illustrated in Fig. 2, from the baseline to the study endpoint, ABC-C inappropriate speech subscale score demonstrated more improvement in the folinic acid compared to the placebo group (change from baseline to study endpoint (mean (standard error (SE))): 1.71 (0.28) and 0.88 (0.28), respectively; $t = 2.04$, $df = 53$, $P = 0.045$, Cohen's $d = 2.98$). Similarly, GLM repeated measures analysis showed significant time × treatment interaction effect on the ABC-C inappropriate speech subscale score ($F = 3.51$; $\eta_p^2 = 0.06$; $df = 1.61$; $P = 0.044$) (Table 3).

Secondary Outcome Measures: *ABC-C Stereotypic Behavior, Hyperactivity/Noncompliance, Irritability, and Lethargy/social Withdrawal Subscale Scores*

Improvements in ABC-C stereotypic behavior (change from baseline to study endpoint (mean (SE)): 3.21 (0.58) and 1.59 (0.45), respectively; $t = 2.17$, $df = 53$, $P = 0.034$, Cohen's $d = 3.12$) (Fig. 3) and hyperactivity/noncompliance (change from baseline to study endpoint (mean (SE)): 8.17 (0.99) and 3.96 (0.95), respectively; $t = 3.05$, $df = 53$, $P = 0.003$, Cohen's $d = 4.33$) (Fig. 4) subscales scores showed significant difference between the folinic acid and the placebo groups from baseline to week 10. GLM repeated measures analysis also revealed a significant effect for time × treatment interaction on the stereotypic behavior ($F = 4.02$; $\eta_p^2 = 0.07$; $df = 1.37$; $P = 0.036$) and hyperactivity/noncompliance ($F = 6.79$; $\eta_p^2 = 0.11$; $df = 1.66$; $P = 0.003$) subscale scores (Table 3). Contrarily, improvements in lethargy/social withdrawal (change from baseline to study endpoint (mean (SE)): 4.10 (0.93) and 3.25 (0.67), respectively; $t = 0.73$, $df = 53$, $P = 0.466$) subscale score showed no significant difference between the folinic acid and the placebo groups from baseline to week 10 (Fig. 5). Likewise, no significant effect for time × treatment interaction was found for lethargy/social withdrawal ($F = 1.06$; $\eta_p^2 = 0.02$; $df = 1.57$; $P = 0.336$) subscale score (Table 3). For irritability subscale score, patients in folinic acid group showed a significantly higher decline compared to the patients in the placebo group (change from baseline to study endpoint (mean (SE)): 10.67 (0.83) and 8.25 (0.58), respectively; $t = 2.35$, $df = 53$, $P = 0.022$, Cohen's $d = 3.37$) (Fig. 6). However, no significant effect for time × treatment interaction was found for irritability subscale score ($F = 2.86$; $\eta_p^2 = 0.05$; $df = 1.91$; $P = 0.064$) (Table 3).

Clinical Complications and Side Effects

If any severe side effects were presented in participants, they were excluded from the study. However, no severe side effects were found, and no patient was excluded for this reason. A total of 8 side effects were identified over the course of study, and no unpredicted signs or symptoms were reported by the patients or their parents. Increased appetite (25%) and diarrhea (17.9%) were the most common adverse events seen in the folinic acid group, and headache (18.5%) was the most common one in the placebo group. Nevertheless, there was no statistically significant difference in the frequency of adverse effects between the two groups (Table 4). Additionally, no significant difference was seen between the folinic acid and the placebo groups in respect of extrapyramidal symptoms.

Table 2 Comparison of scores and score changes between the two trial groups

	Clinical scores	Folinic acid group (n = 28)	Placebo group (n = 27)	Mean difference (95% CI)	P-value (Cohen's d)
Inappropriate speech	Baseline	6.54 (0.327)	6.41 (0.437)	0.128 (− 0.96 to 1.21)	0.814[a]
	Week 5	5.54 (0.319)	6.07 (0.458)	− 0.538 (− 1.65 to 0.57)	0.337[a]
	Week 10	4.82 (0.341)	5.52 (0.481)	− 0.697 (− 1.87 to 0.48)	0.240[a]
	Change from baseline to week 5	1.000 (0.241)	0.333 (0.130)	0.666 (0.11 to 1.22)	**0.020**[a] (3.47)
	Change from baseline to week 10	1.714 (0.285)	0.888 (0.284)	0.825 (0.01 to 1.63)	**0.045**[a] (2.98)
Stereotypic behavior	Baseline	12.32 (0.766)	11.30 (1.057)	1.025 (− 1.58 to 3.63)	0.434[a]
	Week 5	10.11 (0.856)	10.11 (1.055)	− 0.004 (− 2.72 to 2.71)	0.998[a]
	Week 10	9.11 (0.871)	9.70 (1.042)	− 0.597 (− 3.31 to 2.12)	0.662[a]
	Change from baseline to week 5	2.214 (0.418)	1.185 (0.287)	1.029 (0.00 to 2.05)	**0.049**[a] (2.93)
	Change from baseline to week 10	3.214 (0.584)	1.592 (0.456)	1.621 (0.13 to 3.11)	**0.034**[a] (3.12)
Hyperactivity/noncompliance	Baseline	28.25 (0.949)	26.37 (1.595)	1.880 (− 1.81 to 5.57)	0.312[a]
	Week 5	23.89 (1.096)	23.56 (1.656)	0.337 (− 3.62 to 4.29)	0.865[a]
	Week 10	20.07 (1.325)	22.41 (1.688)	− 2.336 (− 6.62 to 1.95)	0.279[a]
	Change from baseline to week 5	4.357 (0.573)	2.814 (0.739)	1.542 (− 0.32 to 3.41)	0.104[a]
	Change from baseline to week 10	8.178 (0.995)	3.963 (0.952)	4.215 (1.45 to 6.98)	**0.003**[a] (4.33)
Lethargy/social withdrawal	Baseline	20.57 (1.156)	20.37 (1.652)	0.201 (− 3.81 to 4.22)	0.920[a]
	Week 5	17.43 (1.012)	18.59 (1.765)	− 1.164 (− 5.20 to 2.88)	0.566[a]
	Week 10	16.46 (1.062)	17.11 (1.483)	− 0.647 (− 4.28 to 2.99)	0.723[a]
	Change from baseline to week 5	3.142 (0.715)	1.777 (0.507)	1.365 (− 0.40 to 3.13)	0.128[a]
	Change from baseline to week 10	4.107 (0.930)	3.259 (0.672)	0.847 (− 1.46 to 3.16)	0.466[a]
Irritability	Baseline	22.82 (0.922)	22.67 (1.122)	0.155 (− 2.75 to 3.06)	0.915[a]
	Week 5	17.39 (1.320)	17.70 (1.346)	− 0.311 (− 4.09 to 3.47)	0.870[a]
	Week 10	12.14 (0.750)	14.41 (1.140)	− 2.265 (− 4.98 to 0.45)	0.101[a]
	Change from baseline to week 5	5.428 (1.012)	4.963 (0.585)	0.465 (− 1.90 to 2.83)	0.695[a]
	Change from baseline to week 10	10.67 (0.835)	8.25 (0.585)	2.41 (0.35 to 4.48)	**0.022**[a] (3.37)

P-value of < 0.05 was considered statistically significant; Data are shown as mean (standard error)

CI Confidence interval;

[a]Independent T-test

Discussion

The current study is the first double-blind, placebo-controlled randomized clinical trial examining the beneficial effects of folinic acid as an adjunctive treatment with risperidone in improving inappropriate speech and other behavioral disruptions in ASD children. For this purpose, we used ABC-C rating scales, including inappropriate speech as the primary outcome measure, and lethargy/social withdrawal, stereotypic behavior, hyperactivity/noncompliance, and irritability as the secondary outcome measures. Our findings substantiated that adjunctive pharmacotherapy with folinic acid leads to significant improvements in inappropriate speech, stereotypic behavior, hyperactivity/noncompliance, and irritability subscales. Notwithstanding, we found no significant effect for adjunctive pharmacotherapy with folinic acid on the lethargy/social withdrawal subscale. Our results suggest that folinic acid could be recommended as a beneficial complementary supplement for alleviating speech and behavioral symptoms in children with ASD. The nutrients containing high levels of folate, such as dark green leafy vegetables, might also lead to some noticeable improvements in ASD patients. However, this should be further examined in future studies. Ultimately, it should be noted that both the folinic acid and placebo groups had significant improvements in all ABC-C subscales over the course of the study confirming the beneficial effect of risperidone in relieving the associated symptoms of ASD.

One of the main associated symptoms of ASD, which have crucial effects on communication, learning, and

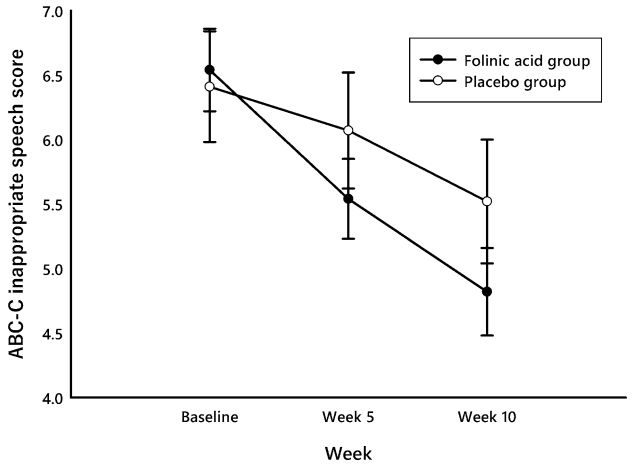

Fig. 2 Comparison of ABC-C inappropriate speech subscale score [mean (standard error)] between the folinic acid and placebo groups

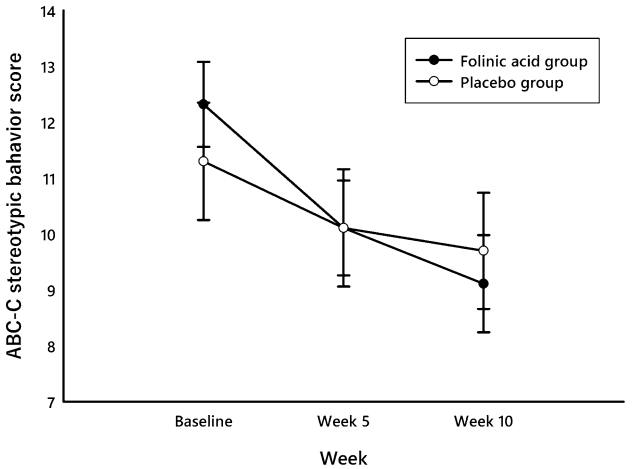

Fig. 3 Comparison of ABC-C stereotypic behavior subscale score [mean (standard error)] between the folinic acid and placebo groups

development in ASD children is inappropriate speech. Language and speech problems in ASD comprise a wide spectrum of disorders including phonological [8], semantic (e.g. idiosyncratic word use and neologisms) [9], productive morphology and syntax [10], prosody (i.e. lexical and affective intonation) [11, 12], and pragmatics (i.e. appropriate responses in social contexts) [11, 12] impairments. Inappropriate speech might interfere with proper social interactions that are required for normal development of the patients; thus, designing new pharmacological treatment strategies for inappropriate speech is a crucial necessity. Up to now, a number of clinical studies have investigated the therapeutic effects of folinic acid in the improvement of adaptive behaviors of ASD patients [39, 46]. However, the design of these studies was not adjunctive, and risperidone was not administered for the patients. In 2013, Frye et al. [46] treated thirty-seven ASD children with 75 mg/Kg methylcobalamin, twice weekly, and 400 mg folinic acid, twice daily, for three months. They reported that all the Vineland adaptive behavior scale (VABS) subscales significantly improved throughout the study. Furthermore,

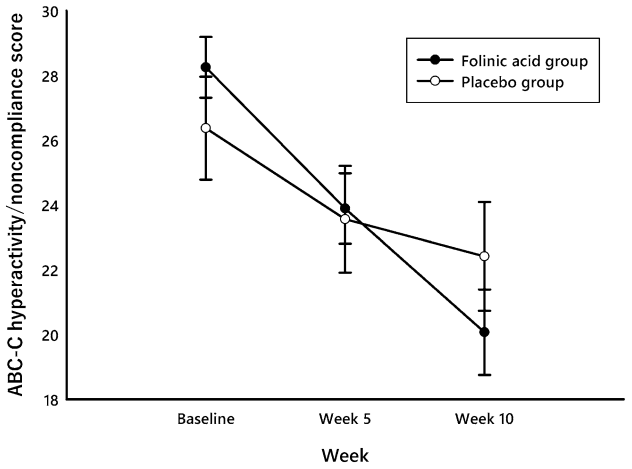

Fig. 4 Comparison of ABC-C hyperactivity/noncompliance subscale score [mean (standard error)] between the folinic acid and placebo groups

Table 3 Results of GLM repeated measures analysis

ABC-C subscales	Factors					
	Time		Treatment		Time × treatment	
	F	η_p^2	F	η_p^2	F	η_p^2
Inappropriate speech	31.02***	0.36	0.048	0.009	3.51*	0.06
Stereotypic behavior	122.46***	0.40	0.012	> 0.001	4.02*	0.071
Hyperactivity/noncompliance	55.67***	0.51	> 0.001	> 0.001	6.79**	0.11
Lethargy/social withdrawal	31.59***	0.37	0.082	0.002	1.06	0.02
Irritability	156.26***	0.74	0.30	0.006	2.86	0.05

***P-value < 0.001; **P-value < 0.01; *P-value < 0.05

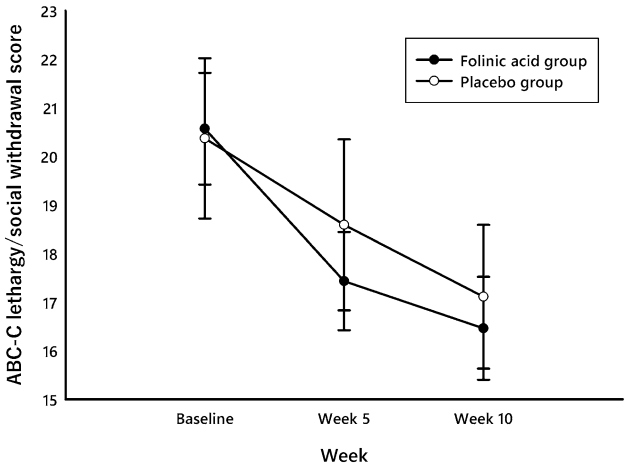

Fig. 5 Comparison of ABC-C lethargy/social withdrawal subscale score [mean (standard error)] between the folinic acid and placebo groups

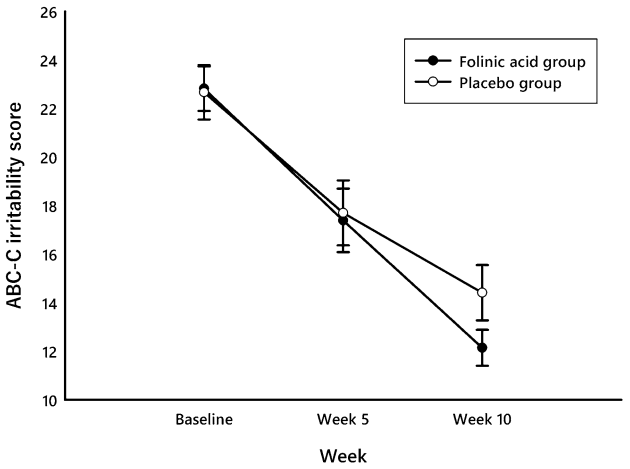

Fig. 6 Comparison of ABC-C irritability subscale score [mean (standard error)] between the folinic acid and placebo groups

Table 4 The frequency of adverse events in the study populations

Side effect	Folinic acid group (n = 28)	Placebo group (n = 27)	P-values
Dizziness, n, %	4 (14.3%)	1 (3.7%)	0.352
Sedation, n, %	3 (10.7%)	2 (7.4%)	1.000
Abdominal pain, n, %	4 (14.3%)	3 (11.1%)	1.000
Increased appetite, n, %	7 (25.0%)	3 (11.1%)	0.295
Headache, n, %	1 (3.6%)	5 (18.5%)	0.101
Diarrhea, n, %	5 (17.9%)	3 (11.1%)	0.705
Constipation, n, %	4 (14.3%)	3 (11.1%)	1.000
Rash, n, %	1 (3.6%)	0 (0.0%)	1.000

P-value of < 0.05 was considered statistically significant

* Fisher's Exact test was used for comparison of all adverse events

it was demonstrated that improvement in GSH redox status was positively correlated with improvement in expressive communication, personal and domestic daily living skills, and interpersonal, play-leisure, and coping social skills. In 2018, the same group of researchers conducted a 12-week double-blind placebo-controlled clinical trial in forty-eight ASD children with language impairment to assess the effects of high-dose folinic acid (2 mg/kg) on verbal communication and behavioral symptoms of ASD [39]. They reported that treatment with folinic acid leads to improvements in all ABC-C scales and daily living subscale of VABS. Our findings are in agreement with these studies showing significant improvements in all ABC-C subscales, except for lethargy/social withdrawal.

Among the underlying mechanisms of ASD, abnormal folate metabolism has been suggested to play a key role, possibly thorough disrupted methylation activity, excessive oxidative stress, and mitochondrial impairment. It has been demonstrated that ASD is linked to polymorphisms in genes encoding folate pathway proteins [47]. Furthermore, maternal treatment with folic acid leads to lower risk of ASD in the offspring [26, 27, 48]. A notable portion of ASD patients might have features of cerebral folate deficiency, whose symptoms improve with folinic acid treatment [24, 49, 50]. Studies showed that FRα dysfunction is probably due to blockade or binding by FRα autoantibodies (FRAAs) [23, 51] and associated with mitochondrial impairment [52]. In a recent study, it was found that blocking and binding FRAAs were present in 60% and 44% of ASD children [28], which is much higher than 3% prevalence reported in non-autistic children with development delay [53]. Moreover, the levels of folate were reported to be low in CSF of 23% of ASD children who had lumbar puncture [54]. Animal studies have also shown that impairments in folate metabolism due to FRAAs during pregnancy lead to ASD-related behaviors in the offspring [35]. Besides, it is documented that folate plays a key role in alleviating oxidative stress through the production of GSH, a major intrinsic antioxidant [55]. In this regard, it was reported that concurrent administration of low-dose folinic acid and methylcobalamin leads to increased generation of glutathione and improvement of development in ASD patients [51]. In our study, the beneficial effects of folinic acid on ASD behavioral symptoms might be through several mechanisms. Folate-dependent one-carbon metabolism can be regulated by folinic acid [56]. In addition to FRα, folinic acid can use alternative routes to cross the blood-brain barrier, such as the reduced folate carrier [28, 51]. This is particularly important when the FRAAs, mitochondrial impairment, or genetic mutations disrupt FRα function [28, 51].

In this study, there was no difference in the frequency and severity of side effects between folinic acid and placebo groups, folinic acid was well tolerated, and no unforeseen

side effects were reported. Similarly, other clinical studies found no increased frequency or severity of side effects in patients receiving folinic acid. However, the most frequent adverse events were agitation [39], insomnia [39, 46], and hyperactivity [39, 46].

The current study is accompanied by a number of limitations. The relatively small sample size of participants is a major limitation of our study, which necessitates further studies with larger sample sizes to verify our findings. Furthermore, the 10-week duration of the study may not reveal the long-term therapeutic effects and side effects of folinic acid. Finally, the findings of this study are limited to adjunctive therapy with folinic acid, and no interpretation could be made regarding monotherapy with folinic acid from the findings of this study.

Summary

Among the associated symptoms of ASD, inappropriate speech has gained considerable attention as it might significantly affect communication, learning, and development in ASD children. The current pharmacotherapy for ASD behavioral symptoms, particularly inappropriate speech, is not ideal. The pathophysiology of ASD is closely linked with abnormal folate metabolism. Thus, in this double-blind, placebo-controlled randomized trial, we investigated the beneficial effects of adjunctive treatment with folinic acid on inappropriate speech and other behavioral symptoms in ASD children. Our findings substantiated that adjunctive treatment with folinic acid led to significant improvements in primary outcome measure (inappropriate speech) and secondary outcome measures (stereotypic behavior, hyperactivity/noncompliance, and irritability). Our findings suggest that folinic acid and probably nutrients containing high levels of folate could be recommended as beneficial complementary supplements for improving speech and behavioral symptoms in children with ASD.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The trial was approved by the institutional review board and the ethics committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.503) and was in agreement with the Declaration of Helsinki. This trial is registered in the Iranian Registry of Clinical Trials with IRCT20090117001556N114 as the registration code.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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